1. INTRODUCTION

1.1. Marine Natural Products

Natural products have led to the excellent drugs for therapeutic purposes. In the period of 1989-1995 over 60% of the approved drugs and pre-NDA (New Drug Application) candidates were of natural origin. Drugs of natural origin have been classified as original natural products, semi-synthetically from natural products or synthetic products based on natural product models. Thereafter natural products played invaluable role in the drug discovery, particularly in the areas of cancer and infectious diseases. Of the 211 pre-NDA anticancer drug candidates (i.e., in preclinical or clinical development for the period above), 61% were the original natural products and about 4% of the candidates were marine-derived (Cragg *et al., 1*997).

The urgency of new antibiotic discovery becomes more serious since often no effective therapies are available and the development of resistance of many pathogens to currently used drugs (Cassell & Mekalanos, 2001; Nussbaum *et al.,* 2006; Saleem *et al.,* 2010). Moreover, bacteria can acquire drug resistance in a multitude of ways, thus to solve this problem is not a straightforward matter (Saleem *et al.,* 2010). A similar situation exists with the need to develop new cancer chemotherapeutic agents with activity against the disease-types still resistant to current therapies and to overcome the development of multidrug resistance, which is increasingly observed in the treatment of many tumors (Cragg *et al., 1*997).

Ironically, in the mid 1990s most large pharmaceutical companies have decreased the screening of natural products for drug discovery in favour of synthetic compound libraries (Lam, 2007). Moreover, some companies have terminated the activities of natural product-based drug discovery (Ortholan & Ganesan, 2004). New technologies such as high throughput screening could not activate the activities again, since the equipments were very costly and the updated data libraries were also not openly to access (Buss & Butler, 2004).

The stated phenomenon was happened for temporary short time. The facts proved that the chemical natural products are incredibly distinct from other medicines and continuously showing fascinating biological activity (Baker *et al.,* 2007; Labischinski & Ruebsamen-Waigmann, 2008). Currently, natural products are hence back to be rising desired in drug discovery. Some reviews demonstrated that natural products play a dominant role in the discovery of lead compounds for drugs development (Newman *et al.,*

2003; Koehn & Carter, 2005; Newman & Cragg, 2007; Saleem *et al.,* 2010). This trend is also precisely happened to marine natural products.

In December 2004 was the first drug from the sea Ziconotide to be approved in the United States for the treatment of pain. Two months later the drug was also approved by the European Commission for the treatment of severe, chronic pain in patients who require intrathecal analgesia. Ziconotide (ω-conotoxin MVIIA) is a peptide originally from a tropical marine cone snail. The drug is presently in the market under the trade name Prialt® (produced by Elan Pharmaceuticals). The second drug was Trabectedin (Ecteinascidin-743/ET-743) that is marketed under the trade name Yondelis® (PharmaMar/Johnson&Johnson/OrthoBiotech). Yondelis® was the first anticancer drug from the sea originally isolated from a tropical sea squirt *Ecteinascidia turbinata.* The drug also has been approved by the European Union in October 2007 for the treatment of soft tissue sarcoma (Molinski *et al.,* 2009; Mayer *et al.,* 2010). Moreover, both drugs are of the first members of new human drug classes (Butler, 2008).

1.2. Fungi as a Source of Natural Products

Fungi produce a vast range of secondary metabolites. Some of the metabolites are high-value products with pharmaceutical applications such as penicillins, a group of structurally related ß-lactam antibiotics isolated from *Penicillium chrysogenum*. Several non-ß-lactam antibiotics are also produced by fungi such as griseofulvin. Griseofulvin which is isolated from *Penicillium griseofulvum* has been used for several years to treat dermatophyte infections of the skin, nails and hair of humans. Some valuable secondary metabolites of fungal origin are listed in Table 1.1 (Deacon, 2006).

Table 1.1. Some valuable secondary metabolites produced commercially from fungi

Metabolite	Fungal source	Application
Penicillins	*P. chrysogenum*	Antibacterial
Cephalosporins	*Acremonium chrysogenum*	Antibacterial
Griseofulvin	*P. griseofulvum*	Antifungal
Fusidin	*Fusidium coccineum*	Antibacterial
Ciclosporins	*Tolypocladium* spp.	Immunosuppressants
Zearalenone	*Gibberella zeae*	Cattle growth promoter
Gibberellins	*Gibberella fujikuroi*	Plant hormone
Ergot alkaloids and related compounds	*Claviceps purpurea* and related fungi	Many effects including: antimigraine, vasoconstriction, vasodilation, antihypertension, anti-Parkinson, psychiatric disorders

Source: Deacon (2006)

Marine fungi are prolific resources of natural products (Liberra & Lindequist, 1995; Pietra, 1997; Jensen & Fenical, 2000; 2002; Ebel, 2010). However, the potential of marine fungi has only been investigated to a limited extent.

