



## Review

## Exploring the role and mechanism of potential probiotics in mitigating the shrimp pathogens

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## ARTICLE INFO

## Keywords:

Shrimp culture  
Pathogens  
Probiotics  
Immune boosting  
Competitive inhibition  
Antimicrobial substances

## ABSTRACT

Shrimp aquaculture has rapidly developed into a significant industry worldwide, providing not only financial gain and high-quality food but also tens of thousands of trained and competent workers. Frequent diseases are now regarded as a significant risk factor for shrimp aquaculture, as they have the potential to significantly reduce shrimp production and result in economic losses. Over the years various traditional methods including the use of antibiotics have been followed to control diseases yet unsuccessful. Probiotic is considered potential supplements for shrimps during farming, they may also act beneficially as disease control and increased production. Probiotics are described as a live microbial supplement that benefits the host by modifying the microbial population associated with the host and its ambient. The present state of research about probiotics demonstrates notable impacts on the immune defences of the host's gastrointestinal system, which play a crucial role in safeguarding against diseases and managing inflammation inside the digestive tract. In the past ten years, many studies on probiotics have been published. However, there is a lack of information about the processes by which probiotics exert their effects in aquaculture systems, with only limited elucidations being offered. This study explores the variety of procedures behind the positive effects of probiotics in shrimp culture. These mechanisms include the augmentation of the immune system, control of growth, antagonistic action against pathogens, competitive exclusion, and modification of the gut microbiota. Mechanisms involved in the probiotic mode of action are mostly interlinked. This provides a greater understanding of the importance of probiotics in shrimp culture as an environmentally friendly practice.

## 1. Introduction

Shrimp farming is a significant factor of the aquaculture industry. Shrimp have been raised for food all over the world for many years, and many countries are still actively involved in shrimp aquaculture today. According to reports, approximately 45 lakh metric tonnes of shrimp were produced globally. Among them, Asian countries produce 80 % of the world's shrimp production (Diwan et al., 2022). The culture system got intensified to meet the rising demands worldwide which in turn increased the risk of various disease outbreaks resulting in huge economic losses for the farmers. Probiotics were considered an alternative remedy for various issues in shrimp farming including disease control, increased production, and water quality maintenance (Fig. 1).

Probiotics in shrimp culture were considered one of the effective and eco-friendly alternative approaches that are capable of not only controlling bacterial and viral pathogens but it also capable of improving

the nonspecific immune system (Fig. 2) (Jamal et al., 2019). Probiotics which have been isolated from shrimp gastrointestinal tract (GIT) are known as putative probiotics for shrimp culture. Commercial sources, on the other hand, are those that have already been synthesized and are readily available in the market. Probiotic bacteria from the genera *Bacillus*, *Lactobacillus*, and *Bifidobacterium* are the most commonly used species (Hai, 2015). Probiotics, as previously stated, can boost immunological responses in fish and shrimp. Furthermore, the indiscriminate use of antibiotics has led to the emergence of novel strains that possess resistance to these medications. One effective strategy for mitigating and combating viral infections involves enhancing the capacity of animals to withstand illnesses and enhancing their immune systems (Lakshmi et al., 2013). A further competitive advantage lies in the efficacy and rapidity with which microorganisms assimilate nutrients. The optimal distribution of resources can lead to the maximisation of growth. In order to optimize growth rates, it could advantageous to use metabolic strategies

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<https://doi.org/10.1016/j.sjbs.2024.103938>

Received 4 December 2023; Received in revised form 10 January 2024; Accepted 19 January 2024

Available online 20 January 2024

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that are contingent upon substrate availability. This includes transitioning from respiration, which yields a larger amount of ATP, to fermentation, even in the presence of adequate glucose.

Probiotics are arising as a popular alternative to antibiotics to prevent disease, and promote growth and regulation of pond water quality in shrimp aquaculture (Knipe et al., 2021). Most probiotics show their activity by producing antibacterial molecules like bacteriocins that may inhibit the pathogens directly, actively fighting infections. Some probiotics work by inhibiting the bacterial movement across the wall of the gut (translocation), enhancing the function of the mucosal barrier by modulating the immune/inflammatory response or increasing innate immune molecules (Lakshmi et al., 2013). Probiotics' considerable impacts on host gut defences, which are crucial for disease prevention and inflammatory treatment of the digestive tract, were shown by an extensive investigation of the probiotics literature (Azimirad et al., 2016; Modanloo et al., 2017). Probiotic species including *Lactobacillus acidophilus* can help mitigate *Vibrio harveyi* and *Vibrio parahaemolyticus* (Natesan et al., 2012). Some are listed below along with their applications and mechanism behind (Table 1).

## 2. Probiotic source and selection

Probiotics can be derived from different sources such as red and brown algae, bacteria, terrestrial fungi, and marine yeast (van Hai and Fotedar, 2010). Most commonly shrimp probiotics are isolated from the shrimp culture pond samples, and the intestine of the shrimp itself (Rengpipat et al., 2000). But, there are also other source for shrimp probiotics that includes fish mucus, curd (Ramachandran, 2014), Chicken gut (Phianphak et al., 1999), fermented soybeans "Natto" (Liu et al., 2010), digestive tract of fish (Sha et al., 2016), fermented pickle (Zokaefar et al., 2012), and filtrates of fruit waste (Nurliana et al., 2020).

Various microbiological techniques may be used to assess the probiotic potential of microorganisms, such as evaluating their ability to tolerate salinity and other relevant factors, as previously indicated (Fig. 3) (Lakshmi et al., 2013). The discovery of potential probiotics using constrained techniques, such as cell-free extracts or diffusion assays, shows a preference for interference-based competition tactics. The selection of shrimp probiotics for testing mostly rests on their capacity based on evaluations made utilising bacterial antagonist assays, to effectively eliminate pathogens. However, the means by which they effectively prevent the entry of viruses into their system are seldom

subjected to thorough investigation (Knipe et al., 2021). The predominant microbial species found in aquatic probiotics are *Bacillus*, *Streptomyces*, and *Lactobacillus*. *Lactobacillus*, which were often used as a probiotic for humans, has also shown probiotic benefits on shrimp (Jamal et al., 2019). After conducting a thorough evaluation using the aforementioned methodology, the strain may be deemed a potential probiotic strain suitable for use in shrimp farming. The methodology used to assess the probiotic capabilities of microbial strains were shown (Fig. 4) (Lakshmi et al., 2013).

