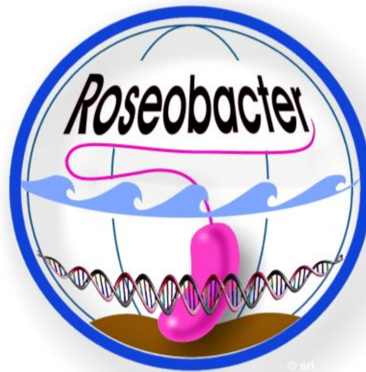
Transregional Collaborative Research Centre (TRR 51)

Ecology, Physiology and Molecular Biology of the *Roseobacter* Group:

Towards a Systems Biology Understanding of a Globally Important Group of Marine Bacteria

Final Report

2010-2022



Coordinating University

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2. Published Results

The 50 most important published results of the entire funding phase

2.1 Publications with scientific quality assurance, i.e. peer-reviewed articles in international journals

1. **Berger, M.**, A. **Neumann**, S. **Schulz**, M. **Simon**, **T. Brinkhoff** (2011). Tropodithietic acid production in *Phaeobacter gallaeciensis* is regulated by *n*-acyl homoserine lactone-mediated quorum sensing. J Bacteriol. 193: 6576–6585 (https://doi:10.1128/JB.05818-11). **[A1, B2, C2]**
2. **Beyersmann, P. G.**, **Tomasch**, J, Son K, Stocker R, **Göker** M, **Wagner-Döbler**, I, **Simon**, M, **Brinkhoff** T (2017). Dual function of tropodithietic acid as antibiotic and signaling molecule in global gene regulation of the probiotic bacterium *Phaeobacter inhibens*. Sci. Rep. 7: 730 (https://doi:10.1038/s41598-017-00784-7). **[A1, A6, B2, B4]**
3. **Billerbeck** S, **Wemheuer** B, **Voget** S, **Poehlein** A, **Giebel** HA, **Brinkhoff** T, Gram L, Jeffrey WH, **Daniel** R, **Simon** M (2016). Biogeography and environmental genomics of the *Roseobacter* group affiliated pelagic CHAB-I-5 lineage. Nature Microbiol. 1: 16063 (https://DOI:10.1038/NMICROBIOL.2016.63) **[A1, Z02]**
4. **Bischoff** V, **Bunk** B, **Meier-Kolthoff** J, Spröer C, **Poehlein** A, **Dogs** M, Nguyen M, **Petersen** J, **Daniel** R, **Overmann** J, **Göker** M, **Simon** M, **Brinkhoff** T, **Moraru** C (2019). Cobaviruses – a new globally distributed phage group infecting Rhodobacteraceae in marine ecosystems. ISME J: 13, 1404–1421 (https://doi:10.1038/s41396-019-0362-7). **[A1, A5, A6, A7, B2, B6]**
5. **Brinkmann** H, **Göker** M, Koblížek M, **Wagner-Döbler** I, **Petersen J** (2018). Horizontal operon transfer, plasmids, and the evolution of photosynthesis in *Rhodobacteraceae*. ISME J 12: 1994–2010 (https://doi:10.1038/s41396-018-0150-9). **[A5, A6, B4]**
6. **Dlugosch** L, **Poehlein** A, **Wemheuer** B, Pfeiffer B, Badewien TH, **Daniel** R, **Simon** M (2022). Significance of gene variants for the functional biogeography of the near-surface Atlantic Ocean microbiome. Nature Comm 13: Article number 456 (https://doi.org/10.1038/s41467-022-28128-8). **[A1, C7, Z02]**
7. **Dogs** M, **Wemheuer** B, **Wolter** L, Bergen N, **Daniel** R, **Simon** M, **Brinkhoff** T (2017). *Rhodobacteraceae* on the marine brown alga Fucus spiralis are predominant and show physiological adaptation to an epiphytic lifestyle. System Appl Microbiol 40: 370-382 (https://doi.org/10.1016/j.syapm.2017.05.006). **[A1, B2, Z02]**
8. **Drüppel** K, **Hensler** M, **Trautwein** K, **Koßmehl** S, **Wöhlbrand** L, **Schmidt-Hohagen** K, **Ulbrich** M, Bergen N, **Meier-Kolthoff** JP, **Göker** M, **Klenk** H-P, **Schomburg** D, **Rabus** RA (2014). Pathways and substrate-specific regulation of amino acid degradation in *Phaeobacter inhibens* DSM 17395 (archetype of the marine *Roseobacter* clade). Environ Microb 16: 218-138 (https://doi:10.1111/1462-2920.12276). **[A6, C1, C3]**
9. **Freese** HM, **Sikorski** J, **Bunk** B, **Scheuner** C, **Meier-Kolthoff** JP, Spröer C, Gram L, **Overmann** J (2017). Trajectories and drivers of genome evolution in surface-associated marine *Phaeobacter*. Genome Biol Evol 9: 3297-3311 (https://doi.org/10.1093/gbe/evx249). **[A6, A7]**
10. **Giebel** HA, Arnosti C, Badewien TH, **Bakenhus** I, Balmonte JP, **Billerbeck** S, **Dlugosch** L, Henkel R, **Kuerzel** B, Meyerjürgens J, **Milke** F, Voss D, **Wienhausen** G, Wietz M, Winkler H, **Wolterink** M, **Simon** M (2021). Microbial growth and organic matter cycling in the Pacific Ocean along a latitudinal transect between subarctic and subantarctic waters. Front Mar Sciences 8: 764383. (https://doi.org/10.3389/fmars.2021.764383). **[A1, A8, C7]**
11. **Giebel** HA, **Wolterink** M, **Brinkhoff** T, **Simon** M (2019). Complementary energy acquisition via aerobic anoxygenic photosynthesis and carbon monoxide oxidation by *Planktomarina temperata* of the *Roseobacter* group. FEMS Microb Ecol 95: fiz050 (https://doi.org/10.1093/femsec/fiz050). **[A1]**

12. **Giebel**, H.-A., D. **Kalhoefer**, R. Gahl-Janssen, Y.-J. Choo, K. Lee, J.-C. Cho, B.J. Tindall, E. Rhiel, C. **Beardsley**, Ö.O. Aydogmus, S. **Voget**, R. **Daniel**, **M. Simon**, **T. Brinkhoff**. (2013). *Planktomarina temperata* gen. nov., sp. nov., belonging to the globally distributed RCA cluster of the marine *Roseobacter* clade, isolated from the German Wadden Sea. Int J Syst Evol Microbiol 63: 4207–4217 (https://doi.org/10.1099/ijs.0.053249-0). **[A1, Z02]**
13. Hördt A, García-López M, **Meier-Kolthoff JP**, Schleuning M, Weinhold LM, Tindall BJ, Gronow S, Kyrpides NC, Woyke T, **Göker M** (2020). Analysis of 1,000+ type-strain genomes substantially improves taxonomic classification of *Alphaproteobacteria* Front Microbiol 11: 468 (https://doi:10.3389/fmicb2020.00468). **[A6]**
14. **Kalvelage J**, **Wöhlbrand L**, Schoon R-A, Zink F-M, Correll C, Senkler J, Eubel H, Hoppenrath M, Rhiel E, Braun H-P, Winklhofer M, Klingl A, **Rabus R** (2023). The enigmatic nucleus of the marine dinoflagellate *Prorocentrum cordatum*. mSphere (https://doi.org/10.1128/msphere.00038-23). **[C1]**
15. **Klingner**, A., **Bartsch** A, **Dogs M**, **Wagner-Döbler** I, **Jahn** D, **Simon** M, **Brinkhoff T**, J. **Becker** J, **Wittmann** TC (2015). Large-scale 13C-flux profiling reveals conservation of the Entner-Doudoroff pathway as glycolytic strategy among glucose-using marine bacteria. Appl Environ Microbiol 81: 2408-2422 (https://doi.org/10.1128/AEM.03157-14). **[A1, B2, B4, B5, C4]**
16. **Liu** Y, **Brinkhoff** T, **Berger** M, **Poehlein** A, **Voget** S, Paoli L, Sunagawa S, Amann R, **Simon** M (2023). Metagenome assembled genomes reveal greatly expanded taxonomic and functional diversification of the abundant marine *Roseobacter* RCA cluster. Microbiome, in press. **[A1, B2, Z02]**
17. **Meier-Kolthoff** JP, **Göker M** (2017). VICTOR: Genome-based Phylogeny and Classification of Prokaryotic Viruses. Bioinformatics 2017: 1-9 (https://doi:10.1093/bioinformatics/btx440). **[A6]**
18. **Meier-Kolthoff JP**, **Göker** M (2019). TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10: 2182 (https://doi:10.1038/s41467-019-10210-3). **[A6]**
19. **Meier-Kolthoff** JP, Sardà Carbasse J, Peinado-Olarte RL, **Göker** M (2022). TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Research: 50: D801-D807 (https://doi:10.1093/nar/gkab902). **[A6]**
20. **Michael** V, **Frank** O, **Bartling** P, **Scheuner** C, **Göker** M, **Brinkmann** H, **Petersen J** (2016). Biofilm plasmids with a rhamnose operon are widely distributed determinants of the ‘swim-or-stick’ lifestyle in roseobacters. ISME J 10: 2498–2513 (https://doi:10.1038/ismej.2016.30). **[A5, A6]**
21. **Milici** M, Deng ZL, **Tomasch** J, Decelle J, Wos-Oxley M, **Wang** H, Jáuregui R, Plumeier I, **Giebel** HA, Badewien T, Wurst M, Pieper D, **Simon** M, **Wagner-Doebler** I (2016). Co-occurrence analysis of microbial taxa in the Atlantic Ocean reveals high connectivity in the free-living bacterioplankton. Front Microbiol. 7: 649 (https://doi.org/10.3389/fmicb.2016.00590). **[A1, B4]**
22. **Milke** F, Meyerjürgens J, **Simon** M (2023). Ecological mechanisms and current systems shape the modular structure of the global oceans’ prokaryotic seascape. Nature Comm 14: 6141 (https://doi.org/10.1038/s41467-023-41909-z). **[A1]**
23. **Milke** F, **Wagner-Doebler** I, **Wienhausen** G, **Simon** M (2022). Selection, drift and community interactions shape microbial biogeographic patterns in the Pacific Ocean. ISME J 16: 2653–2665. (https://doi.org/10.1038/s41396-022-01318-4). **[A1, B4]**
24. **Noriega-Ortega** BE, **Wienhausen** G, Mentges A, **Dittmar** T, **Simon** M, **Niggemann** J (2019). Does the chemodiversity of bacterial exometabolomes sustain the chemodiversity of marine dissolved organic matter? Front Microbiol 10: 215 (https://doi.org/10.3389/fmicb.2019.00215). **[A8]**
25. Osterholz H, Singer G, **Wemheuer** B, **Daniel** R, **Simon** M, **Niggemann** J, **Dittmar** T (2016). Deciphering associations between dissolved organic molecules and bacterial communities in a pelagic marine system. ISME J 7: 1717-1730 (https://doi.org/10.1038/ismej.2015.231). **[A1, A8, Z02]**

26. Osterholz H, **Niggemann** J, **Giebel** HA, **Simon** M, **Dittmar** T (2015). Inefficient microbial production of refractory dissolved organic matter in the ocean. Nature Comm 6: 7422 (https://doi.org/10.1038/ncomms8422). **[A1, A8]**
27. **Patzelt** D, **Wang** H, **Buchholz** I, Rohde M, Gröbe L, **Pradella** S, **Neumann** A, **Schulz S**, **Heyber** S, Münch K, **Münch** R, **Jahn** D**, Wagner-Döbler** I, Tomasch J (2013). You are what you talk: quorum sensing induces individual morphologies and cell division modes in *Dinoroseobacter shibae*. ISME J 2: 2274-2286 (https://doi.org/10.1038/ismej.2013.107). **[A5, B4, B5, INF]**
28. **Petersen J**, **Brinkmann** H, **Berger** M, **Brinkhoff** T, **Päuker** O, **Pradella S** (2011). Origin and evolution of a novel DnaA-like plasmid replication type in *Rhodobacteraceae*. Mol. Biol. Evol. 28: 1229–1240 (https://doi.org/10.1093/molbev/msq310). **[A5, B2]**
29. **Petersen J**, Vollmers J, Ringel V, **Brinkmann** H, Ellebrandt-Sperling C, Spröer C, Howat A, Murrell C, Kaster AK (2019). A marine plasmid hitchhiking vast phylogenetic and geographic distances. Proc Nat Acad Sci USA 116: 20568-20573 (https://doi.org/10.1073/pnas.1905878116). **[A5]**
30. **Pohlner** M, **Dlugosch** L, **Wemheuer** B, Mills H, **Engelen** B, Reese BK (2019). The Majority of active *Rhodobacteraceae* in marine sediments belong to uncultured genera: A molecular approach to link their distribution to environmental conditions. Front Microbiol 10: 659, (doi.org/10.3389/fmicb.2019.00659). **[A1, A2, Z02]**
31. Segev E, Wyche TP, Kim KH, **Petersen** J, Ellebrandt C, Vlamakis H, et al. (2016). Dynamic metabolic exchange governs a marine algal-bacterial interaction. eLIFE 5: e17473 (https://doi.org/10.7554/eLife.17473). **[A5]**
32. **Simon** M*,* **Scheuner** C, **Meier-Kolthoff** JP, **Brinkhoff** T, **Wagner-Döbler** I, **Ulbrich** M, **Klenk** HP, **Schomburg** D, **Petersen** J, **Göker** M. (2017). Phylogenomics of *Rhodobacteraceae* reveals evolutionary adaptation to marine and non-marine habitats. ISME J 11: 1483-1499 (https://doi:10.1038/ismej.2016.198). **[A1, A5, A6, B2, B4, C3]**
33. Smith AF, Silvano E, **Päuker** O, Guillonneau R, Quareshy M, Murphy A, Mausz MA, Stirrup R, Rihtman B, Aguilo-Ferretjans MA, Brandsma J, **Petersen** J, Scanlan DJ, Yin Chen Y (2021). A novel class of sulfur-containing aminolipids widespread in marine roseobacters. ISME J 25: 2440-2453 (https://doi.org/10.1038/s41396-021-00933-x). **[A5]**
34. **Sultana** S, Bruns S, Wilkes H, **Simon** M, **Wienhausen** G (2023). Vitamin B_{12} is not shared by all marine prototrophic bacteria with their environment. ISME J 17: 836-845. (https://doi.org/10.1038/s41396-023-01391-3. **[A8]**
35. **Thole** S, **Kalhoefer** D, **Voget** S, **Berger** M, Engelhardt T, Liesegang H, Wollherr A, Kjelleberg S, **Daniel** R, **Simon** M, Thomas T, **Brinkhoff** T (2012). *Phaeobacter gallaeciensis* genomes from globally opposite locations reveal high similarity of adaptation to surface life. ISME J 6: 2229-2244 (https://doi.org/10.1038/ismej.2012.62). **[A1, B2, A3/Z02]**
36. **Tomasch** J, **Wang** H, Hall ATK, **Patzelt** D, Preusse M, **Petersen J**, **Brinkmann** H, **Bunk** B, Bhuju S, Jarek M, Geffers R, **Lang** AS, **Wagner-Döbler** I (2018). Packaging of *Dinoroseobacter shibae* DNA into Gene Transfer Agent particles is not random. Genome Biol Evol 10: 359-369 (doi: 10.1093/gbe/evy005). **[A5, A7, B4]**
37. **Tomasch** J, Gohl R, **Bunk** B, Diez MS, **Wagner-Döbler I** (2011). Transcriptional response of the photoheterotrophic marine bacterium *Dinoroseobacter shibae* to changing light regimes. ISME J 12: 1957-1968. (https://doi.org/10.1093/gbe/evy005). **[A7, B4]**
38. **Trautwein K**, **Hensler** M, **Wiegmann** K, Skorubskaya E, **Wöhlbrand** L, **Wünsch** D, Hinrichs C, **Feenders** C, Müller C, Schell K, **Ruppersberg** H, Vagts J, **Koßmehl** S, Steinbüchel A, Schmidt-Kopplin P, Wilkes H, Hillebrand H, **Blasius** B, **Schomburg** D, **Rabus R** (2018). The marine bacterium *Phaeobacter inhibens* secures external ammonium by rapid buildup of intracellular nitrogen stocks. FEMS Microbiol Ecol 94: fiy154. (https://doi:10.1093/femsec/fiy154). **[C1, C3, C7]**
39. **Voget** S, **Wemheuer** B, **Brinkhoff** T, **Vollmers** J, Dietrich S, **Giebel** HA, **Beardsley** C, Sardemann C, **Bakenhus** I, **Billerbeck** S, **Daniel** R, **Simon** M (2015). Adaptation of an abundant *Roseobacter* RCA organism to pelagic systems revealed by genomic and transcriptomic analyses. ISME J 9: 371-384 (https://doi.org/10.1038/ismej.2014.134). **[A1, B2, Z02]**

40. **Vollmers**, J., S. **Voget**, S. Dietrich, K. Gollnow, M. Smits, K. Meyer, **T. Brinkhoff**, **M. Simon**, R. **Daniel** (2013). Poles apart: Arctic and Antarctic *Octadecabacter* strains share high genome plasticity and a new type of xanthorhodopsin. PloS ONE. 8: e63422 (https://doi.org/10.1371/journal.pone.0063422). **[A1, B2, A3/Z02]**
41. **Wagner-Döbler** I, Baumgart B, **Brinkhoff** T, Buchholz I, **Bunk** B, **Cypionka** H, **Daniel** R, Drepper T, Gerdts G, **Hahnke** S, Han C, **Jahn** D, **Kalhoefer** D, Kiss H, **Klenk** HP, Kyrpides N, Liebl W, Liesegang H, Meincke L, **Petersen** J, Piekarski T, Pommerenke C, **Pradella** S, Pukall R, **Rabus** R, Stackebrandt E, **Thole** S, Thompson S, Tielen P, **Tomasch** J, von Jan M, Wanphrut N, Wichels A, Zech H, **Simon** M (2010). The complete genome sequence of the algal symbiont *Dinoroseobacter shibae* – a hitchhiker`s guide to life in the sea. ISME J 4: 61-77, (https://doi.org/10.1038/ismej.2009.94). **[A1, A5, A6, A8, B1, B4, C1]**
42. **Wang** H, Beier N, Boedeker C, Sztajer H, Henke P, **Neumann-Schaal M**, **Mansky** J, Rohde M, **Overmann J**, **Petersen** J, Klawonn F, Kucklick M, **Engelmann** S, **Tomasch** J, **Wagner-Döbler** I (2021). *Dinoroseobacter shibae* outer membrane vesicles are enriched for the chromosome dimer resolution site *dif*. mSystems 6: e00693-20 (https://doi.org/10.1128/msystems.00693-20). **[A5, A8, B4, C6]**
43. **Wienhausen** G, **Dlugosch** L, Jarling R, Wilkes H, **Giebel** HA **Simon** M (2022). Availability of vitamin B_{12} and its lower ligand intermediate alpha-ribazole impact prokaryotic and protist communities in oceanic systems. ISME J 16: 2002–2014 (https://doi.org/10.1038/s41396-022-01250-7). **[A1, A8, C7]**
44. **Wienhausen** G, Bruns S, **Sultana** S, Groon LA, Wilkes H, **Simon** M (2022). The overlooked role of a biotin precursor for marine bacteria - desthiobiotin as an escape route for biotin auxotrophy. ISME J 16: 2599-2609 (https://doi:10.3389/fmicb.2017.01985. **[A8]**
45. **Wienhausen** G, **Noriega-Ortega** BE, **Niggemann** J, **Dittmar** T, **Simon** M (2017). The exometabolome of two model strains of the *Roseobacter* group: A marketplace of microbial metabolites. Front Microbiol. 8: 1985. (https://doi:10.3389/fmicb.2017.01985). **[A8]**
46. **Wiegmann K**, **Hensler** M, **Wöhlbrand L**, **Ulbrich** M, **Schomburg** D, **Rabus R (**2014). Carbohydrate catabolism in *Phaeobacter inhibens* DSM 17395, member of the marine *Roseobacter* clade. Appl Environ Microbiol 80: 4725-4737 (https://doi:10.3389/fmicb.2017.01985). **[C1, C3]**
47. **Wünsch D**, **Trautwein K**, **Scheve S**, **Hinrichs C**, **Feenders** C, **Blasius** B, **Schomburg** D, **Rabus R** (2019). Amino acid and sugar catabolism in the marine bacterium *Phaeobacter inhibens* DSM 17395 from an energetic viewpoint. Appl Environ Microbiol 85: e02095-19 (https://doi.org/10.1128/AEM.02095-19). **[C1, C3, C7]**
48. **Ziesche**, L., **Wolter**, L. **Wang**, H, **Brinkhoff**, T, **Pohlner** M, **Engelen** B, **Wagner-Döbler** I, **Schulz** S (2019). An unprecedented medium-chain diunsaturated N-acylhomoserine lactone from marine *Roseobacter* group bacteria. Marine Drugs. 17: 20 (doi.org/10.3390/md17010020). **[A2, B2, B4, C2]**
49. **Ziesche** L, **Bruns** H, **Dogs** M, **Wolter** L, Mann F, **Wagner-Döbler** I, **Brinkhoff** T, **Schulz** *S* (2015). Homoserine Lactones, Methyl Oligohydroxybutyrates, and Other Extracellular Metabolites of Macroalgae Associated Bacteria of the Roseobacter Clade: Identification and Functions. Chembiochem 16: 2094-2107. (https://doi.org/10.1002/cbic.201500189). **[B2, B4, C2]**
50. **Zucker** F, **Bischoff** V, **Heyerhoff** B, **Poehlein** A, **Freese** HM, Ndela EO, Roux S, Enault F, **Simon** M, **Moraru** C (2022). New Microviridae isolated from Sulfitobacter reveals two cosmopolitan subfamilies of ssDNA phages infecting marine and terrestrial Alphaproteobacteria. Virus Evolution 8: 1-18 (https://doi.org/10.1093/ve/veac070). **[A1, A2, A7, B6, Z02]**

A complete list of publications can be found in Annex I, p. 82-104.

In Annex II, the 30 most important publications are available as full text.

3. Overview of Projects

Project code	Principal investigators, institute, location	Title	Research area	Duration
A1	Meinhard Simon, Thorsten Brinkhoff, ICBM, Univ. Oldenburg	Ecological significance, biogeography and physiology of the *Roseobacter* group in pelagic systems	Microbiology, Microbial Ecology	2010-2022
A2	Bert Engelen, (Heribert Cypionka) (2010-17) ICBM, Univ. Oldenburg	The metabolic potential and the distribution of the *Roseobacter* group in marine sediments	Microbiology, Microbial Physiology	2010-2022
A3	Rolf Daniel, Institut für Mikrobiologie und Genetik, Univ. Göttingen	Assessment and exploitation of the metabolic potential and molecular characterization of uncultivated members of the *Roseobacter*	Microbiology, Metagenomics	2010-2013
A5	Hans-Peter Klenk (2010-13) Silke Pradella, Jörn Petersen, DSMZ, Braunschweig	Evolution and significance of plasmids within the *Roseobacter* group	Plasmid Biology, Microbiology, Molecular Genetics, Phylogenomics	2010-2022
A6	Markus Göker Hans-Peter Klenk (2010-13) DSMZ, Braunschweig	Phylogenomics and functional genomics of the *Roseobacter* group	Microbial genomics, Phylogenomics, Computational Biology	2010-2022
A7	Jörg Overmann DSMZ, Braunschweig	Population structure and divergence in the Roseobacter group - implications for the ecology and evolution	Microbiology, Microbial Ecology, Population Genomics	2011-2022
A8	Thorsten Dittmar, Jutta Niggemann, Meinhard Simon, ICBM, Univ. Oldenburg	Linking the exometabolome of selected pelagic organisms of the Roseo-bacter group to marine dissolved organic matter	Marine Microbiology, Marine Organic Geochemistry	2014-2022
B1	Heribert Cypionka ICBM, Univ. Oldenburg	Physiological response to energy limitation in the *Roseobacter* clade	Microbiology, physiology, bioenergetics	2010-2017
B2	Thorsten Brinkhoff, Meinhard Simon, (2010-13) ICBM, Univ. Oldenburg	Ecological significance of secondary metabolite production by members of the *Roseobacter* group	Microbial ecology, chemical ecology, genetics	2010-2022
B3	Jeroen Dickschat Inst. of Organic Chemistry Univ. Braunschweig	Biosynthetic pathways to secondary metabolites of the *Roseobacter* clade	Organic Chemistry, Molecular Biology	2010-2017

B4	Irene Wagner-Döbler, HZI, Univ. Braunschweig	Cell-cell communication of bacteria of the *Roseobacter* group with other bacteria and algae	Microbiology, Genetics, Ecology	2010-2022
B5	Dieter Jahn, Dr. Elisabeth Härtig, Inst. of Microbiology, Univ. Braunschweig	Regulatory networks for the adaptation of *Dinoroseo-bacter shibae* to changes in oxygen, iron and light	Microbiology, Molecular Biology, Bioinformatics	2010-2022
B6	Cristina Moraru, ICBM, Universität Oldenburg	Bacteriophages of the *Roseobacter* group	Microbiology, Phage ecology,	2018-2022
B7	Jeroen Dickschat Institut für Organische Chemie und Biochemie, Universität Bonn	Interactions between bacteria of the *Roseobacter* group and marine algae via sulfur metabolites	Organic Chemistry, Molecular Biology	2018-2022
C1	Ralf Rabus, ICBM, Univ. Oldenburg	Adaptation of metabolic and cellular networks to changing nutrient conditions in *Phaeobacter gallaeciensis* and *Dinoroseobacter shibae*	Microbial physiology, Proteogenomic s	2010-2022
C2	Stefan Schulz Inst. of Organic Chemistry Univ. Braunschweig	Metabolic profiling and extracellular signalling compounds of *Dinoroseobacter shibae* and other members of the *Roseobacter* group	Organic Chemistry, Natural Product Chemistry, Chemical Ecology	2010-2022
C3	Dietmar Schomburg, (2010-17) Kerstin Schmidt-Hohagen Karsten Hiller (2018-22) Dep. for Bioinformatics & Biochemistry Univ. Braunschweig	Metabolome analysis and modelling of the metabolism of *Dinoroseobacter shibae* and *Phaeobacter gallaeciensis*	Microbial metabolome analysis, systems biology, modelling	2010-2022
C4	Christoph Wittmann, Inst. of Biochemical Engineering, Univ. Braunschweig	Metabolic network analysis of the *Roseobacter* clade: Pathways and pathway fluxes in *Dinoroseobacter shibae, Phaeobacter gallaeciensis* and other members	Systems Biology, Biotechnology	2010-2013
C5	Irene Wagner-Döbler, HZI, Univ. Braunschweig, Dieter Jahn Richard Münch, (2010-17) Inst. of Microbiology, Univ. Braunschweig Ralf Rabus, (2018-22) ICBM, Univ. Oldenburg	Systems biology of *Dinoroseobacter shibae`s* environmental adaptation	Microbiology, Molecular Biology, Bioinformatics	2010-2022
C6	Susanne Engelmann, Institut für Mikrobiologie, TU Braunschweig	Adaptation of *Dinoroseo-bacter shibae* to different kinds of oxidative stress	Molecular microbiology, Proteomics	2014-2017
C7	Bernd Blasius ICBM, Universität Oldenburg	Modelling of physiological bioenergetics and global biogeography of the *Roseobacter* group	Theoretical Ecology, Mathematical Modelling ,	2018-2022

			Complex Systems	
INF	Dieter Jahn, Institut für Mikrobiologie, TU Braunschweig	Information infrastructure, database & bioinformatics tool development		2010-2022
MGK	Heribert Cypionka (2010-17) Thorsten Dittmar, (2018-22) ICBM, Universität Oldenburg	Integrated Research Training Group		2010-2022
Z02	Rolf Daniel, Institut für Mikrobiologie und Genetik, Univ. Göttingen	Assessment and exploitation of the metabolic potential and molecular characterization of uncultivated members of the *Roseobacter* group	Microbiology, Metagenomics	2014-2022

Year of funding	Funding in Euro incl overhead
2010	1.739.963
2011	1.785.172
2012	2.243.894
2013	2.101.160
2014	2.734.747
2015	2.656.806
2016	2.730.071
2017	2.758.310
2018	2.947.940
2019	3.070.469
2020	2.852.700
2021	2.912.369
2022 *	1.280.312
total	31.813.913

* Additional funding provided by DFG as support of TRR51 during Corona restrictions.

4. Research Achievements of TRR51

Before the start of this CRC the *Roseobacter* clade had emerged as one of the most important groups of marine bacteria. Initially this lineage was assumed to be monophyletic, but our work (Simon et al. 2017) showed that it is polyphyletic such that we termed it *Roseobacter* group accordingly. It includes basically all marine *Rhodobacteraceae*. Two great assets formed the basis for our research goals: i) isolates of representative members were available, with eight of them genome sequenced in our own preliminary work; ii) based on culture-independent approaches and predominantly on 16S rRNA gene amplicon data, ample reports showed that members of this group were prominent components of microbial communities in coastal and open ocean systems from temperate to polar regions, in the water column and on surface sediments, biofilms and associated to various micro- and macroalgae and invertebrates. Accordingly, this information on the range of habitats, genomic and physiological data, including a diverse array of secondary metabolites, already showed the great diversity of physiological traits of the *Roseobacter* group. Together with important preliminary work by the applying research consortium on this group of bacteria this information formed the basis of the anticipated work in TRR51 and was coined in the following **basic hypothesis** in the initial proposal:

The genetic configuration of the *Roseobacter* group is most suitable for rapid adaptations resulting in efficient growth via a versatile metabolism in multiple habitats. This hypothesis needed to be substantiated by comprehensive, integrative investigations from evolutionary and functional genomics to ecology, physiology and complex systems biology models. The various project areas and projects addressed specific research topics to understand the *Roseobacter* group on the genomic, genetic, physiological, ecological, chemical and systems biological level, in a coordinated, strongly complementary way. Key features were elucidated in two "model" *Roseobacter* organisms (*Dinoroseobacter shibae* DFL12^{T}, *Phaeobacter gallaeciensis* DSM17395, reclassified as *P. inhibens*).

The initial key research questions were:
What is the taxonomic and phylogenomic structure and diversity of the *Roseobacter* group?
How is the genomic organisation of the different sublineages of the *Roseobacter* group, regarding its partitioning into the genome, chromids and plasmids?
What are the ecology, biogeography, taxonomic diversity and functional traits of the *Roseobacter* group in marine communities in the water column of coastal and open ocean regions and in sediments?
What are typical features of the energy metabolism, primary metabolism, carbohydrate and amino acids catabolism, secondary metabolism and the role of signalling for various cellular functions and in general metabolomics in selected model organisms of the *Roseobacter* group (*P. inhibens, D. shibae, Planktomarina temperata*)?
How do typical model organisms of the *Roseobacter* group interact with other pro- and eukaryotic partners regarding mutual interactions, i.e. chemical cross talk?
The latter two questions intended to elucidate metabolic features in model organisms which we assume to be valid to understand general functional features of members of the *Roseobacter* group in marine ecosystems.
As a consequence of the key research questions and different levels of complexity TRR51 was structured into the research areas A, B, C as depicted in Fig. 1:

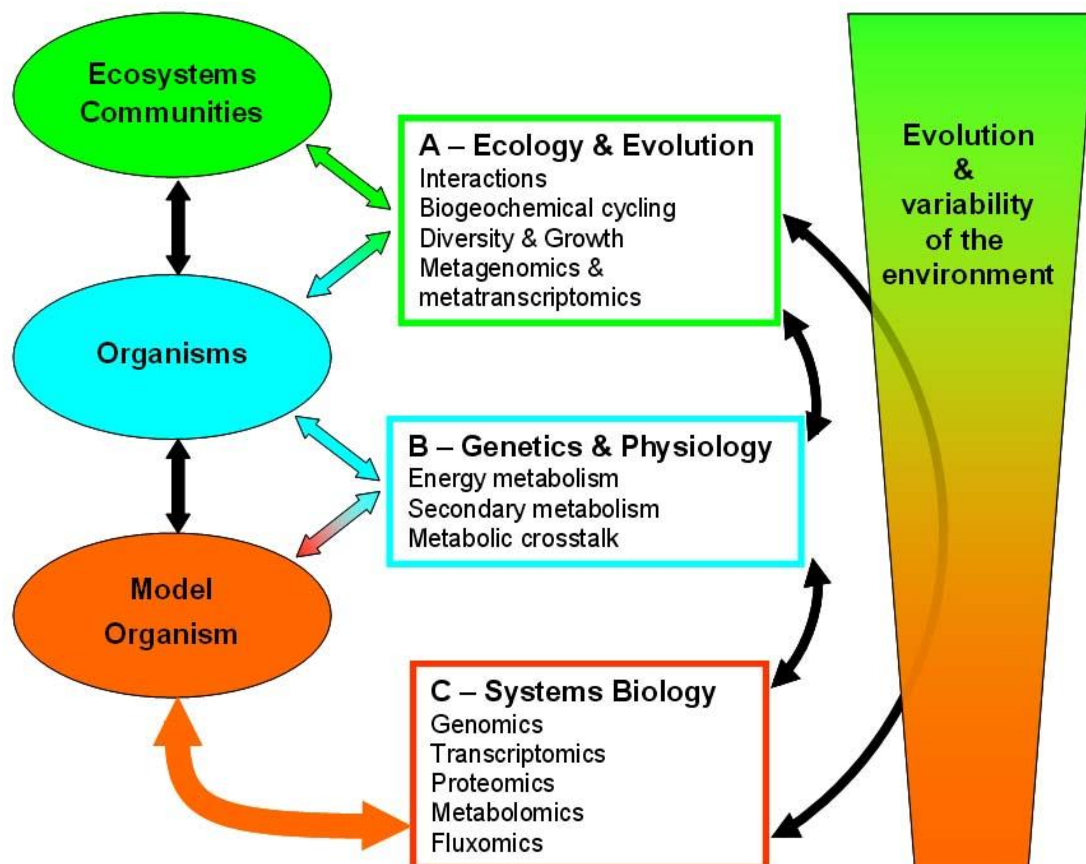

Fig. 1: Structure of TRR51 in its levels of complexity spanning from model organisms to ecosystems and reflected in research areas A, B and C. The levels are interrelated to comprehensively address the basic research questions on the *Roseobacter* group (taken from the original proposal for the first funding phase).

It was clear from the beginning that the research questions could only be properly addressed with a long-term perspective and milestones to be met along this avenue.

Long term goals:
The major long term goals of TRR51 were:
To come to a comprehensive understanding of the phylogeny and taxonomic diversity of the *Roseobacter* group.
To establish a global taxonomic and functional biogeography of the *Roseobacter* group in its major marine systems, the water column and sediments, embedded in the respective microbial communities.
To comprehensively understand principal features of the primary and secondary metabolism of typical model organisms of the *Roseobacter* group.
To successfully apply systems biology approaches to understand the primary metabolism of both model organisms, *P. inhibens*, *D. shibae*.

Medium goals:
For the **initial funding phase** the major medium goals were:
To establish the taxonomic diversity and genome organization of the *Roseobacter* group based on existing organisms and genomic information.
To assess the ecology and biogeography of the *Roseobacter* group in the water column and sediments in the North Sea, the Southern Ocean and the Atlantic Ocean.
To study the primary and secondary metabolism of *P. inhibens* with a special focus on tropodithietic acid (TDA) and of *D. shibae* with a special focus on signalling compounds and the energy metabolism.
To establish *P. inhibens* and *D. shibae* as models for systems biological studies.
For the **second and third funding phases** the major medium goals were:
Extending the phylogenomics and population genomics analyses to novel isolates and genomes of the *Roseobacter* group.
To study exometabolomics of *P. inhibens* and *D. shibae*.
To study starvation and growth under strong energy limitation of *P. inhibens, D. shibae* and *P. temperata.*
To study more specifically chemical cross talk and other interactions between *Roseobacter* organisms and microalgae, i.e. diatoms and dinoflagellates.
To sequence the genome of the two microalgae which serve as models to study interactions with members of the *Roseobacter* group, the diatom *Thalassiosira rotula* and the dinoflagellate *Prorocentrum cordatum* (formerly known as P. *minimum*).
To include the significance and diversity of phages infecting the *Roseobacter* group, roseophages, in the comprehensive investigation of this bacterial group.
To extend the biogeography of the *Roseobacter* group as an important component of the pelagic and sediment-associated microbiome to the Pacific Ocean and thus to form the basis for a global assessment of the biogeography of this group of marine bacteria and the entire oceanic and benthic microbiome.
We are pleased that most of the long term and emerging medium goals and research questions were satisfactorily reached and addressed to a great extent. The **major achievements and results** are presented for the following topics:
Phylogenomics, taxonomic diversity, reclassification, genome organisation (chromids, plasmids), genome size, GC content, population genomics (based on isolates, genomes, metagenome assembled genomes (MAG)).
Taxonomic and functional global biogeography (water column, sediments)

Physiology, primary and secondary metabolism of *P. inhibens* and *D. shibae* with systems biological approaches and of selected other organisms, exometabolomics and B_7 and B_{12} vitamer cycling.
Interactions of *Roseobacter* organisms with other pro- and eukaryotic partners.

Project development in the course of the CRC

There was no need to make any changes to the original concept but we were happy to be able to widen its scope by the addition of projects focussing on i) population genomics in 2011 (A7-Overmann), ii) exometabolomics in 2014 (A8-Niggemann-Simon-Dittmar), iii) roseophages (B6-Moraru) and iv) modelling (C7-Blasius) in 2018. A second project on proteomics (C6-Engelmann) added in 2014 was not as successful as expected and discontinued in the third funding phase. The promising project on metabolic network analyses of the two model organisms (C4-Wittmann) was terminated in 2013 because the PI moved to a different university. Project B1-Cypionka on the physiology and energy metabolism was terminated in 2017 because of the PI's retirement.

Value of collaboration within TRR51

This CRC benefitted greatly from the collaboration of different disciplines and subdisciplines, including molecular and genomic microbiology, microbial physiology and ecology, phage ecology, organic geochemistry, natural product chemistry and modelling. This surplus value is reflected by the fact that 61% of the 272 publications are co-authored by at least 2 projects and 33% by projects from at least two locations.

Phylogenomics, taxonomy and genomic features of the *Roseobacter* group

Our comprehensive analyses of all available information on *Rhodobacteraceae* and related *Alphaproteobacteria* yielded a very comprehensive classification of this family including the *Roseobacter* group. These analyses, based on the Genome BLAST Distance Phylogeny (GBDP) approach (Hördt et al. 2020 [A6]), included information on the genome content, size

Fig. 2: Extracts of Figs. 8 and 9 of Hördt et al. (2020) showing parts of the family *Rhodobacteraceae* of the GBDP tree. Color bars and symbols from left to right: brown: Proteobacteria; green: Alphaproteobacteria; blue triangle: Rhodobacterales; blue diamond: Rhodobactereaceae; light to dark blue square: GC-content, black bar: genome size. For details see Hördt et al. (2020, [A6]).

and GC content of all type strains, many other isolates and a reclassification of quite a few taxa including falsely identified paraphyletic taxa and removal of illegitimate names (Fig. 2). It provides a very important landmark in the classification of described species and isolates of *Rhodobacteraceae* and the *Roseobacter* group. Further reclassification issues of marine and non-marine *Rhodobacteraceae* and thus of the *Roseobacter* group led to the proposal of two new families replacing the illegitimate family *Rhodobacteraceae*, *Paracoccaceae* and *Roseobacteraceae*, the latter encompassing the *Roseobacter* group (Göker 2022b)

The detailed analysis of the genome content of the former *Rhodobacteraceae* revealed that this family originated in the marine environment and that a few sublineages entered non-marine habitats (Simon et al. 2017). These lineages discarded several traits including sodium chloride transport, carbon monoxide oxidation and vitamin B_{12} biosynthesis whereas they gained the Embden-Meyerhof Parnas pathway for glucose catabolism and sulfate transport (Fig. 3). The extant marine *Rhodobacteraceae*, the *Roseobacter* group, gained elaborate traits of osmolyte metabolism.

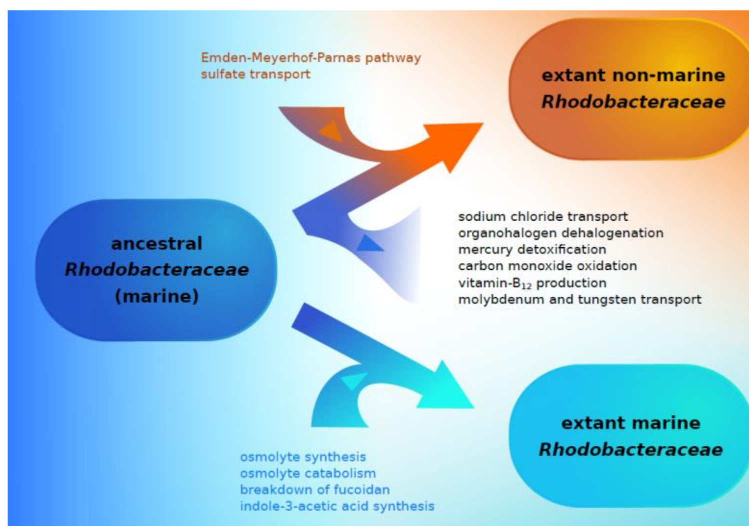

Fig. 3: A summary of the interpretation of the genetic traits of the most recent marine ancestor of *Rhodobacteraceae* significantly gained or lost during adaptation to non-marine and gained during better adaptation to marine habitats. For further details see Simon et al. (2017 [A1, A5, A6, B2, B4, C3]).

The phylogenomic analysis presented above is based on taxa of which isolates were available. Evidence emerged, however, that in particular in open ocean environments (but also in deep sea sediments, Pohlner et al. 2017, 2019) not yet cultured and thus unknown taxa of the *Roseobacter* group exist. In order to capture genomic information on open ocean lineages we made an analysis of 609 high quality MAGs (>90% completeness, <5% contamination) and single amplified genomes (SAGs) recruited from all major metagenomics data sets from the global oceans, including but not limited to Tara Oceans, Malaspina, Biogeotraces, Atlantic Transect 28-4/5, Helgoland Roads and the global ocean reference genome database (GORG). This analysis, carried out in collaboration with Shinichi Sunagawa, ETH Zürich, and Rudi Amann, MPI Bremen, revealed a significantly different size distribution of the genome size and the GC-content of the recruited MAGs as compared to the isolates with a large fraction of

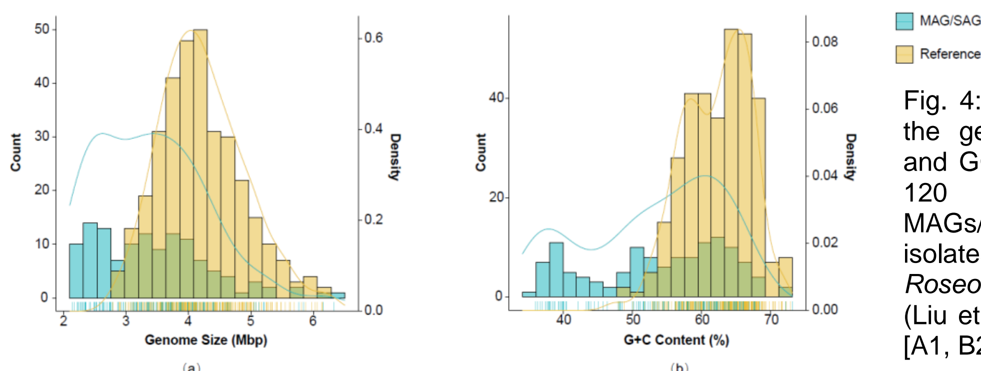

Fig. 4: Density plot of the genome size (a) and GC-content (b) of 120 representative MAGs/SAGs and 345 isolates of the *Roseobacter* group. (Liu et al. unpublished [A1, B2]).

MAGs with lower genome size and GC-content, indicating fundamental differences to the majority of known isolates of the *Roseobacter* group (Fig. 4). This analysis further revealed the existence of three completely novel sublineages of the Roseobactr group, deeply branching and with small genomes and a low GC-content, and a greatly expanded diversification of the *Roseobacter* Clade Affiliated (RCA) cluster (see below) and the Central Oceanic *Roseobacter* (COR) cluster, formerly described as SB2/HIMB11 cluster (Billerbeck et al. 2016; Fig. 5). This analysis indicates that adaptation to pelagic low nutrient environments occurred in several sublineages of the *Roseobacter* group resulting in genome streamlining and a reduction in the GC-content. As most MAGs did not contain a 16S rRNA gene these lineages are not classified by diversity analyses based on 16S rRNA gene amplicon sequencing but lumped together as

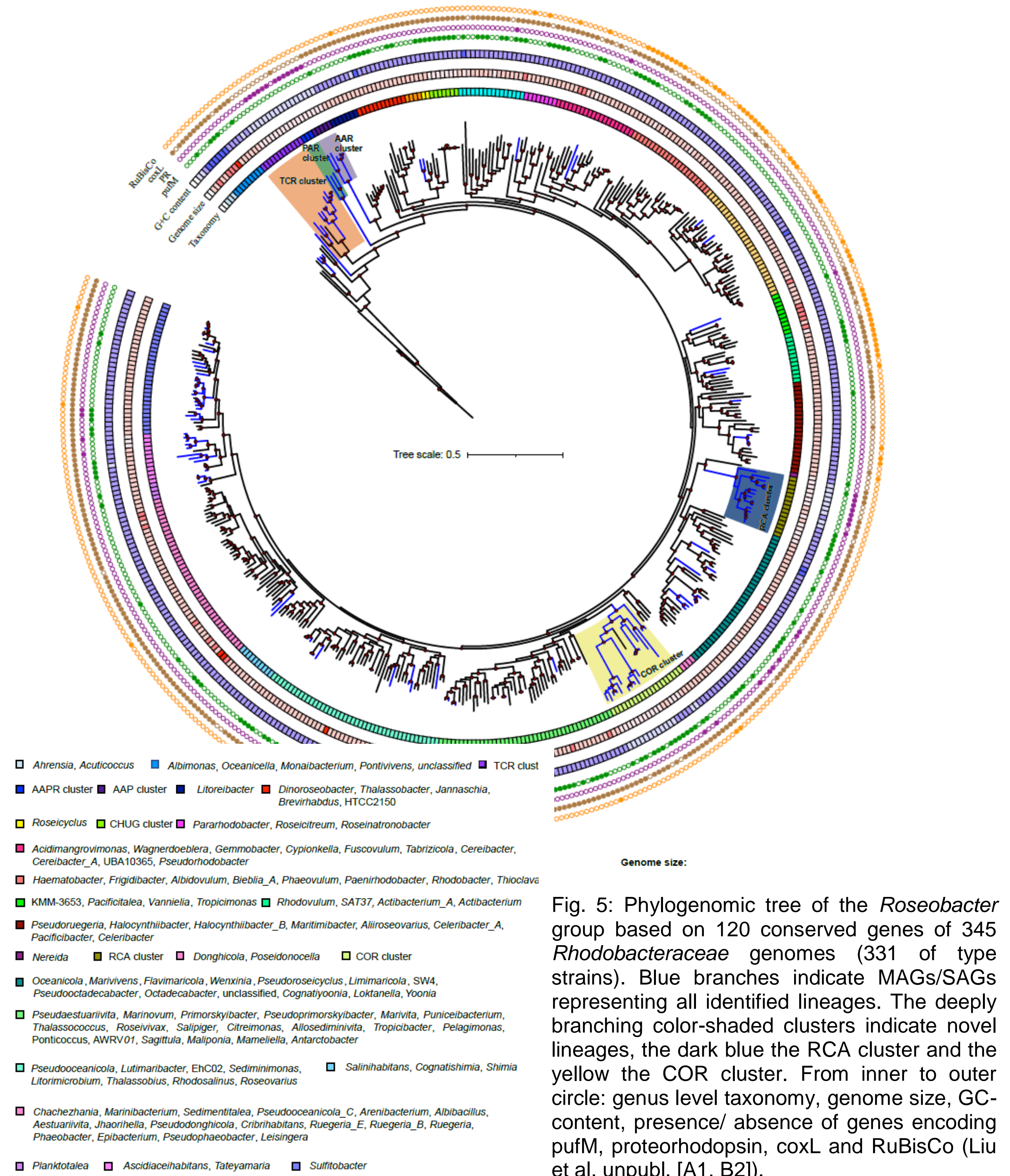

Fig. 5: Phylogenomic tree of the *Roseobacter* group based on 120 conserved genes of 345 *Rhodobacteraceae* genomes (331 of type strains). Blue branches indicate MAGs/SAGs representing all identified lineages. The deeply branching color-shaded clusters indicate novel lineages, the dark blue the RCA cluster and the yellow the COR cluster. From inner to outer circle: genus level taxonomy, genome size, GC-content, presence/ absence of genes encoding pufM, proteorhodopsin, coxL and RuBisCo (Liu et al. unpubl. [A1, B2]).

unknown *Rhodobacteraceae*. The analysis also indicated that acquisition of light energy by proteorhodopsin is much more common among marine pelagic members of the *Roseobacter* group than previously assumed, so far only known from the deeply branching NAC11-7 cluster.

Population genomics and speciation of the genera *Phaeobacter* and *Sulfitobacter*

The genera *Phaeobacter* and Sulfitobacter were subjected to in depth analyses of their speciation. *Phaeobacter* was chosen because the model strain *P. inhibens* belongs to this genus and quite a few isolates were initially available. The genus *Sulfitobacter* is one of the most prominent genera of the *Roseobacter* group and encompasses a great variety of isolates from different habitats.

The population genomics analysis of the genus *Phaeobacter* was based on 88 strains originating from aquacultures in Denmark, France and Spain and coastal marine environments in Australia, France, and Germany. All strains produce the typical brown pigment that is associated with the formation of tropodithietic acid (TDA). The 16S rRNA gene based phylogenetic diversity within this genus is only 0.5%. Two of the three groups of isolates were identified as *P. gallaeciensis* and *P. inhibens* based on their phylogenetic affiliation with the respective type strains, DSM 26640^T and DSM 16374^T. The largest group of strains (47%) belongs to *P. inhibens* and isolates were obtained from all five countries covered by this study and mentioned above. Clustering of the ITS sequences yielded three distinct groups and revealed a 20-fold higher nucleotide substitution rate compared to the 16S rRNA genes within the *P. inhibens* cluster. Phylogenetic distances did not reflect geographic distances indicating absence of allopatric speciation. The genomic comparison, including 32 strains representing unique ITS lineages isolated from different habitats revealed an exceptionally large, highly syntenic core genome. The core chromosome yielded an asymptotic saturation curve indicating that it is robustly predicted based on this set of strains. In contrast, the pan chromosome did not reach saturation. As much as 78 to 87% of the gene content of the *Phaeobacter* strains fell into the large core chromosome that comprised 2920 core genes. The core chromosomal genome phylogeny identified a third clade besides *P. gallaeciensis* and *P. inhibens* (Fig. 6a). This clade is distinct from the two described *Phaeobacter* species and represents a novel species `*P. piscinae*´ (Sonnenschein et al. 2017; Fig. 6a). The phylogenetic network inferred from nucleotide sequences of the core genes showed almost no conflicting phylogenies (Fig. 6b), indicating a low rate of recombination between the different strains. Strains of *P. gallaeciensis* had nearly identical genomes with only 317 polymorphic sites in the *Phaeobacter* core chromosome although they were isolated from different geographic regions and associated with different eukaryotic algae or invertebrates.
The flexible chromosomes are constantly but slightly expanding across all *Phaeobacter* lineages (Fig. 6a). Expansion of the pan chromosome occurs via horizontal gene acquisition rather than gene duplication events, according to a Lambda-Innovation modelling analysis. Between 15 and 25 elements per genome were predicted to be of foreign origin based on tetranucleotide frequency, the analysis of prophages, and of genomic islands. These findings suggest that Gene Transfer Agents (GTA) constitute an important driver of Horizontal Gene

Transfer (HGT) and niche adaptation in the genus *Phaeobacter.* For further details see Freese et al. (2017 [A6, A7]) and Sonnenschein et al. (2017, [A7]).

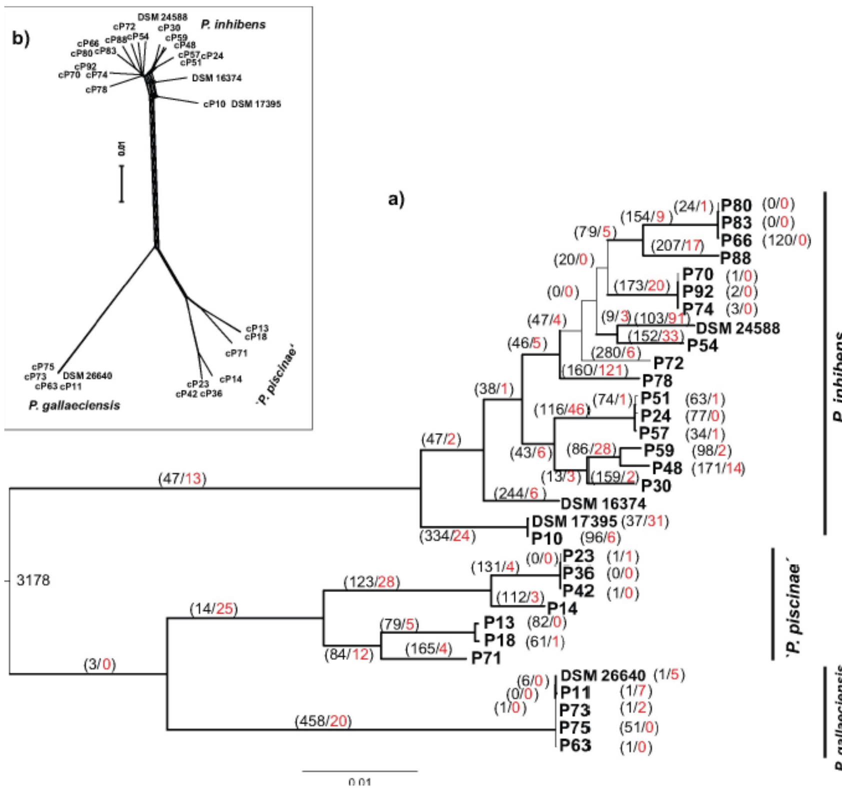

Fig. 6: Phylogenomics of *Phaeobacter*. a) Maxi-mum likelihood (ML) phylogenetic tree inferred from a supermatrix of 1,269,031 aligned amino acid characters. Branches scaled according to the expected number of substitutions per site. Bold edges indicated branches with 100% bootstrapping support. The numbers of inferred gene gains (black) and losses (red) are given next to the corresponding branch. Gene number of the inferred ancestral node is given at the midpoint root. b) Phylogenetic network inferred by split decom-position from the 2,821,782 aligned nucle-otide characters of the concatenated single copy orthologs of 32 *Phaeo-bacter* strains. Scale bar, 0.01 changes per nucleo-tide site (from Sonnen-schein et al. 2017).

For the population genomics study of the genus *Sulfitobacter* the initially available eight closed genomes needed to be supplemented. Therefore, 23 strains from various habitats were isolated and genome sequenced, including samples from cruise SO248 across the Pacific Ocean. Furthermore, closed genomes were generated from 32 strains isolated within TRR51 and by external cooperation partners and available in the DSMZ collection For further details on sequencing of these strains see Freese et al (2022 [A7]).

The phylogenetic analysis of the genomes revealed that a large diversity exists within the genus *Sulfitobacter* (Fig. 7). Many of the investigated strains actually represented novel species, a remarkable fact because this genus already encompassed the second largest number of species within the *Roseobacter* group. The strains representing novel species (Fig. 7) have been characterized and revealed distinct phenotopic differences. For details see report of project A7.

The general genotypic and phenotypic diversity determined for the genus *Sulfitobacter* is much more pronounced than for the surface-associated genus *Phaeobacter* and may reflect its characteristics as generalist. No geographic or habitat preferences could be delineated for the different *Sulfitobacter* clades or species based on their known phenotypic properties except for the *S. porphyrae* clade which occurs in association with phototrophs.

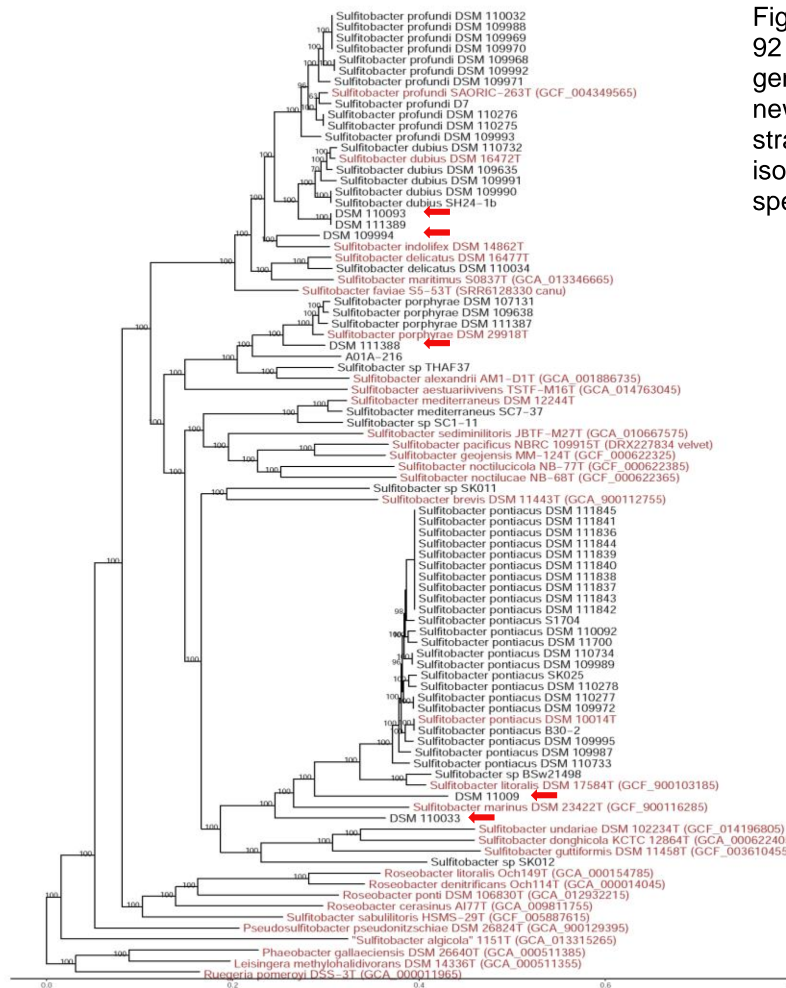

Fig. 7. Phylogenomic tree, based on 92 house-keeping genes, of the genus *Sulfitobacter* encompassing newly sequenced strains and type strains as reference (red). Newly isolated strains representing novel species are indicated by red arrows.

Genomics and diversification of the RCA cluster, the most abundant pelagic *Roseobacter* lineage

The approach to study the diversification of the RCA cluster in the third funding phase by obtaining populations and genomes from this cluster by flow cytometric sorting and applying SAG technology in project A7 was not successful. Only about 70% of the sorted cells showed an amplification signal and the abundance of the RCA cluster in the sorted cells of samples collected in the Pacific Ocean during cruise SO248 was unexpectedly low.

Another approach, based on recruiting MAGs of the RCA cluster from global ocean metagenomics datasets (see above), however, was very successful. On the basis of 82 high quality MAGs and five genomes of isolates three different subclusters were identified as genera based on <70% average nucleotide identity (ANI). They split into 13 sublineages which qualified as distinct species, i.e. >95% ANI). All five genomes from isolates belong to one species, emphasizing that our MAG analysis greatly diversified this important *Roseobacter* cluster. The different genera and species were distinct regarding genome size and GC content with a general trend of greatly reduced genome size and GC content of the newly discovered relative to the known species. Surprisingly, nine of the twelve newly identified species encoded proteorhodopsin (PR). This finding expands the presence of this mode of complementary energy acquisition in pelagic marine bacterial lineages to the majority of the sublineages of the RCA cluster. The different species exhibit distinct global biogeographies (Liu et al. 2023). For further details see report of project A1.

Significance of extrachromosomal replicons

A very distinct feature of the *Roseobacter* group is the wealth of extrachromosomal replicons (ECR) in many species, encompassing up to 13 and about 30% of the genomic information (Petersen et al. 2013, Simon et al. 2017). Therefore one focus was a detailed analysis of the functional role of plasmids. The majority of plasmids was classified as chromids as they encode important constitutive functional features including the production of TDA in *P. inhibens* and the photosynthetic gene cluster in several species (Brinkmann et al. 2018). Other ECR are volatile. The ECR have been classified, mainly by their replication systems, and used for detailed phylogenomic analyses and their role in HGT. In fact, they are instrumental for HGT, as shown for the type IV secretion systems (Petersen and Wagner-Döbler 2017). These features reflect the multipartite genome organization within the *Roseobacter* group enabling a rapid adaptation to changing environmental conditions. These studies have been concerted by project A5 but other projects were involved (A1, A6, A7, B2, B4). For further details see report of project A5.

Roseophages

In the course of the CRC it became more and more evident that phages infecting organisms of the *Roseobacter* group, roseophages, are important members of the marine virome and encoded in quite a few *Roseobacter* genomes. Therefore, a project was launched in the last funding phase which specifically focused on roseophages (B6-Moraru). Large-scale isolation efforts with different bait strains of the *Roseobacter* group including the genera *Sulfitobacter*, *Lentibacter* and *Celeribacter* resulted in the isolation and genomic characterization of more than 250 dsDNA and ssDNA phages. Further, *Roseobacter* genomes and metagenomes were successfully screened for prophages. These results led to the identification of three novel viral subfamilies encompassing roseophages (Bischoff et al. 2019) and quite a few other interesting discoveries including the phage proliferation cycle of a ssDNA roseophage (Zucker et al. 2022) and a new role of the proliferation of a prophage in a *Roseovarius* strain to trigger the release vitamin B_{12}. For further details see reports of projects B6 and A8.

Physiology and secondary metabolites

<u>*Energy metabolism*</u>
Aerobic anoxygenic photosynthesis (AAP) is a feature of the majority of sublineages of the *Roseobacter* group to conserve complementary energy. Many genetic aspects of this trait, the photosynthesis gene cluster (PGC), its phylogenomic distribution, evolution, presence in the genome or on a plasmid of various members of the *Roseobacter* group have been intensely studied (Brinkmann et al. 2018, Petersen et al. 2012). Detailed physiological studies on AAP have been carried out with the model organism *D. shibae*. Its long-time survival of up to 28 days at light-dark conditions and by conserving energy by AAP is greatly favored as compared to dark or continuous light conditions (Fig. 8; Soora and Cypionka 2013). The 72kb chromid, one of the five ECR of *D. shibae*, has a special role for its survival at light-dark conditions as it mitigates the oxidative stress caused by photosynthesis and reflected by upregulating the transcripts of the oxidative stress response at light-dark relative to dark conditions during starvation (Soora et al. 2013). AAP and light do not only promote proton translocation during growth at oxygen respiration but also at nitrate and nitrite respiration, indicating that *D. shibae* is well adapted to greatly changing oxygen concentrations (Kirchhoff et al. 2018).
The beneficial role of AAP during starvation was also shown for *Planktomarina temperata*. Survival during 80 days of starvation was threefold higher at light-dark relative to dark conditions, measured as cell abundance (Giebel et al. 2019). Further, light enabled *P. temperata* to continue to replicate its DNA during the stationary phase relative to a dark control such that when reinoculated into fresh medium growth resumed two days earlier than in control cultures.

These experiments demonstrate the beneficial role of AAP for different lineages of the *Roseobacter* group, exemplified by the model organism *D. shibae* for close interactions with phytoplankton algae and within biofilms, as well as for the pelagic RCA cluster.

