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Addressing research gaps in tropical sea cucumber (*Holothuria scabra*) aquaculture- A microbiome approach

Kunal Mondal

Department of Research and Development in Aquaculture (Marine Division), IFB Agro Industries Limited, Kolkata, India

Corresponding Author: **Kunal Mondal**

Abstract

Sea cucumbers (holothurians) belong to the phylum Echinodermata. They are exclusively marine, slow moving and reside on the sea bottom thereby sucking sediments from the sea-floor rich in organic matter for their survival, growth, and reproduction. Sea cucumbers are an expensive seafood delicacy that forms an integral part of Southeast Asian cuisine. The Chinese people consume sea cucumbers since ancient times due to their medicinal and aphrodisiac properties. The dried and processed form commonly known as *bêche-de-mer* or *trepang*, is currently the highest priced commodity in international markets like China, Hong Kong, Japan, and Korea. To meet the growing market demand, aquaculture of these commercially valuable sea cucumbers

should be considered, though there lies a knowledge gap related to optimized feeding. Studies reveal that sea cucumbers intake microbes (mainly bacteria) from the sea sediment as one of their important sources of nutrition. It is yet unknown how these animals' control and utilize bacteria derived from ocean floor sediment. It also remains unexplained of the probable functional roles of bacteria in sea cucumber nutrition and metabolism (digestive physiology). This report will emphasize on different proposed methods that could improve sustainable farming of this species, by studying the microbiomes and their future implications.

Keywords: Sea-cucumber, Microbiome, Digestive Tube, Respiratory Tree, Coelomic Fluid

Introduction

Taxonomically the holothurians or sea cucumbers belong to the phylum Echinodermata, distributed throughout the world's ocean floor. They are exclusively marine and obtain food by ingesting marine sediment, or by filtering seawater (McElroy, 1990; Ward-Rainey *et al.*, 1996)^[17, 30]. Aspidochirote holothurians play a vital role in sediment bioturbation in marine ecosystems (Massin, 1982; Mac Tavish *et al.*, 2012; Purcell *et al.*, 2016)^[16, 14, 24]. They feed on surface sediment containing microbes, meiofauna, decaying organic debris and inorganic components (Yingst, 1976; Moriarty, 1982)^[31, 18]. However, the bacterial abundance in the sediment is considered either as a direct food source or as a source that indirectly provides them with essential nutrients that are otherwise unavailable (Gerlach, 1978; Deming and Colwell, 1982; Sun and Chen, 1989; Amon and Herndl, 1991; Amaro *et al.*, 2009; Gao *et al.*, 2010)^[10, 5, 28, 2, 1, 9]. Studies reveal that bacteria are important dietary components for sea cucumbers like *Apostichopus japonicus* (Gao *et al.*, 2010, 2014; Sha *et al.*, 2016)^[9, 8, 27], *Holothuria atra*, *Stichopus chloronotus* (Moriarty, 1982)^[18], *Holothuria tubulosa* (Amon and Herndl, 1991)^[2] and *Holothuria scabra* (Plotieau *et al.*, 2013, 2014; Robinson *et al.*, 2016)^[25]. It is now also accepted that holothurians assimilate carbon from bacteria and diatoms, in addition to available carbon from microbial degradation process (Massin, 1982^[16]; Yingst, 1976^[31]; Plotieau *et al.*, 2013; Lopez and Levinton, 1987^[13]; DeMaster *et al.*, 2011^[4]). Hence, bacterial concentration in the foregut of sea cucumber is higher than bacterial concentration of the surrounding sediments that they consume suggesting that holothurians feed in selecting bacteria from the sediment or multiply them in the foregut before digestion. These observations may highlight the ability of holothurians to cultivate specific bacterial strains in their digestive tube, as some endosymbiotic bacterial community could be involved. Sea cucumbers also intake dissolved nutrients from sea water with the help of respiratory trees located near the anal opening, it could exchange water in and out of the cloacal aperture by pumping mechanism (Pierrat *et al.*, 2021; Newell and Courtney, 1965; Brown and Shick, 1979)^[20, 19, 3]. Innovative studies through radiography and stable isotope

technique demonstrated that the epithelium of respiratory tree can assimilate dissolved organic matter in the form of monosaccharides and amino acids from the water column (Fontaine and Chia, 1968) [7]. Digestive enzymatic activity was documented in respiratory tree of *Isostichopus badiionatus*, resembling that it may serve as an accessory organ in digestive process of sea cucumbers (Martinez-Millian *et al.*, 2021) [15]. It is yet unknown about the bacterial community present within the respiratory tree and their probable functional roles in sea cucumber nutrition.

Holothuria scabra, a species of tropical sea cucumber commonly known as sandfish occurs in shallow waters of the Indo-Pacific, preferably with sandy-muddy bottoms colonised by sea-grass. It has many traits that make them ideal species for aquaculture and stock enhancement. They are one of the most commercially valuable of the tropical species of sea cucumbers and fetch highest prices in international markets as trepang or bêche-de-mer, due to their delicacy and high nutritional value (Kiew and Don, 2012) [11]. The research gap still exists and to address those, microbiome approach could be the best possible way. Here some significant methodologies are presented, that could be more appropriate in optimizing future scenario of sustainable sea cucumber (*H. scabra*) farming.

