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Effect of Probiotic Strain *Bacillus firmus* CAS 7 As Feed Supplement for Growth, Survival and Colour Enhancement of Smoke Angelfish *Apolemichthys xanthurus*

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ABSTRACT

The effect of dietary management of probiotic micro organism, *Bacillus firmus* cas 7 on increase, survival and colour enhancement of smoke angelfish *Apolemichthys xanthurus* become evaluated. within the gift take a look at, fishes have been fed with basal diet supplemented with probiotic *B. firmus* cas 7 at 50, 100 and 150 mg kg-1 and manage basal food regimen containing no probiotic. the outcomes discovered that the basal weight-reduction plan supplemented with probiotic at one hundred fifty mg kg-1 produced considerably better weight benefit (80.95 ± 1.5g), precise increase price (0.675), survival charge (one hundred%) and feed conversion ratio (zero.ninety one) than diets supplemented with different concentrations and manage. furthermore, carotenoid content material was relatively better in fishes fed feed supplemented with probiotic in amount a hundred and fifty mg kg-1 (6.24 mg g-1) than a hundred and 50 mg kg-1 and manipulate (2.ninety one mg g-1). the experimental end result proves that the probiotic bacteria b. firmus cas 7 substantially stepped forward growth and color of smoke angelfish *Apolemicthys xanthurus* which might be useful in ornamental fish industry as feed complement. further, studies concerning the synthesis of carotenoid and appropriate dose of probiotic must be executed earlier than the use of probiotics on a massive scale to prevent any undesired consequences.

KEY WORDS: PROBIOTIC; ANGEL FISH; *BACILLUS FIRMUS*; GROWTH; CAROTENOIDS.

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INTRODUCTION

Modern aquaculture demands alternatives that can sustain a healthy environment with best production practices (Ai, Xu, Mai, Xu, Wang, & Zhang, 2011). Probiotics in aquaculture have been reported to provide helpful property and the use of probiotics is an important management tool in fish culture (Balcazar, De Blas, Ruiz-Zarzuela, Cunningham, Vendrell, & Muzquiz,, 2006). The probiotics in aquaculture is use full for reduction of the use of harmful antimicrobial compounds, particularly antibiotics, and also improved the growth performance of the farmed species in an eco-friendly and sustainable manner (Wang, Xu, & Xia, 2005). Probiotics as feed supplements benefit to host with improving the value of feed, enzymatic role to digestion, inhibition of pathogenic microorganisms, medical properties, growth promoting factors and increasing immune response (Harikrishnan, Balasundaram, & Heo, 2010; Gupta, Gupta, & Dhawan, 2014; Gupta, Geetika, & Paromita, 2016). Several reports suggest that probiotics supplementation can cut down the cost of culture by improving the fish growth and feed utilization efficiency. The most typically used probiotics in aquaculture belong to gram-positive spore forming *bacillus spp.* (Wang et al., 2008; Gupta & Dhawan 2011, 2013; Gupta et al., 2014, 2016).

The genus Bacillus as putative probiotics has been used substantially as aquaculture feed components, due to its resistance to excessive temperature and excessive stress (Rengpipat, Rukpratanporn, Piyatiratitivorakul, & Menasa-veta, 2000). Nutritional supplementation of Bacillus spp. advanced the boom performance, immunity and ailment resistance of fish (Ai et al., 2011; Geng, Dong, Tan, Yang, Chi, Liu, & Liu, 2012) and giant freshwater prawn (*Macrobracium rosenbergii* (Gupta & Dhawan 2011, 2012). In rainbow trout (*Oncorhynchus mykiss*), significant development of feed conversion ratio (fcr), specific growth price (sgr) and protein performance ratio (in line with) turned into observed when the fish was fed with diets containing bacillus spp. (Merrifield et al., 2010; Gupta et al., 2014), as color of decorative species is taken into consideration as an essential factor for marketing of the product (Gouveia & Empis 2003; Gouveia & Rema 2005).

