CHAPTER TWO

LITERATURE REVIEW

2.1 Sea Cucumber

The holothurians or sea cucumbers are marine benthic invertebrates, classified under the phylum Echinodermata. Echinoderms include five different classes, which are the Asteroidea (sea stars), Echinoidea (sea urchins and sand dollars), Crinoidea (crinoids or sea lilies), Ophiuroidea (brittle stars) and Holothuroidea (sea cucumbers) (Pagán-Jiménez *et al*., 2019). The holothurians consist of six orders namely Aspidochirotida, Apodida, Dactylochirotida, Dendrochirotida, Elasipodida and Molpadiida (Conand, 2004). Most of the harvestable species of sea cucumbers belong to the seven genera of the Aspidochirotida including *Bohadschia, Holothuria* (Holothuridae*), Actinopyga, Isostichopus, Stichopus, Parastichopus* and *Thelenota* (Stichopodidae). There are also some sea cucumber species that have not yet been systematically identified, since there are relatively small number of holothurian taxonomists (Bruckner *et al.,* 2003; Bordbar et al., 2011).

Sea cucumbers obtain food by ingesting marine sediment, or by filtration of seawater (Sulardiono *et al.,* 2020). The holothurians are distributed in oceans all over the world, and generally inhabit near the coral reefs, rocky ground, or sea weeds in warm shallow waters of the sea (Mehmet *et al*., 2011; Ridzwan, 2007). One of the common tropical holothurian species, *Holothuria leucospilota* is widely found in shallow reef areas such as the reef flats, shallow coastal lagoons and seagrass beds, of the tropical and sub-tropical Indo-Pacific region, including the Red Sea (Pangestuti and Arifin, 2018). Sea cucumbers are an important component that contribute to the well-being of marine ecosystem. They play an essential role as keystone species, bioturbators and recyclers of lagoons (Lampe, 2013).

Sea cucumbers have also been suggested to be involved in protecting coral reefs from the devastating effects of climate change. According to Schneider *et al*. (2011), corals need to survive by accumulating their key component, calcium carbonate at an equal or better rate than losing them. Sea cucumbers contribute by ingesting and digesting carbonate sand and defecate calcium carbonate as part of their digestion by-products, as were demonstrated by *Stichopus herrmanni* and *Holothuria leucospilota* in One Tree Reef, Australia. Additionally, ammonia waste released from the digestion process serves to fertilise the surrounding area, providing nutrient for coral growth (Schneider *et al*., 2011).

2.2 Sea Cucumber in Malaysia

In Malaysian seawaters, approximately 80 species of sea cucumbers have been reported, which are distributed from the intertidal zones to deep sea (Kamarudin *et al.,* 2010). A total of 52 morphospecies of sea cucumbers from four orders comprising of twelve genera were documented. These include 38 sea cucumber species recorded in Sabah followed by 24 species in Peninsular Malaysia, 10 species in both regions, while nine other species required further taxonomic works for their identification. Sea cucumbers of the order Aspidochirotida and genus *Holothuria* are generally the dominant taxon in Malaysia seawater, while *Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835) is the most dominant species (Kamarudin *et al*., 2015). Among the species from the order Aspidochirotida documented in Malaysia seawaters include *Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835), *Holothuria (Mertensiothuria) hilla* (Lesson, 1830)*, Holothuria (Metriatyla) scabra* (Jaeger, 1833)*, Holothuria (Halodeima) atra* (Jaeger, 1833), *Holothuria (Halodeima) edulis* (Lesson, 1830) and *Bohadschia vitiensis* (Semper, 1868) (Kamarudin *et al*., 2015).

Sea cucumbers are highly valuable marine organism having a long history in the fishing industry and are the most consumed echinoderms. Locally, sea cucumbers are known as *timun laut*, *bat*, *balat*, *trepang*, *brunok*, *hoi sum* and *hoi shen* (Yasoda *et al*., 2006). A mass of harvestable species has been exploited from Malaysia seawater to meet the growing market demand due to their food and pharmaceutical uses. The major species being harvested are from the families of Holothuriidae and Stichopodidae (Bruckner, 2003). Hence, sea cucumber aquaculture in Malaysia was initiated in 2012 to meet the market demand (Mazlan and Hashim, 2015) and this has been well established in other countries (Conand, 2004).

