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Role of Probiotics in Culture of Carps with reference to Rohu, *Labeo rohita* (Hamilton)

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Authors' contributions

This work was carried out in collaboration between both authors. Author MS designed and prepared the manuscript. Author AP reviewed and edited the manuscript for publication. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The demand for farmed carp species has increased significantly over the last decade due to their high market value. In culture of omnivorous species such as Rohu, *Labeo rohita* (*L. rohita*), intestine, gonads, culture water, sediments and even commercial products can be a source of appropriate probiotic. Probiotics technique is a promising statergy for improving aquaculture performance and it is known to have many health benefits including the ability to modulate growth responses and immune system on positive side. Also encapsulating probiotics to live feed is an efficient method to deliver probiotics to aquatic animals. Probiotics can be applied as a single strain or a combination of strains. The comprehensive literature search highlights the significant positive role of probiotics in culture of *L. rohita.* It inferred that, use of probiotics enhanced growth

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performance, survival (SUR), protein efficiency ratio (PER), amylase activity (AMY), total serum protein (TP), hemocrit, specific and non specific immune parameters. Furthermore, dietary administration of probiotics also accelerated heat shock protein (MSP) and gene expression in *L .rohita.* These findings encourage the use of probiotics as a valuable tool in optimizing the production of *L. rohita* in culture system. Moreover, dosage and duration of probiotics and age of the host species are the significant factors in achieving desired result. This review article aims to present research findings on the use of probiotics in farming of *L. rohita*. Furthermore, this study attempts to fill the gaps in existing knowledge for the merit of further investigation and developments.

Keywords: Labeo rohita; probiotics; growth performance; immune response; disease control.

1. INTRODUCTION

Aquaculture has become one of the most promising and fast increasing agribusiness sector which provides high-quality animal protein to an ever expanding global population. There has been a change in aquaculture practices, moving from large scale system towards the intensive, super-intensive and semi intensive system. As intensive culture expands, diseases emerge most commonly [1]. Therefore, the use of antibiotics and chemotherpeutics was encouraged to combat the disease; which in turn caused the emergence of drug-resistant pathogens and suppressed immune response of host species, apart from causing environmental risks and food safety problems [2,3,4]. In addition to therapeutic drugs and vaccines, an effective alternate approach to strengthen immune system is by administering probiotics, a special group of microbe used either as feed additives or as nutriceutical in carp species are encouraged [5,6]. In India, *Labeo rohita*, is one of the major freshwater fish farmed by aquaculture, which is the major source of proteins and beneficial lipids [7-10]. Despite the rising demand for this species, the major obstacles faced by the farmers are: i) combacting disease outbreak with ecofriendly feed additives and ii) obtaining maximum targeted growth rate [11]. *Labeo rohita* or Rohu, is a carp belongs to the order Cypriniformes, predominantly occurs in freshwater and rarely found in brackish water. Rohu is a column feeder and an ideal species for aquaculture industry due to its characteristics such as quick growth, compatibility with other fishes, acceptance of artificial feed, good flesh quality, and consumer preference made this species desirable for the aquaculture sector [12].

Furthermore, probiotics are also a live microbial dietry supplements, that effectively improves the microbial balance in the host system to have a positive impact [13]. Food and Agriculture Organization (FAO) and World Health Organization (WHO) reported, probiotics are live micro-organisms that, when given in an appropriate amount provide overall health benefit to the host [14]. At present, probiotics are quite preferable as an eco-friendly approach to control disease [15] and offers a potential replacement for the use of antibiotics and pesticides in aquatic animals [5]. Various factors, such as probiotic dosage, source, and duration of administration can influence the beneficial activities of probiotics including immune modulation. It is essential that an effective administration method contribute to create suitable conditions for probiotics to function effectively [16]. Knowing the mechanism of action together with right application techniques may be the most important factors for using probiotics in aquaculture system. However, probiotics exert significant influence on fish species, it frequently exhibits host-specific and strain-specific variation in their activity [17,18,19]. Therefore, it has been suggested to replace conventional biological control agents with pathogen-inhibitory gut microorganisms found in probiotics [20]. Although, there have been numerous studies on probiotics in fish and shellfish farming worldwide, there were very few articles on probiotics in *Labeo* rohita. As Rohu culture farming shifts from traditional to intensive ways, a number of contemporary technologies, modern perspectives and various techniques, including probiotic or prebiotic have been used to increase the quantity and quality of this species [5,21]. This study tends to review probiotic research findings with regard to omnivorous species of *Labeo rohita*, whereby a critical evaluation of their efficiency for the host is offered from a screening of possible probiotics. Additionally, several ideas from the research findings are noted for further exploration and development, as the use of probiotic can be a practical and sustainable method for successful aquaculture.