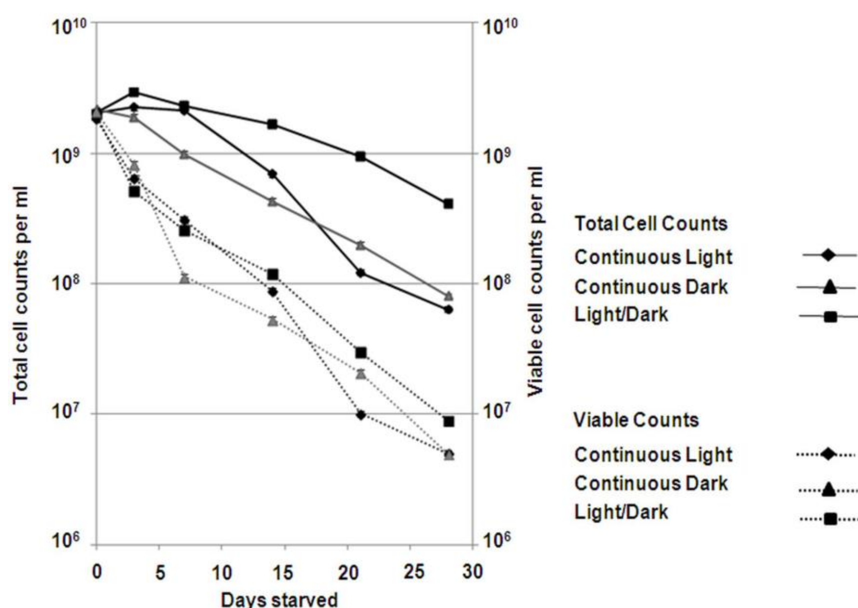

Fig. 8. Changes in total and viable counts of *D. shibae* during starvation. Symbols: total (solid lines) and viable (broken lines) counts of cells starved under light/dark cycles (12/12 h, squares), continuous light (diamonds), or in the dark (triangles). The light intensity was 12 mmol photons m^{-2} s^{-1}. Mean values and standard deviation shown are from two biological replicates. (from Soora and Cypionka 2013)

Chemistry and biology of secondary metabolites, in particular acyl homoserine lactones (AHL) and tropodithietic acid (TDA)

The *Roseobacter* group is well known for the biosynthesis of a variety of secondary metabolites, in particular of AHLs and TDA. These compounds are instrumental in the cell to cell communications of many roseobacters with other microorganisms. Therefore, several projects focused on the chemistry and biological aspects of these compounds. The diversity, structures and synthesis of AHLs among various strains and in particular in the model strains *D. shibae* and *P. inhibens* were comprehensively studied by projects C2-Schulz and B7-Dickschat. AHLs of roseobacters are often distinct and in general relatively long with acyl chains of more than 10 carbons (Wang et al. 2014, Ziesche et al. 2015, 2018). Several AHLs are strain-specific whereas others produced by different strains (Ziesche et al. 2019).
The Roseobacter group is well known to produce and metabolize a variety of organic sulfur compounds, most importantly dimethylsulfoniopropionate (DMSP) which can be further decomposed to dimethylsulfide (DMS) and acrylate (cleavage pathway) or to 3-methiolpropionate and 3-mercaptopropionate (demethylation pathway). Features of the key enzyme of the cleavage pathway, DMSP lyase, have been studied intensely by project B7-Dickschat (Brock et al. 2014, Burkhardt et al. 2017, Dickschat et al. 2015), as well as the decomposition of other organic sulfur compounds produced as well by different strains of the *Roseobacter* group (Chhalodia & Dickschat 2021, 2023). For details see report of project B7.
In several strains of the *Roseobacter* group, especially *Roseovarius* sp., *N*-acylalanine methyl esters (NAMEs) were identified, a new class of compounds (Bruns et al. 2013, Bruns et al. 2018b) whose function is still elusive. The structures were confirmed by synthesis. A genomic analysis of this *Roseovarius* strain yielded no clear evidence for NAME biosynthetic genes.
TDA, produced by several members of the *Roseobacter* group, is one of the compounds most intensively studied by this CRC. Besides its antibiotic activities, it was found to act as signaling molecule in *P. inhibens*, but at 100-fold lower concentrations than the minimal inhibitory concentration against bacteria (Beyersmann et al. 2017). It causes the same regulatory effects in quorum sensing as the common AHL. Furthermore, we revealed concentration-dependent chemotactic effects of TDA, AHLs and extracellular DNA on different roseobacters with

differential responses towards native and foreign compounds, thus further expanding the functions of TDA (Fig. 9). The biosynthesis of TDA was also investigated and the function of the dioxygenase TdaE in the formation of tropone-2-carboxylic acid demonstrated (Duan et al. 2021). The mechanism and enzyme(s) for the incorporation of sulfur still remain to be determined. For further details see report of projects B2 and B7.

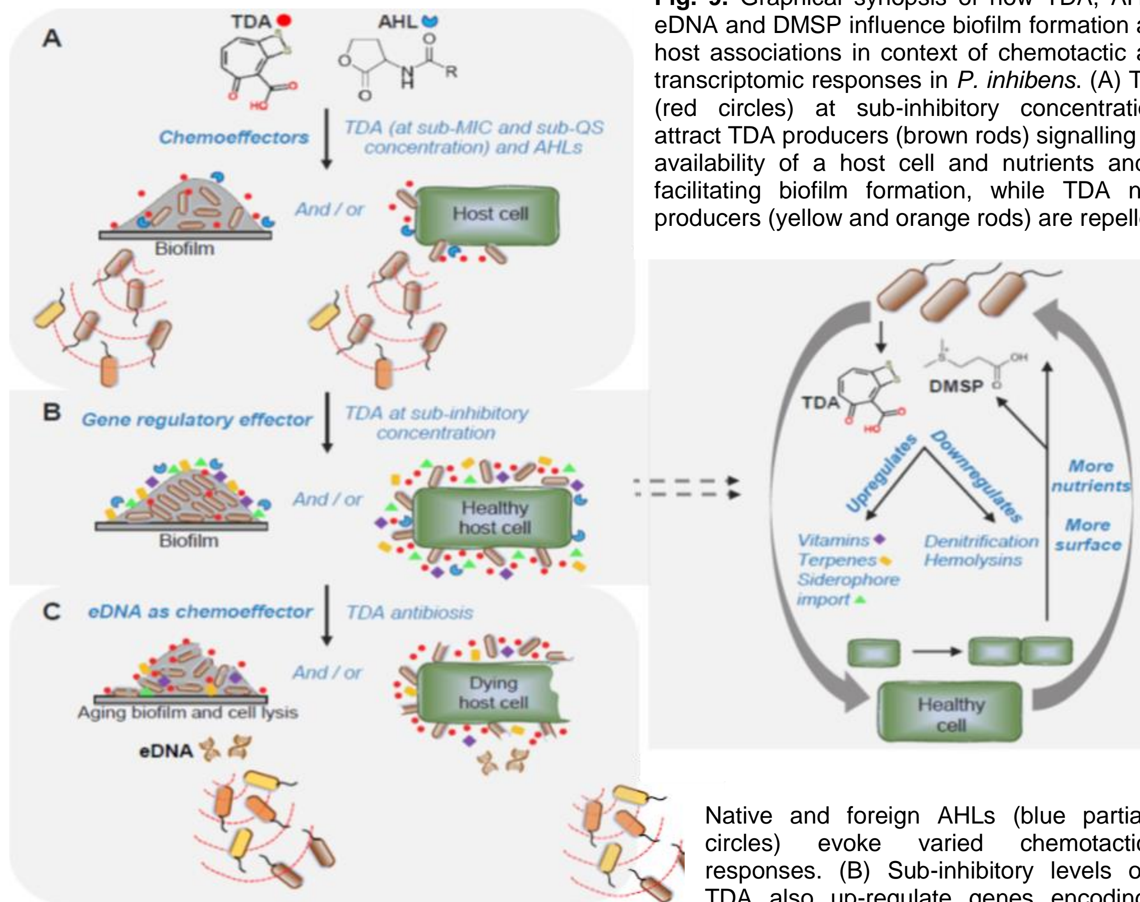

Fig. 9. Graphical synopsis of how TDA, AHLs, eDNA and DMSP influence biofilm formation and host associations in context of chemotactic and transcriptomic responses in *P. inhibens*. (A) TDA (red circles) at sub-inhibitory concentrations attract TDA producers (brown rods) signalling the availability of a host cell and nutrients and/or facilitating biofilm formation, while TDA non-producers (yellow and orange rods) are repelled. Native and foreign AHLs (blue partial circles) evoke varied chemotactic responses. (B) Sub-inhibitory levels of TDA also up-regulate genes encoding vitamin (purple diamond) and terpene (yellow rectangle) biosynthesis as well as siderophore import (green triangle), while down-regulating genes encoding denitrification and hemolysin production. DMSP, a key phytoplankton metabolite, modulates gene expression comparable to TDA. (C) Bacterial surface cell density scales with TDA production. Higher concentrations of TDA repel *P. inhibens*, thus prohibiting further colonization of a matured biofilm. Furthermore, TDA disrupts cell wall integrity, potentially enhancing cell lysis and release of own DNA (brown DNA motifs). Upon sensing own eDNA, *Phaeobacter* cells are repelled, preventing further colonization. Attraction of other bacteria (like non-TDA-producing *Yoonia* spp.) by foreign eDNA (like that of *Phaeobacter* spp.) might indicate a favorable attachment site, e.g., characterized by nutrients released from lysed cells within the dispersing biofilm. For further details see report of project B2.

Biosynthesis and cycling of vitamin B_{12}

Vitamin B_{12} (cobalamin) is an essential cofactor for numerous metabolic functions of prokaryotes and eukaryotes but produced only by about 30% of all prokaryotes. The *Roseobacter* group is one of the major providers of vitamin B_{12} to marine prokaryotes and eukaryotes. The role of different roseobacters as producers and providers of cobalamin and its building blocks to other organisms has been intensely studied in the course of this CRC (Wagner-Döbler et al. 2010, Dogs et al. 2017, Wienhausen et al. 2017). This work was intensified in the last funding phase. In mesocosm studies in three different regions of the Pacific Ocean it was shown that the supply of B_{12} and the activated form of its lower ligand alpha-ribazole, simulating the supply by roseobacters, stimulate growth and changes in the composition of the pro- and eukaryotic microbial communities (Wienhausen et al. 2022). In bioassay studies with co-cultures of 33 B_{12}-prototrophic strains of the *Roseobacter* group and the B_{12}-auxotrophic diatom *Thalassiosira pseudonana*, it was shown that only 18 of the

producers provide B_{12} to the diatom resulting in growth whereas nine retained B_{12} in the cell (Fig. 10). The other strains either shared B_{12} with the diatom only with the addition of substrate or inhibited the growth of the diatom. These interesting results show that B_{12} prototrophs do not necessarily provide other microbes with this cofactor and imply that the providers have an exporter system which is missing in the retainer strains.
In extensive studies we showed that two bacterial B_{12} auxotrophs can salvage different B_{12} building blocks and jointly synthesize B_{12} but only when a prophage is induced in one partner and proliferates. When cultivated in co-culture a *Colwellia* strain synthesizes and supplies a B_{12} lower ligand. The second B_{12}-auxotroph, a *Roseovarius* strain, uses the B_{12} lower ligand to finalize B_{12} but does not share it. A genome-encoded prophage in *Roseovarius* induces its lytic cycle upon the presence of *Colwellia*, leading to growth of the latter, presumably due to B_{12} release. For further details of these experiments see report of project A8.

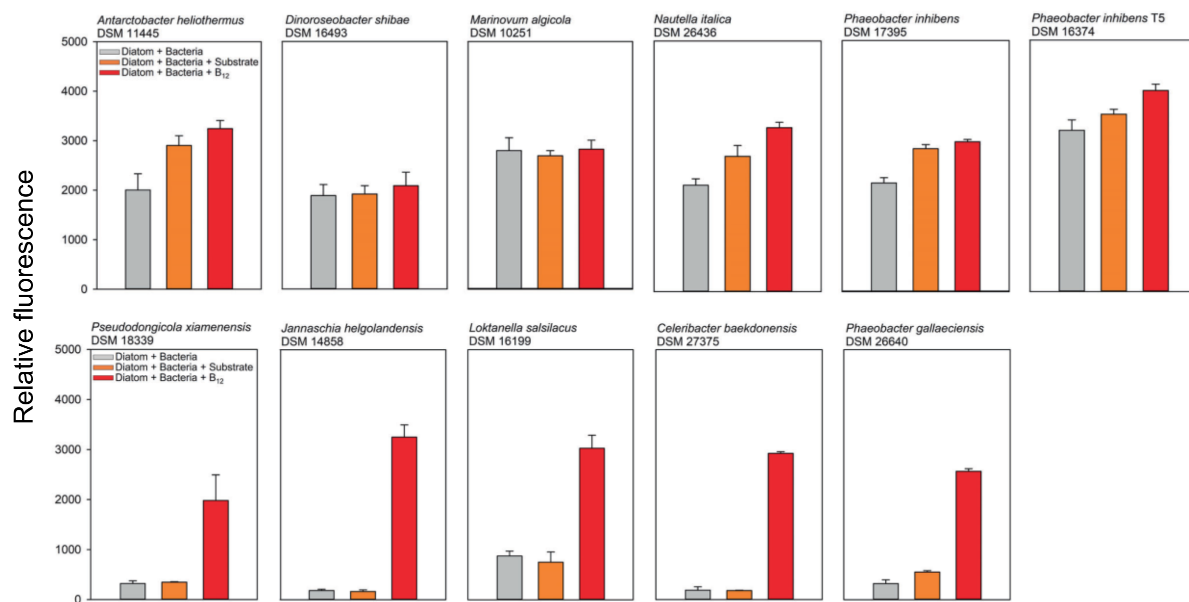

Fig. 10: Maximum growth of *T. pseudonana* in co-culture with B_{12}-providers (upper row) and retainers (lower row). Bars represent the maximum relative fluorescence of *T. pseudonana* during growth in co-culture with B12-providers or retainers under different growth conditions. Grey bars represent maximum relative fluorescence of *T. pseudonana* in co-cultures without further additions, orange co-cultures with an additional substrate mix and red the co-cultures with B_{12} additions. (composite Figure from Sultana et al. (2023). For further details see report of project A8.

Systems biology studies of *P. inhibens* and *D. shibae*

Extensive systems biology studies have been carried out with both model organisms to elucidate specific features regarding substrate spectra and uptake kinetics, elemental growth limitation, energetic costs, growth yield and the role of specific ECR. The establishment of transposon mutant libraries of both organisms was a great asset for these studies.
In the purely heterotrophic *P. inhibens* the metabolism of different monosaccharides and amino acids were intensely studied. Enzymes involved in the catabolic pathways of different monosaccharides were idensified by proteomic and genetic analyses of these pathways and compared to other *Rhodobacteraceae*. It revealed distinct catabolic features of the monosaccharide catabolism of several subgroups of the *Roseobacter* group and other marine bacteria (Fig. 11; Wiegmann et al. 2014). Glucose and other monosaccharides feeding into this catabolic pathways are metabolized via the Entner-Doudoroff (ED) pathway. Detailed analyses by stable isotope labelling showed that this pathway is predominant for glucose catabolism in marine bacteria (Klingner et al. 2015). This is strikingly different from terrestrial model strains, which preferentially use the Embden-Meyerhoff-Parnas (EMP) pathway which

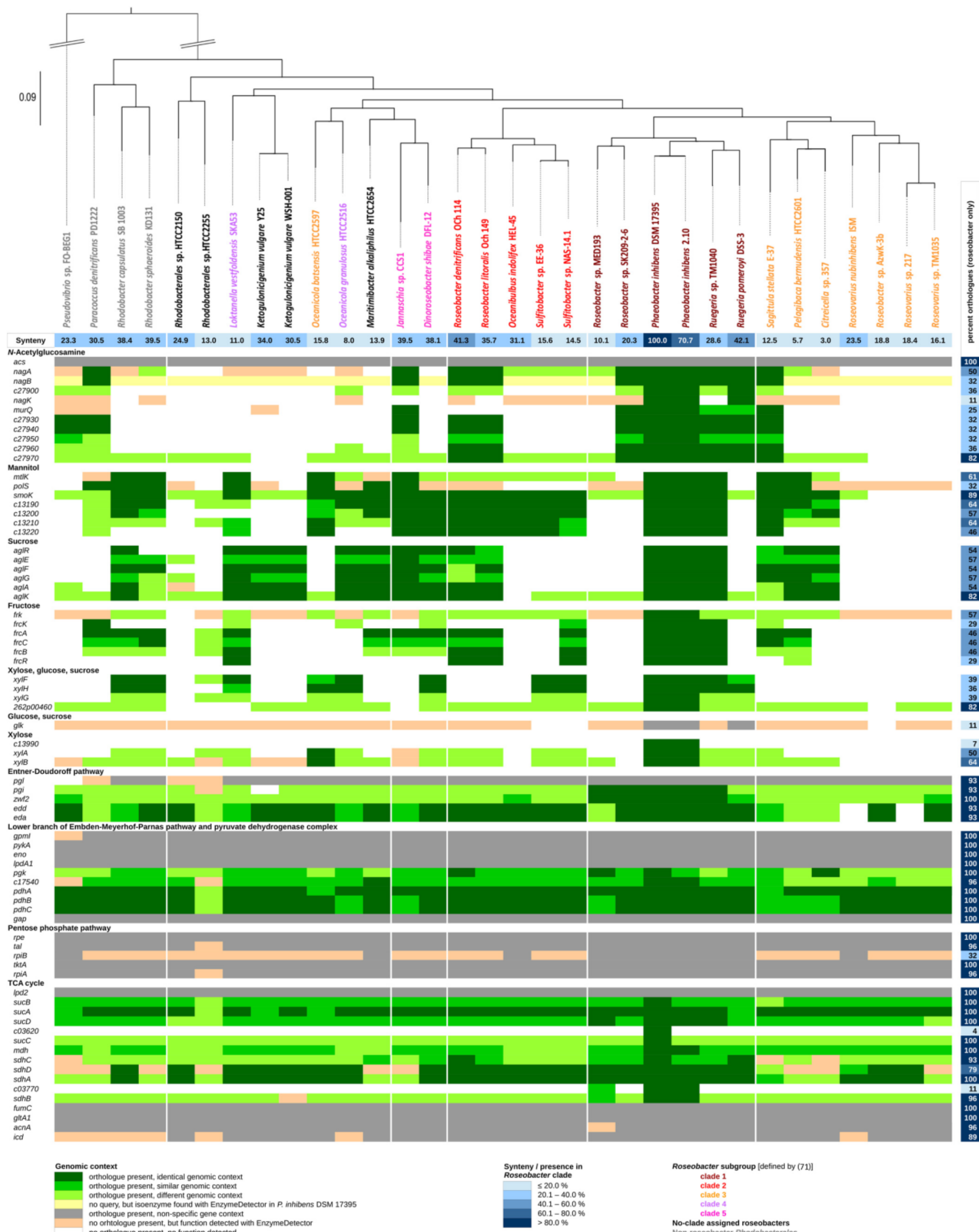

Fig. 11: Genes related to carbohydrate transporters and degradation pathways investigated in *P. inhibens* and other Roseobacter group members in comparison to non-*Roseobacter Rhodobacterales* (for further details and abbreviations see Wiegmann et al. 2014).

yields higher ATP levels, in contrast to higher levels of NADPH obtained by the ED pathway. Marine bacteria such as *P. inhibens* and *D. shibae*, using the ED pathway, however, exhibited a more robust resistance against the oxidative stress typically found in sunlit marine environments. This protective feature may explain the predominance of the ED pathway in marine bacteria.

In another series of experiments monosaccharides and amino acids and their degradation pathways in *P. inhibens* were assessed for energetic efficiencies based on catabolic ATP

yields, calculated from net formed ATP and reducing equivalents. The efficiencies of carbon assimilation into biomass ranged from 28% to 61%, with His/Trp and Thr/Leu yielding the lowest and highest levels (Wünsch et al. 2019). Further, the responsiveness to low, i.e. ambient concentrations of monosaccharides and amino acids were studied yielding rapid responses (Weiten et al. 2022). For details see report of project C1.
The production of the antimicrobial compound TDA was shown to be a substantial metabolic burden to *P. inhibens*. When growth of the wild type was compared to that of a mutant cured of the 262 kb plasmid encoding TDA biosynthesis or a transposon mutant impaired of TDA biosynthesis, the growth yield of the wild type was only 50% of that of the mutants impaired of TDA biosynthesis (Will et al. 2017). Hence, TDA biosynthesis must be of key importance for *P. inhibens* to successfully compete in marine microbial communities.
In the photoheterotrophic *D. shibae* the role of light, ECR and motility for energy demand and growth was assessed in genome-scale metabolic models and experimentally validated at many different environmental conditions (Rex et al. 2013). This comprehensive analysis revealed the key role of light availability on many metabolic processes at growth limiting conditions and the essential role of ECR which encode essential metabolic traits thus complementing other studies on the energy metabolism of this organism (see above). The significance of specific AHLs and role of quorum sensing for cell division, flagellar biosynthesis and type IV secretion system was demonstrated in extensive experimental analyses with the wild type and different mutants of this model organism (Fig. 12; Patzelt et al. 2013)

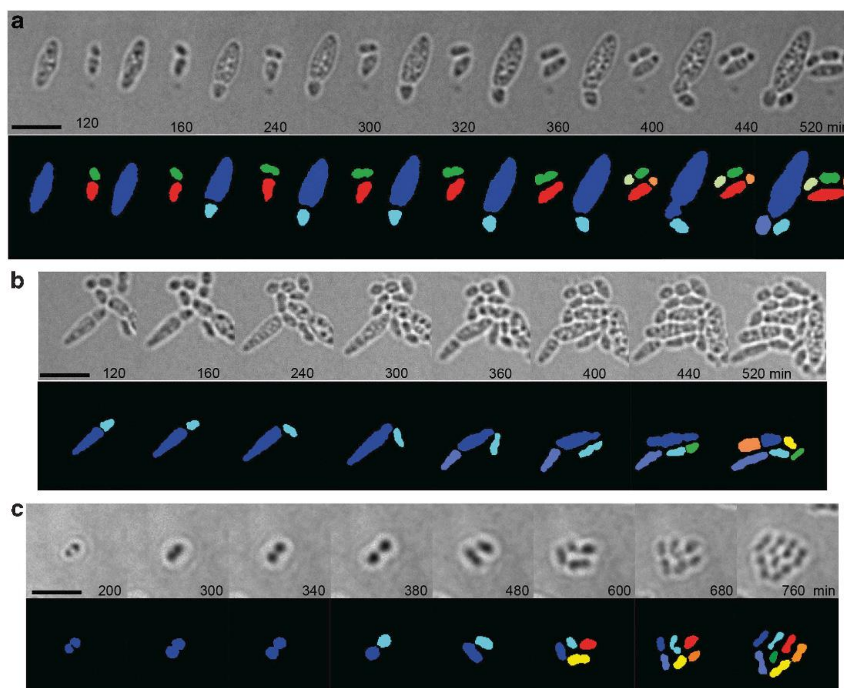

Fig. 12. Growth and division of individual *D. shibae* wild-type and DluxI1 cells visualized by time-lapse microscopy. (a) Symmetric (green) and asymmetric cell division (blue, red) co-occur in *D. shibae* wild-type cultures. (b) Example of a wild-type cell (highlighted blue in the lower schematic) showing alternating budding from both cell poles. (c) QS null mutant cells dividing by binary fission. Daughter cells are indicated by novel colors. Scale bar: 5 mm. (From Patzelt el al 2013)

In addition, other features of *D. shibae* were studied as well by systems biology approaches. The simultaneous application of transcriptomic, proteomic and metabolomics analyses yielded valuable insights into the metabolic adaptation to reduced oxygen conditions, showing rapid transcriptomic and proteomic responses but also a balanced adaptation to these new environmental conditions (Laass et al. 2014). Exposure to salinity stress showed that *D. shibae* is able to adapt by producing novel and unusual compatible solutes, alpha-glucosylglycerol and alpha-glucosylglycerate, in addition to glutamate and putrescine (Kleist et al. 2017).
Other topics on various aspects of the metabolism, including further details of ECR, outer membrane vesicles, the regulation of the biosynthesis of the photosynthetic gene cluster and modelling interactions of *D. shibae* with microalgae were investigated as well. For details of these studies see reports of projects B4, B5 and C3.

Genome sequencing and analysis of the model algae for interaction studies with *Roseobacter* organisms, *Prorocentrum cordatum* and *Thalassiosira rotula*.

A major aim of the third funding phase was genome sequencing and analyses of the model algae used for interaction studies with various organisms of the *Roseobacter* group, the dinoflagellate *P. cordatum* (formerly *minimum*) an the diatom *T. rotula*.

<u>*Prorocentrum cordatum*</u>

The genome analysis of *P. cordatum* was completed successfully as well as transcriptomic, proteomic and metabolomics analyses of this bloom-forming dinoflagellate challenged by temperature stress (Dougan et al. 2023). This success was crucially dependent on a very fruitful collaboration with Dr. Cheon Xin Chan, Australian Center for Ecogenomics, University of Queensland, Brisbane, Australia, and Dr. Debashish Bhattacharya, Rutgers University, New Brunswick, USA. The genome of *P. cordatum* shows large, complex structures and multi-level transcriptional regulation (Fig. 13). The high gene density, long introns, and extensive genetic duplication likely reflect genomic hallmarks of bloom-forming dinoflagellates. Its haploid genome has a size of 4.15 Gbp, at least fourfold larger than any of the other five dinoflagellate

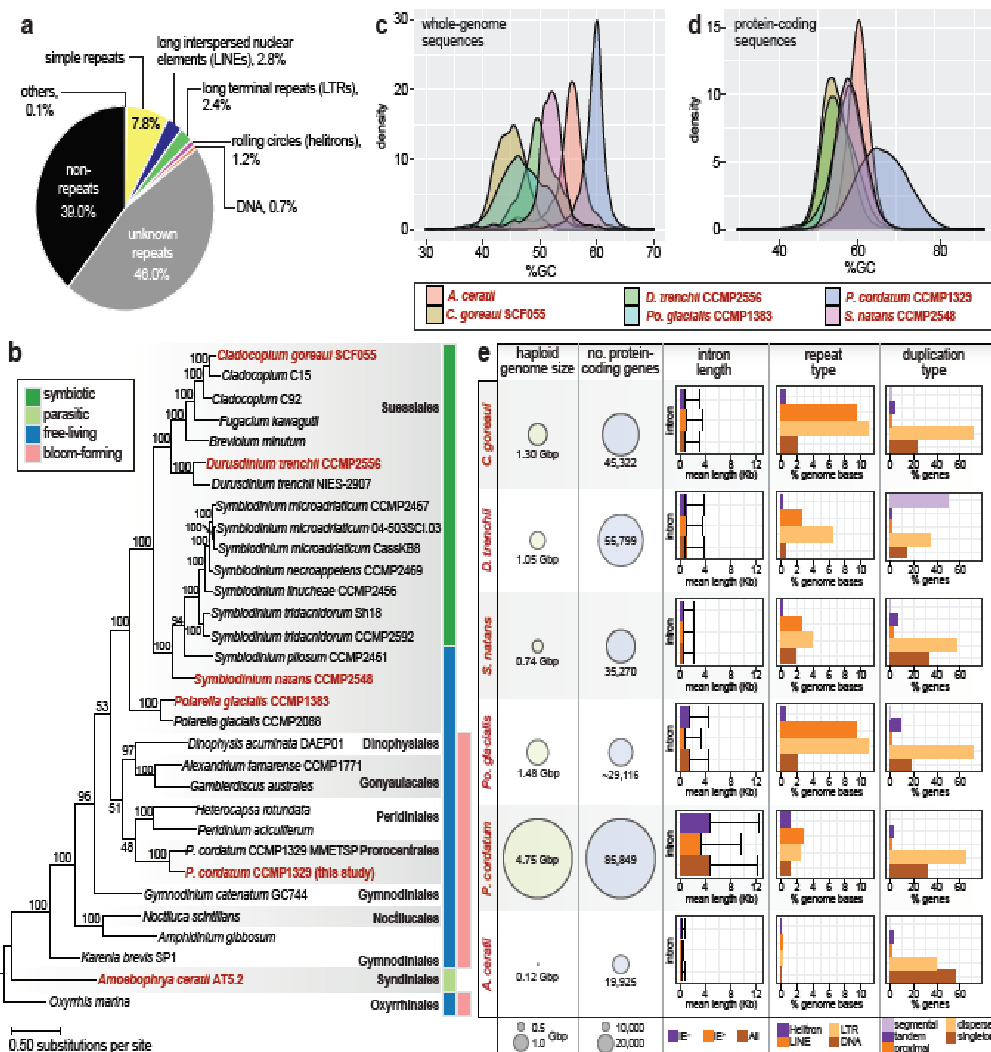

Fig. 13. Genome features of *P. cordatum*. (a) Distribution of repeat types in the genome. (b) Maximum likelihood tree inferred using 3,507 strictly orthologous, single-copy protein sets among 31 dinoflagellate taxa, with ultrafast bootstrap support (based on 2000 replicate samples). The ecological niche for each taxon is shown on the right of the tree. The five representative taxa and *P. cordatum* (this study) are highlighted on the tree in red. Distribution of G+C content for (c) whole-genome sequences and (d) protein-coding sequences relative to

the other five representative genomes. (e) Genome and gene features of *P. cordatum* relative to the other five taxa, showing haploid genome size estimated based on sequence data, number of protein-coding genes, intron lengths, and separately for introns that contain introner elements (IE+), and those that lack these elements (IE–), known repeat types, and types of duplicated genes. For further details see report of project C5 and Dougan et al. 2023)
genomes sequenced so far (Fig. 13e). The genome encodes 85,849 protein coding genes and thus 51% more than the dinoflagellate with the second highest number of protein coding genes, *Durusdinium trenchii*. About half of the genes (52.2%) were of unknown function. *P. cordatum* has the highest G+C content of all genomes of these dinoflagellates, a genomic mean of 59.7% and of 65.9% regarding only the protein coding genes (Fig. 13c,d).
The genome of *P. cordatum*, atypical of any other non-dinoflagellate eukaryote, is organized in highly condensed multiple chromosomes and packed in a dinoflagellate-specific nucleus (dinokaryon). High-resolution FIB/SEM analysis of the flattened nucleus revealed the highest density of nuclear pores in the vicinity of the nucleolus, a total of 62 tightly packed chromosomes (~0.4–6.7 μm^3) and interaction of several chromosomes with the nucleolus and other nuclear structures (Fig. 14)

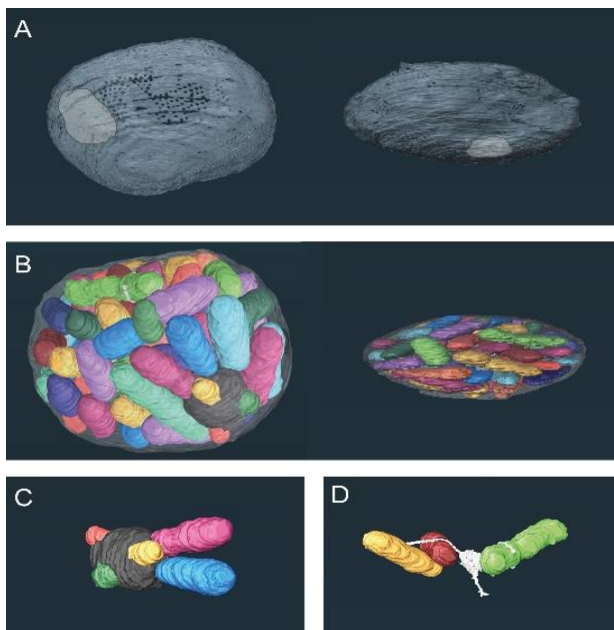

Fig. 14. Three-dimensional reconstruction of the nucleus of *P. cordatum* based on FIB/SEM images. (**A**) Distribution of nuclear pores across the nuclear envelope. Left panel, patch with high number of pores proximal to the nucleolus; right panel, pore-poor region. (**B**) Tight packing of chromosomes in the nucleus. Left panel, top view; right panel, side view. Chromosomes are arbitrarily colored, the nucleolus is marked dark grey and the nuclear membrane displayed transparently. (**C**) Focus on nucleolus with interacting chromosomes. (**D**) Conspicuous structure (white, probably extension of endoplasmic reticulum) interacting with several chromosomes. For further details see report of project C5.

For the results of the heat stress responses of *P. cordatum* assessed by transcriptomics, proteomics and metabolomics analyses, which was reflected in many features of growth, the photosynthesis and central metabolism of this alga, see reports of projects C3 and C5 and Dougan et al. (2023).

Thalassiosira rotula
The genome analysis of *T. rotula* is not yet as far as we had hoped. We sequenced the genome of the strain *T. rotula* CCMP1647 but the annotation and further data evaluation is still ongoing. The genome turned out to be much larger than initially assumed, ~450 Mbp. Sequencing of the genome of another newly obtained axenic strain from the North Sea is still ongoing. For further details see report of projects A1 and Z02.

Global biogeography of benthic and pelagic members of the *Roseobacter* group

Another aim of the third funding phase was to assess global biogeographic patterns of benthic and pelagic members of the *Roseobacter* group in the major ocean basins.
In prokaryotic communities in sediments <20 cm in the Atlantic and Pacific Ocean the *Roseobacter* group constituted less than 1% of the total and about 70% were assigned to unknown representatives as assessed by 16S rRNA gene amplicon sequencing (Pohlner et

al. 2019). In a latitudinal transect across the Pacific Ocean from 30°S to 59°N sampled with RV Sonne the *Roseobacter* group accounted for less than 2% of the total benthic prokaryotic communities as assessed by Catalyzed Reporter Deposition Fluorescence in situ Hybridization (CARD-FISH) and qPCR. 16S rRNA gene amplicon sequencing also showed that the majority of this group affiliated to unknown sublineages (Pohlner et al. 2017). These results indicate that the *Roseobacter* group is only a minor component of marine sediment- associated prokaryotic communities.

Global assessments of the *Roseobacter* group in the epipelagic, in contrast, showed that this group constitutes one of the major components of prokaryotic communities, constituting around 5-10% and in certain environments and during phytoplankton blooms in particular in colder regions of temperate to polar latitudes up to about 30% (Fig. 15). This assessment is based on recruiting 609 quality controlled MAGs (>70% completeness, <5% contamination) from the major metagenomics ocean data sets (see also Fig. 5). These MAGs affiliate predominantly to five sublineages of the *Roseobacter* group which constitute the pelagic *Roseobacter* communities to great extent and exhibit distinct biogeographic patterns. These sublineages are distinct in their genomic features and differ from known lineages of the *Roseobacter* group (see Fig. 4).

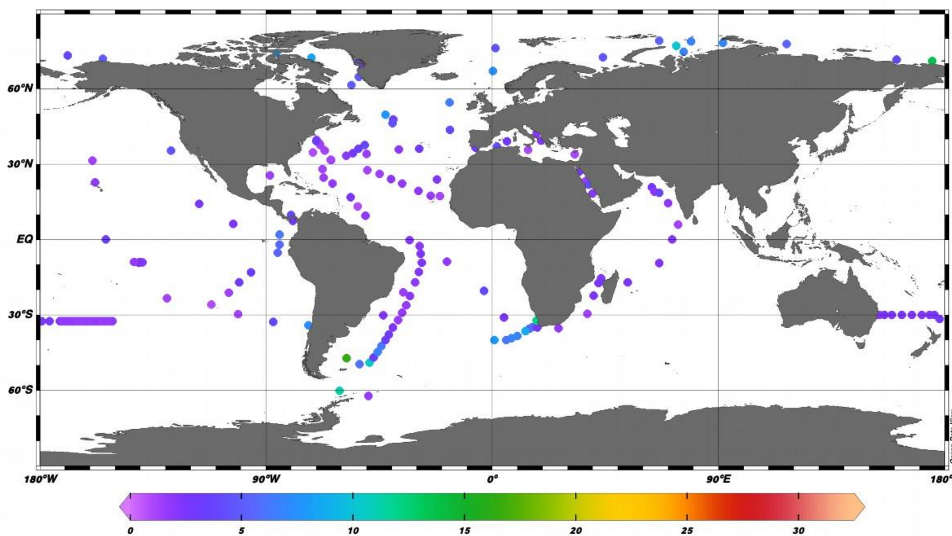

Fig. 15. Distribution and relative abundances of the *Roseobacter* group in the epipelagic of the global oceans. (Liu et al. unpubl.). For further details see Figs. 4 and 5 and report of project A1.

Development of the research field and the role of this CRC

When this CRC started in 2010 research on the *Roseobacter* group was in its early phase and limited by the number of available isolates and genomes. Its significance and relative abundance in various marine ecosystems was mainly based on data assessed by CARD-FISH, clone libraries and fingerprinting of the 16S rRNA gene. Basic features of this group, such as AAP, its significance in decomposing DMSP, first insights into the genomic structure of several members of this group were available. Within the last 13 years, research on the *Roseobacter* group boosted, driven by the increasing interest in this group and technological developments (DNA sequencing, metagenomics, - transcriptomics, -proteomics, data analysis by increasing computational power) and diversified into studies on taxonomy, based on culturing and (meta)genomic analyses, phylogenomics, physiology, secondary metabolites, gene expression by (meta)transcriptomics, proteomics, biodiversity and community studies.

This CRC contributed to the development of these topics and provided valuable information to basically all of them. This CRC was a leading body for this development in phylogenomics, genome organization, secondary metabolites, energy metabolism, systems biology studies of model organisms and biodiversity and community level studies. The topic of the metabolism of organic sulfur compounds was only studied regarding chemical aspects as this topic has been largely covered by another leading group in this field, Mary Ann Moran, University of Georgia, USA.

A taste of the impact of this CRC on research on the *Roseobacter* group is reflected by the fact that it contributed 152 to the 997 references to the keyword *Roseobacter* in the Web of Knowledge (as of July 28, 2023).

4.1 Scientific Events and Science Communication

During all three funding phases TRR51 was very active in having joint status seminars and symposia in Oldenburg/Delmenhorst and Braunschweig which brought together the members of the different locations. The scientific exchange and planning of joint experiments and field studies, also with international experts, was instrumental in creating the successful identity of the TRR51 members.
In total, **TRR51 organized six international symposia, 17 status seminars and a final PhD symposium.** Details on the topics, speakers and programs can be found in Annex I.

The **Integrated Research Training Group** (IRTG, Module Graduierten Kolleg, MGK) organized a broad spectrum of workshops and courses on scientific and soft skills and retreats for the PhD students of each cohort of the three funding phases. Details on the program can be found in Annex I.

Status Seminars of TRR51

1. 2010, 21 October, Helmholtz Center for Infection Research, Braunschweig
2. 2011, 10 February, Helmholtz Center for Infection Research, Braunschweig
3. 2011, 16-17 May, Hanse Institute for Advanced Study, Delmenhorst
4. 2011, 15 September, Helmholtz Center for Infection Research, Braunschweig
5. 2012, 6 February, Helmholtz Center for Infection Research, Braunschweig
6. 2012, 5 June, Helmholtz Center for Infection Research, Braunschweig
7. 2012, 15-16 October, Hanse Institute for Advanced Study, Delmenhorst
8. 2014, 8-9 May, Alter Landtag, Oldenburg
9. 2014, 20 November, Helmholtz Center for Infection Research, Braunschweig
10. 2015, 7-8 May, Alter Landtag, Oldenburg
11. 2015, 9 November, Helmholtz Center for Infection Research, Braunschweig
12. 2016, 23-24 June, Alter Landtag, Oldenburg
13. 2018, 30 November, BRICS Braunschweig Integrated Centre of Systems Biology
14. 2019, 13-14 June, Alter Landtag, Oldenburg
15. 2021, 10, 12, 23 February, online in 3 hours slots
16. 2021, 18 May, online
17. 2022, June 8, BRICS Braunschweig Integrated Centre of Systems Biology

Final PhD symposium of TRR51, 27 April 2022
Main organizers, F. Esser, T. Dittmar, E. Härtig, M. Simon

International Symposia organized by TRR51
Kick-off Symposium of TRR51, 13-15 June 2010,
Main organizer: M. Simon, 80 participants, 6 international and 3 national invited speakers.
International Symposium of TRR51, 24-26 June 2013,
Main organizer: M. Simon, 70 participants, 7 international and 1 national invited speakers.
Kick-off Symposium of TRR51 funding phase 2, 8-9 May 2014
Main organizer: M. Simon, 65 participants, 1 international and 1 national invited speakers.
Kick-off Symposium of TRR51 funding phase 3, 6-7 June 2018,
Main organizer: M. Simon, 80 participants, 3 international invited speakers.
International symposium Marine Microbiota, 28-30 August 2019
Organizers: M. Simon, I. Wagner-Döbler, S. Billerbeck, V. Bischoff, T. Brinkhoff, J. Dickschat, L. Dlugosch, 95 participants, 4 international and 2 national invited speakers.
Closing symposium of TRR51, 4-6 September 2022
Main organizers: M. Simon, I. Wagner-Döbler, 70 participants, 4 international and 3 national invited speakers

TRR51 seminar series

TRR51 was instrumental in organizing the Microbiological Seminar series at ICBM in Oldenburg held every other Wednesday during the semester. Hence during the entire funding phase, including online seminars during most of the Corona lockdown, more than 130 seminar talks were given by invited speakers. For further details on invited speakers see Annex I, p. 10-13. In 2020 and until July 2021 during the Corona pandemic the format was switched to online, which enabled us to invite speakers from other countries and continents who would otherwise never have come to UOL. This format enabled also the non-Oldenburg TRR51 members to participate. Therefore we kept a hybrid format after the online only phase.

During the funding period of TRR51 multiple speakers (ca. 40) covering the topics of the consortium were invited at TUBS in the framework of the different seminar series of biosciences and chemistry. During the Corona lockdown the TUBS groups additionally participated in the online microbiology seminars at ICBM in Oldenburg.

4.2 National and international collaboration

The principal investigators of TRR51 are very well embedded in a national and international scientific community and actively build on these networks. They routinely attended national and international conferences and workshops, partially as invited speakers, and thus contributed to the still increasing visibility of research carried out in the framework of this Roseobacter CRC at ICBM in Oldenburg and at the research institutions in Braunschweig and the other locations. The Hanse Institute for Advanced Study in Delmenhorst (HWK) enabled visits by partners by providing fellowships and thus greatly supported the collaborative research activities. These activities, together with the publications, were a very important support to recognize the Roseobacter group as one of the most interesting and most intensely studied group of marine bacteria.

Most relevant collaboration with the following colleagues going beyond one project were:

Prof. Dr. Rudolf Amann, Max Planck Institute for Marine Microbiology, Bremen. Projects **A1, A8 and B2** collaborated with him regarding a global analysis of metagenome assembled genomes of pelagic members of the Roseobacter group. Publications are submitted and in preparation.

Prof. Dr. Carol Arnosti, University of North Carolina, Chapel Hill (HWK fellow).
She brought in her expertise on bacterial polymer degradation and participated in the research cruise SO248 with RV Sonne across the Pacific Ocean. She coauthored three publications and one submitted manuscript involving projects **A1, B2, Z02**.

Prof. Dr. Debashish Bhattacharya, Rutgers University, New Brunswick, NJ, USA.
Collaboration with him was instrumental in the successful analysis and annotation of the genome of the dinoflagellate *Prorocentrum cordatum*. He is coauthor of the key publication involving projects **A5, A7, B4, B5, C3, C5**.

Dr. Cheon Xin Chan, Australian Center for Ecogenomics, University of Queensland, Brisbane, Australia.
Collaboration with him was instrumental in the successful analysis and annotation of the genome of the dinoflagellate *Prorocentrum cordatum*. He is coauthor of the key publication involving projects **A5, A7, B4, B5, C3, C5**.

Prof. Dr. Yin Chen, University of Warwick, UK.
Collaboration focused on the lipidomic analysis of roseobacters and involved projects **A1** and **A5**, resulting in two publications.

Prof. Dr. Suhelen Egan, University of New South Wales, Sydney, Australia.
Collaboration was built on joint interests on biofilm forming and algal associated roseobacters. She co-authored five publications with projects **A1, B2, C2**.

P**rof. Dr. Jed Fuhrman, University of Southern California, Los Angeles**, USA.
Collaboration included the analysis or proteorhodopsin in marine Flavobacteria, a bacterial group important for comparison to roseobacters, and global analyses of diversity patterns of pelagic prokaryotic communities by projects A1 and B4, resulting in three publications and in a membership in a PhD committee.

Prof. Dr. Lone Gram, Technical University of Denmark, Lyngby, Denmark.
Collaboration included secondary metabolite production by Roseobacters and effects on target organisms and involved projects **A1, A6, A7, B2, B7, C2, Z02** with an outcome of five publications.

Prof. Dr. Michal Koblizek, Czech Academy of Sciences, Trebon, Czech Republic. Collaboration focused on aerobic anoxygenic photosynthesis in members of *Rhodobacteraceae* with projects **A5, A7 and B4** resulting in two publications.

Dr. Yanting Liu, College of Ocean and Earth Sciences, Xiamen University, China. She was a visiting scientist for 18 months, supported by fellowships from DAAD and the Chinese Scholarship Council. Collaboration with projects **A1, A8 and B2** focused on the analysis of metagenome assembled genomes of pelagic roseobacters in the global oceans. One publication is submitted and one in preparation.

Prof. Dr. Hendrik Schäfer, University of Warwick, UK (HWK fellow). Projects **A2 and A1** collaborate with him regarding metagenomics and metatranscriptomic analyses of prokaryotic communities in Wadden Sea sediments with a special focus on metabolism of organic sulfur compounds by roseobacters. Two manuscripts are in preparation.

Prof. Dr. Shinichi Sunagawa, ETH Zürich, Switzerland. Projects **A1, A8 and B2** collaborated with him regarding a global analysis of metagenome assembled genomes of pelagic members of the Roseobacter group. Publications are submitted and in preparation.

Prof. Dr. Andreas Teske, University of North Carolina, Chapel Hill (HWK fellow). Collaboration focused on biofilm-associated roseobacters with projects **A1, A7, B2 and Z02** and resulted in three publications.

Prof. Dr. Torsten Thomas, University of New South Wales, Sydney, Australia.
Collaboration included biofilm forming and algal associated roseobacters and genome analyses of Roseobacter strains. He co-authored seven publications with projects **A1, B2 and Z02**.

Junior Professor Dr. Jan de Vries, University of Göttingen. He is expert in genome analysis of microeukaryotes and thus projects **A1, B2 and Z02** collaborate with him regarding the genome analyses of the diatom *Thalassiosira rotula*. This cooperation started in the final year of the CRC Roseobacter and will continue until these genomes will be fully analyzed an published.

5. Impact on Research Priorities and International Visibility of the Host Institutions

Carl von Ossietzky University of Oldenburg (UOL)
Marine Science is one of the major foci of the university's research topic *Environment and Sustainability*. Its home is ICBM. TRR51 was the university's first CRC in the field of marine

science and therefore of utmost importance for the development of this research focus, in particular after the research unit BioGeoChemistry of the Wadden Sea (FG432) was terminated after eight very successful years in 2009. To further strengthen marine sciences at ICBM the ministry of science and culture of Lower Saxony, in close cooperation with the Max Planck Society, provided extra funds (Aufwuchs Meeresforschung) since 2007 to establish two Max Planck Research groups, to foster marine science at ICBM and to support the acquisition of a CRC in marine sciences. The implementation of a research group on Marine Geochemistry, headed by Thorsten Dittmar, and another one on isotope geochemistry, headed by Katharina Pahnke, and TRR51 were the successful outcomes of this governmental support. Both group leaders were appointed later as professors at UOL and Thorsten Dittmar became a PI of TRR51 in the second funding phase (project A8).
TRR51 was a major asset for the successful competition of UOL for a DFG research center on biodiversity in 2010/11 in which UOL and partners qualified among the final four competitors. Even though UOL was finally not successful, this initiative, headed by Prof. Helmut Hillebrand, fed into the successful application for the Helmholtz Institute of Functional Marine Biodiversity (HIFMB). HIFMB as a joint venture of UOL and the Alfred Wegener Institute for Polar and Marine Research (AWI) was founded in 2017. TRR51 contributed greatly to this success which expanded the visibility and impact of marine science at UOL and of this location as an emerging important player in marine science nationally and internationally. Three of the six founding members of UOL were PIs of this CRC (Bernd Blasius, Thorsten Dittmar, Meinhard Simon). Two of the four newly appointed professors of HIFMB have a focus closely related to marine microbiology, modelling and data science and thus to topics of key importance of TRR51: Prof. Thilo Gross, Theoretical Biology, and Prof. Murat Eren, Ecosystems Data Science.
TRR51 was certainly also helpful in the decision of the state of Lower Saxony that UOL and ICBM became the home of the new Research Vessel *Sonne*, put into service in 2015. The impact of this CRC on the successful development of marine science at UOL was documented most recently by the report of the Wissenschaftliche Kommission Niedersachsen (WKN) in its comparative evaluation of biology research at the universities of Lower Saxony which took place in 2021/22: " *The research profile of UOL biology is characterized by very concise and clear foci...... The committee rates the overall scientific performance of UOL biology as outstanding…….. Members of UOL biology are involved as speakers in seven coordinated research programs (incl. DFG funded SFB1372, TRR51, GRK1885, FOR2716, FOR5094)."*

For the entire report see
(https://www.wk.niedersachsen.de/taetigkeitsbereiche/forschungs_und_strukturevaluation/facherbezogene_forschungsevaluation/facherbezogene-evaluationsverfahren-an-universitaten-191024.html).

Technical University of Braunschweig (TUBS)

Systems biology is a major research focus of the TUBS since about 2010. It originated from the strong modelling focus of the interdisciplinary natural science-engineering projects in the framework of the biotech DFG CRC 578 (From gene to product, 2000-2012) and the systematic biochemistry approach of the DFG Research Unit 1220 (PROTRAIN, 2008 - 2016) with Dieter Jahn serving as speaker/dep. speaker. After a recommendation of the Wissenschaftliche Kommission Niedersachsen (WKN) in 2010 during a SWOT analysis of the biomedical research in whole northern Germany to focus on microbial systems biology at the TUBS, the North German Center of Microbial Genomics (NZMG) was founded with the University of Göttingen and Greifswald. During that time based on the outlined background the Braunschweig Integrated Center of Systems Biology (BRICS), a joint center of the TU, HZI and DSMZ, was planned, build and finally opened in 2016. The central systems biology consortium guiding the research during this exciting phase was TRR 51, since all BRICS founders, TU, HZI, DSMZ, were active members of TRR51. Like in Oldenburg, TRR51 was a major asset for the success of BRICS which greatly expanded the visibility and impact of bacterial systems biology at the Braunschweig research campus, establishing BRICS as an emerging important player in bacterial systems biology nationally and internationally. Quickly, other systems

biology research consortia followed, including the BMBF “UroGenOmics” group, the DFG Research Training Group “PROCOMPAS”, the Lower Saxony Collaborative Research Group “*CDiff* Epidemiology and systems biology of the bacterial pathogen *Clostridium difficile*”. Similar to Oldenburg, the impact of TRR51 on the successful development of research at TUBS was documented most recently by the report of the WKN during its comparative evaluation of biology research at the universities of Lower Saxony which took place in 2021/22: *The research quality in Braunschweig is excellent and the prospects for future innovation (neurometrology, biotechnology) are very promising….The centers have the advantage that they can include research units from other disciplines or even groups from non-university partners (such as the BRICS of TUBS or the HIFMB of UOL for example)* (link, see above).Today, systems biology and BRICS are integral part of TUBS core research area “Engineering for Health” and TRR51 contributed significantly to that.

Measures taken to promote academic achievements accomplished by this CRC

TRR51 has continuously been present as major scientific centers at the home institutions, visible on the home pages as collaborative research projects and on its own home page (https://www.tu-braunschweig.de/brics/forschung/roseobacter-1/). Key publications in high impact journals were always promoted by press releases and contributions in social media (Twitter, Facebook). Major scientific events such as international symposia organized by TRR51 were promoted by press releases. A particular highlight were the continuous blogs of the two cruises with RV *Sonne* in the Pacific Ocean in 2016 and 2017, dedicated almost completely to research of this CRC.
Results achieved by members of this CRC were continuously presented at national and international conferences, such as VAAM annual meetings, ISME symposia, SAME symposia, ASLO Aquatic Sciences meetings, GRC conferences and numerous smaller workshops. Quite a few contributions were invited. Hence, work of this CRC became well known and almost a trade mark of *Roseobacter*-related research in the respective international scientific community.

6. Structural Impact of the CRC

6.1 Staffing

For the successful initial application three decisions were instrumental: Dr. Thorsten Brinkhoff and Dr. Bert Engelen, both UOL, obtained permanent positions. Dr. Jeroen Dickschat returned to TUBS in September 2008 after the successful application for an Emmy Noether group as head of this group.

In 2010, Prof. Jörg Overmann was appointed director of the Leibniz Institute DSMZ and joined TRR51 in 2011 with project A7.

In 2013, Dr. Thorsten Dittmar, head of the Max Planck research group at ICBM, was appointed W3-professor in Marine Geochemistry at UOL, with the status of a Max Planck bridging group (to the MPI of Marine Microbiology Bremen), and joined TRR51 in project A8 for the second funding phase, together with his research associate Dr. Jutta Niggemann.
Dr. Susanne Engelmann was appointed W2-professor for Microbial Proteomics at TUBS/HZI in 2013 and joined TRR51 in 2014 for the second funding phase in project C6.
In 2014, Dr. Jeroen Dickschat, project B3, accepted a W2-Professorship at the University of Bonn but remained a PI in TRR51, of project B7, formerly B3, in funding phase three.
Prof. Christoph Wittmann, project C4, accepted a W3-professorship at Saarland University, Saarbrücken, in 2013 and thus left TRR51 at the end of the first funding phase.
Dr. Thorsten Brinkhoff, project A1 and B2, was appointed adjunct professor at UOL in 2014.
In 2015, Dr. Cristina Moraru was hired at UOL, ICBM, as a young research associate to bring in her expertise on marine virus ecology and to prepare for a new subproject on roseophages, recently identified as a missing topic in TRR51, in the third funding phase, project B6.
In 2018, Professor Heribert Cypionka retired. Therefore his project B1 ended with the second funding phase.
Project C6, headed by Prof. Susanne Engelmann, was discontinued in phase three as a result of a critical internal review process.
Prof. Dietmar Schomburg, project C3, retired in 2018 from his Lower Saxony professorship. His regular position was refilled in 2016 by Prof. Karsten Hiller who took over project C3 in the third funding phase.
In 2018, Professor Bernd Blasius, UOL, joined TRR51 for a modelling project, C7, in the third funding phase.

As all essential topics were covered by existing expertise at the institutions involved in TRR51 or could be filled with research associates, there was no need to fill a specific topic by a targeted opening of a new professorship or attracting a junior group leader, neither at UOL nor at TUBS or other institutions involved. However, several recruitments of scientists listed in the following table were important to TRR51:

Funding phase	Name	Location	Employment level	gender
2010-2013	Overmann, Jörg	DSMZ	W3	male
	Engelmann, Susanne	TUBS, HZI	W2	female
2014-2017	Dickschat, Jeroen	Univ Bonn	W2	male
	Moraru, Cristina	UOL	Junior group leader	female

In phase one, TRR51 expanded by one project (A7) from 18 to 19 projects (including IRTG, INF, Z).
In phase two, TRR51 expanded by two projects (A8, C6) but lost one project (C4) so that in total 20 projects constituted this CRC. As recommended by the evaluation, project A3 was changed into a service project (Z02).

In phase three, TRR51 expanded by two projects (B6, C6) but discontinued two projects (B1, C6) so that the total number of projects remained constant. Project B3 was given a new number, B7, because of the relocation of the PI, Professor Jeroen Dickschat, to the University of Bonn.

TRR51 received favorable ratings at all three on site evaluations. At the initial evaluation, one project was not recommended for funding, but at the two other evaluations funding of every project was recommended.

TRR51 benefitted from the large core of 15 projects continuously funded throughout the entire 13 years. This stability fostered very fruitful and complementary long-term collaborations among projects greatly. These collaborations were instrumental for the success of TRR51, documented by the fact that 58% of the 272 publications were co-authored by at least two projects and in 33% at least two of the four locations were involved.

6.2. Researchers in early career phases

In total 79 dissertations as outcome of research of TRR51 were successfully completed during the three funding phases of this CRC. Six further dissertations are submitted or close to submission. Graduates received their degrees from UOL, TUBS, University of Göttingen and Bonn. For further details on the names, Thesis titles, year of completion, project affiliation and thesis advisor see Annex I, p. 1-10.
All PhD students from UOL, TUBS and DSMZ enrolled in the structured PhD program of the Graduate School of the respective University:
UOL: Graduate School of Science, Medicine and Technology *OLTECH,*
PhD study program *Environmental Sciences and Biodiversity,*
TUBS: Life science part of the Graduate School Grad^{TUBS}.
These study programs offer a large variety of soft skill and research-oriented courses of which interesting ones were taken by the PhD students of TRR51.
More importantly, however, the MGK/IRTG of TRR51 offered tailored courses in soft skills, e.g. presentations, project management, career planning, good scientific practice, scientific writing, genomic analyses, bioinformatics and statistics (program R) at which all PhD students of TRR51 could participate, also those from the Universities of Göttingen and Bonn. They were put together by the coordinators of the IRTG, in the initial funding phase first by Dr. Birte Junge and since 2012 by Dr. Ferdinand Esser, considering also suggestions by the students and in close cooperation with the co-coordinators at TUBS. The great majority of these courses was very well received by the participants and they greatly benefitted, according to their feed-back and structured evaluation after the courses.
Every PhD student signed a dissertation agreement with his or her primary supervisor assuring that freedom of science in research, teaching and studies is guaranteed and their responsibility for fostering the fundamental values and norms of good scientific practice.
Further, each student, together with his/her supervisor formed a PhD thesis committee which consisted of the student, supervisor and up to two other committee members, usually from a different location of TRR51 or from foreign research institutions. The committees met usually twice per year to discuss progress reports and further research plans of the students.
Retreats of the PhD students, usually two days and at least once per year at different locations, were very important events for the students, broadening their perspective beyond their own research topic. They were organized by the coordinators and students and offered a well composed program of presentations by the students and invited experts, scientists from other basic and applied research institutions and companies These retreats were very well received by the students, also because they helped greatly to bring together the students from the different locations.
Hence, the IRTG was a very important asset of TRR51 from which not only the PhD students benefitted greatly but also the research outcome of this CRC.

Every student enrolled in the structured PhD program needed to accumulate 30 Credit Points (CP) subdivided into the following three areas:
Specialized scientific knowledge,
Communicative competence

Systemic competences and soft skills

Within the IRTG we initiated together with collaborating graduate schools in northwestern Germany the format: “Graduate Symposium *Career Paths of Marine and Climate Scientists*. The graduate symposium, held once per year in the Haus der Wissenschaft in Bremen, aims at introducing different job perspectives in marine and climate sciences in their widest sense. It focuses on career paths outside academia including for example jobs in industry, administration, consulting and journalism. Since the Corona restrictions this symposium was transformed into an online format *Career insights online* which takes place every other month. This format became so popular that it was taken over by the Graduate Schools in northwestern Germany and continues.
Mentoring program for young researchers of the IRTG: Mentoring is a proven and effective way of purposefully supporting young scientists and promoting leadership development. Mentoring is based on the informal transfer of experience and knowledge and the individual support of a young professional (mentee) by an experienced professional in the field (mentor). Hence this program was developed and offered on a voluntary basis to PhD students of the IRTG. Each student must search for and selects a mentor. About 30% of the PhD students participated and were very happy with this program. Therefore it was implemented into *OLTECH* and is continued.
Oldenburg and the northwestern region of Germany have an international reputation as an outstanding location for research in the field of marine sciences that successfully attracts both national and international scientists and students. To take advantage of these opportunities PhD students of the IRTG had the possibility to enrol in courses of the following collaborating graduate schools: Helmholtz Graduate School for Polar and Marine Research (POLMAR), International Max Planck Research School of Marine Microbiology (MarMic), Center for Marine Environmental Science (MARUM) and Leibniz Graduate School Center for Tropical Marine Ecology (ZMT).

These collaborations, together with the program of the IRTG, provided an excellent environment for the career development of PhD students of TRR51 in fundamental and applied science and also in other professional areas.

The TRR51 student fellowships given to the IRTG provided most valuable funds for a number of PhD students who continued with a later funding through regular salaries for PhD students (65% TV-L 13).

Table: List of contractual employment duration of all research staff members employed in the last funding period

Duration of contract	Number of contracts for doctoral researchers		Number of contracts for postdoctoral researchers		Number of researchers in total
	male	female	male	female	
up to 12 months	11	8	3	3	**25**
up to 24 months	2	3	0	4	**9**
up to 36 months	4	4	2	0	**10**
up to 48 months	6	6	3	5	**20**

The following table summarizes the number of completed dissertations in the funding periods, institutions and indicating gender aspects. For further details see Annex I, p. 1-10.

Funding period	Institution	female	male
2010-1013	UOL	3	1
	TUBS	2	4
	Univ GÖ		1
2014-2017	UOL	6	4
	TUBS	8	7
	Univ GÖ		1
	Univ Bonn		1
2018-2022 *	UOL	7	10
	TUBS	13	9
	Univ GÖ		1
	Univ Bonn		1

* includes also Theses which were completed in 2023.

How did the CRC affect the teaching portfolio within the institution?

At UOL topics of TRR51 were fed into the master programs Marine Environmental Sciences *(MUWI)* and *Microbiology*, in particular in compulsory research projects, practical courses and in Master Theses in the field of microbiology, bioinformatics and marine geochemistry. The close association of these research oriented modules made the program Microbiology and the biology part of the *MUWI* program very attractive for students. Several students joined TRR51 for their PhD Thesis work. Hence teaching in these Master programs benefitted greatly from TRR51.

At TUBS about 6 years ago the Biology Master program was enriched with the new study focus “Biodiversity”. It is a joint effort with the DSMZ, which provides regular courses on new methods of diversity research (course MI22 – Molecular microbial evolution and diversity, hosted by Jörg Overmann). Since 4 years the TU offers a “Phycology” practical course with an obligatory excursion of one week to Helgoland (BD05 - Phycology hosted by Dieter Jahn). Experiences and new results from research of TRR51 were regularly fed into these courses and attracted students for their Master Thesis work.

6.3. Equal opportunities and work-life balance

DFG generously provided funds to TRR51 to promote gender equality measures. We are grateful for this financial support for activities specifically targeted to advance the skills and self-confidence of female PhD students and young scientists. We used these specially allocated funds in many different ways and highlight in the following several of the major events and supports:

- 2011: Workshop Promotion of Female Early Career Scientists (UOL).
- 2011-2021: child care during holidays for scientists of TRR51
- 2012: Workshop Education and Research Management as part of the Zukunftstag (UOL).
- 2012, 2014-2019 : Coaching and workshops for female early career scientists (UOL)
- 2013: Workshop Arrogance Training and Quick-Wittedness for Female Early Career Scientists (UOL)
- 2017: Workshop Conflict Management (UOL)
- 2018: Workshop Gender Awareness Training (UOL)
- 2018: Workshop Leadership Competence (BS)

- Coaching and workshops of female early career scientists as part of the mentoring program of the IRTG
- Support of female scientists for child care during conferences and visits as guest scientists

UOL and TUBS are strongly committed to promote gender equality and a sustained work-life balance. UOL and TUBS have been continuously certified as a family-friendly university by the Hertie Foundation since 2004 and 2007, respectively.

One measure we had hoped to support more was child care during conferences for female scientists. As the administration required that only a certified kindergarten or day care teacher can take over this job, but only under very special circumstances a family member, e.g. grand parents, this prevented in several cases the participation of early career female scientists from attending conferences.