Even though sea cucumbers are highly important marine organism, their populations are facing great threats caused by overexploitation and pollution in many places around the world including in Malaysia (Purcell *et al*., 2012). In 2013, 16 sea cucumber species were listed as threatened with extinction, in which seven of them considered as endangered and nine as vulnerable on the IUCN Red List (Hamel and

Mercier, 2013). Factors that can lead to species declines include direct exploitation, habitat loss and degradation, and introduced species (Gaston and Spicer, 2004). This becomes worse with the proximity of surrounding islands to the mainland and the negative effects from the tourism developments (Kamarudin et al., 2009). Illegal fishing in Sabah of East Malaysia operated by illegal immigrants from the Philippines has also been suggested to contribute to this problem (Choo, 2004).

The survival rate of sea cucumber in marine water of Pangkor and Langkawi Island of Peninsular Malaysia were also affected by poor management development that cause sedimentation due to land clearing (Pankor, 2004). Therefore, efforts are being taken to conserve and sustain sea cucumbers in Malaysia and other countries. For example, the Langkawi Development Authority (LADA) have started on restoring the sea cucumber species via sea ranching in Teluk Yu, Temoyong and Tuba Island, Langkawi Island, Kedah (Sharif and Osman, 2016). There are also other sea cucumber ranching projects being conducted in a few places in Sabah.

2.3 Morphology and Anatomy of Sea Cucumber

Sea cucumbers generally have leathery skin and their body size range from a few millimetres to a metre (Backhuys, 1977; Lawrence, 1987). They are soft and cylindrical-bodied echinoderms that are related to starfish and sea urchin due to some common characteristics they share that include radial symmetrical body and having a coelomic, water vascular system. Morphological characteristics can be used as a basis to distinguish the orders of sea cucumber such as the tentacles, the presence or absence of respiratory tress, and the presence and distribution of podia on the trunk (Kumar, 2012). The outer surface of its body wall is known as cuticle (Table 2.1) (Yang, 2015), and the mouth of a sea cucumber at its anterior end generally has tentacles that are extended for feeding purposes, mainly to obtain particulate organic matter (Purcell *et al.,* 2012).

The internal body of a sea cucumber houses its digestive system and connected organs. This includes a long and coiled alimentary canal of intestine that is connected to the anus through rectum and cloaca (Kamarudin and Rehan, 2018). The body cavity of sea cucumber is filled with coelomic fluid, covering the internal organs and forms a fluid medium (Shi *et al.,* 2020). Individuals are also characterised by having evolved skeleton recognised as ossicles and the ancient-looked respiratory system called respiratory tree possessed by a few species (Lambert, 1997). The respiratory tree is a specialized organ for gas exchange and excretion in sea cucumbers, which is also connected to the cloaca by connective tissue (Yang, 2015).

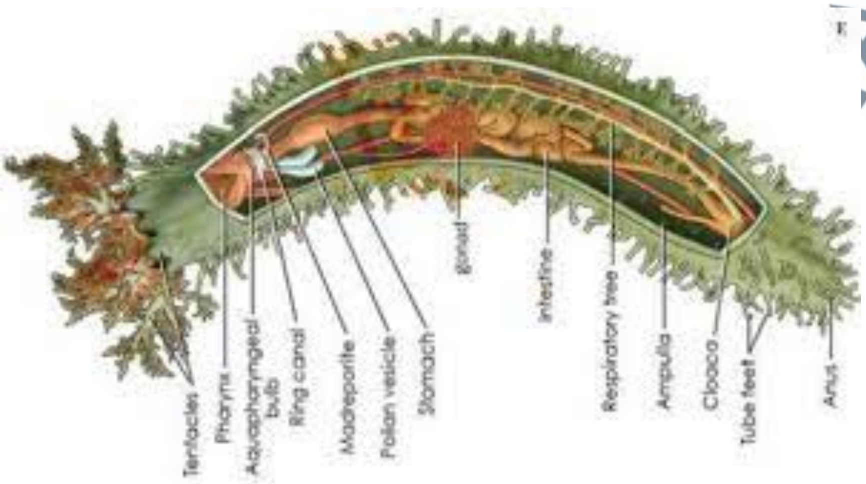

Figure 2.2. Sea cucumber anatomy (Mashjoor and Yousefzadi, 2019)

