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MARINE BACTERIA - A POTENTIAL SOURCE OF NOVEL BIOACTIVE COMPOUNDS

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Marine microorganisms and their natural products, including novel bioactive compounds needed to combat antibiotic-resistant pathogens, are becoming a valuable resource in biotechnology. We have taken part in a Danish global research cruise circumnavigating the earth in 2006-2007. The purpose of the project and our on-going work is to analyses the production of bioactive compounds in culturable marine bacteria and determine the potential use in biotechnology as well as their possible role in microbial ecology.

Seawater samples (122 from two depths) and more than 300 swab samples from surfaces of eukaryotic organisms, wood or stones were collected. We determine total cell counts using SYBR Cold staining and found approx $.10^6$ cells/ml independantly of sampling location. Culturable counts of seawater was between 10 and 1000 cfu/ml and we replica plated all samples with colony growth onto an agar cast with *Vibrio anguillarum.* Following incubation, we isolated colonies that caused clearing zones in the turbid layer of *V. anguillarum;* indicative of antibacterial activity.

Of the approx. 900 strains isolated, we selected a representative subset of 519 strains for further work. Identification was based on partial 16S rRNA gene sequence homology and the organisms belonged to three major groups: *Vibrionaceae* (309 strains), *Pseudoalteromonas* spp. (128 strains) and the *Roseobacter*-clade (29 strains). Several strains, especially *Vibrio* species, had a marked reduced or absent antibacterial activity upon sub-culturing. The *Pseudoalteromonas* clustered in two main groups: pigmented and non-pigmented and especially the former contained strains with pronounced antibacterial activity. These were identified as *Ps. luteoviolaceae, Ps. rubra, Ps. ruthenica*, and *Ps. piscicida*. We are currently studying the chemistry of the secondary metabolite profile of these organisms. *Ruegeria mobilis* was the most common of the *Roseobacter*-clade strains (25 of 29 strains) and all formed a brown pigment and produced antibacterial compound(s). In a sub-set of strains tested by LC-MS, we identified the antibacterial compound tropodithietic acid.

Apart from the direct antibacterial (bacteriostatic or bacteriocidal effect), we are currently studying other bioactivities of these organisms, namely their ability to interfere with AHL-mediated quorum sensing in Gram-negative bacteria (*Pseudomonas aeruginosa*) and their ability to interfere with virulence factors in *Staphylococcus aureus*. In parallel to this, we are also addressing if the secondary metabolites may have a direct effect on eukaryotic immune cells. We have learned, that studies of marine bacteria are difficult due to the low concentration of compounds produced and the requirement for marine growth media which hampers subsequent chemical purification. However, the study is also demonstrating that marine bacteria are, indeed, a source of an array of bioactive secondary metabolites that have not been studied before and some of these may potentially be of biotechnological interest.