



Effect of *Sphingomonas* sp., as a Probiotic on Survival, Growth and Biochemical Constituents of *Vibrio anguillarum* Challenged *Labeo rohita* Fingerlings

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ABSTRACT

This study was attempted to investigate the probiotic effect of a novel locally isolated bacterium from home-made yogurt, *Sphingomonas* sp. AsCh-P3 on growth, survival and biochemical constituents of *Labeo rohita* fingerlings challenged with well-renowned fish pathogenic bacterium *Vibrio anguillarum*. Positive control-C2 as well as experimental groups was challenged with *V. anguillarum* intraperitoneally. Probiotic treated feed with antibacterial potential against *V. anguillarum* was administered to experimental fishes in different groups; G1 (soon after challenge for 30 days), G2 (for 15 days prior and 30 days after challenge) and G3 (for 15 days prior to challenge). The survival rate of fishes was 60% in C2, 66% in G1, 83% in G2 and 76% in G3. Fishes subjected to *V. anguillarum* (C2) showed significant decrease in growth and biochemical constituents as compared to C1 and experimental groups. The incorporation of probiotic isolate enhanced total proteins, total lipids, DNA contents in G2, G3, average weight, weight gain in G1, G3 than both control groups and specific growth rate in G2. On the contrary carbohydrate contents were found to be decreased significantly in all experimental groups. Henceforth, it has been concluded that on the basis of survival data and body profile, *Sphingomonas* sp. AsCh-P3 could be used successfully as probiotic bacterium against the fish pathogen prophylactically in *Labeo rohita* fingerlings.

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Authors' Contribution

AC designed and performed the experiments. QA and AMA helped in data compilation of analysis. MI wrote manuscript. JIQ guided the trial design and manuscript writing.

Key words

Probiotics, Body composition parameters, Fish biochemical constituents, Fish growth performance, *Sphingomonas* sp., Aquaculture.

INTRODUCTION

Prevailing higher outbreaks of fish diseases pose a negative impact to the success of aquaculture especially in developing countries. Pathogens are one of the main causative agents for fish diseases. The prevention of fish diseases by application of live pathogen antagonistic bacteria has received widespread interest (Hoseinifar *et al.*, 2018).

Probiotics have achieved considerable acceptance in aquaculture to control disease causing agents. Probiotics are live microbial cultures when added to feed or rearing water, result in improved health status and growth of host animals (Moriarty, 1999; Ghosh *et al.*, 2002). Probiotics have been used to improve feed digestibility by producing fatty acids, vitamin, digestive enzymes that help to break down indigestible components of food and stimulate appetite of fish (Seenivasan *et al.*, 2012; Lara-Flores and

Olvera-Novoa, 2013; Abdelhamid *et al.*, 2014). They have been employed as feed additive/supplement, water quality enhancers and available commercially consisting of Gram positive bacteria (*Bacillus*, *Carnobacterium*, *Lactobacillus acidophilus*, *Streptococcus*, *Lactococcus*, *Micrococcus*), Gram negative bacteria (*Pseudomonas fluorescens*, *Pseudomonas chlororaphis*, *Aeromonas hydrophyla*, *Enterobacter*, *Enterococcus*), yeast (*Saccharomyces cerevisiae*, *Debaryomyces* and *Phaffia*), microalgae and bacteriophages (Nayak, 2010, 2013; Pandiyan *et al.*, 2013; De *et al.*, 2014; Llewellyn *et al.*, 2014; Hoseinifar *et al.*, 2018; Opiyo *et al.*, 2019). The microbes are involved in resisting pathogenic diseases by secreting antibiotics, bacteriocin, siderophore lysozyme, etc and treat inflammation of gut, modulate immunity, defend the gut by sticking with mucosal epithelia, produce fatty acids, organic acids, and vitamin B12 (Yan *et al.*, 2002; Vine *et al.*, 2006; Luis-Villaseñor *et al.*, 2011; Mahdhi *et al.*, 2012; Sorroza *et al.*, 2012; Azimirad *et al.*, 2016; Modanloo *et al.*, 2017).

