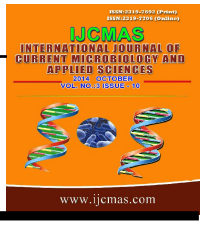
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 657-663**
http://www.ijcmas.com



Original Research Article

Influence of Different Prebiotics and Probiotics on Selective Intestinal Pathogens

Anayata Sharma[1*] and Dadhich[2]

[1]Department of biotechnology, D.A.V College, Abohar-152123, India
[2]Officiating Principal & Dean Academics, Dolphin P.G.College of Life Sciences- Mohali, India
**Corresponding author*

Keywords

Probiotics, Prebiotics, *Escherichia coli* , *Salmonella typhi*, *Lactobacillus casei*, *Lactobacillus rahmnosus*, *Streptococcus*

A B S T R A C T

Probiotics are living micro organism used as food supplements, which provide health benefit when consumed, by improving intestinal microbial balance of host. Probiotics had high antagonistic activity, antimicrobial activity. Prebiotics had the ability to enhance the growth of probiotics. The probiotic strain *Lactobacillus casei*, *Lactobacillus rahmnosus*, *Lactobacillus fermentum* and *Streptococcus thermophillus* were isolated and tested for antagonistic activity. The combination of variable concentration of prebiotics on the growth was observed upto 96 hrs. Among various prebiotics used Garlic and Soya beans showed higher effects on growth of probiotics. Among these microbes *Lactobacillus casei* & *Lactobacillus rahmnosus* showed higher antagonism against *Escherichia coli*& *Salmonella typhi.* Probiotics reduce the risk of intestinal disease like diarrhea and typhoid, probably due to their role in suppressing the activity of certain bacterial enzymes with the production of bacteriocins or with the help of lowering the pH by producing certain acids like lactic acid and acetic acid. Thus probiotic treatment will offer a promising alternative to the use of antibiotics in healthcare. Hence prebiotics are enhancers of our savers (probiotics). The FAOhas defined probiotics as "living microorganisms when consumed in adequate amounts as a part of diet confer a health benefit on the host".

Introduction

Probiotics and prebiotic formulations are proving very popular and it has been documented on television, in consumer press and inshelvs of the health food stores. This is because, unlike many nutrition trends, the evidence that they promote good health is strong (Gibson, 2003). Not only do they help us digest our food, but they may also help reduce the severity of food poisoning and reduce effects of food intolerance. Users report that formulations also help improve general well-being and they may help improve performance in sports due to improved digestion of food and, therefore increased availability of nutrients. Diarrhea occurs in about 20% of patients who receive antibiotics. Antibiotics may directly affect the indigenous gut micro biota by

compromising colonization resistance and favoring the growth of pathogenic micro-organisms. Indigenous intestinal bacteria protect the host from infection by exogenous pathogens and opportunistic bacteria that are present in the gut. Thimechanism of protection is termed colonization resistance.

Prebiotics

Prebiotics can be defined as nutrients and constituents of food which our gut flora feed upon thus increasing their number. Prebiotics include Fructo Oligo Saccharides, which are found naturally in many plants including onion, wheat, garlic, chicory roots and artichokes where they function as storage carbohydrates and some in some pulses, fruits and some cereal products. These compounds are called as non digestible oligosaccharide (NDOs) These effects include serving as sources of energy, regulation of gene expression and cell differentiation and anti-inflammatory properties. Prebiotics have also been incorporated into supplements and functional foods, in order to exert positive effects on digestion, the immune system. Structurally they are mixture of polymers (chain repeats of same unit molecule) and oligomers comprising branching chains between 10-60 repeat units. Other prebiotics as Inulins, Lactilol, Soyoligosaccharides, Xylo oligosaccharides, Lactulose, Pyrodextrins

Materials and Methods

Media Required: MRS Agar (Hi Media) at pH 7±2.

Microbial culture: Four probiotic strains; *Lactobacillus casei, Lactobacillus rahmnosus, Lactobacillus fermentum* and *Streptococcus thermophillus* were obtained from the National Collection of Dairy cultures, National Dairy Research Institute, Karnal (Haryana).