Color changes in fish are regularly related to environmental strain, and illumination might be a number one factor regulating pigment distribution via hormone regulation (Van der Salm, Martnez, Flik, & Wendelaar Bonga, 2004). Shade of fish pores and skin is predominantly depending on the presence of chromatophores containing colored pigments (Fox, 1957). The colour of fish pores and skin is generated via the absorption, mirrored image, and scattering of mild through the pigments and microstructures within the fish integument (Fujii, 2000). carotenoids are evidently going on pigments that variety in shades from yellow to crimson (Hill, 2002) which are lipid soluble pigments, are answerable for pores and skin coloration of ornamental fish, and might determine their commercial feed. The prosperity of the decorative fish industry has precipitated the indiscriminate use of antibiotics and chemo therapeutants for improvement in fitness and vitamins, and this therefore has led to the development of drug resistant traces of pathogenic microorganisms (Amabile-Cuevas, Cardenas-Garcia, & Ludgar,1995). Fishes are unable to perform de novo synthesis of carotenoids (cd) like other animals and consequently rely on nutritional supply to gain their herbal pigmentation (Goodwin, 1984).

Below in depth farming conditions and aquarium rearing, ornamental fish are fed completely with compound feeds, which ought to consequently be supplemented with carotenoids (Wang, Li, & Lin, 2008). The yellow, orange and red colors discovered in fish pores and skin are the end result of the group of carotenoid pigments, each carotenes and xanthophylls (Simpson, Katayama, & Chichester, 1981). Spore-forming bacillus species able to synthesizing carotenoid pigments and the biochemical evaluation at the carotenoids answerable for the yellow/ orange pigmentation found in bacillus sp. has been performed and the identification of the carotenoids was elucidated (Perez-Fons, Steiger, Khaneja, Bramley, Cutting, Sandmann, & Fraser, 2011).

The most typically found pigments had been yellow, orange and red. isolates have been nearly usually participants of the bacillus genus and in maximum cases have been related with recognised species consisting of *B. marisflavi*, *B. indicus, B. firmus, B. altitudinis and B. safensis*. 3 types of carotenoids were found with absorption maxima at 455, 467 and 492 nm, corresponding to the visible colors like yellow, orange and pink, respectively (Perez-Fons et al., 2011). A total of 1471 species of ornamental fish are traded globally (Wabnitz, Taylor, Green, & Razak, 2003) of which, marine angelfishes (Pomocanthidae) are the following maximum crucial organization than damsel and anemone fish (33%), consisting of approximately 25% of the overall change. *Pomacanthus* angelfish are the various most high prized of the coral reef fish (Frische, 1999; Debelius, Tanaka, & Kuiter, 2003). The marine angelfish circle of relatives pomacanthidae consists of 8 genera and 82 species global (Nelson, 2006).

Marine angelfish, *Apolemichthys xanthurus* (Bennett, 1833) are extensively dispensed at some point of the Indian ocean, in regions such as the Maldives and the east coast of India. these fish tend to stay in coral-rich areas singly or in pairs, often found outer reef facet and reef slopes at depths of 15 m with the most size approximately 25 cm, feeds mainly on sponges, sea squirts and small amounts of copepods (Rajeswari, & Balasubramanian,

2014) to the fine of our understanding, very little statistics is available on its replica and early ontogeny, possibly because of the problems of field observations because of low populace densities and massive domestic stages, and also because of the issue in breeding such species in small aquaria (Moyer, 1987). But, there was no tries had been made on increase, survival, coloration enhancement, herbal spawning and larval rearing of *Apolemichthys xanthurus* in captivity. Even though the software of probiotics has been diagnosed in aquaculture by means of some researchers global, no concerted tries have been made on these marine angel fishes to enhance boom and color till date. therefore an attempt turned into made to analyze the impact of probiotic bacteria B. firmus cas 7 on boom overall performance, survival and color enhancement of smoke angelfish *Apolemichthys xanthurus* that's one of the maximum appealing and commercially essential marine angel fish species.