The existing body of research related to probiotics demonstrates notable impacts on the defensive mechanisms of the host's gastrointestinal system, which play a crucial role in safeguarding against diseases and managing inflammation inside the digestive tract (Azimirad et al., 2016). In the past ten years, many probiotic research has been reported. However, there was a lack of comprehensive knowledge on the processes by which probiotics function in aquaculture systems, with few explanations available. This study extensively examined the possible advantages related with the introduction of probiotics.

## 3. Modulation of the gut microbiome

The gastrointestinal microbiota of aquaculture species may undergo alteration by the intake of other microorganisms. Consequently, the change of microbial populations was a potential approach for mitigating or eradicating the occurrence of opportunistic infections (Fig. 5) (Kumar et al., 2016). Probiotics often induce alterations in the makeup of the microbiota's gut, resulting in favourable outcomes. This stands in contrast to the mechanism of action shown by antibiotics, which diminishes the population and variety of bacteria residing in the gastrointestinal tract. The inclusion of a combination of probiotic consortia consisting of *Saccharomyces cerevisiae* and *Bacillus subtilis*, along with prebiotics, in the diet of shrimp resulting in an increase in the population of *Lactococcus* in the shrimp's gastrointestinal tract. Additionally, this dietary intervention resulted in reduction in the availability of vibrio, a pathogenic microorganism (Table 1) (Yao et al., 2021). The favourable aspect of probiotic colonization lies in its ability to rapidly occupy ecological niches and inhibit the colonization of opportunistic strains, hence prolonging the therapeutic benefits (Won et al., 2020).

Probiotics play a crucial role in safeguarding the integrity of the gut's epithelial lining. They improve the barrier function of the gut microbiota against harmful microorganisms through various mechanisms. These include the synthesis of antimicrobial agents such as hydrogen peroxide,

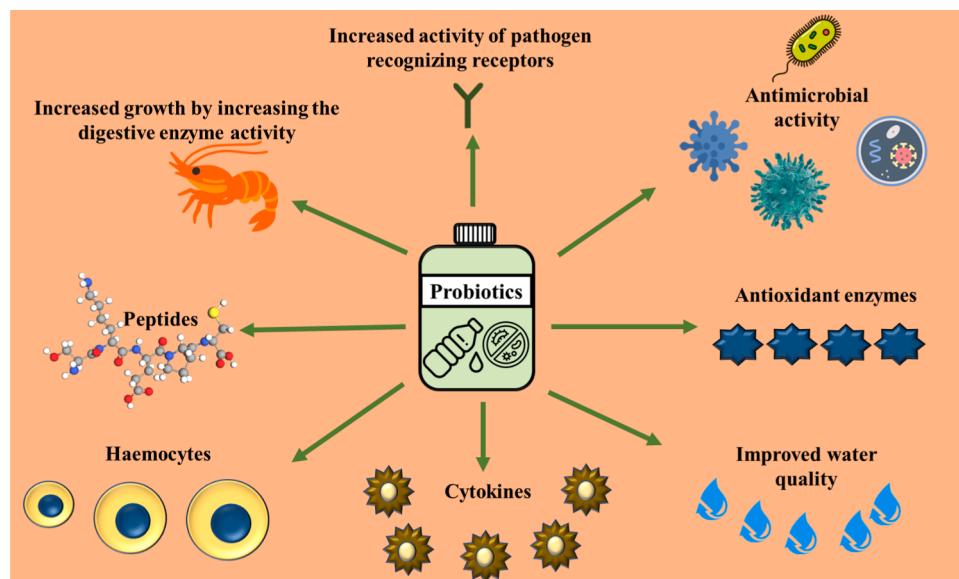


Fig. 1. Applications of probiotics in shrimp.

bacteriocins, lysozymes, and proteases. Probiotics also contribute to the formation of ammonia and diacetyl, and they can alter pH levels. Additionally, probiotics boost the immune response by raising phagocytosis and promoting the production of systemic and local antibodies at mucosal surfaces (Fig. 5) (Hoseinifar et al., 2019). For instance, *Lactobacillus platarum* isolated from the wild shrimp's digestive tract mitigates *Vibrio harveyi* by decreasing the pH or hydrogen peroxide production (Kongnum and Hongpattarakere, 2012). Moreover, the intestinal cuticle of shrimp performs a crucial function in the uptake of probiotics by serving as a safeguarding barrier, promoting the attachment and establishment of probiotics, and sustaining an ideal environment for their useful effects inside the shrimp's GIT (Koga et al., 2022; Yang et al., 2020; Zhang et al., 2009). The use of probiotics in shrimp culture has been shown to modulate the gut microbiome, leading to improved growth and health. Studies found that probiotics can enhance shrimp performance and stimulate beneficial bacteria in the gut (Servin Arce et al., 2021; Xuan Hui Goh et al., 2023). Studies emphasized the potential of gut microbiome manipulation in aquaculture, including the use of probiotics (Holt et al., 2021). The potential mechanisms by which probiotics enhance the integrity of shrimp and any potential unfavourable interactions between probiotics and the shrimp microbiome have not been well investigated.

#### 4. Modifying gastrointestinal morphology

Recent studies have shown that the use of probiotics in shrimp culture can significantly impact gastrointestinal morphology. Studies found that probiotics can enhance the growth performance and body composition of shrimp, while also influencing the intestinal microbiota and morphology (Boonanuntanasarn et al., 2016). The intestinal villi's structure demonstrates the considerable morphological alteration induced by probiotics inside the shrimp's gut. The administration of probiotics led to an augmentation in both the intestinal wall thickness and the mucosa breadth, therefore enhancing the dimensions of the intestinal epithelial cells (Fig. 5) (Kewcharoen and Srisapoom, 2019). The preservation of the structural integrity of the gut surface serves as a

protective barrier against pathogenic inflammation and infections. This barrier was safeguarded by immune proteins and further reinforced by a stable microbiome (Duan et al., 2018). The group that received probiotic treatment was examined using electron microscopy, revealing that the epithelial cells were actively engaged in secretion. Additional findings included the presence of high-density granules in the cytoplasm, as well as increased density and depth of folds (Zuo et al., 2019). The ingestion of probiotics induces the development of an expanded intestinal surface area, hence enhancing the ability of the gut to assimilate nutrients. This effect was achieved by augmenting the quantity of smaller crypts inside the intestinal structure (Van Hai and Fotedar, 2009).

