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ORIGINAL ARTICLE

Evaluation of Candidate Probiotic against Vibriosis: It's Antibiotic and Adherence Activity on Epithelial Intestinal layer

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ABSTRACT
Three promising probiotic strains Lactobacillus paracasei(SGM-4), Lactobacillus rhamnosus, (SGKM-9), Pediococcus acidilactici (SGM-7) selected from previous probiotic property studies were analyzed for further studies. Antagonistic potent strains were analyzed for hydrophobicity, antibiotic activity, enzymatic activity (protease, lipase). SGM-4,SGM-7 and SGKM-9 showed inhibitory activity against Vibriosis.SGM-4, SGM-7, and SGKM-9 showed positive result for protease and lipase activity. And all the isolates are positive for hydrophobicity that indicates their adherence capability to the host. Lactobacillus paracasei showed lipase activity, whereas L.rhamnosus and P.acidilactici showed positive for protease and lipase activity.Furtherin-vivo and field trials studies can be performed with the candidate probiotics for sustainable aquaculture farming.

Keywords: *Vibriosis, Adherence capacity, Antibiotic activity, Enzymatic and Hydrophobicity.*

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INTRODUCTION

In aquaculture India is the large farming country having great potential, whereas when compared to the other countries Indian aquaculture farming is very limited in development and application of the probiotics. Worldwide shrimp aquaculture farming is mainly affected due to the serious outbreak of disease caused by the virus, bacteria, protozoan and fungi. Initially farmers applied different antibiotics and chemicals for the control and eradication of the disease outbreaks. Whereas in some areas farmers used probiotics for water quality management to reduce the pathogenic bacteria effect [1].

Aquaculture probiotics are live microbes, which when administrated confer health benefits to the host by increasing microbial community of gut thus enhancing better feed utilization, improving sought signals towards disease and promoting the quality of its optimum environment condition [2]. Probiotics are live microbial cell culture or cell components which when administrated give health benefits to the host. Probiotic should be nonpathogenic, resistant to gastric acid, non-toxic, produce antibacterial agents, adhesion to the gut epithelial tissue. Main criteria for the selection of the probiotics is they should be tolerance to the gastrointestinal conditions (pH, bile, phenol), they should be capable of attachment to the gastric epithelial tissue, they should be antimicrobial agents, competitive exclusion of pathogens, absence of toxicity and tolerance to technological process, motility and activity in delivery vehicles. Adhesion of probiotic to the host gastric epithelial tissue is main criteria for selection and it is the universal criteria of lactic acid bacteria[3].

The aim of the present research is to determine enzymatic activity and hydrophobicity of the isolates for the control of vibriosis and these promising probiotic strains *Lactobacillus rhamnosus*, *Lactobacillu sparacasei* and *Pediococcus acidilactici* isolated from shrimp gut sample showedhigher tolerance to intestinal environment and higher adhesion capacity to the intestinal cell line.

MATERIAL AND METHODS

Three bacterial cultures SGM-7, SGM-4, SGKM-9 isolated from shrimp gut sample were found to possess probiotic properties. MRS media was used. The bacterial strains isolated from shrimp gut were cultivated in MRS respectively for 24h at 37^0C. After incubation culture was stored and further analysis was done.

Sensitivity to Antibiotic test

By using disc method another *in-vitro* antibiotic sensitivity test was performed to probiotics. These three probiotic isolates susceptibility to antibiotics was analyzed through Bioanalyse® Antimicrobial susceptibility test. Overnight active culture was swabbed on solidified Muller-Hinton agar plates for growth of isolates lawn. At marked and selective distance antibiotic disc were fixed on pre swabbed plates and at 37^0C Petri-plates were incubated for 24h. After incubation period zone of inhibition was recorded using mm scale. Here we have used Ampicillin (10mcg), Chromophenicol (30mcg), Ciproflaxin (10mcg), Erythromycin (15mcg), Kanamycin (30mcg), Penicillin (10 units), Streptomycin (10mcg), Tetracycline (30mcg), and Vancomycin (10mcg) for present study.

