2. REVIEW OF LITERATURE

**Marine Natural products**

Natural products often contain small molecules that have either an extremely polar or non-polar nature. And such are considered as an ideal drug candidate. Natural products chemist Alan Harvey reports that “the use of natural products has been the single most successful strategy for the discovery of new medicines” (Harvey, 2000). For the search of these new bioactive entities, investigations were expanded to marine habitats. Mankind has known for the last several thousand years that marine organisms contain substances capable of potential biological activity. However, the first serious investigation of marine organisms started only half century ago. Since then, almost all forms of life in the marine environment (e.g., bacteria, algae, fungi, etc.) have been investigated for their natural product content (Abad et al., 2011). Marine environment has largely yielded novel classes of compounds with diverse biological activities (Faulkner and Fenical, 1976) yet remains the largest and least chemically investigated source of new chemotherapeutic agents (Burres and Clement, 1989).

Marine organisms contain a plethora of metabolites, some of which will display extreme bioactivity. In particular, when considering marine invertebrates, which are thought to account for >30% of all animal species, comprising >20 different phyla, it is noteworthy that they should have retained an ancestral, nonspecific innate immunodefense system (Edwin et al., 1992; Rinkevich, 1999). Thus, with a dramatically increasing world population and the danger of over utilizing terricolous resource, there is a great urgency to employ these marine organisms, which are potentially untapped source of new bioactive peptides and value-added food production for the search of potential compounds (Liu et al., 2008a).
Antimicrobial peptides

Marine invertebrates are solely dependent on physical barriers and the innate immune system for protection against pathogenic agents and natural antibiotics have been shown to participate in the immune response (Munoz et al., 2004). The knowledge of the self-defense mechanism of molluscs is extremely limited compared to that of vertebrates and arthropods (Iijima et al., 2003). The immune defense of molluscs is non-specific and lacks inducible immunoglobulins, but the responses against microbial organisms are based on both cellular (phagocytosis, encapsulation, respiratory burst, etc.) and humoral (lectins, agglutinins, lysosomal enzymes, antimicrobial factors, etc.) activities (Chu, 1988; Canesi et al., 2002). In which, humoral immunity is strictly related to the presence of antimicrobial agents in blood cells and plasma. In turn, their cellular immunity is based on cell defense reactions, including encapsulation, nodule formation and phagocytosis (Mydlarz et al., 2006).

Hubert et al. (1996) identified the first molluscan AMP, defensin in Mediterranean mussels *Mytilus galloprovincialis* from acidified plasma supernatant through biochemical purification of active peptides following several steps of HPLC. The biological activity of the peptide was directed against both gram-positive and gram-negative bacteria. In the same year, Charlet et al. (1996) has isolated the antibacterial and antifungal peptides from the immune challenged and untreated mussels of *M. edulis*. They were characterized as the two isoforms of novel 34- residue of cysteine rich peptide and 6.2 kDa peptide which concluded that the host defence mechanisms of molluscs mimics that of arthropods by involving the synthesis of AMPs. These defensins appear to have a common ancestral branch that would have evolved from protodefensin existing already at the root of the Cambrian explosion.

Relf et al. (1999) stated the reasons which favour investigation and exploitation of new potential AMPs, are (i) Their innate immunity lies in their ability to function without
either high specificity or memory, (ii) Their small size which makes them easy to synthesize, (iii) Their remarkable specificity for prokaryotes with low toxicity for eukaryotic cells.