Table 1. Comparative analysis on the efficiency of probiotics on physiology, biochemistry and immune modulation of *Labeo rohita*

Probiotic bacterium	Mode of Administration and Dosage Duration	Beneficial Effects	Reference/ Sources
B. subtilis	0.5 × 10^{-7} , 1 × 10^{-7} , 1.5 × 10^{-7} cfu/g for 2 weeks	RB; SB; D.R (↑)	[22]
Bacillus subtilis VSG1, *Lactobacillus plantarum* and *Pseudomonas aeruginosa* VSG2	0.33, 0.5 and 1 × 10^{8} cfu/g for 60 days	GP,W.G and SG(↑), FP FC(↓): I.P S.L; ACp; PA; RB; SOP and $Serum_{IgM}$(↑): ID AH↑	[23]
Bacillus subtilis	0.5 × 10^{-7}, 1.0 × 10^{-7} , 1.5 × 10^{-7} cfu/g for 2 weeks	SUR;W.G; LEV; Hb; TP; GBL↑ AKP; AAT; AAMT (↔)	[24]
Bacillus substilis, L. lactis and *Saccharomyces cerevisiae*	Sixty days	W.G;SR; FC; PE; DMDM; PDC; LDC; APA; LP; ACCCP; ACCL; THBC(↑)	[25]
Bacillus subtilis, Lactobacillus lactis and *Saccharomyces cerevisiae*	10^{-11}cfu/kg for 30 days	W.G; RB; GUL; TP; ALL; AKP ACp; MPD, HSP_{70}, IMP (↑)	[6]
Bacillus subtilis	10^{8} cfu/g — 60 days	TP; GLV; ABL; D.R (↑)	[26]
Lactobacillus plantarum VSG3	10^{-6}, 10^{-8} , 10^{-10} cfu/g for 60 days	SG; FC; LZA; ACp; PGC; RB; SOP; $Serum_{IgM}$; D.R (↑)	[27]
Bacillus amyloliquefaciens CCF7	10^{5} , 10^{7} & 10^{9} cfu/g — 70 & 28 days	HMB GLV, ALL, and TP(↑) & AST and ALT(↓), IP SOP, SL, CTL and $serum_{IgM}$↑; and MDA↓; IDR A.H↑	[28]
Bacillus circulans	1.5 x 10^{4} , 1.5 x 10^{-5} , 1.5 x 10^{-6}, 1.5 x 10^{7} and 1.5 x 10^{8} per 100 g of feed — 60 days	FNW, WG, SG, PER, FC, CPC(carcass protein content) , CLC(Carcass lipid content), PA, AMY (↑)	[29]
S. cerevisiae	0.5, 0.75 and 1% for 60 days	G.P W.G(↑); FUM PER(↑) & FC(↓) , HMB Hb, RBC, WBC, AAMT and AAT(↑), DEZ PA & AMA(↑), IDR A.H↑	[5]
B. amyloliquefaciens COFCAU_P1	10^{-7} , 10^{-8} and 10^{-9} cfu/g for 30 days	HMB ALL, GLV & TP (↑), I.P MPD, SDA and antiprotease(↑); IDR A. H(↑), TNF-α and IL-1β↑; SUR↑	[30]
G. candidum	10^{9} cfu/L for 70 days	GP W.G & SG(↑), DEZ PA, AMA and cellulose(↑), IDR *Staphylococcus aureus(*↑) SUR↑	[31]
Geotrichum candidum QAUGC01 & *bacillus cereus*	10^{9} cfu/g— 11 weeks	GP FNW and SG (↑), DEZ PA, AMA and Cellulase (↑); IDR A. H (↑), GTM(↑); SUR↑	[32]
Enterococcus faecium QAUEF01 + *Geotrichum candidum* QAUGC01	10^{9} cfu/g - 90 days	GP W.G and SG(↑), FUM FCE (↑) & FC(↓), HMB RBC, WBC, Hb, and Lymphocytes (↑), DZE PA & Cellulase(↑), GTM↑	[33]
Bacillus amyloliquefaciens BN06, Bacillus subtilis WN07 & *Bacillus meegaterium CT03*	10^{6} cfu/g , 10^{9} cfu/g feed for 45 days	W.G, SG, FCE, FC, PE, FI, RBC, WBC, MCV, Hb, MCH, SOP, PA, AMY(↑)	[34]
B. aerophilus KADR3	10^{-7}, 10^{-8}, 10^{9} cfu/g for 3& 6 weeks	LZA, NBT, PGC, IgM, ACp, SOP, TP (↑)	[35]
B. methylotrophicus, B. amyloliquefaciens, & *B.*	10^{7} cfu/g for	GP W.G and SG(↑), FUM PE(↑) & FC↓;HMB Hb, RBC, WBC,	[36]