The administration of probiotics led to a notable alteration in the internal structure of the midgut area (Du et al., 2019), elevated quantity of B cells seen inside the hepatopancreatic tubules (Fig. 5) (García-Bernal et al., 2018), and the hepatopancreas of infected shrimp exhibited a reduction in pathological manifestations (Pooljun et al., 2020). Probiotics have been shown to enhance the structural integrity of the hepatopancreas and gut, hence facilitating the animal's digestion and absorption of nutrients. Improving gastrointestinal health was crucial for ensuring the well-being of animals, fostering their development, and providing protection against opportunistic diseases (Xuan Hui Goh et al., 2023).

#### 5. Enhancement of host immunity

Specific immune genes in shrimp employ defensive mechanisms against pathogens by recognition, haemocytes cell-to-cell communication, cytotoxicity, phagocytosis, and melanisation (Butt et al., 2021). Probiotics have been identified as a viable substitute for antibacterial agents, serving as natural immune boosters that stimulate disease resistance within prawn farms (Kumar et al., 2016). Several literatures have reported that certain bacteria produce metabolites/compounds that have immunological effects on shrimp (Fig. 2). Generally, the probiotic could increase immunity in three ways: (i) Enhancing their ability of phagocytosis pathogen by macrophage activation; (ii) Increase in production of systematic antibody; and (iii) Produce increased local

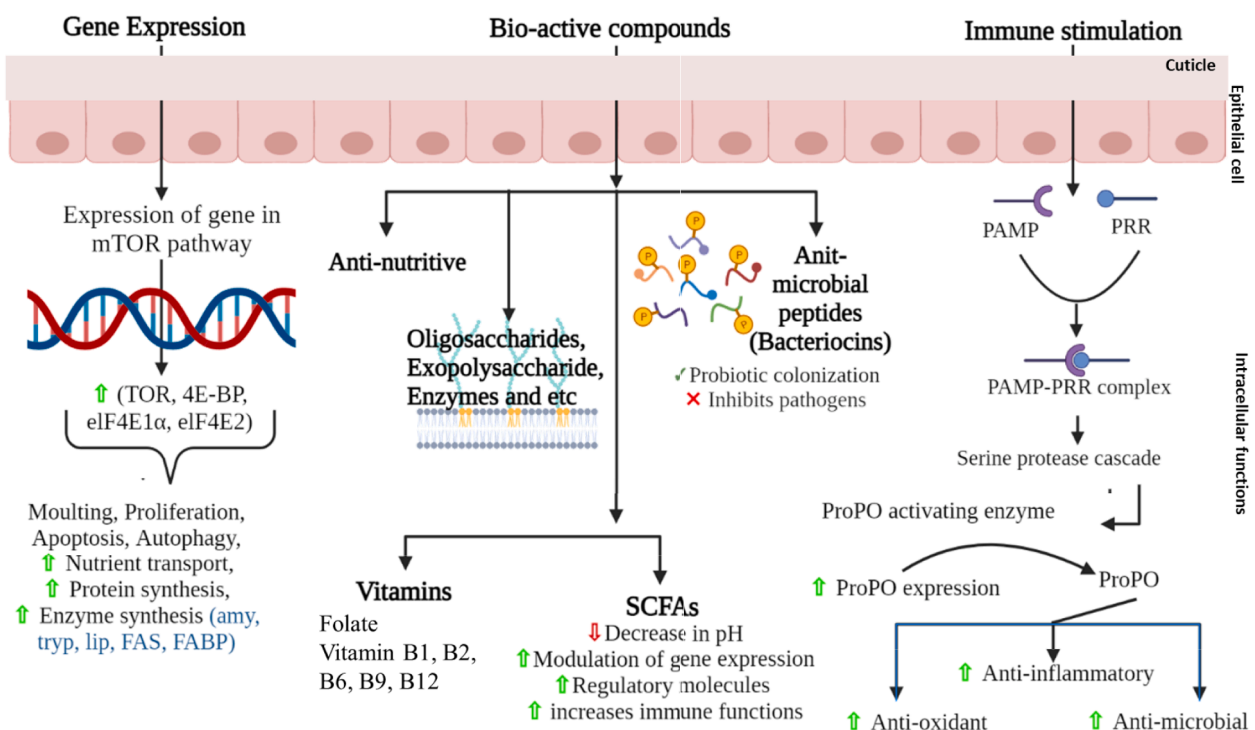
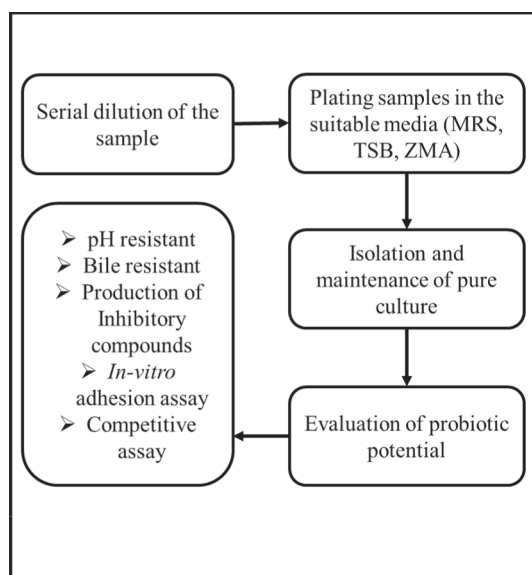


Fig. 2. Probiotics mechanism of action (gene expression, bioactive compounds, immune stimulation).

