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RESEARCH ARTICLE

Specific antibacterial activity of bifidobacteria

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Abstract : Dairy organisms like Bifidobacteria and Lactobacillus are gaining importance as probiotic organisms. They are increasingly used in food as functional foods to improve nutritional benefits. Recently much emphasis is also laid for isolating these organisms from indigenous sources. Hence an attempt is made in his paper to isolate bifidobacterium group of organisms from breast fed infant faeces and study their antimicrobial property.

Key words : Dairy organisms, Bifidobacteria, Antimicrobial property

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INTRODUCTION

The concept of probiotics was introduced in the early 20th century by Elie Metchnikoff (Metchnikoff, 1907). A number of definition of the term probiotic have been used over the years but the one derived by the Food and Agriculture Organization of the United Nations/World Health Organization, best exemplifies the breadth and scope of probiotics as they are today : 'live micro-organisms which when administered in adequate amounts confer a health benefit on the host' .

The probiotic micro-organism consists mostly of the strains of the genera Lactobacillus and Bifidobacteria. Bifidobacterial species are common members of the infant gut where they form up to 91 per cent of the total micro flora in breast-fed babies and up to 75 per cent in formula fed infants (Hadadji *et al*., 2005). Tissier's discovery of Bifidobacteria in breast-fed infants played a key role in establishing the concept that specific bacteria take part in maintaining health.

There are about 29 bifidobacterial species that have been identified and among them eleven species have been isolated from infant faeces. The most frequently isolated *Bifidobacterium* species in infant faeces are *B. bifidum, B. longum, B. infantis* and *B. breve* (Matsuki *et al.*, 2003). These organisms which are gram positive, non motile, non-spore forming anaerobic pleomorphic rods play a significant role as probiotics in controlling the pH of the large intestine through production of lactic and acetic acid thereby restricting the growth of many potential pathogens and putrefactive bacteria (Sullivan and Nord, 2002).

Recently the isolation of *Bifidobacterium* species from infant faeces has assumed considerable importance, as a consequence of interest in the potential health promoting ability of this genus (Arunachalam *et al*., 2000 and Suresh,

2000).

It has been reported that these organisms are able to exert beneficial effects including improvement of intestinal microflora by preventing colonization of pathogens, amelioration of diarrhoea or constipation, activation of the immune system and increasing protein digestion (Ishibashi and Shimamura, 1993). Owing to these properties, Bifidobacteria are now frequently used to prepare probiotic dietary adjuncts.

RESEARCH METHODOLOGY

Fresh faecal samples from healthy newborn infants of both the sexes born through normal delivery in and around Madhavaram, Chennai were examined for bifidobacterial species. The faecal samples were cultured in Yoshioka broth and agar (Yoshioka, 1971) under anaerobic conditions using Anaero gas pack (Hi media cat.no. LE 002F) at a temperature of 37°C for isolation of bifidobacterial cultures. Species identification was confirmed by molecular techniques (mPCR and 16S DNA as per the method of Dong *et al*. (2000)

Screening of Bifidobacteria for inhibitory activity :

Probiotic activity of bifidobacterial species were tested with the following organisms

1.	*Pseudomonas aeruginosa*	-	MTCC 1688
2.	*Escherichia coli*	-	MTCC 452
3.	*Staphylococcus aureus*	-	MTCC 96
4.	*Bacillus cereus*	-	MTCC 430

Inhibitory activity assay :

Inhibitory activity assay was carried out as per the method adopted by Zinedine and Faid (2007). The cell free supernatant of the bifidobacterial cultures was filtered using a millipore filter. Pathogenic bacteria were grown on nutrient agar. Wells were hollowed out in agar and a volume of 80 µl of the supernatant was poured in each well and incubated at 4°C for 30 minutes to facilitate the liquid diffusion in the agar medium. Petri dishes were incubated at 37°C for 24 hours. The antibacterial activity of bifidobacterial strains was determined by measuring the clear zone around the wells. A diameter of 1.5 mm or greater around the well was considered a significant inhibition. The cell free supernatant was also adjusted to pH 7 and the inhibitory assay studied.

RESULTS AND DISCUSSION

The bifidobacterila species identified were *Bifidobacterium longum* (IB10), *Bifidobacterium longum* (IB12), *Bifidobacterium breve* (IB39), *Bifidobacterium bifidum* (IB42)

Inhibitory activity of bifidobacterial isolates against pathogenic bacteria :

Table 1 shows the respective mean inhibitory activity of the bifidobacterial isolates against pathogenic bacteria. The respective mean ± SE values of inhibition zone (in mm) of isolate IB_{10} for *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* are 3.03 ± 0.019, 3.00 ± 0.031, 2.98 ± 0.043, 3.03 ± 0.051. On neutralizing the cell free supernatant (CFS) to pH 7 it still maintained its inhibitory activity against these pathogens.

The respective mean ± SE values of inhibition zone (in mm) of isolate IB_{12} for *S. aureus* and *P. aeruginosa* were 4.00 ± 0.052 and 2.46 ± 0.074. On neutralizing the CFS, it showed a reduced inhibitory effect of 3.98 ± 0.071 and 2.01 ± 0.081 against *S. aureus* and *P.aeruginosa*, respectively. However it did not show inhibition against *B. cereus* and *E. coli.*

The respective mean ± SE values of inhibition zone (in mm) of isolate IB_{39} for *B. cereus, S. aureus* and *P. aeruginosa* were 4.03 ± 0. 030, 2.10 ± 0.052 and 1.58 ± 0.028 On neutralizing the CFS, it did not show zone of inhibition against *P.aeruginosa*.