Table 2.1. General description of sea cucumber body parts (Tehranifard and Rahimibashar, 2012)

Body parts	**Description**
Cuticle	The body surface of sea cucumber was covered by a thin cuticle
Tentacle	Encircled the mouth of sea cucumber are bushy tentacles use to trap small particles of food
Coelomic fluid	Filled body cavity that acts as hydrostatic skeleton
Cloaca	The posterior opening that brings water in and out for respiration
Gastrointestine	The digestive tract that looped within the coelom
Respiratory tree	Attached to posterior cloaca and function in gas exchange
Cuvierian tubules	Specialized defence system that are attached to the basal part of left respiratory tree
Polian vesicle	Sacs that are associated with the ring canal and serve to maintain the fluid pressures within the water vascular system

Sea cucumber also possesses a complicated water-vascular system, which involve a unique arrangement of fluid-filled coelomic passages and associated parts (Li *et al.*, 2013). One of the main accessory structures of its water-vascular system is polian vesicles, which are a bag-like tissue that consists of a thin inner epithelium wrapped by a thick muscular wall (Baccetti and Rosati, 1968). Polian vesicles are also considered to play important roles in inflammatory response of the sea cucumber (Shi *et al.*, 2020). Another important feature of this organism is their defence mechanism called evisceration, the ability to expel their sticky Cuvierian tubules under stress conditions, which are then regenerated (Hashim, 1993). The Cuvierian tubules are present in only several species of family Holthuriidae such as from *Holothuria* and *Bohadschia* species (Flammang *et al.*, 2015).

2.4 Uses and Benefits of Sea Cucumber

Apart from its important roles in the marine ecology, sea cucumbers also provide economic significance in foods and pharmaceutical sectors. Sea cucumber is an edible organism and one of the most commercialised seafood products in the food industry in Asia including in Malaysia. These products have benefited many coastal communities of Sabah as their source of income (Choo, 2004). Some sea cucumber species have long been considered as priced delicacy since they are believed to have aphrodisiac qualities (Al-Haj *et al.*, 2009). The processed sea cucumbers are considered as luxury seafood sold at high price in Asian stores alongside other dried seafood. Nutritionally, sea cucumbers provide a rich source of nutrients such as Vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin) and minerals especially calcium, magnesium, iron and zinc (Rahman, 2014).

In addition to their good flavour, sea cucumbers are also used in traditional and modern-formularized medicines (Lukman *et al*., 2014). In peninsular Malaysia, sea cucumbers are prized for their medicinal properties, and are commonly used for the preparation of traditional medicinal products like *gamat* oil. Their coelomic fluid, also known as *gamat* water, has traditionally been used in post-natal treatment by Malay society (Lukman *et al*., 2014; Al-Haj *et al*., 2009) and is also well known as a tonic by Chinese folks for its efficiency against asthma, rheumatism, tuberculosis, stomach and duodenum ulceration, diabetes, cuts and burns, impotence and constipation (Rahman, 2014). Apart from that, sea cucumbers also have great potential as functional ingredients in cosmetics (Siahaan *et al*., 2017). The manufacturing of *gamat*-based health products through modern technologies is also practised by several multi-level marketing (MLM) companies such as Gamat eMas Sdn Bhd (GESB) and Luxor Network Sdn Bhd.

