

## Research Article



## PROBIOTIC POTENTIAL OF COMMERCIAL AVAILABLE PROBIOTIC PREPARATIONS IN PREVENTION OF ENTERIC BACTERIAL INFECTIONS: AN *IN-VITRO* STUDY

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### ABSTRACT

Probiotics exhibit a beneficial effect on the health of human being by improving the intestinal microbial balance and increase resistance to infection and protect the host from intestinal infections. Some commercial available probiotic preparations such as Sporlac powder, LactoBacil plus, P-Biotics kid, Gastroline, Pre-Pro kid were evaluated for their probiotic potential and determined their antibacterial potential against enteric bacterial pathogens such as *Escherichia coli* (MTCC 443), *Enterobacter aerogenes* (MTCC 111), *Klebsiella pneumoniae* (MTCC 2653), *Proteus vulgaris* (MTCC 426), *Salmonella typhi* (MTCC 734) and *Shigella flexneri* (MTCC 1457). The commercial probiotic preparations P-Biotics kid, Pre-Pro kid and LactoBacil plus were found potent antibacterial against *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi* and *Shigella flexneri*. Study suggested that use of these probiotic preparations can be help to prevent or control the intestinal infections.

**Keywords:** Probiotics preparations, Antibacterial activity, enteric bacterial infection.

### INTRODUCTION

There is considerable interest in including probiotics in dietetic products for children, and such products are marketed in many countries. Probiotics are available in many forms, such as capsules and powders. Probiotic is the group of microbes that may help directly for enhancing resistance against intestinal pathogens and in the prevention of diseases. Probiotic bacteria may produce various compounds, which are inhibitory to the pathogen's growth, which include organic acids (lactic and acetic acids), bacteriocins, and reuterin. The organic acids not only lower the pH, thereby affecting the growth of the pathogen, but they can also be toxic to the microbes. *Lactobacilli* are known to produce many types of bacteriocins like acidophilin, acidolin, lactocidin, bulgarican, lactolin, lactobacillin and lactobrevin<sup>1</sup>.

Infectious diseases are the biggest problem in human being and every year gastrointestinal infections are responsible for significant morbidity and mortality worldwide<sup>2</sup>. World Health Organization<sup>3</sup> estimates there to be more than four billion episodes of diarrheal disease annually, while there were 2.2 million deaths attributable to enteric infection, making it the fifth leading cause of death at all ages worldwide. Enteric bacteria comprised of *Salmonella* species, *Shigella* species, *Proteus* species, *Klebsiella* species, *E. coli*, *Pseudomonas* species, *Vibrio cholerae* and *S. aureus* which are major etiologic agents of enteric infection<sup>4</sup>. There is increasing evidence that probiotics are beneficial in gastrointestinal disturbances, such as diarrhoea, dysentery, typhoid etc<sup>5</sup>. The rise in antibiotic resistant bacteria has awakened the scientific community to the prophylactic and therapeutic uses of probiotics, and to reconsider them as alternatives to antibiotics<sup>6</sup>.

Various *Lactobacilli*, *Bifidobacteria* and *Streptococcus* species have been evaluated for the prevention or treatment of various infectious diseases and these were found to be safe<sup>7</sup>. Various commercial probiotic preparations are also available in the market in the form of capsules, liquid/gel and powdered that claims for prevention of infectious diseases. Commercially available probiotic preparations include *Lactobacillus* alone (Lactiflora, LactoBacil, Lactocap, Lactovit, Sporlac) or in combination with *Streptococcus* (Lacticin) or *Saccharomyces* (Laviest) and showed beneficial effects<sup>8</sup>.

The functional properties and safety of probiotics of particular strains of *L. casei*, *L. lactis*, *L. acidophilus* from various sources have been extensively studied and commercial probiotic preparations also claims its efficiency for prevention of infectious diseases, but probiotics potential with significance in prevention of enteric infection have not been reported so far. The controlled clinical trials that examined the biological effects of different probiotic preparations for human is necessary. Therefore the attempt was made to evaluate the probiotics potential of commercial available probiotic preparations in prevention of enteric bacterial infections.

