AWI Alfred-Wegener-Institut für Polar- und Meeresforschung in der Helmholtz-Gemeinschaft

Helgoland Food Web Project

Diversity and Succession of Bacterial Populations in Microalgae Cultures

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Introduction

Marine bacteria play an important role in food webs especially with regard to nutrient cycling. Latest investigations reveal specific populations of bacteria associated with microalgae. These bacteria live in the phycosphere and feed on algal exudates. But mechanisms controlling the community composition are still poorly understood. In this study we examined bacterial association with microalgae with focus on changes in bacterial community composition during different growth phases of the algae. We investigated 10 freshly isolated key species (diatoms and dinoflagellates) off Helgoland Roads.

Fig. 1: Study site and sampling point in the German Bight, Helgoland

Methods

In order to follow the development of algae we recorded their morphology and their photosynthesis efficiency using pulse amplitude modulation. Diversity and succession of bacteria were analyzed by rDNA internal spacer analysis (RISA) and denaturating gradient gel electrophoresis (DGGE).

Investigated species

Guinardia delicatula
Pseudonitzschia pungens
Corethron hystrix
Thalassiosira rotula
Rhizosolenia pungens
Skeletonema costatum
Diatoms

Ceratium fusus
Ceratium horridum
Gymnodinium sanguineum
Dinoflagellates

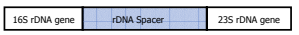
Microalgae cultures
in f/2 at 16 °C, 12h/12h

Cultivation in batch over 8 weeks
Sampling t_0 and after week 1, 2, 3, 4, 8

Successive filtration 3 µm / 0.2 µm
⇨ DNA of organisms > 3 µm ≡ attached bacteria
⇨ DNA of organisms < 3 µm ≡ free living bacteria

Community Analysis

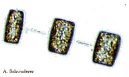
RISA: length polymorphism of intergenic spacer region (IGS)



DGGE: 16S rDNA, 500 bp, excised bands: sequence; denaturing gradients: 15-70 % urea / formamide

Results

Thalassiosira rotula 04/02



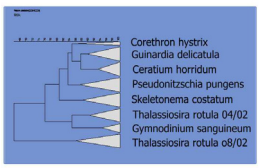


Fig. 2: Dendrogram generated from RISA profiles of 8 studied microalgae cultures containing profiles of fraction > 3 µm and < 3µm > 0.2 µm after week 8 using Pearson Correlation and UPGMA, generell similarity: 77 %
Bacterial communities of different algae cultures show specificity in their composition.

Communities in the examined cultures did not change substantially during sampling period. Although microalgae cells lost fitness over time bacterial composition was stable with little differences regarding starting point.

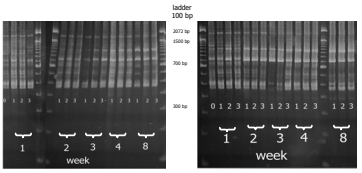


Fig.3: RISA profiles of IGS gene fragments amplified from *T. rotula* isolated in April 2002, left: DNA < 3 µm > 0.2 µm representing free bacteria, right: DNA > 3 µm representing attached bacteria.
0 = starting point, 1 / 2 / 3 = cultures in parallel

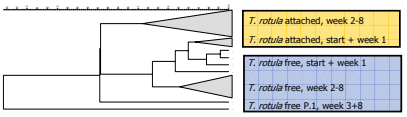


Fig. 5: Dendrogram generated from RISA profiles of *T. rotula* containing profiles of all sampling dates, fraction > 3 µm and < 3µm > 0.2 µm using Pearson Correlation and UPGMA, generell similarity: 78 %
Differences in community structure regarding free living and attached bacteria become obvious at week 2.

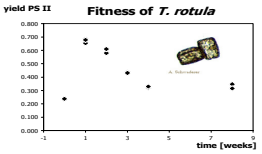
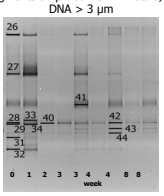


Fig. 4: Photosynthesis efficiency of *Thalassiosira rotula* using PAM

In the case of *T. rotula* 04/02 we found a diversity of 9 to 10 operational taxonomic units. Only two of the 16S rDNA defined populations were found only as attached bacteria. Major phylogenetic groups were α-, γ- Proteobacteria and the Cytophaga-Flexibacter-Bacteroides group.

Fig.6: DGGE profile of 16S rDNA fragments amplified from *T. rotula*, DNA > 3 µm



Tab.1: Closest relatives of 16S rDNA sequences found in a culture of *T. rotula*
yellow: sequences found in both fractions
Blue: sequences found only in the fraction of „attached" bacteria

DGGE band	Phylogenetic group	Closest relative	Similarity [%]	Based positions compared	GenBank accession number of closest relative
26	CFB	*Ulvibacter litoralis*	95	535	AY243096
27	Chloroplast	*Thalassiosira*			
28 40 42	α-Proteobacteria	*Sulfitobacter sp.*	95	482	AJ542658
31	γ-Proteobacteria	*Halomonas venusta*	96	415	AJ306894
34	γ-Proteobacteria	*Pseudoalteromonas sp.*	82	444	AJ244742
41	CFB	*Aequorivita lipolytica*	97	530	AY027805
35 43	α-Proteobacteria	*Sphingomonas flavimaris*	94	532	AY554010

Conclusions

Analysis of RISA profiles revealed specificity of composition of bacterial communities in studied microalgae cultures.

Additionally the fractions of attached and free living bacteria could be distinguished. Generally they seem to be very similar. Only few bacteria occur in one fraction of the culture. Bacteria occuring in both fractions are probably loosely attached, whereas populations found only in the fraction > 3 µm are assumed to be associated more closely.

During culturing of algae the community structure of bacteria did not change according to changes within the cultures. We assume that bacterial populations have to adapt physiologically to different conditions like availability of exudates they feed on.

In order to complete community information important DGGE bands which could not be sequenced will be analyzed after cloning.

Investigating axenic cultures considering the impact for the algae will be the next step. Studies of bacteria – phytoplankton associations *in situ* will follow.

Acknowledgements

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