The respective mean ± SE values of inhibition zone of isolate IB_{42} for *B. cereus* and *P.aeruginosa* were 2.95 ± 0.031 and 3.13 ± 0.030. However this isolate did not show zone of inhibition against *S. aureus* and *E. coli*. On neutralizing the CFS, it did not show zone of inhibition against *P. aeruginosa*.

From the table it is evident that the non neutralized and neutralized CFS of isolate IB_{10} shows inhibition against all the four pathogenic species tested.

The role of probiotics in improving human health has attracted considerable attention and research in this regard is mostly focused on the strains belonging to the genera Bifidobacteria and Lactobacillus. Bifidobacteria have been in the spot light of scientific research since 1990s due to their health promoting effects. Interventions to increase the population of intestinal species of Bifidobacteria in the human gut by administering them as probiotics or administering prebiotics to stimulate their.

The incidence and isolation of Bifidobacteria from breast fed infant faeces in the present study corroborates with Roberts *et al.* (1985); Tamime *et al.* (1995); Silvi *et al.* (1996) and Martin *et al.* (2009) who reported that human milk is favourable for the growth and sustenance of Bifidobacteria in the large intestine of infants.

The opaque, white and concave colonies observed in the present study after anaerobic incubation was similar to the findings of Dubey and Mistry (1996). Wasilewska and Bielecka (2003) also isolated and identified fourteen bifidobacterial strains from faeces harbouring the gut of 3- month old breast fed infant. Vlkova *et al.* (2005) reported the presence of Bifidobacteria from twenty nine infant faeces out of the ninety five faecal samples collected. Hence the breast fed infant faecal sample seemed to be an ideal source of Bifidobacteria.

Screening of Bifidobacteria for Probiotic properties :

Inhibitory activity of bifidobacterial isolates against pathogenic bacteria :

From Table 1, it is evident that the non neutralized and neutralized cell free supernatants of isolate IB_{10} showed inhibition against all the four pathogenic species tested.

The isolates in the present study showed inhibitory activity against selected pathogenic bacteria. On comparing the inhibitory assay of the four isolates, it is noted that isolate IB_{10} (*B.longum*) had an inhibitory zone ranging from 3.03 to 2.95 mm against *B. cereus, S. aureus, E.coli* and *P. aeruginosa* due to acid and antibacterial compounds which was similar to the work of Zinedine and Faid (2007) who reported that *B. longum* showed a wide range of inhibition ranging from 2.7 to 1.6 mm against *L. monocytogenes, E. coli, S. typhimurium* and *S. aureus.*

The findings of the present study are in consonance with the theory of Scardovi (1986) who reported that *Bifidobacterium* species produced lactic and acetic acid which are responsible for the decrease of intestinal pH and inhibition of pathogenic bacteria. The inhibitory activity seen in this study was in agreement to the findings of Anand *et al.* (1985) who showed that *B. bifidum* inhibited the growth of *E.coli, B.cereus, S. aureus* and *P.fluorescens*. The observation in this study that *B.longum* had the most potent antagonistic activity was similar to the findings of Pikina *et al*. (1999) who reported the most potent antagonistic activity of *B.longum* strain 44 against common pathogens. The antagonistic activity of *B.longum* against *E.*coli is in consonance with the findings of Ahmed *et al.* (2009) who reported the inhibitory activity of *B. longum* against the action of vero cytotoxin produced by some strains of *E.coli*. The inhibitory effect of the bifidobacterial isolates in the present study is in agreement to the *in vitro* study of Korshunov *et al.* (1999) who reported that *B.longum, B.bifidum, B.breve* and *B.adoloscentis* were capable of

Table 1 : Inhibitory activity of bifidobacterial isolates (zone in mm) against pathogenic bacteria

Isolate code	*Bacillus cereus*		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*	
	CFS	CFS pH7	CFS	CFS pH7	CFS	CFS pH7	CFS	CFS pH7
IB_{10}	3.03±0.019	3.00±0.020	3.00± 0.031	2.95±0.037	2.98±0.043	2.97±0.051	3.03±0.051	3.00±0.032
IB_{12}	-	-	4.00±0.052	3.98±0.071	-	-	2.46± 0.074	2.01± 0.081
IB_{39}	4.03±0.030	3.99±0.022	2.10±0.052	2.00±0.061	-	-	1.58±0.028	-
IB_{42}	2.95±0.031	2.94±0.029	-	-	-	-	3.13±0.030	--

Average of six trials
CFS – Cell free supernatant
CFS pH7 – Cell free supernatant adjusted to pH7

inhibiting the growth of *S.aureus, E.faecalis, P.aeurginosa* and *E.coli.*

The findings in the present study also met the requisites of antimicrobial activity against potentially pathogenic micro-organisms as per the FAO / WTO Draft Guidelines of 2002 and ICMR-DBT Guidelines 2011 for Evaluation of probiotics in food.

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