MATERIAL AND METHODS

Isolation and identification of probiotic bacteria Bacillus firmus CAS7: Samples were collected from marine sediments of Parangipettai, Tamil Nadu, India and brought to the laboratory aseptically at 4 C. in nutrient broth sea water (NBSW) (salinity 34 g/l and pH 7.89), speared on nutrient marine agar 2216 (Himedia, USA) and incubated at room temperature for 24 h at 30 C. Colonies were spread on nutrient marine agar plates. After Gram staining and catalase testing, only one strain (CAS7) were showed Gram and catalase positive rods were retained. Retained strains were enriched for 24 h at 30 C in NBSW. Colonies were re-isolated on nutrient marine agar and preserved in the laboratory. The purity of such cultures was routinely checked during this work. Stock cultures were frozen at 80 C with 20% (vol/vol) glycerol.

Table 1. Morphological, physiological and biochemical characteristics of the probiotic strain CAS 7

Characteristics	Results
Shape	Rod
Gram stain	Positive
Spore formation	+
Motility	+
Glucose	+
Mannitol	+
Xylose	+
Starch Hydrolysis	+
Gelatin Hydrolysis	+
Fat Hydrolysis	+
Casein Hydrolysis	+
Catalase activity	-
Nitrate reduction	+
Indole	+
Citrate	+

Characterization of the potent strain: Pure cultured colonies were biochemically characterized (Himedia, Mumbai). The identification was confirmed by partial sequencing the 16 S rDNA by kumaran et al., 2009. The isolated bacterial DNA were extracted and 16s rDNA sequences were amplified by polymerase chain reaction (PCR) using universal primers of 8f (3'-AGAGTTTGATCCTGTGCTCAG 5') and 1490r (5'-GACTTACCAGGGTATCTAATCC-3'). The PCR products were electrophoresed on 1% agarose gel and visualized via ultraviolet transillumination. After that the PCR product was purified to remove the primer, primer-dimers and low molecular weight DNA fragments generated by nonspecific amplification. Five volume of binding buffer was mixed with one volume of PCR product and loaded into the purification column. The nucleotide sequences of the PCR products were determined by using the automated DNA sequence with forward and reverse primers (Bio serve pvt. Bio Technologies, India). The sequences was compared with all 16S rRNA gene sequence data stored NCBI by nucleotide BLAST.

Table 2. Formulation and Proximate composition of basal diet

Ingredients	g kg-1
Fish meal	600
Shrimp meal	160
Soybean meal	20
Wheat flour	140
Fish oil	40
Soybean phospholipids	20
Vitamin mineral mix	10
Proximate composition	
Dry matter	941
Crude protein	491
Crude lipids	98
Ash	96

Vitamin mineral mix (EMIX PLUS, Mumbai, India) (Quantity per kg)

Vitamin A: 22 00 000 IU; Vitamin D3: 4 40 000 IU; Vitamin B2: 800 mg; Vitamin E: 300 mg; Vitamin K: 400 mg; Vitamin B6: 400 mg; Vitamin B12: 2.4 mg; Calcium Pantothenate: 1000 mg; Nicotinamide: 4 g; Choline Chloride: 60 g; Mn: 10 800 mg; I: 400 mg; Fe: 3000 mg; Zn: 2000 mg; Cu: 800 mg; Co: 180 mg; Ca: 200 g; P: 120 g; L L-lysine: 4 g; DL-Methionine: 4 g; Selenium: 20 ppm.

Culture of probiotic bacteria B. firmus CAS 7: The strain *B. firmus* CAS 7 was cultured and prepared as described by Sun et al., (2010). 500 mL of fresh nutrient broth was seeded with 1% inoculum (1.50 x 106 CFU mL-1) and kept in a shaker incubator (200 rpm) at pH 7.5, temperature 28 oC, and salinity 30 PSU for 48 h. After incubation

period, the cells were harvested by centrifugation at 5000 xg for 10 min, washed twice with phosphate-buffered saline (pH 7.5) and re-suspended in same PBS buffer for addition to the basal diet and this was administrated for probiotic study. Cell growth was estimated by measuring optical density at 600 nm from the aliquots withdrawn at every 6 h intervals.