Two strains of pathogens were included in study *E.coli,& S. typhi.*

Preparation of Inoculum

Growth of organism appeared on Lactobacillus MRS Agar Medium after 24-48hrs of incubation, and was inoculated with inoculation loop, transferred to 5% peptone water. The inoculum strength was checked at 540nm and set at OD 0.3 to be used as inoculums when OD is 1.0 the dry weight is 0.28 (Rosenmarei *et al*, 1989).

Effect of Prebiotics on Probiotics

Enrichment studies on yakult, Soya Extracts, Garlic were used for given cultures. Various concentrations of akult, Soya Extracts, Garlic 1.25ml, 2.5ml, 5ml were used; in presence of given culture added in media and incubated at 37°C. Results were recorded for four days with 24 hr interval compared with reference to control.

Determination of antimicrobial activity of Probiotics – test pathogens organisms by well diffusion method and over lay method

Well Diffusion Method - Two strains of pathogens were included in study (*E.coli, S. typhi*) selective media were used to test the anti microbial activity against these pathogens . 0.1 ml dilution of each pathogen were tested by pour plate method , four holes was made by using sterile cork borer and then pure cultures were added and results were recorded for 3 days incubation with 24 hrs interval.

Overlay method: Two strains of pathogens were included in study (*E.coli , S. typhi*)

selective media were used to test the anti microbial activity against these pathogens. 0.1 ml dilution of each pathogen were tested by pour plate method , incubated anaerobically for 4 days for growth , then an another layer of media having either pure culture and isolated culture was over laid over it , results were recorded after 4 days anaerobic incubation

Metabolite Characterization

To determine if lactic acid solely responsible for inhibition

The MRS broth supplemented with 1% lactic acid (pH 4 and 6), the MRS broth supplemented with the 1% acetic acid (pH 4), the MRS broth (pH 6.4) and were inoculated test pathogens incubated for 4 days at 27^0 C. Growth was recorded at 550 nm.

To determine effect of pH on anti microbial activity

The cell free supernatants were adjusted to pH 4.5, 5.0, 5.5, 6.0 & were inoculated with test pathogens incubated for 4 days at 27^0C . Growth was recorded at 550 nm.

To determine heat stability

With all standard and optimal conditions, cell free supernatants were treated at 50^0, 70^0, 90^0, and 121^0C for 30 min and were inoculated with test pathogens, and incubated for 4 days at 27^0 C . Growth was recorded at 550 nm.

Culture Supernatant Minimum Inhibitory Concentration

With all standard and optimal conditions, cell free supernatant of probiotic cultures were diluted 4:1, 2:1, 1:1, 1:2and 1:4 in MRS broth and inoculated with test pathogens incubated for 4 days at 27^0 C . Growth was recorded at 550 nm.

Results and Discussion

Effect of Prebiotics on Probiotics

The cell biomass production in response to variable concentrations of yakult, Soya beans and Garlic were examined. The combination of variable concentration of these prebiotics was used. According to data presented in tables there is linear increase in growth upto 96 hr incubation period.

Determination of antimicrobial activity of Probiotics – Test pathogens organisms by Well Diffusion Method and Overlay method

Data presented in table given below involves the use of agar well method with reference to sensitivity of test pathogens in presence of pathogens.Antimicrobial sensitivity showing zone of inhibition against *E.coli*
Result of the agar overlay method showed that all of the probiotic strains were showing inhibiton against test microbial isolates .The spectrum of their antimicrobial activity varied.

Metabolite Characterization

To determine if lactic acid solely responsible for inhibition

Results are summarized in table 4.5.1 (a) demonstrate that low pH alone inhibits growth of test pathogen with either 1% lactic acid or 1% acetic acid To determine effect of pH on anti microbial activity. Table 4.5.2 (a), (b), (c), (d) shows pH neutralization effects on anti microbial properties of cell free supernatant for *L. rhamnosus , L. fermentum, L. casei, S.*

thermophillus against test pathogen respectively. Adjustments were made in pH (0.5 increments) to supernatant between starting pH6.0 to pH4.5 before inoculation with test pathogens. *L.rhamnosus* supernatant retained anti bacterial activity against *E. coli* and *S. typhi* at pH-6. *L. fermentum* supernatant retained anti bacterial activity against *E. coli* at pH-4.5 and against *S. typhi* at pH 5.5. *L. casei* supernatant retained anti bacterial activity against *E. coli* and *S. typhi* at pH-4.5. *S. thermophillus* supernatant retained anti bacterial activity against *E. coli* and *S. typhi* at pH-6.

