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SCREENING OF BACTERIOCIN PRODUCING BACILLUS PUMILUS PB1 ISOLATED FROM THE GUT OF TIGER SHRIMP, PENAEUS MONODON

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ABSTRACT

In the present study, probiotic bacterial isolates were isolated from the gut of Penaeus monodon. The isolated probiotics showed activity against the marine bacterial pathogens. The gut bacterial isolates were further screened for acid production test and only nine bacterial isolates were produced secondary metabolites. Based on morphological, biochemical and physiological factors the selected bacterium was identified as Bacillus pumilus PB1. The secretion of the metabolite was found only at the stationary period of the bacterial growth. It was found that the metabolite was detected at the incubation period of 2 to 3 days and metabolite production started to deplete after 5th day. Hence, optimum fermentation period for secondary metabolite production was 72 h of incubation at room temperature. Of the pH level tested, production of bioactive metabolites and biomass were maxium at pH 7.0. From the experiment it was very clear that the selected bacterial isolate showed maximum growth at 30 °C. mong the carbon sources tested, glucose favoured as good carbon sources for its growth and so preferred as good carbon source for bioactive metabolite production. Among the tested nitrogen sources, glycine enhanced the growth of bacteria and metabolite production. Among the ionic sources, supplemented Ca2+, Mg2+ and Mn2+ supported the growth of the bacterium.

Keywords: Probiotics, Bacteria, Shrimp, Gut, Bacteriocin, Antagonistic

1. INTRODUCTION

The term “probiotic” was introduced in 1974. According to this, probiotics are “substances and organisms which greatly contribute to intestinal microbial balance”. Further it was defined as” microbial live feed supplement which beneficially affects the host organism by improving its intestinal microbial balance. Further, various terms such as, “healthy”, “beneficial”, “friendly” are commonly used to describe probiotics in aquaculture practice. The application of probiotics in aquaculture was started in 1980s. Probiotics possess antimicrobial potentialities and their ability to modify the intestinal microbial flora, secrete antibacterial agents such as, bacteriocins and organic acids, compete against pathogens to prevent their adhesion to the intestine, compete for nutrients necessary for pathogen survival, and stimulate antitoxins thus reducing the requirement of antibiotics in aquaculture sector. Probiotic was used as feed additives to improve stress tolerance and reproductive performances of both ornamental species, including zebrafish and edible species. The application of probiotics in aquaculture was intended as a replacement of the massively used antibiotics [1].

Probiotic microbes have the ability to synthesize extracellular enzymes such as lipases, amylases and proteases well as provide the host with growth factors such as fatty acids, vitamins and amino acids. The physio-chemical

parameter of water such as, dissolve oxygen, pH, dissolved carbon dioxide, minerals and organic load were very important in bacterial colonization. Variation of these physio-chemical parameters, increasing favour on the growth of several facultative or obligate or various pathogenic bacterial strains such as *Edwardsiella*, *Staphlycoccous*, *Aeromonas*, *Pseudomonas, Citrobacter*, *Streptococcus, Proteus* and various species of *Vibrio*, which cause much mortality in both freshwater and brackish water fish [2]. Among several pathogens, *Aeromonas hydrophila* and *Aeromonas salmonicida* are considered to be the most common pathogens in freshwater fish, while *Vibrio anguillarum* and *Vibrio parahaemolyticus* are the most familiar bacterial pathogens in marine environment, which cause different types of fish disease like ulcer disease, carp erythrodermatitis, motile *Aeromonas septicemia* etc [3].

The successful selection of probiotic bacteria has possessed various important features, and few probiotics are available commercially. The selection of probiotic bacteria are differ slightly from one organism to another, as the mode of action of probiotic candidate varies from terrestrial to aquatic environment. The selected probiotic bacterial strain must be non pathogenic to candidate species. The degree of pathogenicity of probiotic mainly depends on toxin producing capability and it varies from one bacterial strain to another bacterial strain. For example, *Aeromonas hydrophila* is considered to be a deadly pathogen in fish, however certain strains of *Aeromonas hydrophila* are used as probiotic candidates in fish. Bio-safety of the selected probiotic candidate is also critically important. The *in vitro* techniques such as manitol utilization ability, haemolytic activity, and other biochemical tests have been widely used to check the bio-safety of the selected bacterial strains. *In vivo* tests also should be performed to confirm the non-pathogenic activity of the selected probiotic candidates. Pathogenicity of any bacterium is mainly determined on the basis of disease symptoms occur and mortality rate of fish [4].