In recent years, marine fungi have been explored more intensely to obtain novel and biologically active compounds. Though compared to marine sponges and bacteria, marine fungi are still less explored. Nevertheless, success stories in marine fungi are quite significant. Cephalosporin C which was originally isolated the first time from *Cephalosporium acremonium* isolated from a sewage outlet off the Sardinian coast have played a key role to the reduction of infectious diseases and suffering of people throughout the world since last three decades (Demain & Elander, 1999). However, it was about incidental discovery and it took another 30 years until marine-derived fungi were investigated more systematically (Ebel, 2010).

1.2.1. Biology of Marine Fungi

Initially fungi were classified as 'cryptogamic plants' and separated into lichenized and non-lichenized groups. For many years fungi were recognized as belonging to kingdom plants until Whittaker's influential five-kingdom classification of living beings (Whittaker, 1969 *in* Kirk *et al*., 2008) and subsequent phylogenetic research has repeatedly confirmed that the fungi are a sister group to the animals rather than the plants.

The kingdom of fungi is the second largest group after insects and widely distributed in nature. Based on the observed ratio between flowering plant diversity and fungal diversity in countries where fungi have been sufficiently well studied, there are 1.5 million estimated fungal species (Hawksworth, 1991; 2001). They inhabit soils, the surface of mountain rocks and seawater (Feofilova, 2001). However, marine environment does not permit the development of large and fleshy fruiting bodies; therefore most of the fungi found in marine habitats are microscopic (Kohlmeyer & Kohlmeyer, 1979).

Marine fungi were from longtime as neglected resources. Unlike their related terrestrial fungi which were initially exploited, marine fungi have attracted great attention as considerable resources since the late 1980s (Ebel, 2010). The term marine fungi itself essentially do not represent a specific taxa, but are a group defined by their ecology. Most fungi isolated from marine samples are not proven to be obligate or facultative marine. Thus, the more general expression "marine-derived fungi" is used.

The generally accepted ecological definition of marine fungi is, "Obligate marine fungi are those which grow and sporulate exclusively in a marine or estuarine habitat; facultative marine fungi are those from freshwater or terrestrial milieus able to grow and possibly also to sporulate in the marine environment" (Kohlmeyer & Kohlmeyer, 1979). Accordingly, there is no taxonomic classification of marine fungi rather to a certain extent of ecological term.

Marine fungi comprise an estimated 1500 species, excluding those that form lichens (Hyde *et al.,* 1998). This number is far fewer when compared to the number of named and undescribed terrestrial fungi which was estimated 250,000 or more (Kohlmeyer & Kohlmeyer, 1979). So far less than 500 of filamentous higher marine fungi have been described and only 79 are associated with algae as parasites or symbionts, and 18 with animal hosts (Kohlmeyer & Volkmann-Kohlmeyer, 2003).

The distribution of marine fungi in the tropics has not been explored as thoroughly as in the temperate areas (Kohlmeyer, 1984; Blunt *et al.,* 2005; 2009). Nevertheless, inventory data for the marine fungi investigated in several tropical countries such as Thailand (Chaeprasert *et al.,* 2010), Palau Islands (Kohlmeyer, 1984; Chatmala *et al.,* 2004), Singapore (Lim & Tan, 1986; Sundari *et al.,* 2010), Brunei (Hyde, 1988), Malaysia (Jones & Hyde, 1988; Alias & Jones, 2000; Zainuddin *et al.,* 2010; Pang *et al.,* 2010) and Siargao Island, Philippines (Besitulo *et al*., 2002) are available. Many tropical regions have been largely unexplored, such as the Indonesian archipelago.

1.2.2. Biosynthesis of Marine Fungal Secondary Metabolites

Extreme conditions such as high salinity, low temperature, lightless and high pressure are supposed to be the inducer of the prolific active compounds of marine microorganisms. Their capabilities to produce unique and unusual secondary metabolites are possibly because of adaptation to a very distinct set of environmental pressure (Jensen & Fenical, 2002). It is also believed that the metabolites act as a chemical defense in competing for substrates (Gallo *et al*., 2004).

Secondary metabolites are defined as small organic molecules that derived from biosynthetic pathways which are not required for maintenance and growth of the respective organism. Besides their role in environmental adaptation, they often contribute to biological defense strategies. By co-cultivation of marine fungi with other microorganisms from the same ecosystem proved to be successful in activating silent gene clusters to produce bioactive secondary metabolites (Brakhage & Schroeckh, 2010). Small changes of cultivation parameters such as media composition were also possible to induce secondary metabolites production (Bode *et al.,* 2002; Calvo *et al*., 2002).

