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Microbial products in terms of isolates, whole-cell biomass, and live organisms as aquafeed ingredients: production, nutritional values, and market potential—a review

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Abstract
Utilizing microbial products could be considered a novel approach to sustain the present growth of the aquafeed sector due to dwindling availability and high cost of fishmeal. Various microbial products in terms of isolates, whole-cell biomass, and live organisms are mainly derived from algae, bacteria, fungi, and yeast through the process of fermentation. They have a balanced amino acid profile and contain several minerals and vitamins. However, essential amino acid index (EAAI) of fungal-based microbial meal was comparatively lower (0.57–0.67) than the other three microbes (0.77–0.90). Microbes were deployed in the production of microbial products ranked as algae > fungus/yeast > bacteria based on their nucleic acid content. The global production of microbial products was valued at US$5.3 billion in 2017 and is predicted to increase by 8.6% in 2018–2023. They could substitute fishmeal by 25–50% in feeds for aquatic species. Notwithstanding, they act as a potential growth promoter, a viable immunostimulant and can control infectious diseases in various aquatic species. The present review reiterates the utilization of microbial products in ameliorating the issues related to the global aquafeed industry, in particular fishmeal demand. However, newer approaches need to be established with regard to fermentation technology and genetic engineering to overcome the present limitations to make them an economical one.

Keywords Aquafeed · Fishmeal substitution · Microbes · Microbial products · Nutritional value

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Introduction

Aquatic species are one of the most potent sources of food, nutrition and livelihood for people living across the globe. In aquaculture, the most essential as well as an expensive component is feed, which alone accounts more than 70% in the variable production cost (Jannathulla et al. 2019c). Protein is an important component in feed, which is usually obtained from animal sources, primarily from fishmeal. Due to uncertainty in fishmeal availability during the past two decades, it is imperative to explore new protein sources for its substitution to a possible extent, which would help to increase or at least sustain the present growth rate of the aquaculture sector. Among the unconventional substitutes, microbial products in terms of isolates, whole-cell biomass, and live organisms appear to be the most promising one, which could replace about 25–50% of dietary fishmeal in aquatic species, including fish and shrimp (Li and Gatlin 2003; Selvakumar et al. 2013; Delamare-Debouteville et al. 2019; Hamidoghli et al. 2019). Microbial products are the whole-biomass or protein extracts of various single or mixed cultures of microorganisms that include algae, bacteria, fungi, and yeast, which usually contain more than 40% of crude protein on dry weight basis (Anupama Ravindra 2000; Garcia-Garibay et al. 2003). In addition, a live microorganism (probiotics) is used as a potential feed supplement in the diet of various aquafeeds (Ferreira et al. 2017). In aquaculture, microbial products not only serve as a good protein/feed source, but also reduce the problems of environmental degradation, which arise due to waste accumulation (Patil and Jadhav 2014).

The global production of microbial products was valued at US$5.3 billion in 2017 and is expected to increase by 8.6% during the period 2018–2023 (Prescient and Strategic Intelligence 2019). Though the various microorganisms (algae, bacteria, fungi, and yeast) contribute to produce microbial products, algae, in particular *Spirulina* and *Chlorella*, were in the dominant category, which share about 33.4% of the total market revenue in 2017 (Prescient and Strategic Intelligence 2019). The approximate revenue share of microbial products for 2016 in the global market by species is given in Figs. 1 and 2, in which North America and Western Europe had a significant revenue share in the global market, while Asia-Pacific and Middle East countries are expected to create a significant growth over the forecast period of

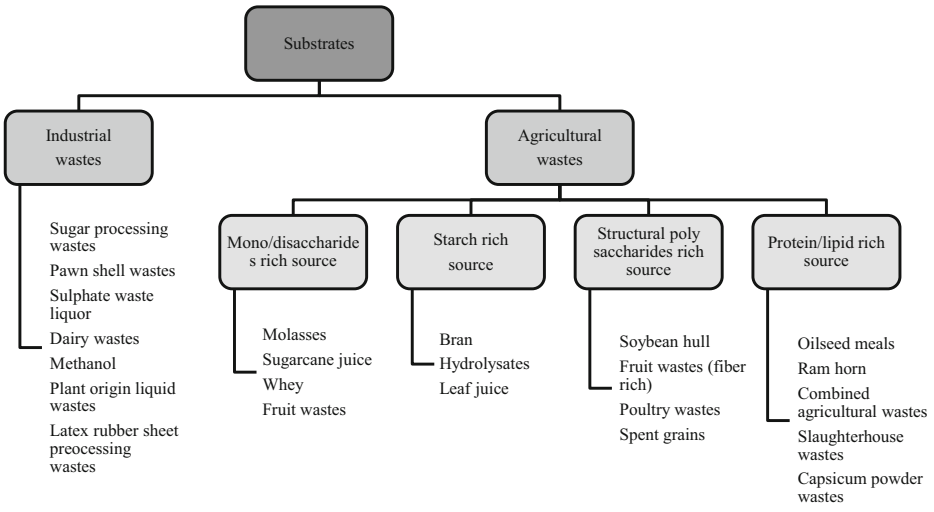

Fig. 1 Substrates used for the production of microbial products

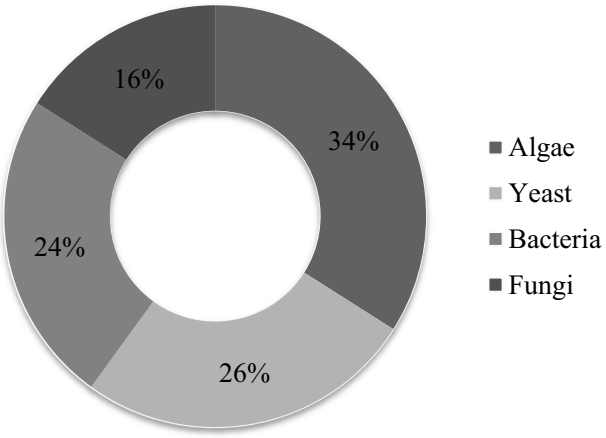

Fig. 2 Approximate revenue share (% in total value) of microbial products in global market by species for the year of 2016 (Source: Transparency Market Research 2019)

2017–2025 (Transparency Market Research 2019). The approximate revenue share of microbial products for 2016 in the global market region-wise is given in Fig. 3. China and Hong Kong produce certain microbial products from the nutrients that are lost during the processing of water for its economic benefits (The Fish Site 2019). China also established various techniques to enhance the production capacity of microbial products to several thousand tons annually and could successfully use them in animal nutrition. A firm from USA invented an appropriate process for the selection, isolation, and cultivation of microbial-derived bacteria called *Clostridia*, with promising implications in the production of microbial products (Tracy 2019). Notwithstanding, the production of microbial products using natural gases through the innovative fermentation process had a great attention recently. Unibio (2016) reported that about 3–4 kg (dry weight basis/m^3 reactor volume/h) of a microbial product produced by the continuous culture of *Methylococcus capsulatus* and marketed in the name of Feedkind. Due to the promising perspectives, the full-scale production of this product reached around 80,000-ton DM/year. Similarly, the production volume and market size of certain other commercialized microbial products is presented in Table 1. However, there is scanty information related to the utility of microbial products in aquaculture compared with several reports in human and

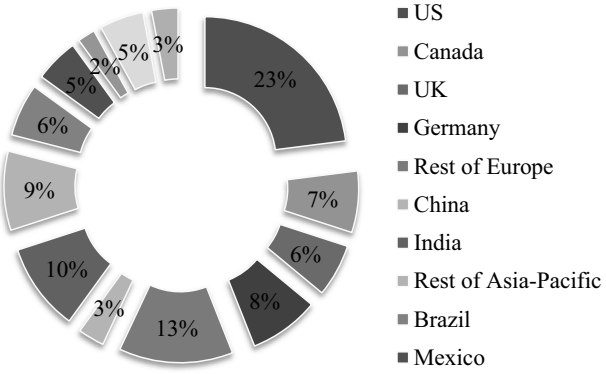

Fig. 3 Approximate revenue share (% in total value) of microbial products in global market by geography for the year of 2016 (Source: Transparency Market Research 2019)

Table 1 Production volume and market size of different commercialized microbial products

Commercial products	Production volume (ton (DM)/year)	Production cost (Euro/kg of DM)	Global market value (Billion Euro)	Yearly growth (%/year)
Baker's yeast	3,000,000	–	9.2	7.9
Quorn	25,000	–	0.214	20
Profloc	5000	1–1.11	–	–
Feedkind	80,000	–	–	–
Valpromic	5000	–	–	–

Source: Matassa et al. (2016)

ruminant nutrition. The present review, therefore, describes the utilization of various microbial products in terms of isolates, whole-cell biomass, and live organisms (probiotics) in the aquafeed industry, so that this basic knowledge can improve the comprehension on the usage of microbial nutrients in the culture of aquatic species.

