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Effect of inulin on growth and antimicrobial activity of *Lactobacillus* spp.

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Abstract

Prebiotics function by acting as selective substrates for probiotic microorganisms, thus enhancing their growth and colonization in the intestine. Inulin, a fructooligosaccharide, is effective in reducing intestinal disturbances, when ingested along with probiotics. The present study evaluated the effectiveness of inulin in enhancing the growth and antimicrobial activity of a *Lactobacillus* spp. Growth and antimicrobial activity of five lactobacilli strains in inulin containing broth were compared with MRS broth. All lactobacilli cultures were able to utilize inulin, however growth and antimicrobial activity were differ from culture to culture. *Lactobacilli acidophilus* NCDC 14 showed maximum growth. *Lactobacillus* cultures antimicrobial activity against indicator organisms in presence of inulin. *Lactobacillus casei* NCDC 298 showed antimicrobial activity against all three indicator organisms. Inulin utilization, growth and antimicrobial activity were variable among the *Lactobacillus* cultures tested.

Keywords: Probiotic, prebiotic, *Lactobacillus*, inulin, antimicrobial activity

Gastrointestinal microflora is increasingly being recognized as one of the factors that determined the state of health and disease in human. This microflora is in a dynamic equilibrium that may be altered by diet, medication, stress, aging and various other environmental factors. Increasing the populations of beneficial organisms such as bifidobacteria and lactobacilli in the gut and suppressing potentially deleterious microorganisms are thought to be important in maintaining optimal intestinal health. The organisms most often included in the probiotic group are members of *Lactobacillus acidophilus* complex, *Lactobacillus casei* complex and species of *Bifidobacterium.* Because of the survivability and colonization difficulties that abound with probiotics, the prebiotic approach offers an attractive alternative.

Prebiotics are non-digestible food ingredient that beneficially affects the human body, selectively stimulating the increase and/ or activity of one or a limited group of colon bacteria. The term synbiotic is used when a product contains both probiotic and probiotic ingredients. The synergism is attained *in vivo* by the ingestion of lactobacilli one hand and by promotion of indigenous bifidobacteria on the other hand (Schrezenmeir and de Verse 2001).

Roberfroid (2000) suggested that these products can also improve the survival of bacteria during the gastrointestinal tract transient also. The non-digestible carbohydrates has a member of functional effects on the gastrointestinal tract (GIT), which include modulation of microbial fermentation with increased short chain fatty acid (SCFA) production reduced pH and ammonia production improvement in mineral absorption, reduced fat absorption etc. Among these, carbohydrates fracto-oligosaccharides (FOS) such as inulin and oligofructose are the most studied and well established prebiotic. This is because of their technological functionality such as low calorie; fat replacing ability improvement in overall texture mouth feel and flavour of

product in addition to selective stimulation of beneficial bacteria. Inulin is an oligosaccharide extracted from commonly consumed plants like onions, asparagus root, Jerusalem artichoke tuber, honey, oat, chicory, etc. (Bengmark *et al*. 2001) and it is a natural food ingredient and is classified as dietary fibre in most European countries (Roberfroid 2000). Probiotic and prebiotic compatibility is essential for development of synbiotic preparations. Therefore, it is imperative to study the effect of prebiotic on probiotic activities like growth characteristics, antimicrobial property, surface adhesion properly etc. to select a probiotic, prebiotic combination in formulation of probiotic functional food i.e. Synbiotic food. The aim of present investigation is to evaluate the effect of inulin supplementation on growth and antimicrobial property of *Lactobacillus* spp.

Materials and Methods

Cultures

Five *Lactobacillus* strains comprising of two strain of *Lactobacillus acidophilus* (NCDC 13 and 14) and three strains of *Lactobacillus casei* (NCDC 17, 297 and 298) were included in the study. For evaluation of antimicrobial activity three indicator strains, viz., *staphylococcus aureus* NCDC 109, *Bacillus cereus* NCDC 240 and *Escherichia coli* NCDC 135 were used. *Lactobacillus* cultures were maintained in chalk litmus milk at refrigeration temperature after their growth at 37°C for overnight. The cultures were sub-cultured at regular intervals in chalk litmus milk and stored under refrigeration conditions. Before use the cultures were sub-cultured twice in MRS broth (37°C/12-15 h). Indicator cultures were maintained and stored at refrigeration temperature in nutrient agar slant. Before use cultures were activated in nutrient broth (37°C/ 12-15 h).

