General Introduction
1. GENERAL INTRODUCTION

Ocean covers 70% of the planet’s surface (Fenical, 1993; Whitehead, 1999). Thus, the marine environment is a natural habitat of an immensely vast and complex array of species and ecosystems having different physiology and capacity to adapt their environment. The enormous pool of diversity resources renders chemical diversity which is a sustainable source of clues for the novel and robust biomolecules. For this reason, the marine environment is considered as one of the current hotspots for the bio-prospection of new bioactive molecules (Montaser and Luesch, 2011). The chemical diversity associated with marine natural products is unanimously acknowledged as the “blue gold” (Leal et al., 2014). Discovery of potent marine natural products (MNPs) is continuously increasing day by day which reached up to 1000 compounds per year (Nadeem et al., 2015). Around 30,000 bioactive products were isolated from marine organisms which are currently either under pre-clinical or clinical trials for drug development (Newman and Cragg, 2014; Schmidtko et al., 2010). Recently, Blunt et al. (2016) reviewed 1340 new natural compounds for the year 2015 and 1378 new compounds in the year 2014 from different marine organisms. Still, exploration is in progress providing a novel and improved therapeutics for human diseases, drug delivery systems along with other innovative products for industrial activities like novel enzymes, nutraceuticals, cosmetics, antifouling paints, biosensors, molecular probes and agrichemicals (Burgess et al., 2003; Leal et al., 2012).

Marine biotechnology is the recently egressing field that explores the diversity of marine environment in terms of the form, structure, physiology and chemistry of marine organisms for their biotechnological applications. It is an interdisciplinary field that employs marine biology, microbiology, chemistry, genomics and
bioinformatics. Through the application of biological knowledge and cutting-edge techniques, marine biotechnology harnesses marine materials for several thousands of new compounds and unlocks the access to their applications.

Coral Reef Ecosystem

Coral reefs are one of the most biologically diverse ecosystems on Earth and are widely recognized as the “rainforest of the ocean” (Reaka-Kudla, 1997). Nearly one-third of the tropical coastlines are fringed with coral reefs (Birkeland, 1997). Coral reefs are estimated to cover 284,300 km² (109,800 sq mi) just under 0.1% of the oceans' surface area. One fascinating animal, the coral polyps are primarily responsible for reef-building which takes many forms such as large reef-building colonies, graceful flowing fans and also small and solitary organisms. The coral reef ecosystem is made up of not only hard and soft corals, asides reefs support extraordinary biodiversity and home to multitude of different lives including sponges, sea anemones, crustaceans, mollusks, sea turtles, sharks, dolphins, bryozoans, flatworms, polychaetes, sea stars, sea cucumbers, sea urchin, fishes, etc. Thus, this ecosystem is a diverse collection of species that interact with each other and the physical environment. Competition for resources such as food, space and sunlight are some of the primary factors which determine the abundance and diversity of organisms on a reef.

Marine Natural Products (MNPs) from Cnidarians

There is a significant increase in the discovery of MNPs from marine invertebrates. Cnidarians occupy the second position as a source of MNPs next to sponges. The phylum Cnidaria is a large, diverse and ecologically important group of marine invertebrates that includes over 11,000 extant species and over 3000 MNPs
have been described (Rocha et al., 2011). The class Anthozoa is the largest of the 4 classes of Cnidaria with over 7500 species. The survey from the year 1990 to 2009 revealed that class Anthozoa comprised 99.0% of new MNPs and the discovery increased 72.0% from 1990 to 2000. There are two subclasses, namely, Alcyonaria (or Octocorallia) and Zoantharia (or Hexacorallia). It is well known that members under the order Alcyonacea (soft corals) and Gorgonacea (sea fans) contributed the most to promising bioactive marine compounds (Leal et al., 2012). On the other hand, the Actiniaria (sea anemones) and Scleractinia (hard corals) have also reported for the presence of MNPs.

Limited discovery of bioactive compounds such as secondary metabolites, proteins, enzymes and genes were reported from the Scleractinian corals around the world (Fontana et al., 1998; Koh and Sweatman, 2000; Ramos and García, 2007; Higuchi et al., 2008; Meyer et al., 2009; Palmer et al., 2009; Anithajothi et al., 2014; Armoza-Zvuloni and Shaked, 2014; Gacesa et al., 2015; García-Arredondo et al., 2016). Interestingly, researchers have demonstrated that the structure of many MNPs produced by the macroorganisms closely resembles those identified from associated bacteria (Piel, 2004, 2009; König et al., 2006). Thus, it remains unclear whether the target metabolites are produced by the macroorganisms themselves or from their associated microbial symbionts.

