Urgency and Mechanism of Biosynthesis of Marine Microbial Secondary Metabolites

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Abstract

Marine microorganism is one of biologically active potential resources of secondary metabolites. Its potency are so promising that the knowledge of how its secondary metabolite occured need to be studied and collected. Those knowledges will enable further study is improving secondary metabolite production in the laboratory. In nature, secondary metabolites synthesis occur when there are effect of both biotic and abiotic factors such as sea water and microbe symbiosis with other living materials. When this is explained in metabolic pathways, secondary metabolite synthesis affected by available nutrient and regulated by autoinducer molecules through quorum sensing mechanism.

Keywords: *Autoinducer, Marine Microorganism, Quorum-Sensing, Secondary Metabolite, Symbiotic.*

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A. INTRODUCTION

The sea is one of the sources of biological and chemical wealth. One of the sources of biological and chemical wealth can be obtained from marine bacteria. Although marine bacteria make up a small portion of marine life, one cell of marine bacteria contains thousands of potential chemical compounds for medicines, nutritional supplements, cosmetics, agrochemicals, chemical probes and enzymes. Generally these potential chemical compounds come from microbial secondary metabolites.

Metabolites are classified into two, namely primary metabolites and secondary metabolites. Primary metabolites formed in limited amounts are essential for the growth and life of living things. Secondary metabolites are not used for growth and are formed from primary metabolites under stress conditions. Examples of secondary metabolites are antibiotics, pigments, toxins, ecological and symbiosis competition effectors, pheromones, enzyme inhibitors, immunomodulatory agents, antagonist and agonist receptors, pesticides, antitumor agents, and animal and plant growth promoters.

There are several hypotheses about the function of secondary metabolites for secondary metabolite producers, such as in the survival of bacteria, fungi, insects, and animals through the production of antibiotics and antifouling (Gudbjarnason 1999). In addition, secondary metabolites also play a role in improving the microbial life of secondary metabolite producers when compete with other species (Tabarez 2005). There are 5 reasons to strengthen this (Tabarez 2005). First, secondary metabolites act as alternative defense mechanisms so that organisms deficient in the immune system will produce abundant and varied secondary metabolites. Second,

secondary metabolites have a sophisticated structure and mechanism of work and their metabolic pathways are complex and energetically expensive. Third, secondary metabolites act when there is competition with microbes, plants, or animals. Fourth, secondary metabolites are produced by a group of biosynthetic genes. Fifth, the production of secondary metabolites with antibiotic activity is usually accompanied by sporulation and occurs in microbial cells that are sensitive to microbes, plants, or animals. Generally these sensitive microbes need special protection when their nutrients begin to deplete.

The formation of secondary metabolites is regulated by nutrition, decreased growth rate, feedback control, enzyme inactivation, and enzyme induction. Nutritional limitations and decreased growth rate will produce signals that have a regulatory effect resulting in chemical differentiation (secondary metabolites) and morphological differentiation (morphogenesis) (Demain 1998). This signal is a low molecular weight inducer that acts as a negative control so that under normal conditions (rapid growth and adequate nutrition) prevents the formation of secondary metabolites and morphogenesis. Unlike primary metabolites, secondary metabolite pathways are not widely understood. Therefore, in this review article we will try to discuss the occurrence of secondary metabolites in nature and the factors that influence the biosynthesis mechanism of secondary metabolites of marine microbes.

B. METHOD

This study used a qualitative approach by collecting data with a literature study on Urgency and Mechanism of Biosynthesis of Marine Microbial Secondary Metabolites. The data is then analyzed and analyzed to produce a research framework.

C. RESULT AND DISCUSSION

1. Biosynthesis of Marine Microbial Secondary Metabolites in Nature

The results of secondary metabolite exploration have shown that marine bacteria are one of the potential sources of secondary metabolites. Based on its way of life, secondary metabolite-producing bacteria can be derived from free-living bacteria, sedimentary bacteria found in sediments, bacteria associated with algae surfaces, or bacteria associated with invertebrates (Burgess et al, 1999). Based on the results of previous research, generally bacteria that live in a way associated with marine life show great potential in the secretion of secondary metabolites with antibacterial properties (Burgess et al, 1999; Armstrong et al, 2001; Yan et al, 2003). Bacteria that live in contact with certain particles produce secondary metabolites 5-10 times higher than free living bacteria (Long 2001). An example of a bacterium producing a secondary metabolite of the sea is Actinopolyspora species AH1 obtained from marine sediment and showing antimicrobial activity (Kokare et al, 2003). Epibiotic bacteria taken from Petrosia ficiformis are able to inhibit the growth of other marine bacteria in vitro (Chelossi et al, 2004). Pseudoalteromonas piscicida

associated with the sponge Hymeniacidon perleve produces the compound norharman (an alkaloid betacabbolin) which has antimicrobial activity (Zheng et al, 2005).

