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Isolation, identification, enzyme productivity and antibacterial activity of intestinal bacteria of Blue morph *Maylandia lombardoi* and its role on growth

Aislamiento, identificación, productividad enzimática y actividad antibacteriana de bacterias intestinales de Blue morph Maylandia lombardoi y su papel en el crecimiento

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ABSTRACT

Four distinct colonies were isolated from the intestine of Blue morph (*Maylandia lombardoi*) through serial dilution, pour plate, spread plate and streak plate method. The isolated colonies were identified by using biochemical and enzyme (Amylase, Cellulase, Lipase and Protease) productivity tests. The isolated bacteria such as *Bacillus sp.,* (BM1), *Enterobacter sp.,* (BM 2*), Escherichia sp*., (BM3) and *Pseudomonas sp*., (BM4) were mass multiplied in nutrient broth. Antibacterial activity of intestinal bacteria of Blue morph was carried out with the help of selective media along with commercial antibiotic Ampicillin. Based on higher enzyme productivity and antibacterial activity two bacteria were sequenced and the identified as BMI (*Lactobacillus casei)* and BM2 (*Lactobacillus acidophilus*).Five different feeds having different concentration of bacteria such as Feed I (Control)(without bacteria), Feed II (1ml of *Lactobacillus casei),* Feed III (1ml each of *Lactobacillus casei* and *Lactobacillus acidophilus),* Feed IV(1 ml each of *Lactobacillus casei, Lactobacillus acidophilus Bacillus sp.,* and *Escherichia sp.,)* and Feed V (1ml each of *Lactobacillus casei, Lactobacillus acidophilus Escherichia sp., and Pseudomonas sp.,)*were prepared by using fish meal, groundnut oil cake, wheat flour and tapioca flour. Feed utilization parameters of Blue morph were estimated after a period of 21 days. Based on the antibacterial test the *Lactobacillus acidophilus* (BM2) has higher inhibition. The feed consumption, feed conversion ratio, feed conversion efficiency, growth, percentage growth, relative growth rate, assimilation, metabolism, gross growth efficiency, and net growth efficiency was higher in feed IV.

Key words: Isolation, identification, enzyme, antibacterial activity, Blue morph, growth

RESUMEN

Se aislaron cuatro colonias distintas del intestino de Blue morph (Maylandia lombardoi) mediante el método de dilución en serie, placa de vertido, placa de extensión y placa de rayas. Las colonias aisladas se identificaron mediante pruebas de productividad bioquímica y enzimática (amilasa, celulasa, lipasa y proteasa). Las bacterias aisladas como Bacillus sp., (BM1), Enterobacter sp., (BM 2), Escherichia sp., (BM3) y Pseudomonas sp., (BM4) se multiplicaron en masa en caldo nutritivo. La actividad antibacteriana de las bacterias intestinales de Blue morph se llevó a cabo con la ayuda de medios selectivos junto con el antibiótico comercial Ampicilina. En base a una mayor productividad enzimática y actividad antibacteriana, se secuenciaron dos bacterias y se identificaron como BMI (Lactobacillus casei) y BM2 (Lactobacillus acidophilus) .Cinco alimentos diferentes con diferente concentración de bacterias como el Alimento I (Control) (sin bacterias), Alimento II (1 ml de Lactobacillus casei), Feed III (1 ml de Lactobacillus casei y Lactobacillus acidophilus), Feed IV (1 ml de Lactobacillus casei, Lactobacillus acidophilus Bacillus sp. Y Escherichia sp.,) Y Feed V (1 ml de Lactobacillus de cada uno) casei, Lactobacillus acidophilus Escherichia sp. y Pseudomonas sp.,) se prepararon utilizando harina de pescado, torta de aceite de maní, harina de trigo y harina de tapioca. Los parámetros de utilización de alimento de Blue morph se estimaron después de un período de 21 días. Según la prueba antibacteriana, el Lactobacillus acidophilus (BM2) tiene una mayor inhibición. El consumo de alimento, la tasa de conversión de alimento, la eficiencia de conversión de alimento, el crecimiento, el porcentaje de crecimiento, la tasa de crecimiento relativo, la asimilación, el metabolismo, la eficiencia de crecimiento bruto y la eficiencia de crecimiento neto fue mayor en el alimento IV.

Palabras clave: aislamiento, identificación, enzima, actividad antibacteriana, morfo azul, crecimiento.

INTRODUCTION

Aquaculture is the fastest growing food producing sector in the world. It has made significant advances in recent years in the production of a wide range of aquatic organism both for human consumption and as ornamental species (Balcazar *et al.,* 2003 and Kesarcodi-Waston *et* al.,2008). Per capita fish consumption in industrialized countries was 26.8kg. Global total capture fishery production in 2014 was 93.4 million tonnes, of which 81.5 million tonnes from marine water and 11.9 million tonnes from inland waters (FAO,2016). Fresh water aquaculture contributes to over 95% of the total aquaculture production. India is the second largest country in aquaculture production. In the global scene, ornamental fish keeping is considered as the second largest hobby next only to photography and popular for its aesthetic beauty. The success of ornamental fish culture

