CHAPTER ONE

INTRODUCTION

1.1 Research Background

Malaysia has been recognised as one of the mega diverse countries in the world endowed with a wealth of marine life. Sea cucumber or the holothurian is one of marine organisms that exist in nearly every marine water around the world (Higgins, 2000). In Malaysia, sea cucumbers are widespread along Sabah seawater and in the East and West Coast of Peninsular Malaysia (Choo, 2004). Sea cucumber is an abundant group of elongated and wormlike echinoderm that belongs to the class Holothuroidea (Ridzwan, 2007). This soft bodied organism is known with various terminology by the locals including *gamat, timun laut* and *balat*, and has been acknowledged as a Malaysia marine heritage (Kamarudin *et al*., 2013). The growing market demand for this organism in the last decade has led to their massive exploitation to meet the needs (Anderson *et al.,* 2011; Ramírez-González *et al.,* 2020). This is because the sea cucumbers are widely consumed as foods and provide a promising source for traditional and modern-formularised medicine in Malaysia.

Apart from their beneficial value to human, sea cucumbers also play a significant role in the ecological system. They contribute to counteract the negative effect of ocean acidification on coral growth (Schneider *et al*., 2011), and facilitate in nutrients recycling as well as decomposing detritus and other organic matters, which are further

degraded by marine bacteria (Du *et al.,* 2012). Sea cucumbers also live on the seabed and gain their foods by ingesting marine sediment or filtrating the seawater, including the microorganisms present in the surrounding (Sulardiono *et al*., 2020). Bacteria have been found within some sea cucumber species including in their gut, which were ingested as part of their food intakes (Pagán-Jiménez *et al*., 2019; Roberts *et al*., 2001; Gao *et al*., 2014). These microorganisms have been suggested to assist in the host's nutrient digestion and absorption, immunization and biological antagonism (Amaro *et al*., 2009; Hess *et al*., 2011; Amaro *et al*., 2012). Furthermore, some bacterial strains isolated from the intestine of sea cucumbers have also been suggested to be probiotic candidates for improving the health of humans and sea cucumbers (Pringgenies *et al.,* 2020; Gao *et al*., 2014; Yang *et al*., 2015).

The surface of sea cucumbers has also been indicated as a hotspot for bacterial colonization, since it furnishes nutrient-rich habitat for bacteria (Wilson *et al*., 2010; Goecke *et al*., 2010). These marine bacterial community and their eukaryotic hosts may have a symbiotic relationship, which play an important role to their survival (Hadfield, 2011; Krediet *et al*., 2013). Furthermore, the surface-attached bacteria have been shown to produce inhibitory compounds against competing species for their survival and to gain an advantage in the highly competitive conditions (Long and Azam, 2001), hence making them a potentially rich source of useful antimicrobial molecules (Wilson *et al*., 2010). Previous studies recorded that microorganism samples are primarily obtained from the external body parts of sea cucumber but study on bacteria from internal body parts are still limited (Chen *et al*., 2021). The symbiotic interaction of bacteria inhabiting the external and internal parts of marine invertebrate host may also constitute

an interesting source of valuable bioactive compounds (Radjasa and Sabdono, 2009), such as pigments and other secondary metabolites.

Pigments are among the diverse bioproducts that have been the focus of many research throughout the years, especially the ones produced by marine bacteria (Venil, 2013). Naturally synthesized pigments by marine bacteria have been suggested to be highly beneficial for human and environmentally safer compared to the synthetic colorants (Soliev *et al*., 2011; Venil *et. al.*, 2013). Furthermore, several marine bacteria have been demonstrated to produce pigments that possess various biological activities including a wide range of antimicrobial, anticancer, immunosuppressive activities, and antioxidant ability (Soliev *et al*., 2011; Balraj *et al*., 2014). In fact, pigmented bacterial strains were revealed to have a strong and broad range of antibiotic activities against other organisms (Holmström *et al*., 2002; Bruhn *et al*., 2007). Compared to other natural sources, microbial pigments have been shown to be more advantageous since microorganisms can grow easily in an inexpensive culture medium and independent from weather conditions (Kumar *et al*., 2015). This characteristic of pigmented bacteria suggested their potential importance in biotechnological applications.

Bacteria with pigment-producing ability have also been isolated from sea cucumbers in Malaysia. For example, an orange bacterial strain identified as *Staphylococcus kloosii* was isolated from *Holothuria leucospilota* respiratory tree 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). However, studies on bacterial communities associated with various sea cucumber species from

Malaysia seawaters are still limited, especially those with pigment producing ability and antimicrobial activity. Therefore, this research aimed to isolate and identify bacterial samples from the external and internal body parts of sea cucumbers from Pangkor Island and Sabah, besides screening for pigment production and antibacterial activity of selected pigmented bacterial isolates.

1.2 Problem Statement

Sea cucumbers of different species are known to exist in Malaysian seawaters, however studies on bacterial communities associated with the whole body of Malaysian sea cucumbers are still scarce. Besides, bacteria associated with sea cucumbers from the Borneo Island including Sabah of East Malaysia are yet to be studied, even though Sabah is among the recognised location of diverse sea cucumber with a total of thirty eight species were documented (Kamarudin et al., 2015). Identification of associated bacteria is important to provide information on the symbiotic interaction between the bacteria and host (Blockley *et al.,* 2017), and the presence of pathogenic bacteria inhabiting the sea cucumbers may also be used as indicators of potential environmental pollution or contamination at the surrounding marine habitat (Morrow *et al.,* 2012).

In fact, the diversity of sea cucumber species at certain location of Malaysia seawaters have also been suggested to be facing threat of declining due to overfishing, direct exploitation, habitat loss and degradation (Kamarudin *et al.,* 2015). Thus, information on the presence of pathogenic bacteria associated with sea cucumbers could be useful to develop intervention program for disease management in aquaculture industries and for consumers protection (Chanderan *et al.,* 2019). Isolation of non-

pathogenic bacteria from the internal body parts of sea cucumbers may also results in cultivable bacteria that can be further characterized to be applied as their probiotic dietary supplements to improve the health of sea cucumbers and provide them with protection against infection (Yan *et al*., 2014; Yang *et al*., 2015), as one of their sustainability measures.

Apart from that, sea cucumber-associated bacteria with pigment producing ability are also still underexplored. Marine bacteria can have important adaptability and function to survive in the extreme marine conditions as compared to those in terrestrial environment, and bacteria that are symbiotic with sea cucumbers may also have the ability to produce important bioactive compounds and secondary metabolites (Joint *et al*., 2010). Due to the marine bacteria's unique metabolism, new metabolites that are different from those synthesized by terrestrial microorganisms may also be discovered (Fitri *et al.,* 2017). This provides a prospect for natural pigment sources with bioactive potential as a substitute to replace synthetic pigments that can be toxic, carcinogenic and may cause damage to the environment (Campanale *et al.,* 2020; Duran *et al*., 2002).

1.3 Research Objectives

The objectives of this research are:

i. To identify sea cucumber specimens collected from two locations of Malaysia seawater using morphological analysis.

ii. To isolate and identify bacteria isolated from sea cucumbers and surrounding sediments and seawater using 16S rRNA gene sequencing and phylogenetic analysis.

iii. To analyse pigments produced by selected bacterial isolates using UV-visible spectroscopy.

iv. To screen for the antimicrobial activity of pigmented bacterial isolates against selected pathogenic bacteria using disc diffusion method.