Production of microbial products

The production of microbial products, in particular microbial isolates and whole-cell biomass, from various microorganisms, including bacteria, fungi, and yeast, is usually achieved either by submerged or solid-state fermentation process. At the end of the process, spores or cells of the respective microorganisms are harvested in an appropriate way and subjected to various downstream processes such as washing, cell wall disruption, protein extraction, and purification (Anupama Ravindra 2000). However, algae can be produced by a number of ways, including indoor/outdoor, open/closed, axenic/non-axenic, and batch/continuous/semi-continuous methods. Though several factors influence the production of microbial products, the substrates required and microorganisms employed in this process need to be standardized to obtain the optimal quantity of production (Table 2).

Screening of microorganisms

Research on microbial products had been initiated a century before; however, its usage became frequent during the 1990s (Nasseri et al. 2011; Suman et al. 2015). The production of microbial products is initiated with a screening of microorganisms during which suitable strains are procured from various sources, viz. soil, water, air, and swabs of certain biological (organic) or non-biological (inorganic) materials. Among the group of microorganisms, superior nutritional qualities make yeast species an important one, and the most commonly used genera are *Candida* and *Saccharomyces.* In addition, having a small particle size and high protein with relatively low production cost catapulted this species as a potential source of microbial products (Kim et al. 1998). However, the rigid cell wall of yeast limits its utilization as a feed source for aquatic organisms due to the poor digestibility, which could be attributed to the external mono-protein layer of the yeast cell wall (Kim and Chung 2001). Vidakovic et al. (2016) reported no negative influence on weight gain and feed utilization in Arctic charr while including pre-lysing yeast cells in the feed. Similarly, an improved protein and amino acid digestibility was observed by Langeland et al. (2016) in both Arctic charr and Eurasian perch while using pre-lysed microbial meal. Nasseri et al. (2011) reported that 100 lbs of yeast

Table 2 Major microorganisms used for the production of microbial products along with their specific substrates

Species	Substrates Pure chemicals	By-products/others
Algal species	–	
Chlorella pyrenoidosa	–	Carbon dioxide, sun light
Chlorella sorskiana	–	Carbon dioxide, sun light
Chondrus crispus	–	Carbon dioxide, sun light
Porphyrium sp.	–	Carbon dioxide, sun light
Scenedesmus sp.	–	Carbon dioxide, sun light
Spirulina sp.	–	
Bacterial species		
Acinetobacter calcoaceticus	Ethanol	–
Achromobacter delvacvate	n-Alkanes	–
Aeromonas hydrophila	Lactose	–
Bacillus subtilis	Cellulose, hemicellulose	–
Cellulomonas sp.	Cellulose, hemicellulose	Agricultural wastes
Flavobacterium sp.	Cellulose, hemicellulose	–
Lactobacillus sp.	Methanol	–
Methylomonas methylotrophus	Non-nitrogenous matters	–
Methylomononas clara	Non-nitrogenous matters	–
Pseudomonas fluorescens	Non-nitrogenous matters	Manure, animal wastes
Thermomonosi porafusca	Cellulose, hemicellulose	–
Fungal species		
Aspergillus fumigatus	Maltose, glucose	–
Aspergillus niger	Cellulose, hemicellulose	Corn cobs, maize, cotton stalk
Aspergillus oryzae	Cellulose, hemicellulose	–
Cephalosporin meichhorniae	Cellulose, hemicellulose	–
Chaetomium cellulolyticum	Cellulose, hemicellulose	Cellulosic wastes
Penicillium cyclopium	Glucose, lactose, galactose	Whey
Rhizopus chinensis	Maltose, glucose	–
Scytalidium acidophilum	Cellulose, pentose	Paper wastes
Trichoderma alba	Cellulose, pentose	Beet pulp
Trichoderma viride	Cellulose, pentose	–
Yeast species		
Amoco torula	Ethanol	Plant origin liquid wastes
Candida intermedia	Maltose, glucose	Plant origin liquid wastes
Candida novellas	n-Alkanes	Plant origin liquid wastes
Candida utilis	Glucose	Plant origin liquid wastes
Saccharomyces cerevisiae	Lactose, pentose, maltose	Plant origin liquid wastes

Sources: Anupama Ravindra (2000); Bhalla et al. (2007); Nasseri et al. (2011)

could generate 250 tons of protein within 24 h. Bacterial-based microbial products have a significant advantage over those produced by other microorganisms, because bacterial cells are not only high in protein but also harbor a significant quantity of photo-pigments and vitamins (Chumpol et al. 2017). Suman et al. (2015) reported that the biomass of bacterial species could double within 20 min to 2 h. In general, phototropic bacterial species are recommended for the production of microbial products (Arora et al. 1991). However, recent research suggests using methanotrophic and other bacterial species also for this purpose. Though algal products are of high quality compared with certain conventional protein sources, technical difficulties and high production costs limit their cultivation. Nasseri et al. (2011) opined that it would take a year to produce about 20 tons of algal products (dry weight) from an acre. The global production of microalgae would be more than 10,000 tons per year, in which nearly 75% is used by the pharmaceutical industries (Becker 2007). As in yeast, the rigid cell wall due to

celluloid components in algae hinders its utilization by affecting digestibility. Hence, post-harvest treatments are advised to improve the digestibility parameters. The filamentous fungi, especially *Aspergillus* species, are mainly explored for the production of microbial products when the complex organic materials, in particular lignocellulosic wastes, are used as a substrate (Jaganmohan et al. 2013). However, molds of certain species are more dangerous in nature; hence, toxicological studies need to be compulsorily performed prior to recommending fungal species as a source for the production of microbial products (Yabaya and Ado 2008).

Potential substrates

A wide range of substrates has been used for the cultivation of microorganisms; however, it is important to consider using biodegradable agro-industrial wastes and their by-products as a source of nutrients to reduce the production cost. The World Bank-Solid Waste Management (2019) reported that about 2.01 billion tons of solid waste had been generated globally in 2016, which accounts for approximately to 0.74 kg/person/day and is further expected to increase by 70% in 2050. The solid waste includes waste of food and green, rubber and leather, glass, paper and cardboard, wood, metal, plastic, etc., in which the waste of food and green alone contributed nearly about 44% in total (World Bank-Solid Waste Management 2019) and could be used as a potential substrate for the cultivation of microorganism. Notwithstanding, the waste of paper and wood can also be considered as a substrate for fungus and yeast species, as they are rich in lignocellulosic materials. Carbon dioxide and sunlight are the most important parameters for culturing algal species (Anupama Ravindra 2000). Both fungus and yeast are mainly cultured on cheap waste, in particular, lignocellulosic materials with varying compositions of cellulose, hemicellulose, and lignin (Jannathulla et al. 2017). However, according to dominant components that are present in the wastes, specific fungi can be selected for this process. By-products of industrial wastes are mainly used for cultivation of bacterial species. The key substrates used for the production of microbial products are categorized in detail in Fig. 1.

Recent advances in producing microbial products

The major objective of the production of microbial products is to increase the end-product yield by increasing cellular growth using an economically viable approach. Though the tremendous feedstocks are available, the recent research had the strongest interest to utilize waste residues and by-products, including waste waters (municipal and industrial), industrial and agricultural residues (off-gases, biogas and cellulosic biomass), bioindustry by-products (brewery residues, dry-grind ethanol co-products, starch processing waters, etc.), and other materials like sugars, corn starch, molasses, certain alcohols, syngas etc., (Jones et al. 2020), in which classic methane, syngas, off-gas, and dry-grind corn ethanol plants are more preferred in the recent trends, as they had more circular economy and lower cost. These feedstocks require different modalities of growth, including autotrophs, photoautotrophs, chemoautotrophs, methylotrophs, heterotrophs, and mixotrophs with various cultivation operations such as aerobic, anaerobic, gas, and photosynthetic bioreactors. By using these recent technologies, microbial products are being now commercially produced by a number of companies worldwide with promising results on aquatic species, including fish and shrimp. Jones et al. (2020) reported different commercial examples for the above-mentioned systems. They are KnipBio, Veramis, Arbiom, Menon, White Dog Labs, Calysta, Unibio, String Bio, Kiverdi, Cellana etc.

