INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
VOLUME-2, ISSUE-2 APRIL-JUNE-2012 ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 20th Feb-2012 *Revised: 24th Feb-2012* *Accepted: 28th Feb-2012*

Research article

COMPARATIVE *INVITRO* AND *INVIVO* STUDY OF THREE PROBIOTIC ORGANISMS, *BIFIDOBACTERIUM* SP., *LACTOBACILLUS* SP., *S. CEREVISIAE* AND ANALYZING ITS IMPROVEMENT WITH THE SUPPLEMENTATION OF PREBIOTICS

S.Venkatesan[1], M.Kirithika[1], I.Roselin[1], R.Ganesan[2] and K.Muthuchelian[3]

[1]Research Department of Microbiology, VHNSN College, Virudhunagar, Tamil Nadu, India.
[2]Department of Microbiology, P.K.N College, Thirumangalam, Tamil Nadu, India.
[3]Vice chancellor, Periyar University, Salem, Tamil Nadu, India.

ABSTRACT : Probiotics are harmless bacteria that provide equilibration of the intestinal flora, and hence have a positive effect on the health of the consumer. Probiotics are live microbes that can be formulated into many different types of products, including foods, drugs, and dietary supplements. Probiotics had high antibiotic resistance activity, antimicrobial activity and antioxidant activity. Prebiotics had the ability to support the growth of probiotics. Probiotics are well being of the host animal and contribute, directly or indirectly to product the host animal against harmless bacterial pathogens. Here the probiotic organisms, *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cerevisiae* were isolated from soil, curd and yeast pellets respectively. Testing the antibiotic resistance activity of probiotic organisms against fourteen antibiotics, *Bifidobacterium* sp. had resistance activity against eleven antibiotics. Considering the antimicrobial activity, it was found that *Bifidobacterium* sp. had the high inhibitory effect against *Salmonella* sp. (18 mm). The prebiotics are helped to support the growth of probiotics. The OD value was measured at 650 nm in 24 hours time using two medium like MRS medium and selective medium. *Bifidobacterium* sp. maximally grown on the prebiotics like Inulin (OD value 0.657 and 1.979) and maximum growth was present in Bifidobacterium broth than in MRS broth. DPPH method results indicated the antioxidant activity of probiotic organisms. The different concentration of probiotic cultures was used and OD measured at 250 nm. The probiotic cultures *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cerevisiae* showed stongest radical scavenging activity. The probiotics were tested *invivo* with the fish and pathogen. From the results obtained it was sure that the probiotic treatment offered a promising alternative to the use of antibiotics in aquaculture.

Keywords: Prebiotics, probiotics, DPPH, *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cerevisiae*

INTRODUCTION

The term “probiotics” was first introduced in 1965 by Lilly and Stillwell. Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host. Probiotics are microbially derived factors that stimulate the growth of other organisms. Probiotics are selected from the strains most beneficial for the most intestinal bacteria, that is bacteria from the genera *Bifidobacterium, Lactobacillus* and *Yeast.* Species of *Lactobacillus* and *Bifidobacterium* are most commonly used probiotics, but the *Saccharomyces cerevisiae* and *Bacillus* species are also used as a probiotics [7]. Among the criteria for microorganisms to be included in the probiotics groups are

- Survival passing through gastrointestinal tract at low pH and on contact with bile;
- Adhesion to intestinal epithelial cells;
- Stabilization of the intestinal microflora;
- Nonpathogenicity;
- Survival in food stuffs and possibility for production of pharmacopoeia lyophilized preparation;
- Fast multiplication, with either permanent or temporary colonization of the gastrointestinal tract; and
- Generic specificity of probiotics [7].

Health benefits of probiotics

- Offers increased resistance to establishment of infection by potentially pathogenic organisms in the intestine.
- Decreased duration of diarrhoea (antibiotic associated, travelers', infective).
- Use in lactose intolerance (promotion of intestinal lactose digestion). Increased nutritional value (better digestibility, increased absorption of vitamins and minerals).
- Regulation of gut motility (constipation, irritable bowel syndrome).
- Maintenance of mucosal integrity of the intestine.
- Reduction in serum cholesterol concentration.
- Reduction in allergy.
- Prevention of colon cancer.
- Reduction in carcinogen /co-carcinogen production [5].

