

Role of GI Microbiota in Spiny Lobsters

Subjects: **Microbiology**

Contributor: Audrey Daning Tuzan , EnYao Lein , Mohammad Tamrin Mohamad Lal , Balu Alagar Venmathi Maran ,

Indigenous intestinal microbiota influences the physiological function of the host GI tract. These influences are evident in scientific articles reporting intestinal microflora abnormalities in a variety of organisms, including germ-free animals, animals, and humans. As observed in higher organisms, humans, terrestrial animals, or aquatic animals, the presence of GI microbiota in spiny lobsters influences the host and brings various benefits to its host. The symbiosis between the GI microbiota and the host is important to ensure the nutrition and health of the host. The human GI tract comprises 1000 culturable microflora species, where 92 species are from eukarya, 8 from archaea, and 957 from bacteria. The complex and diverse microflora community in the GI tract of humans co-exists and contributes to the prevention of metabolic diseases. The GI microbiota in the human body possesses both beneficial and harmful traits. It has been proven that GI microbes are associated with obesity, inflammatory bowel disease, cancers, diabetes, autism, and asthma. The role of fish GI microbiota is based on the host's dietary needs. Herbivorous fishes, such as grass carp, are associated with cellulolytic bacteria, which help in plant fibre intake. Meanwhile, nitrogen-fixing bacteria are the dominant species in the GI tract of wood-eating fish. The GI bacteria of carnivorous fish species consist of mostly lipase- and protease-synthesising bacteria. In aquatic invertebrates, an earlier experiment by Harris proved that the presence of GI bacteria has specific roles and functions.

beneficial microbes

Panulirus

Vibrio

probiotics

aquatic invertebrate

1. Role of GI Microbiota in Digestion

Spiny lobsters feed on live fish, molluscs, other crustaceans, aquatic worms, and some aquatic plants; hence, they are categorised as omnivores. Shrimps and prawns are also classified as omnivores, as their GI microbiota is similar to that of spiny lobsters. The GI tract of spiny lobsters is colonised by bacteria such as *Vibrio*, *Pseudomonas*, *Aeromonas*, *Pseudoalteromonas*, *Photobacterium*, and *Plesiomonas* ^{[1][2][3][4]}. Studies on the digestive relevance of GI microbiota are mostly conducted on freshwater and seawater fish, while crustaceans, especially spiny lobsters, have received little attention. The microbiota present in the GI tract of spiny lobsters mostly consists of proteolytic bacteria, amylolytic bacteria, lipolytic bacteria, and cellulolytic bacteria ^{[5][6]}. Other bacteria, such as the *Flavobacterium* found in the GI tract of spiny lobsters, are capable of hydrolysing complex polysaccharides ^{[7][8]}. All these bacteria are essential to spiny lobsters, as they help in producing enzymes to digest lipase, protease, amylase, and chitin. Spiny lobsters attach to their carnivorous feeding preferences (molluscs, crustaceans, polychaete worms, and echinoderms), which form high proteolytic enzyme activities (trypsin, chymotrypsin, and carboxypeptidase A) and low lipase activities in the digestive tract ^[9]. Kumar ^[5] compared the microbial diversity in the intestine of wild and laboratory-reared spiny lobsters *P. versicolor*. The researchers found

that cellulolytic bacteria were dominant in the foregut of normal spiny lobsters, while proteolytic bacteria were dominant in the foregut of laboratory-reared spiny lobsters. These results indicate that the feeding behaviour of spiny lobsters could change the enzyme-synthesising bacteria in the GI tract.

2. Role of GI Microbiota in Nutrition

Indigenous GI microbiotas possess important roles in the well-being of their host and contribute to nutrient acquisition in humans, terrestrial animals, and aquatic animals [10][11]. The ability of GI-associated microbes to digest and synthesise vitamins from daily diets has mostly been documented in human and animal studies, whereas limited information is available regarding insects, fish, and aquatic invertebrates [10][12][13][14]. More than 90% of the mammalian population on the planet are herbivores, and these animal species are unable to produce enzymes to digest carbohydrates and cellulose. Thus, GI microbes are crucial in the degradation and digestion of food [15].