Assay of Hydrophobicity test by using Congo Red Stain (CRS)

Epithelial binding process of hydrophobicity test was performed by using Congo Red Stain (CRS), to identify hydrophobicity of bacteria culture Triptic Soy Agar petri plates were prepared with 0.03% Congo Red Stain. Note that congo red has to be added after autoclave of TSA media. Each candidate probiotic were streaked on the TSA petri plate in triplicate method and samples were incubated at 37^oC for 24h. Red color colonies were recorded as positive (hydrophobic) and white or colorless colonies were recorded as negative (non-hydrophobic) [4].

Assay of Extracellular enzymatic

To determine protease, amylase and lipase activity the isolates were inoculated into selective media and zone of inhibition was recorded. To detect amylase activity the bacterial culture was inoculated in MRS media incorporated with 0.25% of starch. After incubation the zone of inhibition was observed by addition of Gram's iodine as detecting agent.

To detect the protease activity 50µl of centrifuged cell free extract was inoculated into skim milk agar media (1%) and incubated for 48h. After incubation the zone of inhibition was measured.

Detection of lipase activity was performed by using olive oil (1%). 50µl of centrifuged cell free extract was inoculated into MRS broth supplemented with olive oil (1%) and Arabic gum (1%). After 48h of incubation zone of clearance was observed.

RESULTS AND DISCUSSION

Three isolates SGM-4, SGM-7,SGKM-9 isolated from shrimp gut samples were molecularly identified as *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *and Pediococcus acidilactici.* Previously all the isolates were examined for probiotic characteristics and antagonistic activities against Vibrio species, further hydrophobicity and enzyme activity was performed.

The prominent three isolates were observed resistance to tetracycline (30 µg), streptomycin at 10 µg, slight inhibition of SGM-4 in Neomycin at 30 µg, slight inhibition of SGM-7in chloramphenicol at 30 µg, better inhibition in ciprofloxacin 10 µg, ampicillin 10 µgexcept in SGM-4, in pencillin minimum zone of inhibition was found except SGM-4 and no inhibition in SGM-7 at 10 µg and minimum zone in Cefpodoxime 15 µg, vancomycin, maximum zone in rifampocin, maximum zone of clearance in Amoxyclav and maximum zone of inhibition in Novobiocin except inSGKM-9 (Figure 1, Table 1).A recent report suggests that antibiotic activity of probiotics shows good resistance against the antibiotics. It is concluded that the selected nine isolates have good antagonistic potential as well as antibiotic resistance.

SGM-4, SGM-7, and SGKM-9 showed positive result for protease and lipase activity. And all the isolates showed positive for hydrophobicity that indicates their adherence capability to the host. *Lactobacillus paracasei* showed lipase activity, whereas *L.rhamnosus* and *P.acidilactici* showed positive for protease and lipase activity. Previous studies suggests that the production of extracellular enzymes like proteases and lipases help in the supplement of nutrition to the host [5,6]. Other researchers suggests that over production of these proteases and lipases leads to antagonistic activity, hence pathogenic strains have already high proteolytic activity and extracellular lipolytic activity [7]. Another scientist explains that lactic acid bacteria illustrate production of extracellular enzymatic activity from gut of shrimp and recorded that this mixture of lactic acid bacteria have a beneficiary effect in white leg shrimp (*Litopenaeus vannamei*) against the white spot syndrome virus(WSSV) [8].

An important criteria for the selection of probiotic for beneficial effect to the host is the adhesion to the intestinal mucosa [3]. Probiotic bacteria works as gastrointestinal bacteria colonization against pathogenic agents, immune system modulation, recovering of damaged gastric mucosa, and adhesion capacity [9]. Hydrophobicity positive results explain that probiotic has the capability to attach non-

preciselyto the intestinal epithelium by hydrophobic interaction. As the absence of hydrophobic molecules on the bacterial surface and epithelium layer results in the resist, as they both have negative charge [10, 11, 8]. These probiotic adhesion capacity can also be determined through hydrophobicity test by using congo red stain [4, 8]. In present investigation the promising probiotic bacteria showed positive result to the hydrophobicity. Finally our *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *Pediococcus acidilactici* have the capability to bind to the epithelium of intestine.