Mitta et al. (2000) discovered that the cellular component of marine invertebrate immunity is mediated by hemocytes, motile cells that phagocyte microbes by secreting soluble antimicrobial and cytotoxic substances in the hemolymph. For this reason, most of the AMPs reported in different groups of invertebrates have been isolated from hemocytes which represent an interesting natural source of AMPs. Further, Mitta et al. (2000) proposed the involvement of antimicrobial peptides in mussels constitutes an original model of anti-infectious defence in invertebrates based on: (i) molecular structures (cysteine array and 3D) similar to that of insect defensins, (ii) constitutive expression in circulating hemocytes and storage in different granules of different hemocytes, and (iii) two routes of involvement, immediate intracellular and later as released in plasma. These small cationic antimicrobial peptides are characterised by their high cysteine content and they have been organised into four groups according to the shared features of their primary structure, mainly their consensus cysteine array: defensins, mytilins, myticins and mytimycin. He also described, that the three families of defensin, mytilin, and myticin in Mytilus were characterized by 34–40 amino acids in the active peptide including 8 cysteines engaged in 4 intramolecular disulfide bonds. He not only described the families but also demonstrated the mode of action of mytilins, thereby exerting their microbicidal effect within the cells through the process of phagosome-mytilin granule fusion leading to the co-location of ingested bacteria and mytilins.

Hancock and Patrzykat, (2002) also added an advantage over AMPs that the bacteria are less likely to develop resistance against compounds that target such fundamental structures than conventional antibiotics which target specific motifs in molecules.
Iijima et al. (2003) isolated a novel antimicrobial factor from the body-wall of sea hare Dolabella auricularia including skin and mucus. This peptide dolabellalin B2 consisted of 33 amino acid residues possessing effective cytotoxicity against pathogenic microorganisms which would be advantageous for the survival of the organisms because the animal without the outer shell is in incessant contact with an environment of high amount of microorganisms.

Brogden, (2005) described the two ways by which the AMPs commonly interact with microbes i) AMPs possess a net positive charge and there by interact with the microbes bacterial membrane through electrostatic force and hence converting it to anionic form ii) AMPs form a amphiphatic structures in hydrophobic environments and thus penetrate into the bacterial phospholipid bilayer. However, Suzuki et al. (2005) reported that there are multiple factors that may affect the outcome of AMP discovery such as: seasonal and geographical variations (sampling site), different life stages (pelagic, benthic), age, sex and physiological status (disease, breeding, moulting).

Subsequently, several attempts were made to identify Mytilus-like AMPs. Seo et al. (2005) reported similar APMs in oyster following biochemical purification of an acidified gill extract from unchallenged Crassostrea virginica containing 38 amino acids and 6 cysteines. consequently, other defensin-like AMP were reported from the mantle edge of C. gigas containing 43 amino acids, 8 cysteines by Gueguen et al. (2006), from the whole-body extract (except digestive tracts) and intestines from bay scallop, Argopecten irradians by Song et al. (2006), and from hemocytes of C. gigas challenged with a cocktail of heat killed bacteria containing 43 amino acids, 8 cysteines, 2 isoforms- Cg-defh1 and Cg-defh2 by Gonzalez et al. (2007). Besides these, some other AMPs were also isolated. Gueguen et al. (2009) identified a first proline-rich AMP (Cg-Prp) from the oyster C. gigas. The Cg-Prp in hemocytes was found together with defensins and thus suspected that the antimicrobial activity may be through synergy effect with the former.
Recently, Sathyan et al. (2012) discovered the histone-H2A-derived antimicrobial peptide which is known to actively participate in host defense responses of molluscs from the backwater oyster *C. madrasensis*, rock oyster *Saccostrea cucullata*, grey clam *Meretrix casta*, fig shell *Ficus gracilis*, and ribbon bullia *Bulla vittata* which is of 75 bp. Similarly, De Zoysa (2013) discussed about the role of AMPs, as an essential defence molecule in innate immune system of invertebrate molluscs. AMPs are generally recognized as natural antibiotics which show potential therapeutic power for controlling antibiotic resistant bacteria or superbugs. In addition, AMPs are involved in anticancer, wound healing and immune stimulations. Among molluscs, mussels, oysters, abalones, clams and scallops are the major species in which AMPs have been identified. Defensin is the most common group of AMPs identified in molluscs. Various forms of mollusca defensin have shown growth inhibition of pathogenic bacteria suggesting that they may have potential application in aquaculture as alternative to antibiotics.