Few putative probiotic candidates known are *Bacillus subtilis* subsp. *spizizenii* CC1HG5 and CC2HG7 in Catla (Mukherjee and Ghosh, 2016), *B. tequilensis* in white

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leg shrimp, *Litopenaeus vannamei* (Luis-Villaseñor *et al.*, 2015), *Lactobacillus* in tilapia (Yu *et al.*, 2019), and *B. subtilis* in *Labeo rohita* (Chaudhary *et al.*, 2020). Outbreaks of bacterial diseases contribute to decline in fish production and economic loss that should be controlled properly. In this regard, probiotics appeared as promising candidate to combat pathogens (Hoseinifer *et al.*, 2018). The antipathogenic effects of dietary probiotic supplementation were evident in hybrid bass and striped bass against mycobacterium (Gauthier *et al.*, 2004), to resist vibrio by *Arthrobacter-Enterococcus* consortium in Atlantic cod (*Gadus morhua*) (Lauzon *et al.*, 2010) and to increase colonization by *Lactobacillus* in Atlantic salmon (*Salmo salar*) (Gupta *et al.*, 2019). Probiotic load or dosage is one of the limiting factors in order to achieve desired results with respect to host. The evaluation of optimum concentration of probiotic is therefore suggested to proliferate microflora of gut to modulate immunity (Donnet-Hughes *et al.*, 1999; Bhatnagar and Raparia, 2014). According to Bhatnagar *et al.* (2012), 2×10^5 cells of *B. coagulans* have been proved as effective dose to maximum survival and growth in *Catla catla* and high mortality was reported in *Oncorhynchus mykiss* with high dose of *L. rhamnosus* (10^{12} CFU/g feed) than lower dose (10^9 CFU/g feed) (Nikoskelainen *et al.*, 2001). Same results were observed by *Bacillus cereus* in *Cirrhinus mrigala* (Bhatnagar and Lamba, 2017). There has been found no specific period for feeding the pre/pro-biotics. It depends on feeding either to fingerlings or grows out. But the optimum dose can be species specific.

Vibriosis is hemorrhagic disease caused by *Vibrio anguillarum* in fresh as well as brackish water and marine fish. The disease being responsible for drastic economic loss were successfully controlled by probiotics in different fishes such as salmon, rainbow trout, turbot, sea bass, cod, eel, carps, crustaceans and bivalves (Frans *et al.*, 2011; Chatterjee and Haldar, 2012; Chaudhary and Qazi, 2014).

Among fresh water carps, *Labeo rohita* (rohu) is the most important fish due to good quality meat, rapid growth, consumer's preference and annual production reaches up to 1.7 million tons in Asia (Anonymous, 2018). The appropriate probiotics when administered are considered as putative candidates to combat pathogens, improve growth for sustained production of fresh water carps to cope with consumers demand. Present study dealt with potential of *Sphingomonas* sp. to confront *V. anguillarum* to improve growth performance and body composition of *L. rohita* fingerlings. According to Ali *et al.* (2001) body composition parameters are good indicators of the physiological condition and growth of a fish. Proximate body composition includes fat, protein, water and ash contents of fish (Gupakumar, 1998; Hui, 2001) and can

be affected by physical, abiotic and biotic factors. Body composition is considered as important component on which consumer preference and selection is based (Konca *et al.*, 2009) and will affect the acceptability of food. *L. rohita* is an important member of fresh water fishes and it is commercially important due to its food value. The study aimed to improve growth profile and disease resistance by *Sphingomonas* sp. AsCh-P3 supplemented feed prophylactically with least effect on fish muscle (meat quality). Present investigation is the extension of in vivo assay against vibriosis in *L. rohita* fingerlings to analyze probiotic effect on muscle composition and growth profile when administered by mixing in feed (Chaudhary and Qazi, 2014). In view of growth potential and popularity among fish consumers, present study was undertaken in *L. rohita* fingerlings of Pakistan.