1.3. Marine Natural Products of Fungal Origin

The purpose of finding novel secondary metabolites is usually in order to obtain large numbers of fast growing isolates from marine sources (Kohlmeyer & Volkmann-Kohlmeyer, 2003). Interestingly, the corresponding chemistry was structurally diverse and related to that of terrestrial fungi (Höller *et al*., 2000). Marine environment that is different with freshwater condition is generally responsible for the production of diverse metabolites. Hence, optimization of physical and chemical factors such as salinity, temperature, pH and media components can sometimes be substantially increase the yield of bioactive compounds (Calvo *et al*., 2002).

Marine organisms are a fascinating source of novel and biologically active natural products. Over 14.000 new biologically active compounds have been identified from marine sources and at least 300 patents have been issued (Hunt & Vincent, 2006). Moreover, the number of reported secondary metabolites from marine fungi has steadily increased (Faulkner, 1997; 1998; 1999; 2000; 2001; Blunt *et al.,* 2003; 2004; 2005; 2006; 2007; 2008; 2009; 2010). In the last three years there were over 15 marine-derived secondary metabolites in human clinical trials (Saleem *et al*., 2007). This number increases rapidly since each year on average approximately 700 novel marine natural products have been published and of which 16–18% were of microbiological origin (Blunt *et al.,* 2010).

Fungi are one of the most significant groups of organisms to be exploited for drug discovery purposes. Especially Fungi Imperfecti have provided mankind with wide of different bioactive secondary metabolites such as β-lactam antibiotics, griseofulvin, cyclosporine A or lovastatin. In the last three years, most new natural products described in literature were isolated from fungi (Saleem *et al*., 2007).

Starting in the late 1980s quantities of new secondary metabolites from this long neglected source has been significantly increasing (Ebel, 2010). The highly developed and diverse secondary metabolites are the interesting factors of fungi. Marine fungi are still challenging to explore since only small parts of fungi that have been explored (less than 5% of about 1.5 Mio fungal strains).

The advantages of the investigation of marine fungi as of microorganisms when compared to macroorganisms are obvious, since biotechnological fermentations are possible without ecological exploitation and compounds can be reisolated after recultivation in large amounts which is nearly impossible for marine macroorganisms. Additionally, microorganisms can be easier manipulated genetically and straightforward scale-up of metabolite production (König & Wright, 1997; Stadler & Keller, 2008).

Sponges-derived natural products are presently the most investigated of marine natural products. Correspondingly, the study of natural products from sponges-associated microorganisms, namely bacteria and fungi are also plenty. Interestingly, fungal associates in sponges contribute 65.71% of compounds, almost double compared to the compounds produced by bacteria. In more detail, Ascomycota dominate the proportion of fungal producer by division (Fig. 1.1) (Thomas *et al.,* 2010).

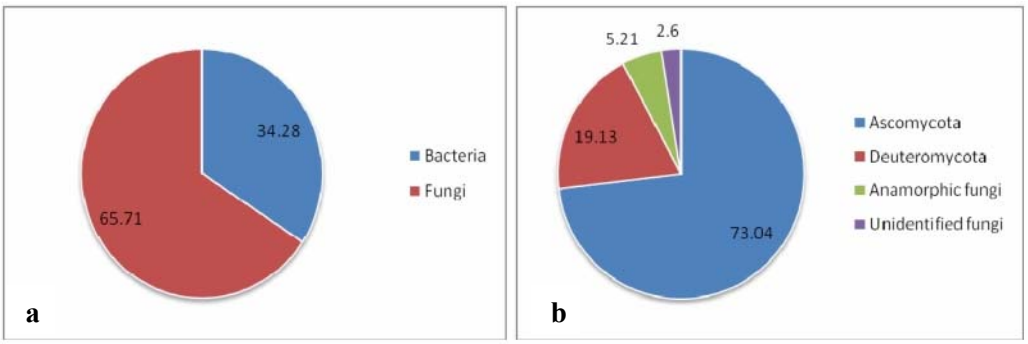

Fig. 1.1. Percentage distribution of compounds produced by (a) sponge-associated bacteria and fungi, (b) associated fungi-division wise (Thomas *et al.,* 2010)

1.3.1. Secondary Metabolites from Algicolous Fungi

Algae have been regarded as a valuable source for the isolation of fascinating marine fungi. Kohlmeyers stated that one third of all known higher marine fungi are associated with alga (Kohlmeyer & Kohlmeyer, 1979). Furthermore, the algicolous fungi may produce unusual and novel metabolites. It is not surprisingly since the fungi have to deal with their host as well as the marine environment.