Nutritional values of microbial products

The range of nutrient composition of microorganisms that are used for the production of microbial products is depicted in Table 3. The protein component in total is comparatively higher in bacterial species (60–83%) than the other three groups (29–65%). A similar trend is observed for true protein, total amino acids (Table 3), and sulfur-containing amino acids, especially methionine; however, the lysine content is comparatively high in fungi followed by bacteria with yeast and algal species (Table 4). Essential amino acid composition of predominantly used microorganisms, from each category, such as algae, bacteria, fungus, and yeast, is depicted in Table 4. It is observed that most of the values that appeared here are comparable with the reference values given by both Food and Agricultural Organization (FAO) and World Health Organization (WHO). However, certain values were found to be high in particular lysine, phenylalanine, and valine in fungus and leucine in algae. Though the quality of protein sources can be assessed according to the amino acid composition, their suitability primarily depends on the amino acid requirement of the candidate species (Jannathulla et al. 2017). The nutrient composition of two commercially available microbial proteins obtained from *Corynebacterium glutamicum* (CMP-1 and CMP-2) was compared with certain marine and plant-based ingredients that are mainly used as a protein source in commercial aquafeed formulations (Tables 5 and 6). The level of crude protein is higher by 12.5% in CMP-1 than CMP-2. This would partly be due to the variation in the production technology that influences the nutritional properties and characteristics of microbial products. However, CMP-2 had similar values with CMP-1 for most of the essential amino acids. Among them, arginine, histidine, and lysine were higher and methionine and phenylalanine lower in CMP-1 compared with CMP- 2, and the other values are comparable with each other. Both the commercial products had better nutritional values than all the plant proteins viz. soybean meal, groundnut cake, rapeseed meal, and sunflower cake. However, the values are more or less similar between the commercial microbial product and marine proteins (fishmeal, mantis shrimp meal, squid meal, and prawn head meal), which clearly indicates that compared with plant proteins, microbial products can play an important role in substituting high-cost marine proteins, in particular, fishmeal. In addition to the nutritional characteristics, certain other parameters also influence the acceptability of microbial products as a feed material (Table 7). Of all the microbial species, algae was found to have lower nucleic acid content (Table 3). While comparing bacteria and yeast, the fungal species is still found to be better due to the lower level of nucleic acids (Table 3) and higher content of

Table 3 The range of nutrient composition of microorganisms used for the production of microbial products (% dry weight basis)

Particulars	Composition of microorganisms			
	Algae	Bacteria	Fungi	Yeast
True protein	40.0–60.0	50.0–80.0	30.0–70.0	–
Total protein	45.0–65.0	60.0–83.0	35.0–50.0	29.0–56.0
Lipid	9.0	–	–	2.0–7.9
Fiber	3.0	–	–	1.0–6.3
Nitrogen free extract	9.0	–	–	21.0–39.0
Ash	3.0	–	–	4.7–13.0
Total amino acids	–	65.0	54.0	–
Mineral salts	7.0	8.6	6.6	–
Bile pigments and chlorophyll	6.0	–	–	–
Nucleic acids	4.0–6.0	15.0–16.0	9.7	7.1–12.0

Sources: Brown et al. (1996); Ziino et al. (1999); Jannathulla (2017)

Table 4 Essential amino acid composition of predominantly used microorganisms for the production of microbial products from each category along with standard values given by FAO and WHO (% dry weight basis)

Particulars	Microorganisms				Reference values	
	Algae (*Spirulina* sp.)	Bacteria (*B. subtillis*)	Fungus (*A. niger*)	Yeast (*S. cerevisiae*)	FAO	WHO
Arginine	4.15	2.40	5.21	2.40	–	–
Histidine	1.09	0.87	1.04	2.70	–	–
Isoleucine	3.21	3.00	0.88	2.50	4.32	4.20
Leucine	4.95	4.80	3.75	3.80	4.90	4.20
Lysine	3.03	3.40	4.50	3.10	4.32	4.20
Methionine	1.15	1.80	0.35	0.65	2.30	2.20
Phenylalanine	2.78	2.20	5.70	2.10	2.88	2.80
Threonine	2.97	2.20	1.11	2.40	2.88	2.80
Tryptophan	0.93	0.38	0.26	0.59	1.44	1.40
Valine	3.51	3.50	4.36	2.80	4.32	4.20

Source: Anupama Ravindra 2000 FAO/WHO (2007); Penuel et al. (2014)

limiting amino acids, in particular lysine (Table 4). In contrast, Adedayo et al. (2011) reported that microorganisms contain high nucleic acid in the form of RNA rather than DNA, which helped to promote rapid protein synthesis by reducing multiplication time, and fish fed with a diet containing high nucleotide had improved hepatic functions and lipid metabolism.

Algae and yeast species generally do not produce harmful toxins, but some toxicity was reported with filamentous fungi and gram-negative bacteria. Therefore, it is necessary to understand about the toxins associated with certain microbial products when they are used as feed sources along with their level and their effects on the candidate species. Xu et al. (2013) opined that the mutagenicity and toxicity nature of fungal species was successfully reduced when they were co-cultured with other microorganisms. The FDA has provided guidance with the maximum permissible level of fumonisins in various animal feeds viz. horse, rabbit (1 ppm), catfish, swine (10 ppm), ruminants (30 ppm), and poultry (50 ppm). On the other hand, most of the fungal species, in particular *Aspergillus niger*, have been categorized in GRAS (generally recognized as safe) notifications (GRN 000296, GRN 000214, and GRN 000183), to which FDA has no objection and also approved to use these species as a host for enzyme-encoding genes (Olempska-Beer et al. 2006). Similarly, gram-positive bacteria produced exotoxins like enterotoxins, erythrogenic toxins, alpha-toxins, and neurotoxins, but the fatal effect of these toxins would be at a nanogram level. However, the endotoxins that are mainly produced by gram-negative bacteria had a severe impact on fed animals. Hence, before using these microorganisms as a source of microbial products, it would be advisable to modify or suppress the genes that are responsible for the production of these unwanted toxins and can nowadays be achieved by specific genetic engineering techniques.

Role of microbial products in aquaculture

Research on the evaluation of suitability of microbial products in the diet of various aquatic species carried out during the past two decades has recommended microbial products as a potential ingredient to the global feed industry (Pike et al. 1990; Bob-Manuel and Alfred-Ockiya 2011). Their utilization in diets for aquatic species was assessed by essential amino

Table 5 Essential amino acid index of different microorganisms used for the production of microbial products in various aquatic species along with their dietary requirements

Particulars	Dietary requirements of essential amino acids (%)										Essential amino acid index (EAAI)			
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Algae	Bacteria	Fungus	Yeast
Shell fish														
P. vannamei	2.32	0.80	1.01	1.70	1.64	0.91	1.40	1.51	0.20	1.41	0.86	0.85	0.63	0.84
P. monodon	1.85	0.80	1.01	1.70	2.08	0.89	1.40	1.40	0.20	1.30	0.85	0.85	0.63	0.84
P. indicus	3.06	1.52	1.55	2.98	2.40	1.05	1.64	1.47	0.20	2.78	0.87	0.87	0.67	0.87
S. serata	1.99	0.55	1.24	1.49	0.48	0.36	1.45	1.02	0.24	1.18	0.90	0.80	0.63	0.86
Finfish														
S. salar	2.30	0.90	1.44	2.34	2.75	1.40	2.61	1.44	0.20	1.76	0.84	0.86	0.67	0.82
O. mykiss	1.88	0.64	0.96	1.76	1.68	0.96	2.08	1.36	0.56	1.24	0.85	0.78	0.59	0.79
L. rohita	2.40	0.70	0.90	1.60	2.00	1.80	2.10	0.90	0.20	1.30	0.80	0.81	0.66	0.77
C. chanos	2.34	0.90	1.80	2.30	1.80	1.13	1.89	2.03	0.27	1.62	0.88	0.86	0.61	0.84
O. niloticus	1.18	0.48	0.87	0.95	1.43	0.75	1.05	1.05	0.28	0.78	0.84	0.79	0.57	0.80
C. carpio	1.60	0.80	0.90	1.30	2.20	1.20	2.50	1.50	0.30	1.40	0.81	0.80	0.62	0.81
I. punctatus	1.00	0.40	0.50	0.80	1.20	0.60	1.20	0.50	0.12	0.71	0.81	0.82	0.65	0.79
A. japonica	1.70	0.80	1.50	2.00	2.00	1.20	2.20	1.50	0.40	1.50	0.85	0.83	0.59	0.84
S. aurata	2.39	0.83	1.35	2.24	2.50	1.20	1.35	1.40	0.31	1.51	0.86	0.86	0.62	0.85