The GI bacteria ferment dietary carbohydrates into short-chain fatty acids (SCFA), which support the host by providing energy and facilitating the absorption of Sodium (Na) and water into cell tissues. Cellulolytic bacteria present in the GI tract assist the host to completely hydrolyse cellulose into glucose, which is the compound available to the host. This process involves the action of exoglycanases, endoglycanases, and β -glucosidases [16]. The host absorbs protein in amino acids degraded by the proteolytic bacteria in the GI tract. The synthesis of B vitamins (complex of 10 water-soluble compounds) is also facilitated by GI microbes. This process is well-documented in fish, where the amount of vitamin B12 varies according to species [17][18]. Based on Nayak [11], the production of vitamin B12 is closely related to the abundance of anaerobic bacteria compared with aerobic bacteria in the GI tract of fish. The GI microbiota is also involved in nutrient-material uptake stimulation, especially in cholesterol metabolism and trafficking in aquatic animals [19][20]. The use of gnotobiotic models demonstrated that germ-free zebrafish larvae failed to degrade and absorb proteins in the intestine, but these functions were performed effectively in the later development stages following the enrichment of the GI microbiota [21]. To date, the nutritional role of the GI microbiota in spiny lobsters remains unclear. The GI microbiota–host interaction could be better understood by utilising gnotobiotic models. Previous studies on gnotobiotic daphnia and gnotobiotic artemia provide a possibility for a better understanding of the interaction and the importance of GI microbiota in spiny lobsters [22].

3. Role of GI Microbiota in Immune System

Gut-associated lymphoid tissues (GALTs) are GI microbiota responsible for the immune system, in which intestinal microbiota is important for the complete development of mature immune cells [23][24]. The intestinal epithelium will secrete and be covered by a layer of mucus, which acts as the first line of defence against harmful microbes. The symbiosis between GALT, mucus, and indigenous intestine microbes will mature the host's gut-associated immune system [11]. According to Lee and Mazmanian [25], the approach on germ-free animals provides a better understanding of the interaction between host GI microbes and the immune system, where the protection from

intestinal mucosa is defective in germ-free mice. The gut-associated immune system mechanism (Payer's patches, lymph nodes, and lamina propria) is smaller and inactive in germ-free animals, while exposure to antigenic stimuli could reverse the action [25][26][27].

The functions of T cells in protecting the host from various infections are also promoted by the GI microbiota. For instance, *Bacteroides fragilis* promotes T cells to protect against *Helicobacter hepaticus* infection, while *Bifidobacterium infantis* diminishes *Salmonella typhimurium* intestinal infection [26]. More than 70% of the total body of Immunoglobulin A (IgA) across the mucosa membrane surface is secreted by the GI microbiota, especially in the intestine [28][29][30]. IgA is an important component of first-line defence and interacts with specific receptors and immune mediators for protective functions [31].

Intestinal immunity in aquatic animals is less advanced compared to terrestrial animals. Nonetheless, aquatic animals are exposed to higher microbial infection challenge, as they inhabit a microbial-rich environment [32][33]. The immunity-associated mechanism of fish is composed of gut-associated lymphoid tissue (GALT), skin-associated lymphoid tissue (SALT), gill-associated tissue (GIALT), and nasopharynx-associated lymphoid tissue (NALT), which were recently uncovered [33]. According to Gomez and Balcazar [34], the gut-associated immune system of aquatic vertebrates and terrestrial vertebrates are similar in that the epithelial cells and mucosa act as a selective barrier and promote T cells and B cells to produce IgA as an intestinal defence mechanism. Indigenous GI microbiota may suppress the foreign microbiota and prevent the colonisation and proliferation of pathogens through the colonisation-resistance process [35]. The indigenous microbes secret and release antimicrobial peptides to win over the competition between the pathogen and the niche space [36].

The immune mechanism in aquatic invertebrates is different compared to vertebrates. In crustaceans, the hard exoskeleton, made of cuticle, acts as the first line of defence against microbial infection [37]. Conversely, when pathogens successfully invade the body or the tissue of invertebrates, the innate immune system will instantly activate and eliminate the intruding pathogen [38]. The innate immune system differs between invertebrates and vertebrates, as evidenced in the lack of antibodies and the critical molecular and cellular players, such as B lymphocytes and T lymphocytes, in aquatic invertebrates [39].

The innate immune system of invertebrates is classified into two groups: humoral immunity (haemolymph agglutination, prophenoloxidase system (proPO), and antimicrobial peptides) and cellular immunity (phagocytosis, encapsulation, and haemocyte nodulation) [40][41][42]. There is a paucity of data regarding the role of GI microbes in the immune system of invertebrates. A recent study found that the gut microbiota of shrimp plays a vital role in maintaining host health, and the colonisation-resistance process may occur in the digestive tract of shrimp [43]. The research conducted by Tepaamorndeche et al. [44] revealed that the introduction of the bacteria strain *Bacillus aryabhattachai* into the GI tract of shrimp could suppress the population of *Vibrio* spp. and stimulate innate immunity and antioxidant activities.

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