and breeding depends on the health status of the candidate species (Hossen, 2009). There are several problems, encountered for achieving successful culture of profitable ornamental fishes. Persistent disease problems, aquatic pollution due to various anthropogenic activities, indiscriminate use of different chemicals and antibiotics, constitute the major obstacles in successful ornamental fish culture (Witte *et al.,*2000). Ornamental fishes are susceptible to bacteria, virus, fungi, protozoa and parasitic organisms and cause loss to the produce. Among the various pathogens affecting the cultured fish species, bacteria cause severe damage. Control of the bacterial disease is made possible by using drugs and antibiotics. The traditional use of antibiotics as growth promoters in aquaculture has been challenged because of the potential development of antibiotic-resistant bacteria. The use of vaccines is laborious, costly and highly stressful to the animals. Since these methods have certain limitations, alternative, productive methods must be examined to reduce the incidence of the pathogen in ornamental fish culture. The bacterial community in the gut of aquatic animals is much more crowded compared to terrestrial animals, as water serves an ideal medium for bacterial growth. The microbial network in the gastrointestinal tract of fish is very complex and plays a vital role in fish nutrition and disease prevention. The composition of the community of microbes in the fish gut is not constant and may change with nutritional status, age, surrounding water and other environmental conditions (Banerjee and Ray, 2017). The normal microflora in the intestinal tract of the fish includes Pseudomonas spp. Aeromonous spp., Enterobacteriaceae spp., Micrococcus spp., Escherichia spp., and Bacillus spp and these bacteria play a vital role in fish nutrition and disease prevention. Bairagi et al. (2002) have reported the existence of several enzymes producing bacterial strains, isolated from different freshwater fishes having different feeding habits. The aim of the present study is the isolation, identification, enzyme productivity and antibacterial activity of intestinal bacteria of Blue morph *Maylandia lombardoi* and its role on growth.

MATERIALS AND METHODS

Fish collection**:** For the present study, Blue morph *Maylandia lombardoi* were collected from Arjun fish farm, Madurai, Tamil Nadu, India and transported to the laboratory in polythene bags filled with aerated water.

Bacterial strain isolation and identification: Intestinal content of Blue morph was collected by dissecting the abdomen of the fish were serially diluted and the appropriate dilution 10^{-5}were selected for the isolation of bacteria. The serially diluted sample was plated over sterilized nutrient agar medium and incubated at 37ºC for 24 hours. (Bergy's manual of Determinative Bacteriology,1994).Nutrient agar was used for present study, different incubation temperature were used in order to obtain a wider range of

isolation and the incubation time range from 24 hours, depending on the incubation temperature , colonies were counted and isolated. After incubation for an appropriate time the colonies on the nutrient agar were enumerated. The predominant colonies on the nutrient agar medium were selected and identified based on the cellular morphology, microscopic and biochemical characteristics. The tests used for examining the colonies were Indole, Methyl Red, Vogues prokauser, Citrate, Catalase, Gelatin hydrolysis, Starch, Sucrose and Lipase test and identified at genus level of bacteria (Rajan and Selvi Christy,2010). The intestinal bacteria of blue morph were examined for the productivity of digestive enzymes like Amylase, Cellulase, Protease and Lipase using selective media. (Muge Aliye Hekimoglun et al.,2014). Based on higher enzyme productivity two bacteria were sequenced and the identified intestinal bacteria are *Lactobacillus casei* (BM1) and *Lactobacillus acidophilus* (BM2). Antibacterial activity of intestinal bacteria: Selected intestinal bacteria were examined for Double layer screening antibacterial activity using selective media. (Jawahar Abraham 2008). The different pathogens selected are Staphylococcus aureus, Shigella sonnei, Enterococcus faecalis, and Pseudomonas aeruginosa. he isolated Bacillus species, Enterobacter species, Escherichia species, Pseudomonas species (10^{-5}cells) were mass multiplied by inoculating into the nutrient broth.

Collection and acclimation of fish:For growth studies, Blue morph (3.66±0.37g) were collected from Arjun fish farm, Madurai, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in glass aquaria (60×45×45 cm) for a period of 10 days at 28±2ºC**.** During acclimation fishes were fed with trainee feed containing fish meal, groundnut oil cake, wheat flour and rice bran in the form of dry pellets.

Experimental feed preparation: The raw material is selected based on their ability to supply nutrients such as protein, carbohydrates and fats at low cast. The major ingredients used in the feed are fish meal, groundnut oil cake, wheat flour and tapioca. After knowing the protein content by Micro-Kjeldahl method (Jeyaraman,1992), one control (without bacteria), four experimental feeds were prepared by using different isolated bacteria(Ali, 1980) (Table 1).The ingredients used for feed preparation was dried, powdered and sieved through 425-micron sieve. The ingredients were weighed and mixed thoroughly with 130-150 ml of distilled water. The mixed feed stuff was put in autoclave for 15mins at 100ºC and cooled. After cooling, fish oil, sunflower oil, supplevite-mix, sodium chloride and sodium benzoate. Five different feeds such as feed I(control-without bacteria), Feed II (1ml of *Lactobacillus casei),* Feed III (1ml each of *Lactobacillus casei* and *Lactobacillus acidophilus),* Feed IV(1 ml each of *Lactobacillus casei, Lactobacillus acidophilus Bacillus sp.,* and *Escherichia sp.,)* and Feed V (1ml each of *Lactobacillus casei,*

Lactobacillus acidophilus Escherichia sp., and Pseudomonas sp.,). The feed stuff was mixed well and then it was extruded with the help of pelletizer. The pellets were dried in room temperature. This formulated feed was kept in the air tight container at -20ºC until used to prevent contamination.