Previous researches have also reported on compounds extracted from sea cucumbers that exhibit antimicrobial, anti-inflammatory, antiviral, antitumor, anticancer and antifertility properties (Bordbar *et al.* 2011). Echinoderms also showed the highest antibacterial activity compared to other marine organisms such as porifera, bryozoan, mollusca and corals (Shakouri *et al*., 2009). The beneficial health properties of Malaysian sea cucumbers have also been reported including their antioxidant activity, analgesic, therapeutic and anti-anaphylaxis effects (Ridzwan *et al*, 1995;

Fredalina *et al*., 1999; Khartini *et al*., 2003; Osama *et al*., 2009). Sea cucumbers are also a rich source of functional ingredients of biomedical products such as bioactive peptides, vitamins, minerals, fatty acids, saponins, carotenoids, collagens, gelatines, amino acids and fatty acids (Kim and Himaya, 2012). Significant amount of carotenoids were also documented from *H. leucospilota* that include β-carotene, β-echinenone, canthaxanthin, phoenicoxanthin, astaxanthin, lutein, zeaxanthin (Pangestuti and Arifin, 2018). The synthesis of these compounds may also be contributed by bacterial communities that live symbiotically with the sea cucumbers (Farjami *et al.,* 2013).

2.5 Marine Bacteria

Bacteria are single cell prokaryotic microorganism without nucleus, whose membranes are made of peptidoglycan (Silhavy, 2010). The existence of microscopic organism, formerly referred as animalcules was discovered by Anthony van Leeuwonhoek in the 17th century (Pace, 1997). Microorganisms are widespread in nature, for example in the soil, air, water, human body and animal gut. Microorganisms also dominate the oceans in the majority of its biomass, since the oceans form the largest habitat on earth that support great diversity of marine life (Glockner *et al*., 2012). Hence, the oceans turned out to be an attractive field for researchers as many mysteries regarding microorganisms and their metabolites have yet to be discovered. This is in line with the development of modern technologies that allow access to the indisputable rich biodiversity environment, leading to a deeper exploration on marine microorganisms by scientists.

Marine bacteria can be different from their terrestrial relatives as a result of adaptation to their respective environmental conditions (Bhakuni and Rawat, 2005). The complexity and dynamic system of the marine environment can be stressful to resident organisms, which makes them acquire the ability to produce secondary metabolites with stronger bioactivities, more novel and contain unique structures that inhibit the growth of other microorganisms (Bultel *et al*., 1999; Rineheart, 2000). These tremendous surroundings have also resulted in the metabolic differences of marine microorganisms as compared to those in terrestrial environment (Schwartsmann *et al*., 2001). Most marine bacteria have been reported to be Gram-negative rods, and it was hypothesised that their outer membrane structure has adapted evolutionarily to the low nutrition, high pressure and high salinity conditions (Soliev *et al*., 2011).

In addition, the variability of resource limitation in nutrient and space have obliged the marine microorganisms to compete among themselves, and acts as a selection pressure that favours production of natural products with medical and industrial values (Armstrong *et al*., 2001). Marine bacteria have become a promising source for a variety of applications in medicines and industrial processing (Maas, 2006). This is because of their ability to produce secondary metabolites with the potential to be developed as anticancer drugs and to treat antibiotic resistant infections (Avendano-Herrera *et al*., 2005). These secondary metabolites are produced as a response against other microorganisms or predators in their environment, as a defence mechanism and for survival (Petersen *et. al.,* 2020). Marine bacteria have also been shown to be antibiotic producers (Wiese and Imhoff, 2019).

Even though bacteria have often been viewed as harmful microorganism, but they have also been acknowledged to provide great contributions in the ecological stability. Bacteria promotes decomposition of matter, mineral cycle and formation of sediments in the marine environment (Das *et al*., 2006). The continuity of living organisms is also dependent on some bacteria that carry out photosynthesis to produce oxygen (Staley *et al*., 1997). Besides, the activities of certain bacteria in the marine ecosystem can also produce phosphorus and nitrogen, which provide mass of nutrients to the reef sediment, and in turns, coral releases mucus in reef metabolism that serves as a growth substance for bacteria (Das *et al*., 2006).