**Table 1**  
Some probiotics along with their applications and mechanism behind.

S. No	Probiotics	Applications	Mechanism	Reference
1	<i>Shewanella algae</i>	(i) Against <i>Aeromonas hydrophila</i> (ii) stress tolerance	(i) Bio-active compound (Pyrrolo [1, 2-a] pyrazine-1, 4-dione,hexa-hydro and Tromethamine) (ii) catalase and hydrolytic enzymes	(Ariole and Ekeke, 2016)
2	<i>Bacillus subtilis</i> E20	Regulate immune molecule and Improved health	Increased digestibility of glutamine which is further used as a fuel for immune cells	(Tseng et al., 2009)
3	<i>Bacillus subtilis</i>	Control <i>Vibrio parahemolyticus</i>	Upregulation of immune-related genes including prophenol oxidase	(Interaminense et al., 2019)
4	<i>Lactococcus lactis</i>	antimicrobial activities against <i>Vibrio parahaemolyticus</i>	Upregulation of the two <i>Litopenaeus vannamei</i> prophenol oxidase ( <i>LvproPO 1</i> , and <i>LvproPO 2</i> ) transcripts.	(Chomwong et al., 2018)
5	<i>Lactobacillus plantarum</i>	Increased the probability of Pacific white shrimp surviving following exposure to <i>Vibrio alginolyticus</i>	Increased lactic acid bacteria count in the intestine without changing total heterotrophic bacteria or <i>Vibrio</i> spp. counts in the midgut	(Constanza Bolívar Ramírez et al., 2017)
6	<i>Enterobacter hominis</i>	Promoting rapid growth	Secretion of digestive enzymes helps in improving digestion and feed absorption ratio	(Zuo et al., 2019)
7	<i>Bacillus</i> sp. NP5 RfR	Growth Performance and Intestinal Microbiota Diversity	By inducing the number of operational taxonomic units (OTU)	(Hasyimi et al., 2020)
8	<i>Bacillus subtilis</i> AQAHBS001	Improved resistance against <i>Vibrio</i> infection	By improving midgut characteristics (increased microvillus and intestinal wall thickness)	(Kewcharoen and Srisapoom, 2019)
9	<i>B. subtilis</i> L10 and G1	Digestive enzyme activity, improved growth, immune response	Increase of the biological activities and induced immune gene expression via manipulating the microbiota of shrimp	(Zokaiefar et al., 2012)
10	<i>Lactococcus lactis</i> D1813	Assessment of Immunomodulatory Role	It has been noted that the expression of the lysozyme gene has been up-regulated, and that anti-lipopolysaccharide factor,prophenoloxidase, superoxide dismutase, and toll-like receptor levels have also increased.	(Maeda et al., 2014)
11	<i>Enterococcus faecium</i> NRW-2	Control of <i>Vibrio</i> infection	Upregulation of immune and digestive-related genes	(Sha et al., 2016)
12	<i>Pediococcus acidilactici</i> GY2	Decreased mortality during <i>Aeromonas hydrophila</i>	Increased digestive enzymes activity and immune response	(Miao et al., 2019)
13	<i>Streptococcus phocae</i> P180	Inhibitory activity against vibriosis	Bacteriocin activity	(Swain et al., 2009)



**Fig. 3.** Microbial procedure for screening probiotic potential.

antibody on the surface of mucosal (Jamal et al., 2019).

### 5.1. Serological immunity

Recent studies have shown that the use of probiotics in shrimp culture can enhance serological host immunity. Studies found that the use of probiotics, specifically *Bacillus subtilis*, led to increased phagocytic activity and disease resistance in shrimp (Pope et al., 2011; Tseng et al., 2009). Use of vitamin C and Chinese herbs as immunostimulants enhanced immune activity in shrimp (Qiao et al., 2011). These studies

collectively suggest that the use of probiotics and immunostimulants can enhance serological host immunity in shrimp culture. Stipulation of serum immunity such as catalase, peroxidase, protease and antiprotease, myeloperoxidase, superoxide dismutase, and lysozyme resulted in antibacterial activity against microbial infections (Nayak, 2010) and stimulation of host immune response (Rauta et al., 2012). Increasing serological immunity using probiotic treatment resulted in disease resistance. Innate immunity of shrimp has been stimulated by immune-related gene expression and modulating humoral immune responses by probiotic treatment (Verschuere et al., 2000).

### 5.2. Mucosal immunity

The use of probiotics in shrimp culture has been shown to enhance mucosal immunity, leading to improved survival and growth. Probiotic *Bacillus S11* increased phagocytic activity and antibacterial responses in black tiger shrimp (Rengpipat et al., 2000). Studies demonstrated that a combination of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* in shrimp feed increased haemocyte parameters and gene expression, leading to enhanced immunity (Pooljun et al., 2020). These findings are consistent with the concept of “immune priming” in shrimp, as suggested by (Pope et al., 2011), and the use of symbiotic to further enhance immune responses, as shown by (Arisa et al., 2015). Compared to serum, probiotics have a potential role in preventing pathogen entry due to their mucosal immunostimulatory activity (Sheikhzadeh et al., 2012). Mucus act as a barrier for infection-causing agent (McNeilly et al., 2008). Probiotics strengthen the mucosa-associated lymphoid tissue against infectious agents (Gomez et al., 2008). Various bacillus species are used as mucosal immune stimulators (Sangma and Kamilya, 2015).