More recently, Sathyan et al. (2014) investigated marine molluscs crude extract in search of antimicrobial peptides. The study revealed that molluscs are potential reservoir of bioactive peptides possessing effective antimicrobial activity. These AMPs would be of promising source for application as next generation antibiotics against drug resistant microbes.

Many authors have emphasised on the technical improvements within liquid chromatography (LC) and especially HPLC technique during the last decades; which has resulted in highly efficient purification procedures for natural products, including peptides. The methods used for purifying marine AMPs are largely based on general methodology developed for peptide purification, and detailed protocols have been published (Hetru and Bulet, 1997; Selsted, 1997; Conlon, 2007; Bulet, 2008; Schroder, 2010). In the case of purification process, RP-HPLC using selected elution conditions (soft slope solvent gradients, based on the hydrophobic nature of the peptides) is the method of choice in most studies.
Antioxidants

Rock *et al.* (1996) described the term oxidative stress as the condition of oxidative damage resulting when the critical balance between the free radical generation and antioxidant defences is unfavourable. Similarly, Mc Cord (2000) reported it as a result of an imbalance between the free radical production and antioxidant defences, which is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids.

During the normal body reactions such as respiration, reactive oxygen species like superoxide anion radicals (O$_2^-$), hydroxyl radicals (OH-) and radical species like hydrogen peroxide (H$_2$O$_2$) and singlet oxygen (¹O$_2$) are formed (Gulcin 2009; 2010). The oxygen derived molecules are O$_2$ (superoxide), HO (hydroxyl), HO$_2$ (hydroperoxy), ROO (peroxy), RO (alkoxy) called as free radical and H$_2$O$_2$ oxygen as non-radical. Nitrogen derived oxidant species are mainly NO (nitric oxide), ONOO (peroxy nitrate), NO$_2$ (nitrogen dioxide) and N$_2$O$_3$ (dinitrogen trioxide) (Evans and Halliwall, 1999). These free radicals and reactive oxygen species are the major sources of oxidative stress in cells, damaging proteins, lipids, and DNA (Orrenius *et al.*, 2007). Thus, antioxidant compounds act as a free radical scavenger (FRS) which postpone the oxidation and block the chain initiated by high energy molecules and other consequent reactions (Halliwell and Aruoma, 1991; Cespecles *et al.*, 2008).

Generally, antioxidants exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation (Krinsky, 1992). Rice-Evans and Diplock (1993) proposed two principle mechanisms of action for antioxidants. The first is a chain breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems.
The second mechanism involves removal of ROS/RNS initiators (secondary antioxidants) by quenching chain-initiating catalyst.

Halliwell, (1995) described an antioxidant, as a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Earlier, Gamble et al. (1995) studied the Selenium-dependent glutathione peroxidase (Se-GPX) and other antioxidant enzyme function of four marine invertebrates, viz. mussel *M. edulis* (euryoxic herbivore), scallop *Pecten maximus* (stenoxic herbivore), crab *Carcinus maenas* (euryoxic omnivore) and starfish *Asterias rubens* indicating that exogenous sources of oxyradical production may also be important in determining levels of antioxidant defences. They exhibited considerably different antioxidant enzyme activities.

Stefanis et al. (1997) postulated the role of oxidative stress in many conditions, including atherosclerosis, inflammatory condition, certain cancers, and the process of aging. Oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematosus, adult respiratory diseases syndrome), ischemic diseases (heart diseases, stroke, intestinal ischemia), hemochromatosis, acquired immunodeficiency syndrome, emphysema, organ transplantation, gastric ulcers, hypertension and preeclampsia, neurological disorder (Alzheimer's disease, Parkinson's disease, muscular dystrophy), alcoholism, smoking-related diseases and many others.

Shi et al. (1999) mentioned that some antioxidants including glutathione, ubiquinol and uric acid are produced during normal metabolism in the body. Other lighter antioxidants
are found in the diet. Although there are several enzymes system within the body that scavenge free radicals, the principle micronutrient (vitamins) antioxidants vitamin E (α-tocopherol), vitamin C (ascorbic acid) and β-carotene cannot be synthesised by the body, so they must be supplied in the diet.