Bacteria in the marine environment can exist as free living or in close association to a variety of marine animal hosts including the invertebrates, such as the sponges and sea cucumbers (Pagán-Jiménez *et al*., 2019; El Samak *et al*., 2018; Gao *et al*., 2014). Likewise, the animal intestines were shown to harbour complex communities of microorganisms (O'hara and Shanahan, 2006). These bacteria were consumed and passed along in the food chain of marine organisms (Das *et al*., 2006). Furthermore, the presence of beneficial bacteria in the gastrointestinal tract of humans and animals facilitate their metabolic processes, and the wide range of diet also shaped the gut microbiome (Ley *et al*., 2008). These bacteria-host interactions and their impacts on animal population and ecosystem have also been discovered with the help of environmental genomics approach (Azam and Worden, 2004).

2.6 Bacteria Associated with Sea Cucumbers

Sea cucumbers are among marine animals that act as a host to a wide variety of bacteria. Sea cucumbers obtain food by the ingestion of marine sediment or by filtration of seawater (Ward-Rainey *et al.,* 1999), and consuming microorganisms, meiofauna, decaying organic debris, inorganic components, and dissolved organic matters that are accessible on surface sediments (Roberts *et al*., 2001; Gao *et al*., 2010). Bacterial communities inhabiting seafloor are extremely diverse in their identities and have been considered either as a direct food source or a source that indirectly offers the sea cucumbers with essential nutrients that are otherwise not available (Amaro *et al*., 2009; Gao *et al*., 2010). These bacteria associated with marine organisms have also been discovered to produce diverse and novel natural products (Konig *et al*., 2006; Brady *et al*., 2009, Piel, 2009).

Previous studies have indicated the association of bacteria with different species of sea cucumbers, mostly on the Japanese spiky sea cucumber *Apostichopus japonicus* (Table 2.2). Characterisation of gut bacterial community in *A. japonicus* collected from Primorskii krai, Russia was reported to consist of facultative anaerobes from the environment (Bogatyrenko and Buzoleva, 2016). Furthermore, bacteria with probiotic potential were isolated from the gut of *A. japonicus* from Shandong Province, China including *Bacillus sp*., lactic acid bacteria (*Lactobacillus*, *Lactococcus* and *Streptococcus*) and *Pseudomonas* (Gao *et al*., 2014). Potentially metabolically active bacteria of *Epsilonproteobacteria* and *Rickettsiales* in the coelomic fluid of *A. japonicus* from Japan were also found (Enomoto *et al*., 2012). It has also been suggested that sampling time and sites are the contributing factor for the fluctuations in microbiota composition in *A. japonicus* samples (Kim *et al.*, 2017). Bacteria that may cause diseases of *A. japonicus* have also been reported, for example, Gram-negative bacteria that belong to the genera *Vibrio*, *Shewanella*, *Serratia*, *Pseudoalteromonas* or *Flavobacteria* were isolated from diseased *A. japonicus* specimen from Dalian, China (Li *et al*., 2010).

Bacteria have also been isolated and detected in other sea cucumber species, mainly focusing on their gut or intestine. Gut bacteria is known to play important roles on the host health (Liu *et al*., 2011). Zhang *et al*. (2012) reported the presence of aerobic culturable bacteria from intestine of *Holothuria leucospilota* in Nagasaki, Japan. High bacterial abundances have also been recorded in the guts of deep-sea holothurians from the north-east Atlantic (Roberts *et al*., 2001). Furthermore, *Gammaproteobacteria* and *Vibrio* sp. were identified as the predominant groups in the intestine of *Stichopus japonicus* from Coast of Dalian, China (Gao *et al*., 2016). On the other hand, bacterial composition of the microbiome of *Holothuria tubulosa* and *Holothuria forskali* showed a predominance of the phylum Bacteroidetes (Leon-Palmero *et al*., 2018).