**MNPs from the Associated Microorganisms**

Microbes in the marine environment are the most diverse and abundant group of organisms that constitute 60% of the total biomass. Especially, the microorganism-eukaryote associations have gained significant attention in the past decade. The associated microorganisms have the necessity to evolve inimitable chemicals capable of protecting from the fierce competition that exists between
microorganisms on the surfaces of marine eukaryotes (Penesyan et al., 2010). Thus, they are proven to be the inexhaustible reserve of chemically diverse and biologically active secondary metabolites that are low molecular weight organic molecules. Moreover, the ‘living surface’ nurtures abundant and diverse microbes (Olson and Kellogg, 2010) than the surrounding water (Hentschel et al., 2006; Rosenberg et al., 2007). The diversity has also exerted a driving force on bacterial selection leading to new adaptive strategies and the synthesis of new metabolites.

The associated microbial metabolites have a very important role in the host biology and ecology particularly in its nutrition, chemical defenses against both predators and competitors, provide defensive mechanisms against stress, facilitate reproductive processes and chemical signaling for interaction among marine organisms (Webster and Taylor, 2012; Ainsworth et al., 2010; Hay, 2009; Paul and Puglisi, 2004). Thus, the diverse secondary metabolites are known for their broad range of biological effects that include antibacterial, antifungal, antiparasitic antiprotozoan, anticancer, anti-inflammatory, neurotoxic, antiviral, cholesterol-lowering agents, immunosuppressants, herbicides as well as antifouling activities that prevent the surface-settlement of various marine organisms are of considerable biotechnological interests (Kobayashi and Ishibashi, 1993; Piel, 2004; Molinski et al., 2009). Nevertheless, the vast biotechnological potentials of marine epibiotic or symbiotic microorganisms remain mostly unexplored.

There are several benefits with the use of microorganisms as a source of bioactive compounds over macroorganisms. The bacteria can be easily cultured in the growth media to scale up a large amount of bioactive compounds in a short time. In addition, the culturing techniques and the optimized culture conditions with other factors could be manipulated to enhance the microbial production of MNPs.
Different strains of same species of bacteria can produce different bioactive conditions under different culture conditions (Armstrong et al., 2000). Therefore, it is reasonable to expect new classes of secondary metabolites with multipotent bioactivity. Since 1990, the number of bioactive metabolites from marine bacteria has been exponentially increased (Fenical, 1993; Kobayashi and Ishibashi, 1993; Bernan et al., 1997; Faulkner, 2001; Hill, 2003; Blunt et al., 2016). Up to now, bioactive agents were isolated extensively from *Streptomyces*, *Altermonas/Pseudoalteromonas*, *Bacillus*, *Vibrio*, *Pseudomonas* and *Cytophaga* (Al-Zereini, 2006).

**Microbial Diversity Associated with Corals**

Tropical reef-building corals are diverse meta-organisms that contain symbiotic dinoflagellate algae *Symbiodinium* and also a microbiome assembled of bacteria, fungi, archaea and viruses, collectively referred to as coral holobiont (Thompson et al., 2014). The compartmentalization of the microbiome generates distinct microbial communities within the surface mucus layer, tissues, skeleton, and gut and some particularly associated with *Symbiodinium* (McDevitt-Irwin et al., 2017). For the last two decades, there were substantial records on the abundance and diversity of microorganisms associated with the healthy and diseased corals. The importance of coral associated microbes is being recognized due to the findings that microbes play an active role in maintaining coral nutrition and health (Bourne and Webster, 2013). Precisely, high coral bacterial species diversity has been emphasized in numerous studies. The diversity attracts many researchers as the ribotypes of these bacterial communities could go up to 6000 (Rohwer et al., 2002).

**Coral Mucus**

Scleractinian corals are covered by a gel-forming mucus layer which is released continuously or periodically from the coral surface into the reef environment
(Bythell and Wild, 2011). This mucus layer has been variously described as the Coral Surface Microlayer (CSM), the Surface Mucopolysaccharide Layer (SML) and the Muco-Poly saccharide Layer (MPSL) (Brown and Bythell, 2005). Coral mucus is a dynamic system, where its molecular organization and its intricate concoction of biochemicals are influenced by species and temporal-specific composition that varies with depth and/or irradiance, with aging and also contamination upon release into the seawater environment (Brown and Bythell, 2005). They are excreted by mucocytes in the epidermis of corals.

The SML protects the corals from foreign matter, allows the exchange of metabolites (organic and inorganic) (Wild et al., 2004; 2008), also nutritious that contributes to its own food chain, provides heterotrophic feeding, protection against fouling, avoids desiccation during air exposure at low tide and sedimentation. In addition, they cleanse themselves by sloughing off continuously or periodically, from the coral surface into the reef environment (Brown and Bythell, 2005) and defense against a multitude of environmental stresses.