Various strains of probiotics are known to produce biological activities such as antibacterial, antifungal, and aitiviral activity. The antimicrobial activity of probitics *invitro* against gram positive and gram negative bacteria.

PREBIOTICS

Prebiotics are range of non-digestible dietary supplements, which modify the balance of the intestinal microflora, stimulating the growth and / or activity of beneficial organisms and suppressing potentially deleterious bacteria. These supplements include lactulose, lactitol, glucose, a variety of oligosaccharides (especially fructo-oligosaccharides or FOS, soybean oligosaccharides or SOS), β-glucan, arabinogalactan, arabinose, D(+) raffinose, rhamnose, and inulin [1]. In particular, prebiotics promote the proliferation of *bifidobacteria* in the colon. Some of them also help in promoting the proliferation of *Lactobacilli* in the small intestine to a certain extent.

Criteria for food material to be a prebiotic

As was found in the probiotics, for a food material to be used as a prebiotic certain criteria must be met [3, 2]. These are as follows

- It must be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract.
- It must be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated.
- It must consequently, be able to alter the colonic flora in favour of a healthier composition.
- It must induce luminal or systemic effects that are beneficial to the host health.

Based on these criteria listed, only a few groups of food ingredients qualify to be used as prebiotics. A good number of food materials because of their chemical structure are not absorbed in the upper part of the GIT or hydrolyzed by the digestive enzymes in humans. Such foods have been called “colonic foods” [3].

MATERIALS AND METHODS

SAMPLE COLLECTION

Sample 1

Soil Sample was collected from the garden at V.H.N.S.N. College in Virudhunagar.

Sample 2

Curd sample was collected from the hostel at V.H.N.S.N. College in Virudhunagar.

Sample 3

Yeast pellets were bought from bakery in Virudhunagar.

ISOLATION OF PROBIOTIC ORGANISMS

1g of soil sample **(**Sample 1**)** was serially diluted upto 10^{-1} to 10^{-7}. Then 0.1 ml of each dilution sample was taken and spreaded on the Bifidobacterium medium. The plates were incubated at 37ºC for 24 hours.

Curd sample (Sample 2) was serially diluted upto 10^{-1} to 10^{-7}. Then 0.1 ml of each dilution sample was taken and spreaded on the MRS plates. The plates were incubated at 37ºC for 24 hours.

Yeast pellets (Sample 3) were inoculated into YPD broth. The broth was incubated at 37º C for 4 days. The loop of broth culture was streaked on to the YPD medium plates and the plates were incubated at 37ºC for 24 hours.

IDENTIFICATION OF THE ISOLATES

The isolated colonies from the plates were used for the further analysis of microscopic and biochemical characteristics

IDENTIFICATION OF ANTIBIOTIC RESISTANCE OF PROBIOTIC ORGANISMS

The antibiotic resistance of isolated *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cerevisiae* probiotic cultures were assay using antibiotic discs on Muller- Hinton agar plates. Resistance was a assessed against clindamysin, neomycin, cloxacillin, trimethoprin, ofloxacin, nalidixic acid, bacitracin, cephalexin, methicillin, ampicillin, cefixime, kanamycin, cefdin and clarithromycin. The antibiotic discs were placed on the surface of the agar plates. Then the plates were incubated at 37ºC for 24 hours [9].

INVITRO STUDY OF PROBIOTICS

IDENTIFICATION OF ANTIMICROBIAL ACTIVITY OF PROBIOTOC ORGANISMS

Test organisms

The test organisms, *Pseudomonas* sp., *Salmonella* sp., *Klebsiella* sp., *Shigella* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Escherichia* sp. bacterial cultures were obtained from MTCC Chandigarh. The organisms were inoculated into nutrient broth and incubated at 37ºC for overnight.

Agar-well diffusion method

Autibacterial activity of isolated *Bifidobacterium* sp., *Lactobacillus* sp. and *saccharomyces cerevisiae* probiotic cultures were determined by agar-well diffusion method. Muller-Hinton agar plates were prepared. The test organisms were spreaded on agar plates with sterile effusion. The well was made and filled with 100µ1 of probiotic culture supernatant. The inoculated plates were incubated at 37°C for 24 hours [4].