In aquaculture industry, the emergence of multi-drug resistant bacteria is a great threat for finfish and shell fishes. The drug resistant property of bacteria comes from mainly the plasmid encoded genes. The successful probiotic bacterial strain must not have any plasmid-encoded antibiotic gene cluster or resistance gene. Under stress condition, mainly the presence of antibiotics, bacteria evolve rapidly due to their high mutation rate and this unique property can be transferred from one bacterial species to another bacterial species through lateral gene transfer mechanism. Hence, before performing a bacterial isolate as promising probiotic candidate in aquaculture practice, broad spectrum antibiotic sensitivity and PCR detection of multi drug resistant gene should be followed [5].

Due to intensive shrimp farming, the health management of cultured species is very important for the development of the industry. Monitoring of various environmental factors and maintaining of good water quality are prime important for disease free fish production. High stocking densities and subsequent over feeding cause water pollution, which enhances the susceptibility of various types of bacterial diseases, viral diseases, worm diseases, protozoan diseases, fungal diseases and crustacean diseases. Parasitic diseases in fish are very common in aquaculture, which cause a huge mortality in fresh water fish. The aquaculture is one of the rapidly growing food producing sectors in countries like India, China, India, Norway, Brazil, Sri Lanka, Malaysia, Sri Lanka, USA and Japan which mainly produce shrimps, molluscs, fish and crabs. At present, various types of probiotics or probiotics mixtures are used, but their applications in aquaculture sectors are still limited. Hence, commercialization of these probiotics candidates is important for sustainable aquaculture practise [6]. In the present study, probiotic bacteria were isolated from the gut of *Penaeus monodon* and screened for its antibacterial activity against the aquatic pathogens. Further, the efficient probiotic organism was subjected to produce secondary metabolites.

2. MATERIALS AND METHODS

2.1. ISOLATION OF BACTERIA FROM THE GUT OF *P. MONODON*

A total of five *P. monodon* was collected from the Manakudy estuary, Kanyakumari District, Tamilnadu, under free-living conditions. The physical parameters showed a water temperature between 28 and 30 °C, 10 ppt and pH of 7.5. *P. monodan* was caught by cast net. Shrimp of medium size (25 ± 2 g) was taken for analysis assuming that they might have a well-established pattern of intestinal microbial flora. Shrimps were transferred to water collected from the estuary and brought into the laboratory in rice. Upon reaching the laboratory, analysis of the intestinal microflora was done on samples consisting of excised washed intestines from five samples. The intestines were gently excised and cut open with a pair of sterile scissors. Gut content were removed by scrapping, and the intestines were washed three times with sterile saline solution to remove non-adherent microflora. The samples were then homogenized with 20 ml of

distilled water in Pestle and Mortar. Serial dilutions were prepared from the homogenates. The samples were spreaded on Zobell Marine Agar plates and individual colony was further isolated by repeat streaking.

2.2. SELECTION OF PROBIOTIC BACTERIA

2.2.1. PRILIMINARY SCREENING BY CROSS STREAK METHOD

Antimicrobial activity of isolated colonies was examined through cross-streak method using indicator strain. The bacteria was streaked on a straight line dividing the agar plate into equal half and incubated at 37 °C 24 h. Then indicator bacterial strains were perpendicularly streaked across the probiotic strains and incubated at 37 °C 24 h. Positive results are indicated by zone of inhibition around the test bacterial isolate. Isolates that did not show any inhibitory activity against indicator strains were discarded.

2.2.2. ACID PRODUCTION TEST

Isolated cultures were allowed for fermentation for 2 days and at 37 °C and the medium were collected. It was centrifuged at 10000 rpm for 20 min at room temperature. The supernatant obtained were analyzed for acid production using bromothymol blue solution. Acid producing colonies changed their color of the solution to yellow while bacteriocin producing colonies did not show any colour change.