Sources: NRC (1993); Lall and Anderson (2005); Yigit et al. (2012)

Table 6 Proximate and essential composition of commercially available microbial products along with certain marine and plant proteins (% dry weight basis)

Particulars	Commercially available feed ingredients									
	Marine proteins				Plant proteins				Microbial proteins	
	Fishmeal	Mantis shrimp meal	Squid meal	Prawn head meal	Soybean meal	Groundnut cake	Rapeseed meal	Sunflower cake	CMP-1	CMP-2
Proximate composition										
Crude protein	63.16	50.31	70.26	49.15	52.40	42.92	4.17	35.60	70.03	57.53
Crude fat	10.53	5.46	2.13	8.27	1.09	2.14	2.64	1.73	8.23	7.44
Crude fiber	0.53	4.41	1.54	16.44	6.95	12.87	10.61	28.85	–	–
Total ash	18.95	31.64	13.36	22.39	7.54	7.73	6.76	7.83	–	–
Essential amino acids										
Arginine	4.37	4.06	4.65	2.35	3.04	3.04	3.35	1.61	3.28	2.61
Histidine	1.69	1.27	1.37	0.85	1.75	0.90	1.59	0.46	1.26	0.96
Isoleucine	2.96	2.28	2.59	1.80	2.72	1.36	1.44	3.36	2.45	2.35
Leucine	5.08	2.28	4.99	3.07	3.92	1.09	1.89	1.45	4.23	4.28
Lysine	5.29	2.28	5.26	2.92	1.24	1.41	1.01	1.18	8.23	1.97
Methionine	1.91	1.25	2.53	1.26	0.74	0.55	0.81	1.70	0.98	1.47
Phenylalanine	2.75	1.95	4.31	3.15	2.02	3.00	0.92	1.60	2.15	2.49
Threonine	2.89	2.00	2.52	1.70	1.71	0.94	1.99	1.02	2.86	2.61
Tryptophan	0.71	0.60	0.69	0.47	0.67	0.43	0.43	0.42	0.58	0.73
Valine	3.45	2.49	2.68	1.91	1.62	2.71	2.18	1.48	3.34	3.55

CMP commercially available microbial proteins

Sources: Zhang et al. (2013); Jannathulla et al. (2018); Jannathulla et al. (2019b)

Table 7 Certain other parameters influence the utilization of various microbial species used for the production of microbial products

Particulars	Micro-organism			
	Algae	Bacteria	Fungi	Yeast
Growth rate	Low	Highest	Lesser than bacteria and yeast	Quite high
pH range	Up to 11	5–7	3–8	5–7
Cultivation methods	Ponds and bioreactors	Bioreactors	Bioreactors	Bioreactors
Risk of contaminants	High and very serious	needed specific precautions	Least if pH <5	Low
Nucleic acid removal	Not needed	Needed	Needed	Not needed
Toxins	–	Endotoxins by gram-negative bacteria	Mycotoxins by many of the species	–

Source: Singh (1998)

acid index (EAAI), and the same was computed from the available data (Dayal et al. 2011) based on the dietary requirement of various aquatic species cultured around the world (Table 5). The data revealed that the EAAI was found to be lower in fungal-based microbial products and ranged from 0.63 to 0.67 in shellfish and from 0.57 to 0.67 in the finfish, whereas EAAI of all other species was reported to be > 0.75 and was in the range of 0.80–90 in algal species, 0.78–0.87 in bacterial species, and 0.77–0.87 in yeast species. Jannathulla et al. (2017) suggested that the difference obtained among the species in EAAI could be attributed to the variation in their essential amino acid content. In addition to fishmeal, microbial products also offer a viable substitute for meat and bone meal, soybean meal, and other major protein sources used in animal/aqua nutrition (Hellwing et al. 2007). In aquaculture, microbial products not only act as a potential feed ingredient but also serve as a viable immunostimulant and probiotic, which aid in improving growth, health, disease resistance, and immunity (Bharti et al. 2014). Kolndadacha et al. (2011) reported that utilizing bacterial species, in particular gram-positive bacteria (*Lactobacillus* sp.), as a probiotic, is an alternative to antibiotics among the disease control strategies in aquaculture. The Fish Site (2019) reported that the utilization of microbial products enhanced the ability of cultured animals to absorb protein, resulting in improved feed conversion ratio in aquaculture applications. In addition, microbial products also play a major role in ornamental fish aquaculture by regulating the color and size of the fish. Bharti et al. (2014) reported that the color of ornamental fish can be manipulated by the application of microbial product, which was mainly derived from algae and bacteria that are rich in pigments, in particular carotenoids.

Inevitability of fishmeal substitution

Fishmeal has been considered as the most reliable protein source in aquafeeds; hence, a substantial quantity of it is utilized by the aquafeed industry in commercial formulations (Jannathulla et al. 2019a). The global fishmeal production was > 6.0 million tons (Mt) during the period 1985–2000 and priced at US$755/ton (US Department of Agriculture 2019). Jannathulla et al. (2019b) reported that the productivity of pelagic fishes was reduced by 50% in North Atlantic Ocean and by 20% globally due to climatic change, which resulted in creating a demand for the raw materials required for fishmeal production. This scenario reduced fishmeal availability to a range of 5.7–4.5 Mt after 2000 and is expected to reduce in the future with a simultaneous increase of its cost to 1596 US$/t (US Department of Agriculture 2019). It has been reported by FAO (2015) that the aquafeed industry accounts only 4% in total industrial feed production, but it consumes > 70% of the global fishmeal by majorly sharing about 29% to shrimp feed followed by the feeds of salmonids (24%), marine fish (23%), and others (24%). This clearly indicates that the aquafeed industry is a primary one among the feed sectors, which would predominantly be affected due to fishmeal demand in the future. The aquafeed industry, therefore, reduced fishmeal inclusion level from a global range of 19–40% in 2000 to 11–23% in 2014 and is further expected to reduce by around 6% in 2025 (Salin et al. 2018) by using various potential alternatives, one among them is microbial products.

Microbial products as a fishmeal substitute

Fishmeal serves as a primary protein source in the diet of aquatic species, but its level varies largely (Table 8) based on food habits of the cultured species (carnivores, herbivores, and omnivores). Reducing considerable quantity of dietary fishmeal is a major objective in the feed industry nowadays to increase or at least to sustain the present growth rate of the aquaculture

Table 8 Minimum and maximum level of fishmeal inclusion in the diet of various aquatic species culturing around the globe

Aquatic species	Fishmeal inclusion level (%)	
	Minimum	Maximum
Carp	0	20
Catfish	3	40
Eel	40	80
Freshwater crustaceans	5	25
Marine fish	7	70
Milkfish	1	5
Salmon	20	50
Shrimp	5	40
Tilapia	0	20
Trout	15	55