Inulin positive broth: Inulin - 20 g; peptone - 10 g; tween 80 - 1.00 ml, sodium dihydrogen phosphate - 2 g; magnesium sulphate - 0.2 g; manganese sulphate - 0.038 g, triammonium citrate - 2 g; sodium acetate - 5.00 g; distilled water -1000 ml (pH 6.4 ± 0.2). The ingredients were suspended and mixed thoroughly in one liter of distilled water, and was heated to boil under frequent agitation. The medium was sterilized by autoclaving at 121°C for 15 min. Inulin negative broth was treated as control.

Growth patterns of Lactobacillus spp. in inulin containing broth

Active *Lactobacillus* culture was prepared in MRS broth (37°C/ 12-15 h) and decimal dilution was made using sterile 9 ml normal saline tubes. Cells suspension from third dilution (50 µl) was inoculated in 5 ml of MRS broth, inulin positive and inulin negative broth, respectively and tubes were incubated at 37°C for 24 h. At 0, 6, 12 and 24 h, 1 ml samples were taken from each tube and plating was carried out by preparing appropriate dilutions. In each plate 12-15 ml of melted and cooled MRS agar was poured and mixed the content properly and allowed for solidification. After solidification of agar plates were incubated at 37°C for 24 to 36 h in an inverted position and colonies were counted and recorded.

Antimicrobial activity

Agar well method of Anand *et al.* (1984) modified by Mandal *et al.* (2010) was followed for evaluation the antimicrobial activity. Three indicator organisms (*Escherichia coli, Bacillus cereus and Staphylococcus aureus*) were activated in nutrient broth at 37°C for 12-15 h. *Lactobacillus* cultures were activated and cultured in three different media such as MRS, inulin positive and inulin negative broth at 37°C for 24 h. To 25 ml of melted and cooled nutrient agar 200 µl of active indicator organism culture was mixed and poured into sterile petridish. After solidification, wells (8 mm dia.) were made. Active *Lactobacillus* culture cell free supernatant (75 µl) was poured in respective wells. Plates were incubated at 37°C for 10-12 h and examined for clear zones around well. The diameter of clear zone around the well was measured.

Results and Discussion

Cultures

All the *Lactobacillus* cultures were Gram positive rod shaped. *L. acidophilus* NCDC 13 was long and slender rods. *L. acidophilus* NCDC 14 and all strains of *L. casei* NCDC 17, 297 and 298 were small thicked rods. When examined with indicator organisms, *B. cereus* NCDC 240 were found gram positive rod shaped, *E. coli* NCDC 135 were appeared as gram negative, coccobacilli shaped and *S. aureus* NCDC 109 were gram positive, cocci in bunched

forms. All the *Lactobacillus* cultures were catalase negative and indicator organisms were found catalase positive (Table 1). The cultures thus were proved to be pure and were used for further studies.

Table 1: Gram's reaction, morphology and Catalase test of cultures

Cultures	Grams' Staining	Cell Morphology	Catalase Test
L. acidophilus NCDC 13 (LA-13	Positive	Rods	Negative
L. acidophilus NCDC 14 (LA-14)	Positive	Rods	Negative
L. casei NCDC 17 (LC-17)	Positive	Rods	Negative
L. casei NCDC 17 (LC-297)	Positive	Rods	Negative
L. casei NCDC 17 (LC-298)	Positive	Rods	Negative
S. aureus NCDC 109 (SA-109)	Positive	Cocci	Positive
E. coli NCDC 135 (EC-135)	Negative	Rods	Positive
B. cereus NCDC 240 (BC-240)	Positive	Rods	Positive

Growth patterns of Lactobacillus cultures

To study the effect of inulin supplementation on the growth lactobacilli, the organisms were cultured in three different growth medium, viz., MRS broth, inulin positive broth and inulin negative broth. Inulin positive broth was prepared by excluding all possible sugar sources and incorporating inulin as complex sugar source. Initial counts in all three media were in between 4.6 – 5.4 log cfu/ml. After 24 h, viable counts were increased to 8.5 to 9.5 log cfu/ ml in MRS broth, where as in inulin positive and negative broth the values were 7.5 to 8.4 log cfu/ ml and 6.3 to 7.2 log cfu/ ml, respectively. In MRS broth, maximum viable count was observed for *L. acidophilus* NCDC 14 and minimum value was observed for *L. acidophilus* NCDC 13. In inulin positive broth, maximum viable count was observed for *L. acidophilus* NCDC 14 and minimum value was observed for *L. casei* NCDC 297. The maximum growth was observed in MRS broth, followed by inulin positive and inulin negative broth (Table 2).