MATERIALS AND METHODS

Formulation of fish feed (25% protein)

Fish feed was formulated by mixing locally available ingredients following feed formula with some modification (Ghosh and Mandal, 2015). The ingredients include fish meal (Faisal Fish Feeds, Pakistan) 5.0%, rice polishing (Faisal Fish Feeds, Pakistan) 34.3%, ground nut oil cake (Faisal Fish Feeds, Pakistan) 53.7%, molasses (Aquatic Shipping Trading Co., Pakistan) 4.0%, di calcium phosphate (Polifar Group Limited) 1.0%, table salt (BDH, Middle East FZ-LLC) 1.0% and vitamin premix (The Bayer, Pakistan) 1.0%. The feed formula fermented with *Bacillus subtilis* AsCh-A4 (MF543124) showed $19.36 \pm 0.45\%$ relative weight gain and 1.68 FCR than control group. *Sphingomonas* sp. AsCh-P3 (MF543123) culture was revived overnight in nutrient broth and then centrifuged. The pellet was suspended in PBS up to cell density of 0.5 ± 0.05 at 600 nm. The probiotic suspension (10% v/w) containing 93×10^6 C.F.U/g was sprayed on autoclaved formulated fish feed. Fish feed was dried in a laminar flow cabinet at room temperature ($\sim 27-30^\circ\text{C}$) up to three days then stored in refrigerator up to 7 days. Fish feed without probiotic isolate served as control feed (Chaudhary and Qazi, 2014).

Source of fish pathogen and probiotic isolate

Fish pathogen *Vibrio anguillarum* (90-11-287; Sero type 01) was obtained from Royal Veterinary and Agricultural University, Copenhagen, Denmark. The literature about the isolated pathogen was also provided with the courtesy of Skov *et al.* (1995). *V. anguillarum* is the most studied etiological agent of vibriosis (Larsen *et al.*, 1994). The O1 and O2 serotypes of *V. anguillarum* are the virulent strains frequently isolated from diseased fish

(Toranzo and Bajra, 1990).

Pathogen caused 40% mortalities by injecting 0.1 ml (56×10^5 C.F.U/ml) with symptoms such as body reddening, belly swelling and reddening and erythma in eyes in pathogenicity study. While *Sphingomonas* sp. AsCh-P3 was isolated from home-made yogurt and selected on the basis of antibacterial potential against *V. anguillarum* in *in vitro* assay (Chaudhary and Qazi, 2014). As yogurt contains certain bacteria that exert immunostimulatory effect on enteric microflora in human being (Meydani and Ha, 2000). On the basis of this hypothesis, bacteria were isolated from yogurt and used as immunomodulant in *L. rohita* fingerlings. Moreover, *Sphingomonas* contain GSL (glyco-sphingolipids) in their cell envelope and are completely devoid of Lipopolysaccharides that are capable of stimulating NKT (Natural killer and T) cells which is a part of innate immune response in *L. rohita* (Sriram *et al.*, 2005).

Experimental set up

L. rohita fingerlings were collected from Department of Fisheries, Government of Punjab, Pakistan. Initially fish were acclimated for 10 days. Then experiment was designed for 45 days. For 15 days the experimental fishes were fed with respective feed, then pathogen was injected and feed trial expanded for more 30 days. *L. rohita* fingerlings were identified on basis of phenotypic traits such as body shape, mouth, eye, fin color, lateral line, head size/shape, scale shape and body color. The fingerling has stream-lined body, mouth small and inferior; lips thick and fringed, eyes dorsolateral in position, head without scale, body with cycloid scales, distinct lateral line, slightly greenish on dorsal surface, reddish tinge on ventral and caudal fins (Sarder *et al.*, 2011). The health status of the fish was examined grossly throughout the acclimatization and experimental period for visible lesions or symptoms of vibriosis *i.e.*, reddening of body, swelling and reddening of belly and hemorrhages. Fishes were fed with known amount of feed according to 3% of fish body weight once a day during the period. Temperature was maintained at 24 to 27°C. The fish were reared in glass aquaria (2' × 1' × 1') in University animal house following closed or recirculating system. Glass aquaria were partially cleaned and two-third water was changed daily. Water was aerated by applying aerators (Soltan, 2016; Xiao *et al.*, 2018).