Table 1. Composition of Different Ingredients in Experimental Feeds (g/100gm)

Ingredients	Experimental Feeds				
	Feed I (Control)	Feed II	Feed II	Feed IV	Feed V
Fish Meal	33.75	33.75	33.75	33.75	33.75
GNOC*	33.75	33.75	33.75	33.75	33.75
Wheat Flour	11.25	11.25	11.25	11.25	11.25
Tapioca	11.25	11.25	11.25	11.25	11.25
Fish oil	2	2	2	2	2
Sunflower oil	2	2	2	2	2
Supplevite-Mix	4	4	4	4	4
Sodium chloride	1	1	1	1	1
Sodium benzoate	1	1	1	1	1
Microbes(10^{-5}cells)	-	1ml of *Lactobacillus casei*	1ml each of *Lactobacillus casei* and *Lactobacillus acidophilus*	1ml of *Lactobacillus casei, Lactobacillus acidophilus and*	1ml of *Lactobacillus casei Lactobacillus acidophilus, Escherichia sp., and Pseudomonas sp.,*

GNOC* -Groundnut oilcake

Experimental design for growth studies: For the present study, uniform size of Blue morph (*Maylandia lombardoi*) (3.66 ± 0.36) was selected and then the fishes were introduced in the rectangular glass tanks (45cm L×22cm B×22cm H) having a capacity of 18 liters. Five fishes were introduced in each tank. For each treatment triplicates were maintained. During rearing, the fishes were fed on ad-libitum diet of the prepared feed twice a day for 1 hour each from 9-10 am and 4-5 pm. The unfed were collected after one hour of feeding without disturbing the fishes. The unfed was dried to constant weight. The faecal matter was collected daily before changing the water with least disturbance to the fishes and dried at 95ºC. Approximately, 70% of water in the tank was replaced with tap water. The experiment was continued for 21 days. On the 21st day length and weight of the fishes were measured in live condition.

Statistical Analysis: The experimental results are presented in the form of tables and graphs using MS EXCEL (Version 2007). Mean, Standard deviation and T-test were

also calculated with the help of the same tool, One-way ANOVA method was used for the analysis using MS EXCEL (Version 2007). The data was input manually and computed. The output results obtained from the software indicate whether the difference is between the treatments and days. Sum of square variations (SS), Degree of freedom (DF), Variability of sample means (MS), Critical probability value (F) and probability (prob.) were also obtained.

RESULTS AND DISCUSSION

The organism isolated from the intestinal content was identified using biochemical tests and enzymatic productivity (Table 2 & 3).(*Bacillus sp.,* are rod shaped, gram positive and either obligate aerobic or facultative anaerobic. *Enterobacter sp*., is also rod shaped but gram- negative bacteria. *Bacillus* can promote survival and growth, by stimulating the immune system and by controlling pathogenic bacteria (Ai, *et al.,*2011 and Vaseeharan and Ramasamy 2003). Test for the production of digestive enzymes like Amylase, Cellulase, Lipase and Protease in intestinal bacteria of Blue morph was carried out with the help of selective media. Based on the test the identified bacteria were *Escherichia sp., Pseudomonas sp., Bacillussp.,* (BM2), (BM3), (BM4) found to be producing higher amount of digestive enzymes. Paramita Das (2014) reported the production of digestive enzymes in brackish water fish species fed by plant food stuffs. Muge Aliye Hekimoglu (2014) reported the production of digestive enzymes in koi carp fed by *Artemia nauplii.*

Table 2. Biochemical characterization of intestinal bacteria of Blue morph

Test	BM 1	BM2	BM3	BM4
Simple staining	Bacillus	Rod	Rod	Cocci
Gram staining	Positive	Positive	Positive	Positive
Indole test	Negative	Negative	Negative	Negative
Methyl red test	Positive	Positive	Positive	Positive
Voges proskawer test	Negative	Negative	Negative	Negative
Catalase test	Negative	Negative	Positive	Negative
Citrate test	Positive	Positive	Positive	Positive
Starch test	Positive	Positive	Negative	Negative
Lactose test	Negative	Negative	Negative	Negative
Sucrose test	Negative	Negative	Negative	Negative
Gelatin test	Negative	Negative	Negative	Negative
Identification result	*Bacillus sp.,*	*Enterobacter sp.,*	*Escherichia sp.,*	*Pseudomonas sp.,*

Table 3. Enzyme productivity of intestinal bacteria of Blue morph

S.No	Intestinal Bacteria	Amylase	Cellulase	Lipase	Protease
1	BM1(*Bacillus sp*.,)	+++	++	++	+++
2	BM2(*Enterobacter sp.,)*	+++	++	+	+++
3	BM3(*Escherichia sp.,)*	+++	+	++	++
4	BM4(*Pseudomonas sp.,)*	+	++	+	+

\+ - Nil (Absent) or (Negative) ++ - Low productivity (Positive) +++ - Higher productivity (Positive)

Antibacterial activity of intestinal bacteria of Blue morph was presented in table 4. Based on the test the *Bacillus sp.,* (BM1) has shown higher resistance towards the pathogenic bacteria. Shubhankar Ghosh *et al* (2014) reported the antimicrobial activity of lactic acid bacteria from marine fish *Rastrelliger kanagurta* against fish shrimp and human pathogen. Anita Bhatnagar and Ritu Lamba (2014) reported the antimicrobial activities of lactic acid bacteria strains separated from Nile tilapia *Oreochromis niloticus.* Karthikeya and Santosh (2009) have isolated a strain of *Lactobacillus plantarum* from gut of *Penaeus monodon* and confirmed its antagonistic activity against a wide range of pathogenic bacteria.

