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Review

Ecobiotechnology of Marine Sponges and Their Symbionts – Review and Present Status

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Abstract Marine sponges are a rich source of highly diversified bioactive compounds. These medicinally valuable molecules represent extreme physiological and ecological functions in sponges, more presumably involving in the resistance to the feeding by other marine organisms like fish and fouling by barnacles, bacteria, fungi, etc. This feature of attaining resistance made sponges as successful poriferans that possess an impressive array of biological properties ranging from antimicrobial to anticancerous activities. The diversified bioactive principle of sponges might be due to their spacio-temporal distribution and although, the gateway for exploiting the sponges for isolating these distinct, potential molecules is open, suitable technical and methodological approaches are yet to be implemented in order to bring the sponges as successful pharmaceutical leads in the field of marine biotechnology. Despite of the identified difficulties of marine sponge research from past few decades, one should concentrate not only on the basic and applied technical/methodological considerations, but also on the novel strategies like *in vitro* sponge cell, fragment and whole sponge culture; sponge symbiont cell culture; *in situ* and *ex situ* sponge cultivation; and sponge bioreactors and metagenomic approaches, for the successful exploitation of marine sponges towards the novelty in sponge biotechnology. The present review narrates the pros and cons of the nowadays-marine sponge research by focusing on the suggestive ecobiotechnological approaches, based on the latest studies for feasible ecological exploitation and biotechnological application of sponges from the sea.

Key words : Marine sponges, bioactive compounds, sponge symbionts, *in situ* and *ex situ*, *in vitro* culture, bioreactor.

Introduction

The chemical diversity of the bioactive compounds ranging from simple linear peptides to the complex macrocyclic polyethers is mainly dependant on the eco-physiological variations of the sea. This chemical diversity draws us towards the discovery of novel marine natural products (MNPs) to apply in various therapeutic areas like cancer, inflammation, microbial infections, and even in various other deadly syndromes like AIDS etc. (Rawat *et al*., 2006; Artan *et al*., 2008; Glaser and Mayer, 2009; Jain, 2009). A Number of marine peptides have also been isolated in recent years such as kahalalide F, hemiasterlin, dolastatins, cemadotin, soblidotin, didemnins, aplidine, cyclodepsipeptides etc. Chemical diversity of MNPs is a better match to that of successful drugs than the diversity of the synthetic compounds (Feher and Schmidt, 2003; Grabowski and Schneider, 2007). Marine resources are immeasurable and extraordinary mines for the discovery of new anticancer drugs and therefore, a more rigorous study is to be conducted to explore MNPs. The development of high throughput screens based on molecular targets had led to a demand for the generation of largely diverse libraries of compounds to satisfy the enormous capacities of these screens (Galm and Shen, 2007; Newman and Cragg, 2007).

Nowadays, owing to their therapeutic potential, ma-

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rine organisms are getting great recognition for their importance to human life (Jain, 2009). When compared to terrestrial organisms, very few of the marine flora and fauna have been reported as potential sources of anti-bacterial, anti-viral (e.g., anti-HIV), anti-cancerous, anti-oxidative, anti-inflammatory and even anti-photo-aging molecules etc (Kim *et al*., 2006; Rajapakse *et al*., 2007; Artan *et al*., 2008; Kim *et al*., 2008; Kong *et al*., 2008; Ryu *et al*., 2009; Arepalli *et al*., 2009; Pallela *et al*., 2010). Information on marine sources is very scarce and thus, an in-depth study of marine life will surely decipher the sustainable exploitation of this environment for human healthcare. One of the richest and important faunal sources of MNPs are marine sponges, a highly diverse component of marine benthic communities, found in all the world's oceans and at all depths (Hooper and Van Soest, 2002; Mc Clintock *et al*., 2005). Although their commercial and evolutionary importance is being increasingly recognized, many pivotal aspects of their basic biology remain enigmatic (Wörheide *et al*., 2005). Despite of a wide range of functional roles performed by marine sponges, they are still poorly represented in many research, monitoring and conservation programmes (Bell, 2008).