Each control as well as experimental group comprised of triplicates with 10 fish per aquarium. Each experimental and positive control fish was injected intraperitoneally (*i.p.*) with 0.1 ml of *V. anguillarum* suspension (56×10^5 C.F.U. /ml). Negative control was injected *i.p.* with comparable amounts of Phosphate buffer saline (PBS).

The negative (C1) and positive control (C2) groups

were fed with formulated sterilized feed without probiotic (control feed) for first 15 days and 30 days after *i.p.* pathogenic injections. In experimental group G1, control feed was administered for 15 days prior pathogenic dose followed by *Sphingomonas* sp. AsCh-P3 treated feed for 30 days whereas G3 was provided with the *Sphingomonas* sp. AsCh-P3 supplemented feed prior (for 15 days) and control feed after (for 30 days) pathogenic dose. Experimental group G2 received *Sphingomonas* sp. AsCh-P3 enriched feed prior (for 15 days) and after (for 30 days) pathogenic dose.

Growth parameters

After pathogen injection and probiotic supplementation, dead fishes were removed out from aquaria and survival data was recorded. Live fishes were weighed to determine weight gain, survival and specific growth rate at start of experiment (initial reading), at time of injection (R0), at 15 (R1) and 30 (R2) days post pathogen injection. Survival percentage was calculated at termination of experiment by following formula described by Opasola *et al.* (2013).

$$\text{Survival (\%)} = \frac{\text{Total No. of survived fish}}{\text{Total No. stocked}} \times 100$$

Specific growth rate (SGR %d⁻¹) and relative growth (RGR) was assessed as reported by Lugert *et al.* (2014):

$$\text{SGR \%d}^{-1} = \frac{\log \text{Final weight} - \log \text{initial weight}}{\text{Experimental periods in days}} \times 100$$

$$\text{RGR (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

Biochemical analysis of muscle tissue

Muscles of three fishes per replicate were homogenized to extract proteins, lipids, carbohydrates, RNA and DNA (Bregman, 2002). The extraction was carried out for estimation of protein by Lowry *et al.* (1951) and Sapan *et al.* (1999), carbohydrates by Dubois *et al.* (1956), Lichtenthaler and Buschmann (2001) and Jain *et al.* (2017), lipids by Zollner and Kirsch (1962) and Anschau *et al.* (2017), and nucleic acids *i.e.* DNA, RNA by Schnieder (1957) and Jain *et al.* (2020) following Lowry *et al.* (1951), phenol sulfuric acid, sulfo-phosphovanillin, diphenyl amine and orcinol methods, respectively.

The ash contents were determined after heating the samples in muffle furnace at 550°C for 3-4 h while moisture contents were determined by drying the sample in oven at 105°C until consistent weight (AOAC, 2016).

Statistical analysis

Values were expressed as mean ± SEM (Standard error mean) for three fish per replicate. All the experimental

data were analyzed using one way analysis of variance (ANOVA) at $p \leq 0.05$ followed by Dunkun's multiple range test (SPSS ver.16.0 software, SPSS, Chicago, IL, USA).

RESULTS

Growth performance of L. rohita fingerlings

The dietary supplementation of formulated fish feed with *Sphingomonas* sp. AsCh-P3 enhanced percent survival rate of *V. anguillarum* challenged *L. rohita* fingerlings significantly in G2 (83.3±3.3) and G3 (76.7±3.3) than both control and G1 groups. The percent fish survival of fingerlings infected with *V. anguillarum* (act as positive control) was 60.0±5.8 than negative control groups as

reported in the previous study (Chaudhary and Qazi, 2014), dead fishes were removed daily. The results were tabulated in Table I.