Table 4. Antibacterial activity (Double layer screening) of intestinal bacteria of Blue morph

Intestinal bacteria	Zone of inhibition(mm)							
	P1	CA	P2	CA	P3	CA	P4	CA
BM1 (*Bacillus sp*.,)	12	6	10	6	8	2	14	7
BM2 (*Enterobacter sp.,)*	10	5	8	4	6	3	16	8
BM3 (*Escherichia sp.,)*	12	6	10	5	6	2	15	9
BM4 (*Pseudomonas sp.,)*	7	4	8	3	9	4	17	7

CA - Commercial Antibiotic (Ampicillin) P1 – Staphylococcus aureus
P2 – Shigella sonnei P3 – Enterococcus faecalis P4 – Pseudomonas aeruginosa

Based on the biochemical test, enzymatic and antibacterial activity the selected *Bacillus sp.,* (BM1) and *Enterobacter sp.,* (BM2) was studied at genus level by genetic sequencing and identified as *Lactobacillus casei*(BM1) and *Lactobacillus acidophilus* (BM2)(Fig.1 & 2).Suganya et al (2014) reported the sequence of intestinal bacteria isolated from the intestine of gold fish. Yuniar Mulyani et al (2018) reported the sequencing of *Bacillus subtilis* isolated from the intestine of common carp.

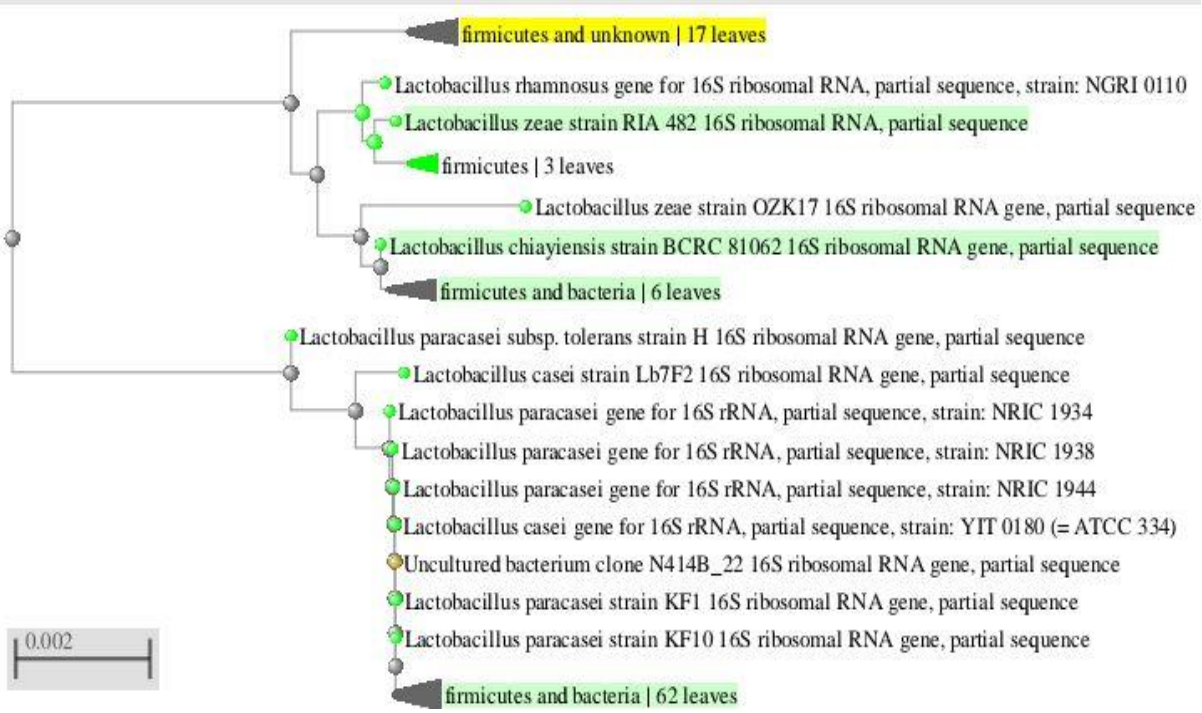
BM 1 -CODING

TGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAGT
TTTGGTCGATGAACGGTGCTTGCACTGAGATTCGACTTAAAACGAGTGGCGGACGGGTGAGTAA
CACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAAAT
CCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGCAAGCTATCGCTTTTGGATGGACCCGCGG
CGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGATGATACGTAGCCCCACTGAGAGGT
TGATCGGCCACATTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCT
TCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAA
AACTCTGTTGTTGGAGAAAAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAACCA
GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGG
ATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAAC
CGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGT
AGCGGTGAAATGCGTAGATATATGGAAGAACACCAGCCAGTGGCGAAGGCGGCTGTCTGGTCT
GTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACATCATTAGATACCCTGGTAGTCCATG
CCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTA
AGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA
CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTT
TTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGTTGTCG
TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATGACTAGTTGC
CAGCATTGAGTTGGGCACTCTAGTAAGACTGCCGGTGAC AAACCGGTGG