Table 2.2. Some studies exploring the association of bacteria with sea cucumbers

Sea Cucumber	**Location**	**Body parts / Source**	**Associated Bacteria**	**Reference**
Holothuria glaberrima	Playa Piñones, Puerto Rico	Intestine	Bacteria from phyla Proteobacteria, Bacteroidetes, Firmicutes	Pagán-Jiménez et al., 2019
Apostichopus japonicus	11 farms located in the suburbs of Dalian, China	Surface mucus, skin ulcerations, coelomic fluid, viscera of sea cucumbers, the nets, water and sediment of culture areas.	31 isolates belonged to the genera of *Vibrio*, *Shewanella*, *Serratia*, *Pseudoalteromonas* or *Flavobacterium*	Li *et al*., 2010
Apostichopus japonicus	Funka Bay and Ainuma fishing port, Hokkaido, Japan	Small intestine, large intestine, coelomic fluid and body surface.	Members of the genera *Roseobacter*, *Shewanella*, *Pseudoalteromonas*, *Vibrio* and *Flavobacterium. Epsilonproteobacteria* and *Rickettsiales* members with antibacterial compounds were found.	Enomoto *et al*., 2012
Apostichopus japonicus	Bottom enhancement area Qingdao, Shandong Province, China	Sediment, foregut and hindgut	The predominant classes include *Holophagae, Gammaproteobacteria*, *Deltaproteobacteria*.	Gao *et al*., 2014
Apostichopus japonicus	Alekseev Bay and Kiyevka Bay, (Primorskii krai, Russia)	The gut of individual and soil from its habitat	Facultative anaerobic bacteria.	Bogatyrenko and Buzoleva, 2016
Apostichopus japonicus	Four different areas with maximum production in Korea	Whole body	Alpha-, Beta- and Gammaproteobacteria were common dominant classes found.	Kim *et al*., 2017

Stichopus japonicus	Coast of Dalian, China	Intestine	*Vibrio* sp., *Pseudomonas* sp., *Shewanella* sp., *Leisingera* sp., *Alistipes* sp., *Propionigenium* sp., *Arcobacter* sp., *Pseudoalteromonas* sp., *Ferrimonas* sp. and *Rhodobacteraceae*	Gao *et al*., 2016
Holothuria leucospilota	Coastal waters of Nagasaki, Japan	Intestine	The phyla *Firmicutes*, *Proteobacteria* and *Actinobacteria*.	Zhang *et al*., 2012
Holothuria tubulosa, Holothuria forskali	iMARE Natural S.L. facilities (Motril, Granada, Spain)	Intestines, coelomic fluid and feces	27 isolated bacteria with antimicrobial activity, dominated by the genus *Bacillus*.	Leon-Palmero *et al*., 2018
Holothuria atra	Pangkor Island, Perak, Malaysia	coelomic fluid, stomach, intestines, inner body tissue, brown gastrointestinal tissue and outer skin layer.	30 bacterial strains including halophilic *Klebsiella* and *Bacillus* sp.	Farouk *et al*., 2007
Stichopus chloronotus, Holothuria leucospilota	Dayang Bunting Island, Yan, Kedah; Tioman Island, Pahang, Malaysia	coelomic fluid	The bacterial communities associated with *H. leucospilota* were dominated by *Pseudomonas* and *Bacillus*, while all bacterial isolates from *S. chloronotus* are *Micrococcus.*	Lukman *et al*., 2014
Stichopus badionotus	West coast of Peninsular Malaysia	Surface- associated bacteria	22 bacteria from three main classes: *Gammaproteobacteria*, *Firmicutes* and *Alphaproteobacteria,*	Alipiah *et al*., 2016
Holothuria leucospilota, Stichopus horrens	Pangkor Island, Perak, Malaysia	Gastrointestines	6 genera i from *H. leucospilota: Bacillus*, *Brevibacillus*, *Lysinibacillus*, *Staphylococcus, Dermacoccus* and *Micrococcus;* 3 genera from *S. horrens* i.e., *Bacillus*, *Brevibacillus* and *Lysinibacillus*	Kamarudin and Rehan, 2018