The constant threat from competitors, by way of overgrowth, poisoning, infection or predation has armed sponges with a storehouse of these potent chemical defense agents (Bakus *et al*., 1986; Thakur and Muller, 2004). Sponges produce a wide array of secondary metabolites ranging from derivatives of amino acids and nucleosides to macrolides, porphyrins, terpenoids, aliphatic cyclic peroxides and sterols (Sarma *et al*., 1993; Faulkner, 1995; Scheuer, 1995; Parameswaran *et al*., 1997; Fontana *et al*., 2000; Tilvi *et al*., 2004; Sipkema *et al*., 2005a; Andavan and Lemmens-Gruber, 2010). However, only few compounds like arabinofuranosyladenine, has been approved for human applications (Muller *et al*., 2000). There are many inhibitory molecules isolated from nature that are very crucial to certain enzymes, e.g., Phospholipase A_2 (PLA_2), which is widely distributed in mammalian systems and in the venoms of snakes, bees, scorpions and spiders. Knowing the therapeutic importance of PLA_2 inhibitors, many pharmaceutical industries and academic institutions are involved in the search for novel and specific PLA_2 inhibitors, which have also been characterized from marine sponges (Rangappa, 2007). Even, there are recent reports on some potential sponges as suitable sources for obtaining novel bioactive pesticidal leads (Rao *et al*., 2008). Poriferan research is advancing nowadays and most recently due to many of the sponge components e.g., silica synthesized enzymatically by siliceous sponges (Demospongiae and Hexactinellida); these sponges have become potential models for environmentally friendly nanoscale material production. These silicon based technologies can be applied for the synthesis of valuable semiconductors, metal oxides such as titanium and gallium that have photovoltaic and semiconductor properties (UCSB, 2004; Muller *et al*., 2009).

Increasingly sophisticated molecular tools are now being applied, with results contributing significantly for a better understanding of Poriferan micro-evolutionary processes and molecular ecology. These aspects should be considered seriously to monitor the ecologically oriented exploitation of marine sponges *in situ*, so that the sponge-biotechnology would gain better advancement in a limited period. Presently, researchers are focusing on the production of novel molecules from marine sponges by *in vitro* sponge cell culture with more advanced culturing and cultivating methodologies (Muller *et al*., 2000; Pomponi and Willoughby 1994; Sipkema *et al*. 2006).

Keeping the importance of pharmaceutical potential of marine sponge associated microbes in view; targeting microsymbionts is also an essential focus nowadays as the sponge-microbial association is a potential chemical and ecological phenomenon that provides sustainable resource for developing novel pharmaceutical leads (Gandhimathi *et al*., 2008; Selvin *et al*., 2009). Recently, Koopmans *et al*. (2009) have proposed novel strategies for sponge metabolite production of how to develop the process of producing metabolites from sponges, based on two important aspects *viz.,* to understand the metabolite production and to choose the improved culturing phenomena in marine sponges.

The occurrence of a partial lag of marine sponge research due to many facts like, inability to exploit the marine research area completely due to ministerial regulations; distance and sample transportation problems till the work station; difficulties in maintaining sponges *ex situ/in vitro* for various biological and chemical evaluations etc. Though it started in 1800s, marine sponge research is quite behind when compared with other allied biological researches. Acquiring fundamental knowledge has also been hurdled in areas that are piv-

otal for commercialization of biomedical products because of marine environmental problems like pollution, ecosystem diseases, and harmful algal blooms (Hobson, 2003). The search for new drugs and agrichemical compounds should be revitalized by using latest innovative methods to acquire more fundamental understanding of the biosynthetic capabilities of pharmaceutically potential marine organisms like sponges.

This review discusses various latest methodological strategies to target potential symbionts producing novel molecules through *in situ* and *ex situ* culture/cultivation and phylogenetic/molecular approaches. These techniques at the bioreactor level have provided latest insights into the future prospects in marine biotechnology. We aim to review previous sponge research towards the direction of producing bioactive molecules through simplest, economical and ecobiotechnology oriented multi-dynamic approaches (Fig. 1).