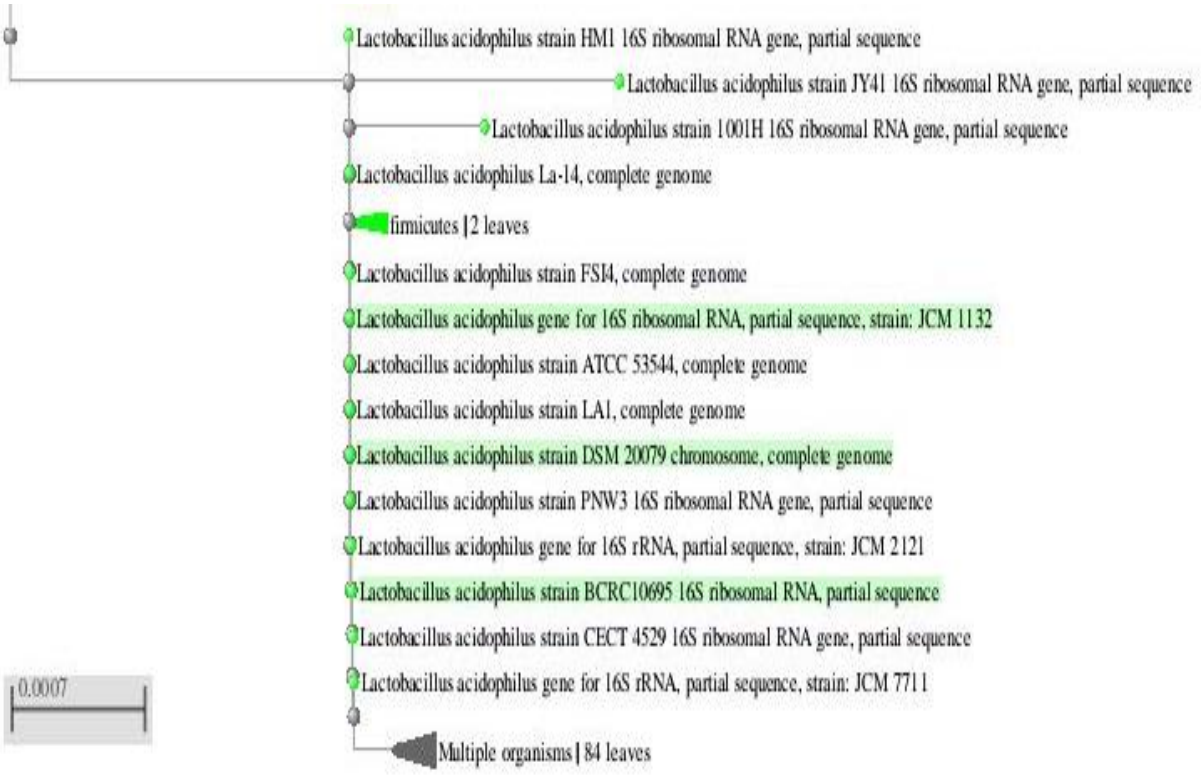
BM 2 - CODING

AGAGTTTGATCCTGGCTCAGGACGAATGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGC
TGAACCAACAGATTCACTTCGGTGATGACGTTGGGAACGCGAGCGGCGGATGGGTGAGTAACA
CGTGGGGAACCTGCCCCATAGTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAA
AGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCGCGGT
GCATTAGCTAGTTGGTAGGGTAACGGCCTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACT
GATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCT
TCCACAATGGACGAAAGTCTGATGGGCCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAA
AGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAG
AAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAT
TTATTGGGCGTAAAGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACC
GAGGAACTGCATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAG
CGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTG
ACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAA
CGATGAGTGCTAAGTGCTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTC
CGCCTGGGGAGTACGTCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCAAT
CCGTAGAGATACGGAGTTCCCTTCGGGGACACTAAGACAGGTGGTGCATGGCTGTCGTCAGCTC
GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCATTAGTTGCCAGCATTA
AGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTC
ATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGTACAACGAGGAGCAAGCC
TGCGAAGGCAAGCGAATCTCTTAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCA
CGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCG GGCCTTG

Fig.1: Genetic code (sequence) of selected *Lactobacillus casei* (BM1)and *Lactobacillus acidophilus* (BM2)from the intestine of Blue morph



BM1



BM2

Fig.2: Phylogenetic trees of selected *Lactobacillus casei*., and *Lactobacillus acidophilus* from intestinal content of Blue morph

Condition factor (K) of Blue morph *Maylandia lombardoi* was estimated for comparative purposes to assess the feed. The final condition factor is increased in all the feeds(Table 5). Mohammad Bodrul Munir *et al*.,(2016) reported that the final condition factor was increased in phase 2. Shankar and Kulkarni (2005) reported that the condition factor provide information on the suitability of environment for survival, growth and reproduction of fish (*Notopterus notopterus*). Sivakumar et al., (2016) reported that the average initial condition factor of yellow molly was 1.84 and the final condition factor increased in feed V (2.65) when fed with *Escherichia fergusonii*. Suganya et al (2018) reported that the final condition factor in Zebrafish fed with Pseudomonas sp. in the feed.

Table 5. Condition Factor (K) of Blue morph

Feeds	Initial	Final
EX. Feed I (Control)	1.35 ± 0.95	1.41 ± 1.06
EX. Feed II	1.48 ± 1.04	1.52 ± 1.13
EX. Feed III	1.52 ± 1.07	1.64 ± 1.15
EX. Feed IV	1.61 ± 1.13	1.70 ± 1.28
EX Feed	1.54 ± 1.08	1.65 ± 0.99