Generally the chemical structure of marine products is often different from terrestrial secondary metabolites especially in halogenation with bromine and or chlorine (Gudbjarnason 1999). These differences are influenced by the unique marine environment. According to Okami (1982), there are 3 facts that prove that the marine environment is unique. First, seawater contains a variety of biologically active substances such as vitamins, and many marine microorganisms are capable of producing vitamins. Second, seawater contains active inhibitor agents for the organism. Some factors that illustrate this fact are that seawater has the ability to inhibit gram-positive bacteria, natural seawater is more inhibitory than artificial seawater, seawater that has been given heat treatment shows a reduction in inhibitor activity compared to fresh seawater, seawater inhibitor activity is not caused by faga or salinity but because there are antibacterial agents in seawater. Third, some microorganisms isolated from seawater show antibacterial activity.

Symbiosis between microbes and invertebrates is a rule used by microbes in producing what kind of secondary metabolites will be produced (Thakur et al, 2003). Generally, microbial-produced secondary metabolites are used by marine invertebrates to fight off other organisms. Based on the above obtained a new concept that states that the symbiosis that produces secondary metabolites can be triggered due to biotic environmental barriers. The model used to explain this concept is a bacterial symbiosis with a sponge.

At first the secondary metabolite cells to complete protection against microbial or eukaryote attack (first direct protection), for example acetylenic compounds. In addition, sponges can also produce secondary metabolites in the form of proteins that can resist bacterial growth (protection with the immune system), for example perforin (Thakur et al, 2003) and tachylectin (Schroder et al, 2003). Functionally, these compounds act as defense molecules. Due to the interaction of secondary metabolites produced with bacteria associated with sponges, it is possible for bacteria to be induced to produce a secondary metabolite. The resulting secondary metabolites have various functions, for example functioning in the defense system as well as activating important pathways for self-defense (metabolite activators). An example of a bacterial secondary metabolite is okadaic acid (okadaic acid) which is produced by bacteria in the sponge Suberites domuncula. Okadaic acid acts as a defense molecule against foreign metazoan attack and simultaneously is a positive modulation of this pathway to enhance the immune response of host cells (Wiens et al, 2003). Bacteria that live on the surface of spongy host cells produce specific secondary metabolites to fight certain bacteria (indirect protection), for example antifouling compounds (Thakur et al, 2003) and tribromophenol compounds (Clare et al, 1999). Examples of secondary metabolites produced as a result of the symbiosis between sponges and bacteria or fungi,

bacteria or fungi are also induced to produce secondary metabolites such as cribrostatin or sorbicillactone.

In addition to spongy symbiosis microbes, also symbiosis with algae such as the green algae Enteromorpha linza symbiosis with the bacteria Flavobacterium spp. and Cytophaga spp. (Shiba & Taga 1980). E. linza produces extracellular products which are absorbed by Flavobacterium spp. and Cytophaga spp. which compose biofilms on algae. The green pigment bacteria Pseudoalteromonas tunicata which also forms biofilms can produce specific target inhibitor compounds against bacteria, algae, fungi, and invertebrate larvae (Prochnow et al, 2004).

2. Secondary Metabolite Biosynthesis is Influenced by the Availability of Certain Nutrients.

Primary metabolites can increase the production of secondary metabolites. In marine microbes, environmental conditions with limited nutrition mean that the use of carbon by marine microbes in cellular metabolism is not used for cell growth but the available carbon will be used for the production of secondary metabolites. The experiments of Barry and Wainwright (1997) proved that the addition of primary metabolite precursors can increase secondary metabolites. The primary metabolite precursors used were ± -ketoglutarate, ²-ketoglutarate, glucose, and oxaloacetate. Marine bacteria J292 / 97 were inoculated in various media, namely Marine Broth Difco (MB), 10X diluted MB (MB 1: 9), MB 1: 9 supplemented with 0.1% ± -ketoglutarate, MB 1: 9 supplemented with 0.1% ²-ketoglutarate, MB 1: 9 supplemented with 0.1% glucose, MB 1: 9 supplemented with 0.1% oxaloacetate. The growth media supplemented with TCA (Tricarboxylic acid) cycle precursors, namely ± -ketoglutarate and oxaloacetate, will induce compounds that have antimicrobial activity. Pseudomonas fluorescens HV37a synthesizes antibiotics in the presence of glucose (Gutterson et al, 1988). In addition, MB media diluted 10x (MB 1: 9) supplemented with 0.1% glucose can also induce antibacterial compounds from Alteromonas sp. K10 and Bacillus sp. K11.

Nitrogen also plays a role in the production of microbial secondary metabolites. In nitrogen-limited conditions, ppGpp synthetase (RelA) associated with ribosomes is required for antibiotic production by Streptomyces coelicolor A3 (2) (Chakraburtty & Bibb 1997). This condition is also needed for the production of cephamycin C by Streptomyces clavuligerus (Jin et al, 2004). However, the mechanism of antibiotic production driven by ppGpp is not yet clear.