In inulin positive broth, after 6 h incubation, it was observed that *L. acidophilus* NCDC 14 showed maximum growth (6.87 log cfu/ml) and lowest growth was observed in *L. acidophilus* NCDC 13 (5.45 log cfu/ml), intermediary growth were observed in *L. casei* NCDC 17 (6.16 log cfu/ml) and *L. casei* NCDC 297 (6.10 log cfu/ml). After 12 h, it was observed that

Table 2: Growth patterns of *Lactobacillus* spp. in MRS, inulin positive and inulin negative broth at 37°C for 24 h

Growth media	Incubation period, h	Viable counts of Lactobacillus cultures (log cfu/ ml)				
		LA-13	LA-14	LC-17	LC-297	LC-298
MRS broth	0	4.60	4.72	4.21	4.61	4.64
	6	5.87	6.54	6.35	6.20	6.08
	12	7.52	8.07	8.59	7.39	8.63
	24	8.51	9.53	9.47	9.21	9.35
Inulin positive broth	0	4.91	4.83	4.37	4.78	5.03
	6	5.45	6.87	6.16	6.10	5.57
	12	6.53	7.56	7.61	6.89	7.42
	24	7.98	8.40	7.88	7.56	7.67
Inulin negative broth	0	4.77	5.06	4.45	4.71	5.43
	6	5.00	6.21	5.11	5.69	4.73
	12	5.31	6.55	5.60	5.84	6.11
	24	7.63	7.18	6.92	6.30	6.90

L. casei NCDC 17 growth was maximum (7.61 log cfu/ml followed by *L. acidophilus* NCDC 14 (7.56 log cfu/ml). The growth of *L. casei* NCDC 197 (6.89 log cfu/ml) was more than *L. acidophilus* NCDC 13 (6.53 log cfu/ml). The intermediary growth was observed in case of *Lactobacillus casei* NCDC 298 (7.42 log cfu/ml). After 24 h, the maximum growth was observed in *L. acidophilus* NCDC 14 (8.40 log cfu/ml), lowest growth was observed in *L. casei* NCDC 297 strain (7.56 log cfu/ml). Intermediary growths were seen in both *L. casei* NCDC 298 (7.67 log cfu/ml) and *L. acidophilus* NCDC 13 (7.63 log cfu/ml). From growth comparison, it was found that *Lactobacillus acidophilus* NCDC 14 used the inulin maximally.

Shin *et al.* (2000) observed a maximum activity of bifidobacteria at 5 percent concentration of FOS. This difference can be explained by the fact that the optimum concentration of probiotic may be strain dependent. The ability of bifidobacterial cultures to ferment inulin had been reported by other researchers also. Dubey and Mistry (1996) could not observe any difference in the maximal counts and generation times of *Bifidobacterium breve, Bifidobacterium infantis* and *B. longum* on growing them in infant formulae supplemented with fructo oligosaccharides (0.5%).

Kaplan and Hut Kins (2000) observed that seven out of eight *Bifidobacterium* were able to ferment inulin and oligofructose on MRS agar. Bielecka *et al.* (2002) studied the influence of fructon type oligosaccharides (as probiotics) on growth of *Bifidobacterium* strains. *In vitro* studies showed that the majority of *Bifidobacterium* species utilized fructo-oligosaccharides and low polymerized inulins; but only 18 out of 30 strains tested (Mostly of *B. longum* and *B. animalis* species) were stimulated. To sum up, all the bifidobacterial cultures used in the present study could use inulin as the sole carbon source. Suggesting better colonization chances of these organisms on consuming them together with inulin (as a synbiotic) as this prebiotic will serve as a ready source of energy for these organisms in the colon. *L. paracasei* EL7 was able to grow in the presence of all five prebiotic substances (FOS, Inulin, IMO, GOS and lactulose) at a concentration of 2% (Pennacchia *et al.*, 2006).

Antimicrobial activity

Among the probiotic properties antimicrobial activity is one of the important criteria of selection of suitable strain of probiotic organism. Antimicrobial activity is antagonistic activity against other bacteria. With the emergence of antibiotic resistant bacteria and natural way of suppressing pathogens, the concept of probiotic has a attractive much attention therefore their antimicrobial activity against pathogen is an important criteria for the selection. *Lactobacillus* produces various antimicrobial substances, which cause the inhibition of pathogenic organisms' growth and activities. Antimicrobial activity, exhibited by different lactobacilli culture, used in the study, was determined by agar well diffusion in terms of zones of inhibition. In order to test whether the incorporation of inulin into the growth media has any enhancing effects on antimicrobial activity of the probiotic lactobacilli culture, a comparison was made among the antimicrobial activities exhibited by the culture grown in MRS broth, inulin positive broth and inulin negative broth. It has been observed that cultures was effective against all the three indicator organisms *Eschericia coli* NCDC 135, *Bacillus cereus* NCDC 240 and *Staphylococcus aureus* NCDC 109 only when grown in MRS broth. No inhibition was observed when cultured in both inulin positive and inulin negative broth.