### 5.3. Pathogen-associated molecular pattern (PAMP)

The immune system can be stimulated by activating the immune function by the introduction of PAMPs. Probiotic bacterial cell surface consists of components such as glucans, peptidoglycans, lipoteichoic

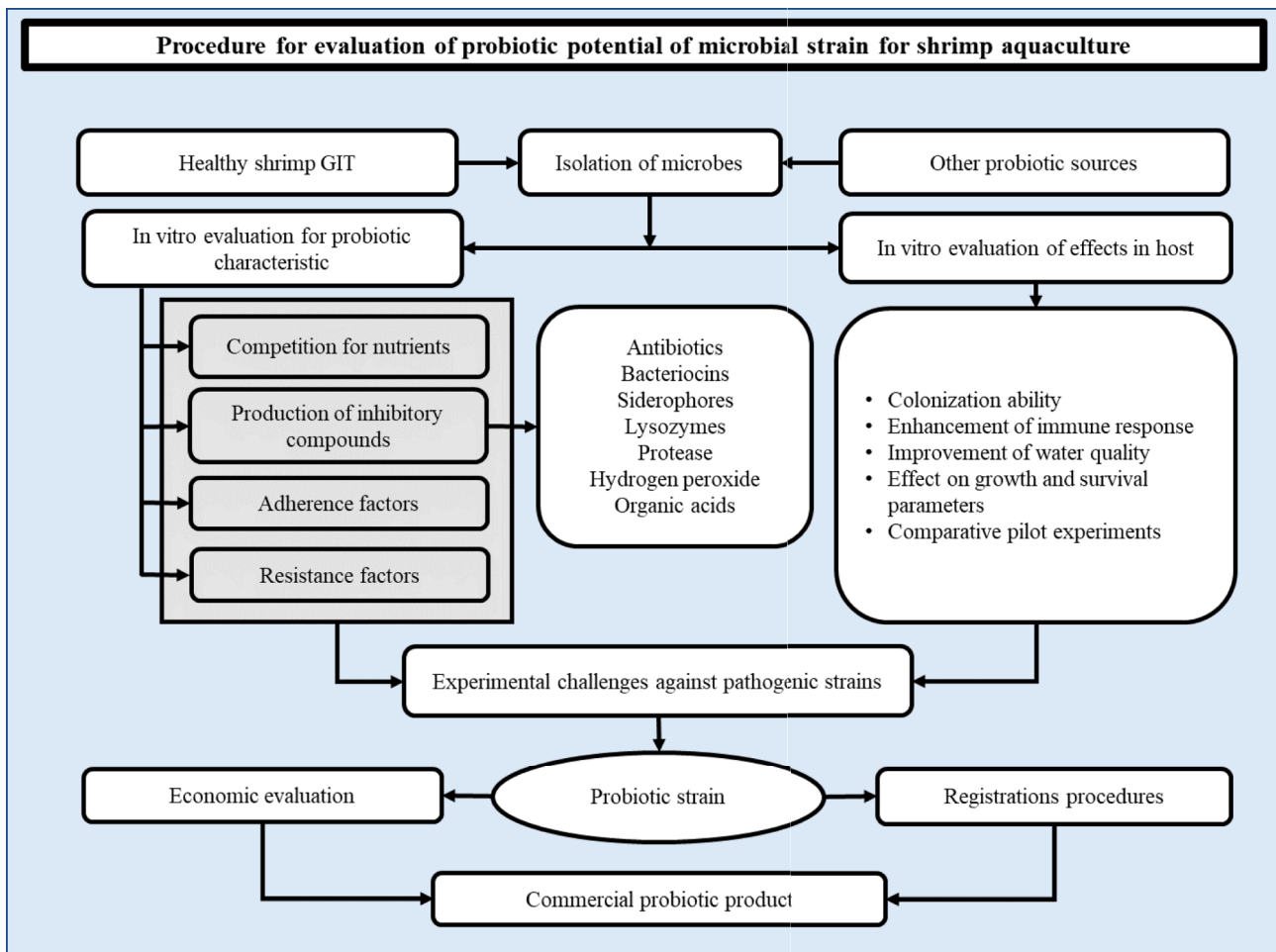


Fig. 4. Procedure to evaluate probiotic strain in Shrimp aquaculture.

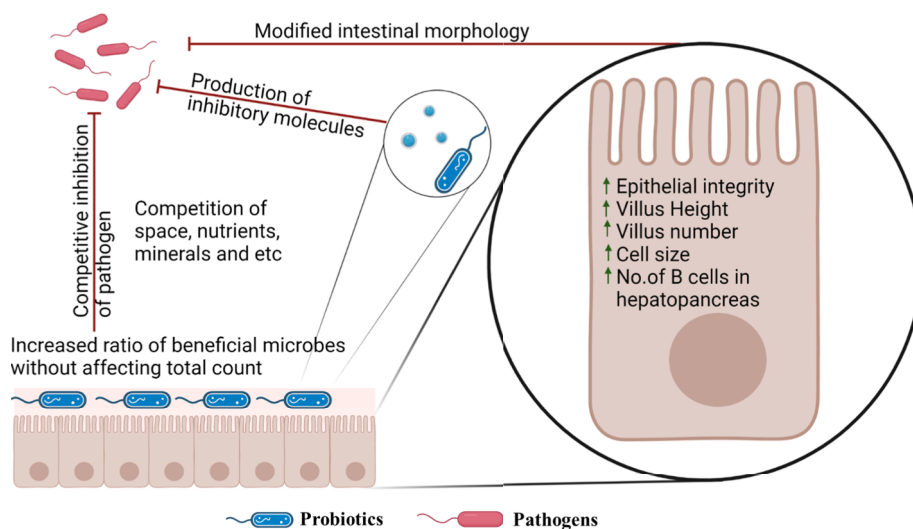


Fig. 5. Modification of gut microbiome and gastrointestinal morphology.

acid, and lipopolysaccharides (LPS) which form a complex with Pattern recognition proteins (PRPs) and act as an immunostimulant in the host. The effector function and binding specificity of each PRP differ (Wang and Wang, 2013). Formation of the PAMP-PRP complex activates the immune response resulting in improved immune function by

upregulation of immune gene expression and helping the animal during pathogen invasion (Xuan Hui Goh et al., 2023).

The PAMP-PRP complex initiates a cascade of serine proteases and triggers the release of phenol oxidase (PO) enzyme via the catalytic cleavage of prophenoloxidase (proPO). The terminal enzyme PO further

mediates several pathways such as melanin synthesis, liberation of cytotoxic reactant, encapsulation, phagocytosis, and nodule formation. Shrimp that are treated with probiotic feed have increased expression levels of peroxyacetic, serine protease, and proPO compared to both the control group and the antibiotic group (Fig. 2) (Won et al., 2020). Lysosome kills the pathogenic microbes by disrupting the peptidoglycan resulting in increased osmotic pressure inside the pathogen leading to cell rupture (Saurabh and Sahoo, 2008). *Bacillus subtilis* E20 is a probiotic from human health food, tested in *Litopenaeus vannamei* (white shrimp) and resulted in elevated gene expression (Table 1) of lysozyme, and prophenoloxidase I and II as well as decreased cumulative mortality in larvae (Liu et al., 2010).