Concerning growth parameters, *L. rohita* fingerlings fed with probiotic supplemented feed in experimental groups showed significant enhancement in average weight, weight gain and specific growth rate than both control. Highest increase in average weight (g) *i.e.*, 3.8±0.9, 4.6±1.0 in G1 and 3.8±0.8, 4.4±0.9 in G3 were observed after 15 and 30 days post-injection, respectively. Same trend was noted in percent RGR in G1 and G3 groups while highest SGR (1.4±0.1 %d⁻¹) was recorded in G2 group after 30 days (Table I).

Table I.- Growth parameters and percent survival rate of *V. anguillarum* challenged *L. rohita* fingerlings fed with probiotic treated diet in different experimental treatments.

Parameters		Negative control (C1)	Positive control (C2)	Experimental groups		
				G1	G2	G3
Morphometry						
Initial average weight (g)	At start of exp.	2.6±0.3 ^a	2.5±0.4 ^a	3.3±0.7 ^b	3.1±0.6 ^b	3.2±0.6 ^b
Final average weight (g)	R0	2.7±0.3 ^a	2.8±0.4 ^a	3.4±0.8 ^b	3.3±0.6 ^b	3.4±0.7 ^b
	R1	3.0±0.4	3.2±0.4	3.8±0.9	3.5±0.7	3.8±0.8
	R2	3.5±0.3 ^a	3.7±0.5 ^a	4.6±1.0 ^b	4.1±0.9 ^b	4.4±0.9 ^b
Initial average total length (cm)	At start of exp.	6.77±0.1 ^a	6.7±0.1 ^a	6.67±0.2 ^b	6.5±0.1 ^b	6.5±0.1 ^b
Final average total length (cm)	R0	7.0±0.1 ^a	7.0±0.1 ^a	7.1±0.2 ^a	6.8±0.1 ^b	6.8±0.1 ^b
	R1	7.4±0.1 ^a	7.3±0.2 ^a	7.5±0.2 ^a	7.1±0.1 ^b	7.1±0.1 ^b
	R2	7.89±0.2 ^a	7.9±0.3 ^a	8.5±0.3 ^b	7.5±0.1 ^c	7.4±0.1 ^c
Initial average width (cm)	At start of exp.	1.4±0.02 ^a	1.37±0.03 ^b	1.4±0.03 ^a	1.35±1.3 ^b	1.36±1.3 ^b
Final average width (cm)	R0	1.5±0.03	1.5±0.04	1.54±0.04	1.4±1.4	1.5±1.4
	R1	1.7±0.04 ^a	1.69±0.03 ^a	1.73±0.1 ^a	1.5±1.4 ^b	1.6±1.5 ^b
	R2	1.9±0.1 ^a	1.87±0.1 ^a	1.94±0.1 ^a	1.65±1.5 ^b	1.74±1.5 ^b
Nutritional indices						
Relative growth rate (%) (RGR)	R0	6.04±0.1 ^a	9.03±0.1 ^b	5.4±0.3 ^a	4.0±0.2 ^c	7.8±0.2 ^{ab}
	R1	12.5±0.1 ^a	16.68±0.1 ^b	11.3±0.1 ^a	7.3±0.2 ^c	10.9±0.2 ^a
	R2	16.2±0.2 ^a	16.6±0.3 ^a	19.4±0.6 ^b	11.8±0.5 ^c	17.1±0.4 ^a
Specific growth rate (% d ⁻¹) (SGR)	R0	0.3±0.1 ^a	0.6±0.1 ^b	0.5±0.1 ^{ab}	0.2±0.1 ^c	0.5±0.1 ^{ab}
	R1	0.7±0.2 ^a	1.3±0.3 ^b	0.6±0.2 ^a	0.5±0.1 ^c	0.7±0.1 ^a
	R2	1.1±0.4 ^a	1.1±0.1 ^a	1.3±0.4 ^b	1.4±0.2 ^c	1.1±0.3 ^a
% Survival rate (SR)	At termination of exp.	100±0.0 ^a	60±5.8 ^b	66.7±3.3 ^b	83.3±3.3 ^c	76.7±3.3 ^{bc}