Ecobiotechnological Approaches in Marine Sponge Research

In situ Marine Sponge Research

The attempts on sponge cell culture have resulted in only a minimum level of understanding on chemical aspects of the sponges rather than biological, as there is huge requirement of the sponge biomass for the isolation and identification of the MNPs and their scientific evaluations. Hence, it is more advisable to perform *in situ* experimentation of the marine sponges rather than by the *ex situ in vitro* methodologies. Achieving a required sponge biomass for MNPs by harvesting the sponges directly from the sea is not feasible in most cases because the massive extraction of sponges would harmfully disturb the marine environment (De Caralt *et al*., 2007a). The approach of *in situ* sponge cell and fragment culture and whole sponge cultivation by establishing temporary or permanent marine research stations will be very effective not only for the exploitation of complete life of a marine sponge species but also to recycle the experimented left over sponge masses in to the sea so that, due to the high regeneration capabilities of poriferans, proper ecological balance will be maintained (Henry and Hart, 2005; Wulff, 2006). These ecologically focused results are economic because of non-transportation of the intact biological samples to work station and as such, there is no much possibility of habitat disturbances, and hence, continuous monitoring of the local oceanic area will then be properly exploited. Hence, the ecological and economic consideration made sponges, a suitable species for marine environmental bioremediation and eutrophication control (Milanese *et al*., 2003; Stabili *et al*., 2006). *In situ* study of sponge cell/fragment/whole sponge culture or culti-

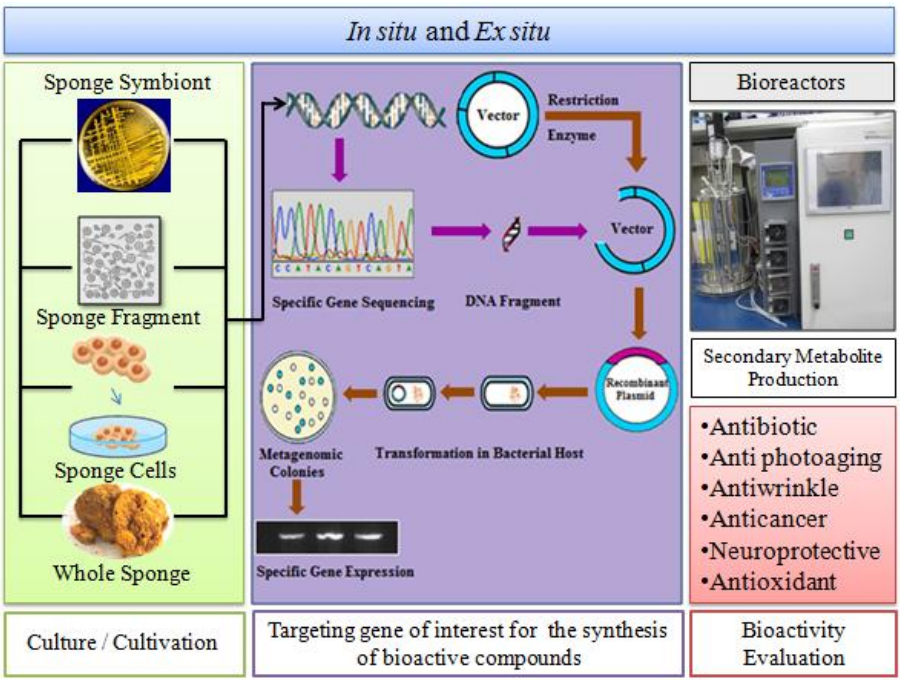

Fig. 1. Schematic ecobiotechnological approach of *ex situ* and *in situ* sponge-symbiont culture/cultivation.

vation always be an intervention in the natural habitat, and high pumping and clearance activity of sponges contribute in decreasing the organic and microbial water pollution (Pronzato *et al*., 2000; Corriero *et al*., 2004). There are several possibilities to scale enough biomass for the purpose of clinical tests and commercial production, at least for certain sponge species through *in situ* cultivation (Page *et al*., 2005). Interestingly, potential role of sponge cell cultures has been studied earlier, to understand the recovery of naturally diseased sponge populations (Castritsi-Catharios *et al*., 2005; Baldacconi *et al*., 2006). *In situ* cultivation of marine sponges (mariculture) and cell culture approaches have been explored as possibilities for large-scale production of sponge-derived compounds (van Treeck *et al*., 2003; Muller *et al*., 2004). However, many studies prove contrasting results concerning the success of *in situ* cultures and thus *ex situ* culture and cultivation attained global concern for the production of novel bioactive molecules (Verdenal and Vacelet, 1990; Corriero *et al*., 2004).

Ex situ Marine Sponge Research

Ex situ approaches of marine sponge cultivation resulted in auspicious growth rates in the established sponges (Osinga *et al*., 1999, 2001, 2003; Munro *et al*., 1999; Brummer *et al*., 2002; Duckworth *et al*., 2003). However, convincing the local forest and environmental agencies for procuring sufficient quantities of marine sponges is a major issue in view of conserving endangered species, natural resources and habitat restriction. In addition, the sponge research projects to be headed by a common government authority that belongs to coastal region, so that ecological monitoring of the sponge exploitation by various academies and institutions will be recorded time to time. This concept of studying a sponge species through ecological or environmental monitoring authorities may control or accelerates the *ex situ* aspects of a particular species that commonly exists in a particular marine habitat available only in specific season.