6.4 Research infrastructure and data management

UOL and ICBM provide infrastructure and core facilities which were important in supporting research of TRR51, predominantly an excellent workshop for mechanics, electronics and maritime equipment, a high performance computing facility and electron microscopy. Substantial investments in personnel and maintaining and updating instrumentation have been continuously made by UOL to keep these services on a high level and make state of the art additions to provide an excellent working environment for high end research in in many different fields, including also those covered by TRR51, microbiology, geochemistry, modelling and marine sciences.
Further, PIs of TRR51 house instrumentations of general importance not only for TRR51 but beyond as service facilities for other research groups at UOL, northern Germany and even internationally:
Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT ICR MS) and related U/HPLC system (marine geochemistry service facility, Prof. Thorsten Dittmar)
Proteomics (Prof. Ralf Rabus)
Flow Cytometry (Prof. Meinhard Simon)
We are very grateful to the DFG funds acquired by TRR51 which helped to support and strengthen these services (FT ICR MS, UPLC), to upgrade and expand their applications (proteomics) and to implement and build up the flow cytometer facilities.
Investments for large instruments, lump sums, and accumulated funds not used for other purposes and overheads were used for these purchases.
In the following some major pieces of equipment acquired by these funds are highlighted:
BD FACS Aria III flow cytometer to sort and enumerate microbial cells.
2 BD Accurri C6 flow cytometers to enumerate microbial cells.
Autosampler for a Waters UPLC system in the environment of the marine geochemistry service facilities.
Nano LC for MALDI coupling and upgrade of ESI-MS/MS (Proteomics service facility)
Major contribution to build in house a CTD rosette sampler equipped with 24 20 L-Niskin bottles, precondition for successful field work during cruises with RV Sonne in the Pacific. The first large-scale CTD in German marine sciences and still the only such equipment in Germany.
Multi corer (MUC) to collect sediment samples, precondition for successful sediment sampling during cruises with RV Sonne, available and used in marine sciences in Germany.

Shortly after the start of TRR51 Blade server infrastructure was established in the framework of the INF project at TUBS. It hosted all the data management systems of the CRC. Currently, it gets migrated to a new system, linked also to the central IT services, and will be available soon afterwards again.

The transposon mutant libraries of more than 4500 mutants of each model organism, *Dinoroseobacter shibae* and *Phaeobacter inhibens*, were a most valuable resource for many

experiments carried out by the TRR51 consortium. They also served as a resource for other colleagues outside the CRC. To make these libraries a sustainable resource for future use they are maintained at DSMZ.

All scientist and in particular the PIs of TRR51 are fully aware of the requirement to ensure the integrity, proper storage and accessibility of primary scientific data, the FAIR principle (findability, accessibility, interoperability, reusability). Therefore, the PIs took particular care that these requirements were met in the projects they conducted and administered. The PIs instructed and supervised the PhD students and postdoctoral researchers of TRR51 in strictly adhering to the "Rules of Good Scientific Practices" as laid down by DFG regulations. In addition, the IRTG offered compulsory courses on good scientific practice and misconduct.

Scientific results generated by the consortium of TRR51 were routinely published in peer-reviewed and in most cases publicly available international scientific journals. Data of (meta)genomics, (meta)viromics, (meta)transcriptomics, (meta)proteomics, plasmids, gene amplicon sequences and environmental variables from field work were routinely deposited in publicly accessible databases (NCBI, ENA, PANGAEA). Newly isolated bacterial strains were either deposited at culture collections, mainly DSMZ, or stored as glycerol stocks and documented in the labs which isolated them.
Back up and replicate samples of many experiments and field work are kept in the labs of the PIs in appropriate form (mainly frozen at -80 or -20°C) for future analyses and to comply with the DFG rules to store data, specimen and samples.
These measures ensure sustainability and future use of the research findings of TRR51.

The computing centers of UOL and TUBS provide daily backup services for data storage facilities ensuring integrity and long-term availability of experimental and field data. IT administrators and IT infrastructure at ICBM. TUBS, DSMZ and Univ Göttingen provided excellent support for all types of computer-based data storage-related work of TRR51. UOL also provided the hardware and software infrastructure (SAP) necessary for the proper management of the financial funds allocated by DFG to TRR51.

6.5 Knowledge transfer

TRR51 was engaged in various directions to foster transfer of knowledge and of gained findings to different target groups outside academic research.
Public lectures on the research cruises with RV *Polarstern* in 2011 and 2012 and *Sonne* in 2016 and 2017 and obtained findings by different participating scientists were held in das Schlaue Haus Oldenburg, Haus der Wissenschaft for public outreach of UOL and the Jade University of Applied Sciences. These lectures were usually part of lecture series attended by the public and very well received.
Similar lectures were held in lecture series for guest students, very often elderly people and high school teachers interested in different fields of science.
Further, during the cruises with RV *Sonne* in the Pacific Ocean, frequent, almost daily blogs from on board research activities were released on the home page of ICBM and also very well received.
The IRTG organized meetings with representatives of different companies, in several cases alumni of TRR51, and in 2015 one three day workshop in Hamburg to meet industry, enterprises, scientific management and governmental and non-governmental research institutes. These activities helped as door openers for graduates to find suitable jobs.
TRR51 took great advantage of the press and public outreach departments of UOL and TUBS to prepare and publish press releases of most interesting results and publications of interest to the public, usually publications in high impact journals. Further, local newspapers published articles on special events of TRR51 such as kick off meetings and symposia.
Meinhard Simon as speaker was invited by the Ministry of Culture and Science (MWK) of Lower Saxony to make a contribution on TRR51 and marine science at the public event

Research Made in Lower Saxony 2017 in the Herrenhausen Castle in Hannover organized by the MWK.
Carsten Reuse, a TRR51 graduate of TUBS, successfully acquired funds from Akademie der Wirtschaftsförderung Braunschweig in 2023 for the start-up company ALGAEPLANT. This company aims at producing pigments, lipids, carbohydrates and hydrogen from algal mass cultures.
Several working groups of TRR51 at UOL and TUBS participated before the Corona lockdown in the annual girls and boys day (Zukunftstage) and hosted middle and high school students for a day to introduce them into their research topics.

All listed activities document the wide portfolio of knowledge transfer of TRR51 which greatly contributed to strengthen the awareness of marine sciences and marine microbiology in the public and even in industries.

6.6 Internal collaboration and management

The very good and trustful collaboration right from the beginning of TRR51 was a result of a successful and close collaboration of seven PIs from UOL, TUBS, DSMZ and Univ Göttingen during a previous project on *Comparative Functional Genome Analysis of Representative Members of the Roseobacter Clade* funded for three years by VW-Vorab Niedersachsen, also with Professor Meinhard Simon as coordinator. This established collaboration of about 40% of the PIs of the initial funding phase formed an excellent basis for a constructive and fruitful collaboration among the CRC consortium.
To foster the collaboration two status seminars were held every year, except in the years of the renewal applications with other joint meetings/retreats, one in Braunschweig and one in Oldenburg/Delmenhorst, with one of them for a full day and the other one for two days. In 2020 during the Corona pandemic we had no status seminars but in 2021 we resumed the status seminars and switched to an online format but were most happy to return to the final status seminar with physical presence in 2022. For the program of these status seminars see Annex I, p. 19-73. Further, the regular workshops and annual retreats of the PhD students helped greatly to develop the collaboration and to integrate new PhD students in each funding phase. The very convenient direct train connection between Oldenburg and Braunschweig facilitated collaborations among projects of different locations.
For preparing every proposal the PIs gathered in a retreat for two days to discuss progress reports, future plans and made a critical evaluation of each existing project and of the ones proposed newly for the next funding phase. In a second step, the CRC board of seven PIs made another two days retreat to critically review the draft proposals of each project for the next funding phase.
Basis for the administration and management of TRR51 was its office at ICBM in Oldenburg. We were most lucky in having excellent personnel in office and to have close collaboration with the general ICBM administration and the department for third party funding of UOL (Dezernat 2). The CRC office managed also organizational and financial issues with the different partners, TUBS, HZI, DSMZ, Univ Göttingen and Bonn. The management and exchange with the respective partners were very efficient and constructive.
Two locations of TRR51 were non-university institutions, HZI and DSMZ. Collaboration and support by these institutions was always without any problem and reflected their long-standing collaboration with universities, mainly TUBS. In fact, TRR51 benefitted from their support, e.g. by having access to their infrastructure such as DNA sequencing facilities.

The smooth and excellent collaboration among all PIs of TRR51 is documented by the fact that 60% of the 272 publications are co-authored by at least two projects and 34% by at least two locations.

7. General information about Project A

7.1 Ecological significance, biogeography and physiology of the Roseobacter group in pelagic systems

7.1.2 Project leaders

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7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1. Report

7.2.1.1 Interactions of roseobacters with the diatom *Thalassiosira rotula*

As outlined in the proposal for this funding phase, direct interactions of roseobacters with microalgae are important and characteristic features of this bacterial group. In addition to the axenic strain of the model diatom *Thalassiosira rotula* from the Bigelow culture collection, CCPM1647, we obtained another strain isolated from the North Sea, CCAC8673B, which we made axenic and which served as another model strain to study its interactions with roseobacters and other bacteria. We focused on physical interactions and applied fluorescently labelled lectins and confocal laser scanning microscopy (CLSM) to characterize the glycocon-jugates of the diatom and the specific bacterial colonization sites of the diatom's surface structures. Different bacterial strains, partially isolated from this diatom, attached to different surface structures with distinct glycoconjugate signatures. Co-cultures of two and more bacterial partners yielded denser colonization patterns than monocultures. Most prominent were fucose-containing threads, which were partially decomposed conjointly by a *Flavobacterium*, *Gramella forsetii*, and a *Roseo-bacter*

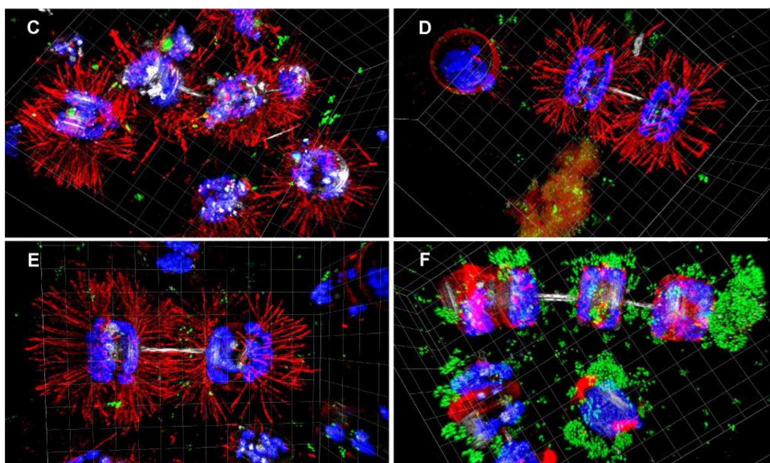

Fig. 1. Colonization patterns of bacterial strains on *T. rotula* cells shown in CLSM micrographs. Surface structures of the diatom were stained by fluorescent glycoconjugates and bacteria by SybrGreen I. C: *T. rotula* and *Pseudophaeobacter* sp.; D: *T. rotula* with *Dinoroseobacter shibae* and *P. frisia;* E: *T. rotula* with *D. shibae*+ *G. forsetii*; F: *T. rotula* with *P. frisia* and *G. forsetii;* Lectin and counterstain, AAL-Alexa568, SybrGreen I; Colour allocation: blue = autofluorescence of chlorophyll *a*, white = reflection, red = lectin AAL-Alexa568, green = SybrGreen I. Grid: 10 µm. For the full image and more details see Tran et al. (2023).

strains, *Planktotalea frisia* (Fig. 1). These results show for the first time such detailed bacterial colonization patterns on a diatom and emphasize their significance in bacterial-diatom interactions (Tran et al. 2023).

7.2.1.2 Genome sequencing and postgenomic analyses of the diatom *Thalassiosira rotula*.

We successfully sequenced the genome of the axenic CCPM1647 strain of *T. rotula* in cooperation with Anja Poehlein and Rolf Daniel (project Z02) based on nanopore and Illumina technology. Sequencing, assembly and annotation took much longer than anticipated, mainly because the genome size is much larger than that of other diatoms, ~450 Mbp. We are still analyzing the genome, in cooperation with Jan de Vries, University of Göttingen, and Thomas Mock, University of East Anglia, Norwich, UK. A manuscript on the genome is in preparation which will be submitted later this year. We are also in the process of sequencing the genome of the new North Sea strain of *T. rotula*, see above, but results are not expected until later this year. This will enable us to make a comparative genomic and transcriptomic analysis of two different strains of this important coastal diatom, however, not any more within the reporting phase of this CRC.

7.2.1.3 Global biodiversity and biogeography assessment of the pelagic *Roseobacter* clusters.

A major focus during the last funding phase was a comprehensive analysis of the samples and data we collected during cruises with RV Sonne in the Pacific Ocean and a synthesis of these data with those of previous cruises to the Atlantic and Southern Ocean. Further, we carried out a global analysis of the pelagic *Roseobacter* clusters and of the structure of pelagic prokaryotic communities including other data sets such as Tara Ocean and the Malaspina Expedition. The great value of our data sets from the Pacific and Atlantic Ocean is that we sampled systematically along a south-north transect between subantarctic and boreal (Atlantic) and subarctic (Pacific) regions.
Regarding the **Atlantic Ocean** our comprehensive metagenomics analyses show that in the near-surface Atlantic and Southern Ocean between 62°S and 47°N microbial communities exhibit distinct taxonomic and functional adaptations to regional environmental conditions (Dlugosch et al. 2022). Richness and diversity showed maxima around 40° latitude and intermediate temperatures, especially in functional genes (KEGG-orthologs, KOs) and gene profiles. A cluster analysis yielded three clusters of KOs but five clusters of genes differing in the abundance of genes involved in nutrient and energy acquisition. Gene profiles showed much higher distance-decay rates than KO and taxonomic profiles. Our results indicate fine-tuned genetic adaptions of prokaryotic communities to regional biotic and environmental conditions in the Atlantic and Southern Ocean. This metagenomics analysis provided an important basis for follow-up studies on the microbial diversity (Milke et al. 2022a), the genetic potential of vitamin B_{12} auxo- and prototrophs (Wienhausen et al. in review, see report of project A8) and metagenome-assembled genomes (MAG) of the *Roseobacter* group (see below).
Regarding the data evaluation of the cruises to the **Pacific Ocean** several studies with different foci were carried out. The basic hydrographic, nutrient and microbial data are presented in two publications (Balmonte et al. 2021, Giebel et al. 2021). Another study tested the effect of vitamin B_{12} and its activated building bock alpha-ribazole on growth and community composition of microbial communities in three biogeographic regions and found pronounced effects (Wienhausen et al. 2022, for details see report of project A8). Further, we elucidated the relative significance of the ecological mechanisms selection, dispersal and drift for shaping the composition of microbial communities between subantarctic and subarctic regions (Milke et al. 2022b). In the epipelagic, homogeneous selection contributes 50-60% and drift least to the three mechanism for the assembly of prokaryotic communities whereas in the upper mesopelagic drift is relatively most important for the particle-associated subcommunities. For eukaryotes >8 µm, homogeneous selection is also the most important mechanisms at two

epipelagic depths whereas at all other depths drift is predominant. As species interactions are essential for structuring microbial communities we further analysed co-occurrence based community metrics to assess biogeographic patterns over the transect. These interaction-adjusted indices explained much better variations in microbial community composition as a function of abiotic and biotic variables than compositional or phylogenetic distance measures like Bray-Curtis or uniFrac. Our analyses, the first ones for an ocean basin, are important to better understand assembly processes of microbial communities in the upper layers of the largest ocean and how they adapt to effectively perform in global biogeochemical processes.
In a synthesis paper including data sets on the composition of prokaryotic communities (16S rRNA gene amplicons, ASV) from all global oceans except the Arctic Ocean and including also the Mediterranean Sea, we show that prokaryotic epipelagic communities exhibit a modular structure. Applying a co-occurrence network analysis, we identified ten clusters, each with different distribution patterns, in different oceanic regions and with different temperature regimes (Milke et al. 2023). Modularity was highest in the major oceanic gyres close to the subtropical fronts where different water masses and thus different clusters mix.
In collaboration with Shinichi Sunagawa, ETH Zürich, and Rudi Amann, MPI Bremen, we comprehensively analyzed MAGs of the *Roseobacter* group recruited from all major metagenomics data sets from the global oceans, including Tara Oceans, Biogeotraces, Malaspina, Helgoland Roads, the global ocean reference genome database (GORG), Hawaiian Ocean and Bermuda Atlantic Times Series (HOTS, BATS). In one study, we analyzed 82 high quality MAGs affiliated to the *Roseobacter* RCA cluster and, together with five genomes of isolates, carried out a comprehensive analysis of genomic features and the biogeographic distribution (Liu et al. 2023). The analyses reveal that these MAGs split into three different subclusters, identified as genera based on <70% average nucleotide identity (ANI), and into 13 different sublineages which qualified as distinct species, i.e. >95% ANI. All five genomes from isolates belonged to one species, emphasizing that our MAG analysis greatly diversified this important *Roseobacter* cluster. The different genera and species were

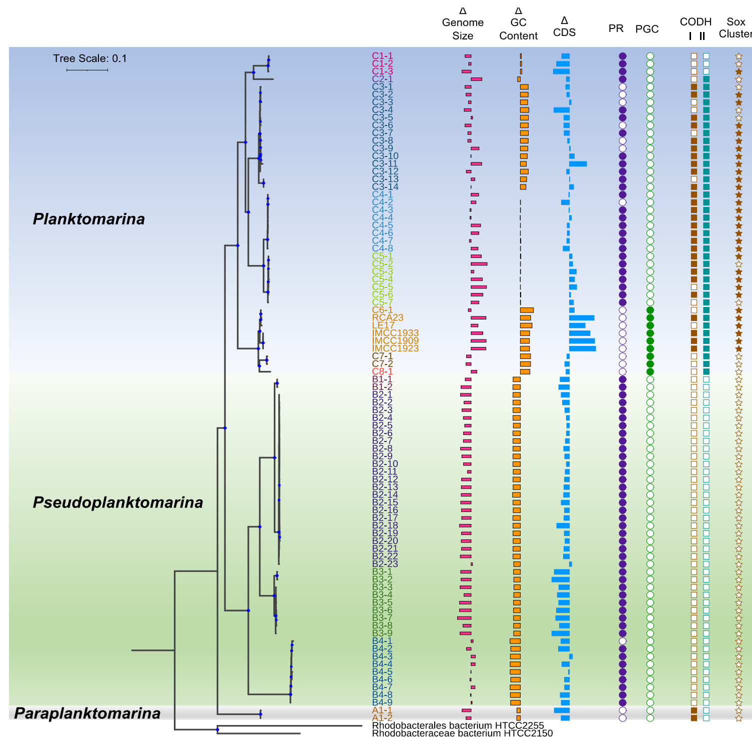

Fig. 2. Genome characteristics of 87 RCA MAGs/genomes of the RCA cluster. Deviation of each genome/ MAG from the overall mean of the genome size, G+C content and CDS and presence/absence of genes for complementary energy acquisition by proteorhodopsin (PR), aerobic anoxy-genic photosynthesis (*pufM*), carbon monoxide dehydrogenase (CODH I and II), and sulfur oxidation (sox cluster). Deviation from the mean is indicated by bar length and presence /absense of genes by filled or empty circles (from Liu et al. 2023)

distinct regarding genome size and GC content with a general trend of greatly reduced genome size and GC content of the newly discovered relative to the known species. Surprisingly, nine of the new species encoded proteorhodopsin (PR). This finding expands the presence of this

mode of complementary energy acquisition in pelagic marine bacterial lineages to the majority of the sublineages of the RCA cluster. Hence, acquiring complementary energy by PR, and not by aerobic anoxygenic photosynthesis as previously assumed (Giebel et al. 2019), appears to be the rule in the RCA cluster.
In a second study, we carried out a comprehensive analysis of 609 MAGs of the entire *Roseobacter* group recruited from these datasets. This assessment enlarged the diversity of various lineages of the *Roseobacter* group greatly. It revealed the existence of three new clusters of pelagic *Roseobacter* lineages not targeted by 16S marker gene analyses (PAR, COR, TCR). The different pelagic *Roseobacter* clusters exhibit distinctly different genome sizes, GC content, coding densities, functional profiles and distinct global biogeographic patterns. A manuscript of these results is in preparation (Liu et al. in prep.).

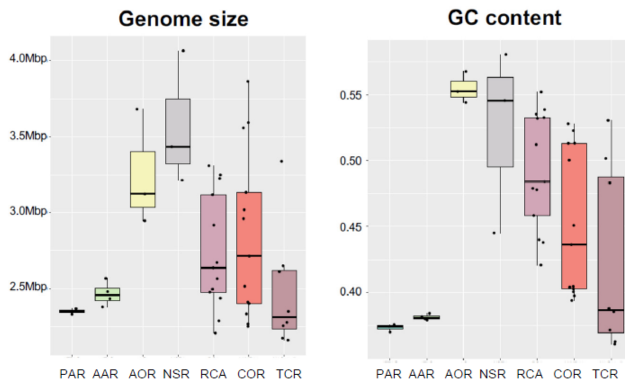

Fig. 3. Genome size and GC content of pelagic Roseobacter clusters assessed on the basis of 609 MAGs recruited from metagenomics datasets from global oceans. Box-Whisker plots are shown for each cluster. Clusters: PAR (Pacific-Atlantic Roseobacter), AAR (Atlantic-Arctic Ocean Roseobacter), NSR (North Sea Roseobacter), RCA, COR(Central

In addition to the outlined results, members of this project were involved in several further studies listed in the publications.

7.2.1 Published project results
Publications with scientific quality assurance
List of publications in peer reviewed journals
(members of A1 in bold, of other CRC projects in italic)

1. **Bakenhus** I, **Dlugosch** L, **Giebel** HA, **Beardsley** C, **Simon** M, Wietz M (2018) Distinct biogeographic patterns of bacterioplankton composition and single-cell activity between the subtropics and Antarctica. Environ Microbiol 20/8, Special Issue: 3100-3108.
2. **Bakenhus** I, *Wemheuer* B, Akyol P, **Giebel** HA, **Dlugosch** L, *Daniel* R, **Simon** M (2019) Distinct relationships between fluorescence *in situ* hybridization- and rRNA gene- and amplicon-based sequencing data of bacterioplankton lineages. System Appl Microbiol 42/5: 126000.
3. Balmonte JP, **Simon** M, **Giebel** HA, Arnosti C (2021) A sea change in microbial enzymes: Heterogeneous latitudinal and depth-related gradients in bulk water and particle-associated enzymatic activities from 30°S to 59°N in the Pacific Ocean. Limnol Oceanogr 66: 3489-3507.
4. **Dlugosch** L, *Poehlein* A, *Wemheuer* B, Pfeiffer B, Badewien TH, *Daniel* R, **Simon** M (2022) Significance of gene variants for the functional biogeography of the near-surface Atlantic Ocean microbiome. Nature Comm 13: 456.
5. **Giebel** HA, Arnosti C, Badewien TH, **Bakenhus** I, Balmonte JP, **Billerbeck** S, **Dlugosch** L, Henkel R, **Kuerzel** B, Meyerjürgens J, **Milke** F, Voss D, *Wienhausen* G, Wietz M, Winkler H, **Wolterink** M, **Simon** M (2021) Microbial growth and organic matter cycling in the Pacific Ocean along a latitudinal transect between subarctic and subantarctic waters. Front Mar Sciences 8: 764383.
6. **Giebel** HA, **Wolterink** M, **Brinkhoff** T, **Simon** M (2019) Complementary energy acquisition via aerobic anoxygenic photosynthesis and carbon monoxide oxidation by *Planktomarina temperata* of the *Roseobacter* group. FEMS Microb Ecol 95/5: fiz050.

7. Koch H, *Freese* HM, Hahnke RL, **Simon** M, Wietz M (2019) Adaptations of Alteromonas sp. 76-1 to polysaccharide degradation: A CAZyme plasmid for ulvan degradation and two alginolytic systems. Front Microbiol 10: 504.
8. Koch H, Germscheid N, *Freese* H, *Noriega-Ortega* BE, *Lücking* D, *Berger* M, Qiu G, Marzinelli E, Campbell A, Steinberg PD, *Overmann* J, *Dittmar* T, **Simon** M, Wietz M (2020) Genomic, metabolic and phenotypic variability shapes ecological differentiation and intraspecies interactions of *Alteromonas macleodii*. Sci Reports, 10: 809.
9. **Liu** Y, **Brinkhoff** T, *Berger* M, *Poehlein* A, *Voget* S, Paoli L, Sunagawa S, Amann R, **Simon** M (2023) Metagenome assembled genomes reveal greatly expanded taxonomic and functional diversification of the abundant marine *Roseobacter* RCA cluster. Microbiome, in press.
10. Majzoub M, *Beyersmann* P, Simon M, Thomas T, **Brinkhoff** T, Egan S (2019) *Phaeobacter inhibens* controls bacterial community assembly on a marine diatom. FEMS Microb Ecol 95/6: fiz060.
11. **Milke** F, *Sanchez-Garcia* S, **Dlugosch** L, McNichol J, Fuhrman JA, **Simon** M, *Wagner-Döbler* I (2022a) Composition and biogeography of planktonic pro- and eukaryotic communities in the Atlantic Ocean: primer choice matters. Front Microbiol. 13: 895875.
12. **Milke** F, *Wagner-Doebler* I, *Wienhausen* G, **Simon** M (2022b) Selection, drift and community interactions shape microbial biogeographic patterns in the Pacific Ocean. ISME J 16/12: 2653–2665.
13. **Milke** F, Meyerjürgens J, **Simon** M (2023) Ecological mechanisms and current systems shape the modular structure of the global oceans' prokaryotic seascape. Nature Comm 14: 6141.
14. Silvano E, Yang M, **Wolterink** M, **Giebel** HA, **Simon** M, Scanlan DJ, Zhao Y, Chen Y (2020) Lipidomic analysis of roseobacters of the pelagic RCA cluster and their response to phosphorus limitation. Front Microbiol 11: 552135.
15. **Tran** Quoc D, Neu T, *Sultana* S, **Giebel** HA, **Simon** M, **Billerbeck** (2023) Distinct glycoconjugate cell surface structures make the pelagic diatom *Thalassiosira rotula* an attractive habitat for bacteria. J Phycol. 59: 309-322. https://doi.org/10.1111/jpy.13308.
16. **Tran** Quoc D, **Milke** F, *Niggemann* J, **Simon** M (2023) The diatom *Thalassiosira rotula* induces distinct growth responses and colonization patterns of *Roseobacteraceae*, *Flavobacteria* and *Gammaproteobacteria.* Environ Microbiol, early online (htpps://DOI:10.1111/1462-2920.16506).
17. *Wienhausen* G, **Dlugosch** L, Jarling R, Wilkes H, **Giebel** HA **Simon** M (2022) Availability of vitamin B_{12} and its lower ligand intermediate alpha-ribazole impact prokaryotic and protist communities in oceanic systems. ISME J, 16: 2002–2014.

7.2. Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.2.1 Project staff in the ending funding period

	Sequen-tial no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Meinhard Simon, Prof. Dr., W3	Microbial Ecology	ICBM	5		UOL
	2	Thorsten Brink-hoff, Prof. Dr.	Microbiology	ICBM	5		UOL
	3	Birgit Kürzel		ICBM	5		UOL

Non-research staff	4	Andrea Schlingloff		ICBM	10		UOL
	5	Rolf Weinert		ICBM	5		UOL
Staff funded with approved grant money							
Research staff	1	Sara Billerbeck, Dr., Postdoc	Microbial Ecology	ICBM	20	E13 50%	
	2	Helge A. Giebel Dr,, Postdoc	Microbial Ecology	ICBM	20	E13 50%	
	3	Felix Milke., Ms. Sc.	Microbial Ecology	ICBM	26	E13 65%	
	4	Mathias Wolterink		ICBM	20	E9 50%	

Job descriptions of staff (supported through existing funds):

1. Simon
   He was principal investigator of the project and responsible for design and coordination of the experiments and sample and data analysis of the Sonne cruises and the global analyses of the metagenome-assembled genomes (MAG) of the *Roseobacter* group. He supervised the PhD student and the postdoc and in addition another postdoc funded by DAAD (Yanting Liu) and two PhD students (Leon Dlugosch, Tran Quoc Den) funded by fellowships (DAAD, VW Vorab).
2. Brinkhoff
   He gave advice in designing and executing experiments and data analysis of the Roseobacter MAG analyses.
3. Kürzel
   She carried out analyses of inorganic nutrients and dissolved amino acids by HPLC.
4. Schlingloff
   She assisted in carrying out physiological growth tests and experiments with isolates, preparing samples for sequencing and molecular biological analyses.
5. Weinert
   He was instrumental in and responsible for growing axenic cultures of *T. rotula*. He also carried out HPLC analyses of dissolved carbohydrates.

Job descriptions of staff (funded with approved grant money):

1. Billerbeck
   She carried out the co-culture experiments with *T. rotula* and bacteria and for genome sequencing
2. Giebel
   He carried out analyses of the Sonne cruises and by flow cytometry for experimental work.
3. Dlugosch
   He carried out bioinformatics analyses of metagenomics and ASV samples of the Atlantic and Southern Ocean
4. Milke
   He carried out the statistical analysis and modelling of the biodiversity and biogeography data from the cruises to the Pacific (RV Sonne) and on the synthetic level of all cruises.
5. Wolterink
   He was instrumental for all technical work and service with flow cytometry

7. General information about Project A2

7.1 The role of the *Roseobacter* group and roseophages in marine sediments

7.1.2 Project leaders

Engelen, Bert, PD Dr., 28.12.1963, German
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Tel. 0441/798 5376
Email engelen@icbm.de

7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report

7.2.1.1 Characteristic compositions of benthic members of the *Roseobacter* group.

As outlined in the proposal for this funding phase, we aimed to finalize our studies on the global distribution and phylogenetic diversity of the *Roseobacter* group in marine sediments. The investigation on the abundance and diversity of *Roseobacter*-affiliated bacteria in Pacific sediments collected on RV Sonne cruise SO248 has been published (Pohlner et al., 2017). We could show that different oceanic provinces with low nutrient content such as both Pacific subtropical gyres were characterized by specific communities of the *Roseobacter* group, distinct from those of the more productive subarctic region of the Pacific and the Bering Sea. The specific quantification of the *Roseobacter* group by CARD-FISH revealed on average a relative abundance of ~2%, and ~6%, determined by quantitative PCR (qPCR). Illumina sequencing of 16S rRNA genes and transcripts showed different compositions containing on average 0.7% and 0.9% *Roseobacter*-affiliated OTUs, mainly assigned to uncultured members of the group. Thus, linking the community structures of benthic roseobacters to specific metabolic processes was hampered by the dominance of so-far uncultured representatives. While the largest proportions of cultured roseobacters were assigned to *Sedimentitalea* and *Sulfitobacter* species, OTUs affiliated to uncultured *Boseongicola, Loktanella, Pseudophaeobacter, Rubellimicrobium, and Ascidiaceihabitans* species were detected in lower abundances or at specific sites only. Our strategy to define clusters of uncultured representatives during the processing of the Illumina dataset helped to assign some OTUs to known genera within the family *Rhodobacteraceae*. The next cultured relatives to these OTUs were within the genera *Loktanella, Pacifibacter, Litorimicrobium, Ruegeria*, and *Halovulum*, a member of the *Amaricoccus* group.
In addition to our own samples from the Pacific transect, we also have analyzed an already existing dataset of bacterial 16S rRNA transcripts, comprising seven oceanic regions and a broad variety of environmental conditions (Pohlner et al., 2019). Thus, by sharing, revisiting and reclassifying already existing gene libraries, we were able to analyze the metabolically active *Rhodobacteraceae* community compositions without redundant sample acquisitions. In this dataset, about 1% of all 16S rRNA transcripts was annotated as *Rhodobacteraceae* with *Sulfitobacter*, *Paracoccus*, and *Phaeomarinomonas* being the most abundant cultured representatives. However, the majority of *Rhodobacteraceae* (78%) was affiliated to uncultured family members, belonging to different subgroups other than the *Roseobacter* group. The general community composition of active *Rhodobacteraceae* was found to be specific for the geographic location, exhibiting a decreasing richness with sediment depth (Fig. 1). At least one-third of these benthic *Rhodobacteraceae* was significantly correlated to prevailing redox conditions ($p \leq 0.05$). They are probably thriving under anoxic conditions and were thus not-yet isolated using the common cultivation-based approaches.

In a molecular-guided cultivation strategy using a *Roseobacter*-specific PCR, we further aimed to isolate novel *Rhodobacteraceae* from different sediments (Master´s thesis A. Bögeholz, 2022). It turned out that the addition of arginine to the growth media enhanced the isolation success for *Roseobacter* species. While a total of 827 of 1096 screened colonies showed a negative result in the *Roseobacter*-specific PCR, 63 colonies from media without arginine

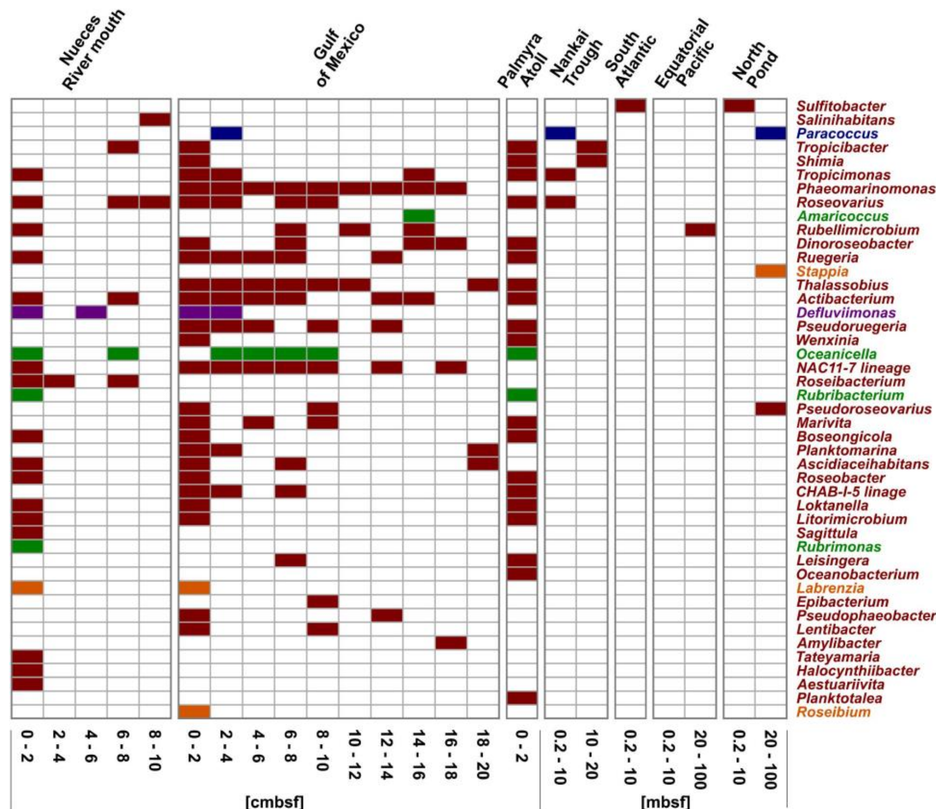

Fig. 1. Distribution of the OTUs affiliated to cultured representatives within the *Rhodobacteraceae*. OTUs are sorted by their relative abundance on the total bacterial community. Sample locations are arranged by water depth and then by sediment depth. The affiliation of OTUs to the phylogenetic subgroups is displayed by red = *Roseobacter*, orange = *Stappia*, green = *Amaricoccus*, purple = *Rhodobacter*, and blue = *Paracoccus*. Figure taken from Pohlner et al. (2019).

addition, and 182 colonies from media supplemented with arginine were positive. Two of them were classified as *Pseudo*- and *Phaeobacter* strains and genomes sequenced. 22 deep-sea isolates from the RV Sonne cruise SO254 were divided into six different *Sulfitobacter* species and also genome sequenced. Despite their positive PCR results, another set of 21 sequenced colonies were mainly classified as Gammaproteobacteria, indicating some unspecificity of the PCR primers.

7.2.1.2 Secondary metabolite production of benthic *Roseobacter* strains.

Another aim of the project was to screen benthic isolates for secondary metabolite production (in cooperation with C2-Schulz) to identify their exometabolome. Three of our benthic *Roseobacter* strains, affiliated to *Shimia* sp. (SK013), *Huaishuia* sp. (SK032) and *Phaeobacter* sp. (SK040) as well as one *Sulfitobacter* strain from the water column (SK038) were positively tested for the presence of N-acylhomoserine lactones (AHLs), bacterial signaling compounds involved in quorum-sensing (Ziesche et al., 2019). We found a wide variance between AHL composition within the strains, but no association of certain AHLs with different habitats. In cooperation with C2-Schulz, two of our benthic isolates affiliated to *Roseovarius pelophilus* (G5II) and *Pseudoruegeria* sp. (SK021), were positively tested for the release of nitrogen-containing volatiles, isobutyl-, isopentyl-, and 2-methylbutylamine (Harig et al., 2017).

7.2.1.3 The exometabolome of *Rhodobacteraceae* strains in relation to viral infections

In cooperation with A8-Niggemann/Dittmar/Simon, we studied Rhodovulum sulfidophilum as model strain to characterize its exometabolome in relation to a viral infection and the respective geometabolome (Heinrichs et al., 2022). This lysogenic (prophage-containing) bacterium is representative for benthic, but also pelagic habitats. In this study, we aimed to identify the molecular signature deriving from virus infections as an imprint on the pool of dissolved organic matter (DOM) in the ocean. Therefore, we induced the prophages of R. sulfidophilum using the DNA-damaging antibiotic mitomycin C to produce a virus-induced cell lysate (vDOM) and compared it with the exometabolome of an uninduced culture. We further analyzed the DOM background of North Sea water (NSW) containing a natural microbial community. To identify the virus signal compared to the natural background, one experimental setup of the NSW was

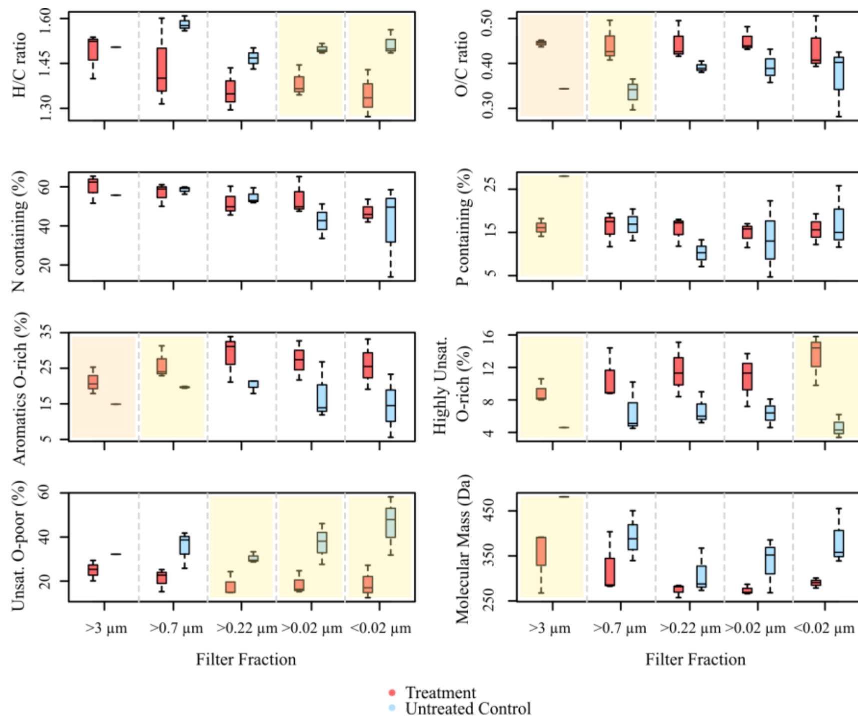

Fig. 2. Molecular compositions of signal intensity-weighted averages of various compound groups, comprised from all masses with assigned molecular formulae detected in the phage-induction treatments and untreated controls The overlay in yellow indicates significant differences between treatment and control ($p < 0.05$), panels overlain in orange represent highly significant differences ($p < 0.005$). Figure taken from Heinrichs et al. (2022).

spiked with vDOM of *R. sulfidophilum*. In all experiments, we used sequential filtration to selectively analyze different DOM fractions, representing particle-associated and free-living bacteria, virus particles as well as a virus-free permeate. Overall, a small DOM subset of the detected molecular formulae correlated significantly with virus abundances in the bacterial cultures (<1%) and <2% of the total signal intensity of the DOM dataset. These were mainly phosphorus- and nitrogen-containing compounds. However, significant differences between the DOM composition of the phage-induction treatment and the untreated control were found in different filter fractions and for various compound groups, indicating specific exometabolomes excreted by the so-called virocells.

7.2.1.4 Probing the DMS/DMSO cycle of coastal marine sediments

The production of the climate-active gas dimethylsulfide (DMS) by the microbial reduction of dimethylsulfoxide (DMSO) is an important factor for the earth climate. This process is catalyzed by DMSO reductases, of which one major reductase type, the dor-type, has been described in *Rhodobacter* species. Thus, we aimed to identify the drivers to organic sulfur cycling in marine sediments by using a combined approach of chemical and molecular analyses of incubation experiments and original North Sea sediments. We hosted a leading expert in benthic organic sulfur cycling, Hendrik Schäfer (University of Warwick, GB) in 2019, as a fellow of the “Hanse Wissenschaftskolleg, HWK” (Delmenhorst) to perform collaborative research within our subproject. During H. Schäfers HWK-fellowship, among other investigations, we could show that DMSO concentrations in the saltmarsh sediments exceeded those from other environments by up to ~300 times. With 16S rRNA amplicon and whole genome shotgun

sequencing, we identified taxa and genes involved in DMSO reduction and the subsequent anaerobic DMS degradation. In our incubation experiments, DMSO reduction was primarily attributed to the *Desulfobacterales,* but with increasing importance of *Rhodobacter* species and the dor-type when sulfate reduction was inhibited. Interestingly, all analyzed genes involved in DMSO reduction, as well as DMSP and DMSO production, were found within the *Rhodobacteraceae*, underlining their key role in organic sulfur cycling in salt marshes.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals
(members of A2 in bold, of other CRC projects in italic)

1. **Heinrichs ME**, **Heyerhoff B**, **Arslan-Gatz BS**, Seidel M, *Niggemann J*, **Engelen B**. (2022) Deciphering the virus signal within the marine dissolved organic matter pool. Front Microbiol 13: 863686,
2. Heinrichs ME, De Corte D, Engelen B, Pan D. (2021). An Advanced Protocol for the Quantification of Marine Sediment Viruses via Flow Cytometry. Viruses 13:102
3. **Heinrichs ME**, **Tebbe DA**, *Wemheuer B*, *Niggemann J*, **Engelen B** (2020) Impact of viral lysis on the composition of bacterial communities and dissolved organic matter in deep-sea sediments. Viruses 12: 922.
4. **Heinrichs ME**, *Dlugosch L*, Mori C (2019) Complex Interactions Between Aquatic Organisms and Their Chemical Environment Elucidated from Different Perspectives. In: Jungblut S, Liebich V, Bode-Dalby M (Eds.), Youmares 9 - The Oceans: Our research, our future. Proceedings of the 2018 conference for YOUng MArine RESearcher in Oldenburg, Germany, Springer International Publishing, p. 279-297,
5. **Heyerhoff B**, **Engelen B**, & Bunse C. (2022). Auxiliary metabolic gene functions in pelagic and benthic viruses of the Baltic Sea. Front Microbiol 13: 863620.
6. *Harig T*, *Schlawis C*, *Ziesche L*, **Pohlner M**, **Engelen B**, *Schulz S* (2017) Nitrogen-containing volatiles from marine Salinispora pacifica and Roseobacter-group bacteria. J Nat Prod ASAP 80: 3289–3295,
7. **Pohlner M**, Degenhardt J, *von Hoyningen-Huene AJE*, *Wemheuer B*, Erlmann N, Schnetger B, Badewien TH, **Engelen B** (2017) The biogeographical distribution of benthic Roseobacters along a Pacific transect is structured by nutrient availability within the sediments and primary production in different oceanic provinces. Front Microbiol 8: 2550,
8. **Pohlner M**, *Dlugosch L*, *Wemheuer B*, Mills H, **Engelen B**, Reese BK (2019) Majority of Active *Rhodobacteraceae* in Marine Sediments Belong to Uncultured Genera: A Molecular Approach to Link Their Distribution to Environmental Conditions. Front Microbiol 10: 659,
9. **Tebbe DA**, Geihser S, *Wemheuer B*, *Daniel R*, Schäfer H, **Engelen B** (2022) Seasonal and Zonal Succession of Bacterial Communities in North Sea Salt Marsh Sediments, Microorganisms, 0(5), 859,
10. Wemheuer F, *von Hoyningen-Huene AJE*, **Pohlner M**, **Degenhardt J**, **Engelen B**, *Daniel R*, *Wemheuer B* (2019) Primary Production in the Water Column as Major Structuring Element of the Biogeographical Distribution and Function of Archaea in Deep-Sea Sediments of the Central Pacific Ocean. Archaea, vol. 2019, Article ID 3717239,.
11. *Zucker F*, *Bischoff V*, Ndela EO, **Heyerhoff B**, *Poehlein A*, *Freese H*, Roux S, *Simon* M, Enault F & *Moraru C* (2022). New Microviridae isolated from Sulfitobacter reveals two cosmopolitan subfamilies of ssDNA phages infecting marine and terrestrial Alphaproteobacteria, *Virus Evolution* 8: veac070,
12. *Ziesche L*, *Wolter L*, *Wang H*, *Brinkhoff T*, **Pohlner M**, **Engelen B**, *Wagner-Döbler I, Schulz S* (2019) An Unprecedented Medium-Chain Diunsaturated N-acylhomoserine Lactone from Marine *Roseobacter* Group Bacteria. Mar. Drugs 17: 20;

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	Sequen-tial no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Engelen Bert, Dr., PD	Microbial Ecology	ICBM	5		UOL
Non-research staff	2	Meyerjürgens Frank, TA		ICBM	5		UOL
	3	Knutzen Mandy, TA		ICBM	5		UOL
Staff funded with approved grant money							
Research staff	1	Pohlner Marion Dr., Postdoc	Microbial Ecology	ICBM	20	E13 50%	
	2	Heinrichs Mara Ms.Sc.	Microbial Ecology	ICBM	20	E13 50%	
	3	Heyerhoff Benedikt, Ms.Sc.	Microbial Ecology	ICBM	26	E13 65%	
	4	Tebbe Dennis, Ms.Sc.	Microbial Ecology	ICBM	26	E13 65%	

Job descriptions of staff (supported through existing funds):

1. Engelen
   He was principal investigator, responsible for design and coordination of the experiments, sample and data analysis of the Sonne cruises, metabolic profiling of Roseobacter strains, organic sulfur cycling and phage experiments, supervised the Postdoc and PhD students
2. Meyerjürgens
   He helped with cultivations of novel Roseobacter strains.
3. Knutzen
   She analysed metabolic compounds by HPLC and Ion-chromatography.

Job descriptions of staff (funded with approved grant money):

1. Pohlner
   She finalized her work on the global distribution of benthic *Roseobacters* and was involved in the analysis of secondary metabolite production of selected isolates.
2. Heinrichs
   He carried out the experiments to identify the exometabolome of Rhodobacteraceae in relation to viral infections in collaboration with Benedikt Heyerhoff.
3. Heyerhoff
   He was working on roseophages and performed all laboratory work on phage-induction experiments and the bioinformatic analysis of metaviromes from various marine origin.
4. Tebbe
   He sampled saltmarshes to measure organic sulfur cycling, performed growth experiments on selected compounds, and did metagenomic studies to identify genes and respective microbial community members that are involved in these cycling processes.

7. General information about Project A5

7.1 Extrachromosomal, extraordinary and essential – the mobilome of the Roseobacter group

7.1.2 Project leaders

Pradella, Silke, Dr., 13.07.1966, German
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7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report

7.2.1.1 Systems biology and the role of ECRs in our model organisms (P. inhibens, D. shibae)

The first two funding phases of the Roseobacter CRC provided many insights into the wealth of extrachromosomal replicons (ECRs) in *Rhodobacterales*, and plasmid-cured mutants of the model organisms allowed us to determine their functional roles. All three ECRs of *Phaeobacter inhibens* DSM 17395 were classified as chromids, and the 262-kb replicon for the biosynthesis of the antibiotic tropodithietic acid (TDA) is probably the most relevant one. Investigation of the global responses of the Δ262 mutant in comparison with the wild type revealed differential expression of about 10% of the genes, but the catabolic network was surprisingly unchanged irrespective of the massive metabolic burden of TDA in the genus *Phaeobacter* (Wünsch et al. 2020). The genome of *Dinoroseobacter shibae* DFL 12 comprises one chromosome, two stable chromids, and three volatile plasmids, reflecting the typical multipartite genome organization of roseobacters. Genome sequencing of an early preserved glycerol stock showed that a sixth ECR, a true plasmid with a size of 102-kb, was spontaneously lost in our model organism (Koppenhöfer et al. 2022). Comparative transcriptome analyses documented a tremendous effect of the sixth element on the gene expression of the chromosome. The analysis of the Δ86 mutant revealed a comparable regulatory role of the 86-kb chromid, thus documenting that gene expression in *D. shibae* is modulated by different ECRs. Beyond the carriage of lifestyle-determining and accessory genes, this finding exemplified the important role of chromids and plasmids in the regulatory network of the cell.

7.2.1.2 Plasmid conjugation across borders of species, genus and order

Sequencing of hundreds of *Rhodobacterales* genomes in the last decade provided the basis for our comprehensive *in silico* analyses and the detection of plasmid-mediated horizontal gene transfer. The presence of syntenous RepABC-2 type plasmids with type IV secretion systems (T4SSs) in *D. shibae* and *Confluentimicrobium naphthalenivorans* NS6 exemplified the role of conjugation in the ocean (Petersen & Wagner-Döbler 2017). A systematic survey of more than 300 (draft-) genomes showed that 96% of the T4SSs of *Rhodobacterales* are located on RepABC-type plasmids, documenting the outstanding role of this replicon type in horizontal gene transfer (Birmes et al., in preparation). The scattered distribution of the capacity for aerobic anoxygenic photosynthesis in *Alphaproteobacteria* was formerly explained by secondary loss of the photosynthesis gene cluster (PGC). However, the comparison of

organismic and PGC evolution clearly showed that the current distribution of photosynthesis in *Rhodobacterales* was mediated by at least seven horizontal operon transfers (HOTs; Brinkmann et al. 2018). Many PGCs of roseobacters were detected on extrachromosomal elements thus proposing that plasmid mobilization was a major driving force for the evolution of photosynthetic *Proteobacteria*.

One focus of this project in the third funding period was the investigation of experimental plasmid transfer and its relevance for the ecology and evolution of roseobacters. Conjugation of a killer plasmid between the model organisms illustrated the functionality of ECRs in different hosts (Tomasch et al. 2022). Our analyses also showed that ECRs mediate the rapid adaptation of roseobacters to marine pollution. Mobilizable RepL plasmids with a conserved backbone and interchangeable gene cassettes conferred resistance against toxic chromate (Petersen et al. 2019). The discovery of 100% identical RepL plasmids in phylogenetically and geographically distant bacteria reflects the relevance of this newly discovered vector. Another case example was the detection of promiscuous RepC_soli plasmids encoding a potent FloR resistance gene against chloramphenicol (**Fig. 1**; Birmes et al. 2021). Its specific occurrence in *Phaeobacter* likely reflects a genetic footprint of antibiotic (mis)use in marine aquaculture, highlighting the connectivity between the open ocean, livestock breeding and public health (One Health concept). The detection of ribosomal operons on ECRs and experimental conjugation of a corresponding *Sulfitobacter* plasmid in *D. shibae* (Ringel, Freese et al., unpublished) challenges the strict vertical evolution of the 16S rRNA gene, previously considered the gold standard of bacterial taxonomy.

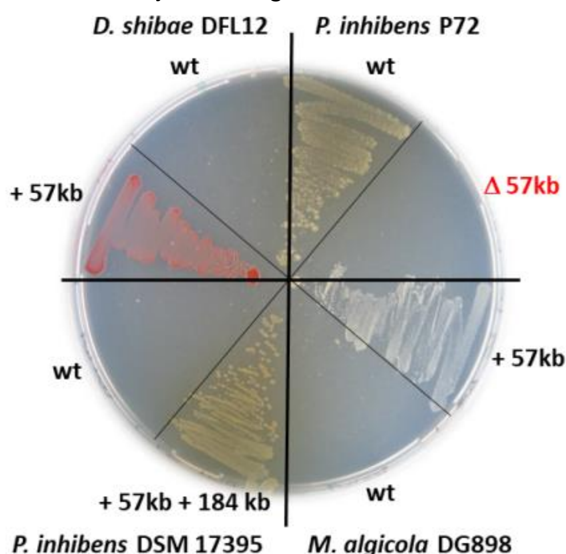

Figure 1. Chloramphenicol resistance of roseobacter strains mediated by the 57 kb RepC_soli-1b plasmid. Growth with 10 µg ml^{-1} chloramphenicol: (i) *P. inhibens* P72 wild type (wt) and its natural plasmid mutant (Δ57kb), (ii, iii, iv) *Marinovum algicola* DG898, *P. inhibens* DSM 17395 and *D. shibae* DFL12 wild type strains as well as their transconjugants (+57 kb).

7.2.1.3 Plasmid biology

Complete genome sequencing allowed the detection of several new plasmid types in *Rhodobacterales* whose function has been experimentally confirmed in *P. inhibens* DSM 17395. The replicase of low copy number (LCN) RepC_soli plasmids is homologous to the equivalent from RepABC-type plasmids (Birmes et al. 2021), but novel medium or high copy number plasmids contain unique replication proteins. All RepQ-, RepY- and RepW-type replicons discovered to date are small cryptic plasmids with unknown function (Freese et al. 2022), while RepL-type plasmids with a replaceable gene cassette illustrate the utility of the Roseobacter mobilome as a genetic backup for rapid adaptations (Petersen et al. 2019).

First insights into the wealth and diversity of ECRs in roseobacters were obtained about 15 years ago by phylogenetic analyses of plasmid replication and partitioning systems. A systematic evaluation of *Rhodobacterales* genomes in the third funding period was the basis for a state-of-the-art classification of LCN plasmids, their quantification, investigation of the host range, and an experimental validation of predictions about their compatibility (Birmes et al., in preparation). The mobilome of roseobacters is dominated by RepABC plasmids (54%), followed by DnaA-like (17%), RepB (16%) and RepA-type ECRs (13%; **Fig. 2**). Phylogenetic analyses and diagnostic palindromes of RepABC plasmids revealed an astonishing diversity of about 20

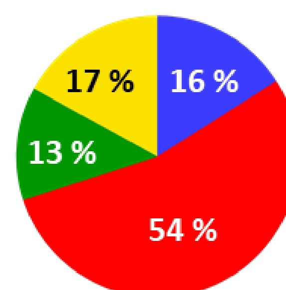

Figure 2. Low copy number plasmid systems of *Rhodobacterales*. Red, RepABC; yellow, DnaA-like; blue, RepB; green, RepA.

distinct lineages. Compatibility tests of cloned RepABC modules in *P. inhibens* DSM 17395 confirmed our *in silico* predictions for the ten most abundant compatibility groups. Host range tests of these *Rhodobacterales*-specific RepABC modules surprisingly showed that all of them are replicated in *Agrobacterium tumefaciens* C58, while rhizobial RepABC plasmids were not functional in roseobacters (Bartling et al. 2017). In cooperation with the group of Anke Becker (Phillips Universität Marburg) a set of four compatible RepABC cassettes from roseobacters was chosen for the development of a series of cloning vectors for future biotechnological applications in rhizobia and *Rhodobacterales* (Körner, Birmes et al., in preparation).

7.2.1.4 Bacterial-algal associations

Co-cultivation of *P. inhibens* DSM 17395 with the dinoflagellate *Prorocentrum cordatum* revealed the probiotic effect of our model organism on microalgae *inter alia* due to vitamin provision (thiamine, cobalamin, biotin). However, conjugation the 191-kb plasmid from *D. shibae* into *P. inhibens* was a 'fatal affair' because it mediated the transition of a probiotic bacterium into an algal killer (Tomasch et al. 2022). The mechanism was further elucidated by the group of Irene Wagner-Döbler (project B4), who showed that the killing is an indirect effect mediated by vitamin depletion from the medium by a plasmid-encoded biotin importer.

We established a bioinformatic pipeline for the analysis of metagenomes from non-axenic algae and cyanobacteria (Marter et al. 2021). The phycosphere of the apicomplexan alga *Chromera velia*, a free-living relative of malaria parasites, was investigated via cultivation and (meta-)genome sequencing. Analyses of the marine alga and four abundant bacterial isolates showed a comparably low overlap of their volatile bouquets (Koteska et al. 2023). Metagenome sequencing of 14 filamentous cyanobacteria (*Coleofasciculus* sp.) from the DSMZ collection resulted in 320 metagenome-assembled genomes (MAGs) reflecting the hidden diversity in cultures of non-axenic phototrophs. The most common housemate was the plasmid-rich roseobacter *Marinovum algicola*, but the most surprising finding was the detection of several alphaproteobacterial MAGs with rhodopsin operons and the complete photosynthesis gene cluster, suggesting dual phototrophy (Marter et al., in preparation).

7.2.1.5 Love is like oxygen – Conquest of the anoxic world via conjugation

We used the phylogenetically broadly sampled dataset of 306 genome-sequenced *Rhodobacterales* to investigate the distribution of operons required for the assembly of four enzymes for complete denitrification (Nar/Nap, Nir, Nor, Nos). The operons showed a very scattered distribution in the phylogenomic tree (**Fig. 3**), but the complete pathway was nevertheless found in seven distinct clades including the eponymous strains *Ruegeria denitrificans* (Clade 1), *Roseobacter denitrificans* (Clade 2), *Pseudooceanicola nitratireducens* (Clade 3A) and *Paracoccus denitrificans* (Clade 8). Phylogenetic analyses of all denitrification operons showed that their distribution cannot simply be explained by vertical evolution and sporadic loss; it was driven by massive horizontal gene transfer. This explanation is supported by the observation that

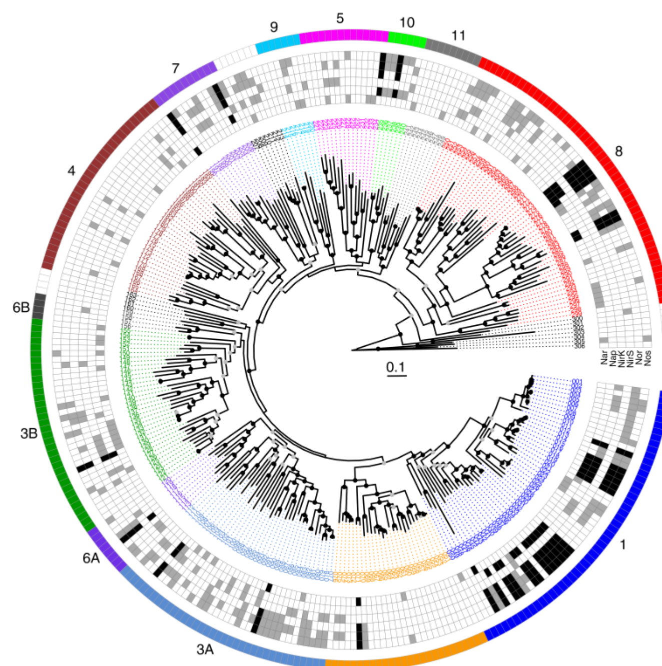

Figure 3. Distribution of denitrification operon in 306 genomes of *Rhodobacterales*. The colored outer ring indicates different phylogenetic clades. Gray, presence of an operon; black, presence of complete pathway.

denitrification operons of *Rhodobacterales* are occasionally located on ECRs, in some cases even on volatile plasmids with T4SSs (Birmes et al., in preparation).
Our model organism *P. inhibens* DSM 17395, which already encodes the enzymes Nir and Nor on its 262-kb chromid, was selected as a recipient for plasmid conjugation experiments to establish the complete denitrification pathway. Tagging of the plasmids with antibiotic resistance genes via transposon mutagenesis allowed selection of transconjugants. Mobilization was performed in two steps, the first transfer based on the conjugation of the 126-kb RepABC-1 with the nitrate reductase (Nar) from *Marinovum algicola* DG898, the second conjugation of the Nos operon was conducted with the 184-kb RepABC-8 plasmid of *P. inhibens* P72. Substrate and growth assays showed that the *P. inhibens* DSM 17395 transconjugant with the complete denitrification pathway gained the capacity to grow anaerobically on nitrate (Birmes et al., in preparation). Thus, we have exemplified the great ecological relevance of plasmid transfer by the conquest of the anoxic world by a formerly strictly aerobic bacterium.
In addition to the outlined results, members of this project were involved in several further studies listed in the publications.

7.2.2 Published project results

Publications with scientific quality assurance
List of publications in peer reviewed journals

1. **Petersen** J, *Wagner-Döbler I* (2017). Plasmid transfer in the ocean - A case study from the roseobacter group. Front Microbiol 8: 1350.
2. **Bartling** P, **Brinkmann** H, *Bunk* B, *Overmann J*, *Göker M*, **Petersen** J (2017). The composite 259-kb plasmid of *Martelella mediterranea* DSM 17316^T - A natural replicon with functional RepABC modules from *Rhodobacteraceae* and *Rhizobiaceae*. Front Microbiol 8: 1787.
3. Tomasch J, *Wang H*, Hall ATK, *Patzelt D*, Preusse M, **Petersen** J, **Brinkmann** H, *Bunk* B, Bhuju S, Jarek M, Geffers R, Lang A, *Wagner-Döbler I* (2018). Packaging of *Dinoroseobacter shibae* DNA into gene transfer agent particles is not random. Genome Biol Evol 10: 359–369.
4. **Brinkmann** H, *Göker M*, Koblížek M, *Wagner-Döbler I*, **Petersen** J (2018). Horizontal operon transfer, plasmids, and the evolution of photosynthesis in *Rhodobacteraceae*. ISME J 12: 1994–2010.
5. **Bartling** P, Vollmers J, **Petersen** J (2018). The first world swimming championships of roseobacters - Phylogenomic insights into an exceptional motility phenotype. Syst Appl Microbiol 41: 544-554.
6. *Bischoff V*, *Bunk* B, *Meier-Kolthoff J*, Spröer C, *Poehlein A*, *Dogs M*, Nguyen M, **Petersen** J, *Daniel R*, *Overmann J*, *Göker M*, *Simon M*, *Brinkhoff T*, *Moraru C* (2019). Cobaviruses – a new globally distributed phage group infecting *Rhodobacteraceae* in marine ecosystems. ISME J 13: 1404-1421.
7. **Petersen** J, Vollmers J, Ringel V, **Brinkmann** H, Ellebrandt-Sperling C, Spröer C, Howat A, Murrell C, Kaster AK (2019). A marine plasmid hitchhiking vast phylogenetic and geographic distances. PNAS 116: 20568-20573.
8. Kallscheuer N, Jeske O, Sandargo B, Boedeker C, Wiegand S, **Bartling** P, Jogler M, Rohde M, **Petersen** J, Medema MH, Surup F, Jogler C (2020). The planctomycete *Stieleria maiorica* Mal15^T employs stieleriacines to alter the species composition in marine biofilms. Commun Biol 3: 303.
9. *Wünsch D*, Strijkstra A, *Wöhlbrand L*, *Freese HM*, Scheve S, Hinrichs C, *Trautwein* K, Maczka M, **Petersen** J, *Schulz S*, *Overmann J*, *Rabus R* (2020). Global response of *Phaeobacter inhibens* DSM 17395 to deletion of its 262-kb chromid encoding antibiotic synthesis. Microb Physiol 30: 9-24.