Co-culture technique

Co - culture assay, another method for determination of antimicrobial effect of probiotics *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cereivisiae.* The bacterial cultures like *Pseudomonas* sp., *Salmonella* sp., *Klebsiella* sp., *Shigella* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Escherichia* sp. were grown in nutrient broth at 37°C for 24 hours. All probiotic cultures were grown overnight in selective broth. All cultures were centrifuged 1000 rpm for 10 minutes 0.2% of each bacterial culture, and different concentration (0.2, 0.4, 0.5, 0.6 µl) of probiotic cultures were co- incubated separately in nutrient broth at 37°C for 24 hours. After the incubation 1ml of cell culture was serially diluted upto 10^{-1} to 10^{-6}. Then 0.1ml of 10^{-6} dilution sample was taken and spreaded on nutrient agar plates. The plates were incubated at 37°C for 24 hours. The probiotic cultures were inhibited the growth of the bacterial culture. The colonies were counted [4]. The CFU Value was calculated using the following equation

$$\text{CFU} = \frac{\text{Number of Colonies}}{\text{Volume of the Sample}} \times \text{dilution factor}$$

IDENTIFICATION OF THE EFFECT OF PREBIOTICS ON PROBIOTICS

Prebiotics

Five different types of prebiotics were used. There are Glucose, Lactose, Inulin, Raffinose and Arabinose.

Effect of prebiotics on probiotics

Testing the effect of the potential prebiotics on the growth of the three probiotic cultures. Two potential medium with carbon source was used. To each of these media any one of the above carbon source was added to the concentration of 1.5%. Then 1% of inoculum of an overnight culture of each probiotic was added to the medium and this was incubated at 37°C for 24 hours. Then without the carbon source the probiotics growth was measured. In this method only probiotic was added and incubated. The growth of each strain was monitered by measuring the OD value of the cultures at 650 nm using spectrophotometer [6].

DETECTION OF ANTIOXIDANT ACTIVITY OF PROBIOTICS- DPPH Method

The antioxidant activity was determined by DPPH method. Add 50µ1 of probiotic culture mixed with 1ml of 0.1mm DPPH in methanol and 450µ1 of 50 mm Tris HCl buffer (pH–7.4). Then 50µ1 of methanol was used for experimental control. The tubes were incubated for 30 minutes at room temperature. After the 30 minutes incubation, the reduction in the number of DPPH radicals was measured at 250 nm using spectrophotometer.

INVIVO ANALYSIS

ANALYSING THE EFFCT OF PROBIOTICS ON INFECTED FISH

Probiotics like *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cereivisiae* were grown at 37ºC for 24 hours in selective broth and pathogens like *Pseudomonas* sp., *Salmonella* sp., *Klebsiella* sp., *Shigella* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Escherichia* sp. were grown at 37ºC for 24 hours in nutrient broth. Ten tanks containing the fish were taken and labelled as I to X. First eight tanks were infected with 0.5ml of bacterial pathogenic culture for 24 hours. To these eight tanks 0.5ml of probiotic culture was added. The ninth fish tank was treated with 0.5ml of probiotic culture alone to confirm the non-pathogenicity of culture. The tenth fish tank without any bacterial pathogenic culture and probiotic culture was used as control. The tanks were kept at room temperature for 15 days. Then nine tanks containing the fish were taken and labelled as I to IX. First eight tanks were infected with 0.5ml of bacterial pathogenic culture for 24 hours. The ninth fish tank without any bacterial pathogenic culture was used as control. The tanks were kept at room temperature for 15 days. The cumulative mortality of the fish were recorded and analyzed. This setup was worked for all the three probiotic organisms separately [10].

RESULTS

IDENTIFICATION OF THE ISOLATES

The isolated colonies from three different medium Bifidobacterium medium, MRS medium and YPD medium were taken separately for future analysis. The microscopic examination of the three isolates was tabulated (Table 1). The various biochemical tests and observed results were tabulated (Table 2, 3 and 4).