2.2.3. AGAR WELL DIFFUSION METHOD

The isolates showed negative results in acid production test were inferred to be bacteriocin producing. Further quantitative analysis of the colonies was done using agar well diffusion. Five indicator strains were used and the activity of the bacteriocin produced by selected isolates was determined. Isolates were inoculated in 100 ml nutrient broth medium and incubated for 48 h at 37 °C. These samples were than centrifuged at 10,000 rpm for 10 min to obtain the cell-free supernatant and 50 µl of which was then was aliquoted in the wells of Meuller Hinton Agar (MHA) plates. These were seeded with indicator strains such as, *Micrococcus* sp., *Bacillus* sp., and *Pseudomonas* sp., *Vibrio* sp. and *Staphylococcus* sp. The zone of inhibition was recorded for further analysis.

2.3. AGAR WELL DIFFUSION METHOD FOR THE DETERMINATION OF ANTIMICROBIAL ACTIVITY

Quantitative analysis of the colonies was done using agar well diffusion. Five indicator strains were used and the activity of the bacteriocin produced by selected isolates was determined. Isolates were inoculated in 100 ml nutrient broth medium and incubated for 48 h at 37 °C. These samples were than centrifuged at 10,000 rpm for 10 min to obtain the cell-free supernatant and 50 µl of which was then were aliquoted in the wells of Meuller Hinton Agar (MHA) plates. These were seeded with indicator strains such as *Micrococcus* sp., *Bacillus* sp., and *Pseudomonas* sp., *Vibrio* sp. and *Staphylococcus* sp. The zone of inhibition was recorded for further analysis.

2.4. OPTIMIZATION OF SECONDARY METABOLITE PRODUCTION TO ENHANCE THE YIELD

Detailed investigation was conducted to establish the most suitable medium for secondary metabolite production by *B. pumilus* PB1. Various parameters were optimized for the maximum production of secondary metabolite. To evaluate the suitable media for growth and bioactive metabolite production, the strain was cultured in different factors such as, fermentation period, pH, temperature, carbon sources, nitrogen sources and ionic sources. The cell growth and the yield of metabolites by the strains were recorded. The medium in which the strain exhibits optimum level of bioactive metabolites was fixed for further studies.

2.4.1. EFFECT OF FERMENTATION PERIOD ON THE GROWTH AND METABOLITE PRODUCTION

To study the influence of fermentation period on the growth and the production of bioactive metabolites of the candidate strain, the production medium was cultured at various fermentation periods (1- 7 days) by keeping the other

factors constant. After every 24 h, the cell growth and the yield of bioactive metabolites by the strains were recorded from the culture supernatant.

2.4.2. EFFECT OF PH ON THE GROWTH AND BIOACTIVE COMPOUND PRODUCTION

To study the influence of pH on the growth and the production of bioactive metabolites of the candidate strain, the selected organism was cultured at various pH values (pH 4.0 – 9.0) by keeping the other factors constconstant. After 96 h, the cell growth and the yield of bioactive metabolites by the strains were recorded from the culture supernatant.

2.4.3. EFFECT OF TEMPERATURE ON THE GROWTH AND SECONDARY METABOLITE PRODUCTION

To study the influence of temperature on the growth and the production of bioactive metabolites of the candidate strain, the production medium was cultured at various temperatures (20-45 °C) by keeping the other factors constconstant. After 96 h, the cell growth and the yield of bioactive metabolites by the strains were recorded from the culture supernatant.

2.4.4. EFFECT OF CARBON SOURCE ON THE GROWTH AND SECONDARY METABOLITE PRODUCTION

To study the influence of carbon sources on cell growth and the production of bioactive metabolites of the strain, the basal medium was supplemented with different carbon sources such as glucose, glycerol, starch, lactose, maltose and sucrose each at a level of 1% (w/v) by keeping the other ingredients constant. The culture medium without any of the above carbon sources was treated as control. After 96 h, the cell growth and the yield of bioactive metabolites by the strains were recorded from the culture supernatant.