**Biochemical composition of coral mucus**

The dynamic and complex layer is composed of macromolecular components such as glycoproteins, lipids and mucopolysaccharides (Ducklow and Mitchell, 1979). The polypeptides of the polymeric glycoproteins also called as mucins are the major components with serine, threonine, aspartate, glutamate and glycine being most common amino acids in different coral species (Krediet et al., 2013a). This polypeptide is decorated with sulfated oligosaccharide side chains O-linked, consists of varying monosaccharides, most common are mannose N-acetyl-D glucosamine, galactose, fucose, glucose and arabinose, with xylose and N-acetyl-D-galactosamine being minor components of coral mucins (Meikle et al., 1987; Brown and Bythell
The composition of sugar moieties in the polymeric glycoprotein changes with species to species. Lipids are the minor component and play a role in the rheological properties along with glycoproteins, i.e., they give the viscous nature to the mucus (Meikle et al., 1988).

**Coral Mucus Associated Bacteria (CMAB)**

It is noteworthy that the organic rich coral mucus on the surface of the coral hosts is attractive for numerous marine organisms than the surrounding seawater. As a consequence, there exists a competition for space and food in this microniche. Moreover, the microbes within this layer actively interact with the potential pathogens and environmental organisms. Hence, this microniche draws more importance to reveal the status of coral health.

Coral mucus layer is the distinctive suite of diverse bacterial communities (Rohwer et al., 2001, 2002; Bourne and Munn, 2005). The associated microbial diversity is greatly influenced by various factors including coral species, colony physiology, continuous and periodical structural changes of mucus, geographical regions, environmental conditions and temporal factors (Rohwer et al., 2002; Hong et al., 2009; Lee et al., 2012; McKew et al., 2012; Nelson et al., 2013; Zhang et al., 2015). The mechanism of dynamic coral mucus associated bacterial population allows for the rapid adaptation of corals to a changing environment (Reshef et al., 2006).

Moreover, high level of specific bacterial communities appear to be ‘core’ symbionts of the holobiont and always associate with a specific coral host highlight the existence of close cross-relationships between the bacterial epibionts and hosts. Thus, the associated bacteria are often metabolically linked with their host. These associated bacteria likely provide important functional benefits to the coral holobiont
(Rohwer et al., 2001; Neave et al., 2017). They are expected to be important for the coral host’s physiology such as biogeochemical cycling and as well as preventing predation of the host via the production of bioactive molecules (Mouchka et al., 2010). For example, evidences suggested the various mutualistic benefits of certain bacterial species in coral holobiont includes fixation and passage of nitrogen and carbon to the coral host, metabolize sulfur and various other biogeochemical cycles (Shashar et al., 1994; Cooney et al., 2002; Rohwer et al., 2002; Lesser et al., 2004; Wegley et al., 2007; Raina et al., 2009; Kimes et al., 2010; Lema et al., 2014).

Generally, bacteria attached to a surface, whether detritus or biotic surfaces are more likely to produce novel antibacterial molecules than free-living bacteria (Burgess et al., 1999; Long and Azam, 2001). It has been hypothesized that the coral mucus associated bacteria also produce secondary metabolites to combat pathogenic microbes (Rohwer and Kelly, 2004; Ritchie, 2006; Nissimov et al., 2009; Rypien et al., 2010; Shnit-Orland and Kushmaro, 2009; Nithyanand et al., 2011; Hussein and Alwaked, 2016). Their antagonistic interactions also prevail among co-occurring coral-associated microbes (Rypien et al., 2010). On the other perspective, the associated bacteria may also produce defense compounds to inhibit the bacterial growth, microfouling and encourage fouling free coral surface (Armstrong et al., 2001; Holmström et al., 2002, Dobretsov and Qian, 2004; Rao et al., 2005). The bioactive compound production can confer a competitive advantage within a biofilm by keeping antibiotic-sensitive isolates from colonizing the community (Tait and Sutherland, 2002). Garren et al. (2009) further emphasized that the resilience capability of bacterial communities within a single coral colony fundamentally contribute to the resilience and health of reef ecosystems. However, the contribution of these distinct and diverse bacterial communities hoarding on the surface layer of
different corals is also not clearly studied. Moreover, the complexity, diversity, specificity and selective pressure on the coral surface associated bacteria have elicited the importance of CMAB as the potential sources of secondary metabolites with diverse biological activities that can be employed for biotechnological applications. The bio-prospecting of CMAB for MNPs is still lacking. In addition to bioprospecting, these studies aid us to understand the status of coral health.