

Richness of Secondary Metabolite-Producing Marine Bacteria Associated with Sponge *Haliclona* sp.

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Abstract: A total of 8 bacterial isolates associated with sponge *Haliclona* sp. collected from Bandengan water, Jepara, North Java Sea, Indonesia, was successfully screened for antibacterial activity against pathogenic bacteria *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Staphylococcus aureus*. Active bacterial isolates were rapidly grouped by using rep-PCR and a dendrogram was constructed. Five isolates were selected based on the constructed dendrogram for subsequent DNA sequencings resulted in the richness of secondary metabolite-producing sponge associated-bacteria having closest similarity to *Vibrio parahaemolyticus*, *Pseudovibrio denitrificans*, *Pseudoalteromonas* sp., alpha proteobacterium and uncultured bacterium clone. The present study highlighted the repetitive-PCR method as a powerful tool for estimating the richness of secondary metabolite-producing parts among sponge colonizers.

Key words: Richness, rep-PCR, sponge-associated bacteria, secondary metabolite, *Haliclona*

INTRODUCTION

The occurrence of large scale of bioactive compounds is not common to all living organisms, but restricted to certain taxonomic groups. Among marine animals, reef's invertebrates are the most prolific producers of secondary metabolites and have become sources of great interest to natural product chemistry, since they provide a large proportion of bioactive compounds with different biological activities.

Sponges (phylum Porifera) are most primitive of the multicelled animals that have existed for 700-800 million years. Of the approximately 15,000 sponge species, most occur in marine environments. Only about 1% of the species inhabits freshwater (Belarbi *et al.*, 2003).

It has been known that sponges produce secondary metabolites to repel and deter predators (Pawlik *et al.*, 2002), compete for space with other sessile species (Davis *et al.*, 1991; Becerro *et al.*, 1997) and for communication and protection against infection. In addition, potentially therapeutic compounds identified in sponges include anticancer agents and immunomodulators. Some sponges seem to produce potentially useful antifouling agents (Hellio *et al.*, 2005).

Recent research progresses reported that many bioactive natural products from marine invertebrates have striking similarities to metabolites of their associated microorganisms including bacteria (Proksch *et al.*, 2002; Thiel and Imhoff, 2003; Radjasa *et al.*, 2007a). Thus, it is important to highlight the possible role of marine bacteria associated with sponges in providing solution to the problem of infection by pathogenic bacteria. Bacteria-sponge association that occurs on the sponges then could be of great interest to search for potential use as new source of antibiotics in particular as a solution of the problem of supply of most bioactive compounds produced by reef's invertebrates.

Advanced techniques of molecular biology such as Polymerase Chain Reaction (PCR), has played important roles in estimating the richness of marine microorganisms. Urakawa *et al.* (1999a and b) and Radjasa *et al.* (2001) used Restriction Fragment Length Polymorphism (RFLP) technique for rapid grouping of large number of marine psychrophilic and psychrotrophic isolates. Very recently, Radjasa *et al.* (2007c) successfully applied repetitive-PCR for grouping marine psychrotrophic bacteria collected from deep-sea waters of Makasar strait, Indonesia. To our knowledge, this technique has not been employed to