Table 1: Results of Gram Staining

Colony form medium	Results observed
Bifidobacterium medium	Gram positive, Rod
MRS medium	Gram positive, Rod
YPD medium	Gram positive, Cocci

Table 2: Results of Biochemical Examination

Colony form medium	Biochemical characters	Results observed
Bifidobacterium medium	Indole production test	Negative
	Methyl - Red Test	Negative
	Voges- Proskauer Test	Negative
	Citrate Utilization Test	Negative
	Catalase Activity Test	Negative
	Oxidase Test	Negative
	Starch hydrolysis Test	Positive
	Casein hydrolysis Test	Positive
	Lipid Hydrolysis Test	Positive

Table 3: Results of Biochemical Examination

Colony form medium	Biochemical characters	Results observed
MRS medium	Indole production test	Positive
	Methyl - Red Test	Positive
	Voges- Proskauer Test	Negative
	Citrate Utilization Test	Negative
	Catalase Activity Test	Negative
	Oxidase Test	Negative
	Starch hydrolysis Test	Negative
	Casein hydrolysis Test	Negative
	Lipid Hydrolysis Test	Negative

Table 4: Results of Biochemical Examination

Colony form medium	Biochemical characters	Results observed
YPD medium	Indole production test	Negative
	Methyl - Red Test	Negative
	Voges- Proskauer Test	Positive
	Citrate Utilization Test	Negative
	Catalase Activity Test	Negative
	Oxidase Test	Negative
	Starch hydrolysis Test	Negative
	Casein hydrolysis Test	Negative
	Lipid Hydrolysis Test	Negative

IDENTIFICATION OF ANTIBIOTIC RESISTANCE OF PROBIOTIC ORGANISMS

The antibiotic resistance of probiotic cultures, *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cerevisiae* were assessed against fourteen antibiotics. The inhibition zone was measured. The antibiotic resistance results were tabulated (Table 5).

Table 5: Results of Antibiotic Resistance of probiotic organisms

Antibiotics	Zone of inhibition in diameter (mm)		
	Bifidobacterium sp.	Lactobacillus sp.	S. cerevisiae
Clindamycin	0.4	-	-
Neomycin	0.4	-	-
Cloxacillin	-	-	-
Trimethoprin	1	-	-
Nalidixic acid	0.3	-	-
Bacitracin	-	-	-
Cephalexin	0.1	-	-
Ofloxacin	0.4	-	-
Methicillin	0.4	-	-
Ampicillin	0.5	-	-
Cefixime	-	-	-
Kanamycin	0.4	-	-
Cefdin	0.2	-	-
Clarithromycin	0.5	-	0.6

The zone of inhibition indicated the antibiotic resistance activity of probiotic strains. The *Bifidobaterium* sp. had the most antibiotic resistance activity than the *Lactobacillus* sp. and *S. cerevisiae*. The *Lactobacillus* sp. was sensitive to all antibiotics. *S. cerevisiae* was found resistant to clarithromycin and sensitive to other antibiotics.

INVITRO STUDY OF PROBIOTICS

IDENTIFICATION OF ANTIMICROBIAL ACTIVITY OF PROBIOTOC ORGANISMS

Agar-well diffusion method

The antimicrobial activity of probiotic cultures, *Bifidobaterium* sp., *Lactobacillus* sp. and *S. cerivisiae* were assayed against eight pathogenic bacterial organisms. The inhibition zone was measured. The antimicrobial activity results were tabulated (Table 6).

Table 6: Results of Antimicrobial activity of probiotic organisms in Agar-well diffusion method

Test organisms	Zone of inhibition in diameter (mm)		
	Bifidobacterium sp.	Lactobacillus sp.	S. cerevisiae
Pseudomonas sp.	13	7	-
Salmonella sp.	18	10	-
Klebsiella sp.	6	2	4
Shigella sp.	4	4	-
Proteus sp.	3	4	-
Streptococcus sp.	15	2	2
Staphylococcus sp.	6	2	-
Escherichia sp.	7	2	1

The zone of inhibition indicated the bactericidal effect of probiotic strains. *Bifidobaterium* sp. had the most inhibitory effect than *Lactobacillus* sp. and *S. cerevisiae*. The *S. cerevisiae* had the poor inhibitory effect against pathogenic bacterial organisms.