2.4.5. EFFECT OF NITROGEN SOURCE ON THE GROWTH AND SECONDARY METABOLITE PRODUCTION

To study the influence of nitrogen sources on cell growth and the production of bioactive metabolites of the candidate strain, the production medium was supplemented with different nitrogen sources such as ammonium chloride, sodium nitrate, peptone, beef extract, yeast extract and glycine each at a level of 1% (w/v) by keeping the other ingredients constant. The culture medium without any of the above nitrogen sources was treated as control. After 96 h, the cell growth and the yield of bioactive metabolites by the strains were recorded from the culture supernatant.

2.4.6. EFFECT OF IONS ON THE GROWTH AND SECONDARY METABOLITE PRODUCTION

To study the influence of ionic sources on cell growth and the production of bioactive metabolites of the candidate strain, the production medium was supplemented with different ionic sources such as Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+} and Hg^{2+}each at a level of 0.1% (w/v) level by keeping the other ingredients constant. The culture medium without any of the above nitrogen sources was treated as control. After 96 h, the cell growth and the yield of bioactive metabolites by the strains were recorded from the culture supernatant.

3. RESULTS

3.1. ISOLATION OF BACTERIAL ISOLATES FROM THE FISH GUT

In the present study, various bacterial isolates were isolated from the gut of *P. monodon*. A total of 13 bacterial isolates were isolated from the fish gut based on morphological characters of bacteria. All cultures were subjected to screen its probiotic properties.

3.2. SCREENING OF PROBIOTIC ISOLATES FOR SECONDARY METABOLITE PRODUCTION

The isolated probiotics showed activity against the marine pathogens such as *Micrococcus* sp. *Bacillus* sp. and *Pseudomonas* sp., *Vibrio* sp. and *Staphylococcus* sp. The zone of inhibition was measured and was tabulated. The isolate M1 exhibited a zone of inhibition of 10 mm against *Micrococcus* sp. 10 mm in *Pseudomonas* sp. But it failed to control the growth of *Vibrio* sp. The probiotic isolate M3 was able to control the growth of *Bacillus* sp. with 11 mm zone of inhibition. But it was not able to control *Pseudomonas* sp. and *Vibrio* sp. The strain M4 was able to control *Micrococcus* sp. with 10 mm, *Pseudomonas* sp. with 12 mm and *Vibrio* sp. with 10 mm zone of inhibition. The isolate M5 exhibited zone of inhibition against *Micrococcus* sp. with 14 mm, *Pseudomonas* sp. with 12 mm and *Vibrio* sp. with 10 mm, and proved good controlling effect against the tested pathogens. The isolate M6 has no activity against the tested pathogens. The probiotic M7 possess activity against *Micrococcus* sp. with 10 mm zone of inhibition, and *Pseudomonas* sp. with 11 mm zone of inhibition, but did not show any activity against *Vibrio* sp. The probiotic M8 did not show any controlling effect on the tested pathogens. The probiotic isolate M9 showed potent zone of inhibition of *Micrococcus* sp. with 15 mm, *Pseudomonas* sp. with 12 mm and *Vibrio* sp. with 14 mm and thus proved as a good probiotic candidate among the isolated gut probiotics (Table 1). The antagonistic activity of the isolated potent strain against marine pathogen *Micrococcus* sp., and *Pseudomonas* sp. (was documented.

Table 1 Antagonistic Activity of the Probiotic Bacterial Isolates (M1 To M9) Against Three Fish Pathogenic Bacteria

Gut isolates	Zone of inhibition (mm)		
	Bacillus sp.	*Pseudomonas* sp.	*Vibrio* sp.
M1	10	10	---
M2	--	--	---
M3	11	--	--
M4	10	12	10
M5	14	12	12
M6	--	--	---
M7	10	11	---
M8	---	---	---
M9	15	12	14

3.3. ACID PRODUCTION TEST

The gut bacterial isolates were further screened for acid production test and only nine bacterial isolates (M1, M2, M3, M4, M5, M6, M7, M8 and M9) were secondary metabolite positive.

3.4. SECONDARY METABOLITE PRODUCTION

The nine bacterial isolates (M1, M2, M3, M4, M5, M6, M7, M8 and M9) were further screened for secondary metabolite production by the agar well diffusion method using pathogenic bacterial strains. The results showed that 9 isolates gave positive inhibition zones (7 – 21 mm). The antimicrobial activity of *Bacillus pumilus* PB1 was relatively higher than the other screened probiotic bacterial isolates. Hence this organism was selected for further studies.