Though it is little cumbersome to establish and maintain the marine environment outside the coastal regions, if the proper methodological set-up is followed through the maintaining of marine environmental conditions, *ex situ* cultivation of marine sponges for a certain period is possible (Sipkema *et al*., 2005b). Present studies support ecologically dependant *ex situ* cultivation of sponges, which significantly concluded that *ex situ* cultivation similar to *in situ* conditions, provides a promising method to keep sponges for the production of bioactive metabolites (Kloppel *et al*., 2008). Recently, flow-through and closed recirculating aquaculture systems are established for marine sponges, which resulted significant implications on the sponge-bacterial communities (Mohamed *et al*., 2008).

In Vitro Sponge Cell and Fragment Culture

As sources of natural products with potential human therapeutic value, marine sponges are important subjects for cell culture studies (Willoughby and Pomponi, 2000). However, sponge cell culture primarily depends on the prevailing ecological conditions (salinity, pH-value, temperature, dissolved oxygen, light, water current velocities, nutrients, waste removal, and silica concentration) as in the natural habitat (Osinga *et al*., 1999; Belarbi *et al*., 2003). Recent studies on sponge cell (or primmorphs) and fragment cultures *in situ* and *ex situ in vitro* (under controlled conditions) pave a way to identify the fundamental requirements for the production of sponge biomass (Gunda and Janapala, 2009). Cultivation of intact sponges and fragments at sea prevents or minimizes the control of environmental conditions, which may not be optimal for sponge growth (Belarbi *et al*., 2003). Thus, culturing of sponges/fragments by regulating external conditions in semi-open and closed systems resulted positive indication of sponge survival (Osinga *et al*., 2003; Nickel and Brummer, 2003). However, survival rates *in vitro*, varies among different sponges, reflecting the need for better culture optimization techniques that are to be developed for fragment/explants culture, by identifying the intrinsic and extrinsic factors promoting their survival (De Caralt *et al*., 2003; Camacho *et al*., 2006).

Earlier, Rinkevich (2005) brought a significant publication database of invertebrate cell cultures and also made a discrimination on short-term and long-term culturing strategies of cell cultures in Mollusca, Porifera and Crustacea (e.g., *Botryllus schlosseri, Crassostrea, Mytilus, Penaeus,* and *Suberites Domuncula*). Though many of the researchers nowadays concentrating much on the Poriferan cell culturing methodologies, no single established cell line was available till now. A critical component of any cell culture system is its growth medium. A new medium formulation that may be more suitable to support cell growth and division, providing an improved cell culture system is needed for advanced

sponge culturing studies. Previously, Sipkema *et al*. (2006) studied the *in vitro* culturing of marine sponges, *Dysidea avara* and *Chondrosia reniformis* on a diet of viable *Phaeodactylum tricornutum* cells and dissolved nutrients with algae and fish powders. The property of regeneration is also decisive for sponge survival and persistence *in vitro* (De Caralt, 2007). *In situ*/*ex situ in vitro* approach is primarily useful for suitable evaluations and optimization of culture methodologies and hence, improving the *in vitro* culture conditions towards the biotechnological potential of marine sponge cell or primmorph culture is the first and foremost consideration (Zhang *et al*., 2004).

Sponge Symbiont Culture

The bacterial association with marine sponges has been well known for a long time and several investigations have explored this association using different approaches (Vacelet and Donadey, 1977; Esquenazi *et al*., 2008; Thomas *et al*., 2010). Even recently, both culture-dependent and culture-independent methods have been used to reveal the diversity in producing the pharmaceutical metabolites of marine microbial symbionts in the China Sea (Li, 2009). However, as sponges harbor diverse bacterial populations (as much as 40-60% of animal biomass), it is under scientific debate whether the sponge and/or its associated bacteria produce these bioactive molecules reported to come from the sponge (Thakur *et al*., 2004). Previously, it was observed that both direct (by producing antibacterial metabolites) and indirect (with the help of associated bacteria) epibacterial defense systems exist in marine sponges (Thakur *et al*., 2003). Most of the sponges have the symbionts in their body and there are few evidences that confirmed the contribution of symbiotic microbes to sponge well-being or survival (Webster and Blackall, 2009). There are few exceptional studies that have shown the translocation of photosynthate from cyanobacteria to the host sponge and a decline in sponge health with a loss of cyanobacteria (Wilkinson, 1979; Thacker, 2005; Taylor *et al*., 2007). However, in general, the presence of microbes with certain metabolic phenotypes e.g., ammonium-oxidizing archaea provides only a circumstantial evidence that they are important for sponge health (Steger *et al*., 2008).

