# INTERACTIONS BETWEEN SPONGES AND MARINE BACTERIA AS A ROUTE TO THE DISCOVERY OF NOVEL BIOACTIVE COMPOUNDS

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NOVEMBER 2006

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A thesis presented for the degree of

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# ABSTRACT

The bacterial community architecture associated with four species of sponges, *Halichondria panicea*, *Suberites domuncula*, *S. carnosus* and *Pachymatisma johnstonia* was investigated using culture-dependent and culture-independent strategies. Marine agar was found to be the best of several media used for cultivation of culturable bacteria associated with sponges. Molecular methods, including denaturing gradient gel electrophoresis (DGGE), 16S rDNA cloning and sequence analysis suggested a bacterial community different from that identified using culture-dependent methods. DGGE can provide a profile of the whole community of the sponge and facilitate screening of large-scale samples. 90% of the bacteria associated with these four sponges were sponge species-specific. *S. carnosus* was also transferred to an aquarium to study kinetic changes of sponge-associated bacterial communities. DGGE analysis showed the consistent presence of some particular bands suggesting the continued presence of species of symbiotic bacteria. Four *Bacillus* species (*B. licheniformis* SC-43, *B. subtilis* SD-8, *B. pumilus* HP-48 and *B. cereus* HP-22) isolated from the sponges exhibited antagonistic activity against isolates of Gram-positive bacteria obtained from the same sponges. All strains tested were active against *Micrococcus luteus*, strain HP-5/6 isolated from *H. panicea*. This suggests that HP-5/6 can be used in the laboratory as a sensitive indicator of activity. A comparison of several media found Nutrient Agar/ Broth containing glycerol and iron (NGF) to be the best medium tested for antimicrobial compound production. *B. licheniformis* (SC-43), *B. subtilis* (SD-8), and *Pantoea* sp., SC-AF, in the presence of glycerol and ferric ion, could produce antimicrobial compounds when grown within biofilms; however, the corresponding shaken flask cultures could not. This effect could be related to oxidative stress defence responses. *Pantoea* sp., SC-AF produced several antimicrobial compounds active against *M. luteus*, HP-5/6 which were different from previously reported Pantocin antimicrobials. In addition, *Pantoea* sp., SC-AF produced 'jelly-like' extracellular polysaccharide (EPS) on NGF and on the nylon membrane in Air-membrane surface bioreactor (AMS) cultures, along with the production of antimicrobial compounds. Only fructose and cellobiose after acid lysis of EPS of *Pantoea* sp., SC-AF have been identified. In addition, my study confirmed that sponges accommodate large amounts of uncultured bacteria, whose metabolic capability cannot be explored without cultivation. New cultivation strategies should be investigated and biofilm-based culture techniques incorporated in the future search for novel antibiotics.