Preparation of control and probiotic feed: The control basal diet was formulated using the ingredients such as fish meal, shrimp meal, soya bean meal, wheat flour, fish oil and vitamin mineral mix (Table1) (Sun, Yang, Ma, & Lin, 2010).All the ingredients were dried overnight at 80° C in a hot air oven and powdered. The powdered ingredients were sieved through a fine-meshed screen (0.5 mm diameter) and mixed well. The dough was prepared by adding required amount of water with the ingredients, sterilized (autoclave at 121° C for 15 min) and incorporated with 3% (v/w) commercial vitamin mineral mix (EMIX PLUS, Mumbai, India) and pelletized using hand pelletizer to obtain 1 mm pellets. The pellets were initially sun dried and then oven dried at 60 ± 5°C for 12 hours to get moisture content. Further, they were manually broken into smaller bits and stored at room temperature in an air tightened sterile polypropylene containers.

The test feeds for the experiments were prepared by gently spraying the required amount of bacterial suspension on the control diet and mixing it part-by-part in a drum mixer to obtain a final concentration of 50, 100 and 150 mg kg-1 probiotic based on preliminary experiments in our laboratory. The probiotic cell suspensions (108cells mL-1) were added to the control diet after the dosage had been autoclaved and subsequently cooled, before pelletizing. The proximate composition (moisture, protein, ash, lipid and fibre) of all probiotic feeds and control feed were determined by the standard procedures of AOAC (1990). The viability counts (CFU/g) of the probiotic incorporate feed before and after drying was enumerated using a pour plate technique using the method described by Reddy et al., (2009) and expressed in % of survival. The probiotic strain-incorporated feeds were packed in sterile polypropylene containers and stored at 4°C for further studies.

Experimental design: The experiment was conducted at Aquaculture breeding center, Centre of Advanced Study in Marine Biology, Annamalai University, India. The juveniles of smoke angelfish, *Apolemichthys xanthurus* were obtained from MAV Breeders (Mandabam, Tamil Nadu, India) and acclimatized for 4 weeks before the trial. The feeding experiment was conducted in (20 L) rectangular fibre glass tanks, with temperature ranging from 26 - 30oC, salinity 28 -30 PSU, pH 7.4 - 7.8; and dissolved oxygen (DO) 4.2 to 5.6 mg L-1. A total of 30 fish seeds were maintained in each tank throughout the experiment (12.89 ± 0.41 g mean weight) and each treatment was conducted in triplicate. In control, the fishes were feed with prepared pellet feed alone. The probiotic bacteria of 50, 100 and 150 mg kg-1 were mixed with experimental feed. The feeding rate was about 3% of biomass per day provided in equal rations at 8.00 AM, 1.00 PM, 6.00 PM for 120 days and the excess diet was collected and dried at 60 °C, put in room temperature for 3 days to restore the natural moisture and then weighed. Feeding rate was adjusted every fortnight during sampling by batch weighing of fish in each tank after a 24 h period of starvation. At the same time, fish survival was also determined by counting the individuals in each aquarium. Experimental tanks were cleaned and water exchange was done once a week.

Growth indices: The growth parameters such as weight gain, specific growth rate (SGR), survival rate and feed conversion ratio (FCR) were assessed at 30, 60, 90 and 120 days. The weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) was evaluated based on standard formula as follows.

Weight gain (g) = (Final body weight (g) – Initial body weight (g)

Specific growth rate (SGR) = 100 (loge final body weight - loge initial body weight) / culture period (days)

Feed conversion ratio (FCR) = total dry feed fed (g) / total live weight gain (g)

Colour enhancement and carotenoid content estimation

The color enhancement was monitored by visual examination and estimation of carotenoid content in the skin of experimental fishes. The carotenoid content of the experimental fish skin was extracted according to the method of Torrissen & Naevdal (1984). Three fishes from each experiment were randomly sampled, anesthetised using clove oil (dissolved in 95% ethanol at 1:10) and used for carotenoid content analysis in triplicate. Briefly, 2 mg of skin were collected from both sides between the abdominal and dorsal regions of the fish and then transferred to 10 mL of pre - weighed glass tubes after the fat layer had been removed from the skin and ground well with acetone containing anhydrous sodium sulphate and made up to 10 mL with acetone. The samples were stored for 3 days at 4oC in a refrigerator, and then extracted three times till no further colour could be obtained and centrifuged at 5000 xg for 5 min. The total carotenoid content of the samples was determined using spectrophotometer (Shimadzu, UV mini 1240) using extinction coefficients (E1%, 1 cm) of 2000 for astaxanthin (Hata, & Hata, 1971) at 475 nm, and 2500 for carotenoids from alfalfa at 450 nm (Schiedt, & Jensen, 1995).