Co-culture technique

Co-culture assay, another method for detecting the antimicrobial activity of probiotic cultures, *Bifidobaterium* sp., *Lactobacillus* sp. *and S. cerevisiae* were assayed against eight bacterial pathogens. In the co-culture method, the probiotic strains inhibit the growth of the pathogenic organisms. Different concentration (0.2, 0.4, 0.5, 0.6 µl) of probiotic cultures were used. The colonies were counted and the CFU value was calculated (Table 7, 8, 9).

Table 7: Results of Antimicrobial activity of *Bifidobacterium* sp. in Co-culture technique

Bacterial pathogens	Different concentration of Bifidobacterium sp. CFU (10-6 dilution)			
	0.2 µl	0.4 µl	0.5 µl	0.6 µl
Pseudomonas sp.	TNTC	273×10^5	63×10^5	32×10^5
Escherichia sp.	192×10^5	145×10^5	81×10^5	36×10^5
Salmonella sp.	172×10^5	122×10^5	91×10^5	69×10^5
Klebsiella sp.	138×10^5	117×10^5	78×10^5	26×10^5
Staphylococcus sp.	81×10^5	45×10^5	39×10^5	17×10^5
Shigella sp.	100×10^5	64×10^5	42×10^5	30×10^5
Proteus sp.	170×10^5	88×10^5	70×10^5	50×10^5
Streptococcus sp.	128×10^5	75×10^5	48×10^5	39×10^5

Table 8: Results of Antimicrobial activity of *Lactobacillus* sp. in Co-culture technique

Bacterial pathogens	Different concentration of Lactobacillus sp. CFU (10-6 dilution)			
	0.2 µl	0.4 µl	0.5 µl	0.6 µl
Pseudomonas sp.	147×105	133×10^5	87×10^5	12×10^5
Escherichia sp.	125×105	117×10^5	93×10^5	70×10^5
Salmonella sp.	130×105	93×10^5	87×10^5	46×10^5
Klebsiella sp.	116×105	84×10^5	73×10^5	66×10^5
Staphylococcus sp.	91×105	79×10^5	62×10^5	48×10^5
Shigella sp.	90×105	49×10^5	38×10^5	30×10^5
Proteus sp.	140×105	75×10^5	40×10^5	36×10^5
Streptococcus sp.	102×105	89×10^5	60×10^5	52×10^5

Table 9: Results of Antimicrobial activity of *S. cerevisiae* in Co-culture technique

Bacterial pathogens	Different concentration of S. cerevisiae CFU (10-6 dilution)			
	0.2µl	0.4 µl	0.5 µl	0.6 µl
Pseudomonas sp.	63×10^5	34×10^5	22×10^5	15×10^5
Escherichia sp.	112×10^5	84×10^5	63×10^5	22×10^5
Salmonella sp.	81×10^5	67×10^5	48×10^5	32×10^5
Klebsiella sp.	93×10^5	62×10^5	47×10^5	28×10^5
Staphyllococcus sp.	130×105	97×10^5	73×10^5	47×10^5
Shigella sp.	180×10^5	112×10^5	95×10^5	70×10^5
Proteus sp.	145×10^5	88×10^5	65×10^5	48×10^5
Streptococcus sp.	98×10^5	79×10^5	45×10^5	39×10^5

From the results observed in table 7, 8 and 9 found that increase in probiotic concentration showed more inhibitory activity on the pathogen. For example 0.6µl showed more inhibitory activity than 0.2µl. The number of colonies also indicated the antimicrobial activity of probiotic strains. *Bifidobaterium* sp. had the higher antimicrobial activity than other probiotic strains.

IDENTIFICATION OF THE EFFECT OF PREBIOTICS ON PROBIOTICS

The effect of potential prebiotics on the growth of probiotic cultures was identified. The OD value was measured at 650 nm in 24 hours time using the two medium like MRS medium and selective medium. The results were compared with the OD value of probiotics without prebiotics and probiotics with prebiotics. The OD value was tabulated (Table 10, 11, 12, 13, 14 and 15).

Table 10: Results of without prebiotics on *Bifidobacterium* sp.

Probiotics	OD value at 650 nm	
	MRS broth (in 24 hours)	Bifidobacterium broth (in 24 hours)
Bifidobacterium sp.	0.089	0.352

Table 11: Results of effect of prebiotics on *Bifidobacterium* sp.