10. **Birmes** L, *Freese HM*, **Petersen** J (2021). RepC_soli: A novel promiscuous plasmid type of *Rhodobacteraceae* mediates horizontal transfer of antibiotic resistances in the ocean. Environ Microbiol 23: 5395-5411.
11. *Wang H*, Beier N, Boedeker C, Sztajer H, Henke P, Neumann-Schaal M, *Mansky J*, Rohde M, *Overmann J*, **Petersen** J, Klawonn F, Kucklick M, *Engelmann* S, Tomasch J, *Wagner-Döbler I* (2021). *Dinoroseobacter shibae* outer membrane vesicles are enriched for the chromosome dimer resolution site *dif*. mSystems 6: e00693-20.
12. *Chhalodia AK*, *Rinkel J*, *Konvalinkova D*, **Petersen** J, *Dickschat J* (2021). Sulfur metabolism of six marine *Celeribacter* strains. Beilstein J Org Chem 17: 420-430.
13. Marter P, Huang S, **Brinkmann** H, *Pradella* S, Jarek M, Rohde M, *Bunk* B, **Petersen** J (2021). Filling the gaps in the cyanobacterial tree of life – Metagenome analysis of *Stigonema ocellatum* DSM 106950, *Chlorogloea purpurea* SAG 13.99 and *Gomphosphaeria aponina* DSM 107014. Genes 12: 389.
14. Smith AF, Silvano E, **Päuker** O, Guillonneau R, Quareshy M, Murphy A, Mausz MA, Stirrup R, Rihtman B, Aguilo-Ferretjans M, Brandsma J, **Petersen** J, Scanlan DJ, Chen Y (2021). A novel class of sulfur-containing aminolipids widespread in marine roseobacters. ISME J 25: 2440-2453.
15. Tomasch J, Ringel V, *Wang H*, *Freese HM*, **Bartling** P, **Brinkmann** H, Vollmers J, Jarek M, *Wagner-Döbler I*, **Petersen** J (2022). Fatal affairs - conjugational transfer of a dinoflagellate-killing plasmid between marine *Rhodobacterales*. Microb Genom 8: 000787.
16. *Freese HM*, Ringel V, *Overmann J*, **Petersen** J (2022). Beyond the ABCs - Discovery of three new plasmid types in *Rhodobacterales* (RepQ, RepY, RepW). Microorganisms 10: 738.
17. Koppenhöfer S, Tomasch J, Ringel V, **Birmes** L, **Brinkmann** H, Spröer C, Jarek M, *Wang* H, **Pradella** S, *Wagner-Döbler I*, **Petersen** J (2022). The sixth element: a 102-kb RepABC plasmid of xenologous origin modulates chromosomal gene expression in *Dinoroseobacter shibae*. mSystems 7: e0026422.
18. Koteska D, *Marter* P, Huang S, **Pradella** S, **Petersen** J, *Schulz* S (2023). Volatiles of the apicomplexan alga Chromera velia and associated bacteria. ChemBioChem 24: e202200530.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	Sequential no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Silke Pradella, Dr.	Microbiology	DSMZ	10		DSMZ
	2	Jörn Petersen, Prof. Dr.	Microbiology	DSMZ	20		DSMZ
Non-research staff	3	Orsola Päuker		DSMZ	19,85		DSMZ
Staff funded with approved grant money							
Research staff	1	Henner Brinkmann, Dr.	Phylogeny	DSMZ	26	E13 65%	

	2	Lukas Birmes, M.Sc.	Molecular Microbiology	DSMZ	26	E13 65%	
	2	Pia Marter, M.Sc.	Molecular Microbiology	DSMZ	26	PhD stipend	
Non-research staff	4	Victoria Ringel, B.Sc.		DSMZ	20	E9 50%	

Job descriptions of staff (supported through existing funds):

1. Silke Pradella
2. Planning and advice of experimental work, in particular involvement in plasmid profiling, comparative genome sequence analyses as well as microbiological and genetic work; participation in data evaluation and writing of manuscripts.
3. Jörn Petersen
4. Planning and advice of experimental work, in particular involvement in plasmid profiling, comparative genome sequence analyses as well as microbiological and genetic work; data evaluation and writing of manuscripts.
5. Orsola Päuker
6. Assistance in microbiological and molecular work, PFGE analyses.

Job descriptions of staff (funded with approved grant money):

1. Henner Brinkmann
   He conducted phylogenetic and phylogenomic analyses and analysed genome sequenced strains.
2. Lukas Birmes
   He carried out plasmid experiments (conjugation, stability, host range, compatibility) and performed genome analyses (ECRs, denitrification).
3. Pia Marter
   She carried out metagenome analysis of the associated marine bacteria from the cyanosphere of *Coleofasciculus* sp.
4. Victoria Ringel
   She conducted microbiological and molecular work, generated transposon mutants, plasmid knock-outs and transconjugants for our collaboration partners and was responsible for the quality control of all strains and mutants.

7. General information about Project A6

7.1 Phylogenomics and functional genomics of the *Roseobacter* clade

7.1.2 Project leaders

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7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022.

7.2.1 Report

7.2.1.1 Phylogenomics, taxonomy and nomenclature of roseobacters and related bacteria

As outlined in the proposal for this funding phase, the classification of roseobacters was in dire need of improvement. It was negatively affected by poor resolution of 16S rRNA gene trees, non-comprehensive sampling in taxonomic studies and sometimes little attention to monophyly in taxonomic studies. Thus, a comprehensive phylogenetic analysis of the group based on genome-scale date was necessary. Further issues recognised during this funding phase were nomenclatural problems such as illegitimate names. While the nomenclature of prokaryotes is officially regulated by the International Code of Nomenclature of Prokaryotes (ICNP), not all names proposed under its auspices actually conform to the rules of this code.

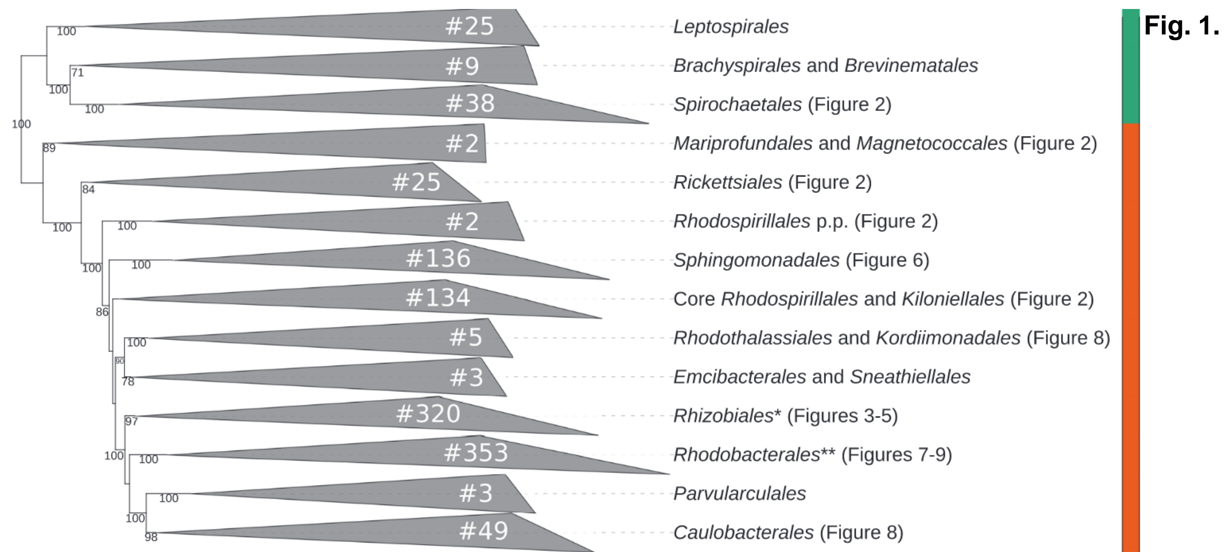

Fig. 1. Comprehensive phylogenomic sampling of the class *Alphaproteobacteria* by Hördt et al. (2020). A variety of the genera taxonomically classified as *Rhodobacteraceae* where phylogenetically scattered over the class, which made it necessary to examine all *Alphaproteobacteria*. Green bar, outgroup; orange bar, *Alphaproteobacteria*; asterisk, group phylogenetically including some taxa taxonomically placed in *Rhodobacterales*; two asterisks, same for *Rhizobiales.* References to other figures in Hördt et al. (2020) are given; numbers within the collapsed branches indicate the respective number of type-strain genome sequences examined.

The study by Hördt et al. (2020), Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of Alphaproteobacteria, included 112 pages, analysed 1104 Alphaproteobacteria and outgroup type-strain genome sequences (largely generated by A6 during the first and second funding periods), cited 925 references and included a comprehensive sampling of taxonomic literature and 16S rRNA gene sequences. It proposed

a huge number of names at the ranks of subspecies (16), species (75), genus (13), family (28) and order (1). Numerous emendations of previously established taxon names at the ranks of subspecies (8), species (594), genus (14) family (24) and order (6) were suggested. Hördt et al. (2020) removed most known instances of non-monophyletic taxa in the class.

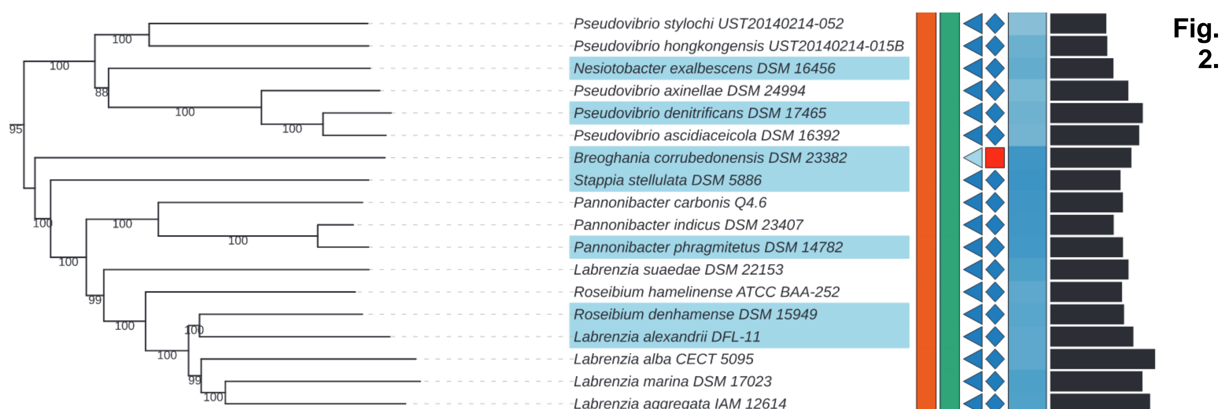

Fig. 2.

Recognition of the new family *Stappiaceae* by Hördt et al. (2020): a home for some of the genera misclassified as *Rhodobacteraceae*. Type species are highlighted in light blue; the columns on the left indicate (from left to right) phylum, class, former order, former family, G+C content and genome size.

As outlined in the proposal for this funding phase, phenotype-genotype correlations were also of major interest for A6. A significant correlation between selected genomic and phenotypic features on the one hand and the genome-scale phylogeny on the other hand was revealed by Hördt et al. (2020). The results shown in Table 1 also assist in explaining the discrepancies between the taxonomic classification and modern phylogenomic approaches. A mechanistic explanation for the scattered occurrence of photosynthesis in roseobacters and other *Alphaproteobacteria* was provided by Brinkmann et al. (2018), a collaboration with A5, who demonstrated that an entire photosynthesis operon can be transferred via a plasmid. A meta-analysis of the relationship between phenotypic data and phylogenetic results for prokaryotes was provided by Göker (2021). For the phylogeny of *Alphaproteobacteria* see also Wiese et al. (2020).

Table 1. Phylogenetic conservation of genomic and phenotypic features in roseobacters and other *Alphaproteobacteria* as measured by Hördt et al. (2020) using the retention index (RI) and assessed for significance by applying a permutation test. Higher RI means higher conservation.

Feature	**Coverage**	**RI**	**P-value**
Percent G+C content	100%	0.736	0.0001
Genome size in bp	100%	0.627	0.0001
Cell length in µm	74%	0.422	0.0001
Cell width in µm	71%	0.303	0.0001
Motility by flagella	72%	0.584	0.0001
Relationship to oxygen	99%	0.511	0.0001
Carotenoids	18%	0.513	0.0001
Bacteriochlorophyll	30%	0.454	0.0001
Isoprene residues	57%	0.476	0.0001

A study by other authors (Liang et al. 2021) unfortunately proposed the family *Roseobacteraceae*, allegedly covering the "*Roseobacter* clade", in a manner that created a paraphyletic family *Rhodobacteraceae*. The name *Rhodobacteraceae* Garrity et al. 2006 is an illegitimate name, as its proposal included the type genus of another family with a validly published name (Rule 51b). *Rhizobiales* Kuykendall 2006 and *Bradyrhizobiaceae* Garrity et al. 2006 also contravene Rule 51b; replacement names were indicated by Hördt et al. (2020). To repace *Rhodobacteraceae*, the family name *Paracoccaceae* was proposed by Göker (2022b).

Some genera of *Rhodobacterales* were not assigned to a family in this study, thereby avoiding the creation of a paraphyletic family.
The name *Alphaproteobacteria* Garrity et al. 2006 was recognized by Göker (2022a) as also being illegitimate. While an emendation of the ICNP made in 2022 caused class names such as *Alphaproteobacteria* to not contravene Rule 8 any longer, the original proposal of Alphaproteobacteria included *Rhodospirillales*, which is the type order of the class with the validly published and legitimate name *Anoxyphotobacteria* Murray 1988. Thus, *Alphaproteobacteria* contravenes Rules 51b and 55(4) of the ICNP. The name instead to be applied to the class would be the hardly known *Anoxyphotobacteria.* In order to stabilize the nomenclature of roseobacters and related bacteria, Göker (2022a) proposed rejecting the name *Anoxyphotobacteria*. The same author, a member of the Judicial Commission of the International Committee on Systematics of Prokaryotes (ICSP), which oversees the ICNP, drafted a Judicial Opinion, which was ratified by the Judicial Commission and the ICSP (Arahal et al. 2023). These measures, together with analyses like those of Hördt et al. (2020), guarantee a modern classification of roseobacters and related groups that is fully in line with the requirements of the international rules of nomenclature (Parte et al. 2020), thereby considerably improving scientific communication about these critically important bacteria.

7.2.1.2 Improvement and easy accessibility of genome-scale methods for phylogeny and taxonomy of Rhodobacteraceae and other prokaryotes

As indicated in the proposal for this funding phase, there is a strong need for more efficient methods for analysing genome-scale data. The pairwise comparison of whole genomes at the amino-acid level currently poses the most significant bottleneck in comparative genomics, because of its huge algorithmic search space, thus resulting in running times at least 1-2 orders of magnitude larger than at the nucleotide level. The software DIAMOND was shown to outperform other approaches competing with BLAST+ without sacrificing sensitivity. Our benchmarking datasets included a comprehensive dataset consisting of the proteome sequences of 172 *Rhodobacteraceae* type strains to compare the phylogenomic results under various settings of BLAST+ and DIAMOND. DIAMOND analyses resulted in virtually identical phylogenies with the same level of support while run times were reduced by a factor of c. 14 when using DIAMOND at optimal settings.
The Type Strain Genome Server (Meier-Kolthoff and Göker 2019; TYGS, https://tygs.dsmz.de) is a highly cited databases and tool set for genome-based taxonomy of prokaryotes. The TYGS was later augmented with proteome-based analyses (Meier-Kolthoff et al. 2022) and clearly benefited further from replacing the standard BLAST+ software by an optimized approach. We subsequently integrated DIAMOND into the TYGS. Users are now able to conduct faster proteome-based analyses without loss of accuracy. The databases developed for TYGS now also forms the basis of the List of Prokaryotic names with Standing in Nomenclature (Parte et al. 2020, Meier-Kolthoff et al. 2022), which also provides up-to-date information on taxon names of roseobacters.

7.2.1.3 Methods for phage phylogeny and classification and their application

In the third funding period project A6 also focused on the development and application of methods for the classification and phylogeny of viruses of *Archaea* and *Bacteria* (phages), which play an enormous role in global life cycles in general, e.g., as antagonists of roseobacters in marine ecosystems. The classification and phylogeny of viruses is a field which was traditionally only little informed by genome sequencing, although single-gene phylogenies are not normally well resolved and phages are genetically enormously divergent. Thus, we developed the VICTOR approach, which is freely and publicly available as a web service (https://victor.dsmz.de) since 2017. VICTOR compares phages using their genome or proteome sequences. The results include phylogenomic trees with branch support, inferred using methods now optimized as described above, as well as suggestions for the classification at the species, genus, subfamily and family level.

Since its release VICTOR has already substantially informed virus research. For instance, VICTOR was successfully applied in collaboration with B6 to clarify the position of newly isolated roseobacter phages and to revise the classification of the virus family *Podoviridae* (Moraru et al. 2019). Jan P. Meier-Kolthoff was invited in 2022 to a workshop organized by the International Committee on Taxonomy of Viruses for discussing approaches for establishing a universal virus taxonomy. The resulting consensus paper (Simmonds et al. 2023) provides four guiding principles for constructing a coherent and comprehensive virus taxonomy. One of these principles is that phylogenomic methods (as provided by VICTOR) are crucial for forming taxa that reflect the evolutionary histories.
A second VICTOR application is the analysis of plasmids (Öztürk et al. 2020) as carried out during a comprehensive analysis of the extrachromosomal replicons (ECRs) of surface-associated Phaeobacter in comparison to other marine microorganisms. This work was done in close collaboration with A7 but could not yet be published. One of the main results is that *Phaeobacter* chromids evolve concertedly with their corresponding chromosomes. This recruitment might reflect an evolutionary innovation which drives divergence in roseobacter subgroups.

7.2.2 Published project results
Publications with scientific quality assurance

List of publications in peer reviewed journals
(members of A6 in bold, of other CRC projects in italic)

1. Arahal DR, Busse H-J, Bull CT, Christensen H, Chuvochina M, Dedysh SN, Fournier P-E, Konstantinidis KT, Parker CT, Rosselló-Móra R, Ventosa A, **Göker M** (2023) Judicial Opinion 128. *International Journal of Systematic and Evolutionary Microbiology* 73: 5797.
2. *Brinkmann H*, **Göker M**, Koblížek M, *Wagner-Döbler I, Petersen J* (2018) Horizontal operon transfer, plasmids and the evolution of photosynthesis in *Rhodobacteraceae*. The ISME Journal 12: 1994-2010.
3. **Göker M** (2022a) Solving the remaining problems with names of classes. Request for an Opinion. *International Journal of Systematic and Evolutionary Microbiology* 72: 5605.
4. **Göker M** (2022b) Filling the gaps: missing taxon names at the ranks of class, order and family. *International Journal of Systematic and Evolutionary Microbiology* 72: 5638
5. Hördt A, García-López M, **Meier-Kolthoff JP**, Schleuning M, Weinhold LM, Tindall BJ, Gronow S, Kyrpides NC, Woyke T, **Göker M** (2020) Analysis of 1,000+ type-strain genomes substantially improves taxonomic classification of *Alphaproteobacteria Frontiers in Microbiology* 11: 468.
6. **Meier-Kolthoff JP**, **Göker M** (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 10: 2182.
7. **Meier-Kolthoff JP**, Sardà Carbasse J, Peinado-Olarte RL, **Göker M** (2022) TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Research*, 50: D801-D807.
8. *Moraru C, Bischoff V*, Bunk B, **Meier-Kolthoff JP**, Spröer C, Poehlein A, *Dogs M*, Nguyen M, *Petersen J, Daniel R, Overmann J*, **Göker M**, *Simon M, Brinkhoff T* (2019) Cobaviruses – a new globally distributed phage group infecting *Rhodobacteraceae* in marine ecosystems. ISME Journal 13: 1404-1421, 2019.
9. Öztürk B, Werner J, **Meier-Kolthoff JP**, Bunk B, Spröer C, Springael D (2020) Comparative genomics suggests mechanisms of genetic adaptation toward the catabolism of the phenylurea herbicide linuron in *Variovorax*. *Genome Biol Evol* 12: 827–841.
10. Parte AC, Sardà Carbasse J, **Meier-Kolthoff JP**, Reimer LC, **Göker M** (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology*, 70: 5607-5612.
11. Simmonds P, Adriaenssens EM, Zerbini FM, Abrescia NGA, Aiewsakun P, Alfenas-Zerbini P, Bao Y, Barylski J, Drosten C, Duffy S, Duprex WP, Dutilh BE, Elena SF, Garcia ML, Junglen S, Katzourakis A, Koonin EV, Krupovic M, Kuhn JH, Lambert AJ, Lefkowitz EJ,

Lobocka M, Lood C, Mahony J, **Meier-Kolthoff JP**, Mushegian AR, Oksanen HM, Poranen MM, Reyes-Munoz A, Robertson DL, Roux S, Rubino L, Sabanadzovic S, Siddell S, Skern T, Smith DB, Sullivan MB, Suzuki N, Turner D, Van Doorslaer K, Vandamme AM, Varsani A, Vasilakis N (2023) Four principles to establish a universal virus taxonomy. *PLoS Biol* 21: e3001922.

12. Wiese J, Imhoff JF, Horn H, Borchert E, Kyrpides NC, **Göker M,** Klenk H-P, Woyke T, Hentschel U (2020) Genome analysis of the marine bacterium *Kiloniella laminariae* and first insights into comparative genomics with related *Kiloniella* species. *Archives of Microbiology* 202: 815-824.

Other publications and published results

1. **Göker** M (2021) What can genome analysis offer for bacteria? Pp. 255-281 in: Bridge, P., Smith, D., Stackebrandt, E. (eds), Trends in the systematics of bacteria and fungi. CAB International, Wallingford.

7.2 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.2.1 Project staff in the ending funding period

	Sequential no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Markus Göker, PD Dr.	Phylogenomics, taxonomy, nomenclature	Bioinformatics and databases	5		DSMZ
Non-research staff	2	Meike Döppner		Services	20		DSMZ
Staff funded with approved grant money							
Research staff	1	Jan P. Meier-Kolthoff, Dr.	Phylogenomics	Bioinformatics and databases	40	E13 100%	DSMZ

Job descriptions of staff (supported through existing funds):

1. Göker
   He was principal investigator and responsible for overall design of the studies.
2. Döppner
   She carried out DNA extraction for type-strain genome sequencing. Unfortunately, she left the position early in 2020.

Job descriptions of staff (funded with approved grant money):

1. Meier-Kolthoff
   He carried out the implementation, further improvement and application of the phylogenomic analysis pipelines established since the previous funding period.

7. General information about Project A7

7.1 Population structure and divergence in the Roseobacter group – implications for the ecology and and evolution

7.1.2 Project leader

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7.2 Project history

This project received funding through TRR51 from August 2011 until December 2022

7.2.1 Report

7.2.1.1 Population structure of generalist *Sulfitobacter*

Only eight closed genomes of *Sulfitobacter* were available in NCBI databases at the beginning of the project. To analyse the population structure of this important genus of the *Roseobacter* group, a large number of high quality, closed genome sequences had to be generated initially. Therefore, we isolated and sequenced 23 strains from various habitats including the Pacific Ocean using samples obtained during the Sonne cruise SO248. Furthermore, we generated closed genomes from 25 strains provided by different cooperation partners (A1, A2, A5, B4, B6, external) and seven strains available in the DSMZ collection. This work showed that a combination of long-read (PacBio) and short-read (Illumina) sequencing is required to establish complete closed genomes comprising all plasmids (Freese et al. 2022).

The phylogenetic analysis of the genomes revealed that a large diversity exists within the genus *Sulfitobacter* (Fig. 1). Many of the investigated strains actually represented novel species, which is remarkable given the fact that the genus *Sulfitobacter* already encompassed the second largest number of species within the *Roseobacter* group (cf. LPSN, https://lpsn.dsmz.de). This indicates that the diversity of *Sulfitobacter* so far was significantly under-represented.

All strains representing novel species (Fig. 1) have been characterized and revealed distinct phentoypic differences. For instance strain DSM 110033 only tolerated pH values between 6-9 while strains DSM 110093 and 109994 could cope with pH 5 – 11. Strain DSM 109994 (isolated from a costal river in Spain) even grew at 40°C while the closest related strains grew only till 30°C. The strains also varied distinctly in the carbon substrates they could utilise. Accordingly, five novel species are currently described by us, including *S. aquaticus* DSM 110093, *S. fluminis* DSM 109994, *S. skagerrakensis* DSM 110095, *S. porticola* DSM 110033 and *S. prorocentri* DSM 111388.

The general genotypic and phenotypic diversity determined for the genus *Sulfitobacter* is much more pronounced than for the surface-associated *Phaeobacter*, which had been investigated in previous funding periods, and may reflect their characteristics as generalist. The analysis of the housekeeping genes revealed that homologous recombination is a factor generating this diversity (*r/m* 1.4) but that it is much less important than for the aquatic generalist *Vibrio* (*r/m* 23.7).

No geographic or habitat preferences could so far be delineated for the different *Sulfitobacter* clades or species based on their known phenotypic properties. The only exemption was the *S. porphyrae* clade which occurred in association with phototrophs. These strains were also characterised by the largest genomes (median 5.3 Mb) and the largest content of biosynthetic gene clusters (9-13), indicating the formation of betalactone, homoserine lactone, ectoine, Nonribosomal peptide synthetase, and specific special adaptations strategies.

A publication of these results is in preparation.

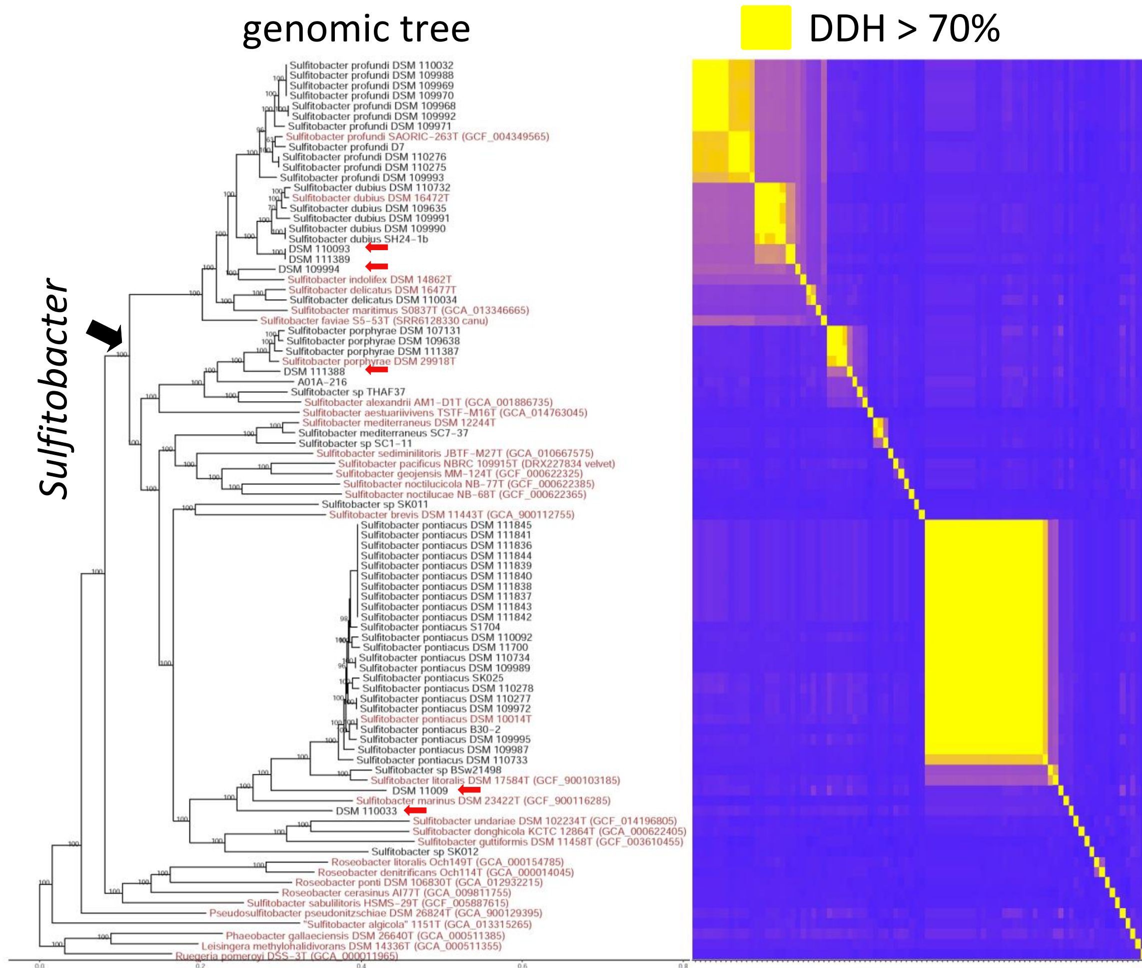

Fig. 1. Phylogenomic tree of the genus *Sulfitobacter* encompassing newly sequenced strains and type strains as reference (red) in comparison to their pairwise digit DNA-DNA-Hybridisation (DDH). DDH value of 70% indicate the threshold for species delineation. Strains isolated by us representing novel species are indicated by red arrows.

7.2.1.2 Population structure of free-living RCA cluster

As outlined in the proposal, comparative population genomics were planned for the pelagic free-living roseobacter population *Planktomarina temperata* (RCA cluster) applying single cell genomics. One precondition was a successful sorting and single cell amplification via multiple displacement amplification which was first tested using the isolate *P. temperata* DSM 22400. However, only 73% of the sorted cells showed a possible amplification signal and nearly none of them could be detected in screening PCRs. Most importantly, and contrary to previous assumptions, bacteria of the RCA cluster showed an unexpected low abundance in the samples collected from the Pacific Ocean (1.3 - 3.1%, Sonne cruise SO248, in cooperation with A1) and North Sea (0.9 - 3.9%, Heincke cruise, in cooperation with A1). Therefore the planned analysis were not feasible and had to be abandoned.

7.2.1.3 Role of extrachromosomal replicons in the evolution of marine bacteria

One focus during the last funding phase was the comprehensive analysis of the extrachromosomal replicons (ECRs) of surface-associated *Phaeobacter* in comparison to other marine microorganisms.
Based on our generated closed high quality genomes we could show that 35 *Phaeobacter* strains comprised 206 circular ECR, which could be differentiated into plasmids and chromids that have chromosome like characteristics. Metabolic adaptations mediated by the 262-kb chromid in *P. inhibens* DSM 17395 were investigated within a CRC cooperation (C1, C2, A5, A7), which included genomic resequencing, transcriptomic and proteomic analyses (Wünsch et al. 2020). This chromid as well as two further chromids were universally present in all *Phaeobacter* strains, which allowed us to test the hypothesis that chromids evolve concertedly with their corresponding chromosome. We could proof this hypothesis for the first time as all three chromids stably co-evolved with the chromosome over speciations. During their evolution, chromids also acquired species-specific genes that reinforced the chromosomal adaptations to different niches on marine surfaces that we had discovered in the preceding funding phase. We also identified a fourth, newly emerging chromid whose acquisition likely initiated the speciation of *P. gallaeciensis.* Our analysis of *Sulfitobacter* strains also revealed a conserved chromid occurring specifically in a distinct clade including *S. dubius* (Freese et al. 2022). Together this indicates that chromids are not just stably co-evolving but that their recruitment reflects an evolutionary innovation which drives divergence in the different *Roseobacter* subgroups.
Genuine plasmids were much more diverse than the chromids. We not only identified several individual novel plasmid types in *Rhodobacterales* which were published (Birmes et al. 2021, Freese et al. 2022), but discovered a high diversity of 25 different plasmid replication systems that all occurred in *Phaeobacter*, indicating that a single cell could theoretically harbour a multipartite genome with two dozens of replicons. In contrast to the conservation of chromids, even closely related *Phaeobacter* plasmids occurring in closely related strains were characterized by a variable mosaic structure revealing that they underwent continuous recombination. Our comparative analysis of the plasmid distribution further revealed that plasmids were frequently horizontally exchanged even across genus borders. In particular, we could identify the marine phycosphere as a hotspot for plasmid transfers. These results will be submitted for publication shortly. The opposite seems to be true for intracellular habitats. Our parallel analysis of the ECR of 73 *Piscirickettsia* strains from marine fish revealed that the plasmids were specific for phylogenomic clades. Here, horizontal plasmid transfer was obviously rare for the bacteria with an intracellular lifestyle.
Additional insights into the ecological relevance of plasmids were gained by cooperation highlighting plasmids dedicated to polysaccharide utilization in marine *Alteromonas* (Koch et al. 2019, 2020). We also documented a recent plasmid-mediated spread and chromosomal integration of functional antibiotic resistance genes in *Phaeobacter*, which was probably driven by anthropogenic pollution. These plasmids can mediate the access of the host to novel ecological niche.

7.2.1.4 Horizontal transfer of ribosomal genes

Our successful sequencing and assembly of 63 closed high-quality genomes of diverse *Sulfitobacter* species also enabled us to test the assumption that ribosomal RNA (*rrn*) genes, which still serve as the gold standard for taxonomic identification and classification, are rarely transferred horizontally. The *Sulfitobacter* strains contain one to four *rrn* operons but 41 of 187 operons analysed were actually found to be located on plasmids. The *rrn* genes varied in number, location and length within species and strains but the intragenomic 16S rRNA diversity was always below 1%. Comparative analysis of the 16S and 23S rRNA genes indicated a discordant evolution of the *rrn* genes. The phylogenetic networks of both genes show complex network structures indicating conflicting signals probably due to recombination. Individual 16S rRNA variants are distributed across species borders and two bacterial strains from different species even contained completely identical 16S rRNAs (Fig. 2, marked by a star), which could also be observed within *Phaeobacter*.

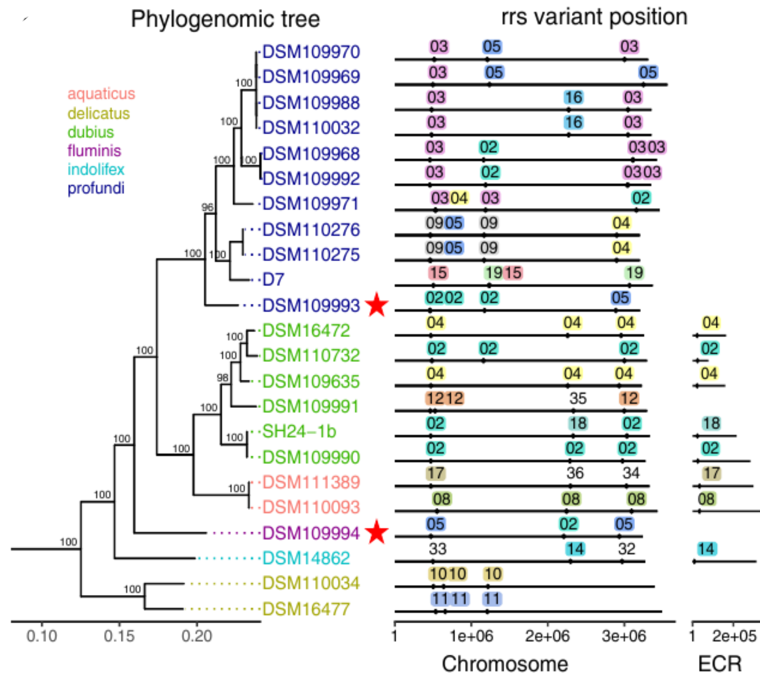

Fig. 2. Genomic position of *rrs* variants in comparison to the phylogeny of a subset of *Sulfitobacter* strains. Non-unique *rrs* variants are indicated by different colors. Strains of different species containing the same *rrs* variants are marked by a star.

In these cases, the usage of *rrn* genes as phylogenetic markers would thus result in a wrong taxonomic classification. In cooperation with A5, we even demonstrated that a *rrn* containing *Sulfitobacter* plasmid could be transferred over genus borders for instance into *Dinoroseobacter shibae*. Furthermore, the impact of homologous recombination on the *rrn* genes (*r/m* 16.1) is an order of magnitude higher than its effect on the housekeeping genes.

Our results indicate that a horizontal transfer of *rrn* genes may be more frequent than expected between strains of closely related species. Our results imply that classifications based on phylogenetic 16S rRNA gene not always are sufficient for diversity assessments at least of the *Roseobacter* group.

7. Published project results

Publications with scientific quality assurance
List of publications in peer reviewed journals
(members of A7 in bold, of other CRC projects in italic)

1. *Klotz* F, *Brinkhoff* T, **Freese** HM, Wietz M, Teske A, *Simon* M & *Giebel* H-A (2018) Description of Tritonibacter horizontis gen. nov., sp. nov., a new member of the Rhodobacteraceae, isolated from the Deepwater Horizon oil spill. Int J Syst Evol Microbiol, 68: 736-744.
2. Koch H, Dürwald A, Schweder T, *Noriega-Ortega* B, Vidal-Melgosa S, Hehemann J-H, *Dittmar* T, **Freese** HM, Becher D, *Simon* M & Wietz M (2019) Biphasic cellular adaptations and ecological implications of Alteromonas macleodii degrading a mixture of algal polysaccharides. ISME J, 13: 92-103.
3. Crenn K, **Bunk** B, Spröer C, **Overmann** J, Jeanthon C (2019) Complete genome sequence of the Silicimonas algicola type strain, a representative of the marine Roseobacter group isolated from the cell surface of the marine diatom Thalassiosira delicatula. Microbiol Resour Announc 8, e00108-19
4. Koch H, **Freese** HM, Hahnke RL, *Simon* M, Wietz M (2019) Adaptations of Alteromonas sp. 76-1 to polysaccharide degradation: A CAZyme plasmid for ulvan degradation and two alginolytic systems. Front Microbiol 10: Article 504.
5. *Bischoff* V, **Bunk** B, *Meier-Kolthoff* JP, Spröer C, *Poehlein* A, *Dogs* M, Nguyen M, *Petersen* J, *Daniel* R, **Overmann** J, *Göker* M, *Simon* M, *Brinkhoff* T, *Moraru* C (2019) Cobaviruses - a new globally distributed phage group infecting Rhodobacteraceae in marine ecosystems. ISME J 13, 1404-1421
6. Koch H, Germscheid N, **Freese** H, *Noriega-Ortega* BE, *Lücking* D, *Berger* M, Qiu G, Marzinelli E, Campbell A, Steinberg PD, **Overmann** J, *Dittmar* T, *Simon* M, Wietz M (2020)

Genomic, metabolic and phenotypic variability shapes ecological differentiation and intraspecies interactions of Alteromonas macleodii. Sci Reports, 10: 809
7. *Wünsch* D, *Strijkstra* A, *Wöhlbrand* L, **Freese** HM, *Scheve* S, *Hinrichs* C, *Trautwein* K, Maczka M, *Petersen* J, *Schulz* S, **Overmann** J & *Rabus* R (2020). Global Response of Phaeobacter inhibens DSM 17395 to deletion of its 262-kb chromid encoding antibiotic synthesis. Microb Physiol, 30: 9-24.
8. *Birmes* L, **Freese** HM & *Petersen* J (2021) RepC_soli: a novel promiscuous plasmid type of Rhodobacteraceae mediates horizontal transfer of antibiotic resistances in the ocean. Environ Microbiol. 23: 5395-5411.
9. *Leinberger* J, Holste J, **Bunk** B, **Freese** HM, Spröer C, *Dlugosch* L, Kück A-C, *Schulz* S & *Brinkhoff* T (2021) High potential for secondary metabolite production of Paracoccus marcusii CP157, isolated from the crustacean Cancer pagurus. Front Microbiol, 12: 1725.
10. *Wang* H, Beier N, Boedeker C, *Sztajer* H, Henke P, Neumann-Schaal M, *Mansky* J, Rohde M, **Overmann** J, *Petersen* J, Klawonn F, Kucklick M, *Engelmann* S, Tomasch J, *Wagner-Döbler* I (2021) Dinoroseobacter shibae outer membrane vesicles are enriched for the chromosome dimer resolution site dif. mSystems 6: e00693-20.
11. Tomasch J, Ringel V, *Wang* H, **Freese** HM, *Bartling* P, *Brinkmann* H, Vollmers J, Jarek M, *Wagner-Döbler* I & *Petersen* J (2022) Fatal affairs – conjugational transfer of a dinoflagellate-killing plasmid between marine Rhodobacterales. Microbial Genomics, 8: 000787.
12. **Freese** HM, Ringel V, **Overmann** J & *Petersen* J. (2022) Beyond the ABCs – Discovery of three new plasmid types in Rhodobacterales (RepQ, RepY, RepW). Microorganisms, 10: 738.
13. *Zucker* F, *Bischoff* V, Olo Ndela E, *Heyerhoff* B, *Poehlein* A, **Freese** HM, Roux S, *Simon* M, Enault F & *Moraru* C (2022) New Microviridae isolated from Sulfitobacter reveals two cosmopolitan subfamilies of ssDNA phages infecting marine and terrestrial Alphaproteobacteria. Virus Evolution, 8: veac070.
14. Schober I, Bunk B, Carril G, **Freese** HM, Ojeda N, Riedel T, Meier-Kolthoff JP, Göker M, Spröer C, Flores-Herrera PA, Nourdin-Galindo G, Gómez F, Cárdenas C, Vásquez-Ponce F, Labra A, Figueroa J, Olivares Pacheco J, Nübel U, **Sikorski** J, Marshall SH, **Overmann** J (2023) Ongoing diversification of the global fish pathogen *Piscirickettsia salmonis* through genetic isolation and transposition bursts. ISME J, accepted.

7.2 Funding

Funding of this project within the Collaborative Research Centre started in August 2011. The project ended by the end of the final funding period.

7.2.1 Project staff in the ending funding period

	Sequential no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Jörg Overmann, Prof. Dr., W3	Microbial Ecology	DSMZ	7		DSMZ
	2	Boyke Bunk, Dr.	Bioinformatics	DSMZ	6		DSMZ
	3	Johannes Sikorski, Dr.	Ecological statistics	DSMZ	5		DSMZ

Non-research staff	4	Anika Methner		DSMZ	6		DSMZ
	5	Franziska Burkart		DSMZ	4		DSMZ
Staff funded with approved grant money							
Research staff	1	Heike Freese, Dr., Postdoc	Population genomics	DSMZ		E13 100%	DSMZ

Job descriptions of staff (supported through existing funds):

1. Overmann
   Planning and advice during experimental work; participation in data evaluation and writing manuscripts.
2. Bunk
   Support during genome assembly and subsequent bioinformatic sequence analysis. Maintaining the database hosting strain-specific information and participation in data evaluation.
3. Sikorski
   Support of population genomic analysis and participation in data evaluation.
4. Methner
   Assistance in microbiological work and molecular biological analyses, growing bacterial cultures for genome sequencing, extraction and purification of genomic DNA and of RNA.
5. Burkart
   Assistance in experimental work, single cell manipulation of natural samples and physiological experiments.

Job descriptions of staff (funded with approved grant money):

1. Freese
   Genome sequencing and assembly, bioinformatic sequence analysis and the subsequent comprehensive and detailed population genomic analyses. In charge of cell sorting, multiple displacement amplification, physiological experiments, and writing of the manuscripts.

7. General information about Project A8

7.1 Linking the exometabolome of selected pelagic organisms of the Roseobacter group to marine dissolved organic matter

7.1.2 Project leaders

Niggemann, Jutta, Dr., 01.04.1974, German
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7.2 Project history

This project received funding through TRR51 from January 2014 until December 2022

7.2.1 Report

7.2.1.1 Bacterial exometabolites in marine dissolved organic matter

During growth, bacteria release a complex mixture of thousands of different compounds. The molecular composition of this exometabolome is modulated by substrate type and growth stage, and even the rather closely related *Roseobacter* model strains *Dinoroseobacter shibae* and *Phaeobacter inhibens* produce very different dissolved organic matter (DOM) when growing under identical conditions (Wienhausen et al. 2017, Noriega-Ortega et al. 2019). In a joint study with project B2, we showed that even single mutations cause significant changes in exometabolome composition (Srinivas et al. 2022). The knock-out of important regulation mechanisms through the two signalling molecules tropodithietic acid (TDA) and the quorum sensing molecule acyl homoserine lactone (AHL) largely impacted the composition of the exometabolome with potential consequences for species interactions in microbial communities. Detecting and tracing bacterial exometabolites in natural seawater is an analytical challenge. We systematically tested and optimized our analytical pipeline of untargeted Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) on solid-phase extracted DOM for the detection of molecular formulae of intact exometabolites in natural seawater samples (Bercovici et al. 2022). Increasing the number of accumulated scans and winnowing a specific mass range where exometabolites are most abundant, we achieved a recovery of >40% of exometabolites in mixtures with natural seawater DOM. We extended our incubation studies to more complex systems with interacting microorganisms, including natural microbial communities growing on zooplankton exudates (DeCorte et al. 2023) and co-cultures of the dinoflagellate *Prorocentrum minimum* and *Dinoroseobacter shibae* (incubations of project B4). In collaboration with project B2, we studied the exometabolome released by the diatom *Thalassiosira rotula*, revealing different molecular composition depending on growth stage and exposure to TDA. For the latter to studies data evaluation is still ongoing (DeCorte et al. in prep., Srinivas et al. in prep.).

7.2.1.2 Chemogeography of marine dissolved organic matter

Microorganisms play a substantial role in creating and sustaining the molecular diversity of marine DOM. In the deep ocean, DOM is the main carbon and energy source for prevailing microbial communities. Yet, vast amounts of DOM reside in the world's ocean, accumulating over thousands of years. A major focus during this last funding phase was on the processing and evaluation of the unique comprehensive data sets collected during the joint CRC Roseobacter cruises covering the Atlantic (Polarstern ANT-XXVIII/2, -/4 and -/5) and Pacific Oceans (SO245, -248, -254), including adjacent Southern Ocean sections (Fig. 1C). We made substantial progress in understanding processes that control the global distribution of marine DOM. We performed a two-endmember mixing analysis on almost 7000 detected molecular formulae in a total of 1126 DOM samples, covering the deep ocean circulation from deep water formation in the North Atlantic via partial upwelling in the Southern Ocean to the North Pacific (Bercovici et al. in rev., Glob Biogeochem Cyc). More than 70% of the detected molecular formulae behaved conservatively in the deep ocean, i.e. their distribution is purely explained by water mass mixing ("*core*" Fig. 1B). We identified dynamic DOM fractions that occurred or disappeared sporadically, indicating local events of DOM production and degradation in the deep ocean such as chemoautotrophy or particle export and solubilization. Newly occurring compounds that were not detected in any of the endmembers ("*new source*" Fig. 1D) accounted for <3% of DOM and positively correlated with DOM lability parameters. The same global DOM data set was used to derive two novel independent process-related molecular indices for biological production (I_{bio}) and photodegradation (I_{photo}). We successfully applied these indices to disentangle and assess the influence of bioproduction and photodegradation, which act simultaneously on the global DOM pool (Bercovici et al. subm., ES&T).

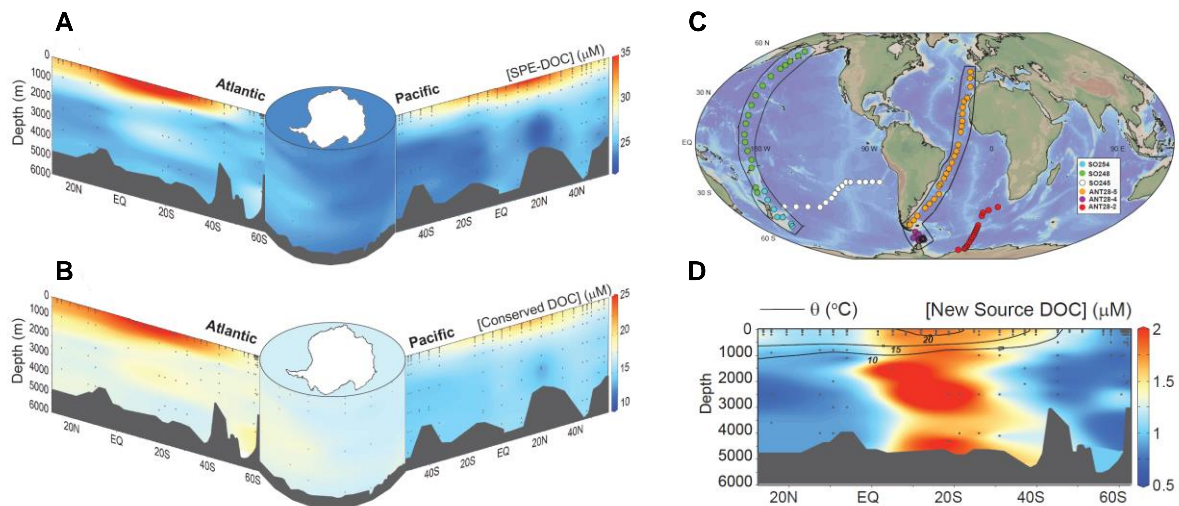

Fig. 1. Latitudinal cross-section of the Atlantic, Pacific and Southern Ocean (sections outlined in global map, C) of the total SPE-DOC concentration (A) and the conserved SPE-DOC concentration (*core* DOC) calculated from the relative peak intensity of the *core* molecular formulae (B). Concentration of *new source* DOC across the Atlantic, with surface temperature in contours (D). Figure modified from Bercovici et al. (in revision for Global Biogeochemical Cycles).

7.2.1.3 Microbial interactions and chemical crosstalk

Based on the previously identified exometabolites in the exometabolome of *D. shibae* and *P. inhibens*, (Wienhausen et al. 2017) we focused our experimental work on interactions of microbial communities and distinct bacteria with vitamin B_{12} and B_7 and respective building blocks. During cruise SO248 across the Pacific Ocean we carried out mesocosm experiments in the south subtropical gyre, the equatorial upwelling and the polar frontal region and supplied the microbial communities with vitamin B_{12} or α-ribazole, the activated form of the lower ligand of B_{12}, dimethylbenzimidazole. We thus simulated the supply of these growth factors, most probably by roseobacters as the main suppliers of B_{12} in the near surface ocean. In all three experiments, the first ones ever addressing this topic for prokaryotes, we found a response of

distinct prokaryotic lineages to the supplementations by shifts in the community composition and on the transcriptomic level (Wienhausen et al. 2022a).
A very important prerequisite for more specific studies with prototrophic and auxotrophic bacterial strains and in situ studies on vitamins B_{12} and B_7 and the respective building blocks was the successful establishment of their quantitative analysis in cooperation with the organic geochemistry group at ICBM (Bruns et al. 2022). By establishing a B_{12} bioassay with the diatom *Thalassiosira pseudonana* we showed that of 33 tested B_{12} prototrophic *Rhodobacteraceae* only 18 shared the produced B_{12} with the diatom whereas 9 retained B_{12} in their cell (Fig. 2, Sultana et al. 2023). This result demonstrates that not more than 55% of prototrophs share B_{12} with other microorganisms, indicating that cellular B_{12} export is a very specific process.
Regarding vitamin B_7, biotin, we showed experimentally that quite a few B_7 auxotrophs can overcome their auxotrophy by using desthiobiotin, the last biosynthetic precursor of biotin which is also present in situ (Wienhausen et al. 2022b). Genomic and metagenomics analyses revealed that this biotin auxotrophy and salvage pathway is quite widely distributed, in particular among *Rhodobacteraceae*, and in prokaryotic communities in the southwest Atlantic and Southern Ocean.

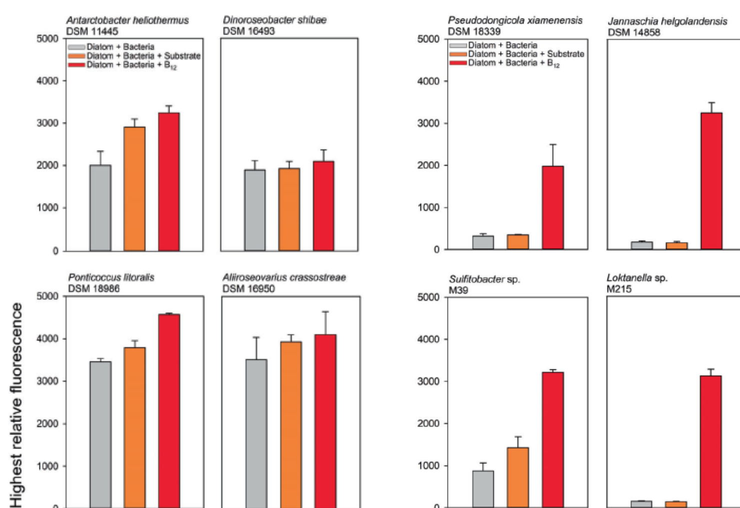

Fig. 2. Growth yield (in fluorescence units) of *Thalassiosira pseudonana* in co-culture with B_{12}-providers (left panels; *Antarctobacter heliothermus, D. shibae, Pontiococcus litoralis, Aliiroseovarius crassostreae*) and B_{12}-retainers (right panels; *Pseudodongicola xiamenensis, Jannaschia helgolandensis, Sulfitobacter* sp. M39, *Loktanella* sp. M215).
Grey bar: diatom+bacterium
Orange bar: diatom+bacterium+substrate
Red bar: diatom+bacterium+B_{12}.
Figure modified from Sultana et al. 2023.

7.2.1.4 Ligand crossfeeding resolves bacterial vitamin B_{12} auxotrophies

In extensive studies we showed that two bacterial B_{12} auxotrophs can salvage different B_{12} building blocks and jointly synthesize B_{12}. This happens only when a prophage is induced in one partner, proliferates and leads to release of phage particles. When cultivated in co-culture a *Colwellia* strain synthesizes and supplies a B_{12} lower ligand. The second B_{12}-auxotroph, a *Roseovarius* strain, possessing corrin ring synthesis genes, can use the B_{12} lower ligand to finalize B_{12} but does not share it. A genome-encoded prophage in *Roseovarius* induces its lytic cycle upon the presence of *Colwellia*, leading to growth of the latter, presumably due to B_{12} release. These complex microbial interactions of ligand crossfeeding and joint B_{12} biosynthesis appear to be widespread in marine pelagic ecosystems. In the western and northern tropical Atlantic Ocean, bacteria synthesizing only the lower ligand outnumber B_{12} producers. This ligand crossfeeding and release of B_{12} to overcome B_{12} auxotrophy, presumably involving a prophage induction, add new players to our understanding of B_{12} supply to auxotrophic pro- and eukaryotic microorganisms in the ocean and possibly in other ecosystems (Wienhausen et al., Nature resubmitted and in review).

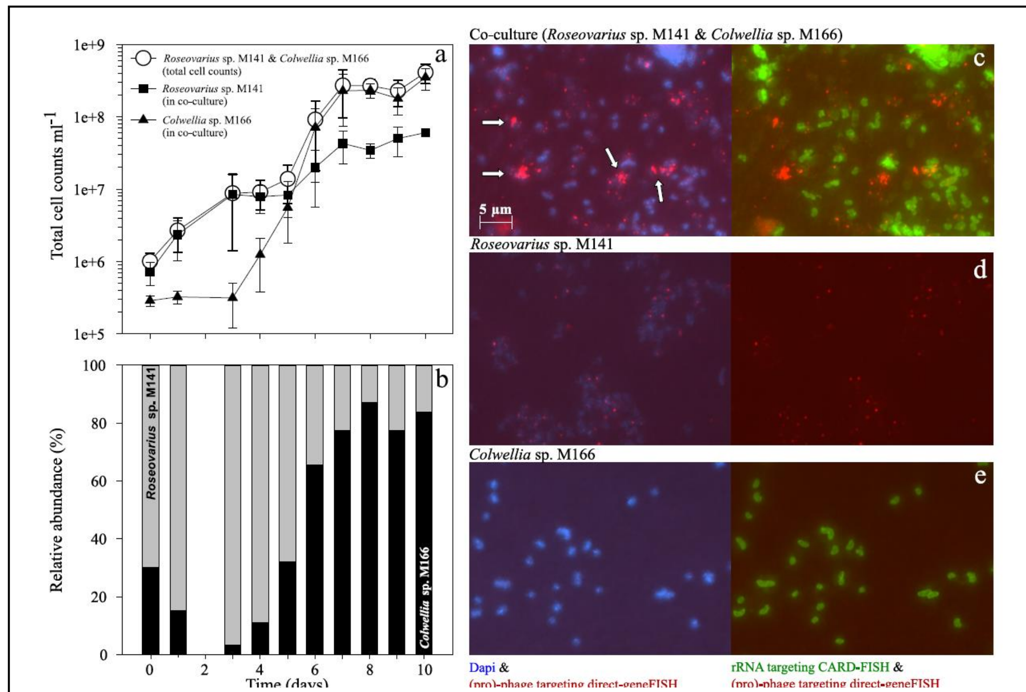

Fig. 3 Bacterial population dynamics and prophage induction in *Roseovarius* sp. M141 and *Colwellia* sp. M166 co-cultures. **a**, total bacterial cell counts (open circles) and cell numbers detected by 16S rRNA targeting CARD-FISH (*Colwellia*; black triangle) and DAPI (*Roseovarius*; black square). Shown are means of triplicates ±SD. **b**, Relative abundances of *Colwellia* and *Roseovarius*. **c-e**, Combined Roseophage ICBM167 targeting direct-geneFISH (red), *Colwellia* rRNA targeting CARD-FISH (green) and all cells counterstain (DAPI – blue) on *Colwellia* and *Roseovarius* growing in co-culture after 120 hours (**c**), and two controls growing in mono-culture with B_{12} supplement: *Roseovarius* after 96 hours (**d**) and *Colwellia* after 96 hours (**e**). Prophage induction events (white arrows) were identified in those co-culture cells with a higher phage signal intensity, indicating an increased number of Roseophage ICBM167 genomes per cell (compare phage signals in c and d). Left column: combined DAPI (all cells) with phage signal. Right column: combined *Colwellia* signal with phage signal. From Wienhausen et al. Nature, resubmitted and in review.

In addition to the outlined results, members of this project were involved in several further studies listed in the publications.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals
(members of A8 in bold, of other CRC projects in italic)

1. **Bercovici** SK, **Dittmar** T, **Niggemann** J (2022) The detection of bacterial exometabolites in marine dissolved organic matter through ultrahigh-resolution mass spectrometry. Limnol Oceanogr: Methods 20: 350-360.
2. **De Corte** D, Varela MM, Louro AM, **Bercovici** SK, Valencia-Vila J, Sintes E, Baltar F, Rodríguez-Ramos T, **Simon** M, Bode A, **Dittmar** T, **Niggemann** J (2023) Zooplankton-derived dissolved organic matter composition and its bioavailability to natural prokaryotic communities. Limnol Oceanogr 68: 336-347.
3. *Heinrichs* ME, *Heyerhoff* B, Arslan-Gatz BS, Seidel M, **Niggemann** J, *Engelen* B (2022) Deciphering the virus signal within the marine dissolved organic matter pool. Front Microbiol 13: 863686.

4. *Heinrichs* ME, *Tebbe* DA, *Wemheuer* B, **Niggemann** J, *Engelen* B (2020) Impact of viral lysis on the composition of bacterial communities and dissolved organic matter in deep-sea sediments. Viruses 12: 922.
5. Koch H, Germscheid N, *Freese* H, **Noriega-Ortega** BE, *Lücking* D, *Berger* M, Qiu G, Marzinelli E, Campbell A, Steinberg PD, *Overmann* J, **Dittmar** T, *Simon* M, Wietz M (2020) Genomic, metabolic and phenotypic variability shapes ecological differentiation and intraspecies interactions of *Alteromonas macleodii*. Sci Reports, 10: 809
6. Merder J, Röder H, **Dittmar** T, Feudel U, Freund JA, Gerdts G, Kraberg A, **Niggemann** J (2021) Dissolved organic compounds with synchronous dynamics share chemical properties and origin. Limnol Oceanogr 66: 4001-4016.
7. **Noriega-Ortega** BE, **Wienhausen** G, Mentges A, **Dittmar** T, **Simon** M, **Niggemann** J (2019) Does the chemodiversity of bacterial exometabolomes sustain the chemodiversity of marine dissolved organic matter? Front Microbiol 10: 215.
8. *Srinivas* S, *Berger* M, *Brinkhoff* T, **Niggemann** J (2022) Impact of quorum sensing and tropodithietic acid production on the exometabolome of *Phaeobacter inhibens*. Front Microbiol 13: 917969.
9. **Sultana** S, Bruns S, Wilkes H, **Simon** M, **Wienhausen** G (2023) Vitamin B_{12} is not shared by all marine prototrophic bacteria with their environment. ISME J; https://doi.org/10.1038/s41396-023-01391-3.
10. **Wienhausen** G, **Noriega-Ortega** BE, **Niggemann** J, **Dittmar** T, **Simon** M (2017) The exometabolome of two model strains of the Roseobacter group: A marketplace of microbial metabolites. *Front Microbiol* 8: 1985.
11. **Wienhausen** G, *Dlugosch* L, Jarling R, Wilkes H, *Giebel* HA **Simon** M (2022a) Availability of vitamin B_{12} and its lower ligand intermediate alpha-ribazole impact prokaryotic and protist communities in oceanic systems. ISME J 16: 2002–2014.
12. **Wienhausen** G, Bruns S, **Sultana** S, Groon LA, Wilkes H, **Simon** M (2022b) The overlooked role of a biotin precursor for marine bacteria - desthiobiotin as an escape route for biotin auxotrophy. ISME J 16/11: 2599-2609.

Other publications and published results

1. **Wienhausen** G, *Moraru* C, Bruns S, *Tran* Quoc D, Wilkes H, *Dlugosch* L, Azam F, **Simon** M. (2023) Ligand crossfeeding resolves bacterial vitamin B_{12} auxotrophies. Nature, 4th resubmission in review.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2014. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	Sequential no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Jutta Niggemann, Dr.	Geochemistry	ICBM	5		UOL
	2	Thorsten Dittmar, Prof. Dr., W3	Geochemistry	ICBM	5		UOL
	3	Meinhard Simon, Prof. Dr., W3	Microbial Ecology	ICBM	5		UOL
	4	Katrin Klaproth		ICBM	10		UOL

Non-research staff	5	Matthias Friebe		ICBM	5		UOL
	6	Ina Ulber		ICBM	5		UOL
	7	Mathias Wolterink		ICBM	5		UOL
	8	Birgit Kuerzel		ICBM	5		UOL
	9	Rolf Weinert		ICBM	5		UOL
Staff funded with approved grant money							
Research staff	1	Beatriz Noriega-Ortega, Ms. Sc.	Geochemistry	ICBM	26	E13 65%	
	2	Sarah Bercovici, Dr., Postdoc	Geochemistry	ICBM	40	E13 100%	
	3	Daniele DeCorte, Dr.	Geochemistry	ICBM	40	E13 100%	
	4	Gerrit Wienhausen, Dr.	Microbial Ecology	ICBM	40	E13 100%	

Job descriptions of staff (supported through existing funds):

1. Niggemann
   She was principal investigator of the project, responsible for the design of the laboratory studies, chemical analyses of the DOM samples, including those collected during the Sonne cruises. She supervised the geochemistry PhD student and postdocs and participated in data evaluation and writing of manuscripts.
2. Dittmar
   He was principal investigator of the project and co-supervised the geochemistry PhD student and PostDocs. Together with JN he was responsible for the design of experiments and chemical analysis, participated in data evaluation and writing of manuscripts.
3. Simon
   He was principal investigator and responsible for the experiments with vitamins B_{12} and B_7, participated in experiment design, data interpretation and writing manuscripts.
4. Klaproth
   She assisted in carrying out analyses on the FT-ICR-MS and processing of the raw data.
5. Friebe
6. Ulber
   They assisted in sample preparation for geochemical analysis and in carrying out analyses of DOC and total dissolved nitrogen on a Shimadzu TOC analyser.
7. Wolterink
   He assisted in enumerating bacteria by flow cytometry of the bacterial growth experiments.
8. Kürzel
9. Weinert
   They carried out analyses of dissolved amino acids and carbohydrates by HPLC.