Data analysis: In the present study, all the data were analyzed by statistical methods. The two way analysis of variance (ANOVA) was performed using SPSS (Statistic Package for social science) version 11.5 software to determine the significant differences among means. For all tests, a criterion of P<0.01 was used to determine statistical significance.

RESULTS AND DISCUSSION

Based on the morphological, physiological, and biochemical characteristics, the strain CAS 7 is a Gram-positive and endospore-forming bacillus, with catalase but without oxidase, which grows in both aerobic and anaerobic environments (Table 1). Further, 16S rRNA gene sequencing and BLAST analysis confirmed that the strain belongs to *B. firmus* and designated as *B. firmus* CAS 7 (GenBank accession no. HQ116805).The 16S rRNA gene sequences of the probiotic strain CAS 7 obtained from the present study was deposited in NCBI with accession number HQ116811.

The results revealed that the weight of fishes increased with the increase in days of culture in all the experimental groups (Table.2). At the end of the experiment, it was found that there was significant weight gain in all the three experiments where fishes were fed with probiotic-supplemented basal feed. The weight gain was comparatively less in the control group. The weight gain was the highest in group 150 mg kg-1(80.95 g) followed by group 100 (67.26 g), 50 (57.37 g) and control groups (45.81 g). The specific growth rate (SGR) was significantly (P<0.01) higher in 150 mg kg-1 group (0.675) compared with fishes in the group of 100 mg kg-1 (0.527), 50 mg kg-1 (0.457) and control (0.382). Furthermore, the most advantageous value for conversion ratio (FCR) classified as follows: 150 > 100 > 50 > control group (Table 3). The survival rate of the fishes was 80 - 100% (in control and 150 mg kg-1group, respectively) (P<0.01).

The results of the carotenoid analysis revealed that the initial carotenoid content of the fish skin varied between 1.24 and 1.28 mg g-1 in all experimental groups and control and increased gradually with increasing days of culture. At the end of the experimental period, carotenoid content was 2.91 - 6.24 mg g-1 (in control and 150mg kg-1 group) (Table 3) (P<0.01) (Table.3). Thus, these results depicted that the carotenoid content of fish group fed with feed was supplemented with 150 mg kg-1 of probiotic was the highest. The research on the use of probiotics in aquatic animals has received heightened attention with the demand for environment friendly health management in aquaculture (Geng et al., 2011). A growing number of studies have demonstrated the ability of probiotics to increase the growth rate and / or feed utilization of farmed aquatic animals (Carnevali et al., 2004; Wang et al., 2008). The benefits of probiotics in fish farming are improvements of growth performances, immunity and pathogen exclusions (Qi, et al., 2009; Sun et al., 2010). Most of the probiotic studies have focused on use of *Lactobacillus* and *Bacillus spp.* (Lee, 2013).

Bacillus species are gaining more and more importance and are widely used in aquaculture due to their longer stability, easy preparedness, antagonistic effects on pathogens and enhancement of immunity (Hong et al., 2005; Gupta et al., 2014). Earlier studies suggested that the Bacillus species significantly improved the growth in Catla catla (2 × 105 B. circulans PB7 cells per 100 g feed) (Bandyopadhyay & Mohapatra ,2009), Labeo rohita (1.5 x 105 *B. circulans*) (Ghosh et al., 2003), Macrobrachium rosenbergii (*Artemia salina nauplii* with *B. subtilis* (108cells mL-1) (Keysami et al., , 2007) and Penaeus monodon (Rahiman, et al.2010). Although several studies have been undertaken to study the beneficial effects of probiotic bacteria on growth and survival rate of various organisms, no studies have been attempted on marine ornamental fishes, especially on marine angel fish, *Apolemichthys xanthurus*. Hence, an attempt was made in this study to ascertain the effect of probiotic, Bacillus firmus CAS 7 as feed supplement on growth, survival and colour enhancement in marine angelfish *Apolemichthys xanthurus*.