Currently, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone and propyl gallate are added to food products to retard lipid oxidation. However, these compounds must be used under strict regulation due to their potential health hazards. Siddhuraju and Becker (2003) and Jeong et al. (2004) reported that synthetic antioxidants possess increased risk of carcinogenicity and toxicity effects like liver swelling. This emphasises the need for new natural antioxidants for the future.

Valko et al. (2006) stated the normal mechanisms that take part in the human body to counteract oxidative stress by producing antioxidants which are either naturally produced in the body, or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as free radical scavengers and therefore can enhance the immune defense and lower the risk of cancer as well as degenerative diseases. In addition, Seifried et al. (2007) insisted that although the cells are equipped with an impressive repertoire of antioxidant enzymes as well as small antioxidant molecules, these agents may not be sufficient enough to normalize the redox status during oxidative stress which thereby emphasise the need of antioxidant supplementation. Thus, marine invertebrate offer a pool of potential antioxidant compounds to be discovered.

Earlier, Sheean et al. (2007) reported a study in which the aqueous extract of purple sea urchin (Heliocidaris erythrogramma) gonad was found to possess significant antioxidant activity against hydrogen peroxide in vitro. The extracts also displayed various levels of anti-
inflammatory properties in connection with inhibition of cyclooxygenase. In addition the dichloromethane extract and aqueous extract of sea urchin significantly inhibited the effect of arthritis symptoms.

Earlier, numerous researches have been undertaken on marine organisms. Gomez-Guillen et al. (2010) extracted gelatin from tuna skins and gaint squid tunics by hydrolysis and separated peptide fractions possessing antioxidant property. Prem Anand et al. (2010) reported the presence of effective antioxidant property in the methanolic extract of Horse conch, Pleuroloca trapezium with its high nutritive value. Qin et al. (2011) has evidenced that all the purified peptide fractions of hydrolysate from the gonad of Purple sea urchin (Strongylocentrotus nudus) exhibited strong antioxidant activity. Interestingly, the peptides below 1 kDa fractions were also found to exhibit highest DPPH radical scavenging capacity.

Jiang et al. (2011) purified CSPS-3 polysaccharide fraction from bivalve molluscs Cyclina sinensis (CSPS). He demonstrated that CSPS-3 fraction had strong scavenging activities in vitro on superoxide radical and hydroxyl radical activities. Similarly, Nurjanah et al. (2012) reported that the extract of mollusc Discodoris sp., which is an important organism for Pamakasan people, used as nutraceutical and functional food, contain effective antioxidant properties.

Nazeer and Naqash (2011) studied the antioxidant property of solvent extracts of two molluscan species and led to a conclusion that molluscs are rich sources of antioxidants which justify their wide use as natural antioxidants and points out other possible therapeutical uses in the different diseases caused by free radicals. Recently, Subhapradha et al. (2013) investigated the methanolic extract of Bursa spinosa and has reported potent antioxidant activity that may be due to the presence of phenols. In recent years, there has been increasing
interest in finding natural antioxidants since the synthetic antioxidants, possess higher risk of health defects.

Liu et al. (2013) purified a novel mannoglucon designated as BEPS-IB isolated from the foot muscle of Bullacta exarata, an economically important aquatic species of China. He deduced the complete molecular structure through several experiments. The results indicated that BEPS-IB was a heptasaccharide backbone ([−(1→6Glcα)61→6Mana−]n), with a tetrasaccharides branch ([−(1→3Mana)31→3Glcα−]n) occurring at the O-3 position of mannose residues in the backbone. The purified polysaccharide also showed significant antioxidant activity in scavenging superoxide radicals (O$_2^•−$) and reducing power.