Algal-fungal relationships were intensively investigated. There is a symbiotic association of fungi with algae in lichens where both the partners benefit. On the extreme end this association is called mycophycobiosis where an obligate symbiotic association exists between systemic fungi and marine macroalgae (Raghukumar, 2006). However, fungi are also potential as pathogens in algae (Johnson & Sparrow, 1961; Kohlmeyer & Kohlmeyer, 1979).

In the last decade secondary metabolites obtained from marine algicolous fungi have shown significant rising of the quantity and moreover diversity of the marine natural products (Blunt *et al.,* 2010). Some algae which have been reported to be hosts of some active compound-producing fungi are red algae *Liagora viscida* (Osterhage *et al.,* 2002), *Plocamium* sp. (Pontius *et al.,* 2008), *Acanthophora spicifera* (Greve *et al.,* 2008); green algae *Ulva* sp. (Osterhage *et al.,* 2000; Gamal-Eldeen *et al.,* 2009); *Ulva pertusa* (Cui *et al.,* 2010); brown algae *Rosenvingea* sp. (Cueto *et al.,* 2001), *Fucus vesiculosus* (Abdel-Lateff *et al.,* 2003) and *Sargassum horneri* (Nguyen *et al.,* 2007).

1.3.2. New Bioactive Natural Products from Marine-derived Fungi

There are several valuable reviews dealing with marine fungal metabolites (Liberra & Lindequist, 1995; Pietra, 1997; Bugni & Ireland, 2004; Bhadury *et al.,* 2006; Saleem *et al*., 2007; Ebel, 2010; Debbab *et al*., 2010). They covered new biologically active natural products of marine-derived fungi published until 2009. In addition, D.J. Faulkner has reviewed marine natural products (including metabolites from marine fungi) annually since 1984 to 2002 in Natural Product Reports. After his death in November 2002, this annual review has been continued by J.W. Blunt and his group.

Natural products isolated from marine fungi were generally classified into seven classes, namely polyketides, alkaloids, diketopiperazines, peptides, terpenoids, lipids, and shikimate-derived metabolites. Several review papers illustrated that polyketides dominate marine natural products of fungal origin (Ebel, 2010; Blunt *et al*., 2010).

Concerning to the previous review as mentioned before, in this currently report are listed new metabolites which have been published in January to September 2010.

1.3.2.1. Antimicrobial Compounds

Five new polyketide derivatives, 7-*O*-methylkoninginin D (**1**) and trichodermaketones A-D (**2**-**5**) were isolated from the marine-derived fungus *Trichoderma koningii*. The compounds were investigated for action against bacteria, fungi and for synergistic antifungal activity. Compound **2** showed synergistic antifungal activity against *Candida albicans* with 0.05 µg/ml ketoconazole (Song *et al.,* 2010).

Investigation for secondary metabolites of *Trichoderma* sp. 05FI48 which was isolated from an unidentified marine sponge revealed three new aminolipopeptides named trichoderins A (**6**), A1 (**7**) and B (**8**). The compounds were reported to be active against *Mycobacterium smegmatis, Mycobacterium bovis* BCG, and *Mycobacterium tuberculosis* H37Rv in both active and dormant states with MIC values in the range of 0.02-2.0 µg/ml (Pruksakorn *et al.,* 2010). The chemical structures of new antimicrobial compounds are presented in Fig. 1.2.

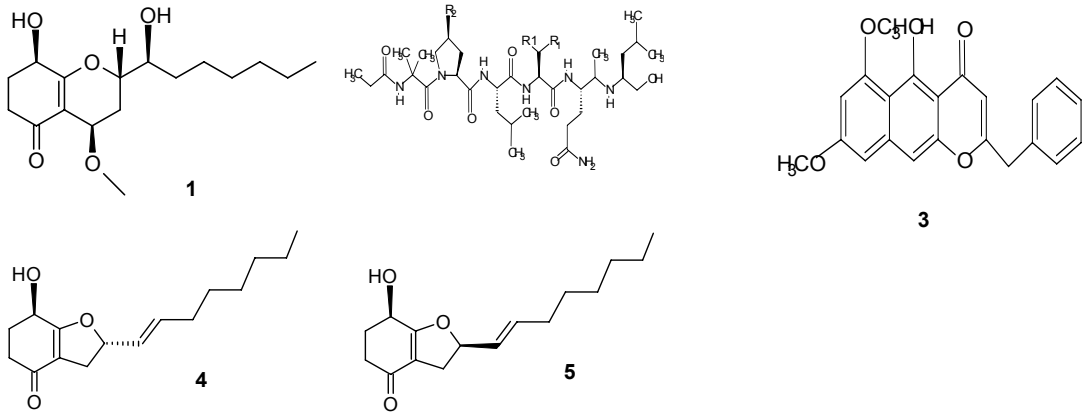

Fig. 1.2. New antimicrobial compounds isolated from *Trichoderma* spp.