### MATERIALS AND METHODS

The commercial available probiotics were purchased from local market of Amravati (Table 1). These preparation contain

**Acid and bile salt tolerance:** Probiotics preparations were inoculated into MRS medium of varying pH, i.e. pH 2, 3, 4 and 5; as well as broth with varying concentrations of bile salt (0.5, 1.0, 1.5 and 2.0%), and incubated at 37°C for 48h. Then 0.1mL inoculums was transferred to MRS agar by pour plate method and incubated at 37°C for 48h. The



growth of LAB on MRS agar plate was used to designate isolates as acid or bile salt tolerant<sup>9,10</sup>.

**Detection of antagonistic activities:** The antagonistic properties of probiotics preparations were determined by modifying the disc diffusion method. Sterile blotting paper discs (10mm) were dipped into 48h incubated culture broth and then placed on solidified Nutrient agar seeded with 3h (10<sup>5</sup>cfu/ml) old culture of test pathogens, which included *Escherichia coli* (MTCC 443), *Enterobacter aerogenes* (MTCC 111), *Klebsiella pneumoniae* (MTCC 2653), *Proteus vulgaris* (MTCC 426), *Salmonella typhi* (MTCC 734) and *Shigella flexneri* (MTCC 1457). The plates were kept at 4°C for 1h diffusion and then incubated at 37°C for 24h. Zones of inhibition were measured<sup>11</sup>.

**Antibiotic resistance:** The antibiotic resistance of probiotic preparations was assessed using antibiotic discs (Hi media Laboratories Pvt. Ltd. Mumbai, India) on MRS agar plates. A 10<sup>5</sup> cfu/mL suspension of freshly grown test organisms was mixed with 5mL of MRS soft agar (0.5% agar) and over layered on bottom layers of MRS agar. The antibiotic discs were placed on the surface of agar and the plates were kept at 4°C for 1h for diffusion, and then incubated at 37°C for 24h<sup>12</sup>. Resistance was assessed against Ampicillin (1µg), Cephalothin (30µg), Co-Trimoxazole (25µg), Gentamicin (10µg), Nalidixic acid (30µg), Nitrofurantoin (300µg), Norfloxacin (10µg) and Tetracycline (25µg).

**Preparation of bacteriocin assay:** The probiotic preparations were selected as potential bacteriocin producers grown in MRS broth at 37°C for 48h. Cell suspensions were centrifuged at 5000 rpm for 15 min. The pH of the cell free supernatant was adjusted to pH 6.5-7.0 with 1N NaOH to neutralize the acids in broth

culture of probiotics. The antagonistic activity of bacteriocin was determined by disc diffusion method<sup>13,14</sup>.

**Heat and pH sensitivity:** To test the heat sensitivity, culture supernatant containing bacteriocin was heated for 10 min. at 60°C, 70°C, 80°C, 90°C, 100°C and 121°C and bacteriocin activity was tested against *E. coli*. Similarly sensitivity of bacteriocins to different pH was tested by adjusting the pH of culture supernatant (containing bacteriocins) in the range of pH 3.0, 4.5, 7.0 and 9.0 then bacteriocin antibacterial activity was detected by disc diffusion method against *E. coli*<sup>15</sup>.

**Susceptibility to proteolytic enzyme:** Action of proteolytic enzyme was tested on culture supernatant by treatment with pepsin and trypsin each at a final concentration of 1.0 mg/ml. It was then incubated at 37°C for 2h and residual activity of bacteriocin was assayed against *E. coli*<sup>14</sup>.

