



RESEARCH ARTICLE

INVITRO EVALUATION OF CERTAIN BIFIDOBACTERIUM BIFIDUM SPECIES FOR THEIR PROBIOTIC CHARACTERISTICS

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ABSTRACT

The objective of the present study was to collect different Lactic acid bacterial strains from culture collection centers and screen their functional probiotic characteristics such as acid tolerance, bile tolerance, antibacterial activity and antibiotic sensitivity for their commercial use. Among the six probiotic strains tested, all showed good survivability at high bile salt concentrations (0.3 to 2.0 % oxgall) and good growth at a low pH of 1.5 to 3.5. These probiotic species showed good survival abilities in acidic pH of 2.0 to 3.5 except *Bifidobacterium bifidum*-232 which did not grow at pH of 2.0. Among the six lactic acid species, *Bifidobacterium bifidum* 231 and *Bifidobacterium bifidum* 236 showed resistant to all antibiotics tested except Streptomycin (Co-Trimoxazole, Gentamicin, Norfloxacin and Tetracycline). All these probiotic organisms were screened for their *in vitro* inhibition ability against pathogenic microorganisms namely, *E.coli* ATCC, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Staphylococcus aureus*. *Bifidobacterium bifidum* 229, *Bifidobacterium bifidum* 232 and *Bifidobacterium bifidum* 233 inhibited the growth of all pathogenic bacteria tested. The study indicated *Bifidobacterium bifidum* 232 and *Bifidobacterium bifidum* 236 as potential functional probiotics for future *in vivo* studies for commercialization in the food industry.

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INTRODUCTION

In recent years, different investigations support the importance of probiotics as a part of healthy diet for humans and animals and as a way to provide a natural, safe and effective barrier against microbial infections. According to the definition given by the World Health Organization, probiotics are "live microbial food supplements which, when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). A number of requirements have been established for lactic acid strains to be effective probiotic microorganisms. They must be of human origin and be able to survive through the gastrointestinal tract (Bauer *et al.*, 1966). Required probiotic characteristics also include inhibition of entero-pathogenic bacteria, tolerance to acid and bile salts, resistance to antibiotic discs, adherence to intestinal epithelial cells and stability to conditions of industrial processes. Therefore, the present study aims to screen and identify certain lactic acid bacterial strains for ideal probiotic characteristics by *in vitro* techniques.

MATERIALS AND METHODS

Experimental design

Six Lactic acid bacterial strains obtained from different sources were evaluated for their probiotic characteristics namely acid tolerance, tolerance to bile salts, antagonistic activity against different food borne pathogens and antibiotic sensitivity.

Biological and Chemicals

The lactic acid bacterial strains *Bifidobacterium bifidum* 229, *Bifidobacterium bifidum* 231, *Bifidobacterium bifidum* 232, *Bifidobacterium bifidum* 233, *Bifidobacterium bifidum* 235, *Bifidobacterium adolescentis* 236, were obtained from National Dairy Research Institute (NDRI, Karnal) and National Institute of Nutrition (NIN, Hyderabad). Media and chemicals were procured from Himedia Laboratories, India and prepared according to standard procedures. The lactic acid bacterial strains were sub-cultured three times before use in sterile de Mann Mclean Rogosa Sharpe broth (MRS) using 1% inoculums and incubated at 37°C for 48 h.

Acid and Bile tolerance

Acid and bile tolerance of selected LAB was determined according to the method (Tambekar and Bhutada, 2010) in which selected Lactic acid bacterial strains. were inoculated into MRS broth of varying concentrations of bile salt (0.5, 1.0, 1.5 and 2.0%) and pH (1.5, 2.0, 2.5, 3.0 and 3.5) and incubated at 37°C for 48 h. The growth of LAB in MRS broth containing different concentrations of ox gall was measured by spectrophotometer at 620nm. The growth of LAB in MRS broth was assessed by visual turbidity.

