

Case Study

Development of Dietary Multi-Strain Probiotics on Banana Peel Powder for Sustainable Shrimp Aquaculture in India

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Abstract

Shrimp production in India has continued to face an increasing challenge in the last two years owing to improper farm and disease management and lack of efficient probiotic availability. India mainly comprises two varieties of shrimp: Pacific white leg shrimp (*Litopenaeus vannamei*) and Indian black tiger shrimp (*Penaeus monodon*). Generally, shrimps lack an adaptive immune system to combat invading pathogens. Probiotics have been suggested as a substitute strategy to improve immune function and prevent or minimize shrimp disease. A variety of imported single- or multi-strain probiotic formulations and products have been available in markets, but the cell viability and shelf life of the strains in shrimp farms are questionable to date. The current perspective is that new dietary multistrain probiotics must be formulated and immobilized on fruit-based solid matrices for long-term viability and increased shelf life in India. Dried banana peel powder is suggested herein as a food matrix or probiotic carrier because it is a low-cost fruit waste that is readily available and contains more total dietary fiber with antioxidant activity. Hence, dietary multi-strain probiotics could be ideally viable in the pond environment by reducing the bacterial and organic loads, which will fetch larger profits with additional savings in the production costs of shrimp farming.

Key words: Shrimp, Probiotics, Disease control, Immunity, Banana peel

The global production of farmed shrimp in 2017 was estimated between 2.9-3.5 million tons (<http://www.fao.org/in-action/globefish/marketreports/resource-detail/es/c/1136583/>). The overall export of shrimp during 2016-17 was pegged at 4, 34,484 metric tons worth USD 3,726.36 million. Shrimp exports improved from 2, 56,699 metric tons to 3, 29, 766 metric tons in 2016-17 (28.46% in quantity). The demand for shrimp and shrimp products in international markets has gradually increased (<https://mpeda.gov.in/MPEDA/admin/files/PressRelease/Press-releaseFinal2016-17.pdf>). Pacific white-leg shrimp (*Litopenaeus vannamei*) and Indian black tiger shrimp (*Penaeus monodon*) are commonly cultured on Indian farms. India is the second-largest shrimp producer in the world. Andhra Pradesh is India's largest *Litopenaeus vannamei* farming area, it has 974 km of coastline and 175,000 ha of brackish water (<http://www.caa.gov.in/uploaded/doc/FAO%20Aqua41-79.pdf>). The Indian shrimp industry has gained relative importance globally over the past 10 years. The global export of shrimp rose from 6% in 2009 to 23% in 2018 at a CAGR of 27% from a level of 97 thousand tonnes to 690 thousand tonnes

(<https://www.televisory.com/blogs/-/blogs/global-shrimp-industry-the-indian-dynamics>).

Aquaculture probiotics

Shrimp culture represents a great opportunity for rural development and has a significant economic impact. The feed industry related to shrimp farming is of great economic importance, as it accounts for 60 to 80% of shrimp production costs. Shrimp aquaculture faces several issues and challenges in achieving sustainability related to low feed conversion ratios and microbial diseases. Several infectious disease outbreaks on shrimp farms have resulted in serious mortality and economic losses. Shrimps lack an adaptive immune system, and rely on effective cellular and humoral innate immune responses to combat invading pathogens. Several chemical compounds associated with antibacterial resistance among opportunistic bacteria have been used to treat microbial pathogens in shrimp farming. In addition, continuous antibiotics use antagonists of beneficial gut microbes and suppress the formation of the natural microbiota of shrimp post-larvae. The accumulation of antibiotic residues in animal tissues and the environment is subsequently passed to the consumer. Probiotics in aquaculture

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are an established farm management tool for improving gut health, the immune system, environmental quality, and the prevention of shrimp diseases. The importance of probiotics in aquaculture is increasing because of outbreaks of infectious diseases and environmental problems. This is the biggest constraint on shrimp farming and is a huge challenge for the future. Commercial formulations of aquaculture probiotics include *Bacillus* spp., lactic acid bacteria, yeast, and nitrifying/denitrifying bacteria. These microorganisms can proliferate and colonize the gastrointestinal tract of shrimps. However, probiotics and other top-dressing chemicals can easily be destroyed by other chemicals, which generally affect their stability and establishment in shrimp culture. This indicates that aquaculture probiotics must be safe, effective, viable, and have a long shelf-life [1].

More than 700 companies produce probiotics that target the aquaculture industry worldwide. The global market for probiotic ingredients, supplements, and food is expected to reach 19.6 billion in 2013 at a CAGR of 4.3%. The Indian probiotics market, valued at \$12 million in 2011, is expected to witness a CAGR of 11% by 2016 (<https://www.bccresearch.com/market-research/food-and-beverage/probiotics-market-fod035d.html>). Farmers apply probiotics directly into pond water (84%) and mix them with aquafeeds (16%) to improve water quality and reduce

environmental stress. The cost of probiotic use accounted for 20% of the production. However, this depends on the efficacy of probiotics, viability, and the cost of imported probiotics, which are too burdensome to farmers in India (<https://thefishsite.com/articles/the-godfather-of-shrimp-probiotics>).