**Marine Associated Bacteria**

Unlike mammals, healthy invertebrates may have bacteria in their body fluid and tissues (Farley, 1977; Stein et al., 1987). Bacteria may constitute a substantial proportion of the diet from marine filter feeders and colonize their integument and gut (ZoBell and Feltham, 1938; Mc Henery and Birkbeck 1985). It has been reported that marine bivalves accumulate large numbers of microorganisms, including gram-negative *Achromobacter, Aeromonas, Alcaligenes, Flavobactenum, Pseudomonas*, and *Vibrio* organisms and gram-positive *Bacillus, Corynebactenum*, and *Micrococcus* organisms as symbionts (Colwell and Liston 1962; Murchelano and Brown 1968) and only minor variations in this flora were found among bivalves from different locations. Farley (1977) identified that the bacteria colonizing the gut or body surfaces of animal possessing a moist integument may enter the tissue through this surface. Olafsen et al. (1993) also discovered bacteria from the soft tissues of two Pacific oysters and horse mussels. He also confirmed that the presence of bacteria in hemolymph and soft tissue of marine bivalves at temperature below 8°C.
**Carotenoids**

Marine invertebrates are known to be natural unique accumulators of specific microbial communities due to their filter-feeding habit as mentioned above. Some of the natural products isolated from them have been shown to be, or are suspected to be, of microbial origin and this is now thought to be the case for the majority of such molecules. External envelops, internal tissues and organs of these animals enriched by nutrient compounds and adhesive substances can be considered as auspicious conditions for colonization, attachment and activity of associated micro organisms. These microbial associates of marine invertebrates have proven to be a rich source of biologically active substances with antimicrobial, cytotoxic or antineoplastic activities and would be significantly used for biotechnological and pharmaceutical application (Austin, 1989; Fenical, 1993; Faulkner, 1999).

Zeaxanthin is a natural, fat soluble, yellowish carotenoid that is found in some plants, algae and photosynthetic bacteria was isolated by Nelis and De Leenheer, (1989) where it serves as an accessory light-gathering pigment, as well as a protectant against the toxic effects of UV radiation and oxygen radicals. Zeaxanthin is currently utilized as an ingredient in feed for fish and poultry to enhance the pigmentation of animal flesh and egg yolk. Apart from these, this pigment has a remarkable potential for use in pharmaceuticals as it prevents age-related macular degeneration and tumour formation.

Fenchel and Bernard, (1993) reported the presence of endosymbiotic purple non-sulphur bacteria in the marine ciliate *Strombidium purpureum* in Kahr harbours. The bacteria were found to contain bacteriochlorophyll *a* and carotenoid spirilloxanthin. The ciliates require light for survival and growth under anaerobic conditions and in the dark the cells prefer microaerobic conditions. The ciliates show photosensory behavior and they accumulate
in light at wave lengths corresponding to the absorption spectrum of the symbionts. Their presence in human diet is being considered positive because of their action as pro-vitamin (Johnson and Schroeder, 1996). Shahmohammadi et al. (1998) identified the properties of bacterioruberin pigment containing 13 pairs of conjugated double carbon bonds produced by Halobacteria that could confer bacteria with resistance to oxidative DNA damage from radiography, UV-irradiation and \( \text{H}_2\text{O}_2 \) exposure. Bacterioruberin also has other equally important roles for membrane fluidity including its function as a water barrier and responsibility for the permeability of oxygen and other molecules, thus enhancing bacterial survival in hyper saline and low-temperature environments (Strand et al., 1997; Fong et al., 2001; 2010).

Asker et al. (2007a) documented a yellow-pigmented, Gram-negative, motile, and strictly aerobic, pleomorphic bacterium (strain TDMA-16T) from a freshwater sample collected at Misasa (Tottori, Japan). Strain TDMA-16T was slightly tolerant to gamma-ray irradiation and produced carotenoids including zeaxanthin, nostoxanthin and an unknown carotenoid. Godinho and Bhosle, (2008) also identified lycopene type carotenoid pigment from orange pigmented colonies of \textit{Microbacterium arborescens-AGSB}. He also stated that the bacteria may be accumulating carotenoids as part of their response to various environmental stresses and thus aiding their survival in the stressed habitat.