Sources: Tacon and Metian (2008, 2015); Jannathulla et al. (2019c)

sector due to decreased availability and increased cost of fishmeal. Research has been initiated to evaluate the nutrient utilization of microbial products as a potential substitute for fishmeal in various aquatic species from the 1980s (Table 9). However, microbial isolates (protein) and/or whole-cell biomass are most predominantly used for this purpose rather than using live microorganisms (probiotics). Viola and Zohar (1984) reported that Nile and blue tilapia fish fed with diets that substituted up to 50% fishmeal using a commercial bacterial-based microbial product (Pruteen with 70% protein) developed from *Methylophilus methylotrophus* showed comparable growth with the control group (no fishmeal substitution). Later, a similar result was observed by Zhong et al. (1992) in *Penaeus chinensis*. However, fishmeal substitution was 40% and 50% with the usage of industrial microbial products (Eurolysine Fodder Protein with 64% protein) obtained from *Micrococcus glutamicus* and yeast-based products obtained from *Saccharomyces cerevisiae*, respectively, in tilapia (Davies and Wareham 1988; Bob-Manuel and Alfred-Ockiya 2011). The different results obtained within the species infer that the variation observed might be due to the variability of the microbial species (*M. glutamicus* and *S. cerevisiae*). Bob-Manuel and Alfred-Ockiya (2011) suggested that yeast species provide superior and better nutritional values in fish diets due to their higher palatability, acceptability, and digestibility compared with the bacterium. Contrary, Rumsey et al. (1990) observed a lower performance in lake trout when fed with a diet containing higher level of *S. cerevisiae*-based microbial product. When the digestibility parameters were estimated between intact and disrupted yeast cells in rainbow trout, the fish fed disrupted yeast cells had better digestibility than those fed the intact ones (Rumsey et al. 1991). This clearly indicated that nutrients from intact yeast cells are not easily available to the aquatic species compared with disrupted cells. Perera et al. (1995) substituted 25% of dietary fishmeal in rainbow trout with bacterial-based microbial product without having any deleterious effects on growth, feed consumption, and absorption efficiency. This is in agreement with Storebakken et al. (2004) and Aas et al. (2006) and with Kiron et al. (2016) while using bacterial-based and algal-based (*Desmodesmus* sp.) microbial products, respectively, in Atlantic salmon. Cobia fish fed a control diet containing 65.9% fishmeal could be successfully substituted by a commercial yeast (*S. cerevisiae*)-derived source (NuPro) to an extent of 40%. However, the dietary fishmeal substitution was increased to 50% while feeding a control diet formulated with 54.4% fishmeal (Lunger et al. 2007). Similar partial substitution of fishmeal was observed

Table 9 Optimization of microbial products as a fishmeal substitute in the diet of various aquatic species from 1980s to 2019

Tested aquatic species	Inclusion level tested (%)	Fishmeal level in control diet (%)	Optimum inclusion level of microbial proteins	Fishmeal substitution (%)	References
O. mossambicus	0, 5, 10, 15 and 20	21.1	20	40	Davies and Wareham (1988)
O. mykiss	0, 17.4, 43.5 and 69.5	69.5	17.4	25	Perera et al. (1995)
D. labrax	0, 11, 21.9, 32.9 and 54.8	59.6	21.9	37	Oliva-Teles and Goncalves (2001)
S. salar	0, 5, 9.9, 19.3 and 37	66.9	19.3	29	Storebakken et al. (2004)
O. mykiss	0, 9, 18 and 27	63.5	27	43	Aas et al. (2006)
R. canadum	0, 19.5, 39, 58.5 and 78	54.4	39	72	Lunger et al. (2006)
R. canadum	0, 40 and 59	58.6	40	68	Lunger et al. (2007)
O. niloticus	0, 10.94, 21.88, 32.77, 43.67 and 49.90	49.82	49.9	50	Bob-Manuel and Alfred-Ockiya (2011)
C. carpio	0, 3.3, 6.6, 9.9, 13.2 and 16.5	25	9.9	40	Korkmaz and Cakirogullari (2011)
C. carpio	0, 6.06, 14.03, 19.67 and 46.03	47.45	19.67	42	Omar et al. (2012)
O. niloticus	0, 11.6, 23.2 and 34.8	34	23.2	50	Al-Hafedh and Alam (2013)
C. carpio	0, 5, 10, 15 and 20	24.2	20	41	Abdulrahman and Ameen (2014)
C. auratus	0, 8.87, 14.78, 20.71 and 26.63	40	27.71	34	Gumus et al. (2016)
D. labrax	0, 11, 21.9, 32.9 and 54.8	59.6	54.8	50	Oliva-Teles and Goncalves (2001)
S. salar	0, 10 and 20	69	20	29	Kiron et al. (2016)
M. rosenbergii	0, 6.25, 12.5, 18.75 and 25	25	12.5	50	Radhakrishnan et al. (2016)
M. rosenbergii	0, 6.25 and 12.5	25	6.25	25	Rajkumar et al. (2017)
P. vannamei	0, 2, 4, 6 and 8	20	4	20	Hamidoghli et al. (2019)
S. ocellatus	0, 7, 14, 21, 28 and 35	28	35	50	Perez-Velazquez et al. (2018)
Hybrid of *M. chrysops* and *M. saxatilis*	0, 7, 14, 21, 28 and 35	28	35	50	Perez-Velazquez et al. (2019)
L. calcarifer	0, 10, 20 and 30	30	20	66	Delamare-Deboutteville et al. (2019)

in *P. vannamei* while feeding with algal-based microbial product derived from *Spirulina platensis* (Hanel et al. 2007). A product of yeast species (*S. cerevisiae* and *Candida utilis*) could substitute dietary fishmeal by 30% in Koi carp (Korkmaz and Cakirogullari 2011), 50% in European sea bass (Oliva-Teles and Goncalves 2001) and carp (Omar et al. 2012), 40% in Atlantic salmon (Overland et al. 2013), and 50% in Nile tilapia (Al-Hafedh and Alam 2013).

Radhakrishnan et al. (2016) reported a maximum growth, survival, and nutritional indices in *M. rosenbergii* while supplementing algal-based meal obtained from *Arthrospira platensis* by substituting 50% dietary fishmeal. However, Abdulrahman and Ameen (2014) and Rajkumar et al. (2017) could substitute fishmeal by 10% in common carp using *Spirulina* and 25% in fresh water shrimp, *M. rosenbergii*- using *Turbinaria ornata*- and *Gracilaria corticata*-derived products. This difference would be due to the variation of nutritional composition of algal species used for the production of the respective microbial products. Growth and feed utilization were not retarded in Arctic charr fed on a diet substituting 40% fishmeal by the microbial product derived from *S. cerevisiae* and *Rhizopus oryzae* (Vidakovic et al. 2016). Recently, Perez-Velazquez et al. (2018) reported that an algal meal obtained from a combination of *Arthrospira* sp. and *Schizochytrium limacinum* showed no significant difference in feed efficiency in red drum after substituting 50% of dietary fishmeal and is in agreement with the findings of Perez-Velazquez et al. (2019) in striped bass fed the same algal meal as a fishmeal substitute. Tilapia fed with a diet containing a meal of *Schizochytrium* sp. by substituting 33% of dietary fishmeal had a higher weight gain and digestibility (Sarker et al. 2016). Hamidoghli et al. (2019) observed a comparable weight gain and feed utilization with a control group in *P. vannamei* reared with diets containing a microbial product obtained from the bacteria, *Corynebacterium ammoniagenes*, by substituting 20% of dietary fishmeal over the course of a 9-week feeding trial. However, Delamare-Debouteville et al. (2019) reported that about 66% of dietary fishmeal could be substituted without any major impact in Asian seabass when reared on a diet containing whole-cell biomass of mixed-culture purple phototrophic bacteria.