The biosynthesis of secondary metabolites such as antibiotics is also influenced by the availability of phosphate (Martin 2004). Generally, the production of secondary metabolites occurs in limited phosphate conditions. The components that play a role are the PhoR-PhoP system. PhoR is the standard membrane protein kinase sensor. PhoP is a response regulatory member protein bound to DNA. PhoP also plays a role in controlling the alkaline phosphatase (PhoP) gene. If there is an inactivation of the PhoP response regulator or deletion of the PhoR-PhoP system, it causes high expression of actinohordin and undesylprodigosin in S. coelicolor and S.

lividans. The proposed Cascade mechanism related to the negative effect of PhoP phosphorylation on AfsS expression is shown in Figure 2 (Martin 2004). Expression of the act (actinorhodin) and red (undesilprodigiosin) genes was upregulated by specific transcription activators, ActII-ORF4 and RedD. Production of ActII-ORF4 and RedD was induced by AfsS. High expression of AfsS will cause high expression of ActII-ORF4 and RedD so that there will also be high expression of Act and Red. AfsS is regulated by the PhoR-PhoP system. AfsS is repressed by the PhoR-PhoP system when PhoP is in a phosphorylated state (PhoP ~ P).

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The regulation of secondary metabolite biosynthesis with a quorum-sensing system. Quorum-sensing is the regulation of gene expression that depends on cell density. Generally, the quorum-sensing mechanism is used by gram-negative bacteria. Bacteria produce signal molecules that are able to diffuse out and into cells. This molecule is usually known as an autoinduser. The autoinducer found in gram-negative bacteria is lactone homoserin acylation (AHL). The quorum-sensing model is shown by the symbiosis of marine bacteria, Photobacterium fischeri with fish or squid (Gonzalez & Marketon 2003). AHL is formed from acyl-ACP and SAM (S-adenosylmetionin) with the help of the AHL synthase enzyme encoded by the LuxI gene. The increase in cell density causes the accumulation of AHL which in turn causes LuxR to be active because AHL will bind to LuxR. This active LuxR will activate lux operon expression but inhibit luxR gene expression.

Pseudomonas fluoresescen NCIMB 10586 produces mupirocin compound. The expression of the Mupirocin gene from Pseudomonas fluoresescen NCIMB 10586 relies on a quorum-sensing regulatory system (El-Sayed et al, 2001). The gene encoding mupirocin is the Mup operon. The amino acids MupR and MupI show similarities to the LasR / LasI and LuxR / LuxI regulatory systems. MupR is important for activating mupI and mup operons. In addition to mupirocin synthesis which follows the quorum-sensing regulatory system, the production of antibacterial compounds from the bacterium Roseobacter 27-4 also follows quorum-sensing regulation (Buhn et al, 2005). This is because these compounds are formed when the cell density is high.

Apart from AHL, another autoinducer is-butyrolactone (butanolide). An example of butanolide is factor A (2-isocapriloyl-3R-hydroxymethyl-◎-butyrolactone) Streptomyces griseus. Factor A S. griseus is produced before streptomycin production and will stop when streptomycin production reaches its maximum level (Horinouchi & Beppu 1992). Factor A induces at least 10 proteins at

the transcription level. One of them is streptomycin 6-phosphotransferase, an enzyme that functions in the biosynthesis of streptomycin and in its resistance to streptomycin.

Induction of microbial secondary metabolite biosynthesis in the laboratory. There are several obstacles encountered in the synthesis of secondary metabolites in the laboratory. Some microbes that live in symbiosis with marine life are difficult to cultivate in the laboratory. Some of the microbes producing secondary metabolites can also lose the capacity to produce secondary metabolites after storage for a short time (Tabarez 2005). The cause of this is the unfulfilled nutritional requirements or the strains that produce secondary metabolites are not under stress and can also be caused by the abiotic environment. Efforts made to overcome the abiotic environment are to make the conditions during cultivation similar to the original environment, for example by modifying conventional cultivation methods. Bacillus licheniformis EI-34-6 produces secondary metabolites when grown in a bioreactor using a semipermeable membrane. B. licheniformis EI-34-6 but did not produce secondary metabolites when grown in shaken liquid culture (Yan et al, 2003). The different types of semipermeable membranes did not show different results, so it could be concluded that the chemical composition of the membranes did not affect the production of antimicrobial compounds. Bacillus sp. which was isolated from the algae Palmaria palmate produced antimicrobial compounds with varying spectra in the modified roller bottle culture method compared to shaken liquid culture or liquid culture on a rotating bottle (Yan et al, 2002).

The production of antimicrobial compounds by marine bacteria can also be induced by the presence of living or dead terrestrial bacteria (Mearns-Spragg et al, 1998). Twelve of the 16 epibiotic bacteria from algae and invertebrates showed increased antimicrobial activity against S. aureus, E. coli, Pseudomonas aeruginosa after exposure to living terrestrial bacteria.

D. CONCLUSION

Marine microbes are one of the promising secondary metabolites producers. Some of the obstacles experienced in the cultivation process in the laboratory are the absence of secondary metabolites. There are several efforts that can be done. First, the media used in the cultivation process varied both in type and concentration. Second, the medium used is added with secondary metabolite precursors such as amino acids and carbohydrates. Third, the cultivation process is attempted to be similar to conditions in nature such as in the cultivation process there is no shaking or bioreactor modification using a semipermeable membrane.

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