Probiotic bacterium	Mode of Administration and Dosage Duration	Beneficial Effects	Reference/ Sources
licheniformis	60 days	TP, platelets, GU, AAT, and AAMT(↑), I.P S.L, ACp, PGC, RB, $Serum_{IgM}$, MPD and SAA(↑), DZE PA, AMA and Lipase (↑), IDR A.H↑	
Pseudomonas aeruginosa VSG-2	10^{-5}, 10^{-7} & 10^{-9} cfu/g for 60 days	LZA, ACp, RB, PGC, $Serum_{IgM}$, SOP, LD_{50} (↑)	[37]
Geotrichum candidum QAUGC01	10^{9} CFU g^{-1} — 11 weeks	FNW, W.G, final biomass, SG, SUR, PA, AMY, RBC, WBC, Hb, HCT, MCV, MCH, MCHC HSP 70 gene expression (↑)	[38]
Lactobacillus spp., S.faecium, Bacillus bifidum, Bacillus subtilis, Sacchromyces spp., *Torulopsis* and *Aspergillus oryzae*	1 and 1.5 g /Kg for 30 days	GP FNW & SG, SUR(↑)	[21]

Note:

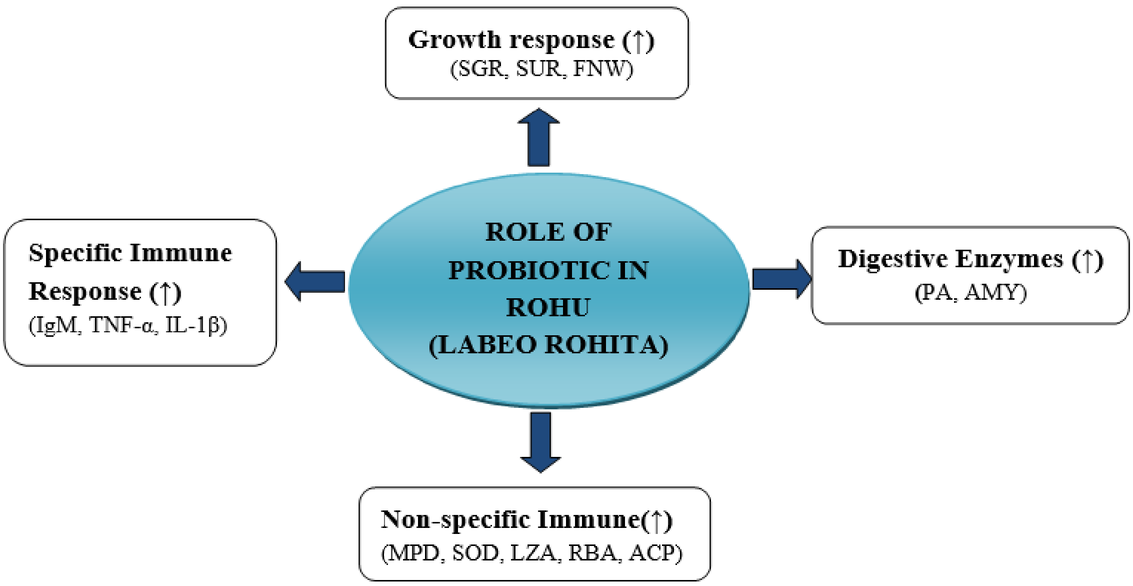
1. *Variation between the treated fish and the control: (↑) Significantly increase: (↓) Significantly decrease & no significant changes (↔).*
2. *CFU: Colony forming unit.*
3. *FC: Feed Conversion ratio, PE: Protein Efficiency ratio, FNW: final weight, SG: Specific Growth, ABL: antibody level, W.G: Weight Gain, GP: Growth performance, WBC: White Blood Cell, RBC: Red Blood Cell, Hb: Hemoglobin, HSP70: heat shock protein, IMP: immunoglobulin production, PGC: phagocytosis, IgM: serum IgM, D.R: Disease Resistance, LZA: Lysozyme Activity, APA: Protease Activity, AMY: Amylase Activity, SUR: survival, TP: Total Serum Protein, RB: Respiratory Brust Activity, SOP: superoxide anion production, GTM: Gut microbiota, SA: Serum bactericidal activity, AKP: Alkaline Phosphatase, AAMT: Alanine Amino transferase, AAT: Aspartate Amino transferase, AMA: Amylase, CTL: Catalase Activity, GLV: Globulin level, LEV: Leucrit value, DMDM: Drymatter digestibility, PDC: Protein digestibility, LDC: Lipid digestibility, LP: Lipase Activity, ACCCP: Carcass composition crude protein, ACCL: Carcass Composition Lipid, THBC: Total heterotrophic bacteria, GU: Glucose, ALL: Albumin level, MPD: Myeloperoxidase, GP: Growth parameters, FUM: Feed utilization parameters, HMP: Haemato biochemical parameters, DEZ: Digestive enzymes, IDR: Infectious Disease Resistance, SDA; Superoxide anion, IRGE: Immune Related Gene Expression, TNF: Tumor necrosis factor, IL: Interleukin, WG: Weight gain, FCE: Feed conversion efficiency, MCW: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, NBT: Nitroblue tetrazolium, I.P: Immunological parameters, S.L: Serum lysozyme, ACp: Alternate complementary pathway, SAA: Serum antiprotease activity, LD_{50}: Lethal dose, MCHC: Mean corpuscular haemoglobin concentration.*

2. PROBIOTICS APPLICATION IN ROHU FARMING

Earlier investigations have examined the impact of probiotics on growth performance, physiology, and over all health of carps [39,37]. Recently, a wide variety of bacteria and yeast such as *Lactobacillus, Bacillus, Leuconostoc, Aeromonas, Lactococcus, Carnobacterium, Vibrio, Enterococcus, Shewanella, Clostridium, Pseudomonas, Enterobacter* and *Saccharomyces* (Table 1) have been used as probiotic in aquaculture [40]. These micro organisms are frequently found in the gastrointestinal system of the host species [41]. Similarly, potential probiotics used in the rohu cultivation are obtained from the intestine of rohu and its culture systems. Probiotics have been extensively and effectively used in human medicine and veterinary medicine. They comprises yeast *Saccharomyces sp.* and the putative *Lactobacillus spp.* [13]. In the similar way, lactic acid bacteria (LAB) are also frequently utilized in rohu culture [27]. Among the probiotic species used in aquaculture, *Bacillus spp*. is probably the most frequently used probiotic to improve proliferation capacity, nonspecific immune response, and disease resistance whereas *B. subtilis* is frequently used [22,25,24,26,42]. *Bacillus aerophilus* KADR3 [35] *Bacillus circulans* [29] are also used as a probiotic for rohu culture.