Romanenko et al. (2008) discovered that marine bivalves can entrap a large amount of indigenous and non-indigenous bacteria on their tissues from marine environment that are of significance from the standpoint of public health. Most intracellular bacterium-animal symbiosis are related to nutrient provision or waste recycling, in particular nitrogenous compounds (Moya et al, 2008) and some marine invertebrates with endosymbiotic bacteria show a reduced body plan.
Muthuselvam et al. (2009) called these microbial cells producing pigments as “Microbial Pigments”. Varieties of pigments extracted from different microbes. Pigments like carotenoids, anthoquinone and chlorophyll have been produced from yeast, fungi, bacteria and algae. There is a growing interest in microbial pigments due to their natural character, safety to use, medicinal properties and nutrients like vitamins. Dieser et al. (2010) proved that these carotenoid pigmentation increases the resistance of heterotrophic bacteria to environmental stressors. Differences in the response to the physical stressors and the varying extent of the treatment effects on pigmented strains suggest that the following factors such as pigment structure, their location within the membrane, and pigment concentration may play a role in protecting cells against these stresses.

Bhat et al. (2013) observed maximum pigments production of bacteria *M. nishinomiyaensis* and *M. luteus* at 35 °C, pH 9 and at 4 % NaCl concentration (w/v) from various food samples procured from local markets of Kashmir. Shatila et al. (2013) isolated an orange pigmented strain of *Exiguobacterium aurantiacum* FH producing carotenoid from air. Both carotenes and xanthophylls were detected in the methanolic extract of carotenoids. The carotenoids produced were considerably stable and demonstrated antifungal activity against *Fusarium* sp., *Penicilium* sp., and *Alternaria* sp. Jafarzade et al. (2013) also identified 18 pigment producing isolates exhibiting antimicrobial activity out of 55 isolates from sponges, seawater, mangrove sediment, sea cucumber and mussel from different coastal areas in Malaysia.

Shindo and Misawa, (2014) searched for carotenoids of orange or red pigment-producing marine bacteria belonging to a rare or novel species. This lead to the discovery of new acyclic carotenoids with a C30 aglycone, diapolycenedio acid xylosylesters A–C and methyl 5-glucosyl-5,6-dihydro-apo-4,4’-lycenoate isolated from the novel gram-negative bacterium *Rubritalea squalenifaciens*, as well as the low-GC gram-positive bacterium
Planococcus maritimus strain iso-3 respectively. The rare monocyclic C_{40} carotenoids, (3R)-aproxanthin and (3R,2'S)-myxol, were also isolated from novel species of gram-negative bacteria belonging to the family Flavobacteriaceae, phylum Bacteroidetes. Among which, C_{30} exhibited strong singlet-oxygen-quenching activities. Naziri et al. (2014) identified carotenoids such as bacteriourberin, lycopene and β-carotene from Halorubrum sp., TBZ126, an extremely halophilic archaeon from Urmia Lake.

**Extraction of bioactive compounds from associated bacteria**

As discussed earlier, marine invertebrate metabolites are clinically important agents. However, there is limitation in the supply due to their small quantities and complicated structures. Since, aquaculture methods for most of these organisms have not yet been adequately developed, the acquisition of the material required for complete biological evaluation is tedious. Moreover, the massive collection efforts involved is time-consuming, expensive and cause potential damage to the fragile tropical reef habitat. In addition, the major hurdle in it is reisolation of previously discovered compounds which has forced many researchers to view the marine environment from different perspectives. This could be solved by investigating the marine microorganism associated or symbiotic with invertebrates making it of practical importance, as well as of ecological interest to determine whether these microorganisms influence secondary metabolite production in invertebrates (Davidson, 1995).