Job descriptions of staff (funded with approved grant money):

1. Noriega-Ortega
   She carried out data analyses of experiments performed during phase II and successfully defended her PhD theses.
2. Bercovici
   She carried out DOM analyses on the FT-ICR-MS and processed results from laboratory studies and a global data set including the Sonne cruises.
3. DeCorte
   He carried out DOM analyses on the FT-ICR-MS and processed data from experimental studies.
4. Wienhausen
   He initiated and took the lead in designing the experimental work with vitamins B_{12} and B_7 and was instrumental in data evaluation and writing manuscripts.

7. General information about Project B2

7.1 Function and ecological significance of secondary metabolites produced by *Roseobacter* spp. for interactive relationships

7.1.2 Project leader

Brinkhoff, Thorsten, Prof. Dr., 17.12.1966, German
Institut f. Chemie u. Biologie des Meeres (ICBM), Carl v Ossietzky-Universität Oldenburg, 26111 Oldenburg
Tel. 0441/798 3269
Email thorsten.brinkhoff@icbm.de

7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report

7.2.1.1 Ecological function of TDA, signalling molecules and extracellular DNA in surface-associated roseobacters

Organisms of the marine *Roseobacter* group were frequently observed being associated in biofilms and with numerous eukaryotes. During the CRC, we studied interactions between roseobacters, isolated from different host organisms or monitored by molecular biological methods (Bakenhus et al. 2018; Steinert et al. 2019; Bischoff et al. 2019; Leinberger et al. 2022). In this context, we found production or the potential for production of a wealth of secondary metabolites, either by chemical or genomic analyses, respectively (e.g. Celik et al. 2017; Breider et al. 2017; Bruns et al. 2018; Breider et al. 2019; Ziesche et al. 2019; Leinberger et al 2021; Wolter et al. 2021; Srinivas et al. 2022), suggesting that the associations are largely mediated by such compounds. In several cases, it was indicated that roseobacters can positively influence growth, but also cause or promote diseases or death of their host, with various metabolites possibly being involved (Dogs et al. 2017; Majzoub et al. 2019; Bergen et al. 2022; Breider et al. 2019).
Tropodithietic acid (TDA), a tropolone derivative, is produced by several members of the *Roseobacter* group, and is one of the most intensively studied compounds during this CRC (e.g. Beyersmann et al. 2017; Srinivas et al. 2022). Besides its antibiotic activities, we found that TDA acts as signalling molecule in our model organism, *Phaeobacter inhibens* DSM 17395, causing the same regulatory effects in quorum sensing (QS) as the common signalling molecule N-acyl-homoserine lactone (AHL), at concentrations 100-fold lower than the minimal inhibitory concentration against bacteria (Beyersmann et al. 2017). Furthermore, we revealed the chemotactic effect of subinhibitory concentrations of TDA, AHLs, and extracellular DNA (eDNA) on different roseobacters, contextualized with the influence of these compounds on gene expression in *P. inhibens* DSM 17395 (Fig. 1). Our results revealed concentration-dependent chemotactic effects of AHLs and TDA with differential response towards native and foreign compounds. TDA attracted TDA-producing *Phaeobacter*, *Tritonibacter* and *Pseudovibrio* strains, but repelled a non-producing *Yoonia* strain, thus expanding the functions of TDA. While strains were overall attracted by foreign and repelled by native DNA, AHLs induced chemotaxis independent from own production (Wolter and Srinivas et al. submitted).
Addition of 10 µM TDA upregulated multiple genes for anabolic processes and mutual interactions (vitamins and terpene biosynthesis, siderophore import) in *P. inhibens*. Native AHL upregulated competence genes and gene transfer agent production, whereas foreign AHL induced the production of hemolysin with confirmed physiological activity. TDA, AHLs and eDNA exerted strong regulatory effects on genes involved in nitrogen metabolism (ammonia assimilation and glutamate synthesis), including the incomplete denitrification pathway (Wolter and Srinivas et al. submitted).

The diverse chemotactic and regulatory responses demonstrate different strategies of surface colonization and chemical crosstalk in roseobacters, with implications for biofilm dynamics and host association. Transcriptomic responses in the chemosensory context support a model where TDA, AHLs and eDNA act in concert and influence biofilm formation, bacterial interactions and host-associations. Thus, our studies have wider implications as eDNA is prevalent in the marine environment, QS is widespread in *Proteobacteria*, and antibiotic, gene regulatory as well as chemotactic effects of TDA could serve as a model for multifunctionality of other secondary metabolites.

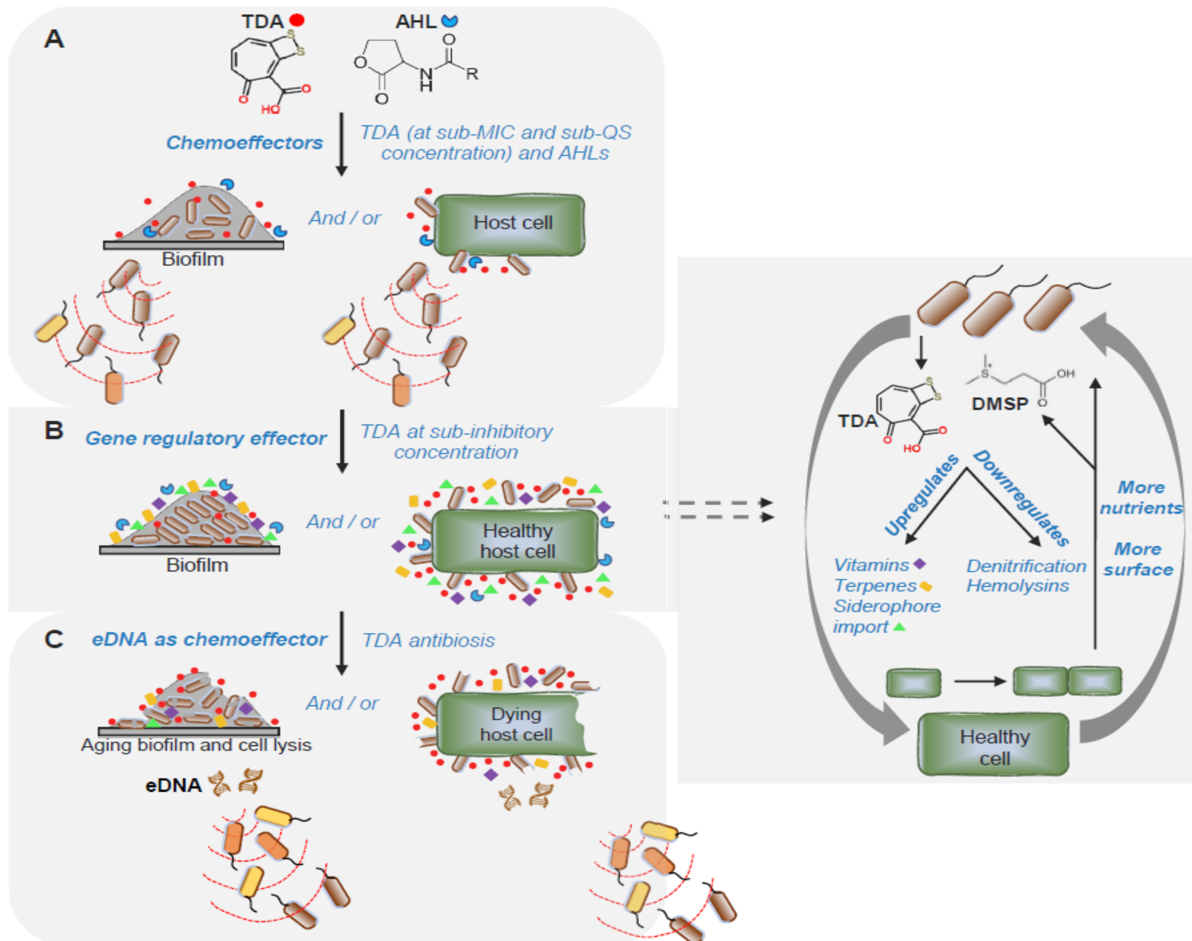

Figure 1. Graphical synopsis of how TDA, AHLs, eDNA and DMSP influence biofilm formation and host associations in context of chemotactic and transcriptomic responses in *Phaeobacter inhibens* DSM 17395. (A) TDA (red circles) at sub-inhibitory concentrations attract TDA producers (brown rods) signalling the availability of a host cell and nutrients and/or facilitating biofilm formation, while TDA non-producers (yellow and orange rods) are repelled. Native and foreign AHLs (blue partial circles) evoked varied chemotactic responses. (B) Sub-inhibitory levels of TDA also up-regulate genes encoding vitamins (purple diamond) and terpene (yellow rectangle) biosynthesis as well as siderophore import (green triangle), while down-regulating genes encoding denitrification and hemolysin production. DMSP, a key phytoplankton metabolite, modulates gene expression comparable to TDA. (C) Bacterial surface cell density scales with TDA production. Higher concentrations of TDA repel *P. inhibens*, thus prohibiting further colonization of a matured biofilm. Furthermore, TDA disrupts cell wall integrity, potentially enhancing cell lysis and release of own DNA (brown DNA motifs). Upon sensing own eDNA, *Phaeobacter* cells are repelled, preventing further colonization. Attraction of other bacteria (like non-TDA-producing *Yoonia* spp.) by foreign eDNA (like that of *Phaeobacter* spp.) might indicate a favorable attachment site, e.g., characterized by nutrients released from lysed cells within the dispersing biofilm.

During the course of this CRC, high relevance of *P. inhibens* in interaction with the diatom *Thalassiosira rotula* was repeatedly observed. We exposed axenic *T. rotula* cultures to bacterial communities from natural seawater in the presence or absence of *P. inhibens* 2.10

or a variant strain that lacks antibacterial activity. We found that after two days the bacterial communities that assembled on the host were distinct from the free-living communities. Furthermore, in the presence of *P. inhibens* a higher abundance of *Alphaproteobacteria*, *Flavobacteriia* and *Verrucomicrobia* was detected attached to *T. rotula* cells, indicating that *P. inhibens* controls the bacterial community assembly on the diatom. We found only minor differences between the communities that established in the presence of either the wild type or the variant *P. inhibens* strain, suggesting that the antibacterial activity of *P. inhibens* is not the primary cause of its influence on bacterial community assembly (Majzoub et al. 2019), supporting the model shown in Fig. 1 that several factors in concert are relevant for the interaction within the biofilm or on the host.

7.2.1.2 Transcriptomic analysis of the diatom *Thalassiosira rotula*

To study the influence of a single bacterial secondary metabolite on a eukaryotic host organism, we chose the axenic diatom *T. rotula* CCPM1647and analysed its transcriptomic response after adding TDA in subinhibitory concentration. In a pre-experiment, we observed that 10 µM and 100 µM TDA are inhibiting growth of *T. rotula*, while 1 µM TDA showed a growth stimulating effect (Fig. 2). To support the analysis of the transcriptomic data, we sequenced the genome of the diatom in cooperation with project Z02. Currently, we analyse the genome, in cooperation with Jan de Vries, University of Göttingen. A manuscript about the genome analysis will be submitted later this year.

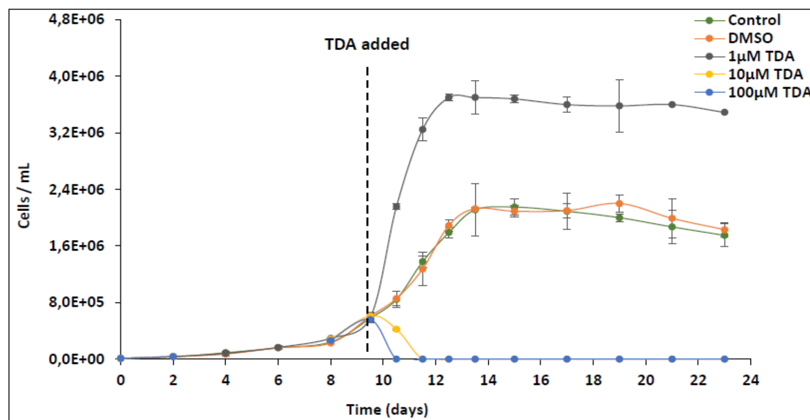

Figure 2. Growth curves of *Thalassiosira rotula* CCPM1647 cultures, i.e. an untreated control and cultures to which different concentrations of TDA (1, 10 and 100 µM) or 14 mM dimethylsulfoxide (DMSO) as solvent control were added. TDA was added after 9.5 hours.

Differential expression of genes in *T. rotula* was analysed at 24 h and 72 h, in cultures to which 1 µM TDA or 14 mM DMSO as solvent control was added, as well as in an untreated culture (culture control, CC) (Srinivas et al. unpublished). Only one and nine differently expressed (DE) genes in DMSO vs the CC were found after 24 h and 72 h, respectively, i.e. that no significant impact of DMSO on *T. rotula* could be observed (Fig. 3). In contrast, the cultures with 1 µM TDA added showed a large number of DE genes, clearly indicating an impact of TDA on *T. rotula*. Whether this is based on a signalling function or an early stress response is currently analysed. Furthermore, more DE genes were found in the cultures at 24 h than at 72 h. This correlates with metabolomics data (not shown) that TDA could not be detected anymore 24 h after addition. A more detailed analysis of the transcriptomic data and a search for possible reasons for a degradation of TDA by the diatom are still in progress.

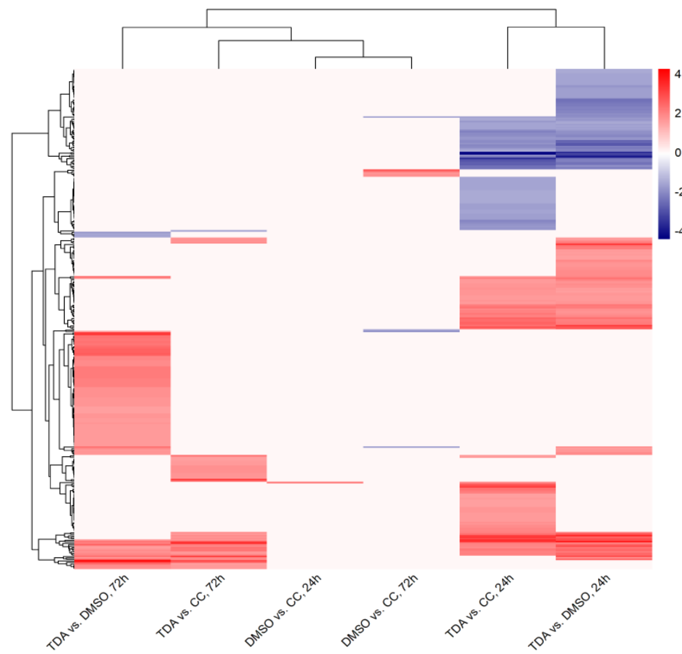

Figure 3: Transcriptomic analysis to investigate differential expression of genes in presence of 1 µM TDA. DMSO was used as a solvent control, CC indicates an untreated culture control. Samples taken 24 and 72 hours after addition of TDA were analysed. Red and blue colours indicate differentially expressed genes (log2 fold change of >1.5 or <-1.5).

Including the results from the transcriptomic analysis of *T. rotula*, it be can summarized that based on the results of project B2, TDA was found to be an excellent example for a multifunctional secondary metabolite. Its production costs the producers a significant amount of their energy, but TDA is obviously highly relevant for the associated lifestyle and to form biofilms, providing a strong advantage in the ecological niche. It could clearly be shown that the function of TDA as antibiotic, signaling molecule and attractant / repellent differs at different concentrations, and varies for different pro- and eukaryotic taxa (Beyersmann et al 2017; Wolter and Srinivas et al submitted; Srinvias et al unpublished), allowing insights how bacteria utilize their metabolites efficiently.

7.2.1.3

In addition to the outlined results, members of this project were involved in several further studies listed in the publications.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals (B2 members bold, of other CRC projects italic)

1. *Bakenhus* I, *Voget* S, *Poehlein* A, **Brinkhoff** T, *Daniel* R, *Simon* M (2018) Genome sequence of *Planktotalea frisia* type strain (SH6-1T), a representative of the *Roseobacter* group isolated from the North Sea during a phytoplankton bloom. Stand Genomic Sci 13, Article No: 7.
2. Bergen N, Krämer P, Romberg J, Wichels A, Gerlach G, **Brinkhoff** T (2022) Shell disease syndrome is associated with reduced and shifted epibacterial diversity on the carapace of the crustacean *Cancer pagurus*. Microbiol Spectr 10(6):e0341922. doi: 10.1128/spectrum.03419-22.
3. **Beyersmann** PG, *Tomasch* J, Son K, Stocker R, *Göker* M, *Wagner-Döbler* I, *Simon* M, **Brinkhoff** T (2017) Dual function of tropodithietic acid as antibiotic and signaling molecule in global gene regulation of the probiotic bacterium *Phaeobacter inhibens*. Sci Rep 7: Article No: 730.
4. *Bischoff* V, *Bunk* B, *Meier-Kolthoff* JP, Spröer C, *Poehlein* A, Dogs M, *Daniel* R, *Overmann* J, *Göker* M, *Simon* M, **Brinkhoff** T, *Moraru* C (2019) Cobaviruses – a newly discovered

phage group infecting protist-associated *Rhodobacteraceae* ubiquitous in highly productive marine ecosystems. ISME J 13: 1404-1421.

5. **Breider** S, *Freese* HM, Spröer C, *Simon* M, *Overmann* J, **Brinkhoff** T (2017) *Phaeobacter porticola* sp. nov., an antibiotic producing bacterium isolated from a harbor in the southern North Sea. Int J Syst Evol Microbiol 67: 2153-2159.
6. **Breider** S, Sehar S, Berger M, Thomas T, **Brinkhoff** T, Egan S (2019) Genome sequence of *Epibacterium ulvae* strain DSM 24752T, an indigoidine-producing, macroalga-associated member of the marine *Roseobacter* group. Environ Microbiome 14: Article Number: UNSP 4.
7. *Bruns* H, *Ziesche* L, Khakin Taniwal N, Wolter L, **Brinkhoff** T, Herrmann J, Müller R, *Schulz* S (2018) N-acylated amino acid methyl esters from marine *Roseobacter* group bacteria. Beilstein J Org Chem 14: 2964–2973. doi:10.3762/bjoc.14.276.
8. *Celik* E, *Maczka* M, Bergen N, **Brinkhoff** T, *Schulz* S, *Dickschat* JS (2017) Metabolism of 2,3-dihydroxypropane-1-sulfonate by marine bacteria. Chem Sci 15: 2919-2922.
9. **Dogs** M, *Wemheuer* B, **Wolter** L, Bergen N, *Daniel* R, *Simon* M, **Brinkhoff** T (2017) *Rhodobacteraceae* on the marine brown alga Fucus spiralis are predominant and show physiological adaptation to an epiphytic lifestyle. Syst Appl Microbiol 40: 370-382.
10. **Leinberger** J, *Holste* J, *Bunk* B, *Freese* HM, Sproeer C, *Dlugosch* L, Kueck AC, *Schulz* S, **Brinkhoff** T (2021) High potential for secondary metabolite production of *Paracoccus marcusii* CP157, isolated from the crustacean *Cancer pagurus*. Frontiers Microbiol 12: Article No: 688754.
11. **Leinberger** J, *Milke* F, Christodoulou M, *Poehlein* A, Caraveo-Patino J, Teske A, **Brinkhoff** T (2022) Microbial epibiotic community of the deep-sea galatheid squat lobster *Munidopsis alvisca*. Sci Rep 12, Article number: 2675.
12. Majzoub M, **Beyersmann** PG, *Simon* M, Thomas T, **Brinkhoff** T, Egan S (2019) *Phaeobacter inhibens* controls microbiome assembly on a marine diatom. FEMS Microbiol Ecol 95: Article No: fiz060.
13. **Srinivas** S, **Berger** M, **Brinkhoff** T, *Niggemann* J (2022) Impact of quorum sensing and tropodithietic acid production on the exometabolome of *Phaeobacter inhibens*. Frontiers Microbiol Vol 13, Article No 917969.
14. Steinert G, *Wemheuer* B, Janussen D, Erpenbeck D, *Daniel* R, *Simon* M, **Brinkhoff** T, Schupp P (2019) Prokaryotic diversity and community patterns in Antarctic continental shelf sponges. Frontiers Mar Sci 6: Article No: UNSP 297.
15. **Wolter** LA, Wietz M, *Ziesche* L, **Breider** S, Leinberger J, *Poehlein* A, *Daniel* R, *Schulz* S, **Brinkhoff** T (2021) *Pseudooceanicola algae* sp. nov., isolated from the marine macroalga *Fucus spiralis* shows genomic and physiological adaptations for an algae-associated lifestyle. Syst Appl Microbiol 44: Article No: 126166.
16. *Ziesche* L, **Wolter** L, *Wang* H, **Brinkhoff** T, *Pohlner* M, *Engelen* B, *Wagner-Döbler* I, *Schulz* S (2019) An unprecedented medium-chain diunsaturated N-acylhomoserine lactone from marine *Roseobacter* group bacteria. Marine Drugs 17: Article No: 20.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period, December 2022.

7.3.1 Project staff in the ending funding period

Sequential no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source

Existing staff							
Research staff	1	Thorsten Brink-hoff, Prof. Dr.	Microbiology	ICBM	5		UOL
	2	Martine Berger, Dr.	Genetics	ICBM	20	Postdoc	UOL
	3	Andrea Schlingloff		ICBM	10	Technician	UOL
Staff funded with approved grant money							
Research staff	1	Sujatha Srinivas	Microbiology	ICBM		E13 65%	
	2	Janina Leinberger	Microbiology	ICBM		E13 65%	

Job descriptions of staff (supported through existing funds):

1. Brinkhoff
2. Principal investigator of the project and responsible for design and coordination of the experiments. Supervision of the PhD students and the postdoc. Design, planning and advice of experimental work, in particular involvement in microbiological, genetic, genomic and transcriptomic work; participation in data evaluation and writing manuscripts.
3. Berger
4. Planning, advice and performance of experimental work, in particular involvement in construction of mutants, expression analysis, genome sequencing and tests of the function of siderophores. Participation in data evaluation and writing manuscripts.
5. Schlingloff
6. Assisting in carrying out physiological growth tests and experiments with isolates, preparing samples for sequencing (Sanger, amplicon) and molecular biological analyses.

Job descriptions of staff (funded with approved grant money):

1. Srinivas
2. Study the ecological relevance of secondary metabolite production by P. inhibens DSM 17395 for interaction with the diatom T. rotula. Co-culture experiments with bacteria and T. rotula and for genome sequencing of T. rotula (with A1-Simon/Brinkhoff). Analyse exometabolomes (with A8- Niggemann/Dittmar/Simon), perform transcriptomic analyses (with Z02-Daniel) and collaborate with the projects A8 and C2-Schulz concerning chemical analyses. Use mutants from the transposon library of DSM 17395, available to TRR51, to identify genes involved in secondary metabolism and study regulatory processes.
3. Leinberger
4. Work on function analyses of identified secondary metabolites and collaborate with C2-Schulz concerning chemical aspects. Investigate various strains producing the compounds, analyse genomes of selected strains to identify genes involved in the production (with A6-Göker and Z02-Daniel) and analyse transcriptomes of strains for which a function in signalling was found (with B4-Wagner-Döbler and C2). Study the role of compounds in interaction with other bacteria to investigate, e.g., the meaning of cross-talk.

7. General information about Project B4

7.1 Physiology of Dinoroseobacter shibae
7.1.2 Project leader

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7.2 Project history
This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report
7.2.1.1 Composition and biogeography of planktonic pro- and eukaryotic communities in the Atlantic Ocean: Primer choice matters

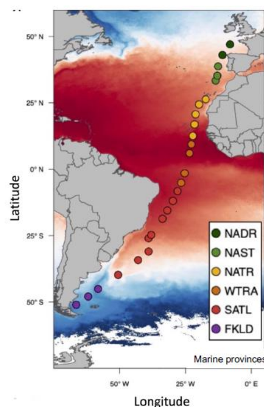

Figure 1. Stations of Polarstern cruise ANT-28-5

We had previosuly analysed a highly resolved transect across the Atlantic Ocean using standard primers for microbiome analysis and had discovered unique biogeographical patterns, namely a U-shaped distance-decay relationship of beta-diversity across the whole transect, and highest alpha-diversity in the temperate regions of the ocean, not at the equator as expected from macro-ecology. Here we re-analysed those samples using primers developed specifically for the marine environment. The data show that the biogeographic patterns observed before are robust with respect to primer choice, lab protocol and bioinformatic pipleline (Milke, Sanchez-Garcia *et al.* 2022). Similar biogeographic patterns were found for a highly resolved transect across the Pacific Ocean and the underlying shaping mechanisms were identified (Milke, Wagner-Döbler *et al.* 2022). The existing data which are based on ASVs rather than OTUs are based on the same sampling strategy and analysis pipeline now cover the two largest global oceans and thus for the first time allow for a global analysis of biogeographical patterns in the open ocean bacterioplankton.

7.2.1.2 The microbiome of the dinoflagellate *Prorocentrum cordatum* in laboratory culture and its changes at higher temperatures

We asked how different the microbiome of *P. cordatum* laboratory cultures might be which had been originally obtained from widely different geographical locations up to 38 years ago, but were maintained since then in the Bigelow culture collection by transferring subcultures to fresh media. The data show that each of the four strains of *P. cordatum* harboured its own microbiome, but there were 14 ASVs which were found in all four cultures and thus could represent a “core microbiome” of *P. cordatum*. Temperature had very different effects on the ASVs in the *P. cordatum* cultures. Interestingly, the growth of the non-axenic *P. cordatum* strains showed a prolonged stationary phase at 20°C, a death phase at 26°C, and at 30°C two of the non-axenic cultures could not grow at all, suggesting that competition for essential nutrients or

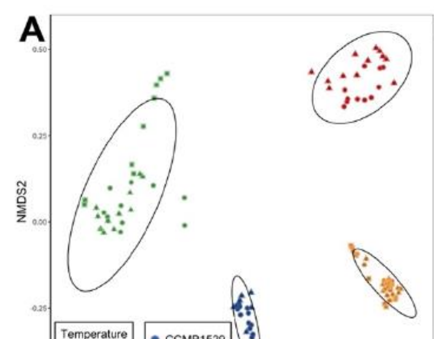

Figure 2. Non-metric multi-dimensional scaling (NMDS) plots of the microbiome of 4 non axenic cultures of *P. cordatum*. Ellipses represent 95% confidence interval of the standard error.

synthesis of toxins by the bacteria impaired dinoflagellate growth at this temperature (Sanchez Garcia *et al.* 2022).

7.2.1.3 Dinoroseobacter shibae Outer Membrane Vesicles (OMV) are enriched for the chromosome dimer resolution site *dif*

Excretion of membrane vesicles is a fundamental trait of all cells. In Gram negative bacteria, they are formed by blebbing of the outer membrane and incorporation of material from the periplasmic space. Their frequency and cargo depend strongly on the cultivation conditions, and accordingly their functions vary widely. DNA has often been found in OMVs, yet a mechanism that could account for transport of DNA from the cytoplasm into the periplasmic space is not known.

We studied the OMVs from *D. shibae* using a multi-omics approach (Wang et al. 2020). One striking finding was the enrichment of the region around the terminus of replication, and specifically the *dif* sequence, in the DNA from the vesicle lumen. *Dif* is the binding sequence for two site specific recombinases, XerCD, which are required for resolving chromosome dimers that can occur during replication of circular chromosomes.

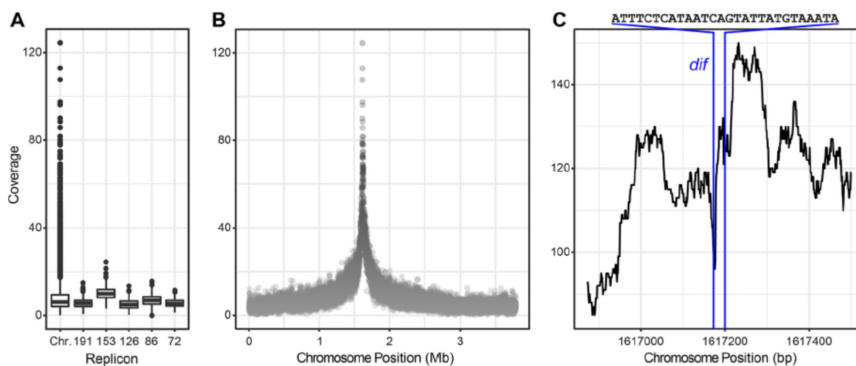

Figure 3. DNA from the lumen of OMVs of *D. shibae* was enriched for the region around the terminus of replication *(ter*). (A) Sequence coverage of chromosome and plasmids. (B) Sequence coverage of the chromosome showing over-representation of the region around *ter*. (C) Sequence coverage of the region around chromosome position 1.6 Mb (*ter*) at single-base resolution. The position and 28-bp nucleotide sequence of *dif* are indicated.

Based on our microscopic data, time-lapse video microscopy, proteome and metabolome data we developed a model for the excretion of OMVs in *D. shibae* during exponential growth. We hypothesize that OMVs are formed during the last stage of cell division when the right and left replichore meet at *ter* and represent a mechanism for removal of over-replicated DNA from the cell. Such over-replication has been regularly observed in *E. coli*, e.g. due to conflict between DNA polymerase and RNA polymerase. If this over-replicated DNA would not be excised and removed from the cell, cell division could not be completed.

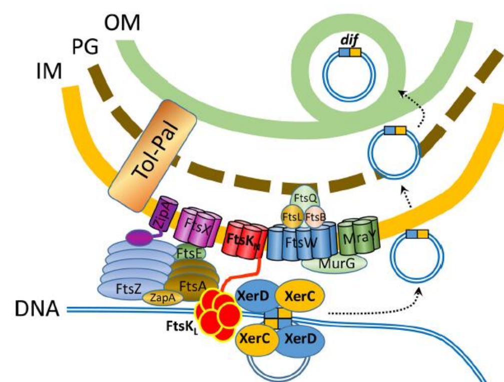

Figure 4. Scheme of the FtsK-dif-XerC/XerD protein complex in the divisome and the hypothetical export of DNA into OMVs. A subset of the proteins comprising the divisome and their localization at the cell envelope is schematically shown. FtsKN is the N-terminal domain of FtsK, and FtsKL, is its C-terminal domain. FtsK is a DNA translocase that moves the replichore toward *dif* and activates the site-specific recombinases XerC/XerD. We hypothesize that these enzymes excise overreplicated genes around the terminus which then enter the periplasm and are enclosed by the outer membrane. It is unknown if the excised DNA is circular or linear. OM, outer membrane; PG, peptidoglycan; IM, inner membrane.

This model provides a possible mechanism for incorporation of DNA into OMVs and it suggests that OMVs represent a regular repair mechanism of growing cells. It was however unclear if the site specific recombinases XerCD play an active role by excising the over-replicated DNA, in which case these recombinases would have a second function in addition to resolving chromosome dimers. To test our hypothesis, we studied two questions: (1) Is the enrichment of the *dif* site specific for *D. shibae*, an Alphaproteobacterium from the *Roseobacter* group, or does it occur in other Proteobacteria as well? We chose *E. coli* as a second model because it is the archetypical, best understood organism regarding the mechanisms of replication and cell division and a library of well-characterized clean gene knockouts is available. (2) Are the XerCD enzymes directly involved in the excision of over-replicated DNA fragments around *ter*? Therefore, we investigated the DNA composition in the lumen of OMVs produced by deletion mutants of XerC and XerD in *E. coli* (Mansky *et al.* under review).

7.2.1.4 The Influence of genes on the "Killer Plasmid" of *Dinoroseobacter shibae* on Its symbiosis with the Dinoflagellate *Prorocentrum minimum*

Dinoroseobacter shibae carries a plasmid (pDS191) which encodes genes that mediate death of the dinoflagellate *Prorocentrum cordatum* in the stationary phase of growth. This "killer plasmid" can be conjugated into another *Roseobacter* strain, *Phaeobacter inhibens*, which is then able to kill the dinoflagellate as well (Tomasch et al. 2022). We therefore investigated transposon mutants of those genes on the killer plasmid which were unique, i.e. not found on the syntenous sister plasmid or the chromosome, for their ability to kill the dinoflagellate in co-culture. Transposon mutants for three genes located in an operon were unable to kill the dinoflagellate. These genes encode bioMNY – a tripartite, modular biotin-uptake transporter. Biotin, vitamin B_7, is present in the co-cultivation medium and is an essential vitamin for both the algae and the bacterium. We therefore hypothesized that *D. shibae* depletes the medium from biotin, causing algal death (Mansky *et al.* 2022).

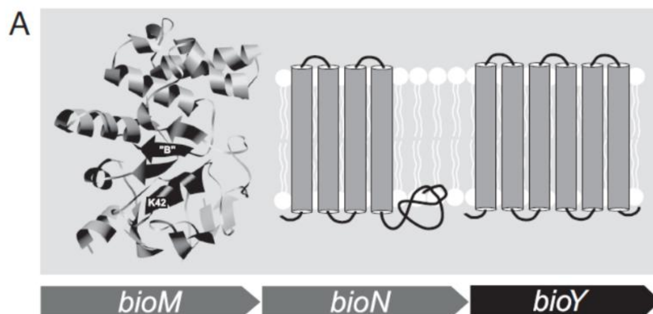

Figure 5. Biotin uptake in prokaryotes by solute transporters with an optional ATP-binding cassette containing module. Hebbeln et al. PNAS 2005
Dshi_3686 bioM ATPase
Dshi_3687 bioN conserved transmembrane protein
Dshi_3685 bioY biotin transporter

To verify this hypothesis, we first showed that excess biotin in the co-culture rescues the dinoflagellate from dying. Second, we constructed a "minimal killer plasmid" containing only the bioMNY genes and showed that these three genes are sufficient to kill the dinoflagellate. Again, the culture can be rescued by addition of biotin.

We then performed a co-culture experiment using three genotypes of *D. shibae* (wild-type, Δ191kb plasmid, bioMNY plasmid) and two concentrations of biotin and determined cell counts of both algae and bacteria for 36 days. From these co-cultures we sampled exponential and stationary phase of growth and extracted RNA for dualRNAseq of both the dinoflagellate and the bacterium.

For *D. shibae*, we found 2570 differentially expressed (DE) genes in total (FDR $\leq$ 0.001) which represents 61.2 % of the 4198 genes of this bacterium. DE genes clustered according to co-cultivation condition, with the most prominent super-clusters containing the photosynthesis operon, denitrification, flagella synthesis and the ctrA regulon.

For *P. cordatum*, we found 18,651 DE genes (log2FC$\geq$2 and FDR $\leq$0.001), representing ~22% of ORFs according to the sequenced genome (Dougan *et al.* 2023). At day 24 of co-cultivation, two super-clusters of DE genes were observed, clearly separating co-cultures with healthy vs. dying algae. Further analysis will concentrate on biotin dependent genes and metacaspases that trigger cell death in algae. The manuscript is currently in preparation.

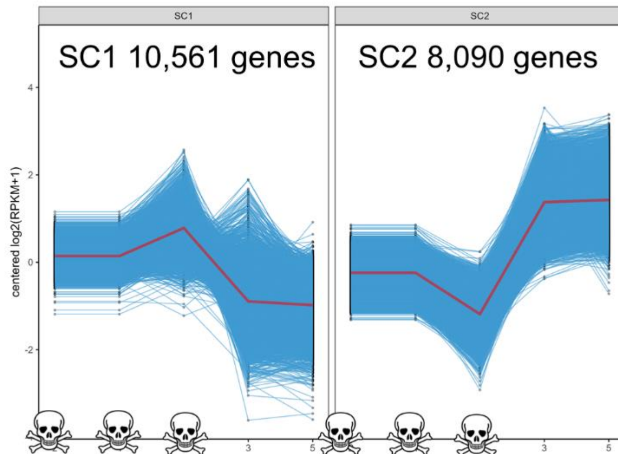

Figure 6. Differentially expressed genes of *P. cordatum* after 24 days of co-culture with killing and non killing strains of *D. shibae* clustered according to similarity of gene expression changes. Supercluster 1 (SC1) represents genes which are up-regulated under killing conditions and down-regulated in healthy algal cultures, while genes in SC2 show the opposite behaviour.

In addition to the outlined results, members of this project were involved in several further studies listed in the publications.

7.2.2 Published project results

Publications with scientific quality assurance
List of publications in peer reviewed journals
(members of B4 in bold, of other CRC projects in italic)

1. *Milke F*, **Sanchez-Garcia S**, *Dlugosch L*, McNichol J, Fuhrman J, *Simon M*, **Wagner-Döbler I.** Composition and Biogeography of Planktonic Pro- and Eukaryotic Communities in the Atlantic Ocean: Primer Choice Matters. Front Microbiol. 2022 13:895875.
2. *Milke F*, **Wagner-Doebler I**, *Wienhausen G*, *Simon M*. Selection, drift and community interactions shape microbial biogeographic patterns in the Pacific Ocean. ISME J. 2022 16: 2653-2665.
3. **Koppenhöfer S**, **Tomasch J**, *Ringel V*, *Birmes L*, *Brinkmann H*, *Spröer C*, Jarek M, **Wang H**, *Pradella S*, **Wagner-Döbler I,** *Petersen J.* The Sixth Element: a 102-kb RepABC Plasmid of Xenologous Origin Modulates Chromosomal Gene Expression in *Dinoroseobacter shibae*. mSystems. 2022; 7: e0026422.
4. **Sanchez-Garcia S**, **Wang H**, **Wagner-Döbler I**. The microbiome of the dinoflagellate *Prorocentrum cordatum* in laboratory culture and its changes at higher temperatures.Front Microbiol. 2022;13: 952238.
5. **Tomasch J,** *Ringel V,* **Wang H**, *Freese HM*, *Bartling P, Brinkmann H, Vollmers J,* Jarek M, **Wagner-Döbler I,** *Petersen J.* Fatal affairs - conjugational transfer of a dinoflagellate-killing plasmid between marine *Rhodobacterales*. Microb Genom. 2022, 8: 787.
6. *Koteska D,* **Sanchez Garcia S**, **Wagner-Döbler I,** *Schulz S*. Identification of Volatiles of the Dinoflagellate *Prorocentrum cordatum*. Mar Drugs. 2022. 20: 371.
7. **Mansky J, Wang H,** *Ebert M*, *Härtig E, Jahn D,* **Tomasch J, Wagner-Döbler I.** The Influence of Genes on the "Killer Plasmid" of *Dinoroseobacter shibae* on Its Symbiosis With the Dinoflagellate *Prorocentrum minimum*. Front Microbiol. 2022. 12: 804767.
8. **Wang H**, *Beier N,* Boedeker C*,* **Sztajer H,** Henke P, Neumann-Schaal M, **Mansky J**, Rohde M, *Overmann J, Petersen J*, Klawonn F, Kucklick M, *Engelmann S*, **Tomasch J, Wagner-Döbler I.** *Dinoroseobacter shibae* Outer Membrane Vesicles Are Enriched for the Chromosome Dimer Resolution Site. mSystems. 2021. 12.
9. **Koppenhöfer S, Wang H,** Scharfe M, Kaever V, **Wagner-Döbler I, Tomasch J.** Integrated Transcriptional Regulatory Network of Quorum Sensing, Replication Control, and SOS Response in *Dinoroseobacter shibae*. Front Microbiol. 2019. 10: 803.
10. *Ziesche L, Wolter L,* **Wang H**, *Brinkhoff T, Pohlner M, Engelen B,* **Wagner-Döbler I,** *Schulz S.* An Unprecedented Medium-Chain Diunsaturated N-acylhomoserine Lactone from Marine Roseobacter Group Bacteria. Mar Drugs. 2018. 17: 20.
11. Kopejtka K, **Tomasch J,** Bunk B, Spröer C, **Wagner-Döbler I,** Koblížek M. The complete genome sequence of *Rhodobaca barguzinensis* alga05 (DSM 19920) documents its adaptation for life in soda lakes. Extremophiles. 2018. 22: 839-849.

12. *Brinkmann H, Göker M,* Koblížek M, **Wagner-Döbler I,** *Petersen J.* Horizontal operon transfer, plasmids, and the evolution of photosynthesis in Rhodobacteraceae. ISME J. 2018. 12: 1994- 2010.
13. **Tomasch J, Wang H,** Hall ATK, **Patzelt D**, Preusse M, *Petersen J, Brinkmann H, Bunk B,* Bhuju S, Jarek M, Geffers R, Lang AS, **Wagner-Döbler I.** Packaging of *Dinoroseobacter shibae* DNA into Gene Transfer Agent Particles Is Not Random. Genome Biol Evol. 2018. 10: 359-369.
14. *Bruns H,* Herrmann J, Müller R, **Wang H**, **Wagner Döbler I,** *Schulz S.* Oxygenated N-Acyl Alanine Methyl Esters (NAMEs) from the Marine Bacterium *Roseovarius tolerans* EL-164. J Nat Prod. 2018. 81: 131-139.

Other publications and published results

1. Mansky J, Wang H, Wagner-Döbler I, Tomasch J. (2023) Role of XerCD in release of over-replicated DNA through Outer Membrane Vesicles in *Escherichia coli*. Microbiology Spectrum, under review.
2. Dougan KE, Deng Z-L, *Wöhlbrand* K, Reuse C, *Bunk B,* Chen Y, *Hartlich J, Hiller K,* John U, *Kalvelage J*, **Mansky** J, Neumann-Schaal M, *Overmann J, Petersen, J,* **Sanchez-Garcia** S, *Schmidt-Hohagen K*, Shah S, Spröer C, **Sztajer** H, Wang H, Bhattacharya D, *Rabus R, Jahn D*, Chan CX, **Wagner-Döbler** I (2023) Multi-omics analysis reveals the molecular response to heat stress in a "red tide" dinoflagellate. BioRxiv, https://doi.org/10.1101/2022.07.25.501386.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	Sequen-tial no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Wagner-Döbler, Irene, Prof. Dr.,	Microbial Ecology	TU, HZI	20		TU, HZI
Staff funded with approved grant money							
Research staff	1	Wang, Hui, Dr., Postdoc	Microbial Ecology	TU, HZI	10	E13	
	2	Sztajer, Helena Dr., scientist	Microbial Ecoloy	TU, HZI	10	E13 50%	
	3	Sanchez-Garcia, Selene, Phd	Microbial Ecology	TU, HZI	10	E13 65%	
	4	Mansky, Johannes, Phd	Microbial Ecology	TU, HZI	10	E13 65%	
Non-research staff	1	Sinem Bektas	Hiwi	TU, HZI	20	E9 50%	

Job descriptions of staff (supported through existing funds):

1. Wagner-Döbler

She was principal investigator of the project, responsible for experimental design, establishment of international cooperation, data analysis, writing manuscripts, and supervisison of PhD students.

Job descriptions of staff (funded with approved grant money):

1. Wang
   She carried out the co-culture experiments, extracted DNA and RNA for sequencing, isolated outer membrane vesicles and supervised the PhD students.
2. Sztajer
   She improved laboratory protocols for extraction of DNA and RNA from cultures or *P. cordatum* and from outer membrane vesicles of *D. shibae*.
3. Sanchez-Garcia
   She cultivated *P. cordatum* under standardized conditions, determined growth using flow cytometry, sequenced 16S rRNA amplicons, studied toxicity of *P. cordatum* using a cell culture assay, and drafted manuscripts.
4. Mansky
   He cultivated *P. cordatum* under standardized conditions, performed co-cultivation experiments, extracted outer membrane vesicles, determined their size and abundance, constructed a minimal killer plasmid, and drafted manuscripts.

7. General information about Project B5

7.1 Regulatory networks for the adaptation of *Dinoroseobacter shibae* to changes in oxygen, iron and light

7.1.2 Project leaders

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7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report

7.2.1.1 The Role of the novel type IscR regulator in iron-dependent gene regulation in *D. shibae*

Results of the former funding period revealed the major role of the IscR regulator in adaptation to iron limitation. The *iscR*::Tn mutant strain showed a drastically growth limitation under iron-limited conditions compared to the wild type strain. Usually, the Rrf2-family transcription factor IscR contains an iron sulfur cluster for iron detection. However, IscR of *Dinoroseobacter shibae* is different to these homologous proteins. It was proposed to measure iron availability via coordination of heme as a cofactor. Coordination of heme was detected by UV/Vis spectroscopy. Purified IscR showed a characteristic absorption maximum of heme at 425 nm. IscR contains three histidine residues. In order to identify the role of these histidine residues for the coordination of heme as a cofactor, each of the three histidine residues of IscR was exchanged to an alanine residue *via* site directed mutagenesis of the corresponding gene. IscR of *D. shibae* and mutant variants were recombinantly produced and purified aerobically. UV/ Vis spectroscopic analysis of the purified mutant protein indicated a loss of absorption at 425 nm indicating heme binding (Fig. 1).

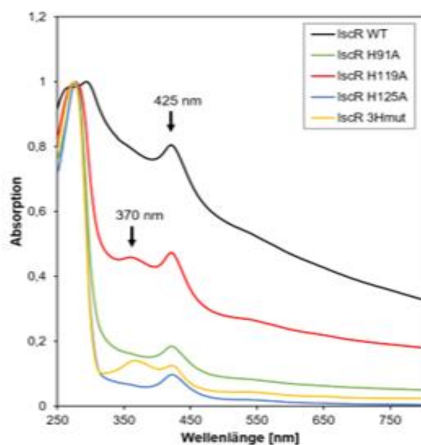

Fig. 1. UV/ vis absorption spectra of the IscR wildtype protein and the IscR mutant proteins H91A, H119A, H125A and 3Hmut

Gene regulatory effects of IscR in response to iron availability were analyzed *via* a *hemB2-lacZ* reporter gene fusion. The *hemB2* gene encodes a TonB dependent heme receptor protein which is upregulated in the absence of iron. Accordingly, *hemB2-lacZ* expression in the wild type strain is increased under iron limited conditions. In the iscR::Tn mutant strain expression of hemB2-lacZ is upregulated indicating a repressor function of IscR (Fig. 2a). Using electro mobility shift assays (EMSA) binding of IscR to the *hemB2* promoter was studied. Using increasing amounts of purified IscR protein two shifted complexes could be determined indicating binding of IscR to the DNA (Fig. 2b). By shortening the DNA fragment used in the EMSA assay the binding region could be limited to a 41 bp region containing the palindromic sequence 5´-TTAA-N11-AATT-3´.

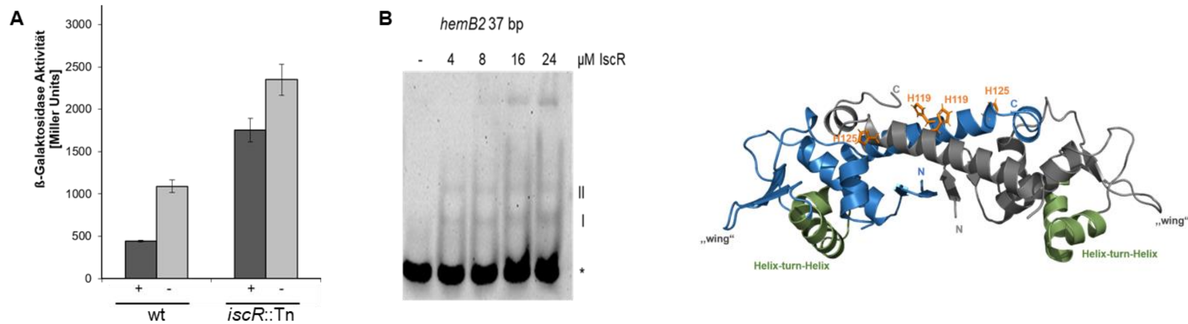

Fig. 2. IscR is acting as a repressor by binding to the *hemB2* promoter A. ß-Galactosidase activities of a *hemB2-lacZ* reportergene fusion measured in the *D. shibae* wild type strain and the iscR::Tn mutant strain grown in the presence (+) or absence (-) of iron. B. EMSA experiment incubating recombinantly produced and purified IscR protein together with a 37 bp *hemB2* promoter fragment. C. Crystal structure of IscR from *D. shibae* (PDB 7ZPN)

Moreover, purified IscR protein was used for crystallization attempts which resulted in IscR crystals. X-ray data up to a resolution of 1.9 Angstrom were collected. The IscR structure showed a dimeric form of IscR with a “winged” helix-turn-helix DNA binding domain (Fig. 3). The structure of IscR was deposited at the Protein Data Bank (PDB 7ZPN).

7.2.1.2 Biochemical characterization of the blue light-dependent regulator LdaP (Dshi_1135) and its functional role in regulation of the photosynthetic gene cluster

In the marine bacterium *Dinoroseobacter shibae* the aerobic anoxygenic photosynthesis involves more than 40 genes which are regulated in a light-dependent manner. Expression of the photosynthetic gene cluster (PGC) in *D. shibae* wild type strain was found upregulated under dark growth conditions and reduced under high light conditions before (Tomasch et al., 2011). A genome-wide screen of a transposon library of *D. shibae* for the loss of pigmentation and specific bacteriochlorophyll absorbance identified the blue light-dependent LOV-protein Dshi_1135. The mutant phenotype could be complemented by the expression of a Dshi_1135-Strep fusion protein *in trans.* Transcript levels of the Dshi_1135:Tn mutant strain were compared with the wild type strain after growth in the dark. In total 437 genes were found higher and 122 genes lower expressed in the Dshi_1135::Tn mutant (log 2-fold change ≥0.8). Among the genes with lower expression all photosynthetic genes localized in the PGC and the *puc* operon were found. Maximal changes up to $\log_2$FC of -6.75 were reached (Fig. 4). This result nicely corresponds to the pale pigmentation phenotype and the reduced bacteriochlorophyll content of the Dshi_1135::Tn mutant strain.

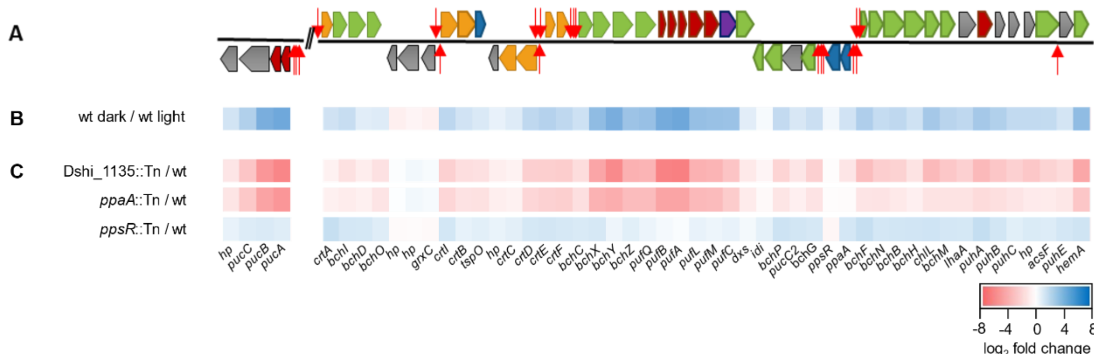

Fig. 4. Transcriptome data comparing transcript levels of the photosynthetic gene cluster (PGC) from wild type strain *D. shibae* DFL12^T with Dshi_1135::Tn, *clpX*::Tn, *ppsR*::Tn and *ppaA*::Tn mutant strains, respectively. The color bar represents the expression level in $\log_2$ fold scale. Red indicates relatively low expression levels in the mutant strains in comparison to the wild type strain; blue indicates relatively high expression levels.

The transcriptome analyses revealed an essential role of Dshi_1135 for expression of the photosynthetic gene cluster. Recombinantly produced and purified Dshi_1135 protein was subjected to biochemical analyses. The UV/ Vis spectrum of the purified Strep-Dshi_1135 protein showed a major absorbance peak at 450 nm and vibronic bands at around 425 nm and 475 nm, which can be attributed to a tight, non-covalent binding of the FMN chromophore to the non-polar binding pocket of the LOV domain (Fig. 5). Illumination of the Dshi_1135 protein with blue light led to a bleaching of the absorption peak at 450 nm, indicating the conversion of the Dshi_1135 LOV domain from the dark into the signalling state, which in turn led to the formation of a covalent cysteinyl-flavin C4(a) adduct with a new absorption peak at 390 nm. This covalent bond is of transient nature since transferring the protein back into the dark reverted it into the ground state again. These measurements demonstrated that the Dshi_1135 protein is capable of a reversible blue light induced photocycle, which is characteristic for LOV proteins (Fig. 5).

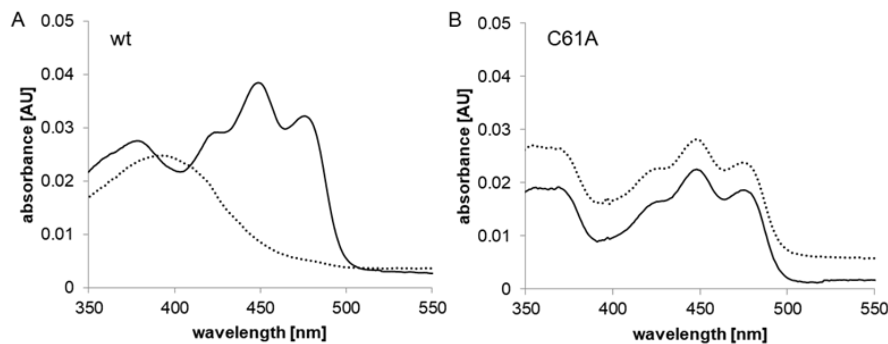

Fig. 5. UV/Vis spectra of purified and reconstituted Dshi_1135 wild type and mutant variant Dshi_1135C61A under dark and blue light conditions Gkround state absorbance spectra of purified and reconstituted TRX-StrepII-Dshi_1135 protein were recorded under dim red light (black line) and showed an absorption maximum at 450 nm and the vibronic structure at 425 and 475 nm, indicative for the non-covalent binding of the FMN cofactor in the dark state. The same sample was illuminated with blue light (λ=450 nm) for 5 min and measured again in the UV/Vis spectrophotometer (dashed line). An absorbance maximum at 390 nm indicated the conversion of Dshi_1135 into the signaling state. B Spectra of purified and reconstituted TRX-StrepII-Dshi_1135C61A mutant protein were recorded under dim red light (black line) and after illumination with blue light (λ=450 nm) for 5 min (dashed line).

Dshi_1135 possesses the highly conserved GXN**C**RLFQ amino acid motif typical for LOV domain proteins. It was proposed that the sulfur atom of the cysteine residue at position 61 is responsible for the observed FMN-cysteinyl adduct formation. To test this hypothesis, the cysteine residue at position 61 of Dshi_1135 was substituted with a non-polar, non-reactive alanine residue by site directed mutagenesis of the corresponding gene sequence. The resulting mutant protein Dshi_1135C61A was analyzed by UV/ Vis spectroscopy and showed the same ground state absorption properties in the dark with peak maxima at 450 nm as the wild type protein (Fig. 5, B). However, after blue light irradiation of Dshi_113C61A, no spectral bleaching was observed indicating that the cysteine at position 61 in the amino acid sequence is the reactive residue for photo adduct formation and photo cycle activity

Expression data of the PGC indicated a potential role of Dshi_1135 as an anti-repressor of PpsR. This function is usually mediated by a stable protein-protein interaction. In order to test this hypothesis co-affinity purification analyses were established to identify the protein interaction partners of Dshi_1135. For this purpose, Dshi_1135 was expressed in the Dshi_1135:Tn mutant strain as fusion protein with a C-terminal StrepII-tag. These Interactomic studies identified the repressor protein PpsR as interaction partner of Dshi_1135. Identification was performed in cooperation with Lars Wöhlbrand and Ralf Rabus (AG Rabus, ICBM, Uni Oldenburg) using nano LC-MS/MS analyses or Western Blot analysis using a PpsR specific antiserum. Co-purification results from bacteria grown under white light, blue light or dark conditions revealed that interaction of Dshi_1135 with PpsR mainly occur under dark conditions.

The physical contact between PpsR and the Dshi_1135 protein was verified *in vivo* using the bacterial adenylate cyclase two hybrid assay. Moreover, it was demonstrated that cobalamin (vitamin B_{12}) is essential for the interaction of PpaA with PpsR. In addition, the antirepressor function of the Dshi_1135 protein was demonstrated *in vivo* using a heterologous expression system based on *Escherichia coli*. Here, the *bchF-lacZ* reporter gene fusion was repressed by production of PpsR in *E. coli*. The expression of the reporter gene increased by simultaneously production of Dshi_1135 protein indicating antirepressor function. A regulatory model including the antirepressor function of Dshi_1135 was developed which contribute to the functional understanding of the light-dependent regulatory circuit for photosynthetic gene expression in *D. shibae* (Fig. 6.). Thus, we suggest to rename the Dshi_1135 protein light-dependent antirepressor of PpsR, LdaP. A manuscript of these results it in preparation (Pucelik et al. in prep.)

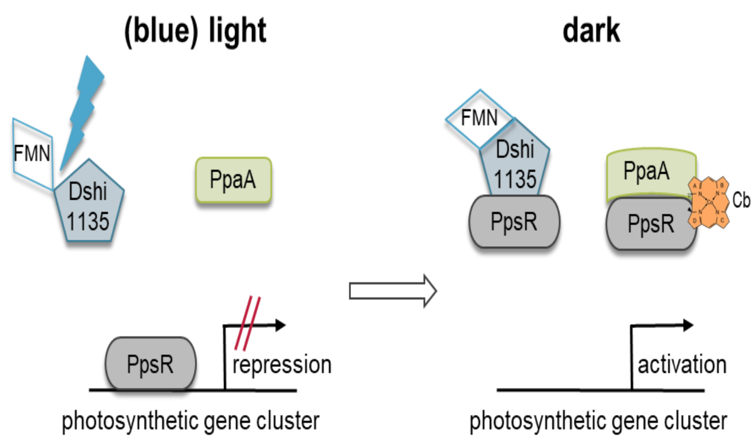

Fig. 6. Model of light-dependent regulation of the photosynthetic gene cluster in *D. shibae.* Under blue light and light conditions PpsR is binding as a repressor to the promoter region inhibiting transcriptional activation. The blue light sensing Dshi_1135 protein is in its inactive form. Under dark light conditions Dshi_1135 is able to interact with PpsR which is no longer able to bind to the DNA and repress transcription. Antirepressor PpaA is able to bind PpsR in the presence of hydroxy cobalamin and supports transcriptional activation.

7.2.1.3 Role of ClpX in regulation of bacteriochlorophyll biosynthesis

The screening of our transposon mutant library of *D. shibae* for loss of pigmentation and Bchl *a* absorbance identified also Dshi_1387, encoding the ATP-dependent protease subunit ClpX of the ClpXP protease of *D. shibae.* This pigmentation phenotype indicated a role of ClpXP in regulating bacteriochlorophyll biosynthesis. Proteome and transcriptome analyses comparing the *clpX*::Tn mutant strain with the wild type were performed. The RNA levels of the photosynthetic gene cluster were significantly lower in the *clpX*::Tn mutant strain compared to the wild type strain grown in the dark which nicely explains the pale phenotype. According to this result the proteins of the bacteriochlorophyll biosynthesis are only produced in the wild type strain under dark growth conditions. This effect on the transcriptome level could be explained by proteolytic digestion of a repressor protein e. g. PpsR, regulating photosynthetic gene expression in the wild type strain.
We established a “trapping” system to identify substrates of the ClpXP protease by co-purification with a mutagenized, functionally inactive ClpPS106A ($ClpP^{trap}$) subunit (Fig. 7).

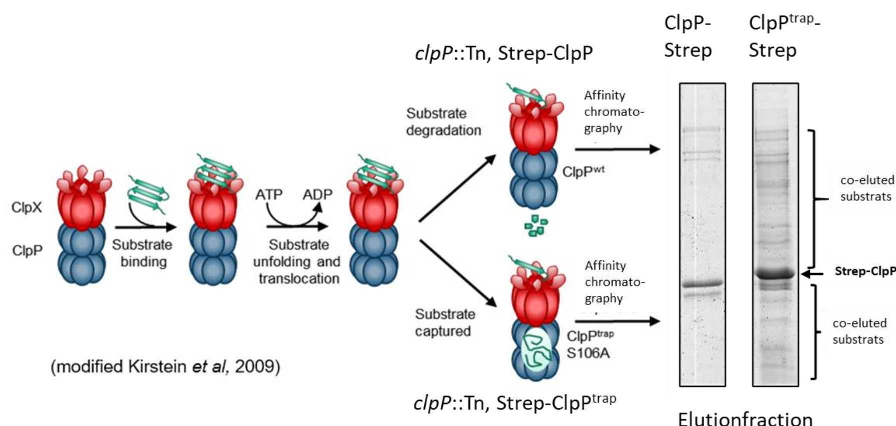

Fig. 7. Trapping system to identify ClpXP specific target proteins.

The *clpP*^{trap} gene was cloned under control of a constitutive promoter. After conjugation into the *ΔclpP* transposon mutant of *D. shibae*, the ClpP^{trap} protein was produced and purified as a Strep-tagged fusion protein. As control, the *clpP*^{wt} gene was also cloned and produced. Potential substrates of the protease were isolated by co-purification with the ClpP^{trap}-Strep fusion protein. The elution fractions of ClpP^{trap}-Strep, ClpP^{wt}-Strep and the vector control were compared on an SDS-PAGE. In addition, the proteins were identified by LC-MS/MS performed in cooperation with Lars Wöhlbrand and Ralf Rabus (AG Rabus, ICBM, Uni Oldenburg).
In total 78 potential substrates of the ClpXP protease were identified. Among these proteins substrates were found that had already been described as substrate of ClpXP in other bacteria, supporting the validity of the ClpP^{trap} system. For example, the transcriptional regulators CtsR and LexA, the chaperone DnaJ, the lipoyl synthase LipA and the cell division protein FtsZ. Newly identified ClpXP substrates include proteins essential for bacteriochlorophyll *a* biosynthesis: 5- aminolevulinate synthase (HemA1, HemA2) and subunit I and H of the magnesium chelatase (BchI, BchH) as well as the regulators Dshi_1135 and PpsR. A manuscript of these results it in preparation (Rommerskirch et al. in prep.). These results led to the hypothesis that the ClpXP protease is responsible for proteolytic digestion of the PpsR repressor.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals
(members of B5 in bold, of other CRC projects in italic)

1. *Mansky, J*, Wang, H, Ebert, M, *Tomasch, J*, **Härtig**, E, **Jahn**, D. & *Wagner-Döbler, I* (2022) The influence of genes on the “killer plasmid” of *Dinoroseobacter shibae* on its symbiosis with the dinoflagellate *Prorocentrum minimum.Front Microbiol.*, 12:804767.
2. *Beier, N*, Kucklick, M, Fuchs, S, Mustafayeva, S, Behringer, M, **Härtig**, E., **Jahn**, D., & *Engelmann*, S. (2021) Adaptation of *Dinoroseobacter shibae* to oxidative stress and the specific role of RirA. *PLOS ONE*, 16, e0248865

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	Sequen-tial no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Jahn, Dieter, Prof. Dr., W3	Micro-biology	Institute of Microbiology	5		TU BS
	2	Härtig, Elisabeth, Dr., E13	Micro-biology	Institute of Microbiology	5		TU BS
Non-research staff	3	Anja Hartmann		Institute of Microbiology	5		TU BS
Staff funded with approved grant money							
Research staff	1	Miriam Becker, Ms. Sc	Micro-biology	Institute of Microbiology	20	E13 50%	

	2a	Lisa Plötsky, Ms. Sc.	Mikro-biology	Institute of Microbiology		E13 50%	
	2b	Alina Rommerskirch Ms. Sc.	Micro-biology	Institute of Microbiology	20	E13 50%	

Job descriptions of staff (supported through existing funds):

1. Jahn
   He was principal investigator of the project and responsible for design and coordination of the experiments. He supervised the PhD student and the postdoc.
2. Härtig
   She gave advice in designing and executing experiments and data analysis of the Roseobacter MAG analyses.
3. Hartmann
   She assisted the experimental work and took care of bacterial culture and conservation.

