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BACTERIAL SYMBIONTS OF REEF'S INVERTEBRATES: A MARINE NATURAL DRUG'S FACTORY

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ABSTRACT

Marine invertebrates that are mainly accumulating within coral reef ecosystems such as soft corals, sponges, tunicates, and bryozoans have long been recognized as the prolific sources of structurally unique and diverse natural products since they provide a large proportion of bioactive compounds with different biological activities.Unfortunately, the supply of these bioactive natural products is usually insufficient to meet the ultimate development of most marine natural products. The concentrations of many highly active compounds in reef's invertebrates are often minute, accounting for less than 10^{-6}% of the wet weight. This problem has been viewed as the most significant threat regarding the development of pharmaceutical from reef's invertebrates. The secondary metabolites from bacterial symbionts, on the other hand,is a rapidly growing field, due to the suspicion that bioactive metabolites obtained from invertebrates may be produced by their bacterial symbionts. In particular, from sustainability point of view, isolating bioactive-producing bacteria is obviously offers a much better approach than cultivating and harvest invertebrates, which are in most cases extremely difficult.Bacteria isolated from living surfaces, in particular from reef's invertebrates, are a promising source of natural products. It is expected that still quite a few parts of unexplored culturable bacterial symbionts exists in the reefs. Such information might be desirable, as these bacterial symbionts may serve beneficial purposes as the source of secondary metabolites including novel marine natural products.

Key Words: bacterial symbionts, marine natural products, reef's invertebrates

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INTRODUCTION

The oceans are the source of a large group of structurally unique natural products that are mainly accumulated in invertebrates that are common to coral reef ecosystems, such as sponges, tunicates, bryozoans, soft corals and molluscs. This diversity has been the source of unique chemical compounds with the potential for industrial development as pharmaceuticals, cosmetics, nutritional supplements, molecular probes, enzymes, and agrichemicals. Thus, coral reef represents a virtually unexploited resource for discovery of even more novel compounds with useful applications.

There are several limitations have been recognized in the utility of marine natural products. Serious obstacle to the ultimate development of most marine natural products that are currently undergoing evaluation and trials is the problem of supply due to their low concentrations. The concentratotions of

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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). The problem of supply has been viewed as the most significant threat regarding the development of pharmaceutical from reef's invertebrates. Providing sufficient amounts of these biologically active substances, hence, may be a difficult task. Limited amounts and low yields of bioactive compounds, further complicate the study of secondary metabolites of aquatic organisms (Radjasa *et al.*, 2007a; Sukarmi and Radjasa, 2007).

Furthermore, source organisms, mainly invertebrates can be difficult to culture or even, may not produce the bioactive compound of interest under the given culture conditions. In the development phase, if the compounds cannot be synthesised or obtained by fermentation technology, there is a pressing need for supply of these compounds through harvesting from the wild. This has been viewed as detrimental to the marine environment.

Understanding of marine invertebrate-microbial associations is a fundamental step in studying biologically potential active, possible medicinal compound from associated microorganisms. In particular, from sustainability point of view, isolating bioactive-producing bacteria is obviously offers a much better approach than cultivating and harvest invertebrates, which are in most cases extremely difficult.

Unfortunately, our knowledge on the chemical ecology of marine invertebrate-associated bacteria in the coral reefs is at present rudimentary. An understanding of chemically-mediated interaction among invertebrate-associated bacteria coupled with biotechnological based-methods could be an alternative choice for the search of marine natural products.

Natural products from reef's invertebrates

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 tropical coral reefs are often rich in secondary metabolites and are preferential targets in the search for bioactive natural products.

Many marine species have been collected in the search for novel bioactive compounds and for developing pharmaceutical drugs (Quinn *et al.*, 2002). The collections tend, however, to focus on organisms containing chemicals, known as secondary metabolites that primarily serve ecological functions in competition for space and in protection from predation, fouling, and ultraviolet light, as well as from bacterial infections (Rohwer *et al*., 2002)

So far, most of novel compounds have been secondary metabolites from soft-bodied, sessile invertebrates, such as Porifera (sponges); Cnidaria (jellyfish, corals, sea anemones); and Urochordata (ascidians) (Hunt and Vincent, 2006). Table 1 lists some examples of bioactive metabolites from reef's invertebrates.

Bacterial symbionts as a sustainable source of natural products

The supply of marine metabolites tested in the pre-clinic and clinic could be provided by several methods, namely open aquaculture of the invertebrates, total synthesis, semi synthesis and fermentation of the producing microbes. It is agreeable that fermentation is seen as the most appropriate method for the production of natural products (Solomon *et al.*, 2004). Furthermore, the metabolites from microorganisms is a rapidly growing

Table 1. Some examples of bioactive metabolites from marine invertebrates

No	Metabolites	Sources	References
1	Ziconitide	Cone shell, *Conus magnus*	Olivera, (2000)
2	Dorisenone A	Nudibranch *Chromodoris obsoleta*	Miyamoto, (2006)
3	Bryostatin	Bryozoan *Bugula neritina*	Hunt and Vincent (2006)
4	Ecteinascidin 743	Ascidian *Ecteinascidia turbinata*	Mendola, (2000)
5	Pseudopterosin	Soft coral *Pseudopterogorgia elisabethae*	Mayer *et al.*, (1998)
6	Halicondrin	Sponge *Lissodendroryx* sp.	Hart *et al.*, 2000

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 (Kelecom, 2002).