Microbial products as a growth promoter

In general, some complementary additives like hormones, antibiotics, ionophores, and certain salts have been used in aquaculture to promote growth and keep the animals healthy. Though these components showed some positive effects on aquatic species, their improper use can cause some adverse effects, which not only affects the cultured species but also the end user. In addition, using these components, in particular antibiotics, leads to pathogenic bacteria developing resistance. Therefore, a number of preventive measures have been explored; one among them is the usage of live microorganisms (probiotics). They should not only be non-pathogenic but also be well characterized both biochemically and physiologically before using them as a feed source. The usage of probiotics began in the early 1970s, and their beneficial effects have been well documented in animal husbandry, in particular cattle, pig, and poultry nutrition (Farzanfar 2006). Therefore, the concept of probiotic usage in aquaculture, in particular shrimp culture, had relatively more attention to create an environment-friendly culturing system. Lara-Flores et al. (2003) evaluated the ability of two different bacterial species (*Streptococcus. faecium* and *Lactobacillus. acidophilus*) and yeast (*S. cerevisiae*) in Nile tilapia. Their results revealed that though fish fed diets supplemented with yeast species exhibited a higher growth, and a combination of bacteria strains was more effective in stimulating the growth of the fish. The authors suggested that this could be due to the

ability of microbes in mitigating the effect of stress factors during culture. This result corroborates the findings of Bogut et al. (1998) in carp reared on *S. faecium*. Noh et al. (1994) reported that supplementation with yeast and bacterial meal yielded a better growth response than those fed with antibiotics. Chumpol et al. (2018) reported that *P. vannamei* reared with a diet supplemented with 1% of purple non-sulfur bacteria had significantly better growth and survival than those reared with the control diet (no bacterial supplementation) and commercial shrimp feed. However, supplementation at 3 and 5% level showed a lower growth, which could partly be attributed to significant reduction of lipid levels in the respective diets. Similarly, *P. monodon* exhibited higher growth and survival from the naupliar to post-larval stages when reared on 2% bacterial cells of *Rhodovulm sulfidophilum* and *Skeletone costatum* (Azad et al. 2002). Selvakumar et al. (2013) studied the efficacy of various strains of *Streptomyces* that had a protein content of > 55% in dry weight basis, in the diet of ornamental southern platyfish. Their results revealed that all the diets supplemented with *Streptomyces* sp. exhibited a higher growth rate compared with the control diet, which could be attributed to the higher protein content of *Streptomyces.* The positive effects of *Bacillus* sp. as a feed source have been reported earlier in a wide range of shrimp such as *P. indicus* (Ziaei-Nejad et al. 2006), *P. monodon* (Boonthai et al. 2011), *P. vannamei* (Zokaeifar et al. 2012), and *M. rosenbergii* (Kumar et al. 2013) and their results also corroborated the findings of Van-Hai and Fotedar (2009) and Adel et al. (2017), who used *Pseudomonas* sp. and *Pediococcus* sp. in the diet of *P. latisulcatus* and *P. vannamei*, respectively. Yanbo and Zirong (2006) documented that mixed probiotics showed better results compared with the individual one and who reported a higher growth rate in common carp while supplementing *Bacillus* sp. with a photosynthetic-bacteria rather supplementing them individually. Similarly, Boonthai et al. (2011) reported that a weight gain of *P. monodon* was increased to 1016.82% when supplementing five different *Bacillus* sp. such as *B. subtillis*, *B. licheniformis*, *B. polymyxa*, *B. megaterium*, and *B. pumilus*, while it was 890.09% with the control group fed no probiotics. The findings of Van-Hai and Fotedar (2009) showed that the SGR was 1.02 with *P. synxantha* and 1.01 with *P. aeruginosa* and was increased to 1.14 while supplementing both the *Pseudomonas* species together in *P. latisulcatus* after 28 days. These results are in agreement with *P. monodon* fed diet with both *Bacillus* sp. and *Enterococcus* sp. (Nimrat et al. 2013) and with *P. vannamei* fed *B. subtilis* and *B. licheniformis* together (Sadat Hoseini Madani et al. 2018). On the contrary, McIntosh et al. (2000) and Shariff et al. (2001) found no effect on growth in *P. vannamei* and *P. monodon*, respectively, when reared with some commercial probiotics, which might be due to the inefficiency of products (Boonthai et al. 2011) and inadequate numbers of microbes (Moriarty et al. 2005). Eshaghzadeh et al. (2015) suggested that the enhanced growth with the probiotics would be due to the increased activities of digestive enzymes, which in turn enhanced feed digestion and absorption. Moriarty (1998) stated that the supplemented probiotics, in particular bacteria, had the capability of secreting a wide range of exogenous enzymes. Though their contribution may be very low to the total enzymatic activity of the gut, they stimulate the secretion of indigenous digestive enzyme in the tract of the farmed species (Ziaei-Nejad et al. 2006). As a result of this increased enzyme activity, probiotics in turn influence the digestive process by increasing the microbial populations that had a beneficial effect to the host species (Bomba et al. 2002), which would enhance the intestinal microbial balance, thereby enhancing the food absorption and digestion and in turn helps to promote the growth. Xie et al. (2019) reported that besides increasing the digestive enzyme activity, supplemented *Bacillus* sp. increased the intestinal villi in *P. vannamei*, which is important for the digestion and absorption of food in the intestine. Similarly, enhanced growth with increased microvilli density and length was observed in tilapia reared on a probiotic-supplemented diet (Standen et al. 2015).

Effect of microbial products on digestive enzyme activity

Though there are reports in relation to the effect of microbial products on growth performance and immune response, very few studies focused on the nutritional effect of microbial products on digestive enzyme activity in aquatic species. Ziaei-Nejad et al. (2006) reported that the specific activity of protease, lipase, and amylase increased due to the supplementation of *Bacillus* sp. in *P. indicus*. This increased enzyme activity could be attributed to the microbial secretion of exogenous enzymes, which in turn contributes to better growth and survival by enhancing food absorption and digestion. Wang (2007) found a gradual increase in protease and amylase activity in the intestine of *P. vannamei* fed a basal diet supplemented with *Rhodobacter sphaeroides* and *B. coagulans* at varied levels of 0.1, 1, and 2%. Though no difference was observed in lipase and cellulose activity between the treatment groups, a significant higher value was noticed in the groups fed bacteria-supplemented diet compared with the control. Similar effects have been reported in tilapia when fed with *L. acidophilus*, *S. faecium*, and *S. cerevisiae* (Lara-Flores et al. 2003) and European sea bass (Tovar-Ramirez et al. 2004) reared with *S. cerevisiae*. Rajkumar et al. (2017) observed an elevated activity of digestive enzymes like protease, lipase, and amylase in *M. rosenbergii* fed *Turbinaria ornata*- and *Gracilaria corticata*-included diet than the control group. Protease is the most prevalent enzyme, mainly responsible for the digestion of dietary protein, and its activity was increased to 0.90–0.98 U/mg protein in the hepatopancreas of *P. monodon* fed *Bacillus* sp.-supplemented diets at the rate of 0.25 and 0.50%, while it was 0.75 U/mg protein in shrimp fed a control diet (Rajaram 2010). Similar results have been reported in *P. monodon* (Nimrat et al. 2013), in *M. rosenbergii* (Gupta et al. 2016), and in Nile tilapia (Lara-Flores et al. 2003). As a result of increased enzyme activity, probiotics in turn influence the digestive process by increasing the microbial populations that had a beneficial effect to the host species (Bomba et al. 2002). Rengpipat et al. (1998) documented that an appropriate selection and application of probiotics would enhance the intestinal microbial balance, thereby enhancing the digestion and absorption process of the cultured species and showed positive results on the growth of the farmed species.

Microbial products as an immunostimulant

The global production of aquaculture is increasing significantly over the years. However, this development has always been accompanied with various adverse impacts that occur due to several problems, mainly diseases that can be attributed to the overuse or misuse of antibiotics and expression of antibiotic resistance genes among opportunistic pathogens, mainly, *Vibrio* species. Due to the development of pathogen-resistant bacteria, the usage of antibiotics in aquaculture has been restricted or in certain cases banned globally (Andrews et al. 2011). The immune system of aquatic species, in particular crustaceans, is incapable of responding to the specific vaccines as it highly depends on the innate mechanisms. This scenario led to the use of live microorganisms (probiotics) as an immunostimulant in order to control infectious diseases in aquatic species, including fish and shrimp (Andrews et al. 2011). In general, the live microbes supplemented as probiotics stimulate both specific and non-specific immune system in the cultured species by promoting phagocytic and lysozyme activities. In addition, probiotics increase the expression of various cytokines that related to the immunity of the host species. Notwithstanding, probiotics enhance the immune system activity in the gut of fish along with increasing immunoglobulin cells and acidophilic granulocytes (Allameh et al.

2017). Agarwal et al. (2011) documented that the dietary administration of probiotics enhanced the natural immune function, which adheres transiently and colonizes the gastrointestinal tract that lead to increases in the antibody level. The positive effect of probiotics on immune responses had earlier been studied in various aquatic species (Thanardkit et al. 2002).

