

Sponge Associated Bacteria from Boreal Sponges – Sources of Marine Natural Products



Ralf Dieckmann^{1,3}, Ines Kaesler², Ingeborg Graeber², Marcel Erhard³, Ulrich Szewzyk² and Hans von Döhren¹

Berlin University of Technology, ¹Biochemistry & Molecular Biology, Sekr. OE2, ²Environmental Microbiology, Sekr. 1-2, Franklinstr. 29, 10587 Berlin, Germany, ³Anagnostec GmbH, Biotechnologiepark, 14943 Luckenwalde, Germany



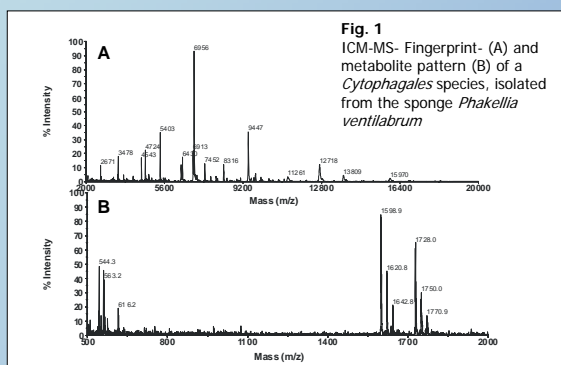
In the BMBF priority program "Marine Natural Products Research" the multidisciplinary project BOSMAN has undertaken the task of examining newly discovered compounds produced by marine porifera and their associated bacteria in boreal habitats. Research in the field of sponges and associated microorganisms have indicated a bacterial source of many compounds with pharmacological properties, formerly attributed to the host organisms. In our search for secondary metabolite producing microorganisms we isolated appr. 1400 strains from marine sponges collected at different locations. After prescreening using rapid mass spectrometric tools more than 60% of selected bacteria were positively tested for bioactivity and suggest the possibility for scientific and industrial applications.

Microbial isolation

Bacterial strains were isolated under aerobic conditions from 12 different sponges belonging to the Demospongiae collected from the Sula Ridge, Norway (deep cold-water reef, at 300-400 m) and the Korsfjord near Bergen, Norway (at 200-300 m). Sponges were homogenized, serially diluted in sterile seawater, and plated on agar plates using the spread plate technique. The strains were recovered from pre-isolation plates or continuous cultures using different kinds of heterotrophic and oligotrophic sea-salt based growth media, supplemented with natural substrates.

Intact-Cell MALDI-TOF mass spectrometric analysis

In natural product discovery, the exclusion of previously probed microorganisms from screening programmes, known as dereplication, is a critical factor. Rapid grouping of bacterial isolates may be achieved by proteotaxonomic clustering using Intact-Cell - MALDI-TOF mass spectrometry. Following sub-cultivation on Marine Broth Agar (MBA), portions of bacterial colonies growing on agar plates were spotted onto wells of the MALDI-TOF MS sample plate and mixed with matrix solution (2,5-Dihydroxybenzoic acid). Microbial isolates are characterized by mass spectrometric „fingerprint“ profiling in the mass range from 2000 Da to 20000 Da (ICM-MS, Fig. 1A). At the same time, information on metabolite production can be obtained by recording the low-molecular-weight mass range (Fig. 1B). The method described is very rapid, uses small samples (subcolony amounts of bacteria growing on agar plates), requires minimal sample preparation and can be automated.



ICM-MS vs. 16S rDNA sequencing

Clustering based on MALDI-TOF MS biomarker profiles and 16S rDNA sequence-based grouping showed remarkable congruencies. Even very closely related strains as different *Pseudoalteromonas* species can be discriminated (Fig. 2). 16S rDNA sequencing of selected group representatives therefore allowed for the assignment of mass spectrometric bacterial groups to respective phylogenetic clusters (Fig. 3). Using this approach, duplicate isolates could be easily dereplicated and only group representatives along with rare isolates were selected for further investigations, e.g. to enhance hit rates in subsequent bioassays (Fig. 4).

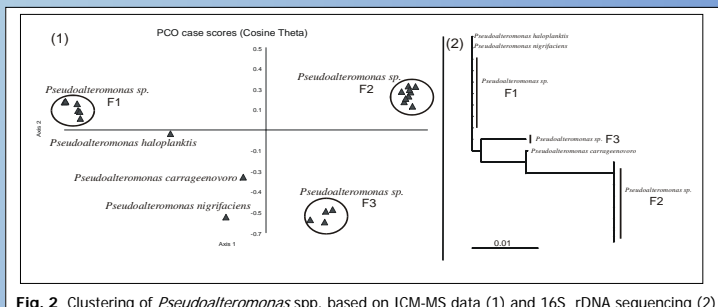


Fig. 2 Clustering of *Pseudoalteromonas* spp. based on ICM-MS data (1) and 16S rDNA sequencing (2)

Conclusions

IC-MALDI-TOF Mass Spectrometry

- is demonstrated to be a valuable tool to rapidly assess the diversity of microbial culture collections
- grouping based on proteomic „fingerprint“ profiles shows remarkable congruence to 16S rDNA based grouping
- allows the dereplication/selection of isolates for further investigations, e.g. to enhance hit rates in subsequent bioassays.