Multiple antibiotic resistance (MAR) were determined as following modified formula

$$\text{MAR Index for a antibiotics} = \frac{\text{Number of antibiotics resistance}}{\text{No. of antibiotics tested} \times \text{No. of}}$$

#### Calculation of probiotic potential

Each property commercial probiotic preparations were scored as under

Probiotic characters	Indication	Score
Acid, bile tolerance sensitivity, antibiotic resistant, acid/ alkali/ heat tolerance of bacteriocin	Sensitivity	0
	Resistance	1
Antibacterial potential of probiotics preparations (zone of inhibition of growth in mm)	14-16 mm	1
	17-20 mm	2
	> 21 mm	3

**Table 1:** Commercial Probiotic preparations used in study

Probiotics preparations	Batch Number	Manufacturer	Composition
	Mfg Date		
Sporlac powder	Batch No. A9SP004 Mfg. Date 01/2009	Uni-Sanyo Ltd. Plot no. B-4, MIDC, Lote Parshuram, Maharashtra-415722.	<i>L. sporogenes</i> (150 million spores)
LactoBacil plus	Batch No. 988024 Mfg Date 11/2009	Swiss Garnier Life Sciences 21-23 Industrial Area, Mehatpur, Dist. UNA, Himachal Pradesh-174315 Organon (India Ltd)	<i>L. acidophilus</i> (0.4 billion), <i>L. rhamnosus</i> (0.4 billion), <i>Bifidobacterium longum</i> (0.4 billion) <i>B. bifidum</i> (0.25 billion), <i>S. boulardii</i> (0.05 billion)
P-Biotics kid	Batch No.60SPK004 Mfg Date 05/2009	Swiss Garnier Life Sciences 21-23 Industrial Area, Mehatpur, Dist. UNA, Himachal Pradesh-174315.	<i>L. acidophilus</i> (350 million), <i>L. rhamnosus</i> (200 million), <i>L. casei</i> (150 million), <i>L. plantarum</i> (150 million), <i>L. bulgaricus</i> (150 million), <i>B. longum</i> (150 million), <i>B. infantis</i> (150 million), <i>B. breve</i> (150 million), <i>S. thermophilus</i> (200 million), <i>S. boulardii</i> (0.05 billion)
Gastroline	Batch No.SG012 Mfg Date 07/2009	Swiss Garnier Life Sciences 21-23 Industrial Area, Mehatpur, Dist. UNA, Himachal Pradesh	<i>L. acidophilus</i> (0.4 billion), <i>L. rhamnosus</i> (0.4 billion), <i>B. longum</i> (0.4 billion), <i>B. bifidum</i> (0.25 billion), <i>S. boulardii</i> (0.05 billion)
Pre Pro kid	Batch No. 155SPR 027 Mfg Date 07/2009	Swiss Garnier Life Sciences 21-23 Industrial Area, Mehatpur, Dist. UNA, Himachal Pradesh.	<i>L. acidophilus</i> (650 million), <i>L. rhamnosus</i> (400 million), <i>B. longum</i> (100 million), <i>B. bifidum</i> (100 million), <i>S. boulardii</i> (50 million)



**Table 2:** Probiotic potential of commercial available probiotic preparations

Probiotics	Acid tolerance		Antibacterial activity of probiotics against enteric pathogens (Zone of Inhibition in mm)							Antibiotic susceptibility							Antibacterial activity of bacteriocin against enteric pathogens (Zone of inhibition in mm)					Heat sensitivity of bacteriocin				Sensitivity to different pH						
	pH 2	2%	E. coli	E. aerogenes	K. pneumoniae	Pr. vulgaris	S. typhi	Sh. flexneri	Ampicillin (A)	Cephalothin (Ch)	Co-Trimoxazole (Co)	Gentamicin (G)	Nalidixic acid (Na)	Nitrofurantoin (Nf)	Norfloxacin(Nx)	Tetracycline (T)	E. coli	E. aerogenes	K. pneumoniae	Pr. vulgaris	S. typhi	Sh. flexneri	60°C	70°C	80°C	90°C	100°C	121°C	pH 3	pH 4.5	pH 7	pH 9
Sporlac powder	R	R	17	20	21	20	23	21	R	R	S	S	R	S	S	R	21	23	23	22	25	23	R	R	R	R	S	S	R	R	R	S
LactoBacil plus	R	R	20	20	17	19	22	18	R	R	R	R	R	R	S	R	22	23	23	23	25	19	R	R	R	R	S	S	R	R	R	S
P-Biotics Kid	R	R	18	20	23	22	23	20	R	R	R	R	R	R	S	R	20	19	20	23	27	25	R	R	R	R	S	R	R	R	S	
Gastroline	R	R	16	16	15	17	19	18	R	R	R	R	R	R	S	R	20	22	22	22	25	23	R	R	R	R	S	S	R	R	R	S
Pre-Pro kid	R	R	16	20	23	25	25	22	R	R	R	R	R	S	S	R	19	22	24	23	27	25	R	R	R	R	S	S	R	R	R	S