3.5. IDENTIFICATION OF THE PROBIOTIC ISOLATE

The identified strain was Gram positive, rod shaped, motile, hydrolyzed starch, catalase- and casein positive. Whereas, this strain showed negative results towards urea hydrolysis, nitrate reduction, indole test and citrate test. Based on morphological, biochemical and physiological factors the selected bacterium was identified as *Bacillus pumilus* PB1.

3.6. OPTIMIZATION OF BACTERIAL GROWTH AND METABOLITES PRODUCTION

3.6.1. EFFECT OF FERMENTATION PERIOD ON THE GROWTH AND PRODUCTION OF SECONDARY METABOLITES

The effect of biomass and secondary metabolite production in relation with fermentation period was studied. The secretion of the metabolite was found only at the stationary period of the bacterial growth. It was found that the metabolite was detected at the incubation period of 2 to 3 days and metabolite production started to deplete after 5th day. Hence, optimum fermentation period for secondary metabolite production was 72 h of incubation at room temperature (30 °C) (Table 2).

Table 2 Effect if Fermentation Period on the Growth and Secondary Metabolite Production from *B. Pumilus* PB1. Data Represent Mean ± SD (N=3).

Fermentation period (days)	Optical Density (at 600 nm)	Zone of inhibition (mm)
1	0.429 ± 0.041	0 ± 0
2	1.672 ± 0.037	2 ± 1
3	2.135 ± 0.041	5 ± 1
4	1.976 ± 0.038	11 ± 2
5	1.782 ± 0.032	17 ± 3
6	1.872 ± 0.031	14 ± 2
7	0.629 ± 0.029	8 ± 1

3.6.2. EFFECT OF PH ON BIOMASS AND METABOLITE PRODUCTION

Of the pH level tested, production of bioactive metabolites and biomass were maxium at pH 7.0. It was observed that the metabolites production of this bacterial isolate was high at wide pH range (pH 6.0 to 8.0). For further experiments, pH 7.0 was used for optimization of metabolites production (Table 3).

Table 3 Effect of Ph on the Growth and Production of Bacteriocin from *B. Pumilus* PB1. Data Represent Mean ± SD (N=3).

pH	Optical Density (at 600 nm)	Zone of inhibition (mm)
4	0.022 ±0.003	0 ± 0
5	0.073 ±0.009	0 ± 0
6	1.776 ± 0.057	2 ± 0
7	1.983 ± 0.061	16 ± 3
8	1.372 ± 0.073	13 ± 2
9	0.873± 0.042	9 ± 2
10	0.526 ± 0.039	0 ± 0

3.6.3. EFFECT OF TEMPERATURE ON BIOMASS AND METABOLITES PRODUCTION

Temperature is one of the critical factors which influence on the growth and production of secondary metabolities. From the experiment it was very clear that the selected bacterial isolate showed maximum growth at 30 °C. The results on the temperature range tested and the activity of bacteriocin were presented in Table 4.

Table 4: Effect of Temperature on the Growth and Production of Bacteriocin from *B. Pumilus* Pb1. Data Represent Mean ± Sd (N=3).

Temperature (°C)	Optical Density (at 600 nm)	Zone of inhibition (mm)

20	1.029 ± 0.047	4 ± 1
25	1.429 ± 0.036	9 ± 2
30	1.996 ± 0.029	12 ± 3
35	1.807 ± 0.032	17 ± 2
40	1.637 ± 0.044	9 ± 2
45	0.401 ± 0.024	4 ± 1
50	0.205 ± 0.019	0 ± 0

3.6.4. EFFECT OF CARBON SOURCES ON METABOLITE PRODUCTION

The effect of carbon sources on biomass and the production of secondary metabolites were measured. Among the carbon sources tested, glucose favoured as good carbon sources for its growth and so preferred as good carbon source for bioactive metabolite production (Table 5).

Table 5. Effect of Carbon Source on the Growth and Production of Secondary Metabolites from *B. Pumilus* PB1. Data Represent Mean ± SD (N=3).