Symbiotic systems in which there is a strong likelihood of microbial bioactive metabolite synthesis offer attractive alternatives to chemical synthesis or extraction from natural sources. Symbionts that can be cultivated in the laboratory and still produce the bioactive metabolite could be subjected to fermentation technology to produce large amounts of the compound (Hildebrand *et al.*, 2004)

Studies regarding screening on secondary metabolites-producing bacterial symbionts are important for understanding biotechnological potentials. In this context, it is of importance to assess the application of sustainable approach on the screening of invertebrate-associated microbial populations (Radjasa and Sabdono, 2003; Radjasa *et al.*, 2007a).

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). In addition, surfaces of many marine invertebrates providing a nutrient rich habitat for heterotrophic bacteria that leading to the formation of biofilm-forming microbial communities.

It has been estimated that less than 2% of microbial flora have been successfully isolated from marine environment as pure cultures. It is expected that still quite a few parts of unexplored culturable invertebrate-associated microorganisms exists in the reef environments. Thus, such information might be desirable, as some of these bacteria may serve beneficial purposes as the source of secondary metabolites including marine natural products (Radjasa and Sabdono, 2003; Radjasa *et al*., 2007a, 2007b).

It has been suggested that natural products from marine invertebrates have striking similarities to metabolites of their associated microorganisms (Proksch *et al*., 2002). Thus, it is important to highlight the possible role of bacteria associated with marine invertebrates as an alternative of biologically active substances. The results may further influence isolation approaches and will show alternative choice in order to obtain representative reef's active metabolites coral reef ecosystems without endangering this precious environment.

Application of Molecular Based-Approaches in the Search of Bacterial Symbiont's Products

The advanced progresses in molecular biology have dramatically changed marine natural product studies. The biological and chemical approaches have now being developed to understand the biosynthesis of completely novel, complex metabolites from

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marine organisms. The molecular perspective has focused on some of the most pharmaceutically useful and structurally interesting microbial metabolites belonging to the biosynthetic classes of polyketides and non-ribosomal peptides (NRPS) (Solomon *et al.*, 2004).

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 biosynthesized by extremely large polyfunctional enzyme systems within the protein. The responsible biosynthetic proteins are known as polyketide synthases (PKS) and non-ribosomal polypeptide synthetases (NRPS).

Recently, PCR amplification of degenerate primers targeted to sequences of genes essential in the biosynthesis of particular secondary metabolites has been used to estimate the genetic ability of microorganisms to produce various compounds belonging to Non-ribisomal peptide synthetases(NRPS) (Marahiel *et al.*, 1997; Ayuso-Sacido and Genilloud 2004), polyketide synthases (PKS) (Metsa-Ketela *et al.*, 2002; Piel *et al.*, 2004) and halogenases (Piraee and Viing 2002). Table 3 lists of selected degenerated primers used for PCR-based screening.

Table 2. Selected PCR primers targeted to amplify NRPS and PKS gene sequences

No	Degenerated primers	Target	References
1	K1 (5'TSAAGTCSAACATCGGBCA3') M6R (5'CGCAGGTTSCSGTACCAGTA3')	PKS gene sequences of Actinomycetes	Ayudo-Sacido and Genilloud (2004)
2	KSDPQQF (5´MGNGARGCNNWNSMNATG GAYCCNCARCANMG3´) KSHGTGR (5´GGRTCNCCNARNSWNGTN CCNGTNCCRTG 3´)	PKS gene sequences of Non-Actinomycetes	Piel, (2002)
3	A3 (5'GCSTACSYSATSTACACSTCSGG3') A7R (5'SASGTCVCCSGTSCGGTAS3')	NRPS gene sequences of Actinomycetes	Ayudo-Sacido and Genilloud (2004
4	A2gamF (5'AAGGCNGGCGSBGCSTAYSTGCC3') A3gamR (5'TTGGGBIKBCCGGTSGINCCSGAGGTG3')	NRPS gene sequences of Non-Actinomycetes	Radjasa *et al.* (2007a)

The facts that many bacterial symbionts are unculturable, subsequently culture-independent approach, widely known as metagenomic library can be prepared from invertebrate total DNA, is preferred option. Both biosynthetic polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) genes can be examined by PCR.

Another favourable aspect is that, unlike their invertebrate hosts, genomes of bacteria and archaea are small and their biosynthetic pathways tend to be organized in continaeus regions of DNA (operons). These features greatly facilitate cloning of these pathways. Expression technology for bacterial genes is well developed, making cloning and expressing biosynthetic genes of bacterial symbionts entirely feasible. In the case of uncultivable symbionts, this provides the only way to produce bioactive metabolites in a culture system. For both

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cultivable and non-cultivable symbionts, cloning and expressing bioactive metabolite genes offer the possibility of providing sufficient amounts of compounds for drug development that could not otherwise be obtained, and open an avenue for combinatorial biosynthesis later on (Hildebrand *et al.*, 2004).

CONCLUDING REMARKS

When a marine bio-product from marine invertebrate has proved to present interesting and promising properties, the commercial source of choice for the pharmaceutical industry is its synthesis, which allows the company to control all aspects of production. But, unlike terrestrial bio-compounds, many bioactive marine natural products from marine invertebrates, particularly those used in the pharmaceutical field, are extremely complex in structure, and require intensive multi-step processes that are not amenable to economic, industrial-scale synthesis.

In this context, it is of importance to assess the application of sustainable approach on the screening of bacterial symbionts of marine invertebrates, with specific consideration of the secondary metabolites-producing part which has been up to now strongly neglected in comparison to the invertebrate. The anticipated results could further show alternative choice in order to protect coral reefs from the search of bioactive compounds and to obtain the representatives of bioactive compounds from these ecosystems. Thus research on the search of bioactive compounds from bacterial symbionts from reef's invertebrates should be given much greater prominence.

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