All values represent means of three replicates ±S.E.M. Two values within respective row not sharing a common alphabet differ significantly from each other at $p \leq 0.05$ at single factor analysis of variance. C1, received sterile PBS 0.1 ml/fish and fed control feed; C2, fish pathogen was injected 0.1ml/fish and fed control feed; G1, the fish were fed probiotic containing feed soon after challenge with fish pathogen for 30 days; G2, the fish were fed probiotic containing feed for 15 days prior and 30 days after challenge with fish pathogen; G3, the fish were fed on the probiotic containing feed for 15 days prior challenge with fish pathogen, thereafter, control feed was given for 30 days ; R0, measurement at time of injection; R1, measurement at 15 days post injection, R1, measurement at 30 days post injection.

Table II.- Effect of *Sphingomonas* sp. AsCh-P3 on biochemical constituents of *L. rohita* fingerlings challenged with *V. anguillarum*.

Biochemical constituents		Negative control (C1)	Positive control (C2)	Experimental groups		
				G1	G2	G3
Total protein (mg/g)	R1	26.29±0.40 ^a	22.88±0.99 ^b	19.75±1.16 ^b	22.75±0.29 ^b	24.99±0.09 ^a
	R2	25.08±0.52 ^a	18.56±0.16 ^b	32.27±2.08 ^c	30.42±2.22 ^{cd}	26.76±0.58 ^d
Total carbohydrates (mg/g)	R1	23.88±2.00 ^a	16.13±0.95 ^b	29.71±2.88 ^c	20.63±1.45 ^{ab}	17.00±2.00 ^b
	R2	26.37±1.04 ^a	23.14±1.09 ^a	24.46±0.56 ^a	16.69±1.10 ^b	24.85±2.30 ^a
Total lipids (mg/g)	R1	244.98±11.77 ^a	141.06±5.21 ^b	209.13±12.94 ^c	158.68±4.57 ^b	270.45±7.35 ^a
	R2	210.78±4.39 ^a	155.99±1.37 ^b	271.18±17.47 ^c	255.59±18.68 ^c	281.94±8.14 ^c
DNA (µg/g)	R1	0.71±0.09 ^a	1.22±0.04 ^b	1.16±0.06 ^b	1.14±0.05 ^b	1.10±0.04 ^b
	R2	1.03±0.07 ^{ab}	0.81±0.06 ^a	1.13±0.03 ^b	1.23±0.07 ^b	1.06±0.05 ^{ab}
RNA (µg/g)	R1	4.40±0.03 ^a	5.16±0.02 ^b	5.32±0.23 ^b	3.90±0.06 ^c	3.85±0.13 ^c
	R2	4.21±0.05 ^a	4.86±0.08 ^b	5.79±0.04 ^c	3.57±0.05 ^d	3.59±0.04 ^d
Moisture contents (%)	R1	68.02±2.53 ^a	74.95±2.21 ^b	74.74±1.96 ^b	76.46±4.43 ^b	82.03±4.00 ^c
	R2	69.53±2.02 ^a	107.38±2.13 ^b	84.69±4.03 ^c	74.24±1.55 ^a	81.43±3.88 ^c
Ash contents (%)	R1	13.15±0.20 ^a	11.44±0.49 ^a	9.87±0.58 ^b	11.37±0.15 ^a	12.45±0.04 ^a
	R2	12.54±0.26 ^a	9.28±0.08 ^b	16.14±1.04 ^a	15.21±1.11 ^a	13.38±0.29 ^a

All values represent means of three replicates ±S.E.M. Two values within respective row not sharing a common alphabet differ significantly from each other at $p \leq 0.05$ at single factor analysis of variance. C1, received sterile PBS 0.1 ml/fish and fed control feed; C2, fish pathogen was injected 0.1 ml/fish and fed control feed; G1, the fish were fed probiotic containing feed soon after challenge with fish pathogen for 30 days; G2, the fish were fed probiotic containing feed for 15 days prior and 30 days after challenge with fish pathogen; G3, the fish were fed on the probiotic containing feed for 15 days prior challenge with fish pathogen, thereafter, control feed was given for 30 days; R1, measurement at 15 days post injection, R2, measurement at 30 days post injection.