Earlier reports on the impact of probiotics on *L. rohita* were inferred that, few of them acts as a growth promoters, while others have no or shown minor and slight impacts on the host (Table 1). Therefore, the specific species of the target host must be considered when using the probiotics. For example, supplementation with *Bacillus subtilis* enhance the growth performances and haematological parameters (haemoglobin, total serum protein, globulin), but not in serum chemistry (aspartate amino transferase and alanine aminotransferase). *L. plantarum* VSG3 showed beneficial impact on Indian major carp, *L. rohita* in enhancing immune response, growth performance and disease resistant [27]. After following 11-week probiotic feeding regimen containing either with *G. candidum QAUGC01* or *B. cereus*, there have been increase in mortality rate, weight gain, health, immunity and disease resistance [32]. Yeast, *Saccharomyces cerevisiae* shown an improved growth performance, innate immunity and gut microbiota of rohu [5]. At different culture temperatures (28, 31, 34, and 37℃), the yeast *S. cerevisiae* combined with *B. subtilis* and *L. lactis* decreased the level of apoptosis, increased the transcription of HSP70, and positively altered haemato biochemistry and nonspecific immunity compared to that of control [6].

3. PROBIOTIC BENEFITS AND ITS ROLE OF ACTION IN ROHU FARMING



3.1 Adhesive Capacity and Colonization

Adhesion to intestinal mucosa is considered as a prerequisite for colonisation and it is essential for positive interactions between probiotic strains and the host [43]. IECs (Intestinal Epithelial Cells) secrete mucin, the primary component of mucous and a complex glycoprotein mixture that inhibits the adhesion of harmful microorganisms [44]. Additionally, mucous gel contain lipids, free proteins, immunoglobulins and salt. This particular interaction has suggested a potential connection between the probiotic bacteria surface proteins and the competitive exclusion of pathogens from the mucus [45].

As with similar benefits of other immunostimulants in aquaculture, probiotics can enhance health status, growth performance, increase disease resistance and body composition, decrease malformations, improve intestinal morphology and microbiome [46]. Assessing both the gut microflora and the host, the epibiotic microbial community has developed into an integral part of the host and may have impact on the host biology [47]. Among its many crucial role, the gut-microbiota may transform feed ingredients into microbial biomass and fermentation end products that the host can be utilized [48]. The health of the host may also be significantly impacted by the gut flora [25]. In addition to probiotics, the opportunistic bacterial pathogens also invades the intestinal flora [49]. Mohapatra et al. [25] showed that the addition of *L. lactis, B. subtilis* and *Saccharomyces cerevisiae* had significantly improved the feed conversion rate, weight gain, specific growth ratio and protein efficiency ratio of major carp, *L. rohita* fingerling after two months of consuming on diet containing 10^{11} CFU kg−1 feed. Furthermore, there is limited information available with regard to other contribution to the digestive system [26].

3.2 Probiotic on Immune Response

One of the main action of probiotics is the control of host’s immune responses through innate and adaptive immune system. Adaptive immune response depends on B and T lymphocytes, because they attach to a particular antigens. In contrast, the immune system reacts to typical structure called pathogen-associated molecular pattern (PAMP), that are common to most of the pathogens [50]. The primary response to pathogens is produced by pattern recognition receptors (PRR), that bind to PAMP. Consequently, pattern recognition receptors comprise TLR (Toll like receptor), which are transmembrane proteins that are expressed on a variety of immune and non-immune cells, such as B cells, natural killer cells, dendritic cells, epithelial cells, fibroblast cells, macrophages and endothelial cells.

Probiotic, *B. licheniformis* KADR5 and *B. pumilus* KADR6 have been demonstrated to augment innate immune responses with enhancing phagocytic activity upon challenge with fish pathogen, *Aeromonas hydrophila* [51]. Also, Dharmaraj Ramesh et al. [35] administered dietary strains of *Bacillus aerophilus KADR3* for 6 weeks @ 10^9 CFU g−1, reported enhanced respiratory burst activity, lysozyme activity and phagocytotic activity in probiotic fed fish groups compared to that of control groups. The activity of serum IgM, alternate complementary pathway and superoxide anion production are the important parameters evaluated when assesing the impact of probiotics on the innate immune response of fish. A dietry supplementation with *Bacillus subtilis* improves innate immune parameters of *L. rohita* [18]. Respiratory burst activity, disease resistance, lysozyme, alternative complement pathway, phagocytosis were enhanced in Rohu, supplemented with a probiotics of *L. plantarum* VSG3 diet for 60 days [44]. In an another investigation by Khan et. al. [30], an isolated strain of *B. amyloliquefaciens* COFCAU P1 were fed for 30 days at a density of 10^{9} cfu g−1 diet, this strain was obtained from the intestine of rohu. Further, they stated that rohu fed probiotic supplemented diet displayed increased gene expression of IL-1b (interleukin 1 beta) and TNF-α (tumour necrosis factor alpha). Such results are suggestive to an enhancement of non specific immunity and the regulatory mechanism underlying mucosal tolerance (Table 1).