In the present study, the fishes were fed basal diet supplemented with probiotic *B. firmus* CAS 7 at 50, 100 and 150 mg kg-1 and the growth performance such as weight gain, survival rate and color enhancement was evaluated for 120 days. Results suggested that the growth, weight gain and SGR were comparatively higher in fish group fed with feed supplemented 150 mg kg-1 of probiotic than other experimental groups and control. Other reports also reported that the probiotic (Bacillus *sp.*) supplemented diet (bio encapsulated *Artemia* nauplii by 3×108 CFU/ L) significantly increased the weight, length and SGR of fish when compared to the control diet. Several studies suggested that the probiotic supplementation has significantly increased the weight gain and SGR in *Rachycentron canadum* (Geng et al., 2012), Labeo rohita (Giri, Sukumaran, & Oviya, 2013) (Giri et al., 2013), *Oreochromis niloticus* (Aly et al., 2008), *Epinephelus coioides* (Sun et al., 2010) and *Larimichthys crocea* (Ai et al., 2011).

The results suggested that the FCR was lower in the fish group fed with feed supplemented 150 mg kg-1 probiotic followed by 100 and 50 150 mg kg-1 and control. It seems that the reduction in FCR of fishes in experimental groups revealed dietary nutrients were utilized more efficiently when the diet was supplemented with probiotics. Similarly, Neja, Rezaei, Takami, Lovett, Mirvaghefi, & Shakouri (2006) reported that *Bacillus spp.* could be used to increase the digestive enzyme activity, survival and growth in the Indian white shrimp.Fishes

are incapable of biosynthesizing carotenoids, so diet is their sole source as only plants, bacteria, fungi and algae have the capacity for its synthesis (Geng et al., 2011). Many reports have demonstrated that skin color change over time depended on the level of carotenoid in the diet and differed among species (Chatzifotis, Pavlidis, Jimeno, Vardanis, Sterioti, & Divanach, 2005; Dharmaraj, & Dhevendaran, 2011; Ho, Zong, & Lin, 2014). The results of the study on color enhancement suggested that the carotenoid content in the skin of fishes fed with probiotic maintained coloration during periods of social interaction, suggesting that the probiotic Bacillus firmus CAS7 may play important roles in maintaining fish skin coloration. Steiger, (2012)& Liu et al., (2009) were suggested that carotenoids are commonly found in pigmented bacteria which are known to have a positive role in the intermediary metabolism of fish that could enhance nutrient utilization.The research on the use of probiotics in aquatic animals has received heightened attention with the demand for environment friendly health management in aquaculture (Geng et al., 2011).

A growing number of studies have demonstrated the ability of probiotics to increase the growth rate and / or feed utilization of farmed aquatic animals (Carnevali et al., 2004; Wang et al., 2008). The benefits of probiotics in fish farming are improvements of growth performances, immunity and pathogen exclusions (Qi et al., 2009; Sun et al. 2010). Most of the probiotic studies have focused on use of Lactobacillus and Bacillus spp. (Cabello et al., 2013; Lee, Kim, Song, Oh, Cha, Jeong, Heo, Kim, & Lee, 2013). Bacillus species are gaining more and more importance and are widely used in aquaculture due to their longer stability, easy preparedness, antagonistic effects on pathogens and enhancement of immunity (Hong, Le Duc, Cutting, 2005; Gupta et al., 2014).