Parameters	Experimental Feeds				
	Feed I (Control)	Feed II	Feed III	Feed IV	Feed V
Feed consumption (FC) (g/g live wt/ 21 days	3.09 ± 2.18[a]	3.12 ± 2.31	3.35 ± 2.52[c]	5.44 ± 3.84[d]	4.55 ± 3.21[e]
Feed Conversion Efficiency (FCE)	0.02± 0.014[a]	0.02 ± 0.014[b]	0.01 ± 0.007[c]	0.03 ± 0.021[d]	0.02 ± 0.014[e]
Feed Conversion Ratio (FCR)	7.41 ± 5.23[a]	4.60 ± 3.25[b]	5.68 ± 4.01[c]	6.43 ± 4.54[d]	4.46 ±3.15[e]
Growth (G) (g/g live wt/ 21 days	0.54 ± 0.38[a]	0.75 ± 0.53[b]	0.93 ± 0.65[c]	1.05 ± 0.74[d]	1.02 ± 0.72[e]
Percentage growth (PG) (%)	40.10± 2.38	36.76± 1.35	43.02± 1.19	46.77± 3.97	35.60± 2.85
Relative Growth rate (RGR)	1.4 ± 0.98[a]	1.2 ± 0.17[b]	1.5 ± 1.06[c]	1.6 ± 1.13[d]	1.45 ± 1.02[e]
Assimilation (A) (g/g live wt/ 21 days	0.43 ± 0.30[a]	0.34 ± 0.24[b]	0.58 ± 0.41[c]	1.64 ± 1.15[d]	0.8 ± 0.56[e]
Metabolism (M) (g/g live wt/ 21 days	0.11 ± 0.07[a]	0.7 ± 0.49[b]	0.4 ± 0.28[c]	0.8 ± 0.56[d]	0.72 ± 0.50[e]
Gross Growth Efficiency (GCF)	4.1 ± 2.89[a]	5.3 ± 3.74[b]	4.9 ± 3.46[c]	6.2 ± 4.38[d]	4.5 ± 3.18[e]
Net Growth Efficiency (NGE) (%)	10.25 ± 7.24[a]	12.2 ± 8.62[b]	11.75 ± 8.30[c]	12.46 ± 8.81[d]	11.85 ± 8.37[e]

Feed utilization and Growth parameters of Blue morph in relation to different bacteria were presented in table 6. ANOVA (Analysis of variance) of Growth parameters are (Feed consumption, growth, Gross Growth Efficiency, Net growth Efficiency) presented in Table 7. Feed consumption (FC) of Blue morph was higher in feed IV (5.44±1.85) containing each 1ml *of Bacillus sp., Enterobacter sp., Escherichia sp., Pseudomonas sp.* and lower in feed I control (3.09±2.18). Chandra and Rajan, (2009) also reported that the

feed consumption of Koi carp was higher in feed V containing 4ml of Lactobacillus.Bisht *et al.,* (2012) reported that the feed consumption in common carp (*Cyprinus carpio)* was higher (95%) in diet D3 and lower (85%) in diet D1.Deepika et al (2019) reported that the feed consumption of Blue gourami was higher in feed IV (5,4 ± 0.13) containing each 1 ml each of *Bacillus* sp, *Enterobactor sp., Aeromonas sp.* and lower in feed I (control) (3.1 ± 0.41)).Feed Conversion Efficiency (FCE) of Blue morph was higher in feed IV (0.03±0.021). In feed I,II, III and V feed conversion efficiency were gradually decreased. Asma Chaudhary and Javed Iqbal Qazi (2007) reported that the feed conversion efficiency of Rohu *Labeo rohita* was higher inSSF2 (44.09±4.25) and lower in control (35.97±4.06). Suganya et al (2018) reported that the feed Conversion Efficiency of Zebra fish was higher in feed V (4.09 ± 0.52) containing 4 ml of Pseudomonas sp Feed Conversion Ratio (FCR) of Blue morph was higher in feed IV (6.43±4.54) and lower in feed I (3.41±0.38).Parthasarathy and Ravi (2011) reported that the feed conversion ratio of *Catla catla* was higher in T1 control (47.6) and lower in T4 (14.52).Rachmawti and Samidjan (2018) reported that the feed conversion ratio of Common carp (*Cyprinus carpio*) was higher in A (1.70±0.07) lower in C (1.39±0.03) fed by phytase. Growth of Blue morph was higher in feed IV (1.05±0.74) and in feed I,II,III and V was decreased. Dhanraj *et al*., (2010) reported that the growth of Koi carp (*Cyprinus carpio var.koi)* was higher in diet 3(SCD) (0.32±0.07) lower in control (0.19±0.02).Suganya et al., (2014) and Sivakumar et al., (2014) reported that the growth was higher in feed V (0.548) and lower in feed I (0.2502) by mixing of Pseudomonas sp.Like the growth, the percentage growth of Blue morph was higher in feed IV (46.77±3.97) and feed I, II, III and V was decreased. Sivakumar *et al.,* (2014) reported that the percentage growth of common carp (*Cyprinus carpio var.communis*) was higher in feed V (51.12±22.30) and lower in feed I control (16.11±9.53).The Relative growth rate of Blue morph was higher in feed IV(1.6±1.13) and lower in feed I (1.4±0.98).Seenivasan *et al.,* (2012) stated that the relative growth rate of fresh water prawn *Macrobrachium rosenbergii* was increased when fed with *Bacillus substilis.* Pornthep Niamphithak et al (2017) reported lower relative growth rate (0.70 ± 0.09 to 0.79 ± 0.13) of Bocourti catfish (*P.bocourti*) with an initial average weight of 132.59 ± 1.20 g $fish^{-1}$ fed with diet containing *Lactobacillus plantarum*. Assimilation of Blue morph was higher in feed IV (1.64±1.15) lower in feed I (0.43±0.30). Rajan and Revathi (2011) reported similar assimilation in Platy. Metabolism of Blue morph was higher in feed IV (0.8±0.56) lower in feed I (0.11±0.07). Same result was also reported by Chandra and Rajan (2009) in Koi carp. Gross and Net growth efficiency of Blue morph was higher in feed IV and lower in feed I. Rajan and Revathi (2011) reported higher gross and net growth efficiency when Platy was fed with higher levels of *Bacillus substilis* in the feed. Sunil Kumar and Vishnu(2011) reported similar result when clown fish was fed with *Lacto bacillus.* Pushparaj et al., (2012) reported higher gross and net growth