Where: R=Resistant, S= Sensitive

Cumulative probiotic potential is the sum of score of acid, bile tolerance, antibacterial potential, acid and alkali and heat tolerance of bacteriocin. Probiotic potential was calculated as observed score divided by maximum score into hundred.

$$\text{Probiotic potential} = \frac{\text{Observed score}}{\text{Maximum score}} \times 100$$

**RESULTS AND DISCUSSION**

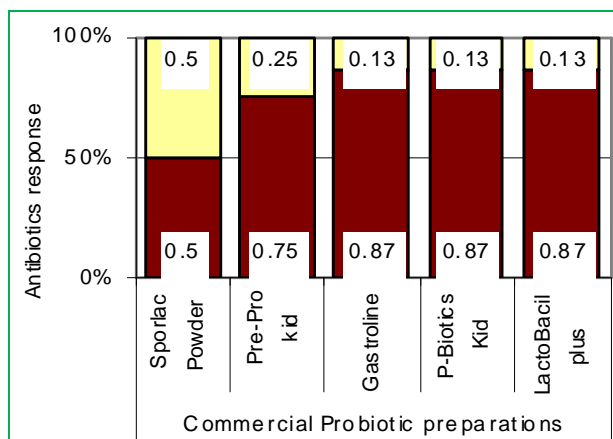
In present study, a total of five probiotic preparations were analyzed for their probiotic potential on the basis of their acid and bile tolerance, antibacterial activity, antibiotic resistance, antibacterial potential of bacteriocin, acid, alkali and high temperature tolerance of bacteriocin.

**Acid and Bile salt tolerance:** Probiotics potential of LAB is necessarily its ability to resist bile salts and acidic pH<sup>16</sup>. In this study, commercial probiotic showed acid tolerance at pH 2 and bile salt tolerance at 2%. Before reaching the intestinal tract, probiotic bacteria must first survive transit through the stomach where the pH can be as low as 1.5 to 2<sup>17</sup>. Tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host<sup>18</sup>. This will help probiotic microorganism to reach the small intestine and colon and contribute in balancing the intestinal microflora<sup>19</sup>.

**Antagonistic activity:** Commercial probiotic preparations Pre-Pro kid, P-Biotics kid, LactoBacil plus, were strong antagonistic (Score 15) where as Gastroline Sporlac powder, containing probiotics (Score 9) were moderate antagonistic against enteric pathogens (fig.1). This may be due to the production of acetic and lactic acids that lowered the pH of the medium or competition for nutrients, or due to production of bacteriocin or antibacterial compound<sup>20, 21</sup>. Our study showed that the probiotic preparation such as useful to prevent enteric infections such as diarrhoea, dysentery, typhoid, food poisoning etc.

**Antibiotic resistance:** Multiple antibiotic resistances (MAR) index (fig.1) of commercial probiotic preparations (MAR index 0.75-0.87). Such resistance to a wide spectrum of antibiotics indicated that probiotics preparation if induced in patients treated with antibiotic therapy may be helpful in faster recovery of the patients due to rapid establishment of desirable microbial flora. Present studies have lent support the use of selected probiotic preparation for the prevention of antimicrobial-associated diarrhoea. Resistance of the probiotic strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections<sup>22</sup>.