## 6. Competitive inhibition of pathogens

One of the primary outcomes that may be attained in the culture system was the suppression of microbial growth (Giri et al., 2013; Jiang et al., 2013; Kesaracodi-Watson et al., 2008) and research has shown that native microbes possess significant potential in this regard, owing to their heightened likelihood of engaging in competitive inhibition as a result of adaptation within an identical environmental area (Laloo et al., 2010). The processes of colonization and adhesion on mucosal surfaces may ultimately serve as defences against infections, despite competition for nutrients and binding sites (Fig. 5).

### 6.1. Competition for space

The pathogen must be connected to the host GIT mucosal layer to cause diseases. Competitive exclusion was when probiotic bacteria compete with the pathogen for the site of adhesion and exclude the pathogen (Jamal et al., 2019). Passive forces, hydrophobic, electrostatic, steric, and lipoteichoic acids are reportedly some of the components that affect probiotic adhesion to sites of attachment (Hoseinifar et al., 2018). In addition, it was crucial to note that attachment plays a vital role in the production of biofilms, despite its potential to reduce mobility (Knipe et al., 2021). The growth of adherent cells within biofilms may also help to eliminate non-adherent cells from the population as an entire group (Schluter et al., 2014).

### 6.2. Competition for nutrients

Exploitation competition encompasses strategies that enhance the efficiency of nutrient acquisition and utilisation in relation to competing organisms. The process of secreting extracellular molecules that possess the ability to degrade complex macromolecules, hence enhancing the accessibility of nutrients, has the potential to enhance nutrient capture. Iron-chelating siderophores that access insoluble iron (Kümmerli et al., 2015) and proteases (Bachmann et al., 2011) are examples of extracellular excretion molecules. Species with the ability to synthesise siderophores with higher iron binding affinities has a competitive edge over those species that produce siderophores with lower affinities for iron. While it was true that siderophores may be seen as a public benefit in situations where all cells share the same receptors, it could be more appropriate to view them as a competitive phenotype (Niehus et al., 2017).

## 7. Production of antimicrobial substances

Probiotics are capable of generating a diverse array of inhibitory chemicals, such as antibacterial substances, antibiotics, bacteriolytic enzymes, protease inhibitors, siderophores, proteases, lactic acids, and other organic compounds (Lee et al., 2000) which are beneficial to the host (Hoseinifar et al., 2018). When probiotics are administered, a variety of bioactive components such as short-chain fatty acids (SCFAs), polyamines, exopolysaccharides, and vitamins are released (Fig. 2). One example could be the production of a bioactive chemical by probiotic

bacterium *Shewanella algae*, which has inhibitory effects on the aquatic disease *Aeromonas hydrophila* (Table 1). The subsequent sections delve into the examination of further significant antimicrobial agents, namely bacteriocin and short-chain fatty acids (SCFAs), in relation to their efficacy against infections affecting shrimp.

Bacteriocins produced from probiotics are antimicrobial peptides that are bactericidal and produced by an organism to kill another (Zacharof and Lovitt, 2012). Probiotic bacteriocins production (Fig. 2) (Stein, 2005), inhibiting virulent gene expression of shrimp pathogens (Miandare et al., 2016), and production of lytic enzymes against a pathogen that block and break down the pathogen's cell wall were predominantly noted (Butt et al., 2021). Additionally, the area of the cytoplasmic membrane where receptor binding occurs on bacterial surfaces contains the bactericidal mode of action of bacteriocin. Furthermore, unlike antimicrobial agents, these bacteriocins are protease-sensitive non-toxic peptides (Mokoena, 2017). For example, *Bacillus cereus*, and *Bacillus thuringiensis* isolated from the GIT of the shrimp help mitigate *Vibrio harveyi* by production of bacteriocins (Masitoh et al., 2016).

Probiotics help in the breakdown of shrimp's undigested sugars into SCFAs like butyrate, acetate, and propionate as well as other by-products like heat and gases (CH<sub>4</sub>, CO<sub>2</sub>, and H<sub>2</sub>) (LeBlanc et al., 2017). Short-chain fatty acids (SCFAs) have a pivotal role in several physiological processes in shrimps, including energy generation, digestion regulation, gut microbiota modulation, disease resistance, and immune response regulation. Furthermore, it was worth noting that the acidic environment generated by short-chain fatty acids (SCFAs) may serve as a deterrent for the proliferation of opportunistic infections inside shrimp (Fig. 2) (Hoseinifar et al., 2017). In order to induce the expulsion of excess intracellular protons via the efflux mechanisms of pathogenic bacteria, short-chain fatty acids (SCFAs) have the ability to permeate the bacterial cell wall and undergo dissociation, resulting in the release of protons. The existing findings provide evidence that short-chain fatty acids (SCFAs) are effective in stimulating the immune response of aquatic animals. However, more mechanistic investigations are required to validate the specific mechanisms by which SCFAs modulate the immune system of shrimp (Xuan Hui Goh et al., 2023). Additionally, SCFA plays a critical role in regulating lipid metabolism and preserving intestinal health.