Table 1.NCBI GENBANK Accession numbers of three isolates

S. No.	Isolates name	Molecular level identification	NCBI Accession number
1	SGM-4	*Lactobacillus paracasei*	MT125880
2	SGM-7	*Pediococcusacidilactici*	MT125882
3	SGKM-9	*Lactobacillus rhamnosus*	MT125886

Table 2.Antibiotic activities of Probiotic isolates

Isolates	T	S	Rif	N	Chl	Cip	Amp	Pen	Cef	Am	Nv	Van
SGM-4	27	18	22	10	26	18	0	9	15	23	21	15
SGM-7	23	17	24	22	0	19	24	0	17	23	18	14
SGKM-9	22	14	20	15	21	22	26	19	22	24	0	12
Mean	24	16.3	22	15.6	15.6	19.6	16.6	9.333	18	23.3	13	13.666
S.E	1.527	1.201	1.154	3.480	7.965	1.201	8.353	5.487	2.081	0.333	6.557	0.881
Stddev	2.645	2.081	2	6.027	13.796	2.081	14.468	9.504	3.605	0.577	11.357	1.527
CV%	11.023	12.744	9.090	38.474	88.060	10.584	86.810	101.833	20.030	2.474	87.367	11.177

T-tetracyclin, S-streptomycin, Rif-riffampicin, N-Neomycin, Chl-chloramphenicol, Cip- ciprofloxacin, Amp-ampicillin, Pen-pencillin, Cef-Cefpodoxime, Am-Amoxyclav, Nv- Novobiocin, Van-vancomycin.

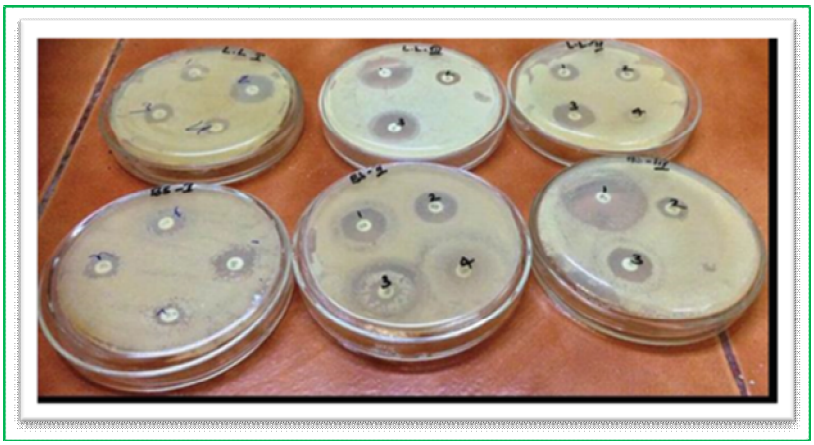

Figure 1. Antibiotic activity of probiotic isolates showing maximum zone of inhibition

Table 3.Hydrophobicity and enzymatic activity of promising probiotic bacteria

S.No.	Probiotic culture	Protease activity	Lipase activity	Hydrophobicity
1	*Lactobacillus paracasei*	Negative	Positive	Positive
2	*Lactobacillus rhamnosus*	Positive	Positive	Positive
3	*Pediococcusacidilactici*	Positive	Positive	Positive

CONCLUSION

Isolated Probiotics have the ability to produce antibiotic activity and extracellular enzymes that decompose organic macromolecules. The identification of these bacterial probiotics with potential probiotic parameters should begin with the isolation of strains from healthy shrimp gut. Subsequently, the antimicrobial, antibiotic and hydrophobicity and enzyme assays are examined and showed great potential activity. We report that isolated bacterial strain of three candidate probiotics *Lactobacillus paracasei* (SGM-4),*Lactobacillus rhamnosus,* (SGKM-9)*, Pediococcus acidilactici* (SGM-7)showed best results based on the antagonistic, enzymatic and hydrophobicity assays and this could be a promising results for shrimp aquaculture that has been affected by vibriosis during the recent years. Further experiment, including the application of candidate probiotics and shrimp challenge with pathogen in field trails, might provide valuable information of probiotics potential abilities of these candidates for shrimp aquaculture facilities.

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