Carbon source (1%)	Optical Density (at 600 nm)	Zone of inhibition (mm)
Glucose	2.108 ± 0.107	18 ± 3
Glycerol	1.629 ± 0.078	8 ± 2
Starch	1.896 ± 0.041	14 ± 3
Lactose	2.007 ± 0.072	10 ± 2
Maltose	1.917 ± 0.042	11 ± 1
Sucrose	1.408 ± 0.039	10 ± 2
Control	2.082 ± 0.071	14 ± 3

3.6.5. EFFECT OF NITROGEN SOURCE ON BIOMASS AND METABOLITE PRODUCTION

The nitrogen sources such as ammonium chloride, sodium nitrate, peptone, beef extract, yeast extract and glycine each at a level of 1% (w/v). Among the tested nitrogen sources, glycine enhanced the growth of bacteria and metabolite production (Table 6).

Table 6. Effect of Nitrogen Source on the Growth and Production of Secondary Metabolites from *B. Pumilus* PB1. Data Represent Mean ± SD (N=3).

Nitrogen source (1%)	Optical Density (at 600 nm)	Zone of inhibition (mm)
Ammonium chloride	1.852 ± 0.072	13 ± 2
Sodium nitrate	1.701 ± 0.052	7 ± 1
Peptone	1.752 ± 0.042	13 ± 2
Beef extract	1.906 ± 0.039	10 ± 2
Yeast extract	1.843 ± 0.041	16 ± 4
Glycine	2.208 ± 0.038	19 ± 3
Control	1.73 ± 0.061	2

3.6.6. EFFECT OF IONS ON BIOMASS AND METABOLITE PRODUCTION

To evaluate the influence of ionic sources on cell growth and the production of bioactive metabolites of the candidate strain, the production medium was supplemented with different ionic sources such as Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+} and Hg^{2+}each at a level of 0.1% (w/v) level. Among the ionic sources, supplemented Ca^{2+}, Mg^{2+} and Mn^{2+} supported the growth of the bacterium. Zone of inhibition was found to be high in the medium containing Mg^{2+} (14 mm) (Table 7).

Table 7. Effect of Ions on the Growth and Production of Secondary Metabolites from *B. Pumilus* PB1. Data Represent Mean ± SD (N=3).

Ions (0.1%)	Optical Density (at 600 nm)	Zone of inhibition (mm)
Ca	2.031 ± 0.04	13 ± 2
Mg	2.001 ± 0.034	17 ± 3
Mn	2.082 ± ± 0.052	14 ± 2
Co	1.906 ± 0.034	8 ± 1
Cu	1.743 ± 0.027	7 ± 2
Hg	1.202 ± 0.039	4 ± 1
Control	1.93 ± 0.082	13 ± 2

4. DISCUSSION

In the present study, various bacterial strains were isolated from the gut of *P. monodon*. A total of 12 bacterial isolates were isolated from the fish gut based on morphological characters of bacteria. Among the bacterial isolates, strain PB1 showed inherent capacity of controlling the marine pathogens and the isolate was found to be *B. pumilis* PB1 based on biochemical properties and 16S sequencing. There are many bacterial strains from the genus *Bacillus* which can produce a wide variety of antibiotics including bacitracin, polymyxin, and colistin. Several bacitracins have been characterized earlier; Members of the *Bacillus* sp. are endospore forming, Gram positive and aerobic bacteria that are catalase production, rod shaped and distributed widely. They found in many environments such as aquatic environments, rocks and gastrointestinal tracts of various organisms [7].