Culture supernatant minimum inhibitory concentration

According to table given below ,dilutions of culture supernatant (4:1,2:1,1:1,1:2,and1:4) were prepared in MRS broth to determine the minimum concentration of metabolites that will inhibit at a 1:2 dilution for *L. rhamnosus* , *L fermentum*, *L .casei* and no inhibition in *S. thermophillus*

Table.2 Biomass concentration using Soya beans

Conc.of SOYA BEANS (%)	1.5%				2.5%				5%			
	L. casei	L. fermentum	L. rhamnosus	S. thermophilus	L. casei	L. fermentum	L. rhamnosus	S. thermophilus	L. casei	L. fermentum	L. rhamnosus	S. thermophilus
Time period(Hr)	Cell Biomass (mg dry wt/ml)											
24	0.40	0.66	0.31	0.21	0.42	0.60	0.31	0.28	0.43	0.65	0.20	0.21
48	0.57	0.65	0.65	0.39	0.73	0.63	0.65	0.57	0.53	0. 63	0.64	0.60
72	0.66	0.84	0.66	0.74	0.64	0.82	0.66	0.69	0.63	0.75	0.81	0.74
96	0.66	1.20	0.68	0.75	0.64	1.21	0.66	0.75	0.64	1.21	0.82	0.78

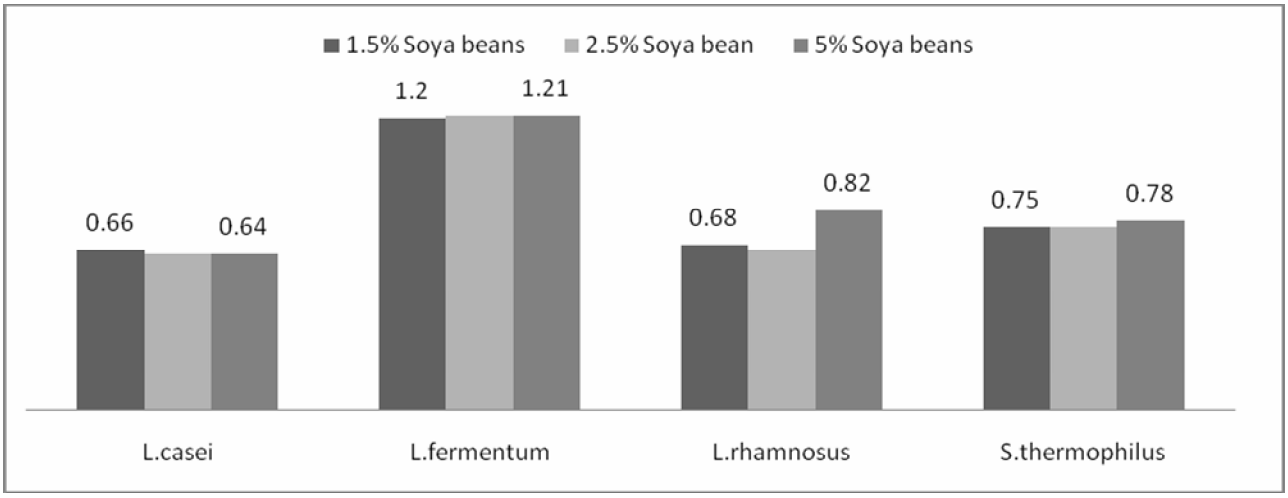


Figure.2 Biomass concentra tion using Soya bean

Table.3 Biomass concentration using Garlic

Conc.of GARLIC (%)	2.5%				5%				10%			
	L. casei	L. fermentum	L. rhamnosus	S. thermophilus	L. casei	L. fermentum	L. rhamnosus	S. thermophilus	L. casei	L. fermentum	L. rhamnosus	S. thermophilus
Time period (Hr)	Cell Biomass (mg dry wt/ml)											
24	0.39	0.44	0.31	0.37	0.37	0.72	0.31	0.35	0.43	0.79	0.28	0.34
48	0.52	0.50	0.64	0.55	0.30	0.61	0.65	0.55	0.53	0.60	0.56	0.53
72	0.61	0.64	0.66	0.55	0.64	0.61	0.66	0.59	0.65	0.59	0.69	0.50
96	0.75	0.64	0.69	0.55	0.66	0.60	0.67	0.62	0.69	0.60	0.73	0.68