efficiency when Platy was fed with higher levels of *Bacillus subtilis* in the feed. Deepika et al(2019) reported that the gross and net growth efficiency of Blue Gourami is higher in feed IV containing 1ml each of Bacillus sp., Enterobacter sp. and Aeromonas sp. From the results, it was concluded that the some of the feed utilization parameters such as Feed consumption, Feed Conversion Efficiency, Growth, Relative Growth Rate, Gross Growth Efficiency and Net Growth Efficiency were higher in feed IV containing each 1 ml each of *Lactobacillus casei, Lactobacillus acidophilus* and *Escherichia sp.*

Table 6. Feed utilization and Growth parameters of Blue morph in relation to different bacteria. Each value is the average (±SD) performance of 5 individuals in triplicates reared for 21 days.

Feed consumption	Growth	Gross growth Efficiency	Net Growth Efficiency
a vs b (p>0.05)S	a vs b (p>0.05) S	a vs b (p>0.05) S	a vs b (p>0.05) S
a vs c (p>0.05) S	a vs c (p>0.05) S	a vs c (p>0.05) S	a vs c (p>0.05) S
a vs d (p>0.05) S	a vs d (p>0.05) S	a vs d (p>0.05) S	a vs d (p>0.05) S
a vs e (p>0.05) S	a vs e (p>0.05) S	a vs e (p>0.05) S	a vs e (p>0.05) S

Table 7. ANOVA (Analysis of Variance) of Growth Parameters (Feed consumption, Growth, Gross Growth Efficiency, Net Growth Efficiency) of Blue morph (*Maylandia lombardoi*)

Parameters	Source	SS	Df	MS	F	Sig
Feed Consumption	Between groups	0.606	4	0.152	6.243	0.009
	Within groups	0.243	10	0.024		
	Total	0.849	14			
Growth	Between groups	0.40	4	0.010	1.387	0.306
	Within groups	0.72	10	0.007		
	Total	0.112	14			
Gross Growth Efficiency	Between groups	177.966	4	44.492	0.616	0.661
	Within groups	722.229	10	72.223		
	Total	900.195	14			
Net growth Efficiency	Between groups	373.153	4	93.288	3.270	0.059
	Within groups	285.303	10	28.530		
	Total	658.456	14			

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REFERENCES

Ai,Q.H., XU.,HG., Mai., K.S., XU., W and Wang, J. (2011). Effect of dietary supplementation of *Bacillus subtilis* and Fructo oligosaccharide on growth performance, survival rate, non- specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquaculture, 317, 155-16.

Ali ,S.A. (1980). Feed formulation method. Manual of research methods for fish and shell fish nutrition. CMFRI special publication, 8, 98.

Asma Chaudhary and Javed Iqbal Qazi (2007). Influence of probiotic *Pseudomonas pseudoalcaligens* fermented feed on growth performance of rohu (*Labeo rohita*) fingerlings. Punjab University Journal of Zoology, 2(1-2), 42-56.

Bairagi, A., Ghosh, K.S., Sen, S.K. (2002). Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International, 10, 109-121.

Balcazar, J.L. (2003). Evaluation of probiotic bacteria strains in *Litopenaeus vannamei.* Final Report, National Centre for Marine and Aquaculture Research, Guayaquil, Ecuador.

Banerjee, S. and K. Ghosh (2014). Enumeration of gut associated extra cellular enzyme producing yeasts in some fresh water fishes. Journal of Applied Ichthyology, DOI:10.1111/jai.12457.

Bergey's Manual of Determinative Bacteriology (1994)**.** Williams and Wilkins, 428 East Preston street, Baltimore, Maryland. 21202, USA.

Chandra, R and Rajan, M.R. (2009). Probiotic effect of intestinal bacteria of koi carp (*Cyprinus carpio var koi*). Journal of Pure and Applied Microbiology, 3(1), 363-366.

Deepika, G., P. Sivakumar and M.R. Rajan(2019) Isolation and Characterization of Bacteria from the Gut of Blue Gourami (*Trichogaster tricopters*) and its Role on Growth. Journal of Pure and Applied Microbiology, 13(4), 2479-2487.

Dhanraj, M., Hannifin, SV., Arun Singh., Jesu Arockiaraj, A., Muthu Ramakrishnan. C., Seetharaman, S and Arthimanju, R.(2010). Effect of probiotics on growth performance of Koi carp *(Cyprinus carpio)* Journal of Applied Aquaculture, 22, 202-209.

FAO (2016) The state of fisheries and aquaculture contributing to food security and nutrition
for all. World fisheries and Aquaculture 5: ISBN 978-92-5-109185-2

Hossen, M. (2009). Isolation of pathogenic bacteria from the skin ulcerous symptomatic Gourami (*Callisa lalia*) through 165 rDNA analysis. University Journal of Zoology, Rajshahi University, 27, 21 - 24.

Jawahar Abraham, T. (2008). Antagonistic fish gut bacterium *Lactobacillus sp.* as biocontrol agent in ornamental fish culture, Indian Journal of Fisheries, 45 (2), 223 - 228.