Multi-strain probiotics

Aquaculture probiotics have beneficial effects in enhancing infection resistance, digestive enzyme activity, growth performance, and plankton growth. It induces innate immune responses including transglutaminase, LvTAB2, and syntenin [2-5] for both bacterial and viral infections. Several water-soluble, dietary, and multi-strain probiotics have been investigated and commercialized for use in aquaculture probiotic formulations (Table 1). In addition, *B. licheniformis* [6], *B. subtilis* [7], *B. subtilis* E20 [8], *Bacillus* PC465 [9], mixed *Bacillus* spp. (*B. licheniformis* MAT32, *B. subtilis* MAT43, and *B. subtilis* subsp. *subtilis* GATB1) [10] are water-soluble probiotics in shrimp culture. *Vibrio alginolyticus*, *V. gazogenes* [11], *Arthrobacter* sp. CW9 [12], *Ectothiorhodospira shaposhnikovii* [13], *Saccharomyces cerevisiae* [14], *E. arabatum* W-01 [15], *Enterococcus lactis* [16], *Lactobacillus plantarum* [17-18], and *L. lactis* subsp. *lactis* [19] are dietary probiotics used in shrimp aquaculture.

Table 1 Details of commercial probiotics formulations and dominant bacteria used for shrimp aquaculture

Trade name	Company	Microorganisms	Microbial counts	Packing size
Vibrionix	SRR Aqua	<i>P. acidolactis</i> , <i>L. acidophilus</i> , <i>L. casei</i> , <i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. mesentericus</i> , <i>B. polymyxa</i> , <i>B. amyloliquifaciens</i> , <i>C. butyricum</i> , and <i>S. faecium</i>	20 Billion CFU/g	1kg
BIOTAB	New IHC Aqua	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>T. viride</i> , <i>N. europaea</i> , <i>N. winogradskyi</i> , <i>A. oryzae</i> , <i>Rhodococcus</i> , <i>R. rubrum</i> , <i>Cyanobacteria</i> , <i>P. denitrificans</i> , and <i>P. oxalaticus</i>	50 Billion CFU/g	1kg
Profs Power	BIOSTADT	<i>Bacillus</i> sp., <i>Pediococcus</i> sp., <i>Nitrosomonas</i> sp., and <i>Nitrobacter</i> sp.	>8 Billion CFU/g	1kg
Environ AC power	BIOSTADT	<i>Bacillus</i> sp., <i>Nitrosomonas</i> sp., <i>Nitrobacter</i> sp., and <i>Nitrosomonas</i> sp.	> 5 Billion CFU/g	10kg
UB-SPORE	Unique Biotech	<i>L. sporogenes</i>	1.5 Billion CFU/g	500g
UB-Aquaremaid	Unique Biotech	<i>Bacillus</i> sp., <i>Lactobacillus</i> sp., <i>Saccharomyces</i> sp., and <i>Aspergillus</i> sp.	NLT 4 Billion CFU/g	500g
UB-Pondcare	Unique Biotech	<i>Rhodococcus</i> sp., <i>Rhodobacter</i> sp., and <i>Thiobacillus</i> sp.	10 Billion CFU/L	1L
Bacimor FP	Hi-Line Aqua	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>B. polymyxa</i> , and <i>B. pumilus</i>	50 Billion CFU/g	100g
Nutrifeast	Waterbase	<i>B. licheniformis</i> , <i>B. pumilus</i> and <i>B. subtilis</i>	>1.5x10 ⁹ CFU/g	500g
BIOSTIM	AABT	<i>S. cerevisiae</i> , <i>L. sporogenes</i> , <i>L. acidophilus</i> , <i>B. subtilis</i> , and <i>B. licheniformis</i>	9.5 Million CFU/g	500g
BIO-PROB	AABT	<i>Lactobacillus</i> , <i>Bacillus</i> , <i>Nitrobacter</i> , <i>Nitrosomonas</i> , <i>Photosynthetic bacteria</i> , <i>Rhodobacter</i> and Yeast etc.,	150 Billion CFU/g	500g
Fe Clear	SRR Aqua	<i>Lactobacillus</i> sp., <i>Bifidobacteriu</i> sp., <i>Streptococcus</i> sp., and <i>Clostridium</i> sp.	2 Billion CFU/ml	100ml
Nitro Con	SRR Aqua	Denitrifying bacteria (<i>P. denitrificans</i> and <i>Lactobacillus</i> sp.)	NLT 5 Billion CFU/ml	5L
Aquasol-PS	SRR Aqua	<i>Rhodococcus</i> , <i>Rhodobacter</i> , <i>T. denitrificans</i> , <i>Cellulomonas</i> , and <i>Bacillus</i> sp.	3 Billion CFU/ml	5L
AquaPro F	Aquaintech inc.	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>B. pumilus</i> and <i>B. polymyxa</i>	2 Billion CFU/g	1 kg