Probiotic strains were also found to stimulate the release of defenins from epithelial cells. These tiny proteins or peptides have strong antibacterial, antifungal and antiviral activity. Additionaly, these tiny peptides or proteins maintain the function of the intestinal barrier [52]. Observations have indicated that the host activates its first line of chemical defence in response to an attack by pathogenic bacteria by producing more antimicrobial proteins (AMP), including alpha and beta-defensins, C-type lectins, cathelicidins and ribonucleases. Numerous AMP are enzymes that destroy bacteria by attacking their cell wall enzymatically or by disturbing their membranes non-enzymatically.

3.3 Probiotic Influence on Digestive Enzyme Activity

The digestive system of the fish are extremely sensitive to the composition of diet and thereby cause abrupt alterations in the digestive enzyme activity [53,11], which in turn affects the health and well-being of the fish. The enzymes released by probionts help to increase the digestive utilization of feed or in detoxifying toxic compounds released by the pathogenic microflora. However, the alterations in microbial metabolism are influenced by either elevated or lowered enzyme activity. The primary enzymes involved in digesting carbohydrate and fats are amylase and lipase, respectively. The beneficial effect of probiotic supplemented with a combination of *Bacillus subtilis, Lactococcus lactis* and *Saccharomyces cerevisiae* for 30 days, reported an increased level of digestive enzymes (proteolytic, amylolytic and lipolytic) activities in *L. rohita* [25]. Ghosh et al. [54] openined that an extracellular enzyme enhance better growth and survival of Rohu. Improved protease, amylase and cellulose activites were noticed after adding *G. candidum* QAUGC01 to culture water [31] of rohu fingerlings. Supplementation with *Geothichum candidum* QAUGC0, *Bacillus cereus* and *Geothichum candidum* QAUGC01 + *Bacillus cereus* @ 10^9 cfu/g enhanced gut digestive enzymes [32]. Furthermore, study by Saravanan et al., [34] also found that dietry supplementation of combination strains at a density of 10^6 and 10^9 cfu/g for 45 days, improved amylase and protease activities when compare to that of control group.

3.4 Probiotic on Vigor and Disease Resistance

Carp species continue to be vulnerable to various bacterial and viral diseases as well the stresses associated with husbandry, including transportation and handling which is significant at various phases of production and growth. A diet supplemented with *Lactobacillus plantarum* improved several immunological responses in the freshwater tropical fish, *Labeo rohita* when challenged with *Aeromonas hydrophila* [27]. After being exposed with *Aeromonas hydrophila*, cumulative mortality was reduced in *L. rohita* when supplemented with a diet having probiotic mixture of *B. subtilis* VSG1, *Pseudomonas aeruginosa* VSG2 and *Lactobacillus plantarum* VSG3 [23]. Supplementing with *Bacillus methylotrophicus, Bacillus licheniformis* and *Bacillus amyloliquefaciens* either individually or in polyculture improved immune, digestive enzyme activities and significantly reduced the risk of infections [36]. Probiotic *B. amyloliquefaciens* COFCAU_P1 turn as a promising candidate species for reducing *Aeromonas hydrophila* infection against *L. rohita* [30]. The duration of the probiotic feeding before the challenge is an another factor to take into account in disease challenge investigations. The impact of feeding frequency on probiotic effectiveness remains scarcely studied in all cultivable species including *L. rohita.*

3.5 Probiotics on Growth and Survival Rates

Probiotic increase appetite and leads to promote growth and improve feed conversion ratio [41]. After introducing *Geothichum candidum* QAUGC01 to the rearing water, Rohu fingerlings showed improved activity of protease, amylase and cellulase leading to better growth [31]. The good growth rates, feed intake and utilisation of feed were registered in *L.rohita* supplemented with a mixture of three probiotics [25]. Probiotic *Lactobacillus plantarum* improved the growth performance of *L. rohita* [27]. Similar parameter modification with accelerated growth rate were noticed after supplementation of *Enterococcus faecium* QAUEF01 and its mixture with *Geotrichum candidum* QAUGC01 [33]. Similarly, only *Bacillus subtilis* increased the growth performance, intestinal microbiota and survival rates of rohu [24]. Yeast, *Saccharomyces cerevisiae* enhanced a best growth performance, protein utilization efficiency ratio, feed efficiency ratio and increased digestive enzyme activities of *L. rohita* [5].