Prebiotics (1.5 %)	1% Bifidobacterium sp. (OD value at 650 nm)	
	MRS broth (in 24 hours)	Bifidobacterium broth (in 24 hours)
Glucose	0.250	1.854
Lactose	0.437	1.924
Inulin	0.657	1.979
Raffinose	0.150	1.909

Table 12: Results of without prebiotics on *Lactobacillus* sp.

Prebiotics (1.5 %)	1% Lactobacillus sp. (OD value at 650 nm) MRS broth (in 24 hours)
Glucose	1.406
Lactose	0.065
Inulin	0.349
Arabinose	0.327

Table 13: Results of effect of prebiotics on *Lactobacillus* sp.

Probiotics	OD value at 650 nm MRS broth (in 24 hours)
Lactobacillus sp.	*0.046*

Table 14: Results of without prebiotics on *S. cerevisiae*

Probiotics	OD value at 650 nm	
	MRS broth (in 24 hours)	YPD broth (in 24 hours)
S. cerevisiae	0.089	0.352

Table 15: Results of effect of prebiotics on *S. cerevisiae*

Prebiotics (1.5%)	1% S. cerevisiae (OD value at 650 nm)	
	MRS broth (in 24 hours)	YPD broth (in 24 hours)
Glucose	1.025	1.061
Lactose	1.579	1.969
Inulin	0.722	0.784
Arabinose	0.521	0.558

From the results observed in table 10 and 11 it was very well understand that the prebiotics improve the growth of probiotics and was found that the OD value of *Bifidobaterium* sp. without prebiotics in both medium were 0.089 and 0.352. While using the prebiotics such as glucose, the OD value in both medium was 0.250 and 1.854. *Bifidobaterium* sp. maximally grown on the prebiotics like Inulin (OD value 0.657 and 1.979) and maximum growth was present in Bifidobaterium broth than in MRS broth.

From the results observed in table 12 and 13 it was very well understand that the prebiotics improve the growth of probiotics and was found that the OD value of *Lactobacillus* sp. without prebiotics in MRS broth was 0.046. While using the prebiotics such as glucose, the OD value was 1.406. *Lactobacillus* sp. grow maximally when supplemented with the prebiotics like glucose.

From the results observed in table 14 and 15 it was very well understand that the prebiotics improve the growth of probiotics and was found that the OD value of *S. cerevisiae* without prebiotics in both medium were 0.189 and 0.472. While using the prebiotics such as glucose, the OD value in both medium were 1.025 and 1.067. *S. cerevisiae* grow maximum on the prebiotics like lactose (OD value 1.579 and 1.969) and maximum growth was present in YPD broth than in MRS broth.

DETECTION OF ANTIOXIDANT ACTIVITY OF PROBIOTICS-DPPH method

The probiotic cultures, *Bifidobaterium* sp., *Lactobacillus* sp. and *S. cerevisiae* showed stongest radical scavenging activity. The different concentration of probiotic cultures were used. The OD value was measured at 250 nm. The results were tabulated (Table 16).

Table 16: Results of Antioxidant activity of probiotics

Probiotics	In different concentration of probiotics (OD value at 250 nm)				
	150 µl	160 µl	170 µl	180 µl	190 µl
Bifidobacterium sp.	2.002	1.952	1.895	1.889	1.852
Lactobacillus sp.	2.492	2.492	2.194	2.068	1.964
S. cerevisiae	2.752	2.436	2.353	2.315	2.297

The results indicated that probiotic cultures contain high level of free radical scavenging activity. The probiotic culture concentration was increases, the number of DPPH free radicals was decreased. The OD value decreased in low concentration to high concentration of the sample. *Bifidobaterium* sp. had high antioxidant activity than *Lactobacillus* sp. and *S. cerevisiae*.

INVIVO ANALYSIS

ANALYSING THE EFFCT OF PROBIOTICS ON INFECTED FISH

Treatment was done with both probiotics and pathogens and the results obtained were tabulated (Table 17, 18, 19, 20 and 21).