Sun et al. (2011) reported that the dietary administration of *Psychrobacter* sp. (bacteria) improved the immune response of grouper fish. A similar effect was reported in *P. vannamei* (Chiu et al. 2007) and rainbow trout (Perez-Sanchez et al. 2011) while using *Lactococcus garvieae.* A promising effect of *B. subtillis* on disease management could be noticed in shrimp aquaculture by Keysami et al. (2012). Korenblum et al. (2005) documented that *Bacillus* species had the capability to produce a variety of antimicrobial peptides and extracellular substances against various diseases. Rohu fed brewer's yeast, and *Spirulina* exhibited an increase in serum proteins, in particular albumin and globulin after challenging with *Aeromonas hydrophila*, compared with the control group (Andrews et al. 2011). This increase of the serum proteins is associated with a stronger innate response in fish. It was reported earlier that the phagocytic activity of channel catfish was found to be high when supplementing *Spirulina* in low dose (Duncan and Klesius 1996). However, Andrews et al. (2011) observed lesser immunostimulant activity with the higher dose of supplementation. This could partly be attributed to the suppression of the defense mechanisms of the fish due to a higher dose of probiotics and infers that the defense mechanism of the fish would be dose specific. Andrews et al. (2011) reported lower leucocyte count with an increased inclusion of commercially available yeast brewers and *Spirulina* as a dietary immunostimulant in rohu and also suggested that the fish with lower leucocyte count were more susceptible to disease. Hence, leucocyte count can be considered as an important factor to identify the health status of fish as it plays a major role in non-specific and innate immunity. This result is in agreement with the findings of Chumpol et al. (2018) in *P. vannamei* reared with a diet containing purple non-sulfur bacteria. Chiu et al. (2007) observed an enhanced resistance in *P. vannamei* against *V. alginolyticus* by upregulating prophenoloxidase activity when fed with bacterial (*L. plantarum*) supplemented diet. The impaired health status of shrimp that related to microbial infection causes lesions in the hepatopancreas (Chupani et al. 2016), and there were no structural changes in the tubule epithelial cells, in particular secretory (B-cells), absorptive (R-cells), brillar (F-cells), and embryonic (E-cells) cells of *P. vannamei* fed a mix of two bacterial strains (*R. sphaeroides* and *A. marina*) even at a higher inclusion level (Fig. 4). However, the number of B-cells was high in shrimp hepatopancreas reared with microbial product–supplemented feed compared with a group fed a commercial feed as a control. Wang et al. (2016) documented that the numbers of B-cells are directly proportional to the absorption and digestion of nutrients. As B-cells of hepatopancreas increase, the nutrient utilization of feed is also elevated thereby enhancing the growth of shrimp. Thus, the findings of Chumpol et al. (2018) clearly indicated that diets with probiotic supplementation are more effective in aquatic species, in particular shrimp. In general, the live microorganisms are widely used as an immunostimulant rather than the specific extracted matter, which not only reduces the cost of the production but also confers better protection against the microbial infection in aquatic species. Scholz et al. (1999) reported that *P. vannamei* fed diets with *Phaffia rhodozyma* (yeast) exhibited better health status than those fed diets containing glucan derived from yeast species of *S. cerevisiae*.

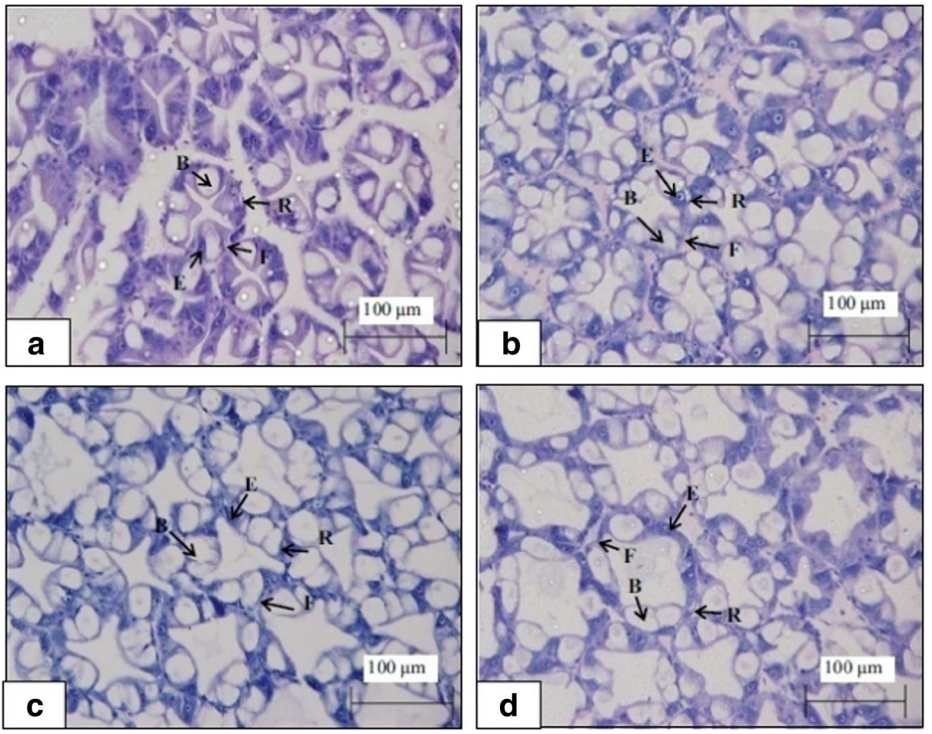

Fig. 4 Histological analysis of tubule epithelial cells of hepatopancreas of *Penaeus vannamei* fed diets supplemented with bacterial-based microbial product (Source: Chumpol et al. 2018)

Effect of microbial products on immune gene expression

Recent studies have demonstrated that dietary inclusion of live microorganisms plays an important role in regulating certain genes, which are related to the immune response of the aquatic species. In general, hemocytes play an important role in cellular defense mechanism, which enhance the innate immune response of the aquatic species, when exposed to the pathogens, by producing humoral defense molecules. However, it is too difficult to collect hemocytes from the larvae, post larvae, and juvenile shrimp to analyze these parameters. Hence, the respective immune-related genes are investigated by the researchers using the real-time PCR. Prophenoloxidase-activating system secretes inactive form of phenoloxidase (prophenoloxidase) and is converted into the active form (phenoloxidase) by serine protein, which in turn enhances the resistance of the aquatic species against the pathogen by recognizing and responding to them. Peroxinectin increases various biological activities that are related to the immune response of the aquatic species such as cell adhesion, opsonin, degranulation, proxidase, and encapsulation, thereby enhancing the defense mechanism of the host species. As like phenoloxidase, lipopolysaccharide and β-1, 3 glucon-binding protein also play a role in recognizing and responding to the pathogen, but it plays a crucial role during early stage. All these parameters are directly and/or indirectly related to the immune response of the aquatic species, and their activities are increased due to the dietary administration of live microorganisms (probiotics). Zokaeifar et al. (2012) studied the expression of prophenoloxidase (proPO), peroxinectin (PE), lipopolysaccharide and β-1, 3 glucon-binding

protein (LPBP), and serine protein (SP) genes in *P. vannamei* fed with diets containing high (10^8 cfu/g) and low (10^5 cfu/g) dose of *B. subtillis* compared with a control diet (no bacterial supplementation). Their results revealed that all the four genes were upregulated in all three groups, including control. However, the expression ratio was comparatively higher in *P. vannamei* fed a diet containing 10^8 cfu/g of bacteria followed by a diet with 10^5 cfu/g of bacteria than the control diet (Fig. 5). Upregulation of proPO gene was also found in *P. vannamei* fed diet supplemented with *L. plantarum* (Chiu et al. 2007), which had an enhanced resistance against *V. harveyi*. Johansson et al. (1995) observed an elevated activity of degranulation, peroxidase, and encapsulation in crayfish due to the upregulation of PE gene. Zokaeifar et al. (2012) reported that SP gene always has a similar pattern as in proPO gene in expression. This could possibly be due to the necessity of serine protein in converting prophenoloxidase to phenoloxidase. However, a different pattern of SP gene expression was noticed by Liu et al. (2010) in *P. monodon* when reared with *B. subtillis*-supplemented feeds. Similarly, tumor necrosis factor (TNF)-α plays a major role in regulating inflammation in early stage of the fish. This mediates powerful antimicrobial responses, including killing infected cells, inhibiting intracellular pathogens, and upregulating diverse host response. Likewise, interleukins (ILs) are a subgroup of cytokines, involved in the intracellular regulation of the immune system. The probiotic potential of fungus *Aspergillus oryzae* was tested at two different concentrations (1×10^6 and 1×10^8 CFU/g) in Nile tilapia by Dawood et al. (2020). The results showed an upregulation of relative messenger RNA of certain genes related to immune response (TNF-α, IL-1β, and HSP70) in fish fed fungus-incorporated diets irrespective of the levels in both before and after hypoxic stress condition. These results are consistent with Niu et al. (2019), who evaluated the effect of multistrain probiotics such as *B. licheniformis*, *B. amyloliquefaciens*, *B. subtilis*, *L. brevis*, *L. plantrarum*, and *S. cerevisiae* in olive flounder. The expression of immune-related genes, including IL-1β, IL-6 and TNF-α, was markedly upregulated in fish fed a low fishmeal (45.5%) diet supplemented with multi strains compared with those fed a control diet containing 65% fishmeal. These results corroborated with the findings of Hosseini et al. (2016) in rainbow trout fed on