4. METHODS OF PROBIOTICS ADMINISTRATION

Probiotics can be administered singly or in polyculture [23,33,25]. Likewise, single supplementation of either *Bacillus methylotrophicus* or *Bacillus amyloliquefaciens* and or *Bacillus licheniformis* through feed, protected Rohu with increased growth performances and immunity [36]. Additionally, probiotics based on polyculture are more effective than those based on a single strain [24,31]. Multiple probiotic supplementation in fish species reared in outdoor tanks results in greater survival of hatchlings and fingerlings at 8 and 38 days respectively. However, after 68 days such changes were no longer noticeable [21].

Probiotics have been practiced in Rohu culture at a various level of dosage, of which combining with the diet at 10^7,10^8 & 10^9 CFU/g is the most popular method of delivering probiotics to the host species. Dietary supplementation of *Bacillus subtilis* @ 10^8 CFUg−1, improved antioxidant and disease resistance in rohu. Probiotic are administered directly into the culture water as water additives at 10^9 cfu/L were treated with *Geotrichum candidum* QAUGC01 for 70 days against *Staphylococcus aureus* [55]. Yet, dosage and frequency of probiotic application was found to be influenced various intrensic and extrensic factors, that need to be investigated indepth.

5. SUMMARY AND CONCLUSION

Aquatic probiotics are often found among the micro-organisms that live in the gastrointestinal tract of healthy aquatic animals, and it has been discovered that their existence confirms beneficial improvements including survival, greater growth and increased immunity. However, the idea of encouraging better health by changing the structure of the gut-microbiome's population has gained popular, the underlying mechanism are still remain unclear. Through a variety of poorly known mechanisms, probiotic organisms reduce the possibility that culture species may be harmed or killed by pathogens. A more in-depth study of these mechanisms is highly warrented. The interaction between probiotic species and the intestinal cells of host species need advanced investigation and additional analysis. Futher more, preparation of feed acts as an crucial role in creating a favourable environment for probiotic and prebiotic to function effectively on the host species. Probiotic may be supplemented as food additive or water additive, and their application can be applied during long-term cultivation periods. Hence, it is essential to understand the ideal settings (Temperature and Time duration) for feed storage and preparation is crucial because they affect the effectiveness of probiotics when admixed as additive.

Rohu, an omnivorous fish, associate with a wide variety of micro-organisms in its habitat. Achievements from the use of probiotic provide a viable substitute to antibiotics and pesticides in aquaculture, providing significant advantages in terms of greater health, growth and survival rates as well as the organic products that are safe for consumers. Although, further studies on probiotics focusing on other molecular components to better understand their mechanisms of action. These may include quorum sensing, various staining techniques, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Polymerase Chain Reaction (PCR), gnotobiotic animals and through genome technologies.

Gaining from the usage of probiotic in rohu are same as in aquaculture. Modern probiotics can improve this two essential factors of growth performance and disease resistance, as they will satisfy the needs of sustainable aquaculture. Without the use of potential probiotics, intensive and extensive Rohu aquaculture will not be viable.

Despite the possible health advantages and vital functions as they have been linked into a number of terrestrial species, less is known about how probiotics affect the fish. The effects of probiotics on growth performance, efficiency of feed, gut microbiom, disease resistance, innate immune response, mucosal barrier, cell damage and morphology have extensively studied. Although, their detailed mechanism of action is clearly unknown. Additionally, probiotic species/ stage/ strain, specificity of probiotics is a major concern that needs to be addressed carefully and promptly. Also, this review indicated that combination of probiotics certainly influenced the physiological, biochemical and immunological parameters at a more visual level than that of individual probiotics. Yet this concent of, use of polyculture of probiotics need to be investigated indepth so as to unreveal their host species dependent action.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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