Table 3. Growth performance and survival rate of smoke angel fish Apolemichthys xanthurus fed with basal diet (control -without probiotic) and basal diet supplemented with 50, 100 and 150 mg kg-1 of probiotic strain B. firmus CAS7. Values are presented in mean ± SD, n = 3, FCR - feed conversion ratio; SGR - specific growth rate (% d-1)

Days of culture	Growth Parameters	Control	50 mg kg^{-1}	100 mg kg^{-1}	150 mg kg^{-1}
0 - 30	Initial weight (g)	12.33±0.38	12.57±0.29	12.63±0.32	12.89±0.41
	Final weight(g)	20.27±1.60	25.94±1.20	29.93±1.40	30.13±1.10
	Weight gain (g)	7.94±1.60	13.37±0.29	17.3±0.30	17.24±1.20
	SGR	0.265±0.03	0.446±0.04	0.577±0.02	0.641±0.03
	FCR	2.33±0.04	1.38±0.03	1.07±0.03	1.07±0.02
	Survival rate (%)	80	90	90	100
	Carotenoid content (mg g-1)	1.24±0.06	1.28±0.08	1.25±0.04	1.25±0.06
0 - 60	Initial weight (g)	12.33±0.38	12.57±0.29	12.63±0.32	12.89±0.41
	Final weight(g)	32.83±1.50	39.92±1.60	45.57±1.10	51.56±1.30
	Weight gain (g)	20.5±1.50	27.35±1.20	32.94±1.40	38.67±1.40
	SGR	0.342±0.03	0.456±0.02	0.549±0.01	0.763±0.02
	FCR	1.80±0.05	1.35±0.02	1.12±0.03	0.96±0.05
	Survival rate (%)	80	90	100	100
	Carotenoid content (mg g-1)	2.54±0.03	3.14±0.03	3.79±0.05	4.38±0.06
0 - 90	Initial weight (g)	12.33 ± 0.38	12.57 ± 0.29	12.63 ± 0.32	12.89 ± 0.41
	Final weight(g)	46.24±0.80	55.32 ±1.50	61.02 ±1.30	72.56 ± 0.90
	Weight gain (g)	33.91±1.60	42.75 ±1.50	48.39±1.30	59.67±0.90
	SGR(% day-1)	0.377±0.04	0.475±0.02	0.552±0.05	0.730±0.02
	FCR	1.64±0.05	1.30±0.03	1.15±0.06	0.93±0.01
	Survival rate (%)	80	90	90	100
	Carotenoid content (mg g-1)	2.76±0.05	3.55±0.07	4.01±0.04	5.69±0.004
0 - 120	Initial weight (g)	12.33±0.38	12.57±0.29	12.63±0.32	12.89±0.41
	Final weight(g)	58.14±1.3	69.94±1.20	79.89±0.90	93.84±1.40
	Weight gain (g)	45.81±1.4	57.37±1.30	67.26±1.40	80.95±1.50
	SGR	0.382±0.03	0.457±0.03	0.527±0.02	0.675±0.04
	FCR	1.61±0.03	1.29±0.03	1.10±0.04	0.91±0.02
	Survival rate (%)	80	90	90	100
	Carotenoid content (mg g-1)	2.91±0.08	4.26±0.06	5.74±0.03	6.24±0.05

Earlier studies suggested that the Bacillus species significantly improved the growth in Catla catla (2 × 105 B. Circulans PB7 cells per 100 g feed) (Bandyopadhyay & Mohapatra ,2009), Labeo rohita (1.5 x 105 *B. circulans*) (Ghosh et al., 2003), Macrobrachium rosenbergii (*Artemia salina nauplii* with *B. subtilis* (108cells mL-1) (Keysami et al., 2007) and *Penaeus monodon* (Rahiman et al., 2010). Although several studies have been undertaken to study the beneficial effects of probiotic bacteria on growth and survival rate of various organisms, no studies have been attempted on marine ornamental fishes, especially on marine angel fish, *Apolemichthys xanthurus*. Hence, an attempt was made in this study to ascertain the effect of probiotic, *Bacillus firmus* CAS 7 as feed supplement on growth, survival and colour enhancement in marine angelfish *Apolemichthys xanthurus*.