It was observed that *L. acidophilus* NCDC 13 and 14 were not effective against *Escherichia coli* NCDC 135, *Bacillus cereus* NCDC 240 and *Staphylococcus aureus* NCDC 109 when cultured in inulin containing broth. *L. casei* NCDC 17 strain was not effective against *Escherichia coli* NCDC 135, no measurable zone of inhibition was observed. However, it was effective against *Bacillus cereus* NCDC 240 and *Staphylococcus aureus* NCDC 109. *L. casei* NCDC 297 strain was not effective against *Escherichia coli* NCDC 109 and *Staphylococcus aureus* NCDC 109, but was effective against *Bacillus cereus* NCDC 240. *L. casei* NCDC 298 was effective against all the three indicator organisms, *Eschericia coli* NCDC 135, *Bacillus cereus* NCDC 240 and *Staphylococcus aureus* NCDC 109 and there was 11 mm, 10 mm and 12 mm in diameter of

Table 3: Antimicrobial activity of *Lactobacillus* spp. cultured in MRS, inulin positive and inulin negative broth at 37°C for 24 h

Growth media	Indicator organisms	Diameter of Zone of Inhibition (mm)*				
		LA-13	LA-14	LC-17	LC-297	LC-298
MRS broth	EC-135	12	10	14	14	13
	BC-240	12	14	20	21	14
	SA-109	14	12	20	17	15
Inulin positive broth	EC-135	—	—	—	—	11
	BC-240	—	—	15	15	10
	SA-109	—	—	15	—	12
Inulin negative broth	EC-135	—	—	—	—	—
	BC-240	—	—	—	—	—
	SA-109	—	—	—	—	—

*including 8 mm well diameter

— no zone detected

zones of inhibition were observed, respectively. From above comparison it was found that *L. casei* NCDC 298 has better antimicrobial properties in presence of inulin (Table 3).

Lacobacillus spp. effectively inhibited the *Shigella*, *Salmonella, Vibrio* and *Bacillus* spp. (Goyal, 2007). Klayraung *et al*. (2008) reported the antimicrobial potential of *Lactobacillus fermentum* strains against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. *Lactobacillus plantarum* Lp9 showed antibacterial activity against *E. coli, L. monocytogenes, S. typhi, S. aureus* and *B. cereus* (Kaushik *et al*., 2009). Oyarzabal and Conner (1995) evaluated the ability of *B. bifidum, Lactobacillus* and *Salemonella spp* to grow in media containing FOS (FOS-50 or FOS pure formulation) through *in vitro* experiments.

They observed clear inhibition of growth of all salmonella serotypes grown in media containing the pure formulation of FOS as the only carbohydrate source. Bamba *et al.* (2002) investigated the influence of administration of *L. paracasei* and meltodextrin KMS X-70, on *E. coli* adhesion in the gastro intestinal tract of gnotobiotic piglets. They observed a stimulatory effect of malto dextrin KMS X-70 on the inhibitory effect of *L. paracasei* on the adhesion of *E. coli* to the jejunal mucosa of gnotobiotic piglets. As in this case we also observed inhibition of growth of *E. coli, Salmonella typhimurium, S. aureus* and S. drysenteriae by culture supernatants obtained by growing B-420 culture in the medium containing inulin as the sole carbon source. Inhibition of growth of some human enteropathogen such as salmonella has been reported in the presence of FOS (Oyarzabal and Conner, 1995).

Conclusion

All the *Lactobacillus* cultures grow faster in MRS broth, medium in inulin positive broth and slowest in inulin negative broth. *Lactobacillus acidophilus* NCDC 14 was more active in inulin positive broth. All lactobacilli cultures showed antimicrobial activity against all the indicator strains when cultured in MRS broth and no activities were found in inulin negative broth. In inulin positive broth both the strains of *Lactobacillus acidophilus* (NCDC 13 and NCDC 14) showed no antimicrobial activity against all the indicator strains. All the *Lactobacillus casei* NCDC 17, NCDC 297 and NCDC 298) showed antimicrobial activities in inulin positive broth. *Lactobacillus case* NCDC 298 was active against all the indicator strains. Inulin was variably utilized by different strains of lactobacilli and growth and activity differ from culture to culture.

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