Journal of Coastal Development ISSN: 1410-5217
Volume 7, Number 2, February 2004 : 65-70 Accredited: 69/Dikti/Kep/2000

Review

MARINE INVERTEBRATE-ASSOCIATED BACTERIA IN CORAL REEF ECOSYSTEMS AS A NEW SOURCE OF BIOACTIVE COMPOUNDS

Ocky Karna Radjasa [1,2*)]

[1] Department of Marine Science, Diponegoro University, Semarang 50275, Indonesia
[2] Center for Tropical Coastal and Marine Studies, Diponegoro University,
Semarang 50275, Indonesia

Received: November 28, 2003 ; Accepted: January 12, 2004

ABSTRACT

Coral reefs are the most species-rich environments in the oceans. Reefs cover 0.2% of the ocean's area and yet they provide home to one-third of marine fishes and to tens of thousands of other species. Coral reefs provide essential fish habitat, support endangered and threatened species, and harbor protected marine mammals. Despite the obvious ecological value of these habitats, most coral reefs around the world, including Indonesia's, are threatened or already being destroyed by human activities. The search for bio-active compounds extracted from coral reef invertebrates which is emerging as an area of increasing interest among biotechnological companies, further threatens the integrity of the reef ecosystem.

It would be of great interest to find alternative sources of these compounds, in order to preserve this precious environment and also to obtain higher amounts of these bi-active molecules. Increasing observations suggest that a number of bio-active metabolites obtained from invertebrates are in fact produced by associated microorganisms: this has prompted research into the rapidly expanding field of study of metabolites derived from microorganisms associated with reef invertebrates. The possibility to culture relevant microorganisms in bioreactors would enable the production of large amounts of the bio-molecules of interest, at the same time preserving the marine ecosystem from exploitation.

Key words: Marine invertebrate-associated bacteria, coral reefs, bioactive compounds

***) Correspondence**: Phone: +62-24-7460038; Fax: +62-24-7460039:
E-mail: ocky_radjasa@yahoo.com

INTRODUCTION

Coral reef ecosystems are unique, biologically diverse systems recognized as valuable economic and environmental resources. The occurrence of large amounts of secondary metabolites is not common to all living organisms, but restricted to certain taxonomic groups. Among animals, arthropods, coral reef coelenterates and other marine invertebrates are the most prolific

producers of secondary metabolites. Thus, these organisms have been the main targets in the search for secondary metabolites with various biological activities from coral reef ecosystems.

Serious obstacle to the ultimate development of most marine natural products that are currently undergoing evaluation and trials is the problem of supply. The concentrations of many highly active compounds in marine invertebrates are often minute, sometimes accounting for less than 10^{-6}% of the wet weight (Procksch *et al*, 2002). In addition, it has often been proven extremely difficult, and some cases impossible, to provide from invertebrates or macroalgae sufficient amounts of many of these substances due to limited amounts found in the producing organism, or to limited quantity of the organism itself, or to geographic, seasonal or sexual variations in the amounts and in the nature of produced secondary metabolites. Thus, new ways have to be found.

It has been clearly established that microbial cells attached firmly to almost any surface submerged in marine environments. The cells grow, reproduce, and produce extracellular polymers that provide structure to the assemblage termed as biofilm. Paul *et al* (1986); Coffroth (1990) and Kim (1994) mentioned that it is not surprisingly mucus-covered coral surfaces are often covered by microorganisms mostly undestructive.

In relation to bacteria associated with reef invertebrates, particularly their interaction among surface colonizers, it is reasonable to expect that the indigenous microbial population may be adapted to a competitive conditions, at least in part, due to the competition on the available nutrients. Thus, one possible mechanism for these bacteria to survive is by producing secondary metabolites that enhance their existence.

Studies regarding screening on secondary metabolites-producers among invertebrate-associated bacteria are important for understanding principal processes of inhibitory interaction among coral-associated bacteria as well as their biotechnological potentials.

It is also expected that there are still a number of unexplored culturable coral-associated microorganisms in the reef environments. Such information might be desirable, as some of these bacteria may serve beneficial purposes as a source of secondary metabolites including novel natural products such as antibiotics, lipids, pigments, and pharmaceuticals.

THE GLOBAL STATUS OF RESEARCH

Since 1995, there are signals of decreased interest in the search of new metabolites from traditional sources such as macroalgae, molluscs, tunicates, soft corals and octocorals, and the number of annual reports on marine sponges stabilized. On the contrary, the metabolites from microorganisms is a rapidly growing field, due, at least in part, to the suspicion that a number of metabolites obtained from algae and invertebrates may be produced by associated microorganisms(Fenical, 1993; Pietra, 1997; Bernan et al, 1997; Faulkner et al, 2000; Jensen and Fenical, 2000). Although it is still too early to define tendencies, it may be stated that the metabolites from microorganisms are in most cases quite different from those produced by invertebrate hosts(Kelecom, 2002).