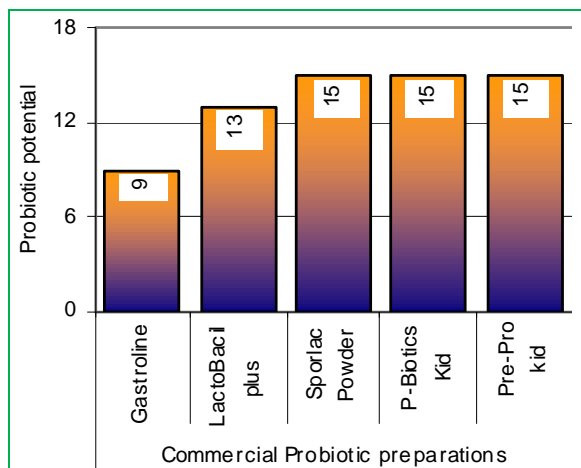
**Figure 1:** MAR Index of commercial probiotic preparations



**Antibacterial activity and characterization of bacteriocin:**

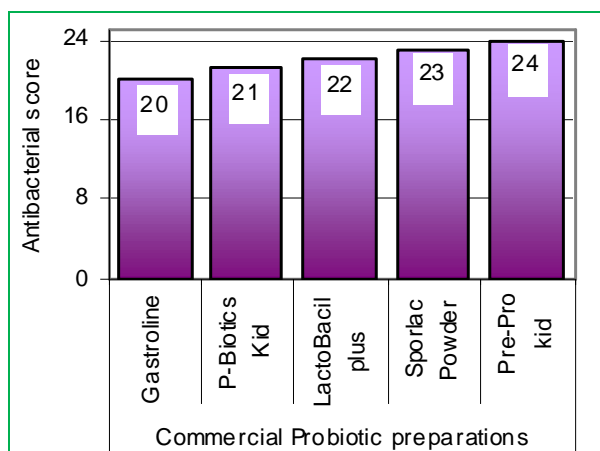
Antibacterial activity (fig.2) of bacteriocin produced by commercial probiotic preparations Pre-Pro kid, Sporlac powder, LactoBacil plus, P-Biotics kid, Gastroline containing probiotics (Score 20-24). Bacteriocins of all the selected commercial probiotic preparations were stable at 90°C and acidic to neutral pH i.e. (3 to 7) except P-Biotics kid was stable up to 100°C. Bacteriocins of standard probiotic strains were stable up to 100°C and pH 3 to 7.



**Figure 2:** Antagonistic activity of commercial probiotic preparations against enteric pathogens

Where: Antibacterial Score between 20- 24 moderate and 27-31 Strong antibacterial.

Among the commercial probiotic preparations Pre-Pro kid was found efficient probiotic followed by P-Biotics kid, Sporlac powder, LactoBacil plus, Gastroline (fig. 3). Most commercial products at the time of purchase have low numbers of viable organisms with little clinical benefit. Production and quality control are not regulated as per the guidelines of probiotic products, but few products are likely to achieve the benefits identified in formal clinical trials. Even with better-characterized isolates, trial results can vary reflecting production and stability of the products<sup>23</sup>. The present study revealed that probiotics preparation were acid tolerant at pH 2, bile tolerant at 2% bile salt, antibacterial against enteric pathogens, antibiotics resistant to most of the antibiotics and their bacteriocin stable at temperature 121°C and pH 3 to 9 and strong antibacterial against enteric bacterial pathogens.

**Figure 3:** Antibacterial activity of bacteriocin produced by commercial

Where: Antibacterial Score between 20- 24 moderate and 27-31 Strong antibacterial.

## CONCLUSION

The present study revealed commercial probiotic were acid tolerant at pH 2, bile salt tolerant at 2% bile salt concentration, antibacterial against enteric pathogens, antibiotics resistant to most of the antibiotics and their bacteriocin stable at temperature 121°C and pH 3 to 9. It was concluded the commercial probiotic preparation can be used for oral therapy and as prophylactic to prevent the enteric infections such as diarrhoea, gastroenteritis, dysentery, urinary tract infections, food poisoning, typhoid, irritable bowel syndrome etc. Study will support their use in prevention of enteric bacterial infection and for the betterment of the health of the public.

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