Microsymbionts may have essential functions in sponge biology by serving as food, or producing bioactive metabolites (Wilkinson and Garrone, 1980; Schmidt *et al*., 2000; Sfanos *et al*., 2005). Earlier, it was thought that secondary metabolites are produced by the sponge, but were in fact synthesized by the symbiotic microorganisms (Elyakov *et al*., 1991; Unson *et al*., 1994; Bewley *et al*., 1996). Later studies confirmed that the symbiotic bacteria produce the target compound in many of the sponges and the culture of symbiotic microorganisms became an alternative method for supplying the bioactive compounds (De Caralt *et al*., 2007b). Isolation, *in vitro* culturing and characterization of various sponge symbionts is a suitable approach to maximize the development of microbial and their respective chemical libraries so that, identification of the bioactive molecules will be made easier to future researchers (Dharmaraj *et al*., 2010). However, isolation of those microorganisms from the sponge cells make this approach unreliable, because of the low viability (only less than 0.1% of the total symbiotic bacterial community is amenable to culture) of these symbionts outside their host (Webster and Hill, 2001; Dharmaraj and Sumantha, 2009). Hence, chemical as well as genetic characterization of the *in vitro* cultured sponge symbionts is a greatest hidden asset, which when revealed, forms a strong new basis for targeting potential pharmaceutical leads to combat deadly diseases (Thakur *et al*., 2004).

Fundamental information on the recently identified sponge disease and its causative agents, identification of reservoirs/vectors of disease and the role of environmental stressors are also to be more emphasized or taken into consideration for understanding the immunological responses of the sponge cells for different microbes. Future sponge microbiology research should, therefore, also include a focus on developing suitable assays and indicators for assessing sponge health, determining the prevalence and etiological agents of sponge disease, and evaluating innate sponge immunity and pathogen virulence mechanisms, by which sponges can make critical distinctions between three important factors symbionts, food and pathogen (Webster and Blackall, 2009).

Bioreactors

Secondary metabolite production through bioreactors brings little success in the field of marine biotechnology and there is a lack of research into bioreactor engineer-

ing and fermentation protocol design in the field of marine bacterial antibiotic production (Marwick *et al*., 1999; Zittelli *et al*., 1999). As the value of the sponge associated or symbiont associated bioactive molecules is increasing day by day, large volume production of these important molecules became a fundamental target for researchers. To achieve this goal, the development of bioreactors was required, where culturing of at least a few liters of cells and media is possible at a time (Edwards and Kauffman, 2003). Although bioactive compound production through sponge cell lines and getting them to produce the targeted molecules under optimal conditions is a promising approach, however, it is not easy to breed and keep sponge cells in a bioreactor to achieve a controlled production of biologically active substances. Several research groups in the fields of bioengineering, biochemistry, and chemical engineering develop and utilize bioreactors for large scale culturing of sponges and their symbiont microorganisms. In some sponge cell and tissue growth experiments, nutritious particles like microorganisms of either batch cultures or continuous cultures were continuously fed to a sponge bioreactor containing a few sponge explants (Castritsi-Catharios *et al*., 2005).

Depending on the type of secondary metabolite, growth phase of the microbe is to be particularly considered e.g., antibiotics are often produced during the stationary growth phase (or idiophase) of a bacterial culture. In addition, a variety of parameters like substrate, slow or fast utilization, Oxygen and Nitrogen requirements, amino acid supplements, temperature, pH, pressure are also important to influence the production of desired secondary metabolites in bioreactor cultures (Madigan *et al*., 2009).