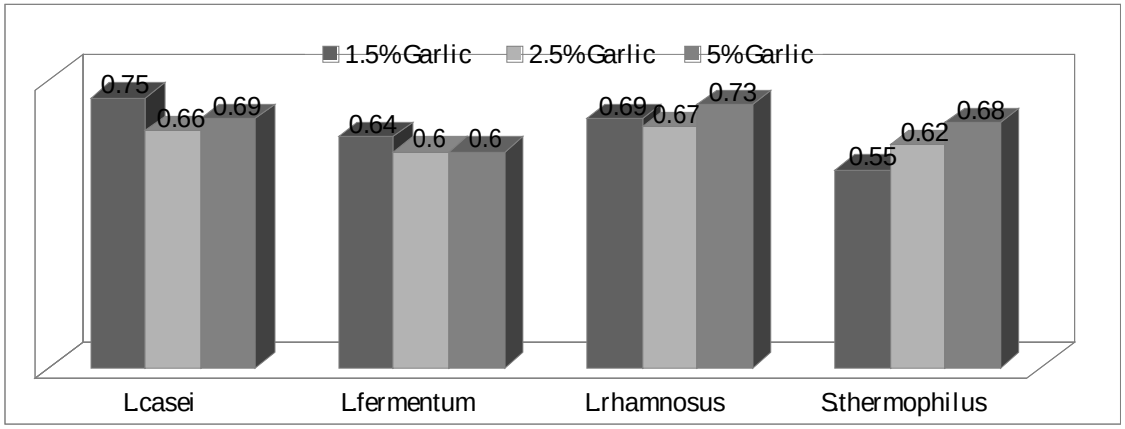
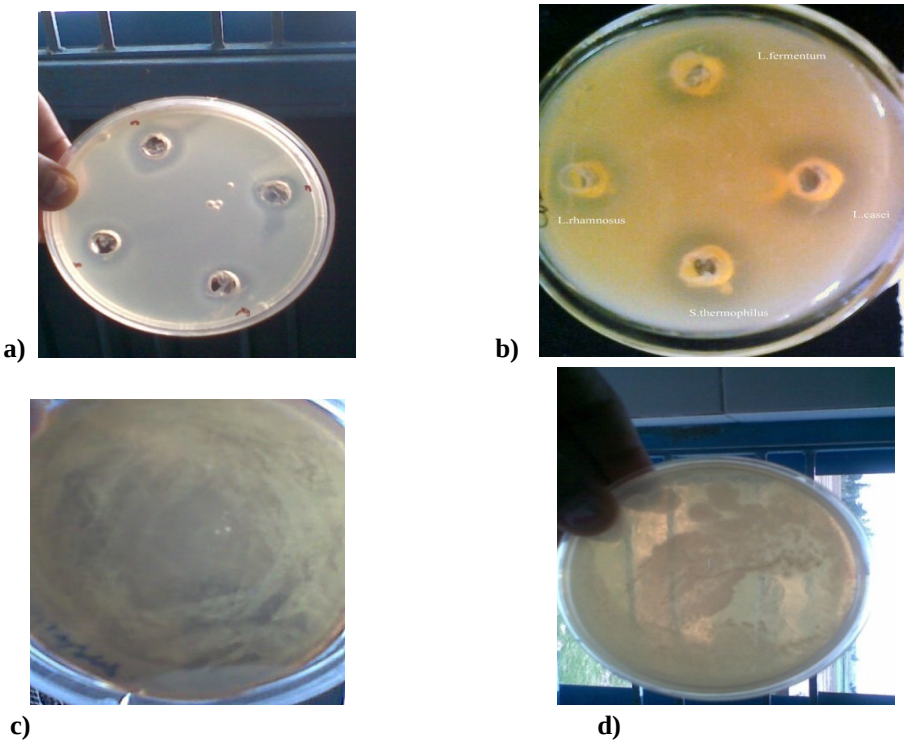