Table 17: Results of Treatment of fish with probiotics

Sample	Probiotics	No. of days survived
Fish	*Bifidobacterium sp.*	15
	Lactobacillus sp.	13
	S. cerevisiae	10

Table 18: Results of Treatment of fish with pathogens

Sample	Pathogens	No. of days survived
Fish	*Pseudomonas sp.*	3
	Escherichia sp.	5
	Salmonella sp.	2
	Staphyllococcus sp.	4
	Klebsiella sp.	4
	Shigella sp.	2
	Proteus sp.	4
	Streptococcus sp.	2

From the result obtained it was sure that the probiotic treatment offered a promising alternative to the use of antibiotics in aquaculture. The fish survived high number of days when treated with *Bifidobaterium* sp. (15 days).

Table 19: Results of Treatment of fish with *Bifidobacterium* sp. and pathogens

Sample	Probiotics	Pathogens	No. of days survived
Fish	*Bifidobacterium sp.*	*Pseudomonas sp.*	8
		Escherichia sp.	9
		Salmonella sp.	5
		Staphyllococcus sp.	7
		Klebsiella sp.	6
		Shigella sp.	7
		Proteus sp.	6
		Streptococcus sp.	5

Table 20: Results of Treatment of fish with *Lactobacillus* sp. and pathogens

Sample	Probiotics	Pathogens	No. of days survived
Fish	*Lactobacillus sp.*	*Pseudomonas sp.*	5
		Escherichia sp.	7
		Salmonella sp.	4
		Staphyllococcus sp.	6
		Klebsiella sp.	3
		Shigella sp.	5
		Proteus sp.	4
		Streptococcus sp.	3

Table 21: Results of Treatment of fish with *S. cerevisiae* and pathogens

Sample	Probiotics	Pathogens	No. of days survived
Fish	*S. cerevisiae*	*Pseudomonas sp.*	5
		Escherichia sp.	7
		Salmonella sp.	4
		Staphyllococcus sp.	6
		Klebsiella sp.	5
		Shigella sp.	4
		Proteus sp.	6
		Streptococcus sp.	3

The fish treated with pathogens, survived low number of days when compared with the supplementation of probiotics. The fish treated with probiotics and pathogens showed better survival time. Here also *Bifidobaterium* sp. showed better effect than *Lactobacillus* sp. and *S. cerevisiae.*

DISCUSSION

[9] Reported that the probiotic species of *L. plantarum* (G95a and G96a) and *L. rhamnosus* (G119b) were showed resistant to eight test antibiotics while *L. rhamnosus* (G92, G99c) were sensitive to tetracycline only but resistant to remaining seven antibiotics. In our present study, probiotic species *Bifidobacterium* sp. was showed better resistant activity against eleven test antibiotics and it had the most antibiotic resistance activity than the *Lactobacillus* sp. and *S. cerevisiae*. The *Bifidobacterium* sp. had the high antibiotic resistance activity against Ampicillin and Clarithromycin (0.5 mm) while *Lactobacillus* sp. was sensitive to all antibiotics. *S. cerevisiae* was found resistant to Clarithromycin (0.6 mm) and sensitive to other antibiotics.

[4] Reported that the antimicrobial effect of the Cell Free Supernatant (CFU) of probiotic *Lactobacilli* by well diffusion method. His results showed that the strong inhibition zone of S. *arueus* was obtained by *L. plantarum*, 13mm. Standard *S. arueus* ATCC 25923 is nearly resistant to CFU of all *Lactobacillai*, inhibitory effect against this bacterium 9mm. [9] reported that *L. plantarum* (G95a and G96a) and *L. rhamnosus* (G92, G99c and G119b) were showed strong antibacterial activity against six bacterial pathogens. In our present study, probiotics like *Bifidobacterium* sp. and *Lactobacillus* sp. had the antibacterial activity against eight bacterial pathogens. *Bifidobacterium* sp. had the strongest bactericidal activity than *Lactobacillus* sp. and *S. cerevisiae* and *Bifidobacterium* sp. had the high inhibitory effect against *Salmonella* sp. (18 mm). *Lactobacillus* sp. had minimum inhibitory effect against all eight pathogens and it had the high inhibitory effect against *Salmonella* sp. (10 mm). But *S. cerevisiae* had antibacterial activity against only three bacterial pathogens.