Marine bacteria and fungi are of great interest as novel and rich sources of biologically active products. They live in close association with soft-bodied marine organisms that lack obvious structural defence mechanisms and thus rely on chemical defence by production of bioactive secondary metabolites, either by themselves or by associated microflora, to survive in their extreme habitat (Jensen and Fenical, 1994). These bacterial species are generally not real symbiotic to the host instead be regarded as associated bacteria (Bultel-Ponce et al.,
1999) with consanguineous relationship. Armstrong et al. (2001) described that these bacteria acquire the necessary nutrition such as vitamin, polysaccharide and fatty acid from their animal hosts and in turn they excrete products such as amino acid, antibiotic and toxin propitious for the development and metabolism of the hosts, or to improve the chemical defence capability of the hosts.

Earlier, He (2001) isolated lomaiviticins $a$ and $b$ substances possessing antitumour activity from squids for the first time. In later experiments it was found that, the bacterium Micromonospora lomaivitiensis isolated from squid was the bacteria which are responsible and that they are the real producers. As discussed earlier, Hentschel et al. (2002) insisted that the interactions between marine invertebrates and microbes are symbiotic relationships. The symbiotic functions that have been attributed to microbial flora include nutrient acquisition, processing of metabolic waste and secondary metabolite production. Engel et al. (2002) stated that these bacteria could chemically defend the host against microbial infections. Thiel and Imhoff (2003) reported the Vibrio sp. isolated from Mediterranean sponges was found to produce antibiotic against other microbes.

Zheng et al. (2005) have isolated 341 strains from sea water, sediment and marine organisms in different coastal areas of China, of which 12% of them showed antimicrobial activity and most of the active bacteria’s are associated with marine invertebrates and seaweeds. He also inferred that different species could produce different antimicrobial metabolites by screening using TLC autobiographic agar overlay assay. The results were impressive that some marine bacteria could probably produce different types of antibiotic compound.

Prem Anand et al. (2006) isolated seventy five marine bacteria associated with four marine invertebrate sponge species. Among the isolated bacteria, twenty one per cent strains
were effective antibiotic producers ranging from broad to species specific. The strain SC3 found to be more active was mass cultured and extracted using ethyl acetate. The active fraction was further purified and identified using reverse phase HPLC. The potential strain was identified as gram positive rod shaped bacteria. This was the first report on phylogenetic identification of antibiotic producing bacteria associated with sponges from Indian waters. Simmons et al. (2007) reported that many of the biologically active molecules resulted in the evolution of diverse genetically encoded small molecules having potential utility in modern medicine and biomedical research. These promising natural products often are derived from organisms richly populated by associated microorganisms.

Romanenko et al. (2008) isolated 149 strains from the marine ark shell Anadara broughtoni. Most bacteria’s isolated were Gammaproteobacteria, Alphaproteobacteria, Firmicutes, Actinobacteria, Cytophaga-Flavobacterium-Bacteroides (CFB) group. Among which the butanol extract of six strains exhibited potent antimicrobial, haemolytic and surface activities. Substances with haemolytic and surface activities were isolated from strain Bacillus pumilus An 112 purified using reverse-phase HPLC and characterized as cyclic depsipeptides with molecular masses 1021, 1035, 1049, 1063 and 1077 Da. Martín et al. (2013) identified a new thiazolyl peptide, kocurin displaying antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA) isolated from the culture broths of a marine derived Kocuria palustris. Similarly, Palomo et al. (2013) isolated kocurin from the sponge-derived Kocuria sp. and Micrococcus sp. Normally, the methods of bacterial culture and identification have become very promising especially, those done through molecular techniques which enables to possibly identify the respective strain. And also, the study of marine mollusc associated bacteria is important for our understanding of their ecological role in the interaction with animals and also for their biotechnological application as producers of bioactive compounds.
The review clearly states the importance of marine natural products been recognized as the most promising source of bioactive substances for drug discovery research. Thus evident, the field of marine invertebrate bioactive peptides is underdeveloped and provides the opportunity for a breadth of research on isolation and identification of the specific peptides that are responsible for various activities from the marine bivalve and associated endophytic microbes should be voted.