Job descriptions of staff (funded with approved grant money):

1. Becker
   She carried out the biochemical characterization of the Dshi_1135 regulator
2. Plötzky
   She performed the biochemical analysis of the IscR regulator, the experiments for iron-dependent transcriptional regulation and the crystallization of IscR
3. Rommerskirch
   She carried out the co-purification experiments to identify targets of the ClpXP protease.

7. General information about Project B6

7.1 Bacteriophages of the *Roseobacter* group
7.1.2 Project leaders

Cristina Moraru, 05.05.1981, Romanian

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Email: lilianacristina.moraru@uni-due.de

7.2 Project history
This project received funding through TRR51 from January 2018 until December 2022

7.2.1 Report
Similar to other bacteria in the marine environment, members of the *Roseobacter* Group are impacted by bacteriophages. However, our knowledge of the diversity of roseophages (phages infecting roseobacters) lags behind that of the roseobacters themselves. Therefore, throughout this project, a lot of effort has been invested into the isolation, taxonomic, genomic, and biogeographical characterization of new roseophages.

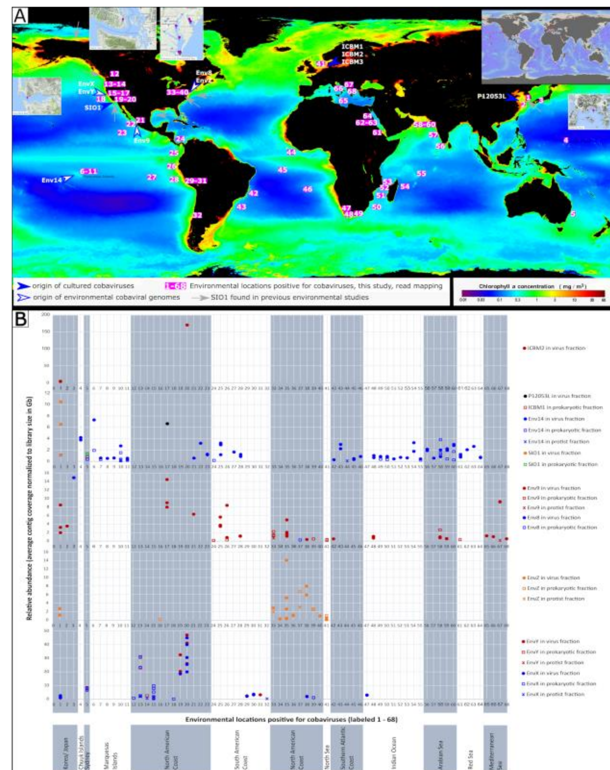

Figure 1: Global distribution of Cobaviruses (a) and their abundance (average contig coverage per Gb metagenome) in metagenomic samples from marine environments (Bischoff et al. 2019).

7.2.1.1 *Cobavirinae* – a globally distributed subfamily of lytic roseophages infect free living and protist-associated marine roseobacters
At the begining of this funding phase, we already had isolated and characterized two lytic roseophages infecting *Lentibacter* sp. SH36, namely ICBM1 and ICBM2. Our initial analyses using whole-genome-based phylogeny indicated that these phages, together with cultivated and environmental relatives, form a highly supported, sub-family level cluster within at the time still valid *Podoviridae* family of the *Caudovirales* order (Bischoff *et al.* 2019). However, major changes in the structure and criteria used for viral taxonomy took place in the years since, resulting first in the abolishment of the *Podoviridae*, and then also of the *Caudovirales*. To keep up with these changes and to clarify the taxonomic placement of our roseophages, we performed further taxonomic analysis (single gene phylogenetic trees, nucleotide-based intergenomic similarities) and finally, established the now officially recognized *Zobellviridae* family – a family of dsDNA, tailed phages, with podoviral morphology, infecting marine *Proteobacteria* (Bischoff *et al.* 2020). Within this family,

both cultivated and environmental roseophages, infecting *Lentibacter, Sulfitobacter,* and *Celeribacter*, clustered within the *Cobavirinae* subfamily.

Our preliminary results based on BLAST searches in the NCBI and IMG/VR databases indicated a potentially global distribution of cobaviruses and their hosts. To investigate this further, through this funding period we have performed an extensive screening of more than 5000 marine metagenomes, by mapping reads to cobaviral genomes. We found cobaviruses in the euphotic zone worldwide, from temperate to tropical waters, mainly in bays and estuaries, but also in the open ocean (Figure 1). Cobaviruses were found not only in the free-living fraction but also in the particulate fraction, which brings further support that roseobacters and their viruses are associated with phototrophic or grazing protists (Bischoff *et al.* 2019).

7.2.1.2 Large-scale isolation of roseophages reveals new diversity

One major undertaking during this funding period was the large-scale isolation of new roseophages, either through direct platting or through phage enrichment procedures. For this, we used as hosts ~390 bacterial strains from the Roseobacter group (most of them received from the groups of Rudolf Amann, MPI Bremen, and Ramon Rossello-Mora, IMEDEA, Spain). We obtained 260 dsDNA roseophage isolates, infecting 65 roseobacter strains. From these, 140 had unique RAPD-PCR patterns, and we obtained the complete genome sequence of 109 phage isolates (sequencing performed in collaboration with project Z02). Clustering based on nucleotide-based intergenomic identities placed these isolates within 51 species-level clusters (95% identity) and 13 genus-level clusters (70% identity).

The new roseophages infected roseobacters in the *Lentibacter, Sulfitobacter,* and *Octadecabacter* genera. Most of the sequenced isolates (85 out of 109 isolates) belonged to a single genus-level cluster and infected strains of a new, species-level *Sulfitobacter* clade. This collection represents an excellent dataset for the study of microdiversity and its influence on phage-host interactions. Part of this collection is used in a new DFG grant (MO 3498/2-1), which is running since February 2022 and is entitled “Impact of phage microdiversity on marine heterotrophic bacteria – a single cell perspective”.

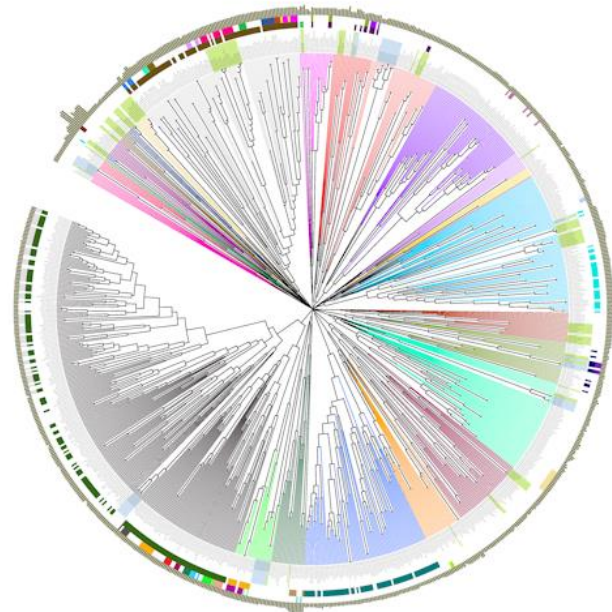

Figure 2: Hierarchical clustering of the new dsDNA roseophages and their relatives. The circles encode from inner to outer: ICTV-recognized phage families, ICTV-recognized phage subfamilies, genome size. Previously cultivated roseophages have their names marked in green. New roseophages have their names marked in blue.

To place the new roseophage isolates into the taxonomic context of the dsDNA phages, we clustered their genomes hierarchically with VirClust (Moraru 2023), based on their protein-cluster content. This placed the new roseophages into eight *Caudoviricetes* families, of which four are new to this study (Figure 2). To finalize the taxonomic description of the new roseophages, we are currently performing a major-capsid protein based-tree. Genomic analysis indicated that most of the new roseophages have a lytic lifestyle, with some being potentially temperate. A manuscript describing the taxonomic and genomic characterization of the new isolates is currently in preparation.

7.2.1.3 The "Tainavirinae" and "Occultatumvirinae" - two new subfamilies infecting *Alphaproteobacteria* comprise ssDNA viruses with a complex life style

Most of the known roseophages have a dsDNA genome and a tailed virion, belonging thus to the *Duplodnaviria* realm. At the beginning of this project, we isolated and characterized ICBM5,

a ssDNA phage with an icosahedral capsid and circular genome, infecting *Sulfitobacter dubius* SH241-b. Our initial analysis showed that this phage was distant but related to previously known *Microviridae*. Through this project, we used a combination of ICBM5-targeted direct-geneFISH, plaque assays, and long-read sequencing (PacBio) to reveal that ICBM5 has a complex lifestyle. In addition to the lytic cycle, it enters a carrier state, where is maintained as a circular chromosome in host cultures that otherwise appear unaffected and exhibit resistance toward reinfection with ICBM5. In addition, when we searched publically available bacterial genomes using a protein-based method, we found 65 ICBM5-like prophages and episomes in bacteria from *Rhodobacterales* and *Hyphomicrobiales*. This indicated that ICBM5-like phages are able to undergo a temperate life cycle also. Furthermore, we uncovered related contigs in environmental viromes. Both genome clustering based on protein content and phylogenetic analysis showed that ICBM5 and it's related prophages and environmental genomes form two new subfamily-level clusters within the *Microviridae*, named "Tainavirinae" and "Occultatumvirinae" (Figure 3). A biogeographic assessment showed that tainaviruses and occultatumviruses are spread worldwide, in terrestrial and marine environments. The isolation of ICBM5 and its relatives uncovered new and diverse branches of the *Microviridae* tree (Zucker *et al.* 2022).

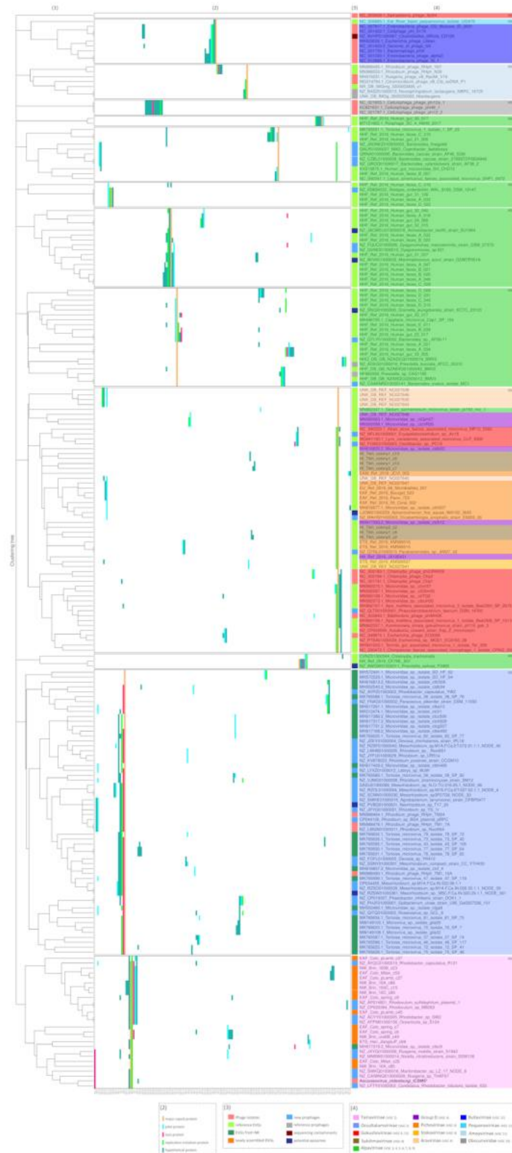

Figure 3: Hierarchical clustering of the ssDNA (pro)-phage genomes, based on their PSSC. The annotations show the following: (1) hierarchical clustering tree and (2) distribution of the PCs (PSSCs) in each viral genome. PCs not shared with any other phage genome are not shown. The color encodes different protein annotations; (3) phage genome category; (4) the label given to each phage genome, consisting of accession numbers and names of the phage isolate or the environmental contig or of the bacterial host in which a (pro)-phage was predicted (Zucker *et al.* 2022).

7.2.1.4 VirClust – a tool for the hierarchical clustering of viruses based on their protein content

To enable the taxonomic classification and the genomic characterization of the new roseophage isolates, especially in light of the major changes affecting viral taxonomy, through this project we developed VirClust (Moraru 2023). This software tool is a reference-free tool for hierarchical clustering of viral genomes, core protein detection, and annotation of viruses (Figure 4). At its core are several algorithms for protein clustering, either based on BLASTp or Hidden Markov Models similarities. Once the proteins have been clustered, VirClust calculates intergenomic distances based on the presence/absence of the protein clusters in the viral genomes. These distances are then used to cluster the viral genomes hierarchically. Various intergenomic distances can be used to split the genome tree into viral clusters at different taxonomic levels (from genus to family, or even order). Benchmarking on a phage dataset showed that the genome trees produced by VirClust match the current ICTV classification at family, sub-family, and genus levels. VirClust is freely available, as a web-service and stand-alone tool (http://rhea.icbm.uni-oldenburg.de/virclust/).

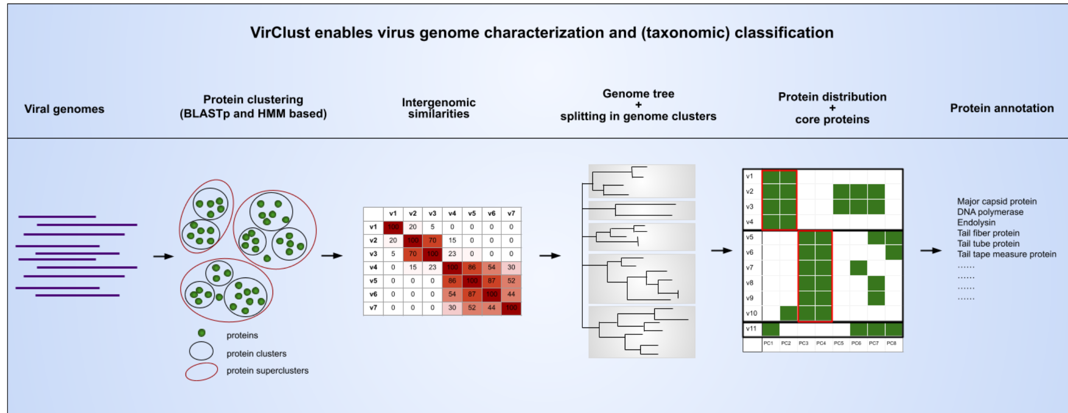

Figure 4: VirClust — graphical visualization of the workflow.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer-reviewed journals (members of B6 in bold, of other CRC projects in italic)

1. **Bischoff**, V., *Bunk*, B., *Meier-Kolthoff*, J.P., Spröer, C., *Poehlein*, A., *Dogs*, M., Nguyen, M., *Petersen*, J., *Daniel*, R., *Overmann*, J., *Göker*, M., *Simon*, M., *Brinkhoff*, T., and **Moraru**, C. (2019) Cobaviruses - a new globally distributed phage group infecting Rhodobacteraceae in marine ecosystems. The ISME Journal 13(6): 1404–1421.
2. **Zucker**, F., **Bischoff**, V., Olo Ndela, E., *Heyerhoff*, B., *Poehlein*, A., *Freese*, H.M., Roux, S., *Simon*, M., Enault, F., and **Moraru**, C. (2022) New Microviridae isolated from Sulfitobacter reveals two cosmopolitan subfamilies of single-stranded DNA phages infecting marine and terrestrial Alphaproteobacteria. Virus evolution 8(2): veac070.
3. **Moraru**, C. (2023) VirClust-A Tool for Hierarchical Clustering, Core Protein Detection and Annotation of (Prokaryotic) Viruses. Viruses 15(4):1007.

Other publications and published results

1. **Bischoff**, V., **Zucker**, F. and **Moraru**, C. (2021): Marine Bacteriophages. In Encyclopedia of Virology 4th edition, Bamford, D., and Zuckerman, M.A. (eds). Amsterdam: Academic Press, pp. 322-341.
2. **Bischoff**, V., Adriaenssens, E.M., Kropinski, A.M., Duhaime, M., and **Moraru**, C. (2020) ICTV-proposal Zobellviridae 2020.187B: Create one new family (Zobellviridae) including one new subfamily (Cobavirinae), seven new genera and 12 new species (Caudovirales). [WWW document]. https://ictv.global/ictv/proposals/2020.187B.R.Zobellviridae.zip.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2018. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	No.	Name, academic degree, position	Field of research	Department of university or non-university institution	Commitment in hours/ week	Category	Funded through:
Available							

Research staff	1.	Cristina Moraru, PhD, Senior Scientist	Microbial Ecology, Virology	ICBM	20		Univ OL
	2.	Meinhard Simon, Prof. Dr., W3	Microbial ecology	ICBM	5		Univ OL
Non-research staff	3.	Andrea Schlingloff		ICBM	8		Univ OL
Requested							
Research staff	1.	Vera Bischoff, Ms. Sc	Virology	ICBM	26	E13, 65%	
	2.	Falk Zucker, Ms. Sc.	Virology	ICBM	26	E13, 65%	

Job descriptions of staff (supported through existing funds):

1. Moraru
2. She was the Principal Investigator, responsible for the design and coordination of the experiments, finalization of the data analysis, and manuscript writing. She designed and developed several bioinformatics pipelines for the assembly, annotation, and biogeographical assessment of the roseophage genomes. She developed tools for the taxonomic classification of phages, including VirClust. She supervised two Ph.D. students: Vera Bischoff and Falk Zucker.
3. Simon
4. He guided the principal investigator through this SFB project, he provided the laboratory infrastructure and he was the official supervisor of the two PhD students.
5. Schlingloff
6. She assisted in carrying out 16S rRNA PCR and sequencing, media preparation, and cultivations.

Job descriptions of staff (funded with approved grant money):

1. Bischoff
   She carried out the roseophage isolation experiments, either directly or by coordinating master students. She has done the screening of the phage isolates, prepared the phage cultures, and extracted the DNA for genome sequencing. She carried out host-range experiments. She has performed taxonomic and genomic analyses of sequenced roseophages. She participated in data analysis and manuscript writing.
2. Zucker
   He carried out the characterization of the ICM5 roseophage infection cycle, using direct-geneFISH, plaque assays, and NanoPore sequencing. He searched for ICBM5-prophages and performed further taxonomic and genomic characterization. He participated in data analysis and manuscript writing.

7. General information about Project B7

7.1 Interactions Between Bacteria of the *Roseobacter* Group and Marine Algae via Sulfur Metabolites

7.1.2 Project leader

Dickschat, Jeroen, Prof. Dr., 3 June 1977, German
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7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report

7.2.1.1 Investigations on AHL synthases

N-Acylhomoserine lactones are important mediators in the intraspecific communication of many bacteria, including bacteria from the *Roseobacter* group. By regulation of processes such as biosynthesis of tropodithietic acid (TDA) in *P. inhibens* they may indirectly also be relevant for the interaction between marine bacteria and algae. The *N*-decanoyl/*N*-dodecanoylhomoserine lactone synthase from *P. inhibens* was characterised and the recombinant enzyme not only catalyses the reaction between the coenzyme A thioesters (**1**) of decanoic/dodecanoic acid and *S*-adenosylmethionine (**2**) in vitro, but also accepts phosphopantetheine derivatives of the fatty acids (Ziesche et al., 2018). The reaction is a two-step process of *N*-acylation to **3** and ring closure by intramolecular attack of the carboxylic acid function to the γ-carbon of the methionine portion in SAM with formation of the AHL (**4**) and extrusion of *S*-methyl 5'-thioadenosine (**5**, Figure 1A). To investigate the stereochemical course of AHLs that may either proceed with retention or inversion of configuration in the ring closure of **3** to yield **4**, stereoselectively deuterated (4*S*)- and (4*R*)-(4-^{2}H)-L-methionine (**6a** and **6b**) were synthesised. Unfortunately, their conversion with SAM synthase and AHL synthase into AHLs was too unefficient for conclusive results. Therefore, the synthesis of (4*S*)- and (4*R*)-(4-^{13}C,4-^{2}H)-L-methionine is currently performed (Figure 1B). In these precursors the additional ^{13}C-labels will allow for a highly sensitive detection of the outcome by HSQC spectroscopy.

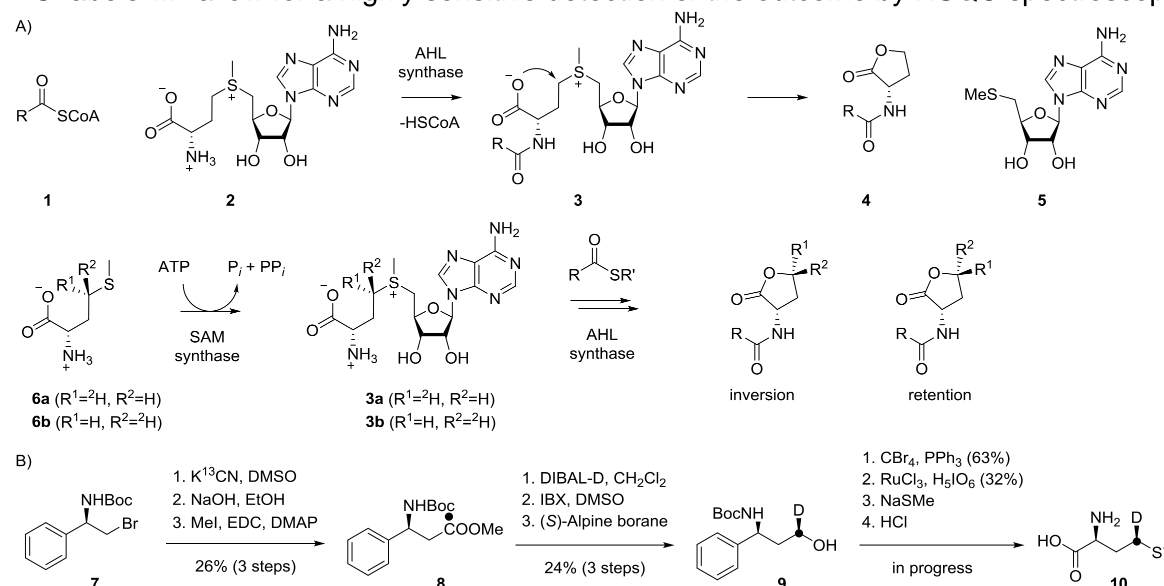

Figure 1. AHL biosynthesis. A) Proposed mechanism for the biosynthesis of AHLs from acyl-CoA derivatives and SAM and labelling experiments to investigate the stereochemical course. B) Synthesis of (4*R*)-(4-^{13}C,4-^{2}H)-L-methionine. The (4*S*) stereoisomer can be synthesised analogously using (*R*)-Alpine borane. Black dots indicate ^{13}C-labelled carbons.

7.2.1.2 Investigations on DMSP lyases

Our investigations on dimethylsulfoniopropionate (DMSP) lyases that were already performed during the first two funding periods were continued in the third funding period. DMSP lyases are enzymes that cleave the algal metabolite DMSP into dimethylsulfide (DMS) and acrylate (**12**). Several different enzymes are known for this transformation including DddL, DddP, DddQ, DddW, DddY, DddK and Alma1, while DddD and DddX act mechanistically differently (Figure 2A). In 2019 Pohnert reported on the new marine sulfur metabolite DMSOP that can be degraded to DMSO and acrylate in a reaction analogous to DMSP degradation. We have shown that the known DMSP lyases DddL, DddP, DddQ, DddW, DddY and DddK can all cleave DMSOP to yield DMSO (Figure 2B). Enzyme kinetic measurements revealed that DddQ is more efficient in the conversion of DMSOP than of DMSP, suggesting that this enzyme is best described as a DMSOP lyase (Chhalodia and Dickschat, 2023). Considering the enormous amounts of sulfur that is metabolised through DMSP and DMSOP, these reactions are of global importance for the global sulfur cycle.

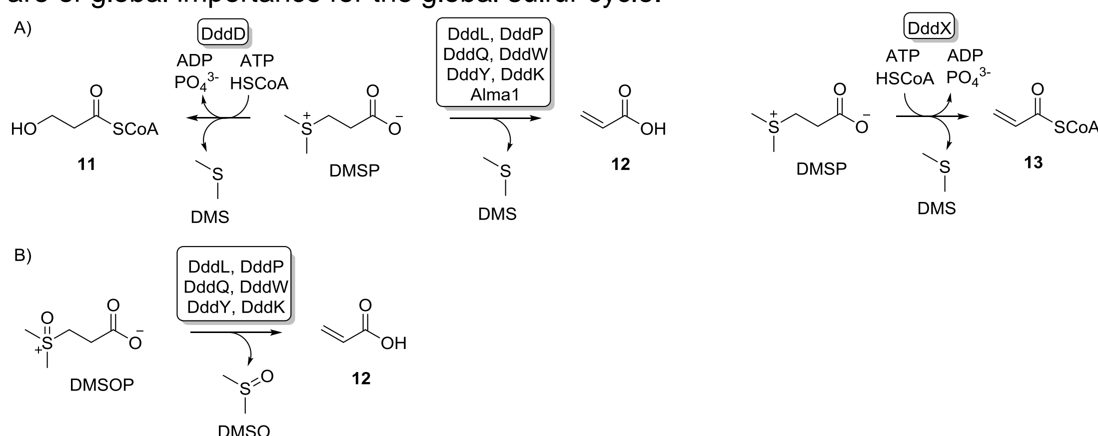

Figure 2. DMSP lyases. A) Known enzymes and their mechanisms for DMSP degradation to DMS. B) Cleavage of DMSOP into DMSO and acrylate.

7.2.1.3 Investigations on the stereochemical course of the DMSP demethylation pathway

The DMSP demethylation pathway that is widespread in bacteria from the *Roseobacter* group is known to proceed by the tetrahydrofolate (FH_4) dependent demethylation to methylthiopropionic acid (**14**) by DmdA, followed by conversion into the corresponding CoA ester **15** by DmdB, FAD-dependent oxidation to **16** by DmdC and hydrolysis to acetaldehyde (**17**), MeSH, HSCoA and CO_2 by DmdD (Figure 3A). The stereochemical course of the reactions along this pathway is unknown. In order to investigate the stereochemical course, stereoselectively deuterated **22** was synthesised according to Figure 3B. Also its enantiomer was synthesised using the same route in conjunction with (–)-DIPT in the Sharpless epoxidation. Furthermore, both enantiomers of (3-^{13}C,3-^{2}H)-**22** were synthesised. All four compounds are now available for enzyme incubations with DmdB and DmdC for which currently the conditions are optimised.

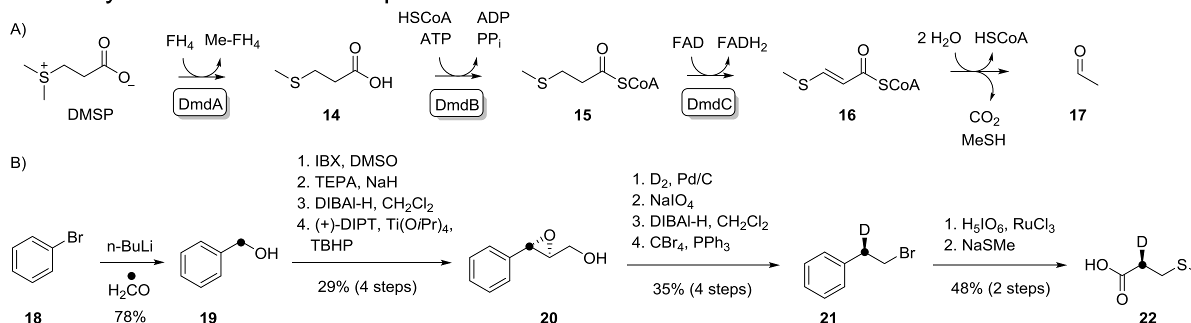

Figure 3. DMSP demethylation. A) The DMSP demetylation pathway. B) Synthesis of (*R*)-(2-^{13}C,2-^{2}H)-3-methylthiopropionic acid (22). The (*S*)-enantiomer was synthesised analogously using (–)-DIPT.

7.2.1.4 Investigations on the biosynthesis of tropodithietic acid (TDA)

The antibiotic TDA from *P.* inhibens is arguably one of the most interesting natural products made by an organism from the *Roseobacter* group. Its biosynthesis was studied by us throughout all three funding periods, and during the third funding period a breakthrough was achieved. In this recent work it was shown that TdaE is an unusual flavoenzyme that catalyses a multistep reaction in which first **4** is oxidised to the tropone derivative **16**. Reactivation of the reduced flavin cofactor with molecular oxygen allows for oxidative cleavage of the thioester to release tropone-2-carboxylic acid (**17**) that becomes subsequently oxidised to **18**. This final compound is highly unstable and can spontaneously degrade to tropolone (**9**) by epoxide opening and decarboxylation, explaining the biosynthesis of **9** in *Phaeobacter* and other bacteria. Therefore, a labelling strategy was developed to identify the structure of **18**. Its absolute configuration was determined by derivatisation through catalytic hydrogenation and esterification to obtain **19** that was compared to synthetic reference standards of racemic and enantiomerically pure **19**.

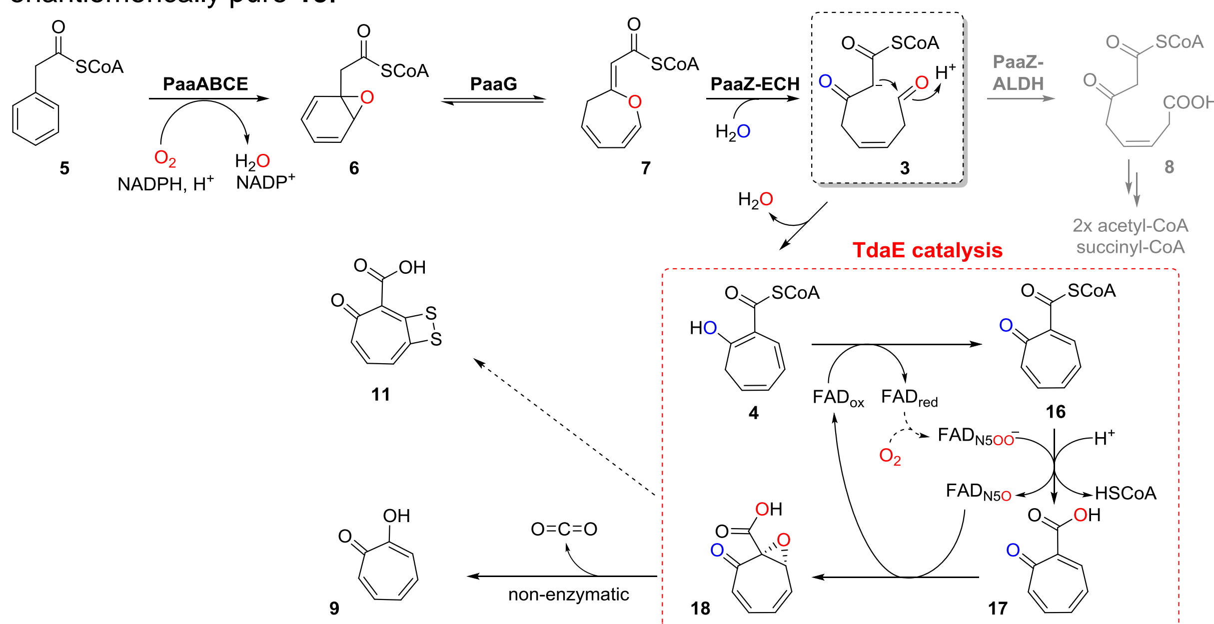

Figure 4. The role of TdaE in TDA (11) biosynthesis.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals
(members of B7 in bold, of other CRC projects in italic)

1. **Chhalodia** AK, **Dickschat** JS (2023) Discovery of Dimethylsulfoxonium Propionate Lyases – A Missing Enzyme Relevant to the Global Sulfur Cycle. Org Biomol Chem, 21, 3083-3089.
2. Duan Y, Toplak M, Hou A, **Brock** NL, **Dickschat** JS, Teufel R (2021) A flavoprotein dioxygenase steers bacterial tropone biosynthesis via coenzyme A-ester oxygenolysis and ring epoxidation. J Am Chem Soc 143: 10413-10421.
3. **Chhalodia** AK, **Dickschat** JS (2021) Breakdown of 3-(Allylsulfonio)propanoates in Bacteria from the Roseobacter Group Yields Garlic Oil Constituents. Beilstein J Org Chem, 17: 569-580.
4. **Chhalodia** AK, **Rinkel** J, Konvalinkova D, *Petersen* J, **Dickschat** JS (2021) Identification of volatiles from six marine *Celeribacter* strains. Beilstein J Org Chem, 17: 420-430.
5. Richter AA, Kobus S, Czeck L, Höppner A, Zarzycki J, Erb TJ, Lauterbach L, **Dickschat** JS, Bremer E, Smits SHJ (2020) The architecture of the diaminobutyrate acetyltransferase active site provides mechanistic insight into the biosynthesis of the chemical chaperone ectoine. J Biol Chem, 295: 2822-2838.

6. Czech L, Höppner A, Kobus S, Seubert A, **Riclea** R, **Dickschat** JS, Heider J, Smits SHJ, Bremer E (2019) Illuminating the catalytic core of ectoine synthase through structural and biochemical analysis. Sci Rep, 9: 364.
7. *Ziesche* L, **Rinkel** J, **Dickschat** JS, *Schulz* S (2018) Acyl group specificity of AHL-synthases involved in Quorum-sensing in *Roseobacter* group bacteria. Beilstein J Org Chem, 14: 1309-1316.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period. (B3 from 2010-2013 and B7 from 2018)

7.3.1 Project staff in the ending funding period

	Sequential no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Dickschat, Jeroen, Prof. Dr.	Bioorganic and Natural products Chemistry	Kekulé-Institute for Organic Chemistry and Biochemistry	5		University of Bonn
	2	Nozinovic, Senada, Dr.	NMR specialist	Kekulé-Institute for Organic Chemistry and Biochemistry	2		University of Bonn
	3	Engeser, Marianne, Dr.	MS specialist	Kekulé-Institute for Organic Chemistry and Biochemistry	2		University of Bonn
Non-research staff	4	Schneider, Andreas		Kekulé-Institute for Organic Chemistry and Biochemistry	2		University of Bonn
Staff funded with approved grant money							
Research staff	1	Immo Burkhardt	Natural products Chemistry	Kekulé-Institute for Organic Chemistry and Biochemistry		PhD student	
	2	Jan Rinkel	Natural products Chemistry	Kekulé-Institute for Organic Chemistry and Biochemistry		PhD student	
	3	Anuj Chhalodia	Natural products Chemistry	Kekulé-Institute for Organic Chemistry and Biochemistry		PhD student	
	4	Anwei Hou	Natural products Chemistry	Kekulé-Institute for Organic Chemistry and Biochemistry		PhD student	

Job descriptions of staff (supported through existing funds):

1. Jeroen Dickschat
   He was principal investigator of the project and responsible for design and coordination of the experiments, data analysis, and publication of the results. He supervised the PhD students.
2. Senada Nozinovic
   She is the head of the NMR department at the University of Bonn and was responsible for all NMR data aquisition.
3. Marianne Engeser
   She is the head of the mass spectrometry unit at the University of Bonn and was responsible for all MS data aquisition.
4. Andreas Schneider
   He is a lab technician at the Kekulé-Institute who operates the HPLC pool of our institute. He was responsible for HPLC purifications of compounds.

Job descriptions of staff (funded with approved grant money):

1. Immo Burkhardt
   He was responsible for the investigations on DMSP lyases. After his leave of the group this work was continued by Anuj Chhalodia.
2. Jan Rinkel
   He carried out the work on AHL synthases.
3. Anuj Chhalodia
   He took over the work on DMSP lyases from Immo Burkhardt and was also responsible for the investigations on the stereochemical course of the demethylation pathway
4. Anwei Hou
   He was responsible for the work on the biosynthesis of TDA.

7. General information about Project C1

7.1 Interactions of *Phaeobacter inhibens* DSM 17395 and metaproteomics of *Roseobacter*-dominated bacterial communities

7.1.2 Project leader

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7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report

7.2.1.1 Chemostat experiments with *P. inhibens* DSM 17395

As outlined in the proposal for this funding phase, chemostat experiments were planned to be conducted with *P. inhibens* DSM 17395, in order to explore substrate-controlled growth efficiency and energetics as basis for subsequent systems biology-level experiments to unravel the underlying molecular mechanisms. To study energetic aspects of growth, adenosine triphosphate (ATP) and NAD(H) are widely applicable molecular proxies for an organism's viability and activity. To this end, we established a luciferase-based assay in combination with a microplate reader (96-well) for *P. inhibens* DSM 17395 (*Escherichia coli* K12 served as reference). The ATP and NAD(H) yields determined for *P. inhibens* DSM 17395 at ¼ OD_{max} were found to reside well within the range previously reported for *E. coli* and other bacteria, e.g., 3.28 µmol ATP (g cells dry)$^{-1}$ (Fig. 1) (Wünsch et al. 2022).

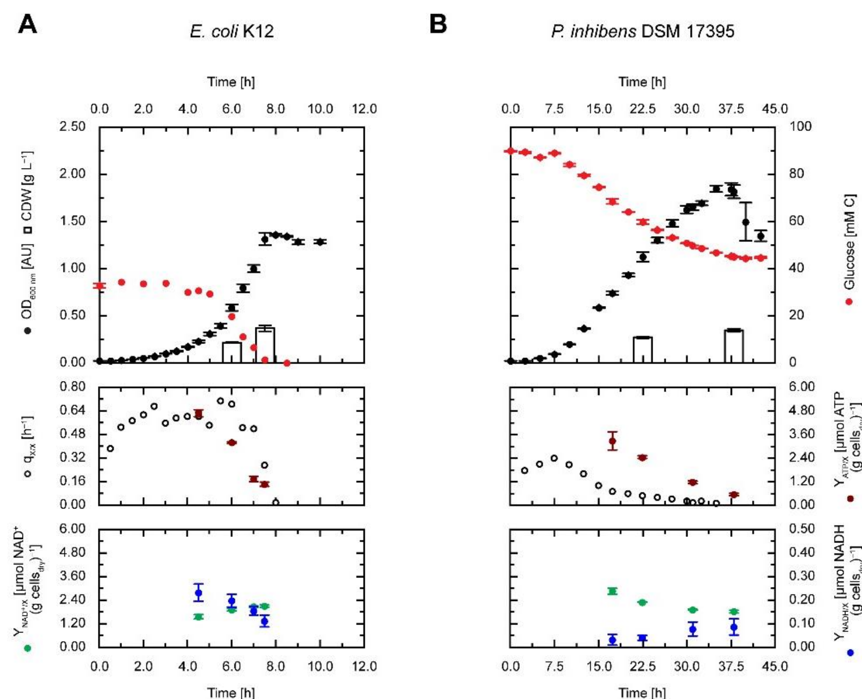


Fig. 1. Growth behaviour of the standard bacterium *E. coli* K12 (A) and *P. inhibens* DSM 17395 (B) during batch cultivation in defined mineral medium containing glucose as sole source of carbon and energy. Basic growth parameters are determined by measurement of optical density (OD_{600}) (black, filled dots), concentration of cellular dry weight (CDW) (black, open bars) and glucose consumption (converted to carbon; red, filled dots). The growth energetics are assessed by the biomass specific growth rate ($q_{X/X}$) (black, open dots) and the biomass demand for ATP ($Y_{ATP/X}$) (brown, filled dots), NAD^+ ($Y_{NAD+/X}$) (green, filled dots) and NADH ($Y_{NADH/X}$) (green, filled dots).

The chemostat experiments were set up with 2-liter double-jacket glass vessels, equipped with sensors for pH, temperature, dissolved oxygen tension and foam control. In and outflowing air was analyzed online for O_2, CO_2, and Ar using a quadrupole mass spectrometer. In flowing medium and outflowing culture broth was weight-controlled. Following an initial promising start, the following two adverse effects were observed with the wild type of *P. inhibens* DSM 17395.

First, after several generation times the cultures turned from the typical TDA-derived brown coloring to white, indicating a loss of the large 262 kbp plasmid harboring the genes for TDA biosynthesis. This was confirmed e.g. by streaking chemostat samples onto agar plates. Second, during prolonged operation, the chemostat cultures developed extensive biofilms that covered the inner face of the glass vessel, most probably reflecting respective gene activities encoded in the 65 kbp plasmid. Both unexpected, adverse effects repeatedly occurred during several rounds of chemostat cultivation, impeding the reproducibility and informational value of the experiments with the wild type. Since these constituted the fundamental basis for all other planned chemostat experiments, after thorough consideration, we decided not to pursue this path any longer. Instead, we investigated the responsiveness of *P. inhibens* DSM 17395 to selected growth substrates as outlined below.

7.2.1.2 Responsiveness of *P. inhibens* DSM 17395 to selected growth substrates.
Phaeobacter inhibens DSM 17395, as a heterotrophic member of the *Roseobacter* group, is specialized in the aerobic utilization of carbohydrates and amino acids via pathways widespread among roseobacters. The in vivo responsiveness of *P. inhibens* DSM 17395 was studied with nonadapted cells (succinate-grown), which were exposed to a single pulse (100–0.01 µM) each of *N*-acetylglucosamine, mannitol, xylose, leucine, phenylalanine, or tryptophan (effectors) (Fig. 2). Responsiveness was then determined by time-resolved transcript analyses (quantitative reverse transcription-PCR) of “degradation” and “uptake” genes selected based on previously reported substrate-specific proteome profiles. The transcriptional response thresholds were: 50–100 nM for *nagK* (*N*-acetylglucosamine kinase), *paaA* (ring 1,2-phenylacetyl-CoA epoxidase), and *kynA* (tryptophan 2,3-dioxygenase), 10–50 nM for *xylA* (xylose isomerase), and around 10 nM for *mtlK* (mannitol 2-dehydrogenase). A threshold for leucine could not be determined due to the elevated intrinsic presence of leucine in the exometabolome of succinate- grown cells (no effector addition) (Fig. 3). Notably, the response thresholds for presumptive carbohydrate-binding proteins of ABC-transporters were in the same range or even lower: 0.1–1 µM for *c27930* (*N*-acetylglucosamine) and even below 10 nM for *c13210* (mannitol) and *xylF* (xylose). These findings may contribute to the “emergent recalcitrance” concept of dissolved organic matter (DOM). First, the rapidness (within min) of detectable transcriptional response even to effector concentrations in the nanomolar range agrees well with the instantaneous remineralization of DOM by microbial communities in oligotrophic Atlantic surface waters. Second, the determined thresholds of transcriptional responsiveness (<10–100 nM) of degradation” and “uptake” genes are orders of magnitudes higher than the assumed below picomolar concentrations of individual DOM components in the marine system. The observed thresholds reflect the potential of *P.*

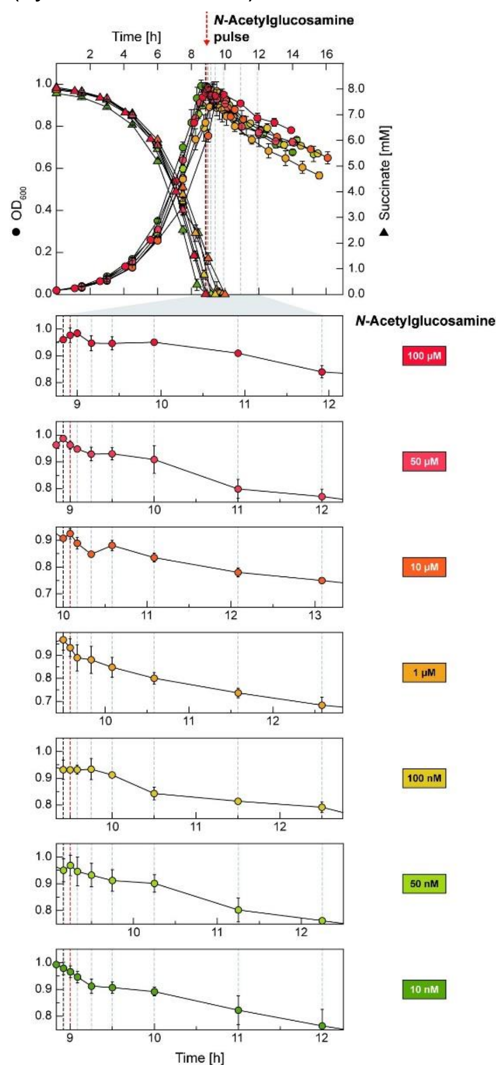
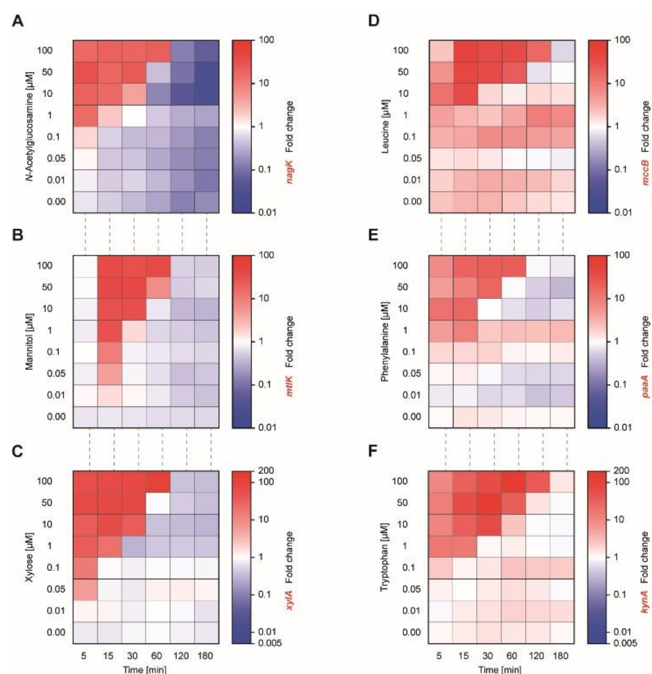


Fig. 2. Cultivation of *P. inhibens* DSM 17395 for targeted transcript analysis in response to *N*-acetylglucosamine. Upon depletion of the primary growth substrate succinate after ~9 h, *N*-acetylglucosamine was added (red dashed line) yielding a distinct concentration as indicated in the zoom-in boxes. In each case, triplicate cultures were performed (note error bars, standard deviation). The grey dashed lines indicate the sampling time points for transcript analysis. Analogous growth experiments were performed for the other five tested compounds.



inhibens DSM 17395 to rapidly respond to sudden increases in nutrient supply. Such "feast" scenarios are regularly encountered, e.g., in the microenvironment surrounding phytoplankton cells, during the collapse of seasonal algal blooms or in nutrient- and species-rich marine upwelling ecosystems. Below the determined response thresholds of *P. inhibens* DSM 17395 ("famine" scenario in oligotrophic systems), growth- or maintenance-supporting DOM components may simply escape biodegradation due to non-expression of genes related to their uptake and degradation (Weiten et al. 2022).

Fig. 3. Transcriptional response of "degradation" genes of *P. inhibens* DSM 17395 in response to defined pulses with different substrates. Tested compounds were: *N*-acetylglucosamine (**a**), mannitol (**b**), xylose (**c**), leucine (**d**), phenylalanine (**e**), and tryptophan (**f**). The analyzed transcripts represent genes coding for enzymes involved in the degradation of these selected six substrates (Fig. 1b, highlighted in red). Relative transcript abundance was determined by qRT-PCR (reference: 5 min prior to substrate addition) and based for every data point on three biological replicates with three technical replicates each.

7.2.1.3 Metaproteomics

A specific project newly developed and conducted during the third funding period was the re-enacting of a North Sea algal bloom in a so-called planktotron experiments (each hosting a 500 l volume). We contributed to the planning of this comprehensive experiments and participated with 2 co-workers in the actual 6-weeks experiment (24-7) to closely monitor the course of the algal bloom and to collect samples for a large variety of analyse. In our case samples size fraction filtered (>3 µm, 0.2-3 µm), shock frozen and stored for metaOMICS analyses at –80°C in our laboratory. Overall, from 4 planktotrons operated in parallel and sampled each at nine different time points, a total of 136 filters were extracted and analysed by nanoLC-ESI-MS/MS, generating a dataset based on 476 MS measurements. Since we are still waiting for a suitable metagenomic database for mapping of the metaproteomic peptide data, this exciting and unique study will, however, unfortunately, not be completed within the reporting phase of the CRC.

From the previous research cruise across covering the North-South extension of the Pacific Ocean in total 498 samples (300 pelagic, 102 sediment and 96 day-night cycle) have been stored at –80°C in our laboratory. We have processed and analysed the 52 out of the 102 collected sediment samples (13 stations, each 0 and 20 cm depth, each 2 replicates) applying 2 different extraction methods (phenol vs. SDS & phenol). Subsequent analyses by nanoLC-ESI-MS/MS generating a dataset based on 312 MS measurements. Since we are still waiting for a suitable metagenomic database for mapping of the metaproteomic peptide data, analysis of this exciting and unique dataset will, however, unfortunately, not be completed within the reporting phase of the CRC.

7.2.1.4 Proteomic contributions to collaborative projects with the CRC

Together with project A5 (Jörn Petersen, DSMZ), we studied the exoproteome of eleven different genotypes of *P. inhibens* DSM 17395 to investigate the biosynthesis, regulation and

functionality of flagella. To this end, we conducted 66 MS measurements by nanoLC-ESI-MS/MS. The experimental side of this study is completed and a manuscript is in preparation, which is lead by Jörn Petersen and co-workers.

In case of the ClpX theme in *Dinorosoebacter shibae* (lead: project B5, Jahn/Härtig, TU BS) we analyzed two different sets of samples by nanoLC-ESI-MS/MS. First, wild type and ΔclpX mutant cultivated under three different light regimes (with three replicates each; in total 36 samples) were analysed with shotgun as well as membrane protein focused approaches, resulting in 162 MS measurements. Second, purification and cross-linking of the purified ClpP, ClpX and Dshi_1135 proteins were analyzed, resulting in 72 MS measurements. The experimental side of this study is completed and a manuscript is in preparation lead by the Elisabeth Härtig and Dieter Jahn group.

The project was challenged during the 1st year of the 3rd funding period by a construction projects due to fire protection measures in the building section the group is located in. This in the led to an interruption of lab work for almost half a year.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals
(members of C1 in bold, of other CRC projects in italic)

1. **Wünsch** D, **Scheve** S, **Weiten** A, Kalvelage K, **Rabus** R (2022) Luciferase-based determination of ATP/NADP(H) pools in a marine (environmental) bacterium. Microb Physiol 32:122–134.
2. **Weiten** A, **Kalvelage** K, *Neumann-Schaal M*, Buschen R, **Scheve** S, Winklhofer M, **Rabus** R (2022) Nanomolar responsiveness of marine *Phaeobacter inhibens* DSM 17395 toward carbohydrates and amino acids. Microb Physiol 32:108–121.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	No.	Name, academic degree, position	Field of research	Department of university or non-university institution	Commitment in hours/week	Category	Funded through:
Available							
Research staff	1	Ralf Rabus, Prof. Dr., W2	Microbiology	ICBM	5		Univ OL
	2	Lars Wöhlbrand, Dr.	Microbiology	ICBM	10		Univ OL
	3	Daniel Wünsch, Dr.	Microbiology	ICBM	10		Univ OL
Non-research staff	4	Christina Hinrichs		ICBM	10		Univ OL
	5	Sabine Scheve		ICBM	30		Univ OL
	6	Martina Gehler		ICBM	5		
Requested							
Research staff	1	Arne Weiten., Ms. Sc.	Microbiology	ICBM		PhD student	
	2	Kristin Kalvelage., Ms. Sc.	Microbiology	ICBM		PhD student	

Job descriptions of staff (supported through existing funds):

1. Rabus
   Planning and advice of experimental work; participation in evaluation of physiological (incl. bioreactors) and proteomic data; active involvement in data exchange and interpretation within the Braunschweig-centered systems; participation in conceiving and writing of manuscripts.
2. Wöhlbrand
   Mass spectrometry (MS) based protein identification (MALDI-TOF MS and nanoLC-ESI-iontrap MS) for metaproteomics, as this requires advanced mass spectrometry; analysis and evaluation of MS-data; planning and advice of targeted transcript analysis; participation in writing manuscripts
3. Wünsch
   Active participation in and coordination of the bioreactor-based (in particular chemostat) studies with *P. inhibens* and the establishment of the luciferase-based ATP/NADH assay; participation in writing manuscripts.
4. Hinrichs
   She assisted in carrying out physiological growth tests and experiments with isolates, preparing samples molecular biological analyses until her parental leave.
5. Scheve
   She was instrumental in physiological growth tests, HPLC analysis as well as RNA work.
6. Gehler
   Parental leave substitution of Hinrichs; she was instrumental for sample preparation for metaproteomics. He also carried out HPLC analyses of dissolved carbohydrates.

Job descriptions of staff (funded with approved grant money):

1. Weiten
   He participated in the establishment of the luciferase-based ATP/NADH assay, the chemostat experiments, and had his main project on the responsiveness of *P. inhibens* DSM 17395 towards selected carbohydrates.
2. Kalvelage
   She participated in the establishment of the luciferase-based ATP/NADH assay, the chemostat experiments, and had her main project on the responsiveness of *P. inhibens* DSM 17395 towards selected amino acids.

7. General information about Project C2

7.1.1 Chemistry of secondary metabolites and their interactions within the *Roseobacter* group and with other organisms

7.1.2 Project leader

Schulz, Stefan, Prof. Dr., 6.9.1957, German
Institut f. Organische Chemie, TU Braunschweig, 36106 Oldenburg
Tel. 0531/391-7353
Email s.schulz@tu-bs.de

7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report

7.2.1.1 *N*-Acylhomoserine lactones of the *Roseobacter* group (*Roseobacteraceae)*

In the project my group developed sensitive, GC/MS-based methods for the identification of important signaling compounds within the *Roseobacter* group, *N*-acylhomoserine lactones (AHLs), as well as synthetic methods to synthesize various acylated homoserine lactones (HSL). AHLs mediate several physiological changes mediated by quorum-sensing in roseobacters, such as formation of the antibiotic tropodithietic acid (TDA) in *Phaeobacter gallaeciensis*, using (*R*)-3-hydroxydecanoyl-HSL (**1**, Fig. 1) (Berger et al. 2011). The major AHL of the model strain *Dinoroseobacter shibae* was identified to be *N*-((2*E*,11*Z*)-2,11-ocatdecadienoyl)homoserine lactone (2E,11Z-C18:2-HSL, **2**), the first identified diunsaturated AHL (Neumann et al. 2013). Because this compound was produced only in small amounts in the wild type, the respective AHL-synthase gene *luxI*$_1$ was heterologously expressed in *E. coli* to get enough material for full chemical characterization. Surprisingly, the major product was *N*-((Z)-7-tetradecenoyl)homoserine lactone (Z7-C14:1-AHL). Therefore, *luxI*$_1$ was re-introduced together with a promoter in a *luxI*$_1$ deletion mutant of the wild-type *D. shibae*, yielding now large amounts of 2E,11Z-C18:2-HSL. A robust synthesis to this compound was developed to confirm the structure and for biological assays. This method proved to be instrumental for the synthesis of a large number of AHLs during the whole funding phases, providing material for other groups and our own. In *D. shibae*, C18-HSLs induce changes in cell morphology and division in a AHL-type selective manner. Therefore, this strain can react not only to own AHLs, but also sense those of the near environment (Patzelt et al. 2013). Further studies showed that the CtrA-phosphorelay correlates cell differentiation *in D. shibae* with cell to cell communication, making it a density dependent process (Wang et al. 2014). Various AHLs synthesized by us showed only low or no activity in antibiotic assays. In contrast, Z7-C14:1-AHL showed high antialgal activity against *Skeletonema costatum* (Ziesche et al. 2015),

Generally, AHLs of the roseobacters have longer acyl chains with more than 10 carbons, with the exception of 3OH-C10-HSL, which is present in *Phaeobacter gallaeciensis*. In a comparative study we showed that strains of the *Roseobacter* group show moderate differentiation in AHL structures, with changes mostly in chain length and the degree of unsaturation (Ziesche et al. 2015), although 19 different AHLs were detected in 20 strains (Ziesche et al. 2015), some of them found also released by *Paracoccus* strains (Leinberger et al. 2021). The most widespread AHL within roseobacters is Z7-C14:1-HSL. In several roseobacters, *N*-((2*E*,5*Z*)-dodeca-2,5-dienoyl)homose-rine lactone (**3**), the shortest diunsaturated AHL reported so far, was detected. Position isomers were also detected, revealing the instability of the compound that

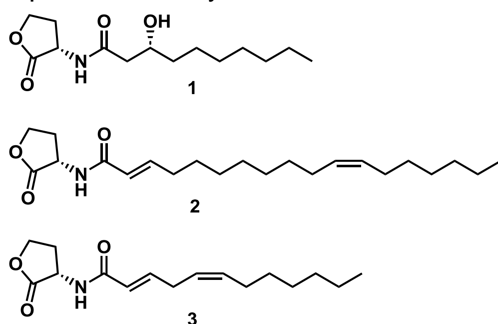


Fig. 1. Homoserine lactones R3OH-C10:0-HSL (**1**), 2E,11Z-C18:2-HSL (**2**),

degrades to the thermodynamically most stable isomer 2,4-C12:2-HSL (Ziesche et al. 2019). In sensor assays, the chain length determines mostly activity, while the double bond position is less important.

A comparative analysis of all known AHL structures showed the HSL part to be conserved, but the acyl chain length varies between C4 and C18, with a carbonyl or 3-hydroxyl group present at C-3. A double-bond at ω-7 with Z-configuration (Schulz and Hötling 2015), while the second one is only found at C-2. An interesting finding outside the roseobacters was the presence of Z7-C14:1-HSL in the axenic algae *Prorocentrum minimum*, because AHLs are not known from algae so far. This result needs independent confirmation.

We further investigated the substrate specificity of two AHL-synthase *luxI* from *D. shibae* using synthetic analogs prepared by us using a whole bishomolog series of fatty acid cysteamine conjugates mimicking the first part of coenzyme A. Both enzymes had a widely different substrate specificity. While $luxI_1$ accepted all conjugates except C_4-acyl groups, $luxI_2$ preferred markedly C_{16}- and C_{18} substrates. This indicates that specificity in AHL bouquets of certain strains is obviously only partly dependent on substrate selection by the enzyme, but requires also activity of other enzymes adding additional selectivity, e.g. by the production of the fatty acid ACP conjugates or further upstream processes. Furthermore, the bouquet composition of *D. shibae* is not directly linked to the fatty acid profile of *D. shibae.* The AHL part was mostly investigated together with groups B2 and B4, but also with other groups.

7.2.1.2 N-Acylaminoacid methyl esters (NAMEs)

In some specific strains of roseobacters, especially *Roseovarius* sp., *N*-acylalanine methyl esters (NAMEs) were identified, a new class of compounds (Bruns et al. 2013). The structures were confirmed by synthesis and made available for bioassays for the cooperating partners. The model strain *R. tolerans* contained both AHLs and NAMEs in high concentration, but different in acyl chain length. Thus, Z7-C14:1-AHL occurs together with Z9-C16:1-NAME (**4** in Fig. 2), suggesting differentiation of the biosynthesis on the acid chain length level. We therefore suggested a genomic analysis of the producer strain, revealing *luxI* genes responsible for AHL production in the genome, but gave no clear evidence for NAME biosynthetic genes (Voget et al. 2015). Furthermore, terminally oxidized NAMEs were identified, a structural feature not observed in AHLs. These include terminally oxidized NAMEs such as 18-hydroxy-11-octadecenoylalanine methyl ester (**5**) (Bruns et al. 2018c). Although a large number of samples containing AHLs were analyzed by us, we never found terminally oxidized AHLs. *Loktanella* and *Roseovarius* strains also contained respective esters with other amino acids, glycine (**6**), 2-aminobutyric acid (**7**), and valine (**8**), including compounds with terminally branched acyl groups (Bruns et al. 2018b).

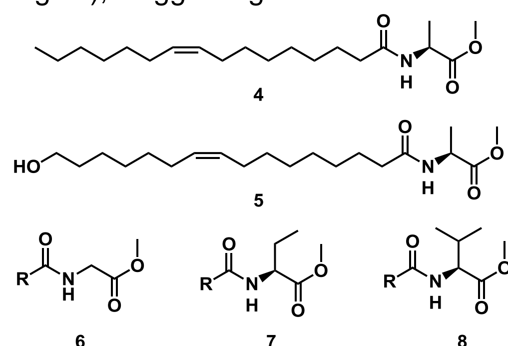


Fig. 2. Various *N*-acylamino acid methyl esters of *Roseovarius* and *Loktanella* sp.

Preliminary experiments for the identification of specific *Roseobacter* siderophores were performed with project B2. Specific isolation procedures for siderophores were learned in the laboratory of B. Moore at Scripps Institution of Oceanography, La Jolla, USA, and resulted in a first author publication of H. Bruns performing isolation of a new siderophore from a non-*Roseobacter* species (Bruns et al. 2018a). Nevertheless, before the learned methods could be applied for the isolation of roseochelin, an important siderophore of the roseobacters, the reviewers did not endorse the proposed continuation of the project in the third phase, and the project had to be terminated. Meanwhile, this siderophore had been isolated by the Seyedsayamdost group and their importance for the ecology of the roseobacters shown. These project was mainly performed together with project B2.

7.2.1.3 Volatile and other compounds of the Roseobacteraceae

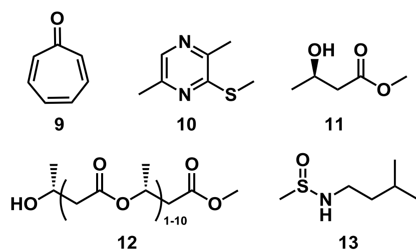


Fig. 3. Volatiles and related compounds of roseobacters.

In preliminary experiments before the start of TRR51 we have shown that roseobacters can produce a wide range of volatile organic compounds, especially sulfur compounds and metabolites of the phenylacetate pathway, such as tropone (**9** in Fig. 3) and its hydrate. Various of these volatiles were tested together with volatiles originating from other bacteria for cell proliferation inhibition, antibiotic activity and activity in quorum-sensing sensor systems (Schulz et al. 2010). In summary, compounds produced by many unrelated bacteria, so called common volatiles (Weisskopf et al. 2021) showed low activity, including dimethyl disulfide. In contrast, more specialized compounds such as 2-methylsulfanyl-3,6-dimethylpyrazine (**10**) from *Loktanella* sp. showed high inhibition of quorum-sensing sensor systems. We furthermore could identify novel amides including the first natural sulfonamides such as **13** in *Roseovarius pelophilus* and other roseobacters, but also in other marine bacteria (Harig et al. 2017). Although roseobacters cannot be regarded as prolific producers of volatile compounds, our analysis revealed the importance of especially sulfur compounds, also proving the release of methanethiol directly, which is the precursor of the ubiquitous dimethyl disulfide. Specifically roseobacters can metabolize 2,3-dihydroxypropane-1-sulfonate into sulfur volatiles, which in turn comes from sulfolipids containing sulfoquinovose (Celik et al. 2017). The analysis of volatiles of about 50 strains revealed that roseobacters do produce a wide range of volatiles, but are usually not producers of specific compounds, with few exceptions. Most of the compounds can be regarded as common components, also found in non-related other bacteria (Weisskopf et al. 2021). *Pseudooceanicola* sp. is of special interest, because it is one of the few roseobacters that releases volatile terpenes such as farnesol, a quorum-sensing inhibitor, but also other compounds known for signaling activity in other bacteria such as 2-aminoacetophenone, making chemical interactions of these strains by volatiles likely (Wolter et al. 2021). Volatiles are fast moving in water because their lack of a hydration sphere, and are therefore the first compounds detectable when bacterial cells are nearing each other. The mechanism of release of apolar volatiles into water is not well understood, but we could show for the first time that outer membrane vesicles of bacteria can carry volatiles; *D. shibae* thus transports (*Z*)-5-dodecenal and ensures a local high concentration when the membrane disintegrates (Koteska et al. 2023).