Molecular Approaches

Until now, several research studies have revealed that sponge populations are genetically well structured, and the evolutionary and historical processes might play an important role in determining such highly diversified structure. Recent advances in cultivation-independent techniques and DNA dependant molecular approaches have provided an interesting perspective to study bioactive natural product biosynthesis in complex microbial communities as well as the symbiont hosts like marine sponges without cultivating the host and symbiont (Hildebrand *et al*., 2004; Piel, 2004; Salomon *et al*., 2004; Schmidt, 2008). At the same time, the genetic diversity in the symbionts of aquaculture also providing additional strength to the biotechnological potential of sponges and sponge symbionts. For example, clone libraries of symbiont 16S rRNA from sponges significantly confirmed that the bacterial communities are different during aquaculture than those growing in natural inhabitant sponges (van Treeck *et al*., 2003).

Genetic and molecular approach of screening and identifying sponge cells within the cultures could result an appropriate way of developing sponge cell bioreactors (Sipkema *et al*., 2003). Hence, to maintain these optimal conditions and to implement molecular screening approaches for producing potent, novel bioactive molecules, marine sponge bioreactors need to be developed both for sponges and sponge associated symbionts. Previously, certain sponge symbionts have been characterized successfully using different molecular techniques like fluorescence *in situ* hybridization (FISH) and 16S rRNA studies (Webster *et al*., 2001).

Another attractive advancement in the microbial biotechnology is to transfer the natural product pathway of interest into a well-developed surrogate host therefore it became an alternative to produce sufficient quantities of the desired compound through a subsequent combinatorial approaches. Basing on this approach, many bioactive natural products have been isolated from slow growing or even unculturable microorganisms (e.g., cyanobacteria, myxobacteria etc.) that are difficult to handle (Harada, 2004; Fortman and Sherman, 2005; Wenzel and Muller, 2005). The pathways for various secondary metabolites should also be tracked to obtain the target molecules more effectively; and the respective gene level studies can then result a suitable approach to obtain required molecules or peptides e.g., polyketides/non-ribosomal peptides or microbial consortia of marine sponges (Fieseler *et al*., 2007; Fisch *et al*., 2009). This family of compounds has received much recent attention due to their potent anti-cancer potential and their representative biosynthetic genes are targeted by proper cultivation or molecular/metagenomic approaches, and it has been suggested earlier that at least some of the bioactive secondary metabolites isolated from sponges are produced by functional enzyme clusters, originated from the sponges and their symbionts (Muller *et al*., 2004; Uria and Piel, 2009).

Metagenomics is the genetic analysis of a complex

microbial mixture that can be used to analyze sponge-microbial associations. This approach is very effective, as shown by Venter's group, who cloned all the genes from 1 m^3 of seawater (Venter *et al*., 2004; Wijffels, 2008). Hence, development of metagenomic approaches for targeting genes or entire pathways in the synthesis of bioactive molecules is very essential nowadays (Haygood *et al*., 1999; Moore and Piel, 2000; Piel *et al*., 2004).

Conclusions and Future Perspectives

Knowledge of historical, bio-geographic affinities and biodiversity distribution patterns of marine sponges is rudimentary, and there is a limited data about genetic variation among sponge populations. Rapid advances and recent biotechnological approaches on the culture/cultivation of marine sponges are importantly responsible for the identification of novel molecular targets. Keeping the challenges for the next millennium, as mentioned recently by Pomponi (2009), it is very important to consider the aspects *viz.,* identifying new sources of marine bio-products; development of novel screening technologies; providing a sustainable sources of MNPs; and optimizing the production and recovery of the bio-products.

Economic, technical and potential production methods like mariculture, *ex situ* culture (in tanks and aquariums), and *in vitro* cell culture of sponges are directional to the future biotechnological sponge research (Sipkema *et al*., 2005b). As mentioned earlier, once the collected sponges are subjected to either *in situ* or *ex situ* studies, proper ecological monitoring is needed to balance the sponge species and also, the establishment of bioreactors at either areas could result potential bioactive molecules from both sponges as well as sponge symbionts. However, several questions remain, where molecular approaches promise great potential to target potential molecules from marine sources e.g., concerning sponge connectivity and bio-geographic relationships. Recent genomic and expressed sequence tag (EST) analyses of sponges pave a way to functional genomic approaches, which can be applied to a wide range of ecological and population genetic processes including fertilization, dispersal, and colonization dynamics, host-symbiont interactions and more importantly, the secondary metabolite production (Muller *et al*., 2004; Degnan *et al*., 2008). Hence, by considering the ecological implications, latest biotechnological characterization of marine organisms like sponges and their symbionts could form a strong basis to target the required bioactive molecules as well as peptides for the treatment of various diseases that are newly evolving in the present era (Thakur *et al*., 2008).

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