In the present study a *B. firmus* CAS 7 isolated from marine environment was cultured in nutrient broth for 48 h and used as feed supplement to evaluate the improvement in growth and colour enhancement on marine smoke angel fish. The growth kinetic study revealed that the maximum cell growth of the probiotic bacterium was achieved at logarithmic phase (24th h). Elayaraja et al., (2011) studied the effect of amylase produced by *B. cereus* on the growth of polycheate and maximum cell growth as well as enzyme production was found at late logarithmic phase (36th h). The short incubation period for achieving maximum growth makes this a potential probiotic candidate species which could be used in the aquaculture industry. The dietary supplementation of *B. firmus* CAS 7 in the present study exhibited good growth performance and significantly increased survival rate of *Apolemichthys xanthurus*. In a previous study, Bacillus administration has also been shown to increase survival by enhancing resistance to pathogens by acting both cellular and humoral immune defence in shrimp and prawn (Rengpipat et al., 2000; Gupta, & Dhawan, 2013).

Abraham et al., (2007) suggested that the probiotics greatly helps in achieving natural resistance and high survivability of fish. In the present study, the fishes were fed basal diet supplemented with probiotic *B. firmus* CAS 7 at 50, 100 and 150 mg kg-1 and the growth performance such as weight gain, survival rate and color enhancement was evaluated for 120 days. Results suggested that the growth, weight gain and SGR were comparatively higher in fish group fed with feed supplemented 150 mg kg-1 of probiotic than other experimental groups and control. Jafaryan et al., (2008) also reported that the probiotic (*Bacillus sp*.) supplemented diet (bio encapsulated *Artemia nauplii* by 3×108 CFU/ L) significantly increased the weight, length and SGR of fish when compared to the control diet. Several studies suggested that the probiotic supplementation has significantly increased the weight gain and SGR in *Rachycentron canadum* (Geng et al., 2012), Labeo rohita (Giri et al., 2013), *Oreochromis niloticus* (Aly et al., 2008), *Epinephelus coioides* (Sun et al., 2010) and *Larimichthys crocea* (Ai et al., 2011).

The results suggested that the FCR was lower in the fish group fed with feed supplemented 150 mg kg-1 probiotic followed by 100 and 50 150 mg kg-1 and control. It seems that the reduction in FCR of fishes in experimental groups revealed dietary nutrients were utilized more efficiently when the diet was supplemented with probiotics. Similarly, Nejad et al., (2006) reported that Bacillus spp. could be used to increase the digestive enzyme activity, survival and growth in the Indian white shrimp. Fishes are incapable of biosynthesizing carotenoids, so diet is their sole source as only plants, bacteria, fungi and algae have the capacity for its synthesis (Geng et al., 2011). Many reports have demonstrated that skin color change over time depended on the level of carotenoid in the diet and differed among species (Chatzifotis et al., 2005; Dharmaraj, & Dhevendaran, 2011; Ho et al., 2014). The results of the study on color enhancement suggested that the carotenoid content in the skin of fishes fed with probiotic maintained coloration during periods of social interaction, suggesting that the probiotic Bacillus firmus CAS7 may play important roles in maintaining fish skin coloration. Steiger, (2012) & Amar et al., (2001) were suggested that carotenoids are commonly found in pigmented bacteria which are known to have a positive role in the intermediary metabolism of fish that could enhance nutrient utilization and may ultimately result in improved growth. in improved growth.

CONCLUSION

The results confirmed that Apolemicthys xanthurus fed with 150 mg kg-1 probiotic bacteria B. firmus CAS 7 exhibited significantly improved growth performance, survival and colour enhancement. Therefore, 150 mg kg-1 of probiotic B. firmus CAS 7 should be used for formulating nutritionally balanced diet of marine angel fish for its better growth and survival. The mode and mechanism of actions about carotenoid content increase in the fish skin and carotenoid production by this probiotic strain need further investigation.

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Highlights

The fishes were fed basal diet supplemented with probiotic of B. firmus CAS 7 at 50, 100 and 150 mg kg-1

The growth performance such as weight gain, survival rate and color enhancement was evaluated for 120 days.

The probiotic effect of fish growth and disease resistant were showed promising activity.