More specific for roseobacters is the release of methyl (*R*)-3-hydroxybutyrate (**11**) and its oligomers, identified up to undecamers (**12**) especially from *Loktanella* sp. (Ziesche et al. 2015). While antioxidant properties of such compounds have been reported recently, we could not find activity in antimicrobial bioassays. The same was true for other important volatiles present, such as indole, which is present in almost all investigated roseobacters, and for various small acetamides (Ziesche et al. 2015).

We also tested the influence of volatiles of hosts on roseobacters, in this case in the toxic microalgae *Prorocentrum minimum/cordatum*. This alga produces only low amounts of volatiles, dominated by apocarotenoids, when grown in non-axenic, but much more but different one under axenic conditions (Koteska et al. 2022a). Therefore, the physiological state of the alga is reflected in the volatile bouquet. The apicomplexan algae *Chromera velia* was used to investigate volatiles of specific, algae associated bacterial strains with those of then non-axenic algae. Several attempts involving commercial companies to obtain axenic *C. velia* failed. Nevertheless, while several bacteria are good producers of volatiles when cultivated alone, the volatiles of the xenic algae was dominated by algal compounds, mostly apocarotenoids, but different from *P. cordatum*. Therefore, the influence of bacteria, including roseobacters which play an important role in the bacterial consortia of *C. velia,* on the composition of the algal volatile bouquet is low (Koteska et al. 2022b).

In addition to the outlined results, members of this project were involved in further studies of the TRR51 performing and developing methods for analysis of the antibiotic TDA (Majzoub et al. 2018; Majzoub et al. 2021; Trautwein et al. 2017; Wünsch et al. 2020). They were also

involved in two reviews on volatiles of bacteria (Schulz et al. 2020a; Schulz et al. 2020b). Research on volatiles was mostly performed in cooperation with group B2, A5, C3, and B4.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals (members of C2 in bold, of other CRC projects in italic)

1. *Berger M*, **Neumann** A, **Schulz** S, *Simon M*, *Brinkhoff T* (2011) Tropodithietic acid production in *Phaeobacter gallaeciensis* is regulated by *N*-acyl homoserine lactone-mediated quorum sensing. J Bacteriol 193:6576–6585
2. **Bruns** H, **Thiel** V, *Voget S, Patzelt D, Daniel R, Wagner-Döbler I,* **Schulz** S (2013) *N*-acylated alanine methyl esters (NAMEs) from *Roseovarius tolerans*, structural analogs of quorum-sensing autoinducers, *N*-acylhomoserine lactones. Chem Biodivers 10:1559–1573.
3. **Bruns** H, Crüsemann M, Letzel A-C, Alanjary M, McInerney JO, Jensen PR, **Schulz** S, Moore BS, Ziemert N (2018a) Function-related replacement of bacterial siderophore pathways. ISME J 12:320.
4. **Bruns** H, **Ziesche** L, Taniwal NK, *Wolter L, Brinkhoff T*, Herrmann J, Müller R, **Schulz** S (2018b) *N*-Acylated amino acid methyl esters from marine *Roseobacter* group bacteria. Beilstein J Org Chem 14:2964–2973.
5. **Bruns** H, Herrmann J, Müller R, *Wang H, Wagner Döbler I*, **Schulz** S (2018c) Oxygenated *N*-Acyl Alanine Methyl Esters (NAMEs) from the Marine Bacterium *Roseovarius* tolerans EL-164. J Nat Prod 81:131–139.
6. *Celik E*, **Maczka** M, *Bergen N, Brinkhoff T*, **Schulz** S, *Dickschat JS* (2017) Metabolism of 2,3-dihydroxypropane-1-sulfonate by marine bacteria. Org Biomol Chem 15:2919–2921.
7. Harig T, Schlawis C, Ziesche L, *Pohlner M, Engelen B*, Schulz S (2017) Nitrogen-Containing Volatiles from Marine *Salinispora pacifica* and *Roseobacter*-Group Bacteria. J Nat Prod 80:3289–3295.
8. **Koteska** D, *Sanchez Garcia S, Wagner-Döbler I*, **Schulz** S (2022a) Identification of Volatiles of the Dinoflagellate *Prorocentrum cordatum*. Mar Drugs 20:371.
9. **Koteska** D, *Marter P, Huang S, Pradella S, Petersen J*, **Schulz** S (2022b) Volatiles of the Apicomplexan Alga *Chromera velia* and Associated Bacteria. ChemBioChem 24:e202200530.
10. **Koteska** D, *Wang H, Wagner-Döbler I,* **Schulz** S (2023) Outer membrane vesicles of *Dinoroseobacter shibae* transport a volatile aldehyde. Front Ecol Evol 11:1102159.
11. Majzoub ME, McElroy K, Maczka M, Thomas T, Egan S (2018) Causes and Consequences of a Variant Strain of *Phaeobacter inhibens* With Reduced Competition. Front Microbiol 9:2601.
12. Majzoub ME, McElroy K, **Maczka** M, **Schulz** S, Thomas T, Egan S (2021) Genomic Evolution of the Marine Bacterium *Phaeobacter inhibens* during Biofilm Growth. Appl Environ Microbiol 87:e0076921.
13. *Leinberger J*, **Holste** J, *Bunk B, Freese HM, Spröer C, Dlugosch L, Kück A-C*, **Schulz** S, *Brinkhoff T* (2021) High Potential for Secondary Metabolite Production of *Paracoccus marcusii* CP157, Isolated From the Crustacean Cancer pagurus. Front Microbiol 12:1725.
14. **Neumann** A, *Patzelt D, Wagner-Döbler I*, **Schulz** S (2013) Identification of new *N*-acylhomoserine lactone signalling compounds of *Dinoroseobacter shibae* DFL-12^T by overexpression of *luxI* genes. ChemBioChem 14:2355–2361.
15. *Patzelt D, Wang H, Buchholz I, Rohde M, Gröbe L, Pradella S*, **Neumann** A, **Schulz** S, *Heyber S, Münch K, Münch R, Jahn D, Wagner-Döbler I, Tomasch J* (2013) You are what you talk: Quorum sensing induces individual morphologies and cell division modes in *Dinoroseobacter shibae*. ISME J 7:2274–2286.
16. **Schulz** S, Hötling S (2015) The use of the lactone motif in chemical communication. Nat Prod Rep 32:1042–1066.

17. **Schulz** S, *Dickschat JS*, Kunze B, *Wagner-Dobler I*, Diestel R, Sasse F (2010) Biological Activity of Volatiles from Marine and Terrestrial Bacteria. Mar Drugs 8:2976–2987
18. **Schulz** S, Biwer P, Harig T, **Koteska** D, Schlawis C (2020a) Chemical Ecology of Bacterial Volatiles. In: Liu H-W, Begley TP (eds) Comprehensive natural products III. Elsevier, Amsterdam, pp 161–178
19. **Schulz** S, Schlawis C, **Koteska** D, Harig T, Biwer P (2020b) Structural Diversity of Bacterial Volatiles. In: Ryu C-M, Weisskopf L, Piechulla B (eds) Bacterial Volatile Compounds as Mediators of Airborne Interactions. Springer Singapore, Singapore, pp 93–121
20. *Trautwein K,* Feenders C, Hulsch R, Ruppersberg HS, Strijkstra A, Kant M, Vagts J, *Wünsch D, Michalke B*, **Maczka** M, **Schulz** S, *Hillebrand H, Blasius B, Rabus R* (2017) Non-Redfield, nutrient synergy, and flexible internal elemental stoichiometry in a marine bacterium. FEMS Microbiol Ecol 93:fix059.
21. *Voget S*, **Bruns** H, *Wagner-Döbler I*, **Schulz** S, *Daniel* R (2015) Draft Genome Sequence of *Roseovarius tolerans* EL-164, a Producer of *N*-Acylated Alanine Methyl Esters and *N*-Acylhomoserine Lactones. Genome Announc 3:15.
22. *Wang H,* **Ziesche** L, *Frank O, Michael V, Martin M, Petersen J,* **Schulz** S, *Wagner-Döbler I, Tomasch J* (2014) The CtrA phosphorelay integrates differentiation and communication in the marine alphaproteobacterium *Dinoroseobacter shibae*. BMC Genomics 15:130.
23. Weisskopf L, **Schulz** S, Garbeva P (2021) Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. Nat Rev Microbiol 19:391–404.
24. *Wolter LA, Wietz M,* **Ziesche** L, *Breider S, Leinberger J, Poehlein A, Daniel R*, **Schulz** S, *Brinkhoff T* (2021) Pseudooceanicola algae sp. nov., isolated from the marine macroalga Fucus spiralis, shows genomic and physiological adaptations for an algae-associated lifestyle. Syst Appl Microbiol 44:126166.
25. *Wünsch D, Strijkstra A, Wöhlbrand L, Freese HM, Scheve S, Hinrichs C, Trautwein K*, **Maczka** M, *Petersen J,* **Schulz** S, *Overmann J, Rabus R* (2020) Global Response of *Phaeobacter inhibens* DSM 17395 to Deletion of Its 262-kb Chromid Encoding Antibiotic Synthesis. Microb Physiol. 30: 309-324.
26. **Ziesche** L, **Bruns** H, *Dogs M, Wolter L*, Mann F*, Wagner-Döbler I, Brinkhoff T*, **Schulz** S (2015) Homoserine Lactones, Methyl Oligohydroxybutyrates, and Other Extracellular Metabolites of Macroalgae-Associated Bacteria of the *Roseobacter* Clade: Identification and Functions. ChemBioChem 16:2094–2107.
27. **Ziesche** L, *Wolter L, Wang H, Brinkhoff T, Pohlner M, Engelen B, Wagner-Döbler I*, **Schulz** S (2019) An Unprecedented Medium-Chain Diunsaturated *N*-acylhomoserine Lactone from Marine *Roseobacter* Group Bacteria. Mar Drugs 17:20.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	Sequential no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Stefan Schulz, Prof. Dr., W3	Organic Chemistry	IOC	5		TUBS
	2	Jonas Holste, PhD	Organic Chemistry	IOC	10		TUBS

Non-research staff	3	Jasmin Müller		IOC	5		TUBS
	4	Serdar Dilek		IOC	5		TUBS
Staff funded with approved grant money							
	1	Diana Koteska	Organic Chemistry	IOC	20	E13 50%	

Job descriptions of staff (supported through existing funds):

1. Schulz
   He was principal investigator of the project and responsible for design and coordination of the experiments. He supervised the PhD student and two PhD students funded by TUBS sources and working part time in the project (Jonas Holste, Peter Biwer)
2. Holste
   PhD student that worked on *Paracoccus* chemical analysis and on toxins of roseobacter associated algae. Other parts of his PhD project were not related to the CRC.
3. Müller
   Grant money managment, secretary.
4. Dilek
   Technician assisiting in chemical synthesis as well as GC/MS analyses.

Job descriptions of staff (funded with approved grant money):

1. Koteska
   She carried out all algal analysis as well as analyses of NAMEs. Furthermore, she synthesized labelled NAMEs for experiments with B4.

7. General information about Project C3

7.1 Metabolic characterization of *Prorocentrum cordatum/minimum*
7.1.2 Project leaders

Hiller, Karsten, Prof. Dr., 04.10.1975, German
Institut f. Bioinformatik und Biochemie, Technische Universität Braunschweig, 38106 Braunschweig
Tel. 0531 / 39155200
Email karsten.hiller@tu-braunschweig.de

Schmidt-Hohagen, Kerstin, Dr., 26.01.1979, German
Institut f. Bioinformatik und Biochemie, Technische Universität Braunschweig, 38106 Braunschweig

7.2 Project history
This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report
In the first two funding-phases of the CRC, metabolism of *Dinoroseobacter shibae* and *Phaeobacter inhibens*, serving as model organisms of the Roseobacter clade, was characterized under a variety of different environmental conditions (Drüppel *et al.*, 2014, Laass *et al.*, 2014, Kleist *et al.*, 2016, Bill *et al.*, 2017). Extensive studies were performed and the obtained data were used to generate metabolic models to simulate the effects of different environmental conditions (Rex *et al.*, 2013). In the third phase, interaction of Roseobacter bacteria with microalgae was in the focus.

7.2.1.1 Changes in the metabolome and proteome of *P. minimum* within a day/night-cycle
To gain an in-depth understanding of the symbiosis between the Roseobacter group of bacteria and microalgae, it is essential to thoroughly examine the morphology and physiology of both entities. Previous research has identified a symbiotic relationship between *P. minimum* and *D. shibae* (Wang *et al.*, 2015). As a result, our study prioritizes the metabolic characterization of the microalgae *P. minimum* under a range of environmental conditions. For this purpose, we investigated an axenic strain of *P. minimum* (CCMP 1329) under varying environmental parameters such as light regimes, salinities, and temperatures. We developed a cultivation device and a protocol for metabolite extraction, followed by Gas Chromatography-Mass Spectrometry (GC-MS) measurement, to facilitate this process. To enhance the reliability of our results, we needed an effective cell counting method. This presents a challenge for automatic cell counters, especially when dealing with samples with high salt concentrations or mobile cells. To address this, we utilized a lenseless microscope with cell tracking ability, developed by Scholz *et al.* 2019, which has demonstrated success with *P. minimum*.
Our exploration of the diurnal cycle in *P. minimum* was conducted in collaboration with Jana Kalvelage and Dr. Lars Wöhlbrand at the laboratory of Prof. Dr. Ralf Rabus (ICBM Oldenburg). The proteome analysis carried out by our CRC collaborators revealed that pigment-binding proteins made up the majority of detected peptides. Notably, lower levels during the light period compared to the dark period may be indicative of reduced energy transfer during periods of light surplus to evade photodamage. This was particularly observed in proteins of photosystem II in *P. minimum* (Figure 1a), appearing to curb chloroplastic ATP synthesis, which could potentially be balanced by an escalation in the activity of the mitochondrial electron transport chain (Figure 1b). Utilizing bioinformatics tools, we visualized the central carbon metabolism and discovered increased peptide counts and metabolite levels across all central metabolic pathways, including carbon fixation, the Calvin cycle, and glycolysis (Figure 1c). The metabolome and proteome data indicate the TCA-cycle may exhibit increased activity during the later light period and the early dark period.

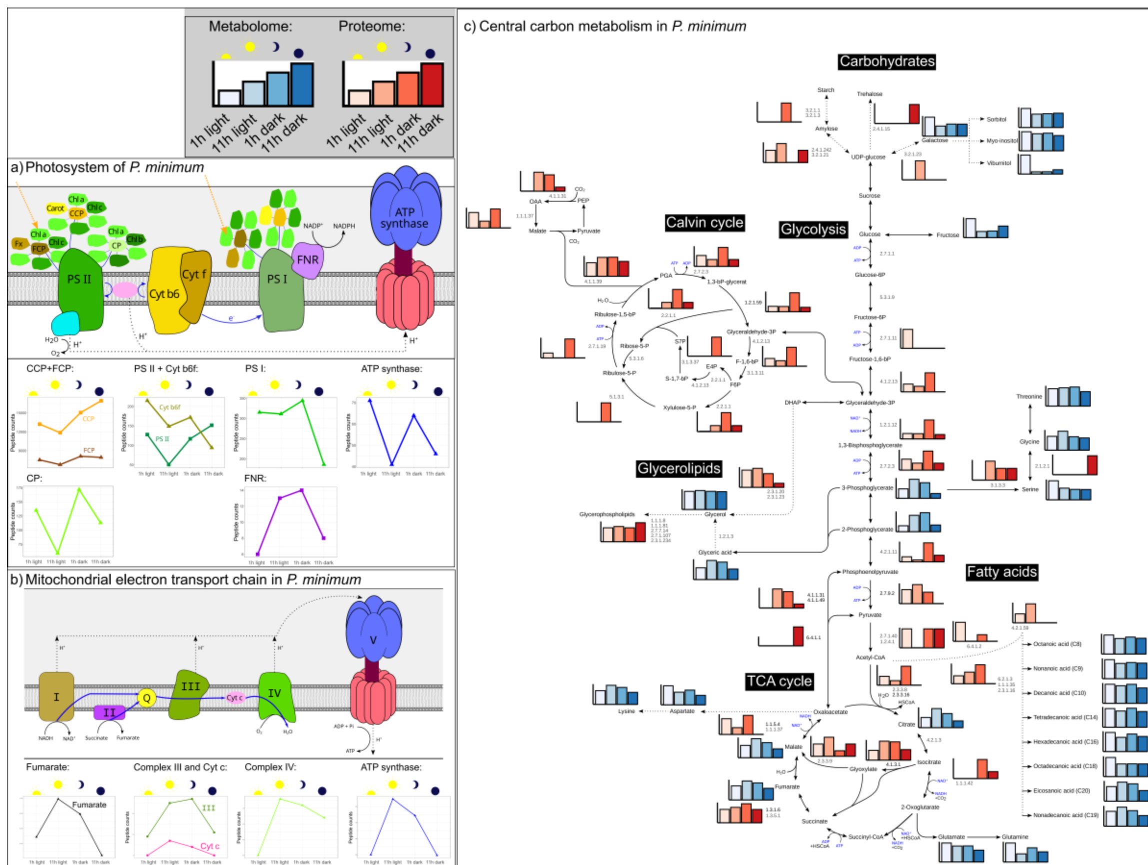


Figure 5: Figure 1Metabolome and proteome results of the investigation of the (a) photosystem, (b) mitochondrial electron transport chain and (c) central carbon metabolism in *P. minimum*.

7.2.1.2 Growth, morphology and metabolism of *P. minimum* at different salinities

Salinity is a critical environmental parameter affected by climate change, resulting in changes in two directions. Increased evaporation elevates salinities, while ice-melting leads to their reduction. Fluctuating salinities influence osmotic pressure, impacting intracellular salt and water concentrations in microorganisms. Salt stress is known to inhibit enzyme activity and trigger an escalation in Reactive Oxygen Species (ROS) formation in marine microorganisms. In our approach to understand the adaptive mechanisms of P. minimum to salt stress, we explored the growth and morphology of cultures adapted to salinities both below and above standard concentrations. To eliminate the impact of short-term effects, cultures were conditioned to various salt concentrations for a minimum of six weeks. We employed staining assays to detect carbohydrates and fatty acids, and conducted GC-MS measurements to study the metabolism of P. minimum under standard, hypo-, and hyper-saline conditions. Notably, hyper-saline conditions posed challenges in the GC-MS measurements of medium samples. In response to this, we developed a robust method, duly adjusted to measure medium samples with extremely low pH-values. This novel approach was used to investigate the metabolome of the first stable co-culture of a member of the Micrarchaeota together with its thermoplasmatales host (Krause et al., 2021). By combining genomic analyses with comparative metabolomics, lipidomics, and Extracellular Polymeric Substances (EPS) composition determination, we discovered that the growth of Micrarchaeum harzensis is dependent on its interaction with its host Scheffleriplasma hospitalis, who appeared largely unaffected by the symbiont.
The staining assays for carbohydrates and fatty acids provided initial insights into the metabolism of P. minimum under varying salinities (Figure 2a). Both hypo- and hyper-saline conditions resulted in elevated carbohydrate levels (Figure 2b). P. minimum's fatty acid content

was significantly increased in hypo saline conditions at 15 g/L and 5 g/L compared to standard conditions at 34 g/L (Figure 2c). A deeper understanding was gleaned through metabolome analysis via GC-MS. Most carbohydrates, including glucose, galactose, and mannose, critical for cell wall synthesis, were significantly increased in 5 g/L and 45 g/L compared to 34 g/L (Figure 2d). Other carbohydrates, previously identified as participants in salt stress responses like sorbitol, glycerol, or myo-inositol, were found in abundance under hyper-saline conditions of 45 g/L.
Our data implies a metabolic shift in P. minimum from enhanced carbohydrate and fatty acid synthesis under hypo-saline conditions to increased carbohydrate synthesis, including compatible solutes under hyper-saline conditions. The study results suggest a mechanism of adaptation to hypo- and hyper-saline conditions, relying on carbohydrate and fatty acid levels, potentially incorporating cell wall adaptation via changes in fatty acid content and synthesis of compatible solutes. The marked increase in ATP-levels in P. minimum cultures grown under hypo- and hyper-saline conditions points to a heightened energy demand during salinity stress (Figure 2e). This could be related to increased carbohydrates, fatty acids, and other vital metabolites.

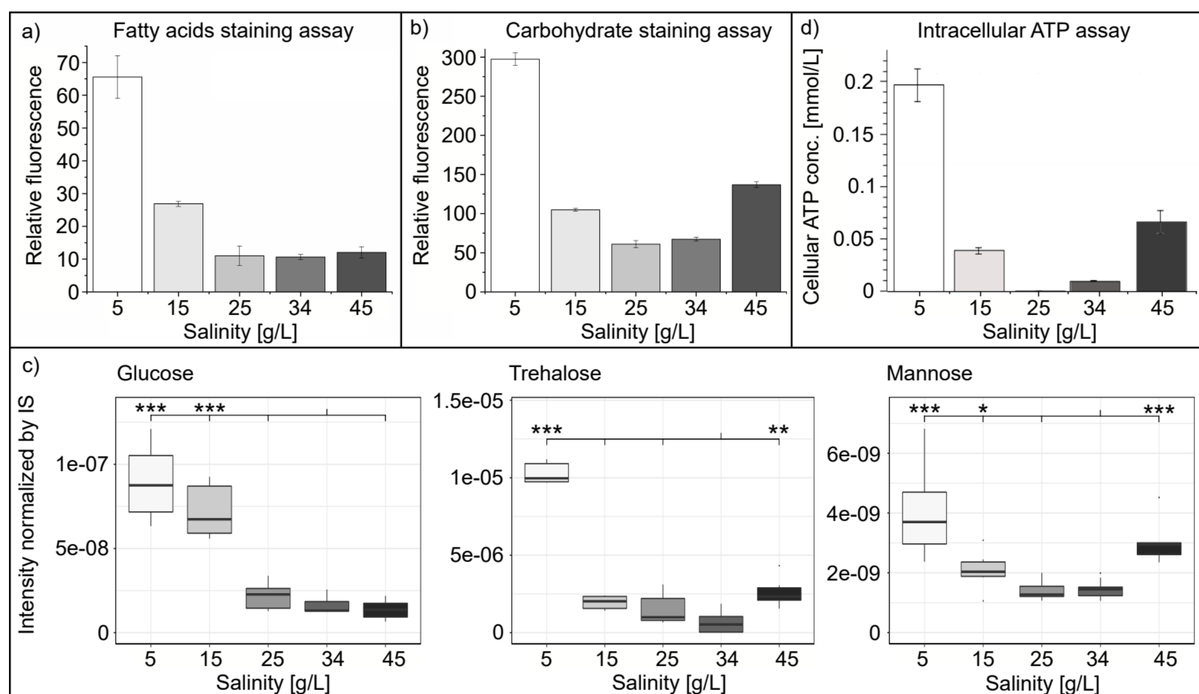


Figure Figure 6: Investigation of (a and b) fatty acid and carbohydrate content by staining assays, (b) carbohydrates by GC-MS and (c) intracellular ATP concentration.

7.2.1.3 Genome-scale atom mapping model of *Dinoroseobacter shibae*

To gain a better understanding of the metabolic interaction of *Dinoroseobacter shibae with microalgae,* we aimed to combine genome-scale metabolic modeling with experimental data obtained from stable-isotope-assisted metabolomics. To that end, we first converted the genome-scale metabolic model of *Dinoroseobacter shibae,* obtained from the previous funding period (Rex *et al.*, 2013) to an atom-mapping model. An atom mapping describes the one-to-one correspondence between substrate and product atoms in a metabolic reaction. Thus, one can follow every atom throughout the metabolic network within an atom mapping model. Because the creation of such a model is a time-consuming process and requires detailed knowledge about the reaction mechanisms, we decided to develop MetAMDB (Metabolic Atom Mapping Database), a freely accessible, web-resource for metabolic atom mappings (https://metamdb.tu-bs.de/) (Starke *et al.*, 2022).

MetAMDB provides atom mappings for around 43,000 reactions present in BRENDA, KEGG, and MetaCyc (Figure 3A) and supports the automatic generation of atom mapping models

based on a given metabolic model (Figure 3B). As such, MetAMDB will greatly facilitate the use of bigger (up to genome-scale) metabolic atom mapping models, as they can now be generated easily, even by non-experts. We then used MetAMDB to create the genome-scale atom mapping model of *Dinoroseobacter shibae,* consisting of around 1500 reactions.

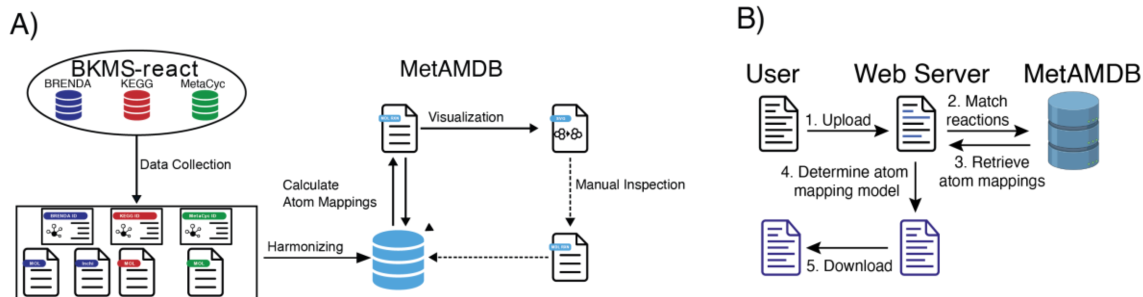


Figure Figure 7: (A) All data were collected from BKMS-react. To avoid duplicates, metabolites were matched internally using a synonym list from BRENDA, and reactions were linked to a single reaction in MetAMDB based on BKMS-react reaction groups. With all reaction data available, atom mappings for each reaction were calculated and visualized. (B) Workflow for automated generation of atom mapping models.

7.2.2 Published project results

1. **Bill** N, *Tomasch* J, Riemer A, Müller K, Kleist S, **Schmidt-Hohagen** K, *Wagner-Döbler* I, **Schomburg** D (2017) Fixation of CO2 using the ethylmalonyl-CoA pathway in the photoheterotrophic marine bacterium Dinoroseobacter shibae. Environ Microbiol doi:10.1111/1462-2920.13746.
2. *Drüppel* K, **Hensler** M, *Trautwein* K, *Koßmehl* S, *Wöhlbrand* L, **Schmidt-Hohagen** K, *Ulbrich* M, Bergen N, *Meier-Kolthoff* JP, *Göker* M, *Klenk* HP, **Schomburg** D, *Rabus* R (2014) Pathways and substrate-specific regulation of amino acid degradation in Phaeobacter inhibens DSM 17395 (archetype of the marine Roseobacter clade). Environ Microbiol 16:218-38.
3. Dudek, C. A., **Reuse**, C., Fuchs, R., Hendriks, J., Starck, V., & **Hiller**, K. (2020). MIAMI–—a tool for non-targeted detection of metabolic flux changes for mode of action identification. Bioinformatics, 36(12), 3925-3926.
4. **Kleist** S, **Ulbrich** M, **Bill** N, **Schmidt-Hohagen** K, Geffers R, **Schomburg** D (2016) Dealing with salinity extremes and nitrogen limitation – an unexpected strategy of the marine bacterium Dinoroseobacter shibae. Environ Microbiol, doi: 10.1111/1462-2920. 13266.
5. Krause, S., Gfrerer, S., Kügelgen, A., **Reuse**, C., Dombrowski, N., Villanueva, L., *Bunk*, B., Spröer, C., Neu, T.R., Kuhlicke, U., **Schmidt-Hohagen**, K., **Hiller**, K., Bharat, Tanmay A. M., Rachel, R., Spang, A. & Gescher, J. (2022). The importance of biofilm formation for cultivation of a Micrarchaeon and its interactions with its *Thermoplasmatales* host. Nature Communications, 13, 1735.
6. *Laass* S, *Kleist* S, **Bill** N, *Drüppel* K, *Kossmehl* S, *Wöhlbrand* L, *Rabus* R, Klein J, Rohde M, *Bartsch* A, *Wittmann* C, **Schmidt-Hohagen** K, *Tielen* P, *Jahn* D, **Schomburg** D (2014) Gene regulatory and metabolic adaptation processes of Dinoroseobacter shibae DFL12T during oxygen depletion. J Biol Chem 289:13219-31.
7. **Rex** R, **Bill** N, **Schmidt-Hohagen** K, **Schomburg** D (2013) Swimming in light. A large-scale computational analysis of the metabolism of Dinoroseobacter shibae. PloS Comput Biol 9(10):e1003224
8. Scholz, G., Mariana, S., Dharmawan, A. B., Syamsu, I., Hörmann, P., **Reuse**, C., & Waag, A. (2019). Continuous live-cell culture imaging and single-cell tracking by computational lensfree LED microscopy. Sensors, 19(5), 1234.

9. **Starke** C, Wegner A (2022). MetAMDB: Metabolic Atom Mapping Database. Metabolites, 12(2):122
10. *Trautwein*, K., Will, S. E., *Hulsch*, R., *Maschmann*, U., *Wiegmann*, K., **Hensler**, M., et al. (2016). Native plasmids restrict growth of *Phaeobacter inhibens* DSM 17395: Energetic costs of plasmids assessed by quantitative physiological analyses. Environmental microbiology 18, 4817–4829.
11. *Wang*, H., *Tomasch*, J., *Michael*, V., Bhuju, S., **Jarek**, M., *Petersen*, J., *Wagner-Döbler*, I. (2015). Identification of genetic modules mediating the Jekyll and Hyde interaction of Dinoroseobacter shibae with the dinoflagellate Prorocentrum minimum. Frontiers in Microbiology, 6: 1262
12. **Will**, S. E., Neumann-Schaal, M., Heydorn, R. L., *Bartling*, P., *Petersen*, J., and **Schomburg**, D. (2017). The limits to growth – energetic burden of the endogenous antibiotic tropodithietic acid in *Phaeobacter inhibens* DSM 17395. PLoS One 12. doi: 10.1371/journal.pone.0177295
13. Zech H, **Hensler** M, *Koßmehl* S, *Drüppel* K, *Wöhlbrand* L, *Trautwein* K, Colby T, Schmidt J, Reinhardt R, **Schmidt-Hohagen** K, **Schomburg** D, *Rabus* R (2013) Dynamics of amino acid utilization in Phaeobacter gallaeciensis DSM 17395. Proteomics 13(18-19):2869-85.
14. Zech H, **Hensler** M, *Koßmehl* S, *Drüppel* K, *Wöhlbrand* L, *Trautwein* K, *Hulsch* R, *Maschmann* U, Colby T, Schmidt J, Reinhardt R, **Schmidt-Hohagen** K, **Schomburg** D, *Rabus* R (2013) Adaptation of Phaeobacter gallaeciensis DSM 17395 to growth with complex nutrients. Proteomics 13(18-19):2851-68.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.4 Project staff in the ending funding period

	Sequen-tial no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Karsten Hiller, Prof. Dr., W3	Biochemie/ Bioinformatik	Inst. f. Biochemie, Biotechnologie, Bioinformat	2		TU BS
	2	Kerstin Schmidt-Hohagen, Dr.	Biochemie/ Bioinformatik	Inst. f. Biochemie, Biotechnologie, Bioinformat	6		TU BS
	3	Andre Wegner, Dr.	Biochemie/ Bioinformatik	Inst. f. Biochemie, Biotechnologie, Bioinformat	5		TU BS
Staff funded with approved grant money							
Research staff	1	Carsten Reuse, Ms. Sc.	Biochemie/ Bioinformatik	Inst. f. Biochemie, Biotechnologie, Bioinformat	26	E13 65%	

	2	Collin Starke, Ms. Sc.	Biochemie/ Bioinformatik	Inst. f. Biochemie, Biotechnologie, Bioinformat	20	E13 50%		

Job descriptions of staff (supported through existing funds):

1. Hiller
   He was the head of sub-project C3 and supervised the doctoral students and postdocs.
2. Schmidt-Hohagen
   She was responsible for design and coordination of the experiments of *P. minimum* and supervised the PhD student C. Reuse.
3. Wegner
   He was responsible for design and coordination of the experiments and computational work of *D. shibae*. He supervised the PhD student C. Starke.

Job descriptions of staff (funded with approved grant money):

1. Reuse
2. He carried out experiments with the algae P. minimum (e. g. day/night cycle, salinity, temperature).
3. Starke
4. He carried out experiments with the bacterium D. shibae (e. g. stable-isotope labeling), created a tool for the generation of genome-scale atom mapping models and compared modeled data with experiments.

7. General information about Project C5

7.1 Systems biology of the interaction between Roseobacters and algae

7.1.2 Project leaders

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7.2 Project history

This project received funding through TRR51 from January 2018 until December 2022

7.2.1 Report

7.2.1.1 *De novo* sequencing the genome of the “red tide” dinoflagellate *Prorocentrum cordatum*

Thus far, genome studies of dinoflagellates have targeted members of the family *Symbiodiniaceae* which form coral symbiosis and their free-living relatives in the genus Polarella (genome sizes ≤ 3Gbp). Past studies lack proteome and metabolome data, which are necessary to elucidate the molecular mechanisms that underpin gene-expression regulation.

Prorocentrum cordatum (formerly *Prorocentrum minimum*) is an invasive, potentially toxic species that is found globally and is regularly detected in the North Atlantic. The tolerance of *P. cordatum* (formerly *minimum*) to a wide range of salinities and temperatures facilitates its increased bloom frequency. We present the genome and multi-omics data from *P. cordatum*, targeting the algal heat stress response in axenic cultures. Our results provide an integrated view of how a HAB-forming species may respond to ocean warming induced by global climate change.

The genome of *P. cordatum* (Fig. 1) shows large, complex genome structure and multi-level transcriptional regulation. The high gene density, long introns, and extensive genetic duplication likely reflect genomic hallmarks of bloom-forming dinoflagellates, consistent with data from *A. gibbosum*. Habitats of *P. cordatum* are known to have rapidly fluctuating tempera-

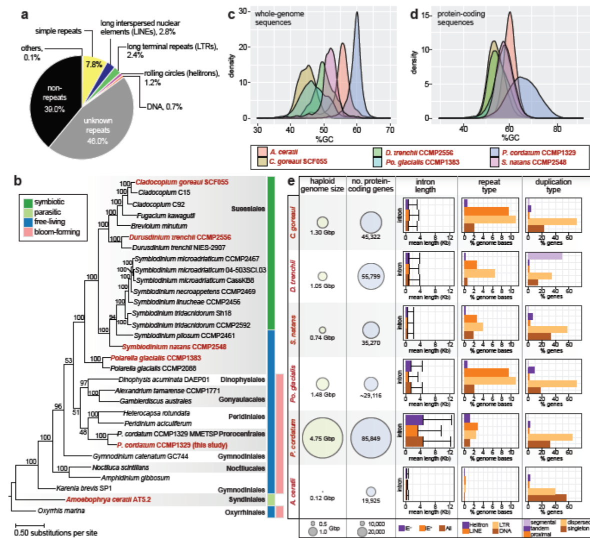

Fig. 1. Genome features of *P. cordatum*. (a) Distribution of repeat types in the genome. (b) Maximum likelihood tree inferred using 3,507 strictly orthologous, single-copy protein sets among 31 dinoflagellate taxa, with ultrafast bootstrap support (based on 2000 replicate samples). The ecological niche for each taxon is shown on the right of the tree. The five representative taxa and *P. cordatum* from this study are highlighted on the tree in red text. Distribution of G+C content for (c) whole-genome sequences and (d) protein-coding sequences relative to the other five representative genomes. (e) Genome and gene features of *P. cordatum* relative to the other five taxa, showing haploid genome size estimated based on sequence data, number of protein-coding genes, intron lengths, and separately for introns that contain introner elements (IE+), and those that lack these elements (IE–), known repeat types, and types of duplicated genes.

tures. The elevated G+C content of the genome may be favored by selection to ensure high fidelity of transcription. The long introns and presence of introner elements (non-autonomous DNA transposons) point to active transposition, contributing to the extensive rearrangement and duplication of genes, and to the large genome size with many functionally redundant but slightly different transcript isoforms that provide rich adaptive resources in frequently changing environments.

We present the first evidence of a complementary mechanism of post-transcriptional regulation, involving RNA editing and exon usage. This mechanism likely maximizes the functional diversity of gene products in dinoflagellates. In single-exon genes that are common in dinoflagellates, a third system we describe is through the adjustment of the number of coding units. All these mechanisms are set against the backdrop of polycistronic transcription followed by trans-splicing.

7.2.1.2 Multi-omics reveals the molecular response to heat stress in *P. cordatum*

To investigate the heat-stress response in *P. cordatum* CCMP 1329, axenic cultures were grown in defined media at the optimal temperature (20°C) before they were exposed to either 26°C or 30°C. We observed similar growth rates under all three conditions, but relative to the final cell density observed at 20°C, algal biomass was reduced to 62% and 41% at 26°C and 30°C, respectively. Transcriptome, proteome, and metabolome data were generated from cells harvested independently at exponential and stationary growth phases in the three temperature conditions.

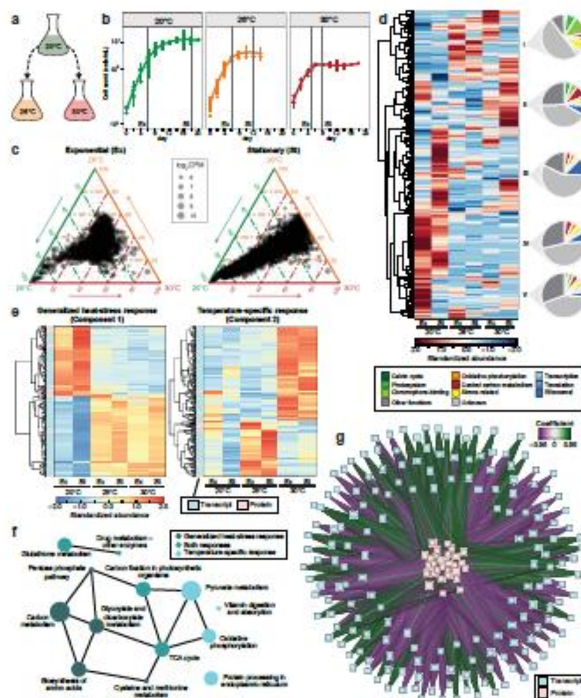

Fig. 2. Integrated analysis of the transcriptome and proteome response of *P. cordatum* to heat stress. (a) Experimental design. (b) Growth of P. cordatum at 20°C, 26°C and 30°C. Collection of cells for multi-omics analysis is indicated by dashed vertical lines (Ex: exponential, St: stationary phase). (c) Ternary plots of highly expressed gene models with mean log2(count per million) >5 in response to temperature and growth phase (8,593 transcripts in each plot). (d) Clustering of 2,098 differentially abundant proteins in response to temperature and growth phase. Abundances of proteins were calculated from standardized peptide counts. (e) Heatmap of transcripts and proteins showing significant correlations for generalized heat stress response (component 1) and temperature-specific response (component 2). (f) Over-represented KEGG pathways in the networks of generalized and temperature specific heat-stress response. (g) DIABLO network of generalized heat stress response (component 1) revealing positive and negative correlations (coefficient ≥0.7) between transcripts and proteins.

We integrated the transcriptome and proteome data using DIABLO to reveal shared multi-omics signatures of the Ex and St phases. This analysis revealed two types of heat stress response: a generalized response (component 1) with abundance changes common to both elevated temperatures (26°C and 30°C), and a temperature-specific response (component 2) with abundance changes specific to 26°C or to 30°C (Fig. 2e). KEGG pathways for carbon metabolism such as the pentose phosphate pathway, glyoxylate and dicarboxylate metabolism, the biosynthesis of amino acids, and the metabolism of cysteine and methionine were enriched in the generalized response (component 1; Fig. 2f). In contrast, oxidative phosphorylation, protein processing in endoplasmic reticulum, vitamin digestion and absorption, and pyruvate metabolism pathways were enriched in the temperature-specific response (component 2). Our results also reveal both positive and negative correlations of expression between transcripts and proteins in component 1 (Fig. 3g) and component 2. For instance, gene expression of glutamine synthetase was positively correlated to the expression

of chlorophyll a-b binding protein and the light-harvesting complex I LH38 proteins, whereas it was negatively correlated to protein expression of pyruvate dehydrogenase, and the sulfate and formate transporters.

7.2.1.3: Heat stress response of central modules of energy and carbon metabolism in *P. cordatum*

Given the lower biomass observed at elevated temperatures, we studied the recovery of biomolecules specific to three metabolic modules that drive growth and primary production in *P. cordatum*: photosynthesis, central metabolism, and oxidative phosphorylation. A global visualization of the relevant expressed transcripts (753) and proteins (278) revealed differential expression at elevated temperatures, with a marked increase in photosynthesis proteins but a decreased abundance of proteins related to central metabolism and oxidative phosphorylation (Fig. 3A). This result is supported by reduction in the accumulation of 38 metabolites associated with central metabolism at elevated temperatures.
The detected components of the photosynthetic electron transport chain (PETC) showed varying abundance profiles (Fig. 3B). Whereas chlorophyll-binding proteins (CPs), light harvesting complex (LHC), stress related proteins and photosystem I (PS I) proteins increased at elevated temperature, subunits of the chloroplast ATP synthase did not change. Notably, PS II, and the associated oxygen evolving complex (OEC) and enzyme components of the response to reactive oxygen species, appeared unchanged, but complex IV and mitochondrial ATP synthase of oxidative phosphorylation showed reduced abundance at higher temperature (Fig. 3D). Taken together, during heat stress *P. cordatum* faces energy deprivation arising from a less efficient PETC, which should directly impact protein synthesis and central metabolism. Accordingly, amino acid synthesis was reduced, demonstrated by decreased levels of phosphoserine phosphatase (PSPH) and glutamine synthetase (GLS), amino acids (*e.g.* serine and glutamate), and TCA-cycle intermediates (*e.g.* succinate) (Fig. 3C). These results suggest lower levels of enzymes involved in concentrating CO_2 (phosphoenolpyruvate carboxylase [PEPC]) and in ATP-consuming reactions of the Calvin cycle, *e.g.* phosphoribulokinase (PRK) and phosphoglycerate kinase (PGK) (Fig. 3C). The generally unchanged profile of CO_2-fixing ribulose 1,5-bisphosphate carboxylase (Calvin cycle) may be misleading in this context, because activity of this enzyme is known to decrease at higher temperatures. Enzymes involved in the synthesis of amylose, *e.g.* starch synthase (SS) and granule-bound starch synthase (GBSS), remained stable (Fig. 3C). In contrast to the declining levels of TCA metabolites and amino acids, some carbohydrates and fatty acids increased at elevated temperature, suggesting the recycling metabolites and re-organization of cellular processes and structural elements (*e.g.* lipids) to protect cells from heat stress, or as a countermeasure against increased membrane fluidity. These results demonstrate the severe impact of heat stress on essential metabolic processes that attenuated *P. cordatum* growth (Dougan et al. 2023).

7.2.1.4 Enigmatic nucleus of *P. cordatum.*

P. cordatum has a genome atypical of eukaryotes, with a large size of ~4.15 Gbp, organized in plentiful, highly condensed chromosomes and packed in a dinoflagellate-specific nucleus (dinokaryon). Here, we apply microscopic and proteogenomic approaches to obtain new insights into this enigmatic nucleus of axenic *P. cordatum*. High-resolution FIB/SEM analysis of the flattened nucleus revealed highest density of nuclear pores in the vicinity of the nucle-olus, a total of 62 tightly packed chromosomes (~0.4–6.7 μm^3) and interaction of several chromosomes with the nucleolus and other nuclear structures (Fig. 4). A specific procedure for enriching intact nuclei was developed to enable proteomic analyses of soluble and membrane protein-enriched fractions. These were analyzed with geLC and shotgun approa-ches employing ion-trap and timsTOF mass spectrometers, respectively. This allowed identi-fication of 4,052 proteins (39% of unknown function), out of which 418 were predicted to serve specific nuclear functions; additional 531 proteins of unknown function could be allo-cated to the nucleus. Compaction of DNA despite very low histone abundance could be accomplished by highly abundant major basic nuclear proteins (HCc2-like). Several nuclear processes including DNA replication/repair and RNA processing/splicing can be fairly well explained on the

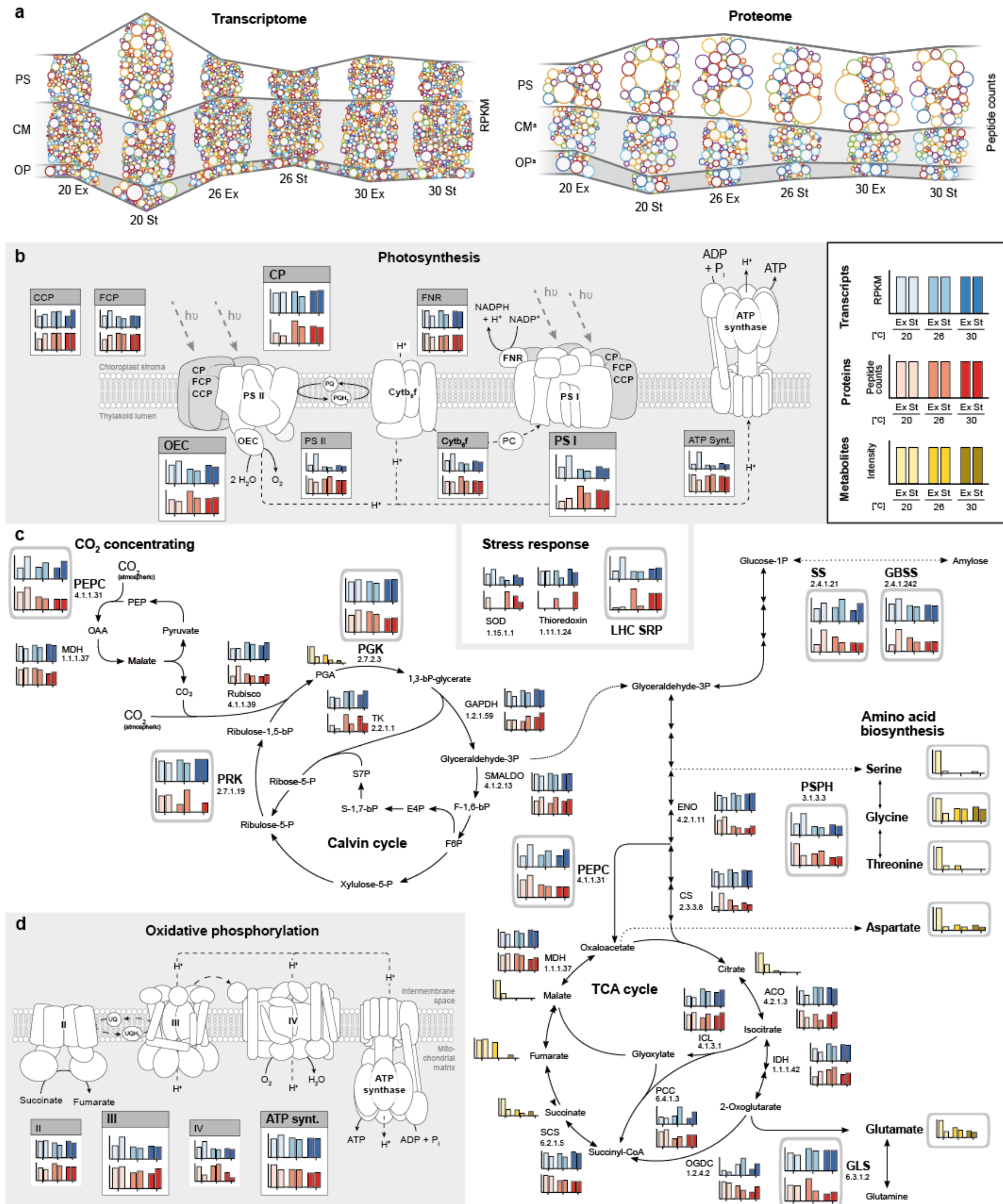

Fig. 3. Heat stress response of central modules of energy and carbon metabolism in *P. cordatum*. (A) Temperature-dependent dynamics of sub-transcriptomes (left) and sub-proteomes (right) associated with photosynthesis (PH), central metabolism (CM), and oxidative phosphorylation (OP). Colored circles represent individual transcripts and proteins, respectively, with their areas proportional to the determined abundances. Expression of transcripts, proteins, and metabolites is shown for functions specific to (B) light reaction of photosynthesis, showing CCP, carotenoid/chlorophyll-binding protein; CP, chlorophyll-binding protein; FCP, fucoxanthin/chlorophyll-binding protein; FNR, ferredoxin:NADP oxidoreductase; OEC, oxygen evolving complex; and PS, photosystem; (C) central metabolism including CO_2-concentrating Calvin cycle, central carbon metabolism, and selected biosynthesis of amino acids, showing PEPC, phosphoenolpyruvate carboxylase; GBSS, granule-bound starch synthase (NDP-glucose-starch glycosyltransferase); GLS, glutamine synthetase; PGK, phosphoglycerate kinase; PRK, phosphoribulokinase; PSPH, phosphoserine phosphatase; and SS, starch synthase; and (D) oxidative phosphorylation.

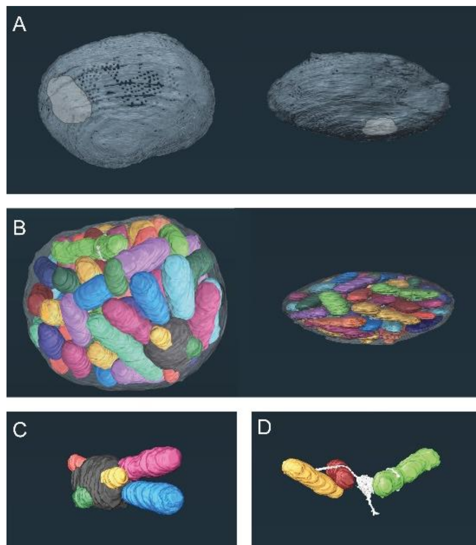

Fig. 4. Three-dimensional reconstruction of the nucleus of *Prorocentrum cordatum* based on FIB/SEM images. (**A**) Distribution of nuclear pores across the nuclear envelope. Left panel, patch with high number of pores proximal to the nucleolus; right panel, pore-poor region. (**B**) Tight packing of chromosomes in the nucleus. Left panel, top view; right panel, side view. Chromosomes are arbitrarily colored, the nucleolus is marked dark grey and the nuclear membrane displayed transparently. (**C**) Focus on nucleolus with interacting chromosomes. (**D**) Conspicuous structure (white, probably extension of endoplasmic reticulum) interacting with several chromosomes.

proteogenomic level. By contrast, transcription and composition of the nuclear pore complex remain largely elusive (Fig. 5). One may speculate that the large group of potential nuclear proteins with currently unknown functions may serve yet to be explored functions in nuclear processes differing from those of typical eukaryotic cells (Kalvelage et al. 2023).

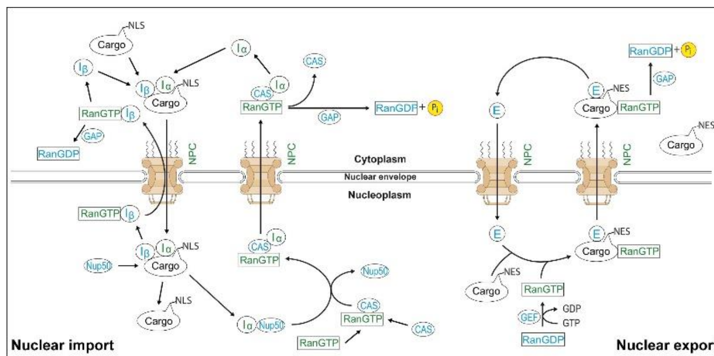

Fig. 5. Reconstruction of nuclear import and export in *P. cordatum*. Coloring: green, proteins identified; blue, proteins predicted only; grey, proteins not predicted. Abbreviations: CAS, CRISPR associated proteins; E, exportin; GAP, GTPase-activating protein; GEF, guanine exchange factor; I, importin; NES, nuclear export signal; NLS, nuclear localization signal; NPC, nuclear pore complex; NUP, nucleoporin (nuclear pore complex protein); RanGTP, ras-related nuclear protein; GTP, guanosine triphosphate.

7.2.1.5 The photosynthetic apparatus of *P. cordatum*

High-resolution FIB/SEM analysis of the chloroplast revealed an irregular, barrel-like structured single chloroplast that aligned with the flattened cell structure of *P. cordatum* and enclosed all other subcellular structures, e.g. a single reticular mitochondrium, the Golgi-appartus, multiple lipid and phosphate storage granules, as well as the aforementioned the nucleus.
The chloroplast-enriched fraction was enriched by differential centrifugation as basis to reconstruct the photosynthetic apparatus by proteogenomics. Due to the membrane embedment of its large protein complexes, we applied 2D BN PAGE to reveal the composition of native complexes (Fig. 6). The proteogenomic reconstruction was challenged by a two-fold:

First, only few predicted proteins were assigned to photosynthesis in the annotated genome. Second, distinct structure and arrangements of the *P. cordatum* genes may hamper protein identification. To meet these challenges, the 149 known photosynthesis genes of the well-studied model plant *Arabidopsis thaliana* were used for manual reannotation. Overall, the composition of the PSII of *P. cordatum* and *A. thaliana* are fairly similar based on sequence analysis. Notably, associated subunits identified in *P. cordatum* were previously assigned to the large pool of proteins of unknown function. In case of the PSI-NDH supercomplex of *P. cordatum* only low similarities to *A. thaliana* could be found, which is also mirrored by the identified proteins. ATP synthase is composed of proteins originally annotated as such, as well as of a considerable share of identified proteins previously also assigned to the group of "unknowns". Pigment-binding proteins constitute the largest group of identified proteins in this study, indicating a prominent role in functionality and adaptation of photosynthesis in *P. cordatum*. Furthermore, the proteomic data benefited the functional assignment to a large extent.

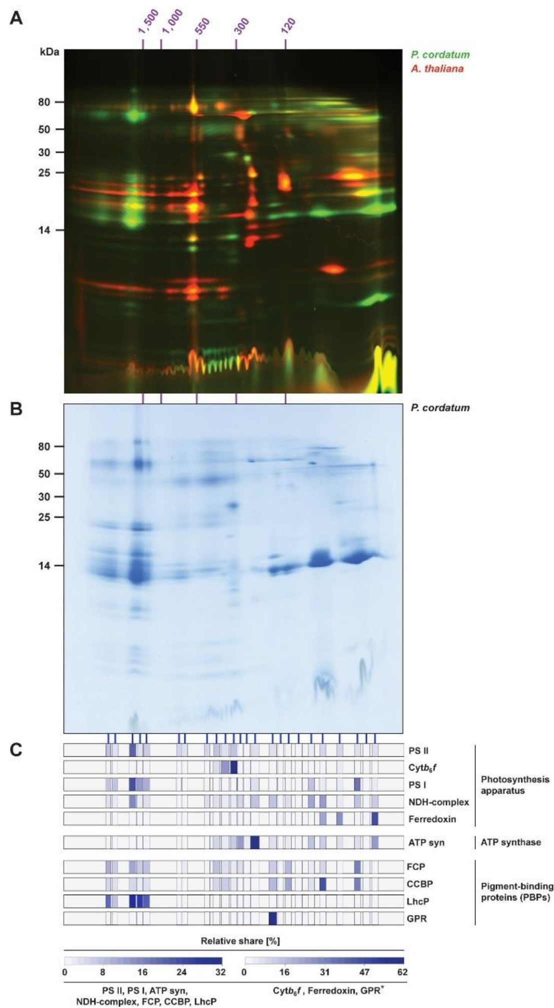

Fig. 6. Proteomic analysis of the photosynthetic apparatus of *P. cordatum* by 2D BN PAGE. (A) Co-separation of fluorescently labeled samples from *P. cordatum* (green) and *A. thaliana* (red). (B) Coomassie-stained gel used for protein identification. (C) Cross-gel identification of components from the photosynthetic apparatus, ATP synthase and pigment-binding proteins.

The experiments and data analyses on this theme are completed and a manuscript is currently being prepared.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals (members of C5 in bold, of other CRC projects in italic)

1. Kalvelage J, Wöhlbrand L, Schoon R-A, Zink F-M, Correll C, Senkler J, Eubel H, Hoppenrath M, Rhiel E, Braun H-P, Winklhofer M, Klingl A, Rabus R (2023) The enigmatic nucleus of the marine dinoflagellate *Prorocentrum cordatum*. mSphere e0003823 (https://doi.org/10.1128/msphere.00038-23).

Other publications

1. Dougan KE, *Deng Z-L*, Wöhlbrand K, *Reuse C*, *Bunk B*, Chen Y, *Hartlich J*, *Hiller K*, John U, Kalvelage J, Mansky J, *Neumann-Schaal M*, *Overmann J*, *Petersen*, *J*, Sanchez-Garcia S, *Schmidt-Hohagen K*, Shah S, *Spröer C*, Sztajer H, Wang H, Bhattacharya D, Rabus R, *Jahn D*, Chan CX, Wagner-Döbler I (2023) Multi-omics analysis reveals the molecular response to heat stress in a "red tide" dinoflagellate. BioRxiv, https://doi.org/10.1101/2022.07.25.501386 ..

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	No.	Name, academic degree, position	Field of research	Department of university or non-university institution	Commitment in hours/week	Category	Funded through:
Existing staff							
Research staff	1	Ralf Rabus, Prof. Dr., W2	Microbiology	ICBM	5		Univ OL
	2	Lars Wöhlbrand, Dr.	Microbiology	ICBM	15		Univ OL

	3	Wagner-Döbler, Irene, Prof. Dr.	Microbial Ecology	TU-BS	20		TU-BS
Non-research staff	4	Christina Hinrichs		ICBM	5		Univ OL
	5	Sabine Scheve		ICBM	10		Univ OL
	6	Martina Gehler		ICBM	5		
Staff funded with approved grant money							
Research staff	1	Jana Kalvelage., Ms. Sc.	Microbiology	ICBM		PhD student	
	2	Wang, Hui, Dr., Postdoc	Microbial Ecology	TU, HZI	10	E13	
	3	Sztajer, Helena Dr., scientist	Microbial Ecoloy	TU, HZI	10	E13 50%	
	4	Sanchez-Garcia, Selene, Phd	Microbial Ecology	TU, HZI	10	E13 65%	
	5	Mansky, Johannes, Phd	Microbial Ecology	TU, HZI	10	E13 65%	

Job descriptions of staff (supported through existing funds):

1. Rabus
   Planning and advice of experimental work; participation in evaluation of physiological and proteomic data; involvement in data exchange and interpretation with the collaborating groups; participation in conceiving and writing of manuscripts.
2. Wöhlbrand
   Mass spectrometry (MS) based protein identification (nanoLC-ESI-iontrap MS), as this requires advanced mass spectrometry; analysis and evaluation of MS-data; planning and advice of subcellular fractionation strategies; participation in writing manuscripts
3. Wagner-Döbler
   She was principal investigator of the sequencing of *P. cordatum* and the systems biology experiment. She established the international co-operation for assembly and analysis of the sequencing data and oversaw the writing of the manuscript.
4. Hinrichs
   She assisted in carrying out physiological growth tests and was instrumental for sample preparation for proteomics until her parental leave.
5. Scheve
   She assisted in carrying out physiological growth tests.
6. Gehler
   Parental leave substitution of Hinrichs; instrumental for sample preparation for proteomics.

Job descriptions of staff (funded with approved grant money):

1. Kalvelage
   She established the cultivation of *P. cordatum*, established the protocols for subcellular fractionation of *P. cordatum*, conducted light and electronmicroscopic examinations, performed the proteomic analyses and participated in writing manuscripts.
2. Wang
   She improved protocols and extracted DNA for genome sequencing of *P. cordatum,* determined DNA fragment size, and sequenced genomic DNA and RNA of *P. cordatum* with nanopore device. She supervised the experimental work of the PhD students.
3. Sztajer
   She improved laboratory protocols for extraction of DNA and RNA from *P. cordatum.*
4. Sanchez-Garcia
   She cultivated *P. cordatum* for systems biology experiments and determined growth rates.
5. Mansky
   He cultivated *P. cordatum* for the systems biology experiment under standardized conditions at three temperatures and obtained samples for transcriptome, proteome and metabolome analysis. He determined growth rates of dinoflagellate cultures by flow cytometry.

7. General information about Project C7

7.1 Modelling of physiological bioenergetics and global biogeography of the Roseobacter group

7.1.2 Project leaders

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7.2 Project history

This project received funding through TRR51 from January 2018 until December 2022

7.2.1 Report

7.2.1.1 Bioenergetic model of marine heterotrophic bacteria

To gain a mechanistic understanding about the functioning and habitat success of *Roseobacter* group bacteria we developed a bioenergetic model that captures the population dynamics, biomass, and growth of a heterotrophic bacterial consumer, together with the connected consumption of substrates in a batch reactor. For this we extended our previously developed Monod-type growth models by adding a total energy reserve, which is maintained by the acquisition of different substrates and then used for expenditures proportionally allocated for cell maintenance and the growth of structural biomass. In cooperation with project C1 (Rabus) we applied the model to experimental data obtained from growth experiments in defined cultures of *P. inhibens* DSM 17395. Thereby, we were able to demonstrate that the growth dynamics and regulation of a heterotrophic bacterium in a culture medium can be adequately described within the framework of dynamic energy budget theory. In particular, we were able to capture bacterial growth on a mixture of eleven organic substrates (sugars and

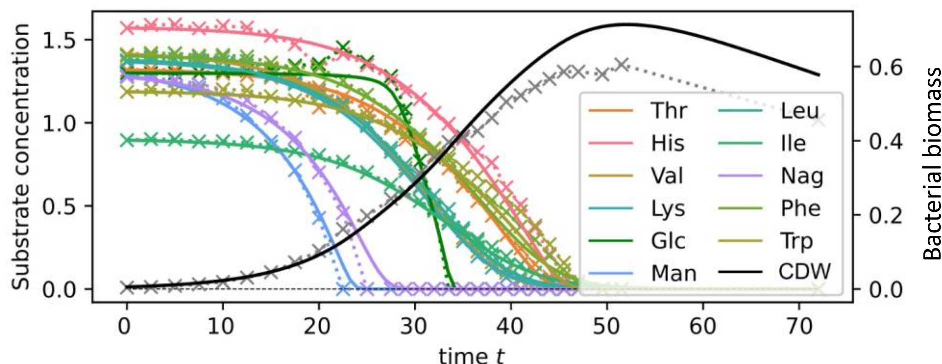

Fig. 1. Reproduction of experimental growth curves of a heterotrophic bacterium living on a mixture of 11 organic substrates in a batch reactor (Lücken et al., in prep). Bacterial biomass in black, depletion of substrates in colors. Model results shown as solid lines and experimental data as symbols.

amino acids organic (see Fig.1). The model accurately predicts the build-up of bacterial biomass and the decline of all substrates, including pronounced auxic shifts in the uptake of certain substrates. In our model this is explained by a dynamic metabolic regulation in form of a substrate inhibition process, whereby *P. inhibens* reduces the uptake rate for specific substrates dependent on the presence and concentration of other substrates in the medium. To investigate this more systematically, we developed a general protocol for inferring inhibitory relationships between substrates in growth experiments on complex substrate mixtures, by successively testing pairwise interdependencies between the supplied substrates. Using this approach revealed a dynamic dependence of the glucose uptake of *P. inhibens* on the availability of other sugars, such as mannitol and N-acetylglucosamin. This metabolic

versatility probably plays an important role in the ecological success of *P. inhibens* and allows for an optimal exploitation of varying conditions in the ocean, e.g., during plankton blooms. Our combined data analysis and modelling approach thus helped us to better understand the regulation of substrate uptake in complex environments containing a rich variety of different substitutable resources.