[4] Reported that the inhibitory effect of probiotic *Lactobacilli* in co-culture with *S. arueus* and standard *S. arueus* ATCC 25923. The best result was obtained when *S. arueus* and standard *S. arueus* ATCC 25923 co-incubated with *L. plantarum*, 87% and 77% respectively. In our present study, the probiotic strains *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cerevisiae* were co-cultured with eight bacterial pathogens. The number of colonies indicated the antimicrobial activity of probiotic strains. From the results obtained increase in probiotic concentration showed more inhibitory activity on the pathogen. For example 0.6ml showed more inhibitory activity than 0.2ml. *Bifidobacterium* sp. had the higher antimicrobial activity than other probiotic strains.

[6] Reported that the effect of different prebiotics on the growth of the probiotics tested two individual basal media MRS broth and RCM broth. The OD value was measured at 600 nm. His result showed that the *L. acidophilus* grows maximally on SOS, glucose and raffinose followed by FOS and inulin. In our present study, the effect of different prebiotics on the probiotics tested two individual basal media. The OD value was measured at 650 nm. The results were compared with the OD value of probiotics without prebiotics and probiotics with prebiotics. The results indicated prebiotics are helped to support the growth of probiotics. The *Bifidobacterium* sp. maximally grown on the prebiotics like inulin and the maximum growth was present in Bifidobacterium broth than in MRS broth. The *Lactobacillus* sp. grow maximally when supplemented with the prebiotics like glucose. The *S. cerevisiae* grow maximum on the prebiotics like lactose and the maximum growth was present in YPD broth than in MRS broth.

[8] Found that the stimulated growth of *Bifidobacteria* in the colon could lead to the inhibition colon carcinogenesis. In our present study, DPPH method was used. The probiotic species, *Bifidobacterium* sp., *Lactobacillus* sp. and *S. cerevisiae* showed stongest radical scavenging assay. Different concentrations of probiotic strains were used. The OD value measured at 250 nm. The probiotic cultures *Bifidobacterium* sp., *Lactobacillus* sp. *and S. cerevisiae* showed stongest radical scavenging activity. The probiotic culture concentration was increases the number of DPPH free radicals was decreased. The OD value decreased in low concentration to high concentration of the sample. *Bifidobacterium* sp. had high antioxidant activity than *Lactobacillus* sp. *and S. cerevisiae.*

REFERENCES

[1].Collins MD and Gibson GR. 1999. "Probiotics, Prebiotics, and Symbiotics: approaches for modulating the microbial ecology of the gut". *Am. J. Clin. Nutr*. 69: 1052S-7S.

[2].Gibson GR. 1999. Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *J. Nutr.* 129: 1438S-1441S.

[3].Gibson GR and Roberfroid M 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr*. 125: 1401-1412.

[4].Nazila Arbab Soleimani, Rooha Kasra Kermanshahi, Bagher Yakhchali and Taher Nejad Sattari. 2010. Antagonistic activity of probiotic *Lactobacilli* against *Staphylococcus aureus* isolated from bovine mastitis. *African Journal of Microbiology Research*. 4(20): 2169-2173.

[5].Orrhage K and Nord CE. 2000. "*Bifidobacteria* and *Lactobacilli* in human health and other colonic bacteria. *Journal of Applied Bacteriology*. 77(4): 412-20.

[6].Ping Su, Anders Henrikssona and Hazel Mitchell. 2007. Selected prebiotics support the growth of probiotic mono-cultures *in vitro. Food microbiology*. 13: 134-139.

[7].Przemyslaw Jan Tomasik and Piotr Tomasik. 2003. Probiotics and Prebiotics. *Cereal Chem*. 80(2): 113-117.

[8].Reddy BS. 1999. Possible mechanisms by which pro- and prebiotics influence colon carcinogenesis and tumor growth. *Journal of Nutrition*. 129: 1478S-1482S.

[9].Tambekar DH and Bhutada SA 2010. Acid bile tolerance, Antibacterial activity, Antibiotic Resistance and Bacteriocins Activity of Probiotic *Lactobacillus* sp. *Recent Research in Science and Technology*. 2(4): 94-98.

[10].Tovar-Ramirez D, ZamboninInfante J, Gastesoupe FJ and Vazque-Juarwz. 2004. Influence of dietary live yeast on European sea bass (*Dicentrarchus larbrax*) larval development. *Aquaculture*. 234: 415-427.