## 8. Enzymatic activity

Common antioxidant enzymes in aquaculture species are glutathione peroxidase (GPx), catalase (CAT), and superoxidase dismutase (SOD) (Butt et al., 2021). Harmful oxygen molecules (O<sup>2-</sup>) are decomposed into H<sub>2</sub>O<sub>2</sub> with the help of SOD (Li et al., 2015). The H<sub>2</sub>O<sub>2</sub> generated were converted into H<sub>2</sub>O and O<sub>2</sub> by CAT (Wang et al., 2017). Antioxidant enzymes also help in the counteraction against the damages caused by reactive oxygen species such as oxidative stress (Abarike et al., 2018). The shrimp's capacity to breakdown some complex carbohydrates, such as starch, was constrained as a result of the lack of particular enzymes, notably  $\alpha$ -1,6-glucosidase. The introduction of probiotics may provide enzymes that facilitate the digestion of nutrients that shrimp are unable to normally process (Leonel Ochoa-Solano and Olmos-Soto, 2006). Probiotic enzymes can break down the antinutritive components of the feed, making shrimp more receptive to plant-based fish meals. Due to their lower costs and greater accessibility, corn, soybeans, and wheat are becoming more and more popular aquafeed ingredients. This makes it crucial to take advantage of this property (Xuan Hui Goh et al., 2023). Also, probiotic enzymes mitigated the shrimp pathogens experimentally. For instance, a mixture of probiotics like *Bacillus subtilis* and *Bacillus licheniformis* produces enzymes that can break down harmful compounds in the shrimp's environment. The probiotic enzyme mixture has the ability to decrease the growth of *Vibrio parahaemolyticus* and *Vibrio harveyi*, two common shrimp pathogens, by up to 90 % vibriosis (Chumpol et al., 2017; Ina-Salwany et al., 2019).

## 9. Growth promoters

In the context of shrimp health, the administration of probiotic-supplemented diets has proven to be instrumental in fostering significantly higher growth rates within experimental groups compared to control groups. This positive impact extends beyond mere growth, as evidenced by observations indicating a pronounced mitigation of the negative consequences induced by stressors (Pooljun et al., 2020). The multifaceted mechanisms underpinning the growth-promoting effects of probiotics encompass various facets of shrimp physiology. Notably, modifications in the gut microbiome, increased enzymatic activity, enhanced immune function, and alterations in intestinal and hepatopancreatic morphology have been identified as contributing factors (Lara-Flores et al., 2003).

Moreover, probiotic supplementation has been associated with a discernible modulation in gene expression, further elucidating the intricate pathways through which these microorganisms exert their beneficial influence on shrimp growth. One noteworthy outcome is the heightened resistance exhibited by shrimp to a spectrum of environmental stress factors, including oxidative and ammonia stress (Ringo et al., 2022). This increased resilience not only safeguards the organism against potential threats but also directly translates into the observed growth enhancement (Lara-Flores et al., 2003). It is imperative to note that these documented mechanisms provide a robust foundation for understanding the growth-promoting effects of probiotics in shrimp. Despite these advancements, it is crucial to acknowledge the existence of potentially unexplored mechanisms that may further contribute to enhanced animal growth (Huynh et al., 2017). A recent study suggest that additional layers of complexity may underlie the observed effects, warranting continued exploration and research in this domain. This calls for a comprehensive approach to unravelling the intricate interplay between probiotics and shrimp physiology, ensuring a thorough understanding of their potential as growth promoters (Xuan Hui Goh et al., 2023).

## 10. Improving water quality

Gram-positive bacteria have always been linked to improved water quality due to their ability (Fig. 1) to convert organic materials back to CO<sub>2</sub> more efficiently related to Gram-negative bacteria (Balcázar et al., 2006). High quantities of Gram-positive bacteria can decrease the accumulation of dissolved organic carbon and particulate during the culture period. Furthermore, the use of bioremediation techniques has been extensively employed in the field of aquaculture, with the aim of enhancing water quality via the utilisation of either native or introduced probiotics (Wang et al., 2005).

Bacterial species belonging to the genera *Acinetobacter*, *Bacillus*, *Cellulomonas*, *Pseudomonas*, *Rhodospseudomonas*, *Nitrosomonas*, and *Nitrobacter* have been identified as proficient bio remediators of organic waste (Kumar et al., 2016). In the conducted investigation, it was shown that *Bacillus* spp. emitted amylase, lipase, and protease enzymes, which effectively decreased the presence of pathogenic *Vibrio* spp. without causing any negative effects on the post-larvae of shrimps. As a result, it is suggested that the utilisation of *Bacillus* spp. in *Penaeus monodon* farming may serve as a beneficial bio-remediation (Devaraja et al., 2013).

Recent studies have highlighted the potential of probiotics in improving water quality in shrimp culture. (Carbajal-Hernández et al., 2013) developed a Water Quality Index that can be used to monitor and prevent negative effects in shrimp farms. (El-Saadony et al., 2022) emphasized the role of probiotics in protecting shrimp from pathogens and improving water quality. (Wu et al., 2016) found that the application of probiotics reduced the levels of pH, nitrite, and soluble reactive phosphorus in shrimp culture water, thus improving water quality. (van Hai and Fotedar, 2010) also noted the positive effects of probiotics in improving water quality and reducing pathogenic bacteria in shrimp

aquaculture. These findings underscore the potential of probiotics as a sustainable solution for improving water quality in shrimp culture.

## 11. Probiotic mode of application in shrimp farming industries

The appropriate use of probiotics, doses, timing, physiological circumstances, and administration techniques must all be carefully studied. In order to mitigate losses associated with diseases, it could be advisable to include probiotics into culture systems prior to the occurrence of disease outbreaks. In the context of the culture phase, the regular administration of probiotics in larval shrimp-producing systems has been seen to enhance the viability rate of shrimp (Guo et al., 2009). Probiotics can be taken alone, in combination, or as a mixture of prebiotics and probiotics. Farmers were to administer probiotics at consistent intervals throughout the culture period in order to augment disease resistance and various immune responses. Furthermore, discontinuation of probiotic supplementation may lead to untreated conditions and render the animal vulnerable to infections (Lakshmi et al., 2013). The investigation of the most effective mode of introduction, appropriate dosage, and necessary technological solutions, specifically for the preservation of probiotics inside dry pellets, has significant importance. The inclusion of this criteria was of utmost importance due to the prevalent occurrence of substantial reductions in viability that are often observed throughout the stages of processing and storage (Knipe et al., 2021).