Biochemical analysis of muscle tissue

The muscle tissue of sampled fish was processed for biochemical characterization of protein, carbohydrates, lipids, RNA, DNA, ash and moisture contents of negative as well as positive control and experimental groups. Healthy fishes with no disease symptoms were sampled after 15 days (R1) and 30 days (R2) for biochemical analysis (Table II). Experimental groups showed increasing trend in total protein, lipid, DNA, moisture and ash contents. The varying degree of increase or decrease contents in experimental fishes after 15 and 30 days than both controls may be due to stress posed by competitive role of probiotics against pathogen. At termination of experiment, experimental fishes showed increasing trend as $G1 > G2 > G3$ in protein (significant) and ash (non-significant) whereas significant improvement as $G3 > G1 > G2$ in lipid contents when compared with control groups. Carbohydrate contents were significantly lower in G2 group. Moisture contents were higher significantly in C2, G1 and G3 than C1 as well as G2 groups. The values for RNA contents were higher in G1 and C2 groups after 15 and 30 days post pathogen challenge. The higher DNA contents in C2 after 15 days led to significant lower value

at end of experiment as compared to C1 and experimental groups (Table II).

DISCUSSION

L. rohita is regarded as an imperative member of fresh water fishes and is commercially important due to its food value. In view of growth potential and popularity among fish consumers, present study was undertaken to monitor and compare the effects of pathogen and probiotic supplementation on growth performance and body composition of *L. rohita* fingerlings. Body composition parameters are considered as excellent indicators of the physiological condition and growth of a fish (Ali *et al.*, 2001). These parameters help to assess the level of stress and iterations in metabolic cycles (Wang *et al.*, 2005). The biochemical constituents such as proteins, carbohydrates, lipids, moisture, ash, RNA and DNA contents were determined in muscle of *L. rohita* fingerlings.

The expansion of aquaculture industry increases the variety of fishes. These fishes are prone to different pathogenic bacteria which causes a great loss. During years, efforts have been made on the reduction of diseases

caused by vibrio. Vibriosis is caused by *Vibrio anguillarum* is reported in fresh water and marine fishes (Frans *et al.*, 2011; Cui *et al.*, 2014). The percent survival rate of *L. rohita* fingerlings varied from 66.7±5.8 to 83.3±3.3 in experimental fish than positive control 60.0±5.8 in present study as Gram *et al.* (1999) observed decreased mortality in vibriosis in rainbow trout by probiotic *Pseudomonas fluorescens* (AH2). According to Welker and Lim (2011) and Hai (2015), *Saccharomyces cerevisiae* and *Bacillus subtilis* improve immunity by producing bacitracin and subtilin that contribute towards better survival rate.

This investigation revealed the effect of viable microorganism supplementation into diet, improvement of disease resistance and body composition. Probiotics are microbes to enhance the nutritional values of food. Isolate *Shingomonas* sp. AsCh-P3 was evaluated for its probiotic potential *L. rohita* fingerlings. Sriram *et al.* (2005) described the presence of glycosphingolipid on the cell envelope of *Sphingomonas* that stimulate natural killer cells and T lymphocytes of *L. rohita* to respond as innate immunity. Experimental groups were supplied with *Sphingomonas* sp. AsCh-P3 fortified feed in different feed patterns and effect was observed in biochemical constituents and growth performance of fingerlings. Probiotics improve protein, lipids and ash contents in experimental groups than both controls.

The administration of *V. anguillarum* in positive control C2 resulted in decrease in average weight, and specific growth rate than experimental groups G1, G2. The decreased growth parameters may be due to pathogenic stress that leads to decreased growth rate because stresses are one of the factors of decreased growth performance in fish (Arthi-Manju *et al.*, 2011). Similarly, *V. anguillarum* in C2 caused the decrease in protein, lipids, DNA, ash except moisture and RNA contents at termination of experiment.