Methodology 1: To do a baseline study of the various microbiomes in *H. scabra* (identification and quantification)

The microbiome refers to the genome of microbial life forms inhabiting a living host, and their interactions with the host (Walter and Ley, 2001) [29]. They play significant roles in the host metabolism. A current knowledge of digestive tube microbiome and its benefits are still lacking as far as *H. scabra* is concerned. Very few investigations highlight about the quantitative data in *H. scabra*. In previous research studies, it is highlighted that during sediment ingestion by *H. scabra*, the bacterial number significantly increased in the digestive tube compared to sediment in the substrate. Values analyzed with optical DAPI counts reached 11.5×10^9 bacteria per gram in the foregut. The number of bacteria then significantly decreased in the midgut and remained stable in the rest of the digestive tube through the faeces, with values around 4×10^9 bacteria per gram (Plotieau *et al.*, 2013, 2014). Yet, by the method of cloning and sequencing, 11 sequences were obtained from seawater, 18 from substrate sediment, 94 from the digestive tube of *H. scabra* and 4 from the faeces. The microbiomes of seawater, sediments and the three segments of the digestive tubes highlighted by this method were very different. In these studies, although we got a first gross idea of the composition of some microbiomes encountered within *H. scabra* and at the level of their potential sources (seawater and sediment), but the degree of resolution of these methods did not allow us to address the gaps such as (i) the bacteria preferentially digested in the digestive tube, (ii) the bacteria associated with the wall of the digestive tube potentially representing mutualistic or symbiotic bacteria, and (iii) the concentration of bacteria in the foregut is really higher indicating that *H. scabra* preferentially selects areas richer in bacteria or cultivates bacteria in its foregut. Another interesting organ in sea cucumbers that is respiratory trees, which we know now that they can be organs where nutrients and bacteria could enter the animal, no investigation is done till date and there also remains a

considerable knowledge gap. With the advancement of molecular sequencing methods (nanopore sequencing technology), it is now possible to identify some internal important microbiomes. The microbiomes of the sediment, seawater near sea cucumbers, faeces, digestive tube (foregut, midgut, hindgut), respiratory trees and coelomic cavity should be primarily focused. These microbiomes needs to be compared to suggest the internal pathways of transit of bacteria to the coelom. To quantify the microbiomes, flow cytometry is essential to unravel the concentration of different bacterial taxa in various compartments within the animal body. This way we may figure out the key bacterial taxa digested and their functional role in *H. scabra* nutrition (as probiotic).

Methodology 2: To emphasize on the bacterial community dynamics of marine sediment during *H. scabra* farming

Studies report that impact of intensive *H. scabra* farming is significant on the sediment (upper layer) bacterial population. They demonstrated that the proportion of the finest grain size fraction ($<250\mu\text{m}$) decreased from 5% to 14%, the carbonate proportion decreased by 5% and the concentration of bacteria decreased by up to 50% within sea farm pen, whereas the total organic matter content was insignificant (Lavitra *et al.*, 2010) [12]. Here lies another gap where nothing had been analyzed concerning the variation of bacterial taxa during farming, both qualitative and quantitative. The sediment structure in terms of bacterial load is fully dependent on the population or stocking density of sea cucumber juveniles. It is observed that after a full farming cycle of *H. scabra* (12-14 months), the fertility of the sediment is lost that means bacterial population dynamics is altered which is not in favor of initiating the next farming cycle as documented by Mondal and Kunzmann, 2018 (unpublished data). Hence emphasis on bacterial population dynamics (taxa-wise) should be given to optimize the stocking density of juveniles for better farming output.

Methodology 3: To study the effect of biofloc probiotics on *H. scabra*

The biofloc systems basic principle is to recycle and transform waste and excessive nutrients, in particular inorganic nitrogen ($\text{NH}_3\text{-N}$ and $\text{NO}_2\text{-N}$), generated from faeces and uneaten feed into microbial biomass. At the end, the biofloc systems produce muds charged with bacteria that must be discarded from the aquaculture ponds and is a by-product that is useless. In this mud are found many bacteria of the genus *Bacillus*, this type of bacteria is used in China as a probiotic to promote the production of the sea cucumber *Apostichopus japonicus*. The production of *H. scabra* in aquaculture, is carried out in three phases such as hatchery, the nursery and the grow-out. During this nursery phase, the health status can be optimized and the survival and production of juveniles can be increased by adding probiotics to the sediments in the ponds as recently reported by Sembiring *et al.*, (2023) [26]. To address these gaps, digestive enzyme studies are also required to find out the effect of endogenous probiotics like *gamma proteobacteria*, *Bacillus subtilis*, etc. on metabolism and physiology of the animal under farming conditions. A detailed investigation is also needed on the response of coelomocytes (immune cells) in sea cucumber *H. scabra* when fed on probiotics, that

remains unattempted.

Conclusion

Sea cucumber *H. scabra* farming is gaining attention worldwide due to their medicinal and aphrodisiac value, export value, high delicacy, and nutritional properties. Many countries have started the commercial farming of this species. The three different methods discussed in this report may contribute to sustainable sea cucumber farming in future (a way towards blue economy).

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