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About the microdiversity of *Phaeobacter* spp. from German harbors

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Introduction

Microdiversity is defined as the diversity of phylogenetically closely related but physiologically distinct populations of bacteria. *Phaeobacter* spp. show, based on their 16S rRNA gene sequences, a similarity of above 99%. However, comparison of the genomes of different species revealed DNA-DNA-hybridization values only around 30%, indicating low relatedness. We extended the isolation technique used in a previous study for samples from the North Sea. Here we report about the newly isolated *Phaeobacter portensis* and compare diverse *Phaeobacter* spp. for their plasmid content, genome rearrangement and ability to utilize substrates.

Methods & Results

Fig 1: Sampled surfaces.
A: Harbor in Bensersiel
B: Wooden pole;
C: Barnacles, algae and biofilm

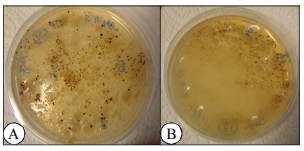

Fig 2: Agar plates with 1:10 diluted samples from biofilm (A) and barnacles (B). Dark colonies indicate *Phaeobacter* spp.

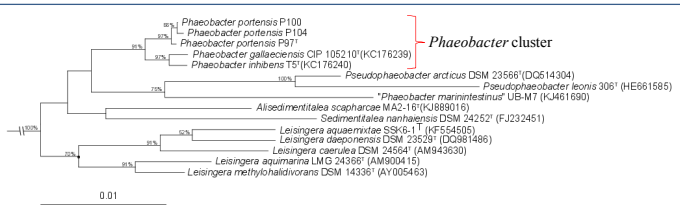

Fig. 3: Neighbour-joining tree based on 16S rRNA gene sequences. Bootstrap values: 1000 replications; only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

Tab. 1: Substrate utilization of different *Phaeobacter* spp. using BIOLOG. +, positive; -, negative; w, weak.

	DSM 17395	CIP 105210	T5	P97	P100	P104
Sodium Formate	w	w	-	w	+	+
Acetic Acid	+	+	+	-	+	+
D-Aspartic Acid	-	+	-	+	w	-
Tween 40	+	+	+	-	w	w
Tween 80	w	+	w	-	w	w
a-Hydroxy-Butyric Acid	+	+	-	w	-	w
Gly-Asp	+	+	+	+	+	-
Propionic Acid	+	+	+	-	+	+
D-Cellobiose	+	+	+	+	+	-
L-Alanine	+	+	+	-	+	+
p-Hydroxy-Phenylacetic Acid	-	+	+	+	+	+
Tyramine	+	-	+	w	+	+
L-Lyxose	w	-	w	+	w	+
D-Arabitol	w	w	+	-	+	w
Melibionic Acid	+	+	+	w	+	-
Sorbic Acid	w	-	-	+	w	w
N-Acetyl-L-Glutamic Acid	+	w	+	w	w	-
L-Valine	w	+	+	w	w	-
D,L-Octopamine	w	-	-	w	+	w
Caproic Acid	-	-	-	w	+	-

Tab. 2: Differences in plasmid size [kbp] and content of *P. gallaeciensis* CIP 105210^T, *P.inhibens* T5^T, *P. inhibens* 2.10, *P. inhibens* DSM 17395 and *P. portensis* P97^T.

P. gallaeciensis CIP 105210^T	*P. inhibens* T5^T	*P. inhibens* 2.10	*P. inhibens* DSM 17395	*P. portensis* P97^T
255			262	
	227	238		218
133				126
109				
		94		
	88			85
2 x 77	78		78	
		70		71
68	69		65	
				53
40				
Σ= 7	4	3	3	5

Samples were taken at harbors located at the southern North Sea. Isolation was done using a serial dilution of crushed samples. We were able to repeatedly isolate *Phaeobacter* spp. from natural habitats at the southern North Sea. There we found strains of *P. inhibens* and *P. portensis* in the same samples. *P. portensis* is a new species within the genus *Phaeobacter*. The compared strains showed distinct differences in their plasmid content, genome arrangement and the ability to utilize substrates.

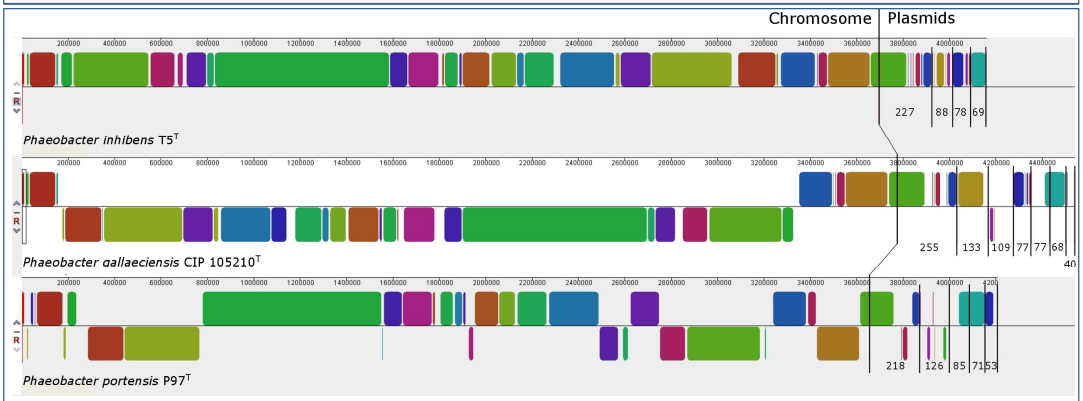

Fig. 4: Whole-genome alignment of *P. inhibens* T5^T, *P. gallaeciensis* CIP 105210^T and *P. portensis* P97^T. The progressive MAUVE alignment tool identifies nucleotide matches, depicted as boxes with the same color. Inverted regions are identified by boxes below the centre line. Replicons are separated by black lines.

	P. portensis P97^T	*P. inhibens* T5^T	*P. gallaeciensis* CIP105210^T
P. portensis P97^T	-	30.1 / 99.4	26.2 / 99.42
P. inhibens T5^T	30.1 / 99.4	-	36.2 / 99.71
P. gallaeciensis CIP105210^T	26.2 / 99.42	36.2 / 99.71	-

Tab. 3: Results of DNA-DNA-hybridization and 16S rRNA similarities of type strains of *P. portensis* P97^T, *P. inhibens* T5^T and *P. gallaeciensis* CIP 105210^T.

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Conclusions & Outlook

The isolation strategy was successful at the southern North Sea and the Baltic Sea (Gram *et al.*, 2015). *Phaeobacter portensis* is the first new characterized *Phaeobacter* species since ten years. Now that the habitat is known, it can be screened for further *Phaeobacter* species. The new collection of *Phaeobacter* strains isolated from natural environments will allow a comparison with relatives obtained from aquacultures and other habitats. We will conduct seasonal studies to get a broader understanding of the ecological role of *Phaeobacter* spp. in their natural habitat. Our study provides the cornerstone for further research.

Reference: Tropodithietic Acid producing *Phaeobacter inhibens* from the *Roseobacter*-clade have a natural environmental niche as surface colonizer in Danish harbors. Gram, L., Rasmussen, B.B., Wemheuer, B., Bernbom, N., Yin Ng, Y., Porsby, C.H., Breider, S., Brinkhoff, T. (Syst Appl Microbiol 38 (7), 483-493).

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