The antibacterial activity of the selected bacterial strain against indicator strains such as *Micrococcus* sp. *Bacillus* sp. and *Pseudomonas* sp., *Vibrio* sp. and *Staphylococcus* sp. was analyzed. The isolated bacterial isolates showed potent activity against these indicator bacteria. Among the screened gut bacterial isolates, only nine showed positive result for bacteriocin production after acid hydrolysis test. The negative strains were discarded and these nine isolates were further subjected to antibacterial assay by well diffusion assay. The isolated strain M9 showed potent activity against the tested pathogenic bacteria. It was previously reported inhibitory activity of bacteriocin from various bacterial species against fish pathogens. In the search for active molecules produced by *Bacillus* species, especially *Bacillus cereus*, *Bacillus subtilis* and *Bacillus licheniformis*, produced several antifungal compounds, mainly peptide molecules [8]. In this present study, the various process parameters were optimized to enhance the bacterial growth and secondary metabolite production. The optimum fermentation period for secondary metabolite production was 72 h of incubation at room temperature (30 °C). In *B. subtilis* 14B, the production of bacteriocin (Bac14) started after a 24-h of incubation then increased exponentially and reached its maximum within 96 h cultivation in submerged fermentation [9]. In bacteria, bacteriocin production was maximum during the exponential growth phase [10].

pH is one of the important factors for bacteriocin production because it critically affects the adsorption of bacteriocin to their surface and the aggregation of the producing cells. The pH value of the medium plays critical role in the proteolytic degradation of bacteriocins. In the present investigation *B. pumilis* PB1 metabolites was detected only when this organism was grown significantly at pH 7.0 and 8.0. Medium pH is one of the critical factors for bacteriocin production. The impact of pH on bacteriocins production was reported by from *L. mesenteroides* FR52, *Bacillus* spp. P11 and *L. plantarum* 17.2b [11].

Bacteriocins produced in alkaline range of pH are now gaining much more attention in food industries. It was earlier reported that nisin is the only bacteriocin used in industries as a food additive which is stable in acidic conditions while it is highly unstable at alkaline pH range. In *B. licheniformis*, bacillocin 490 showed antibacterial activity between acidic to alkaline pH [12]. Temperature is one of the critical factors which influence on the growth and production of secondary metabolities. The selected bacterial strain *B. pumilus* PB1 grown and produced maximum amount of secondary metabolites at 30 °C. The optimal temperature for halocin production by *Micrococcus* sp. GO5 was found to be 37 °C [13]. Among the carbon sources tested, glucose favoured as good carbon sources for bioactive metabolites. Glucose is one of the critical nutrient factors, which positively influenced on the production of bacteriocin from *Bacillus* sp. GU057 [14], Bacteriocin production may be regulated by the amount of glucose in the medium. Increased bacteriocin production in

the presence of glucose was recorded in *L. plantarum* AMA-K [15]. The present findings suggest that the glucose moiety of sucrose was one of the essential nutrients for bacteriocin production [13, 14].

Among the nitrogen sources tested, glycine significantly enhanced the growth of bacteria and metabolite production. However, the other supplemented nitrogen sources also enhanced on the growth and metabolite production. Production was lowest when glycerol was opted as the nitrogen source. This result was in accordance the observations made previously with various organisms. In *B. cereus* XH25 and probiotic lactic acid bacteria, glycine enhanced the production of bacteriocin [16].

In the present study supplemented Mg^{2+} ions significantly enhanced bacteriocin production. Ionic sources are very important components for biological production process. Due to specific ionic and water binding capacity, metal ions significantly affect the bacterial metabolic activity. In *L. plantarum*, low concentration of $MnSO_4.H_2O$ (0.014%) found to stimulate growth and bacteriocin production while $MgSO_4.7H_2O$ was not able to increase considerable amount of bacteriocin production, similar to results observed in this study. In *Micrococcus* sp. GO5, eight fold increase in micrococcin GO5 production was achieved when concentration of K_2PO_4 was increased from 0.2% to 2-5% in the culture medium [17].

5. CONCLUSIONS

The present study was carried out to isolate and screen secondary metabolite producing probiotic bacteria from the gut of *Penaeus monodon*. A total of 12 bacterial isolates were isolated from the fish gut based on morphological characters of bacteria. These organisms were subjected for their antibacterial potential against pathogenic bacteria such as *Micrococcus* sp. *Bacillus* sp. *Pseudomonas* sp., *Vibrio* sp. and *Staphylococcus* sp. by cross streak method and well diffusion method. The intestinal microbial strain, which produced a clear inhibitory zone against the pathogenic strains with a diameter greater than 5 mm was judged to be an antibacterial substance producer or potent probiotic strain.

CONFLICT OF INTERESTS

None.

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