PrimaLac®, AquaStar®Pond, and AquaStar®Growout are commercial multi-strain probiotic formulations for *L. vannamei* cultures [20]. Multi-strain probiotics have more beneficial effects on multiple health conditions, while boosting

the overall gut and immune performance of *L. vannamei* than single-strain probiotics [21]. Mixed dietary multi-strain probiotics can promote growth performance and enhance non-specific immunity [22-23]. Probiotic strains isolated from the

intestinal tract of *L. vannamei* show strong digestive enzyme production activity and antagonistic effects against common bacterial pathogens in shrimp [24]. Additionally, some ammonia-oxidizing bacteria, including *Nitrosomonas marina*, *Nitrospira moscoviensis*, and *Nitrosopumilus maritimus*, have been used to reduce ammonia in shrimp ponds [25]. The availability of single- and multi-strain probiotics provides insights into the formulation of new multi-strain probiotics with overall health benefits and improved pond environments in India. However, the development of probiotic immobilization matrices could ensure the feasibility of such multi-strain probiotics with the desired benefits.

Table 2 Chemical proximity of dried banana peel powder

Constituents	Concentration
Moisture (% dw)	0.54 ± 0.04
Ash (% dw)	16.07 ± 0.55
Fat (% dw)	7.85 ± 0.34
Protein (% dw)	7.17 ± 0.06
Dietary Fiber (% dw)	42.39 ± 0.80
Carbohydrate (% dw)	25.98 ± 0.76
Calcium (% dw)	34.02 ± 0.01
Sodium (% dw)	3.01 ± 0.01
Vitamin C (% dw)	91.30 ± 0.01
Phenol (mg/g)	1.21 ± 0.02
Flavonoid (mg/g)	1.06 ± 0.23
Solubility (%)	73.71 ± 1.44
Tannin (mg/g)	1.27 ± 1.00
Saponin (mg/g)	0.09 ± 0.01
Water holding capacity (g/g)	5.94 ± 0.29
Oil holding capacity (g/g)	1.04 ± 0.04

Matrix-assisted cell immobilization

Cell immobilization on a solid carrier can be defined as the localization of intact cells via physical adsorption between the carrier and cell membrane. Previously, multi-strain

probiotics were immobilized on various solid carriers, such as apple [26], quince [27], cereal [28], bacterial cellulose [29], watermelon rind [30], and pear [31]. Recent studies have shown that total dietary fibers from fruit waste or sugarcane bagasse can be used as food matrices to enhance the viability of multi-strain probiotics [32-33]. This suggests the suitability of fruit waste as a food or solid matrix for increasing the viability and shelf life of multi-strain probiotic formulations. Concerning the commercialization and production cost of probiotics, inexpensive, abundant, and dietary fiber-enriched fruit waste is of great interest for the immobilization of multi-strain probiotics.

Immobilization on banana peel powder

Banana (*Musa acuminata*) is one of the most produced and consumed fruits worldwide, and its by-products (peel, banana fiber/trunk) are discarded in large quantities. Banana peel powder is widely used for the synthesis of nanomaterials, removal of synthetic dyes as absorbents, amelioration of tablet properties as binders, and probiotic carriers. It contains approximately 43–49 g of total dietary fiber, 1 g of inulin, 6 g of fructooligosaccharide, and 10–20 g of pectin per 100 g of dry matter, in addition to significant amounts of α -linolenic acid, essential amino acids, and micronutrients (Table 2) [34-35]. It also contains antioxidant compounds, including polyphenols, catecholamines, and carotenoids [36-38]. The administration of hot-water banana peel extract to diets enhances the resistance of a giant freshwater prawn (*M. rosenbergii*) to pathogenic infections [39-42]. Green banana powder improves the stability of *L. paracasei* LBC 81 enriched fermented milk during storage [43]. Freeze-dried banana powder also serves as a safe probiotic food carrier for microencapsulation of *L. acidophilus* and *L. casei* [44]. Thus, the potential bioactive compounds in banana peel powder are beneficial for human health and aquaculture. The process flow for the development of multi-strain shrimp probiotics using banana peel powder is shown in (Fig 1).



Fig 1 The process flow for the development of multi-strain shrimp probiotics on banana peel powder

Current perspective

Matrix-assisted techniques have been widely used because of their capability to enhance and maintain the viability of multi-strain probiotics during product processing and storage. The functional properties of banana peel powder include water-holding, swelling, gel-forming, bile acid-binding, and cation-exchange capacity. Banana peel powder is a potential agro-based resource that can be exploited as a food/immobilization matrix for the formulation of multi-strain probiotics with prolonged viability and shelf life. Such dietary multi-strain probiotics will gain importance in recycling nutrients, optimizing the food conversion ratio, and conditioning pond environments. The production cost of multi-strain probiotics on banana peel powder is comparatively lower

than that of other immobilization matrices. Overexploitation from banana peel (as a probiotic carrier) will signify its scope for entrepreneurs to endeavour to this field of activity, which in turn provides noteworthy revenue to the country.

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Declaration

I have no conflict of interest.

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