The importance of terretrial bacteria and fungi as sources of valuable bioactive compounds has been very well established for more than half a century. As a result, over 120 of the most important

medicines in use today (penicillins, cyclosporin A, adriamycine, etc) are obtained from terrestrial microorganisms. At first sight thus, the expectable enormous biodiversity of marine microorganisms might have been the reason for the interest in the study of marine bioactive compounds (Kelecom, 2002).

Due to cultivation biases only a minor fraction of heterotrophic microorganisms in the coral reefs has yet been isolated. More information on coral-associated bacteria might be desirable, as many of these bacteria serve as sources of secondary metabolites including novel antibiotics.

THE CURRENT STATUS OF RESEARCH IN INDONESIA

The oceans are the source of a large group of structurally unique natural products that are mainly accumulated in invertebrates such as sponges, tunicates, bryozoans, soft corals and molluscs (Proksch et al, 2002). Several of these secondary metabolites showed pronounced pharmacological activities and are interesting candidates for new drugs.

Indonesia is the world's largest archipelagic state with approximately 17,508 islands containing valuable coastal and marine resources such as coral reefs. Coral reefs are some of the most productive ecosystems on earth (Grigg et. al, 1984), and are certainly the most productive and species-rich environments in the oceans.

One of the most important issues faced by Indonesian reef ecosystems is the search for bioactive compound from coral reef ecosystems. Thus research on the search of bioactive compounds from invertebrate-associated bacteria should be given much greater prominence.

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 (Kioerboe et al. 2003). Recently many coral-associated bacteria have been characterized as sources of marine natural products (Moore, 1999), especially since the coral surface is more nutrient rich than seawater or even sediments (Unson et al, 1994; Bultel-Ponce et al, 1999). However, colonization of coral surfaces by bacteria and other microorganisms is mostly nondestructive to corals (Paul et al, 1986; Coffroth, 1990 and Kim, 1994).

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. There has been less report been documented on exploration of coral-associated bacteria in Indonesia as source of secondary metabolites.

PCR-BASED APPROACH FOR INCREASING THE SCREENING EFFICIENCY

The 16S rDNA-based approaches have become a standard for studying the phylogeny and diversity of marine microorganisms (Bowman *et al*, 1997, 1998; DeLong *et al*, 1997; Nogi *et al*, 1998; Radjasa *et al*, 2001a, 2001b; Urakawa *et al*, 1999a, 1999b). In addition, 16S rDNA-based approach has been very

successful in the search of secondary metabolites in particular by using specific degenerated primers (Konz and Marahiel, 1999; Seow, 1997).

Polyketides and non-ribosomal peptides represent large families of secondary metabolites and numerous natural products belonging to these groups are widely used as pharmaceuticals, industrial agents or agrochemicals (Silakowski et al, 2000). Both types are biosythesized by extremely large polyfunctional enzyme systems within the protein. The responsible biosythetic proteins are known as polyketide synthetases (PK-Ss) and non-ribosomal polypeptide sythetases (NRP-S) (Cane, 1997).

In this context, it is important to assess the application of PCR-based approach on screening of coral-associated microbial populations with specific consideration of the secondary metabolites-producing parts which have been up to now strongly neglected in comparison to the invertebrate parts.

The application of molecular approach through PCR using specific primers provides rapid detection and is suitable to greatly improve the screening efficiency for secondary metabolite-producer among invertebrate-associated bacteria. Understanding the genetic basis as well as the biochemistry of specific group of bacteria will facilitate genetic engineering aimed at improving design of bioactive substances.

CONCLUDING REMARKS

Coral reefs are in the center of global interest concerning diversity and climatic changes. The significance of coral reefs as the source for microbial bioactive substances is still under development. Studies regarding screening on secondary metabolites-producers among invertebrate-associated bacteria are important for understanding principal processes of inhibitory interaction among invertebrate-associated bacteria as well as their biotechnological potentials.

The possibility to culture relevant microorganisms in bio-reactors would enable the production of large amounts of the bio-molecules of interest, at the same time preserving the marine ecosystem from exploitation. The results of the works on the search for bioactive compounds from reef invertebrate-associated bacteria will further influence isolation approaches and will show alternative choice in order to obtain the representatives of secondary metabolites-producing bacteria from coral reefs.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Paul Harrison of McMaster University for the inspiring discussion. Contributions were provided by Dr. Paul W Sammarco of Louisiana Universities Marine Consortium (LUMCON) that making this review possible.

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