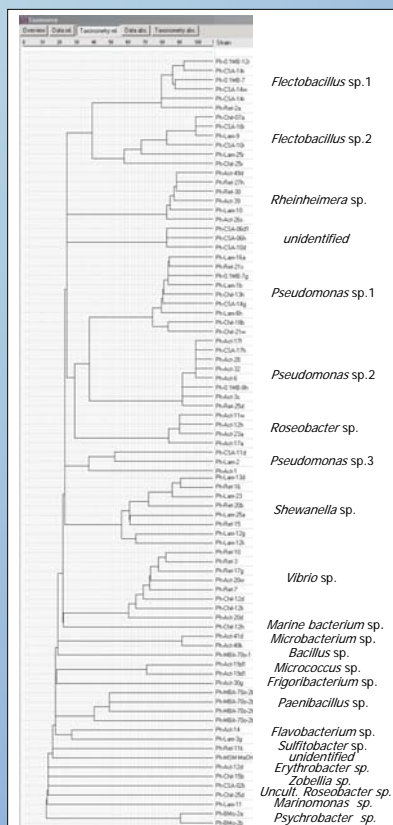


Fig. 3 Dendrogram of a subset of the strain collection isolated from *Phakellia ventrilabrum* based on ICM-MS data. Dendrograms were calculated using the taxonomy feature of the SARAMIS software package (Spectral Archiving and Microbial Identification System, Anagnostec GmbH, Luckenwalde). Phylogenetic assignment was based on partial 16S rDNA sequencing.

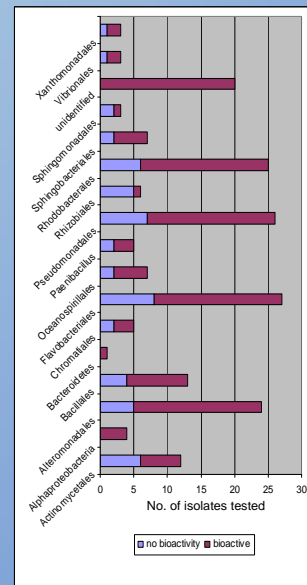


Fig. 4 Distribution of bioactivities exhibited by microbial extracts (orders shown). Bioassays were conducted at the IBWF, Kaiserslautern including antimicrobial, antifungal, nematocidal, phytotoxic and cytotoxic (HeLa S3, Jurkat) activities. Of 190 isolates tested, more than 60 % displayed bioactivity in at least one test.

This strategy is also used to rapidly assess the microbial diversity isolated from different spongal sources and/or sampling locations (Fig. 5a), to optimize isolation strategies using different media (Fig. 5b) and to monitor time-dependent shifts in the cultivated bacterial diversity e.g. from continuous cultures (Fig. 5c).

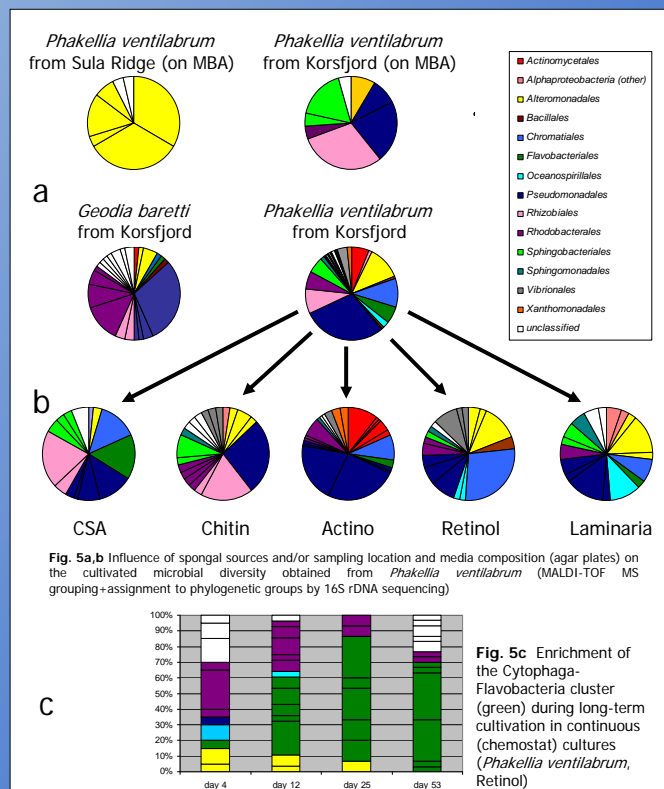


Fig. 5a,b Influence of spongal sources and/or sampling location and media composition (agar plates) on the cultivated microbial diversity obtained from *Phakellia ventrilabrum* (MALDI-TOF MS grouping+assignment to phylogenetic groups by 16S rDNA sequencing)

Fig. 5c Enrichment of the Cytophaga-Flavobacteria cluster (green) during long-term cultivation in continuous (chemostat) cultures (*Phakellia ventrilabrum*, Retinol)

This work is part of the research program BOSMAN II (03F0358B). Financial support was provided by the Bundesministerium für Bildung und Forschung (BMBF) and the the Improving Human Potential Program from the EU, contract No. HPRI-CT-1999-00056, Bergen Marine.

E-mail: ralf.dieckmann@tu-berlin.de