There are several methods for introducing probiotics into shrimps, including supplementation with feed, oral administration, submersion, and immersion. The use of feed supplementation or the in-feed approach has been shown to be more efficient and feasible compared to other methods (Azad et al., 2005) majority of probiotics are formulated to be included with animal feed (Gomes et al., 2009). Oral delivery was seen to be the most feasible way for shrimp probiotics (Table 2) (Huang et al., 2006). The integration of probiotics into one's dietary regimen was a straightforward process; nevertheless, it can be essential to assess the viability of these microorganisms in feed pellets. In the event of a low viability count, it may be necessary to augment the frequency of incorporations. Probiotics are often included in animal feed in the freeze-dried culture format. Lipids may also serve as top coatings in animal feed formulations (Kumar et al., 2016). Spray drying has also become a viable alternative to other encapsulation methods for improving probiotic utilization with potential industrial applications because it could be continuous, and cost-effective process. As a result, spray drying has the potential to be an effective encapsulation method (Vivek et al., 2023).

## 12. Future perspective

In the future, research endeavours should place a paramount emphasis on the intricate characterization of probiotics, delving into the nuances of their direct physiological impacts and the potential mediation through metabolites. Leveraging advanced techniques, such as in-depth gut microbial studies and comprehensive gene/protein expression analyses, is pivotal for unravelling the intricate mechanisms governing the behaviour of probiotic strains. A specific focus on immune-boosting probiotics, whether deployed individually or as consortia, is essential. These probiotics hold the promise of not only effectively combating a spectrum of pathogens but also fostering optimal growth in shrimp populations. By adopting this targeted research approach, we can systematically address existing knowledge gaps, paving the way for a more nuanced and informed understanding of probiotic functionalities. This comprehensive understanding, in turn, will lay the foundation for the development of precise and effective strategies for administering probiotics in the management of shrimp health.

While our current understanding of the role and mechanisms of probiotics in mitigating shrimp pathogens has provided valuable insights, the exploration of related avenues, such as prebiotics, holds promise for future research. Prebiotics, defined as non-digestible

**Table 2**  
Therapeutic probiotics in shrimp diseases.

S. No	Probiotics	Mode of admission	dosage	Host species	Application	Reference
1	<i>Pseudoalteromonas piscicida</i> 1UB	Oral (mixed with feed in final concentration $10^9$ CFU $g^{-1}$ )	The feed ratio is 5 % of body mass (4 times a day for 40 days prior experiment)	<i>Litopenaeus vannamei</i>	Action against <i>Vibrio harveyi</i> and White spot syndrome virus (WSSV) coinfection	(Nababan et al., 2022)
2	<i>Pediococcus pentosaceus</i> MR001	Oral (prepare $10^{10}$ CFU/ml and add 1 ml of probiotics for 1 g of shrimp feed)	3 times a day for 30 days before the experiment	<i>Litopenaeus vannamei</i>	Protection against WSSV	(Wanna et al., 2023)
3	<i>Lactobacillus plantarum</i>	Oral ( $10^9$ CFU per gram of diet)	3 times a day for 28 days where infection was caused during day 14	<i>Penaeus vannamei</i>	Increased chance of surviving when faced with <i>Vibrio alginolyticus</i>	(Tseng et al., 2023)
4	<i>Bacillus subtilis</i> E20	Encapsulation of <i>B. subtilis</i> E20 was prepared with $10^6$ CFU/kg of feed	5 % of body weight, twice day, for 56 days, on the experimental diets.	<i>Litopenaeus vannamei</i>	Challenge against <i>Vibrio alginolyticus</i>	(Adilah et al., 2022)
5	<i>Bacillus subtilis</i> P2.24	Microcapsules prepared with marine probiotics as $10^6$ CFU/kg of feed	30 days of twice-daily feeding	<i>Litopenaeus vannamei</i>	controlling vibriosis disease	(Aribah et al., 2022)
6	<i>Paenibacillus polymyxa</i>	Oral ( $10^9$ CFU per gram of diet)	Fed twice for 8 weeks	<i>Litopenaeus vannamei</i>	Increased disease resistance against <i>Vibrio parahaemolyticus</i>	(Amoah et al., 2020)

substances that promote the growth and activity of beneficial microorganisms in the host, can potentially complement the effects of probiotics in shrimp health. Investigating the synergistic interactions between prebiotics and probiotics may unveil novel strategies for enhancing the efficacy of microbial interventions in shrimp aquaculture.

Moreover, as we delve deeper into the realm of probiotic applications, it becomes imperative to scrutinize the growth conditions that can optimize their effectiveness. The performance of probiotics is intricately linked to environmental factors, including water quality, temperature, and nutrient availability. Future research endeavours should focus on delineating the specific growth conditions that facilitate the proliferation and sustained activity of probiotic strains, ensuring their robust functionality within the shrimp gut. Understanding the intricate interplay between prebiotics, probiotics, and the environmental milieu is pivotal for developing comprehensive strategies to bolster shrimp health. By broadening our scope to include prebiotics and refining the growth conditions for probiotics, we can unlock new dimensions in the mitigation of shrimp pathogens, paving the way for more effective and sustainable aquaculture practices.

### 13. Conclusion

Probiotics seem to be an eco-friendly alternative to the disease-controlling methods already available in the market. The application of probiotics leads to a new prospect for the growth and resistance against infections, stress, and other issues faced by shrimp farmers. Even with the lack of data on large-scale implementation, studies reported the positive impact of probiotic usage including increased growth performance and rate of survival. Different probiotics trigger different positive impacts through different mechanisms which are yet underexplored. With the data for available mechanisms, we recommend probiotics with different mechanisms can be a better solution in case one does not work out well with the different environments on the farm. Once the probiotics have been identified it has to be characterized and every possible mechanism of the probiotic strain should be studied.

In conclusion, the integration of prebiotics into the discourse on shrimp health represents an exciting frontier for future research. This expansion of our focus beyond probiotics underscores the dynamic nature of aquaculture research and the continuous pursuit of innovative strategies to ensure the well-being of shrimp populations in the face of pathogenic challenges. A holistic approach to future research in shrimp probiotics involves not only expanding our focus to include prebiotics

but also meticulously examining growth conditions. By integrating these elements, we pave the way for sustainable aquaculture practices that prioritize shrimp health and resilience against pathogens. The dynamic interplay between probiotics, prebiotics, and optimal growth conditions represents a promising frontier, ensuring the continued advancement of knowledge in shrimp health management.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgment

The authors thank Vellore Institute of Technology (VIT), India for providing necessary facilities to carryout the studies.

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