Few studies have also been carried out to determine bacteria associated with sea cucumbers in Malaysia. A total of 30 bacterial strains was isolated from lollyfish, *Holothuria (Halodeima) atra* Jaeger, 1833 from Malaysian waters (Farouk *et al*., 2007). Lukman *et al*. (2014) studied microbes inhabiting the coelomic fluid of *Holothuria leucospilota* and *Stichopus chloronotus*, two local species of sea cucumbers from Malaysian waters. From the coelomic fluid of *H. leucospilota,* five bacterial genera were isolated, which are *Bacillus*, *Exiguobacterium*, *Pseudomonas*, *Stenotrophomonas*, and *Vibrio,* whereas from *S. chloronotus* three bacterial genera were isolated namely *Kytococcus*, *Micrococcus* and either *Kocuria* or *Rothia*. Alipiah *et al*. (2016) assessed the potential of surface-associated bacteria isolated from *Stichopus badionotus* species from Port Dickson, west coast of Peninsular Malaysia. Six genera were identified namely *Vibrio, Planococcus, Exiguobacterium, Psychrobacter, Pseudoalteromonas and Sphingomonas.* Recently, Kamarudin and Rehan (2018) reported the culturable bacteria from the gastrointestine of *H. leucospilota* and *Stichopus horrens*. The bacterial isolates were affiliated to the genera *Bacillus, Brevibacillus*, *Lysinibacillus*, *Staphylococcus*, *Dermacoccus* and *Micrococcus* for *H. leucospilota.*

Bacteria inhabiting the internal fluid of sea cucumbers have also been shown to exhibit antimicrobial activity. For instance, Farouk *et al.* (2007) have isolated bacteria from the coelomic fluid of *H. atra* Jaeger, which shows antimicrobial activity against pathogenic bacteria that include *P. vulgaris, K. pneumoniae* and *S. typhimurium.* These activities were proven to be from the bacterial secretions, and not from the sea cucumber's coelomic fluid that showed no activity. Furthermore, pigmented bacteria were also suggested to be present in sea cucumbers from Malaysia seawaters. For example, *Staphylococcus kloosii* exhibiting orange colony colour was isolated from respiratory tree of *H. leucospilota* from Pangkor Island, Perak (Kamarudin *et al*., 2013), while a red pigmented bacteria that showed antimicrobial activity against *Escherichia coli* and *Candida albicans* was isolated from a sea cucumber in Tinggi Island, Johor (Jafarzade *et al.,* 2013).

2.7 Pigment-Producing Bacteria

Bacteria are known to produce secondary metabolites that include pigments, and are considered as a source of natural colours among other organisms such as ores, insects and plants (Venil *et al.*, 2013). Pigments from microbial sources have great potential and benefits over artificial and inorganic colours, and are preferred compared to other natural sources (Francis *et al*., 2000). This is because microbial pigments have some advantages due to their independence from seasonal production and weather conditions, can be rapidly grown on inexpensive medium, eco-friendly colours and many are also safe to use (Bat and Khan, 2013).

Pigments from natural sources such as flavonoids and anthraquinones have been documented since prehistoric times. In 1856, the world's first synthetic colour, mauve, was accidentally discovered by Sir William Henry Perkin and led to the emergence of the synthetic colour industry (Walford, 1980). The extensive history of synthetic colour usage is due to their ease of production, sustainability, inexpensive and superior colouring properties (Tuli *et al.,* 2014). However, over a period of time, these man-made colorants were conferred as harmful to human beings and imparting non-environment friendly impact (Malik *et al.,* 2012). These negative effects have increased the global demand for natural sources.

In countering the ill effect of synthetic colorants, many researchers have shown immense interests in understanding bacterial pigments since it offers promising opportunities for various applications such as in food, cosmetics, pharmaceuticals and nutraceutical industries (Charu *et al.*, 2011; Azman *et al.,* 2018). Bacteria have been shown to produce many types of pigment molecules such as carotenoids, melanin, flavins, phenazines, quinones, bacteriochlorophylls, monascins, violacein, prodigiosin or indigo (Dufosse, 2009). These coloured pigments possess by bacteria obliges important roles in cellular physiology and survival (Soliev *et al.*, 2011). The pigments also exhibit antibiotic activity against other bacteria and sometimes can act as a shield against natural antimicrobial compounds produced by other bacteria (Azman *et al.,* 2018). The antimicrobial activities of bacterial pigments have also been reported act against a wide range of pathogens, hence have the potential for medical applications (Narsing Rao *et al.,* 2017). Although numbers of research has been conducted, the exact mechanisms of antibacterial activity of this pigment is still poorly understood (Celedon & Diaz, 2021).

