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Antagonistic Activity of a Marine Bacterium *Pseudoalteromonas luteoviolacea* TAB4.2 Associated with Coral *Acropora* sp.

[1,2]Ocky Karna Radjasa, [3]Torben Martens, [3]Hans-Peter Grossart,
[3]Thorsten Brinkhoff, [1,2]Agus Sabdono and [3]Meinhard Simon
[1]Department of Marine Science, Diponegoro University, Semarang-50275, Central Java, Indonesia
[2]Center for Tropical Coastal and Marine Studies, Diponegoro University,
Widya Puraya, Semarang-50275, Central Java, Indonesia
[3]Institute for Chemistry and Biology of the Marine Environment,
Carl von Ossietzky Universität Oldenburg, Carl-von-Ossietzky.Str. 9-11, 266111 Oldenburg, Germany

Abstract: A coral-associated bacterium was successfully screened for secondary metabolite production based on biological activity and PCR amplification of the non-ribosomal peptide synthetase (NRPS) gene and was identified as closely related to *Pseudoalteromonas luteoviolacea* based on its 16S rDNA. The bacterium was found to inhibit the growth of other coral-associated bacteria and pathogenic bacteria. To characterize the inhibiting metabolite, a 279 bp long DNA fragment was obtained and the deduced amino acid sequence showed conserved signature regions for peptide synthetases and revealed a high similarity to NosD (40% identity), a multifunctional peptide synthetase from *Nostoc* sp. GSV224 and NdaB (44% identity), a peptide synthetase module of *Nodularia spumigena*. To estimate the possible role of secondary metabolites, analyses on the quantitative proportion of *Pseudoalteromonas* was carried out. The results revealed that *Pseudoltermonas* group was indeed present within surfaces of coral *Acropora* sp.

Key words: Coral-associated bacterium, antagonistic activity, *Pseudoalteromonas*, *Acropora* sp.

INTRODUCTION

Coral reefs are the most diverse marine ecosystems, however, little is known about the microbial diversity in these ecosystems. It is well understood that corals harbor diverse microbial communities (William *et al*., 1987; Shashar *et al*., 1994; Kim 1994; Kushmaro *et al*., 1996; Rohwer *et al*., 2001). Their surface is covered by mucopolysaccharides, which provides a matrix for bacterial colonization leading to the formation of biofilm-forming microbial communities (Kushmaro *et al*., 1997).

Marine organisms including those from coral reef ecosystems have become sources of great interest to natural product chemistry, since they provide a large proportion of bioactive metabolites with different biological activities (Faulkner, 2000). In particular, marine invertebrates with high species diversity in the Indo-Pacific regions (Coll and Sammarco, 1986) are often rich in secondary metabolites and are preferential targets in the search for bioactive natural products (Sammarco and Coll, 1992).

Perhaps the most significant problem that has hampered the investigation of secondary metabolites is their low concentration (Munro *et al*., 1999). In marine invertebrates many highly active compounds contribute to $<10^{-6}$% of the body-wet weight (Proksch *et al*., 2002). Providing sufficient amounts of these biologically active substances, hence, may be a difficult task. Limited amounts found in the producing organism, limited quantities of the organism itself and geographic or seasonal variations in the produced secondary metabolites (Kelecom, 2002), further complicate the study of secondary metabolites of aquatic organisms.

It is a widely observed phenomenon that microbial cells attach firmly to almost any surface submerged in marine environments, grow, reproduce and produce extracellular polymers that provide structure to the assemblage termed as biofilm (Kiorboe *et al*., 2003). Due to the close spatial vicinity of these biofilm-forming bacteria, it can be expected that the indigenous microbial population is adapted to competitive conditions, e.g. for available nutrients and space (Slattery *et al*., 2001). The production of secondary metabolites is a common adaptation of these bacteria to compete in such microenvironments (Long and Azam, 2001; Grossart *et al*., 2004). Many coral-associated bacteria have been characterized as sources of a great variety of marine natural products (Moore, 1999), especially since the coral

Corresponding Author: Ocky Karna Radjasa, Department of Marine Science, Diponegoro University, Semarang-50275, Central Java, Indonesia Tel: 62-24-7474698/7460038 Fax: 62-24-7474698/7460039