Karthikeya, V and Santosh, S.W (2009). Isolation and partial characterization of bacteriocin produced from *Lactobacillus plantarum*. African Journal of Microbiological Research, 3,233–239.

Kesorcodi - Watson., Kaspar, A. H., Lategan, M.J and Gibson, L. (2008). Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. Aquaculture, 274, 1 - 4.

Mohammad Bodrul Munir., Roshada Hashim., Mohammad Suhaimee., Abdul Manaf and Siti Azizah Mohd Nor (2016). Dietary prebiotics and probiotics influence of the growth performance, Feed utilization and Body indices of snakehead (*Channa striata*)fingerlings. Tropical Life Sciences Research, 27(2),111-125.

Muge Aliye Hekimoglu., Cuneyt Suzer., Sahia Saka and Kursat Firat.(2014). Enzymatic characteristics and growth parameters of ornamental koi carp (*Cyprinus carpio* Var. koi) larvae fed by *Artemia nauplii* and cyst. Turkish Journal of Fisheries and Aquatic Science, 14, 125 - 133.

Parthasarathy, R. and Ravi, D. (2011). Probiotic bacteria as growth promoter and biocontrol agent against *Aeromonas hydrophila* in *Catla catla* (Hamilton, 1822), Indian Journal of Fisheries, 58(3), 87 - 93.

Pornthep Niamphithak., Siripavee Chareonwattanasak and Sompong Doolgindachbaporn. (2017). Effect of Dietary Supplement of Probiotic (*Lactobacillus plantarum*) on Growth Performance, Feed Utilization and Survival Rate in Bocourti Catfish (*Pangasius bocourti* Sauvage, 1880). Journal of Pure and Applied Microbiology, 11(2), 803-809.

Pushparaj, A., Ramesh, U. and Ambika, P. (2012). Effect of probionts on the growth and food utilization of clown fish *Amphiprion sebae*. International Journal of Applied Biology and Pharmaceutical Technology, 3(1), 309-314.

Rachmawati, D. and I. Samidjan. (2018). Performance efficiency of feed utilization, relative growth rate, survival rate of common carp (*Cyprinus carpio*) through the addition of phytase in the feed. Earth and Environmental Science, DOI: 10.1088/1755- 13/5/137/1/012027.

Rajan, M.R and Revathi, U. (2011). Role of probiotics in Ornamental fish Platy *Xiphophorus maculates*. Journal of Pure and Applied Microbiology, 5(2), 81823.

Rajan, S and Selvichristy, R. (2010). Experimental procedure in Life Science, Anjana Book House, Old.28, New Delhi.16, South Mada street, Koyambedu, Chennai – 600 107.

Seenivasan, C., Saravana Bhavan, P., Radhakrishnan, S and Shanthi, R (2012) Enrichment of *Artemia nauplii* with *Lactobacillus sporogenes* for enhancing the survival, growth and levels of biochemical constituents in the post-larvae of the freshwater prawn *Macrobrachium rosenbergii*. Turkish Journal and Aquaculture Science, 12,23-31.

Shankar, D.S. and Kulkarni, R. S. (2005) Somatic condition of the fish *Notopterus notopterus* during different phases of the reproductive cycle. Journal of Environmental Biology, 26(1), 49-53.

Shubhankar Ghosh, A., D.G.Selvam., C.S. Neethu., A.V.Saramma and A.A.M. Hatha. (2014). Diversity and antimicrobial activity of Lactic Acid Bacteria from the gut of marine fish *Rastrelliger kanagurta* against fish, shrimp and human pathogens. Marine Biology Association of India, 55(2), 22-27.

Siva Kumar, P., Rajan, M.R and Ramachandran, P. (2014). Effect of probiotics on growth performance of common carp *Cyprinus carpio var communis.* International Journal of Pharma and Bioscience, 5(1)(B), 835- 839.

Siva Kumar, P., Rajan, M.R and Balakrishnan, R (2016). Enzymatic productivity and Molecular Characterization of Intestinal bacteria of Yellow molly (*Poecilia latipinna*) in relation to Growth. International Journal of Information Research and Review, 3(6), 2552-2555.

Suganya, D., Rajan, M. R. and Siva Kumar, P. (2014). Isolation, identification, enzyme and molecular characterization of intestinal bacteria of goldfish (*Carassius auratus*) and its role on growth. Indian Journal of Applied Research, 4 (7), 9 - 11.

Suganya, D., S. Anisha Banu., P. Sivakumar and M. R. Rajan. (2018**).** Impact of Probiotics on Growth Performance of Zebra fish (*Danio rerio*). International Journal of Current Research and Modern Education,3(1),473-478.

Sunil Kumar, P. and Vishnu, P. S. (2011). Effect of *Lactobacillus acidophilus* on growth of *Etroplus suratensis.* Fishing Chimes, 31(7), 54 - 55.

Vaseeharan, B. and P. Ramasamy. (2003). Control of pathogenic V*ibrio spp,* by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp (*Penaeus monodon*). Applied Microbiology, 36, 83-87.

Witte, W. (2000). Selective by antibiotics use in livestock. International Journal of Antimicrobial Agents, 16,19-24.

Yuniar Mulyani., I. Nyoman., P. Aryantha., Sony Suhandono and Adi Pancoro. (2018). Intestinal Bacteria of Common Carp (*Cyprinus carpio* L.) as a Biological Control Agent for *Aeromonas*. Journal of Pure and Applied Microbiology, 12(2), 601-610.

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