Pigments produced by bacteria in the marine environment are of current interest to be explored for their production of clinically and industrially important secondary metabolites and their antimicrobial, anticancer, photoprotective, antiparasitic, and immunosuppressive activities (Ramesh *et al.,* 2019; Velmurugan *et al.,* 2021). Furthermore, secondary metabolites produced by marine bacteria can be different as compared to their terrestrial counterparts due to their unique metabolic activities to adapt and function in the marine environments, especially bacteria associated with marine host (Fitri *et al.,* 2017; Joint *et al*., 2010). The marine-derived bacterial pigments were also shown to have broad-ranging pharmacological activities and may exhibit unique colours (Soliev *et al.*, 2011).

One of the primary tools for characterization of pigments especially carotenoids is to study the UV-visible absorption spectrum of the organic solvent extract (Rodriguez-Amaya *et al*., 1999; Godinho and Bhosle, 2008). Pigments can selectively absorb some wavelengths of light while allowing others to be reflected, and their absorption profile depends on their underlying chemistry (Hill *et al.,* 2006; Shawkey and D'Alba, 2017). Variation in chemical structure compositions and the presence of specific chromatophores resulting in pigment diversity.

2.8 Bacterial Identification

Bacteria isolated from various sources can be identified using different approaches, commonly via phenotypic and molecular-based methods. Bacterial identification and classification based on phenotypes can be considered classical analyses that involve morphological examination, physiological analysis, and biochemical profiling. Such methods include Gram-staining, observation of colony morphology, growth on selective media and numerous biochemical reactions (Bergeron and Oullette, 1998; Felske *et al*., 1998). Even though phenotypic bacterial classification is favourable by some researchers due to their low cost, they have their own limitations. For example, phenotypic methods have low reproducibility and are rarely able to identify the bacteria up to species level (Aguilera-Arreola, 2015). Therefore, molecular techniques have been developed to overcome these limitations and hence provide higher accuracy in bacterial identification. In practice, both techniques are usually applied, with phenotypic identification done for preliminary screening of numerous isolates prior to confirmation through genetic-based methods.

Molecular-based bacterial identification employs a variety of markers. Markers derived from ribosomal genes especially 16S rRNA gene is the most frequently used to characterize and determine the phylogenetic and taxonomic status, as well as relationship of the bacterial isolates since it is universally present in prokaryotes (Márta, 2012; Normand *et al*., 1996). Ribosomal RNA is a major component for ribosome and ribosomes have the same function in protein synthesis in all cells. Therefore, the 16S rRNA gene tends to be conservative enough and possesses a faster evolutionary rate than nuclear genomes. This gene is highly conserved with nine hypervariable regions which allow researchers to study and identify bacterial taxonomy (Ahmadian *et al.,* 2006). The 16S rRNA gene is also regarded as a reliable marker because of its functional consistency and its length of sequence (approximately 1,500 bp) amplified by universal primers is large enough to conduct the bioinformatics analysis (Janda and Abott 2007).

Bacterial identification is usually done using the polymerase chain reaction (PCR) amplification of the 16S rRNA gene, DNA sequencing, sequence alignment with known sequences in public database and interpretation of results (Barghouthi, 2011). PCR is used to amplify the target 16S rRNA gene using sequence specific universal primers (Lima-Bittencourt *et al*., 2007). The presence of the appropriate amplified PCR product confirms the presence of the organisms. Information obtained from the sequences is then analysed and compared with other known sequences in public databases such as GenBank using Basic Local Alignment Search Tool, BLAST

(Altschul *et al*., 1990). The DNA sequence derived from phylogenetic studies are settled in public databases to facilitate rapid identification of bacterial community from different ecological niche (D'Auria *et. al.,* 2006).