7.2.1.2 Modelling interactions between marine DOM and heterotrophic bacteria

One major research focus was the development of a model framework that captures the interactions between heterotrophic microorganisms and Dissolved Organic Matter (DOM). This investigation was performed together with project A8 (Dittmar, Niggemann). The mathematical model describes the carbon concentration of a mixture of DOM compounds and a community of heterotrophic microorganisms. Thereby, organic matter is externally supplied to the DOM pool, taken up by micro-heterotrophs, or abiotically degraded, the consumed carbon is split among the pathways of respiration, microbial growth and release of transformed compounds, and finally consumers can die by lysis:

$$\dot{B}_i = \eta \sum_k U_{ik} \, f(D_k) B_i - \mu B_i$$

$$\dot{D}_j = \underbrace{S_j}_{\text{supply}} - \underbrace{\sum_i U_{ij} \, f(D_j) B_i}_{\text{DOM uptake}} + \beta \sum_i R_{ij} \Big[\underbrace{\sum_k U_{ik} \, f(D_k) B_i}_{\text{release of transformed DOM}} + \underbrace{\mu B_i}_{\text{mortality}} \Big] - \underbrace{\delta D_j}_{\text{degradation}}$$

The DOM-microbe interaction model successfully reproduces experimental findings in incubation studies, as well as large-scale patterns of DOC concentration in the ocean. Based on a constant inflow of single substrates the model predicts a strong diversification of the supplied organic matter. After an initial bloom of consumer biomass, the system typically evolves to a state of highly diluted and diverse DOM in which micro-heterotrophs live at the edge of their fitness range. The resulting reduced degradation rates at low substrate concentrations and low abundance of microbial consumers yields the emergence of DOM recalcitrance without imposing intrinsic recalcitrant molecular structures (Dittmar et al. 2021). One unexpected finding was the model outcome when applied to deep sea environmental conditions of low external DOM supply. In this regime our numerical simulations revealed the emergence of large-amplitude oscillations. That is, the model exhibits a dynamic instability, even in stable environments and especially under conditions of energy scarcity, that give rise to self-sustained chaotic fluctuations in relative abundance and COM concentration (Fig. 2). In the fluctuating regime, microbial communities and organic matter organize into a hierarchy of layers, reminiscent to trophic layers in food-webs, that are characterized by drastic differences in biomass, matter flows, and oscillation periods. Fluctuations are most pronounced for rare taxa, they can span several decades, and consist of short high-abundance periods, interspersed by long periods of very low population numbers. We were able to identify specific interaction network motifs that promote instability and provide testable hypotheses for experimental approaches. Our results suggest a new paradigm of the dark ocean microbiome as a highly dynamic system, where even under conditions of low environmental variability microbial communities might exhibit a larger intrinsic variability than previously thought.

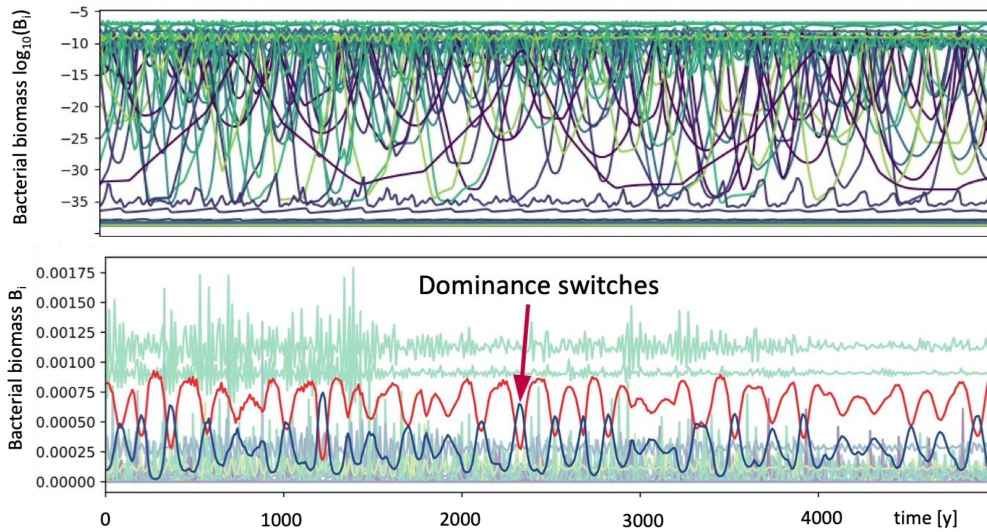

Fig.2. Self-sustained chaotic dynamics in the DOM-microbe interaction model that arise for small external DOM supply. Plotted are simulated bacterial biomass trajectories on a logarithmic (top) and linear (bottom) scale. Blue and red lines highlight antagonistic fluctuations between two bacterial consumers.

In a further investigation we studied how complex cross-feeding networks of heterotrophic marine microbes may develop from a simple initial community, given some elemental evolutionary mechanisms of resource-dependent speciation and extinctions. To investigate this, we implemented processes of species invasion and extinction into the DOM-microbe interaction model (Lücken et al. 2022). Using numerical experiments we observed rich co-evolutionary dynamics, where even in stable environments, the system is subject to persisting turnover in community structure, indicating an ongoing co-evolution. Our results imply that high microbial and molecular diversity is an emergent property of evolution in cross-feeding networks, which affects transformation and accumulation of substrates in natural systems, such as soils and the ocean, but also with potential relevance to biotechnological applications.

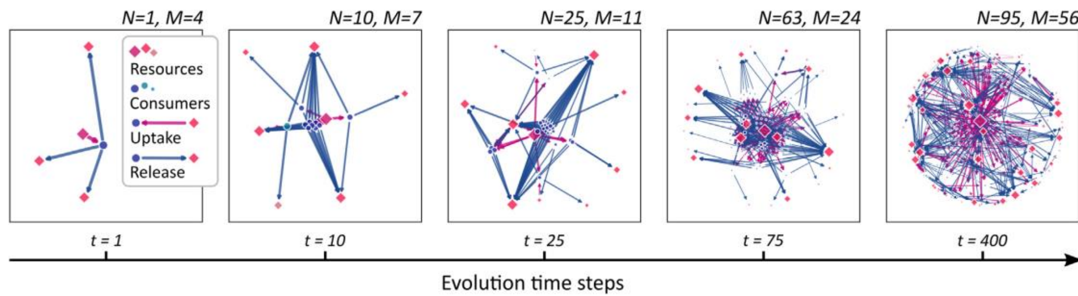

Fig. 3. A growing microbial cross-feeding network. Starting from a founding consumer (leftmost panel), new microbial consumers (blue dots) are added sequentially leading to an increasing community size N. As novel metabolic capacities evolve, the number M of different resources (red squares) increases. The symbol sizes are scaled proportional to the stationary flow passing through the corresponding network node. Uptake links are drawn as magenta arrows, release links as blue (Lücken et al. 2022).

7.2.1.3 Modelling host-phage dynamics and optimal lysogenic strategies

In this subproject, we investigated phage-host dynamics and phage life-cycles of both strictly lytic and temperate bacteriophages. For this we formulated and analyzed a phage-host model of marine heterotrophic bacteria. In particular, we studied the temporal dynamics of the bacteriophage-host system in presence of fluctuating environmental conditions modeled via changes in the explicit resource supply. Our main aim was to study the replication benefits through the choice of different lysogenic strategies under changing environmental conditions. For this purpose, we developed a competition approach for phages of different lysogenic strategies using the method of invasion analysis. Applying this approach, we investigated static and adaptive viral strategies, such as ‘Killing the Winner’ (KtW) and ‘Piggyback the Winner’

(PtW), with respect to their replication advantages in a varying environmental setting. Our analysis showed that both, a fluctuating environment and an adaptive strategy of phages, are required for successful lysogenic replication. Only fluctuations at constant non-adaptive lysogenic strategies are not sufficient, whereas successful lysogenic replication only occurs when the KtW strategy, and not the PtW or constant strategy, is included. This work can be used as a starting point for further investigations to understand the mechanisms and evolutionary benefits of lysogenic replication, especially under fluctuating environmental conditions.

7.2.1.4 Analysis of large-scale biogeography of the *Roseobacter* group and other oceanic microbes

A major focus was a comprehensive analysis of the samples and data which were collected during cruises in the Atlantic, Pacific and Southern Ocean with RV Polarstern and Sonne. In close collaboration with projects A1 and A8 bioinformatics and modelling pipelines (R, random forest models) were adopted and applied to assess the biogeography on levels of the taxonomy, KEGG orthologs and functional genes in a latitudinal transect across the Southern and Atlantic Ocean. The results show that the biogeography on the latter level was pronounced in greatest detail (Milke et al. 2022, Dlugosch et al. 2022). Variants of selected functional genes occurred in distinct regions reflecting their temperature ranges and preferences. This project was also involved in the analysis of the large data set of the cruises across the Pacific Ocean from subantarctic to subarctic regions (Giebel et al. 2021) providing the first comprehensive investigation of microbial features regarding the abundance, growth dynamics and community composition in this greatly understudied oceanic region. In mesocosm experiments, carried out at three locations in different oceanic regions during Sonne cruise SO248 and examining the effect of supply of vitamin B_{12} and its activated lower ligand α-ribazole on growth and community composition of pro- and eukaryotic communities, this project contributed to evaluating metatranscriptomic data. These analyses, carried out for two of the three experiments, showed that the entire prokaryotic community responded to enhanced B_{12} supply by downregulating the transcription of the B_{12} importer (Wienhausen et al. 2022). The greatest direct positive effect of B_{12} supply was upregulation of the transcription of photosynthesis-related genes of *Prochlorococcus*.

In another study we showed that the average gene length of various pelagic prokaryotic families occurring in the near surface Atlantic and Southern Ocean is a function of latitude and nitrate concentrations (Fig. 4, Dlugosch et al. 2021). This very interesting study is still ongoing because comparable gene length analyses from full genomes of the major oceanic prokaryotes such as *Prochlorococcus* and SAR11/*Pelagibacteraceae* are still ongoing.

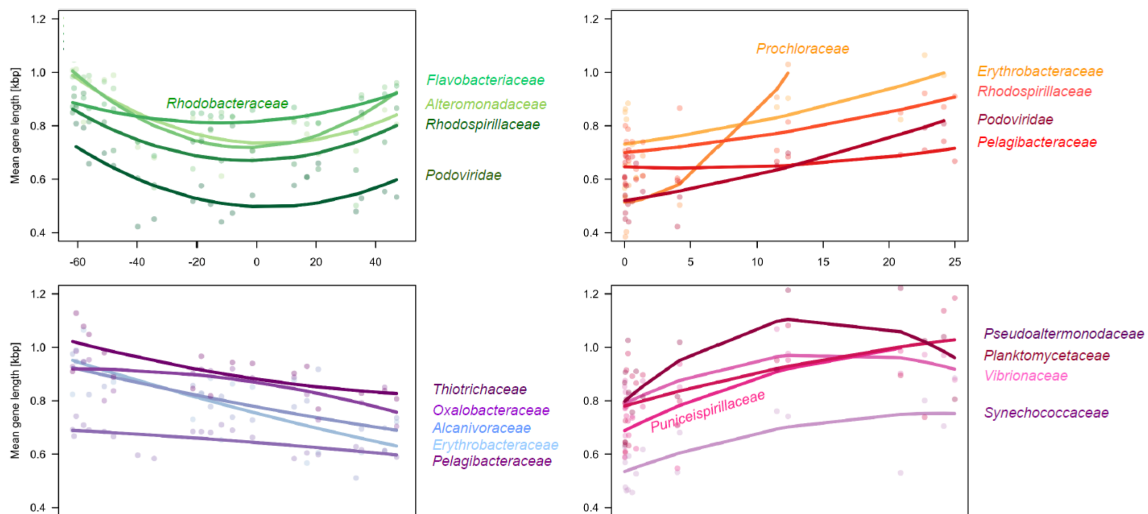

Fig. 4: The average gene length of selected prokaryotic families and Podoviridae of the near surface microbiome of the Southern and Atlantic Ocean between 62°S and 47°N as a function of latitude (left panel) and nitrate concentration (right panel). Dlugosch et al. unpublished data.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals
(members of C7 in bold, of other CRC projects in italic)

1. **Dlugosch** L, *Poehlein* A, *Wemheuer* B, Pfeiffer B, Badewien TH, *Daniel* R, *Simon* M (2022) Significance of gene variants for the functional biogeography of the near-surface Atlantic Ocean microbiome. Nature Comm 13: Article number 456.
2. *Giebel* HA, Arnosti C, Badewien TH, *Bakenhus* I, Balmonte JP, *Billerbeck* S, **Dlugosch** L, Henkel R, *Kuerzel* B, Meyerjürgens J, *Milke* F, Voss D, *Wienhausen* G, Wietz M, Winkler H, *Wolterink* M, *Simon* M (2021) Microbial growth and organic matter cycling in the Pacific Ocean along a latitudinal transect between subarctic and subantarctic waters. Front Mar Sciences 8: Article 764383.
3. *Milke* F, *Sanchez-Garcia* S, **Dlugosch** L, McNichol J, Fuhrman JA, *Simon* M, *Wagner-Döbler* I (2022) Composition and biogeography of planktonic pro- and eukaryotic communities in the Atlantic Ocean: primer choice matters. Front Microbiol. 13: 895875.
4. *Wienhausen* G, **Dlugosch** L, Jarling R, Wilkes H, *Giebel* HA *Simon* M (2022) Availability of vitamin B_{12} and its lower ligand intermediate alpha-ribazole impact prokaryotic and protist communities in oceanic systems. ISME J, 16: 2002–2014.
5. *Dittmar T*, Lennartz ST, Buck-Wiese H, Hansell DA, Santinelli C, Vanni C, **Blasius** B, Hehemann JH. (2021) Enigmatic persistence of dissolved organic matter in the ocean. Nature Reviews Earth & Environment. 2: 570-83.
6. **Lücken** L, Lennartz ST, Froehlich J, **Blasius** B (2022) Emergent diversity and persistent turnover in evolving microbial cross-feeding networks. Frontiers in Network Physiology. 2: 4.

Other publications and published results

7. **Dlugosch** L, *Poehlein* A, *Wemheuer* B, Pfeiffer B, *Giebel* HA, *Daniel* R, *Simon* M (2021) Nitrogen availability drives gene length of dominant prokaryotes and diversity of genes acquiring Nitrogen-species in oceanic systems. bioRxiv **h**ttps://doi.org/10.1101/2021.01.10.426031.
8. Kerimoglu O, Hintz NH, **Lücken** L, **Blasius** B, Böttcher L, Bunse C, *Dittmar T*, Heyerhoff B, Mori C, Striebel M, *Simon M*. (2022) Growth, organic matter release, aggregation and recycling during a diatom bloom: A model-based analysis of a mesocosm experiment. bioRxiv. 2022:2022-05.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2018. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	Sequential no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Bernd Blasius, Prof. Dr., W3	Mathematical Modelling	ICBM	5		UOL

	2	Christoph Feenders, Dr.	Mathematical Modelling	ICBM	10		UOL
	3	Alexey Ryabov, Dr.	Mathematical Modelling	ICBM	5		UOL
Staff funded with approved grant money							
Research staff	1	Leonhard Lücken, Dr., Postdoc	Mathematical Modelling	ICBM	40	E13 100%	
	2	Leon Dlugosch Dr. Postdoc	Bioinformatics	ICBM	40	E13 100%	

Job descriptions of staff (supported through existing funds):

1. Blasius
   He was principal investigator of the project and responsible for design and coordination of the models and data analysis. He supervised the postdoc and participated in the model investigations and in writing manuscripts.
2. Feenders
   He assisted in the development of the conceptual models of species interactions and the multivariate data evaluation (results of cultivation experiments).
3. Ryabov
   He assisted in the design and implementation of the bioenergetics model, as well as the modelling of bacteria-DOM interactions.

Job descriptions of staff (funded with approved grant money):

1. Lücken
   He performed the bioenergetic modelling of *Roseobacter* culture experiments and the investigation of the dynamic outcome of the DOM-microbe interaction network.

2 Dlugosch
He carried out bioinformatics analyses of metagenomics and ASV samples of the Atlantic, Pacific and Southern Ocean cruises.

7. General information about Project Z02

7.1 Assessment and exploitation of the metabolic potential and molecular characterization of uncultivated members of the *Roseobacter* clade

7.1.2 Project leaders

Daniel, Rolf, Prof. Dr., 20.09.1963, German
Genomische und Angewandte Mikrobiologie & Göttingen Genomics Laboratory (G_2L), Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstr. 8, 37077 Göttingen
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7.2 Project history

This project received funding through TRR51 from January 2010 until December 2022 (as A03 2010-2013 and as Z02 from 2014)

7.2.1 Report

7.2.1.1 Assessment of the *Roseobacter* group and microbial communities

We collected and analyzed marine samples from the North Sea, Atlantic Ocean (transect), Southern Ocean, Pacific Ocean (transect) and oceanic provinces throughout the previous funding phases. In this context, optimized methods for isolation of nucleic acids from marine community samples on filters and marker gene-based functional community analysis were published (Hollensteiner et al. 2023; Wemheuer et al. 2020). In the last funding phase, a comprehensive metagenomic analysis of communities and functions of the Atlantic Ocean (transect) microbiome was performed in cooperation with A1 Simon/Brinkhoff (Dlugosch et al 2022). It was shown that microbial communities in the near-surface Atlantic and Southern Ocean between 62°S and 47°N exhibited distinct taxonomic and fine-tuned functional adaptations to regional environmental conditions. Richness and diversity showed maxima around 40° latitude and intermediate temperatures, especially in functional genes (KEGG-orthologues, KOs) and gene profiles. Biotic factors contributed significantly to the detected functional patterns, whereas temperature and biogeographic province contributed mainly to taxonomic patterns.

In cooperation with A2-Engelen a comprehensive study on benthic microbial communities focusing on frequently neglected minor groups such as the *Rhodobacteraceae* was carried out (Pohlner et al. 2019) To unravel the distribution and diversity of benthic, metabolically active *Rhodobacteraceae*, we analyzed bacterial 16S rRNA transcripts derived from 154 individual sediment samples comprising seven oceanic regions and a broad variety of environmental conditions. Across all samples, a total of 0.7% of all 16S rRNA transcripts was annotated as *Rhodobacteraceae.* Among these, *Sulfitobacter*, *Paracoccus*, and *Phaeomarinomonas* were the most abundant cultured representatives, but the majority (78%) was affiliated to uncultured family members, indicating a large part of hidden diversity within the benthic *Rhodobacteraceae* with so far unknown functions. The general composition of active *Rhodobacteraceae* communities was specific for the geographical location and exhibited a decreasing richness with sediment depth. An adaption to anoxic conditions was indicated for roughly one-third of *Rhodobacteraceae* community members.

Regarding Pacific Ocean, to show differences between entire (DNA-based) and active (RNA-based) communities. We analysed marker data sets covering bacteria and archaea as well as the free-living and particle-associated communities using corresponding samples derived from 20 m, the deep chlorophyll maximum (DCM) and 300 m at the different stations. Community composition, distribution of taxa and extent of differences between both community types were recorded at family and genus level, and dependent on regional environmental conditions and lifestyle. In an accompanying study (cooperation with A2-Engelen) using Pacific deep-sea sediments, we showed that the composition of the entire and the active archaeal communities

was strongly linked to primary production. Latitude was significantly correlated with functional profiles of the entire community, whereas those of the active community were significantly correlated with nitrate and chlorophyll content (Wemheuer et al. 2019). In addition, we used the 80 metagenomes generated from the before-mentioned different samples of the Pacific cruise to generate metagenome-assembled genomes (MAGs). A total of 2,257 quality-filtered prokaryotic MAG bins were generated and taxonomically classified (Fig.1). MAGs from new and known species and strains of the *Roseobacter* group belonging e.g., to *Planktomarina* and *Pseudophaeobacter* were detected. Interestingly several MAGs representing new species of *Rhodobacteraceae* were detected, especially in the deep samples. We plan to come up with a manuscript later this year.

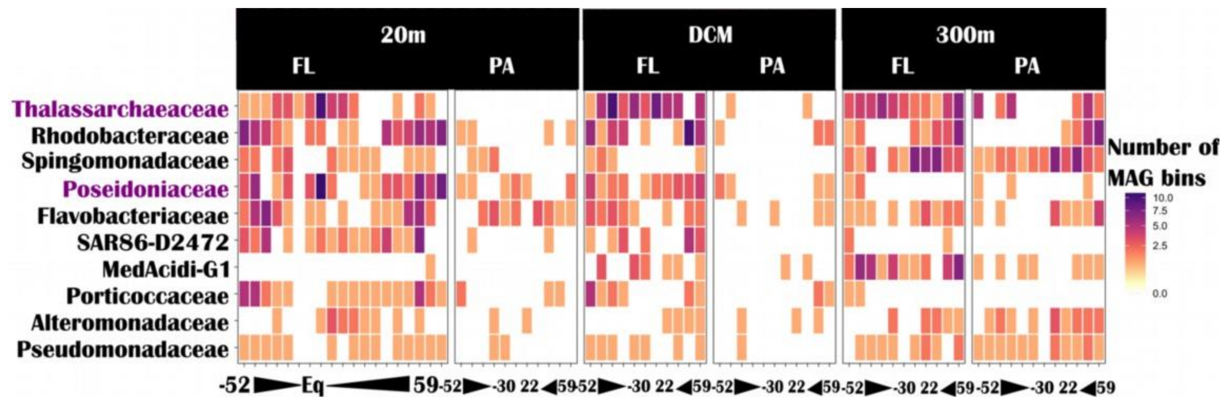


Fig. 1. The 10 families harbouring the highest numbers of MAGs. In purple archaeal families are shown. PA, particle-associated; FL, free-living.

7.2.1.2 Functional genomics of isolated strains

In a cooperation with B2-Brinkhoff, we analysed members of the genus *Paracoccus,*which are known for high abundance in many marine environments (Hollensteiner et al. 2023). The genus *Paracoccus* was phylogenomically analyzed (n = 160), allowing species level classification of 16 so far unclassified *Paracoccus* sp. strains and detection of misclassifications. We performed pan-genome analyss of *Paracoccus* type strains, isolated from a variety of ecological niches

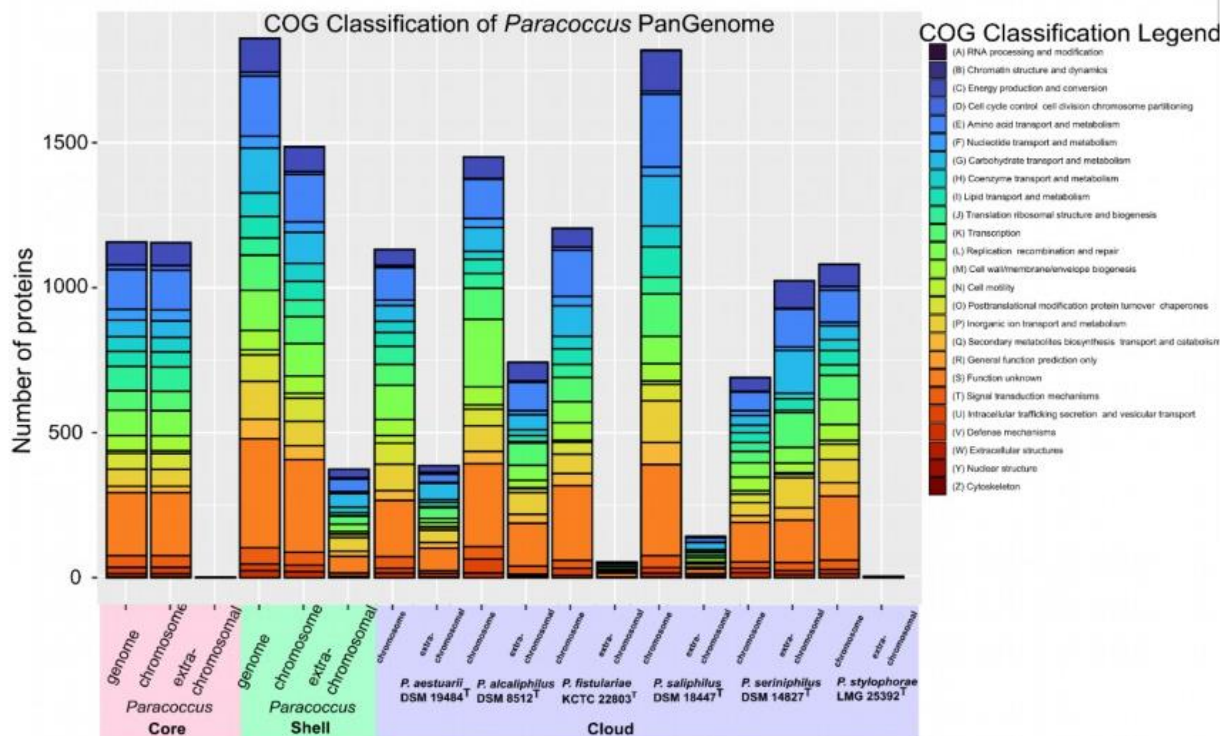


Fig. 2 COG classification of the pan-genome of *Paracoccus*.

(Fig. 2). Pan-genome analysis revealed an open pan-genome composed of 13,819 genes with a minimal chromosomal core (8.84 %) highlighting the genomic adaptation potential and the impact of extra-chromosomal elements. In general, in contrast to host-associated genomes, the ones from free-living members tend to have larger genomes and more extra-chromosomal elements and mobile genetic elements. Another functional genomics studies was on *Roseobacter ponti* (Hollensteiner et al. 2020). Phylogenomic placement and analysis of the genome indicated that it is the missing link between the *Sulfitobacter* and *Roseobacter* genus rather than a new species within the *Roseobacter* genus. Genome analysis indicated also high versatility and high niche adaptation potential of the strain. Other functional genomic studies performed in cooperation with CRC projects comprised *Planktotalea frisia, Maribacter dokdonensis (Bakenhus et al. 2018, Wolter et al. 2021a; both with A1-Simon-Brinkhoff), Pseudooceanicola algae (Wolter et al. 2021b; with B2-Brinkhoff and C2-Schulz) and others.*

7.2.1.3 Genome analysis of the diatom *Thalassiosira rotula*.

We successfully sequenced and assembled the genomes of *T. rotula* strains in cooperation with B2-Brinkhoff and A1-Simon/Brinkhoff. We used a hybrid sequencing strategy employing Oxford nanopore and Illumina technology. The genome size (approximately 450 Mbp) is much larger than that of other diatoms. The completeness was more than 96%. The genomes are currently analysed in cooperation with experts outside TRR51 (Jan de Vries, University of Göttingen, and Thomas Mock, University of East Anglia, Norwich, UK). Partial results are presented in the report of project B2-Brinkhoff. We want to come up with a manuscript on the genome later this year. The additional genome of the new North Sea strain will allow a comparative functional genome analysis of two different strains of this important coastal diatom.

7.2.1.4 Viral communities and distribution of *Rhodobacteraceae-associated viruses*

In cooperation with B6-Moraru, A1-Simon/Brinkhoff, A5-Petersen/Pradella, A6-Göker and A7-Overmann the phylogeny, genomic organization, biogeography and ecology of cobaviruses infecting marine *Rhodobacteraceae* were analysed (Bischoff et al. 2019). Investigation of the genomes suggests that viral lysis of the cell proceeds via the canonical holin-endolysin pathway. Cobaviral hosts include members of the genera *Lentibacter*, *Sulfitobacter* and *Celeribacter* of the *Roseobacter* group within the family *Rhodobacteraceae*. Analysis of more than 5,000 marine metagenomes revealed that cobaviruses were worldwide distributed. The presence of cobaviruses in protist metagenomes suggested that they could infect bacteria associated with phototrophic or grazing protists. The distribution analysis of 'Ascunsovirus oldenburgi' ICBM5, a novel ssDNA phage distantly related to known *Microviridae* and infecting *Sulfitobacter dubius* SH24-1b yielded 47 environmental viral genomes (EVGs) from various viromes. A biogeographical assessment showed that ICBM5 and its relatives are spread worldwide, including terrestrial and marine environments (Zucker et al. 2022; with B6-Moraru, A1-Simon/Brinkhoff, and A7-Overmann). We also investigated the effect of viruses on the composition of bacterial communities and the pool of dissolved organic matter (DOM) by employing virus-induction experiments with sediments from the seafloor of the Bering Sea (Heinrichs et al. 2020, with A2-Engelen and A8-Dittmar/Niggemann). Ultra-high resolution mass spectrometry revealed an imprint of viral-based cell lysis on the molecular composition of DOM. We showed also that viral lysis is an important driver in sustaining bacterial diversity, consistent with the “killing the winner” model.

In addition to the outlined results, members of this project were involved in several further studies listed in the publications.

7.2.2 Published project results

Publications with scientific quality assurance

List of publications in peer reviewed journals
(members of Z02 in bold, of other CRC projects in italic)

1. *Bakenhus* I, **Voget** S, **Poehlein** A, *Brinkhoff* T, **Daniel** R, *Simon* M (2018) Genome sequence of *Planktotalea frisia* type strain (SH6-1^T), a representative of the *Roseobacter* group isolated from the North Sea during a phytoplankton bloom. Stand Genomic Sci 13: 7.
2. *Bakenhus* I, **Wemheuer** B, Akyol P, *Giebel* HA, *Dlugosch* L, **Daniel** R, *Simon* M (2019) Distinct relationships between fluorescence *in situ* hybridization- and rRNA gene- and amplicon-based sequencing data of bacterioplankton lineages. System Appl Microbiol 42: article 126000.
3. *Bischoff* V, *Bunk* B, Meier-Kolthoff JP, Spröer C, **Poehlein** A, *Dogs* M, Nguyen M, *Petersen* J, **Daniel** R, *Overmann* J, *Göker* M, *Simon* M, *Brinkhoff* T, *Moraru* C (2019) Cobaviruses – a new globally distributed phage group infecting ***Rhodobacteraceae*** in marine ecosystems. ISME J 13: 1404–1421.
4. **Daniel** R, *Simon* M, **Wemheuer** B (2018) Editorial: Molecular ecology and genetic diversity of the ***Roseobacter*** clade. Front Microbiol 9, 1185.
5. *Dlugosch* L, **Poehlein** A, **Wemheuer** B, Pfeiffer B, Badewien TH, **Daniel** R, *Simon* M (2022) Significance of gene variants for the functional biogeography of the near-surface Atlantic Ocean microbiome. Nature Comm 13: Article number 456.
6. *Heinrichs* ME, *Tebbe* DA, **Wemheuer** B, *Niggemann* J, *Engelen* B (2020) Impact of viral lysis on the composition of bacterial communities and dissolved organic matter in deep-sea sediments. Viruses 12: 922.
7. **Hollensteiner** J, **Poehlein** A, **Daniel** R (2019) Complete genome sequence of *Marinobacter* sp. strain JH2, isolated from seawater of the Kiel Fjord. Microbiol Resour Announc 8: e00596-19.
8. **Hollensteiner** J, Schneider D, **Poehlein** A, **Daniel** R (2020) Complete genome of *Roseobacter ponti* DSM 106830T. Genome Biol Evol 12: 1013-1018
9. **Hollensteiner** J, Wemheuer F, Schneider D, Pfeiffer B, **Wemheuer** B (2023) Extraction of total DNA and RNA from marine filter samples and generation of a universal cDNA as universal template for marker gene studies. Methods Mol Biol 2555: 13-21
10. *Pohlner* M, *Dlugosch* L, **Wemheuer** B, Mills H, *Engelen* B, Reese BK (2019) The majority of active *Rhodobacteraceae* in marine sediments belong to uncultured genera: a molecular approach to link their distribution to environmental conditions. Front Microbiol 10: 659. (
11. Steinert G, **Wemheuer** B, Janussen D, Erpenbeck D, **Daniel** R, *Simon* M, *Brinkhoff* T, Schupp PJ (2019) Prokaryotic diversity and community patterns in Antarctic continental shelf sponges. Front Mar Sci 6: 297.
12. Wemheuer F, Taylor JA, **Daniel** R, Johnston E, Meinicke P, Thomas T, **Wemheuer** B (2020) Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. Environ Microbiome. 15: 11.
13. Wemheuer F, von Hoyningen-Huene AJE, *Pohlner* M, Degenhardt J, *Engelen* B, **Daniel** R, **Wemheuer** B (2019) Primary production in the water column as major structuring element of the biogeographical distribution and function of Archaea in deep-sea sediments of the Central Pacific Ocean. Archaea 2019: ID 3717239.
14. *Wolter* LA, Mitulla M, Kalem J, **Daniel** R, *Simon* M, Wietz M (2021a) CAZymes in *Maribacter dokdonensis* 62-1 from the Patagonian shelf: Genomics and physiology compared to related flavobacteria and a co-occurring *Alteromonas* strain. Front Microbiol 12: 628055
15. *Wolter* LA, Wietz M, *Ziesche* L, *Breider* S, *Leinberger* J, **Poehlein** A, **Daniel** R, *Schulz* S, *Brinkhoff* T (2021b) *Pseudooceanicola algae* sp. nov., isolated from the marine macroalga *Fucus spiralis*, shows genomic and physiological adaptations for an algae-associated lifestyle. Syst Appl Microbiol 44: 126166.
16. *Zucker* F, *Bischoff* V, Ndela EO, *Heyerhoff* B, **Poehlein** A, *Freese* HM, Roux S, *Simon* M, Enault F, *Moraru* C (2022) New Microviridae isolated from *Sulfitobacter* reveals two cosmopolitan subfamilies of single-stranded DNA phages infecting marine and terrestrial Alphaproteobacteria. Virus Evol 8: 1-18

Other publications and published results

1. Hollensteiner J, Schneider D, Poehlein A, *Brinkhoff* T, Daniel R (2023). Pan-genome analysis of six complete *Paracoccus* type strain genomes from hybrid next generation sequencing. (https://doi.org/10.1101/2023.06.19.545646)

7.2 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period. (A03 2010-2013 and Z02 from 2014)

7.2.1 Project staff in the ending funding period

	Sequen-tial no.	Name, academic degree, position	Field of research	Department of university or non-university institution	Project commitment in hours per week	Category	Funding source
Existing staff							
Research staff	1	Rolf Daniel, Prof. Dr., W3	(Meta)genomics, Microbiol.	Gen. & Appl. Microbiol. & G_2L	4		Univ. GÖ
	2	Heiko Liesegang, PD Dr., Research associate	Microbiol. Bioinformatics	Gen. &Appl. Microbiol. & G_2L	4		Univ GÖ
	3.	Heiko Nacke, Dr., Research asso-ciate (until 09/19)	Microbiol. Metagenomics	Gen. &Appl. Microbiol. & G_2L	4		Univ GÖ
	4.	Bernd Wemheuer, Dr., Research associate	(Meta)genomics, Bio-informatic	Gen. & Appl. Micro. & G_2L	4		Univ GÖ
	5	Dominik Schneider, Dr, Research associate	Bioinformatics, (Meta)genomics	Gen. & Appl. Microbiol. & G_2L	3		Univ GÖ
	6	Jacqueline Hollensteiner, Dr., Research associate	(Meta)genomics, Mic ecology	Gen. & Appl. Microbiol. & G_2L	12		Univ GÖ
	7.	Anja Poehlein, Dr. Research associate	FunctionalGenomics	Gen. &Appl. Microbiol. & G_2L	Consul-tancy		Univ Gö
Non-research staff	8	Mechthild Bömeke, technician		Gen. &Appl. Microbiol. & G_2L	4		Univ Gö
	9	Melanie Heinemann, technician		Gen. &Appl. Microbiol. & G_2L	3		Univ Gö
Staff funded with approved grant money							

Research staff	1	Jacqueline Hollensteiner, Dr., Research associate	(Meta)Genomics, Micr ecology	Gen. & Appl. Microbiol. & G_2L	100%	E13	
	2	Dominik Schneider, Dr, Research associate	Bioinformatics, (Meta)genomics	Gen. & Appl. Microbiol. & G_2L	30%	E13	

Job descriptions of staff (supported through existing funds):

1. Daniel
   Principal investigator of the project and responsible for planning and design of experimental work; supervision of the postdocs; participation in data evaluation and writing manuscripts
2. Liesegang
   Maintaining and extending bioinformatic tools; participation in bioinformatic processing of functional genomic data. He gave advice in designing and executing experiments and data analysis of the Roseobacter MAG analyses.
3. Nacke
   Responsible for sequencing; participation in analysis of functional metagenomic data
4. Wemheuer
   He gave advice in designing and executing experiments and participated in community, metagenominic data analysis and generation of analysis pipelines
5. Schneider see below
6. Hollensteiner see below
7. Poehlein
   Advice with respect to sequencing, genomics, transcriptomics and functional data analysis; instrumental for all genomic work related to *T. rotula*
8. Bömeke
   Assistance in preparation of samples for sequencing and running of sequencers as well as preparation of nucleic acids for (meta)genome and (meta)transcriptome analyses.
9. Heinemann
   Assistance in preparation of samples for sequencing and running of sequencers as well as preparation of nucleic acids for (meta)genome and especially for the *T. rotula*

Job descriptions of staff (funded with approved grant money):

1. Hollensteiner
   Performed functional genomics of isolates; involved in data generation; performed evaluation and statistical analysis of diversity and community composition and functional profiles throughout the samples and isolates, MAG analysis
2. Schneider
   Establishment and updating of bioinformatic and statistical analysis pipelines for functional (meta)genome and MAG analysis; Participated in functional and statistical analysis of data and MAGs.

7. General information about Project INF

7.1 Data Management, Databases, Bioinformatics Tool Development and Genome Projects

7.1.2 Project leader

Jahn, Dieter, Prof. Dr., 01.08.1959, German
Institut für Mikrobiologie, Technische Universität Braunschweig, 38106 Braunschweig
Tel. 0531/391 55101
Email d.jahn@tu-braunscheig.de

7.2. Project history

This project received funding through TRR51 from January 2010 until December 2022

7.2.1 Report

7.2.1.1 Database 1: ROSY – Roseobacter systems biology database and data management

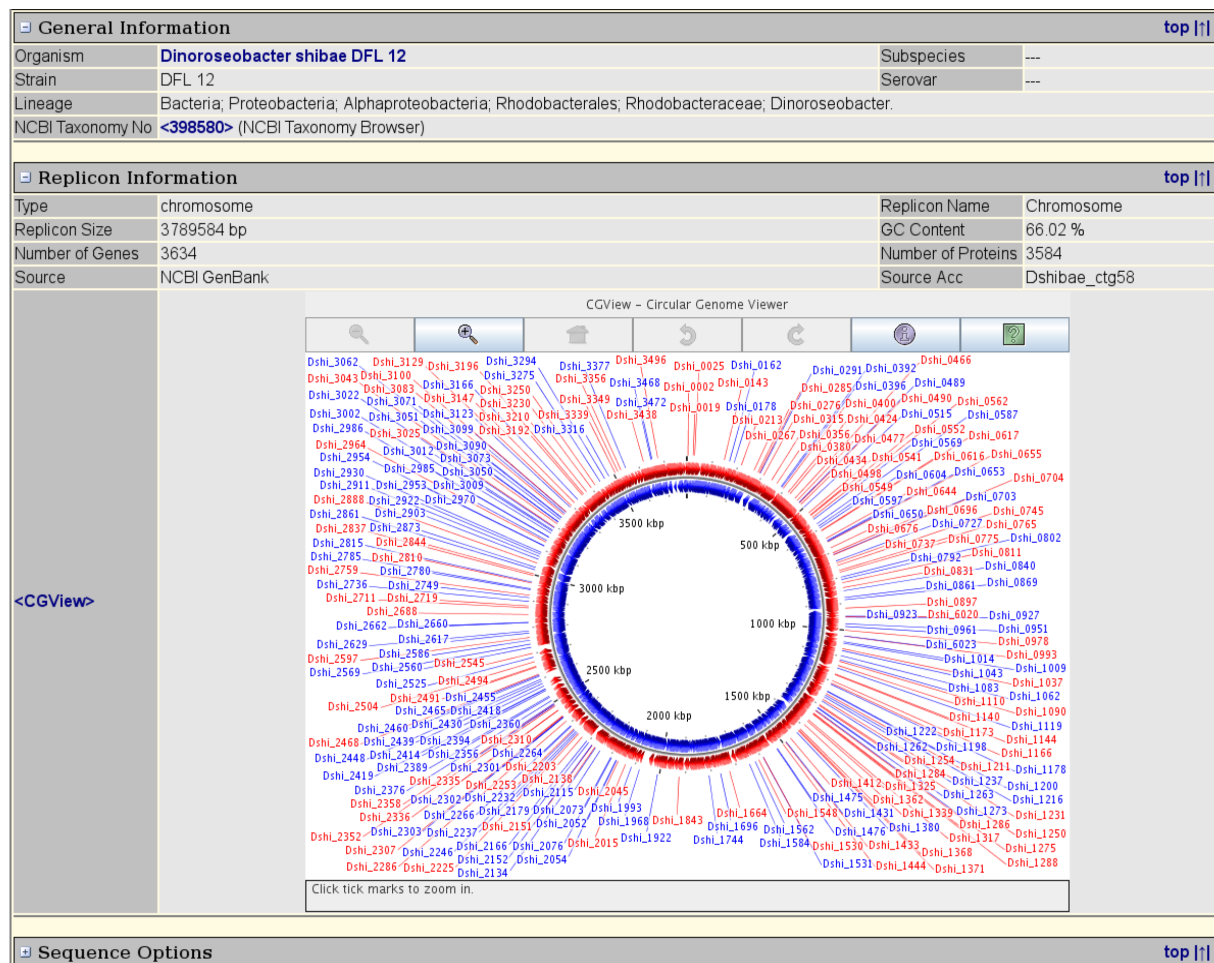


Fig. 1: Screenshot of the ROSY database.

ROSY provides an integrative view on comprehensive data collections such as KEGG, GenBank, RoseoBase, BRENDA, and PRODORIC as well as mediates the use of connected tools for promoter analysis (Virtual Footprint), genome and pathway visualization (CGView, PathCompare), and prediction of signal peptides (PrediSi). Moreover, metabolome, transcriptome, and proteome data can be stored in ROSY, supplying an integrated platform

for comparative genomics and systems biology. This entire database system along with the data retrieval, comparative analysis, and website presentation tools (http://rosy.tu-bs.de) can be easily adopted for the systems biological analysis of other bacterial groups.
The database was updated and curated during the last funding period (Beier *et al*., 2021, Mansky *et al*., 2022).

7.2.1.2 Database 2: PRODORIC (bacterial gene regulation database) with Virtual Footprint

PRODORIC (https://www.prodoric.de) is worldwide one of the largest collections of prokaryotic transcription factor binding sites from multiple bacterial sources with corresponding interpretation and visualization tools developed in the framework of the CRC. With the introduction of PRODORIC2 in 2017, the transition to a modern web interface and maintainable backend was started. With this latest PRODORIC release in 2023 the database backend is now fully API-based and provides programmatical access to the complete PRODORIC data. The visualization tools Genome Browser and ProdoNet from the original PRODORIC have been reintroduced and were integrated into the PRODORIC website. Missing input and output options from the original Virtual Footprint were added again for position weight matrix pattern-based searches. The whole PRODORIC dataset was reannotated. Every transcription factor binding site was re-evaluated to increase the overall database quality. During this process, additional parameters, like bound effectors, regulation type and different types of experimental evidence have been added for every transcription factor. The database was updated during the last funding period (Behringer *et al.,* 2020, Dudek & Jahn, 2022, Dudek *et al*., 2023).

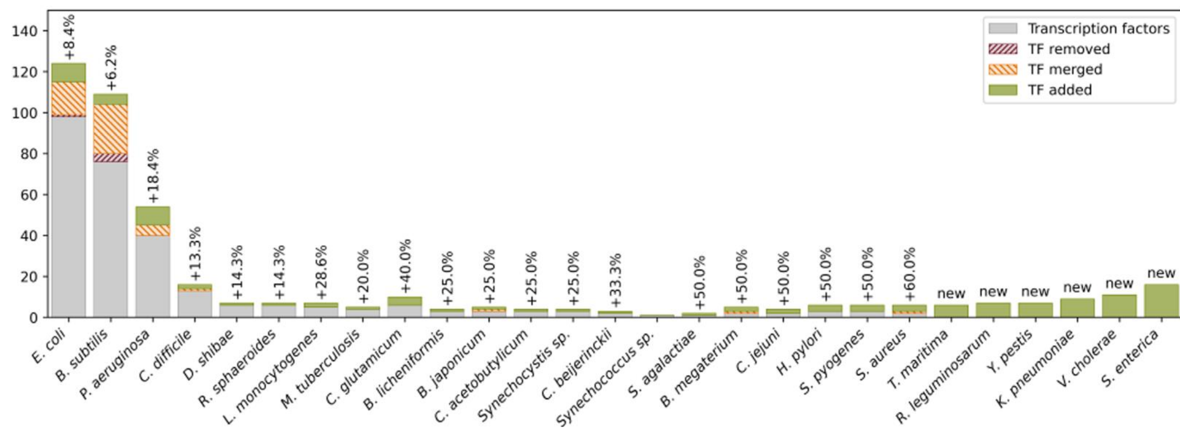


Fig. 2: Number of transcription factors per organisms and their increase since the last release in 2017.

7.2.1.3 Database 3: ProdoNet – identification and visualization of prokaryotic gene regulatory networks

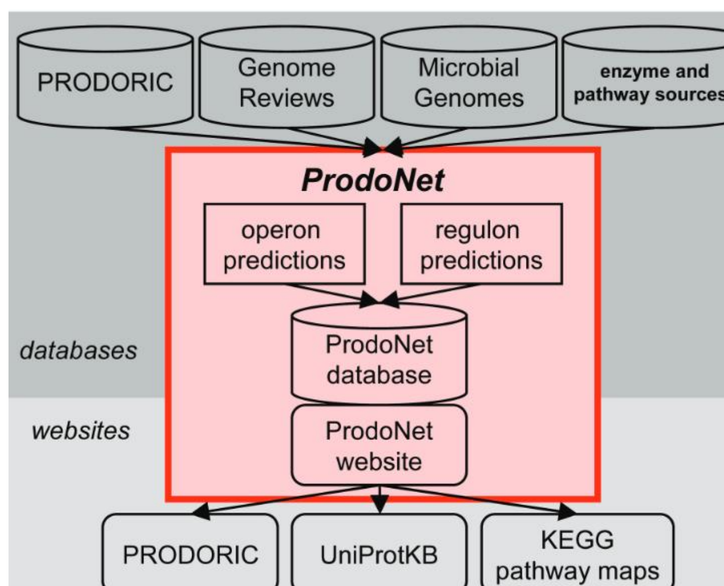


Fig. 3: Structure and bioinformatic integration of ProdoNet.

ProdoNet (http://www.prodonet.tu-bs.de) is a web-based application for the mapping of prokaryotic genes and the corresponding proteins to common gene regulatory and metabolic networks developed for the CRC. For a given list of genes, the system detects shared operons, identifies co-expressed genes and deduces joint regulators. In addition, the contribution to shared metabolic pathways becomes visible on KEGG maps. Furthermore, the co-occurrence of genes of interest in gene expression profiles can be added to the visualization of the global network. In this way, ProdoNet provides the basis for functional genomics approaches and for the interpretation of transcriptomics and proteomics data. The database was updated and curated during the last funding period.

7.2.1.4. Database 2: BRENDA

The BRENDA enzyme database (https://www.brenda-enzymes.org), established in 1987, has evolved into the main collection of functional enzyme and metabolism data. In 2018, BRENDA was selected as an ELIXIR Core Data Resource. BRENDA provides reliable data, continuous curation and updates of classified enzymes, and the integration of newly discovered enzymes. The main part contains >5 million data for ~90 000 enzymes from ~13 000 organisms, manually extracted from ~157 000 primary literature references, combined with information of text and data mining, data integration, and prediction algorithms. Supplements comprise disease-related data, protein sequences, 3D structures, genome annotations, ligand information, taxonomic, bibliographic, and kinetic data. BRENDA offers an easy access to enzyme information from quick to advanced searches, text- and structured-based queries for enzyme-ligand interactions, word maps, and visualization of enzyme data. The BRENDA Pathway Maps are completely revised and updated for an enhanced interactive and intuitive usability. The new design of the Enzyme Summary Page provides an improved access to each individual enzyme. A new protein structure 3D viewer was integrated. The prediction of the intracellular localization of eukaryotic enzymes has been implemented. The new EnzymeDetector combines BRENDA enzyme annotations with protein and genome databases for the detection of eukaryotic and prokaryotic enzymes (Chang *et al.,* 2020).

7.2.1.5 Homepage “www.roseobacter.de”

Fig. 4. Screenshot of the CRC homepage.

At first a web server was set up comprising a comprehensive bilingual website covering general information about research and teaching of the involved groups within the SFB/TRR51. For PhD students detailed information about the graduate school including PhD program, courses and research fellowships was provided. In order to ensure open and flexible access, the framework was implemented in form of a content management system (CMS) that allows publishing, editing and modifying of web contents by all members of the collaborative research centre. For internal purposes like deposition and exchange of unpublished data a restricted intranet with user authentication was established. Here, the applied platform was a Wiki system which offers both a structured and file based organizing of community-based data. The intranet covered presentations of the status seminars, conference posters, lab protocols, transposon libraries, various experimental data and cruises log files of the RV Heincke and RV Polarstern, amongst others. Although many users had contributed to the data, the major part of web content organization was carried out continuously by the INF project. This included web design and HTML programming of the whole content. In order to preserve the system, continuous administration, including server maintenance, software updates and data backup, was periodically performed.

7.2.1.6 Bioinformatic tools – InFiRe - a novel computational method for the identification of insertion sites in transposon mutagenized bacterial genomes

For the screening of transposon libraries of *Dinoroseobacter shibae* a new bioinformatics method and software for the localization of transposons was developed – InFiRe (www.infire.tu-bs.de). This approach allows the computational identification of transposon insertion sites in known bacterial genome sequences after transposon mutagenesis experiment. After endonuclease digestions and Southern blot hybridizations with a transposon specific probe, the fragment size distribution pattern allows the mapping of the transposon location by computational analysis. It was shown, that this approach can be superior to other protocols and that this method is capable for inexpensive high-throughput screening of transposon libraries. In collaboration with B5 it was successfully applied and refined by screening the transposon library of *D. shibae*. The software was updated and permanently used during the last funding period.

7.2.1.7 Training courses

The developed analysis methods were shared within the whole consortium. In regular time intervals courses with the subject “data analysis” were offered in Braunschweig and Oldenburg. The focus of these courses was statistics, mathematical modelling and building of

professional graphs in science using the software package R. These courses were offered as part of the graduate school for PhD students and interested Postdocs. The course topics were adapted according to the needs of the participating students and covered widespread topics in the field molecular biology, microbiology, marine biology and systems biology.

7.2.1.8 Single cell analysis using time-lapse fluorescence microscopy and image processing via the new software toll TLM tracker (Time-Lapse Movie Tracker)

In collaboration with the subprojects B4 and C5 we started the establishment of single-cell analyses and time-lapse microscopy using *D. shibae*. Despite its small size and low proliferation rate we showed that *D. shibae* is an extraordinary suitable organism for this kind of analytical technique. In previous experiments live cell imaging was possible without difficulty for a period of about 36 hours. For further analysis, we developed an image processing pipeline for fluorescence microscopic images which offers the measurements of cell size, fluorescence, cell age and migration in cell lineages of a growing microcolony. In this context we developed the new software TLM-Tracker (**http://www.tlmtracker.tu-bs.de**, Time-Lapse Movie Tracker) that covers various sophisticated image processing algorithms necessary for the segmentation and tracking of cells. TLM-Tracker has a dialog based graphical user interface that allows an interactive analysis of time-lapse movies with manual intervention at every step. Hereby, image processing is organized in user-defined pipelines that allow a flexible adaptation to various image qualities like background, contrast, focus, object sizes, amongst others. In a first study of this organism in collaboration with B4 and C5 we analysed the morphological diversity of *D. shibae*. In a batch culture enormous cell size variations between 0.3 – 10 µm were observed. Using time-lapse microscopy, we showed that some cells divide in a budding like behaviour while others show symmetrical binary fission. However, if the quorum sensing system is silenced, this morphological heterogeneity is completely lost. In another approach we measured the intracellular bacteriochlorophyll levels in a growing microcolony using time-lapse microscopy with infrared fluorescence channel (Fig. 5A). Previously, it was shown that during dark-light cycles bacteriochlorophyll is depleted under light exposure and resynthesized in the dark after a lag phase of 6 hours. We confirmed and followed the process of bacteriochlorophyll fluctuation on single cell level using time-lapse microscopy. Moreover, we were able to quantify the dynamics of bacteriochlorophyll levels in the cell lineage trees using the image processing algorithms of the TLM-Tracker software. Hereby, we realized that bacteriochlorophyll is not just diluted by cell division but degraded exponentially (Fig. 5B). The software was updated during the last funding period (Kirchhoff *et al.,* 2018, Behringer et al., 2020).

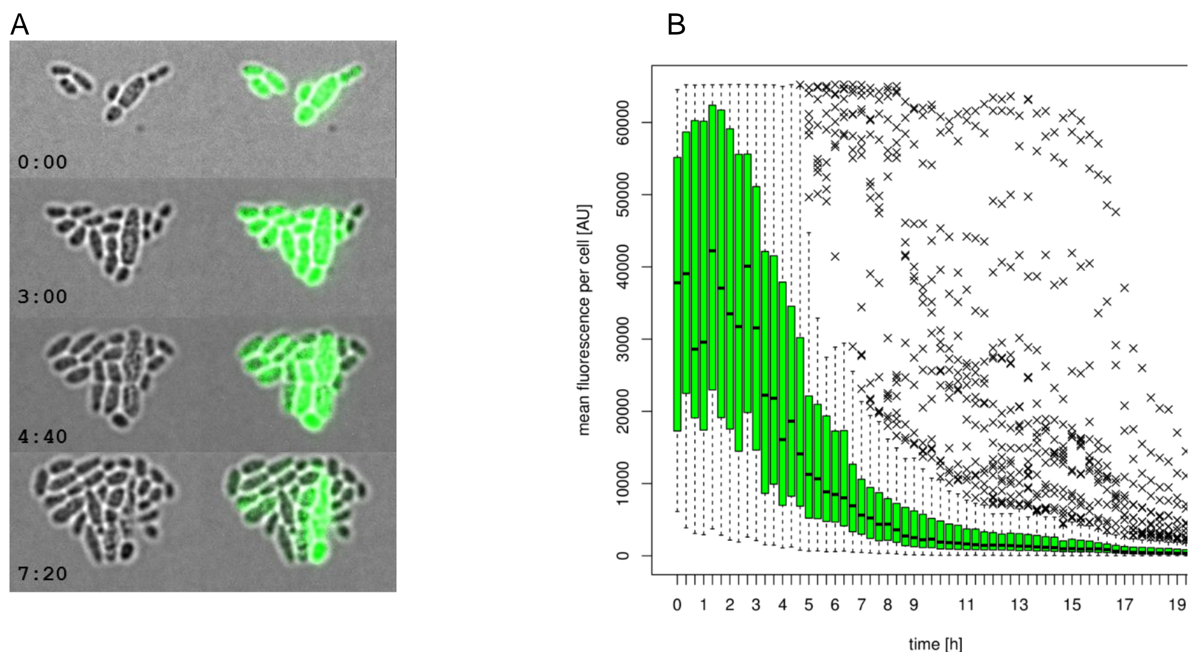


Fig. 5: A. Time-lapse movie with stages of a growing microcolony showing different sized *D. shibae* cells as brightfield image (left column) and with bacteriochlorophyll fluorescence (right column). Bacteriochlorophyll is gradually depleted. B. Boxplot analysis of this time-lapse movie showing the time course of bacteriochlorophyll content on single cell level.

7.2.1.9 Successful genome project of the dinoflagellate *Prorocentrum cordatum*

We determined the *de novo* assembled genome (~4.75 Gbp with 85,849 protein-coding genes), transcriptome, proteome, and metabolome from *Prorocentrum cordatum*, a globally abundant, bloom-forming dinoflagellate. Using axenic algal cultures, we studied the molecular mechanisms that underpin response to temperature stress, which is relevant to current ocean warming trends. We present the first evidence of a complementary interplay between RNA editing and exon usage that regulates the expression and functional diversity of biomolecules, reflected by reduction in photosynthesis, central metabolism, and protein synthesis. These results revealed genomic signatures and post-transcriptional regulation for the first time in a pelagic dinoflagellate. Our multi-omics analyses uncover the molecular response to heat stress in an important HAB species, which is driven by complex gene structures in a large, high-G+C genome, combined with multi-level transcriptional regulation. The dynamics and interplay of molecular regulatory mechanisms may explain in part how dinoflagellates diversified to become some of the most ecologically successful organisms on earth This investigation was the major focus of the INF project during the last funding period (Dougan *et al.*, 2023).

7.2.2 Published project results

Publications with scientific quality assurance

1. *Kirchhoff, C., Ebert*, M., **Jahn**, D. & *Cypionka, H.* (2018) Chemiosmotic energy conservation in *Dinoroseobacter shibae*: Proton translocation driven by aerobic respiration, denitrification and photosynthetic light reaction. *Front. Microbiol.*, 9:903.
2. Chang, A., Jeske, L., *Ulbrich*, S., Hofmann, J., Koblitz, J., Schomburg, I., *Neumann-Schaal, M.*, **Jahn**, D. & *Schomburg*, D. (2020) BRENDA, the ELIXIR Core Data Resource in 2021: New Developments and Update. *Nucleic Acids Res.*, 49:D498-D508.
3. Behringer, M., Plötzky, L., Baabe, D., Zaretzke, M.-K., Schweyen, P., Bröring, M., **Jahn**, D., *Härtig, E.* (2020) RirA of *Dinoroseobacter shibae* senses iron via a [3Fe-4S]1+ cluster coordinated by three cysteine residues. *Biochem. J.*, 477,191-212.
4. *Beier, N*, Kucklick, M, Fuchs, S, Mustafayeva, S, Behringer, M, *Härtig*, E., **Jahn**, D., *Engelmann*, S. (2021) Adaptation of *Dinoroseobacter shibae* to oxidative stress and the specific role of RirA. PLOS ONE, 16, e0248865.
5. Dudek, C.-A. & **Jahn**, D. (2022) PRODORIC: state-of-the-art database of prokaryotic gene regulation. *Nucleic Acids Res.*, 50(D1):D295-D302.
6. *Mansky, J*, Wang, H, *Ebert*, M, *Tomasch, J*, *Härtig*, E, **Jahn**, D. & *Wagner-Döbler, I* (2022) The influence of genes on the “killer plasmid” of *Dinoroseobacter shibae* on its symbiosis with the dinoflagellate *Prorocentrum minimum.* Front Microbiol.,12:804767.

Other publications and published results

1. Dudek, C.-A., *Overmann, J.* **Jahn,** D. (2023) Vorhersage bakterieller Genregulation. BIOspektrum, 19**:**252-254.
2. Dougan, K. N., Deng, Z.-L., *Wöhlbrand, I., Reuse, C., Bunk, B.,* Chen, Y., Hartlich, J., *Hiller*, K.. John, U., *Kalvelage*, J., *Mansky*, J., *Neumann-Schaal, M.*, *Overmann, J.*, *Petersen, J., Sanchez-Garcia,* S., *Schmidt-Hohagen, K.*, Shah, S., Spröer, C., *Sztajer*, H., *Wang, H.,* Bhattacharya, D., *Rabus, R.,* **Jahn**, D., Xin Chan, C., *Wagner-Döbler, I.* (2023) Multi-omics analysis reveals the molecular response to heat stress in a “red tide” dinoflagellate. BioRxiv, https://doi.org/10.1101/2022.07.25.501386.

7.3 Funding

Funding of this project within the Collaborative Research Centre started in January 2010. The project ended by the end of the final funding period.

7.3.1 Project staff in the ending funding period

	Sequ en-tial no.	Name, academic degree, position	Field of researc h	Department of university or non-university institution	Project commit ment in hours per week	Categor y	Fundin g source
Existing staff							
Research staff	1	Jahn, Dieter, Prof. Dr., W3	Micro-biology	Institute of Microbiology	5		TU BS
	2	Louisa Roselius, Dr., E13	Mathem atics	Institute of Microbiology	40		TU BS
Staff funded with approved grant money							
Research staff	3a	Denitsa Eckweiler, Dr., Postdoc	Bioinfrm atics	Institute of Microbiology	20	E13 50%	
	4	Juliane Hartlich, Ms. Sc., PhD student	Bioinfor matics	Institute of Microbiology	20	E13 50%	

Job descriptions of staff (supported through existing funds):

1. Jahn
   He was principal investigator of the project and responsible for design and coordination of the experiments. He supervised the PhD student and the postdoc.
2. Roselius
   She gave advice in designing and executing the different programming procedures

Job descriptions of staff (funded with approved grant money):

1. Eckweiler
2. She carried out all the programming and updating of the various databases and software tools.
3. Hartlich
4. She was responsible for the bioinformatics of the *Prorocentrum* genome project. For that purpose. she went for several month to Australia to our cooperation partner Prof. Dr. Cheong Xin Chan.

8. Comments on the CRC program

The CRC program line of the DFG with its variations of one main location or two or three locations, the transregional CRC like TRR51, appears to us, the consortium of researchers of TRR51, as a great instrument to investigate very comprehensively a particular research topic with a long-term prespective. Only very few program lines of funding agencies in a few other countries provide such an opportunity, in most cases an adoption of this DFG program line. The high flexibility of fund allocation and the lump sum funds of the CRC program line are further tools for high-end research and to react quickly to emerging research aspects of high interest to the CRC. These great opportunities were the basis for the inspiring and fruitful work and the achievements of TRR51 to contribute with several seminal findings and many in-depth studies to a great advancement of the understanding of the *Roseobacter* group within the last 13 years.

One very attractive subproject line of CRCs is the Integrated Resesarch Training Group (IRTG) for a targeted and tailored education in specific research fields and soft skill. Our experience was that in particular in a TRR like ours this IRTG is a very suitable and successful tool for the education and networking of early career scientist in their most important formative career phase. In TRRs this is even more important than the graduate schools at the different locations.

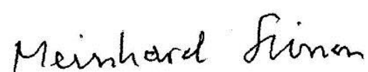
We are most grateful to the leadership of UOL, TUBS, ICBM and the MWK for their continuous support of TRR51. We are also very grateful to the financial administration of Dezernat 2 of UOL and the partner institutions for their support and to the office staff of TRR51, Angelika Hasselbring and Katinka Hoppe for their invaluable contribution to the smooth running of TRR51.

We would like to thank the DFG and in particular Dr. Thomas Münker and Ursula Hüllen for their continuous excellent support during the three funding phases of TRR51. This help was particulary important and most helpful during the preparation of the proposals and the on site review procedures.

The last two years of the final funding phase, 2020 and 2021, were heavily affected by the Corona pandemic. We really appreciate very much that DFG made it rather easily possible to extend the funding for another six months and the formal third funding phase cost-neutrally for another year.

We greatly appreciate the time and efforts taken by numerous reviewers to evaluate our initial and two renewal research proposals, the generous time they spent during reviewing the three reviewing visits in Oldenburg and Braunschweig and their competent and friendly advice they have generously given us over the years. The advice and constructive cristicism given us by the reviewers were invaluable to strengthen and focus the research profile of TRR51.

On behalf of all members of TRR51,



Meinhard Simon Oldenburg, September 2023

(Speaker of TRR51

Group photo at the kick-off symposium at the Hanse Institute of Advanced Study in Delmenhorst, 13-15 June 2010

Group photo at the closing symposium at the Alte Landtag in Oldenburg, 4-6 September 2022