Figure.3 Biomass concentration using Garlic



Figures.4a, b, c & d Results of Agar Well method (figures a&b)and overlay method (Figures c&d)

Table.4 Zone of inhibition against test pathogens

Test organism	ZONE (Diameter) OF INHIBITION			
	L.casei	*L.fermentum*	*L.rahamnosus*	*S.thermophillus*
E.coli	17mm	19mm	15mm	12mm
S.typhi	15mm	10mm	12mm	09mm

Table.5 effects of acid on growth of test pathogens.

Test Organism	Lactic acid				Acetic acid	
	pH4		pH6		pH4	
	0 hr	After 4 days incubation	0 hr	After 4 days incubation	0 hr	After 4 days incubation
S.typhi	0.56	0.47	0.57	0.55	0.49	0.39
E.coli	0.53	0.38	0.50	0.50	0.49	0.34

Table.6 Effects of pH on growth of *E.coli*

pH	Test pathogen **E .coli**							
	L.rhamnosus		L.fermentum		L.casei		S .thermophillus	
	0 hr	3 days	0 hr	3 days	0 hr	3 days	0 hr	3 days
4.5	0 .331	0.279	1.585	1.25	0.88	1.58	0.95	1.33
5.0	0.331	0.46	1.43	1.26	0.88	1.70	0.92	1.37
5.5	0.33	0.52	1.26	1.70	0.92	1.33	0.86	1.70
6.0	0.33	0.27	1.36	1.307	0.90	0.74	0.90	0.55

Table.7 Effects of pH on growth of *S.typhi*

pH	Test pathogen **S.typhi**							
	L.rhamnosus		L.fermentum		L.casei		S .thermophillus	
	0 hr	3 days	0 hr	3 days	0 hr	3 days	0 hr	3 days
4.5	0 .331	0.279	1.62	1.10	0.91	1.67	0.82	1.70
5.0	0.331	0.46	1.50	1.90	0.94	1.64	0.86	1.87
5.5	0.33	0.52	1.32	1.02	1.01	1.70	0.91	1.90
6.0	0.33	0.27	1.21	1.10	1.06	0.86	0.89	0.73

Table .8 showing Minimum inhibitory concentration L. rahmnosus, L.fermentum, L.casei & S .thermophillus against *E.coli*

Ratio	Test pathogen **E .coli**							
	L.rhamnosus		L.fermentum		L.casei		S .thermophillus	
	0 hr	2days	0 hr	2 days	0 hr	2 days	0 hr	2 days
1:2	0.88	0.38	0.937	2.38	0.94	0.71	0.81	3.55
1:4	1.15	1.11	0.99	3.18	1.03	3.76	1.5	3.41
1:1	1.09	0.43	1.22	2.4	0.58	2.2	0.81	3.95
2:1	1.21	1.04	1.5	2.62	1.28	2.8	0.77	2.28
4:1	0.93	0.92	1.41	2.87	1.41	2.55	0.69	2.04

Table .9 showing Minimum inhibitory concentration *L. rahmnosus, L.fermentum, L.casei & S .thermophillus* against *S.typhi*

Ratio	Test pathogen **E .coli**							
	L.rhamnosus		*L.fermentum*		*L.casei*		*S .thermophillus*	
	0 hr	2days	0 hr	2 days	0 hr	2 days	0 hr	2 days
1:2	1.05	0.49	1.1	2.98	0.87	0.74	0.791	3.52
1:4	0.59	0.53	1.2	2.87	0.4	3.5	1.46	3.35
1:1	0.87	0.4	1.331	2.02	0.58	3.74	0.717	3.09
2:1	0.7	0.69	1.4	2.1	1.22	2.30	0.789	2.71
4:1	0.84	0.401	1.33	2.36	0.95	2.44	0.798	2.16

References

Gibson, G.R. 2003. Probiotics & Prebiotics and their Function. Functional Nutrition 2 (2): 11–13.