By keeping in view the growth performance of *L. rohita* fingerlings, higher average body weight and SGR (%d⁻¹) were recorded in G1 (provided probiotic after infection) and G2 (probiotics prior and after infection), respectively. The results were corroborated with finding of Bairagi *et al.* (2004) who reported significantly increased final body weight and SGR of common carp than control group by *B. subtilis* and *B. circulans* supplemented feed. Similar results were observed by Bagheri *et al.* (2008), Boonthai *et al.* (2011) and Parthasarathy and Ravi (2011). Higher relative growth rate (%) was observed in G1 (19.4±0.6) and G3 (17.1±0.4) which were administered probiotics after pathogen injection and prior infection correspondingly. Group G2 received probiotics for long period that is before and after infection showed lower (11.8±0.5) percent RGR. Beghari *et al.* (2008) reported the decreased growth rate in rainbow trout fry fed with higher

levels of *B. subtilis* and *B. licheniformis* in combination. Higher probiotic load in feed or dosage attributed reduced growth which may indicate decreased nutrient utilization due to experimental condition variation and experiment duration of probiotic intake. Opiyo *et al.* (2019) suggested that higher probiotic intake may not be essential in improvement of fish growth and intermediate probiotic dosage may contribute to better health and survival rate instead of enhanced growth.

Protein and lipid contents were improved in experimental groups than positive controls while lower carbohydrates were recorded in C2 as well as experimental groups as compared to C1. In C2, higher moisture and lower lipid as well as ash contents were noted. According to Love (1980), the fish at first consumes lipids from liver and starts to mobilize muscle protein only when fat derived energy has been nearly used up. After that, as protein is utilized, water moves into take its place. Such a shift resulted in the increased water contents that were inversely correlated with protein and fat reserves of their meats (Shimma and Sato, 1985; Mahboob and Sheri, 1997). Carbohydrates being organic nutrients were considered to degrade first due to imposed stress on animal (Gobinath and Ramanibai, 2013). The results of present study were on contrary to findings of Bagheri *et al.* (2008) who described increase in protein and reduced lipid contents in probiotic enriched diet in *Oreochromis mykiss* and *O. niloticus*. According to El-Haroun *et al.* (2006), increased protein contents can be due to deposition of nutrients. The improvement in protein contents could be resulted by secretion of more proteins by probiotics in Nile tilapia (Opiyo *et al.*, 2019) and conversion of ingested diet effectively into structural protein to build muscles (Rosovitz *et al.*, 1998; Mehrabi *et al.*, 2012; Lara-Flores and Olvera-Novoa, 2013).

At 15 days post pathogen injection, RNA and DNA increased than negative control and experimental groups. At termination of experiment, RNA values stabilized near to negative control and G2, G3 while values of DNA decreased than C1 and experimental groups. In another study, higher DNA contents were observed in *L. rohita* fingerlings administered with *B. subtilis* AsCh-A4 fermented fish feed (Chaudhary *et al.*, 2007). Nucleic acid analysis is considered a valuable indicator to assess physiological condition of fish and growth (Smith and Buckley, 2003; Mukherjee and Jana, 2007; Ravikiran and Kulkarni, 2017). In C2, highest values DNA and RNA to some extent are agreed with Khan and Jafri (1991) and Krishnaveni *et al.* (2013).

CONCLUSION

By evaluating body composition and growth profile,

Sphingomonas sp. AsCh-P3 showed highest protein, carbohydrates and lipid contents followed by average weight and relative weight gain in G1 group when provided after infection in *L. rohita* fingerlings. Survival and SGR were observed in G2 group fed probiotics prior and after pathogen challenge. The data generated from present study provide information about probiotic incorporation in commercial aquaculture and fish feed supplement for better growth and immunity.

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Statement of conflict of interest

The authors have declared no conflict of interests.

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