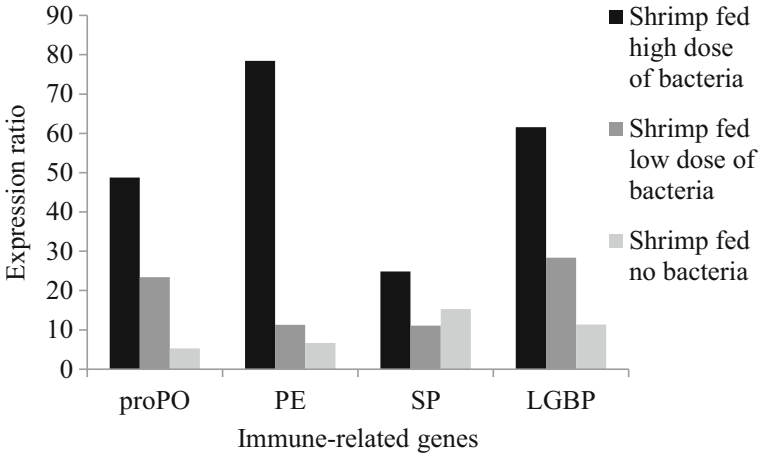

Fig. 5 Relative gene expression of various immune-related genes of *Penaeus vannamei* fed diets with varied level of bacterial-based microbial product (*B. subtillis*) after 55 days challenged with *Vibrio harveyi.* There was a significant ($P < 0.05$) difference among the treatments for all the genes, and their P value was $P = 0.019$, 0.011, 0.025 and 0.013 for proPO, PE, SP, and LGBP genes (Source: Zokaeifar et al. 2012)

lactic acid bacteria. But in contrast, no effect was observed in TNF-α, IL-8 genes by Jami et al. (2019) in trout fed *L. plantarum*, along with certain prebiotics (mannan oligosaccharides and β-glucon). However, information is scanty when fed with algae and yeast species.

Effect of microbial products on carcass composition

Carcass composition of fish fed diets containing microbial products derived from various microorganisms had been reported earlier. The dietary change is due to the supplementation of a microbial product derived from yeast (*C. utilis*) in tilapia (Olvera-Novoa et al. 2002) and commercial yeast-based product (NuPro) in rainbow trout. However, higher carcass protein was observed in European sea bass fed on *S. cerevisiae* (Oliva-Teles and Goncalves 2001) and in rainbow trout fed on a commercial yeast-based product (NuPro) (Hunt et al. 2014). This could be due to the effective utilization of protein from the feed by the respective farmed animals, since carcass protein is clearly related to dietary protein (Lara-Flores et al. 2003). In addition, the protein content of yeast species was from 29 to 56% dry weight basis (Table 3) and would also be a reason for variation in the results among the studies. Hardy et al. (2018) had found increased nitrogen retention with increasing *Methylobacterium extorquens* (bacteria) level in the diet of rainbow trout. The authors suggested that this improved retention could be attributed to better efficient metabolism of host species due to bacterial supplementation, which resulted in lowering catabolic losses. The contribution of non-protein nitrogen, due to the presence of nucleic acid in microbial products, increases the dietary protein, which might also be a reason to influence the carcass protein in fish. Among the microorganisms, the nucleic acid content was predominant in bacterial species and was in the range of 15–16%, while it was 4–6% in algae, around 10% in fungi, and 7.1–12% in yeast (Table 3). Tilapia fish fed diets containing microbial products obtained from the yeast species showed higher body lipid than those fed a control diet (Bob-Manuel and Alfred-Ockiya 2011). This could be due to the deamination of protein by the fish, in which the nitrogenous compound, in particular protein, is eliminated as a by-product such as ammonia, and the non-nitrogenous or carbonaceous portion is deposited as lipid. Bob-Manuel and Alfred-Ockiya (2011) stated that this phenomenon mainly occurred prior to breeding, as fish solely relies on the deposited fat by stopping feeding especially in the maternal mouth-brooders such as Nile tilapia. However, in contrast, low body lipid was found in carp fed on *Spirulina*-supplemented diets. Decreased body moisture and ash content is the good indices of fish growth. This is in agreement with the findings of Bob-Manuel and Alfred-Ockiya (2011) in tilapia. Lara-Flores et al. (2003) observed no significant difference in carcass moisture in tilapia fed on *S. faecium*, *L. acidophilus*, and *S. cerevisiae* diets, while other proximate principles were influenced by dietary treatments. Perez-Velazquez et al. (2018) observed an increased proportion of docosahexaenoic acid and palmitic acid in red drum fed algal meal obtained from *S. limacinum* compared with the control, whereas linolenic, linoleic, and oleic acid levels were found to be low. Whole-body composition of proximate and essential amino acids was not affected due to the supplementation of *Bacillus* sp. in *P. monodon* (Rajaram 2010), and this result is corroborated with the findings of Adel et al. (2017), who reported that there was no significant difference in whole-body composition of moisture, crude protein, crude lipid, and total ash content in *P. vannamei* fed diets with *P. pentosaceus* at various concentrations (1×10^6, 1×10^7, and 1×10^8 CFU/g). But this finding is inconsistent in *P. vannamei* fed a mix of *B. subtilis* and *B. licheniformis* (Sadat Hoseini Madani et al. 2018) and who found an increased crude protein, dry matter, and total ash. This is amply clear from the available studies that there is no possibility to establish a relation between the body composition of the aquatic species and microbial products that are used as a potential protein/feed source.

Conclusion

It can therefore be inferred and concluded from this review that the microbes of algae, bacteria, fungi, and yeast can be considered as suitable feed ingredients to be used as potential feed sources in the diet of aquatic species. As a result of the inclusion of microbial products, it is possible to substitute nearly 50% of dietary fishmeal without having any deleterious effects on the growth of the cultured species. Notwithstanding, microbial products, in particular live microorganisms, would act as a viable immunostimulant by upregulating various immune-related genes and as a growth promoter by enhancing the digestibility mechanisms. The extensive use of value-added microbial products could definitely ameliorate the issues that are related with the global fishmeal availability by bridging the gap between demand and supply in the future. Some of these microbial products can be considered as functional ingredients as their beneficial roles are beyond the nutrient composition. However, despite all the benefits, the utilization of microbial products is not getting adequate importance, which is mainly due to the high production cost, high nucleic acid content, non-digestible cell wall, contamination risk, unacceptable color, and flavor. However, the recent advances in fermentation technology and genetic engineering could pave the way to re-evaluate microbial products by overcoming the limitations and making this substrate as an economical resource for the aquafeed industry at commercial scale in the future.

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Authors' contributions Dr Jannathulla R—written rough draft of the manuscript for the effect of microbial products in aquafeed.

Mrs Sravanthi—written rough draft of the manuscript for production and market potential of microbial products.

Dr Moomeen S—written rough draft of the manuscript for the nutritional value of microbial products.

Dr Gopikrishna G—drafted the manuscript (Fair draft).

Dr Dayal SJ—study design, data collection and over all coordination.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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Consent to participate All the authors agree to participate in this research.

Consent to publication The authors grant to the publisher the sole and exclusive license of the full copyright in the contribution.

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