Antibiotic sensitivity test

The antibiotic resistance of Lactic acid bacterial strains was assessed using different antibiotic discs on MRS agar plates seeded with the test probiotic organism. 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 24 h. Zone of suppression of growth was assessed against the different antibiotic discs namely Co-Trimoxazole (25 mcg), Gentamicin (10 mcg), Norfloxacin (10 mcg) Ampicillin (10 mcg), Nalidixic acid and Tetracycline (10 mcg). The zone size (mm) interpretative chart for antibiotics was measured according to Performance Standards for Antimicrobial Disk Susceptibility Tests as described by (Bauer *et al.*, 1966) method.

Antibacterial activity

A modified method described by (Barefoot and Klaenhamme, 1984) was followed. An overnight culture of pathogenic microorganisms namely, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Salmonella paratyphi* and *Staphylococci aureus* were grown in nutrient broth. A lawn of an indicator strain i.e. pathogenic organisms were made by spreading the cell suspension over the surface of MRS agar plates with a sterile cotton swab. The plates were allowed to dry and a sterile cork borer of diameter 7.0 mm was used to cut uniform wells in the MRS agar plates. Each well was filled with 0.1 ml of lactic acid bacterial inoculums from MRS broth and incubated at 37°C for 36-48 h. After incubation, the diameter (mm) of the inhibition zone around the well was measured (Bauer *et al.*, 1966).

RESULTS AND DISCUSSION

Acid and Bile tolerance

The bile and acid tolerance are important characteristics of probiotic bacterial strains. Bile tolerance is required for bacterial growth in small intestine (Lee and Salmenen, 1995) and acid tolerance is required for the bacteria to survive passage through the stomach (Henriksson *et al.*, 1999) as well as to survive in food (Lee and Salmenen, 1995). All the six Lactic acid bacterial strains showed good survival abilities in the tested acidic pH of (1.5-3.5) except *Bifidobacterium bifidum 232* and *Bifidobacterium bifidum 235*, which did not grow at pH 2.0.

Table 3 *Bifidobacterium bifidum* strains showing sensitivity/resistant with different antibiotics (zone of inhibition diameter in mm).

Name of the probiotic organism	Gentamycin	Co-Trimoxazole	Norfloxacin	Tetracycline	Ampicillin	Nalidixic acid
<i>Bifidobacterium bifidum-229</i>	15mm(S)	R	17mm(S)	17mm(I)	23mm(S)	R
<i>Bifidobacterium bifidum-231</i>	R	R	R	R	R	R
<i>Bifidobacterium bifidum-232</i>	14mm(I)	R	19mm(S)	33mm(S)	21mm(S)	R
<i>Bifidobacterium bifidum-233</i>	18mm(S)	R	R	19mm(S)	22mm(S)	12mm(R)
<i>Bifidobacterium bifidum-235</i>	R	30mm(S)	14mm(I)	22mm(S)	15mm(I)	20mm(S)
<i>Bifidobacterium adolescentis 236</i>	14mm(I)	R	19mm(S)	R	15mm(I)	R

Note: In brackets R=Resistant S=Sensitive,I=Intermediatory according to the performance standards for antimicrobial disc suspension tests (CLSI, 2007).

In the present study, it was observed that all the Lactic acid bacterial strains survived and tolerated bile salts (0.3 - 2%) quite effectively. But a marginal decrease in the viability of all the strains was found as bile salt concentrations was increased from 0.3 - 2%. Similar observations were also reported by Barakat *et al.*, (2011).The differences in the level of bile tolerance between strains in the present study might be due to differences in their ability to grow and colonize and is in accordance with the findings of Shukla *et al.*, (2010). Among all the *Bifidobacterium spp* the difference in the level of bile tolerance is minimum and comparatively the strains *Bifidobacterium bifidum 233* and *Bifidobacterium adolescentis 236* have shown the highest absorbance at 620nm in all

tested bile salt concentrations is an indicative of their good probiotic characteristics (Fig 1).

Table 1 Survivability of *Bifidobacterium* species at different pH levels in MRS broth

Name of the probiotic organism	pH				
	1.5	2.0	2.5	3.0	3.5
<i>Bifidobacterium bifidum 229</i>	-ve	+ve	+ve	+ve	+ve
<i>Bifidobacterium bifidum 231</i>	-ve	+ve	+ve	+ve	+ve
<i>Bifidobacterium bifidum 232</i>	-ve	-ve	+ve	+ve	+ve
<i>Bifidobacterium bifidum 233</i>	-ve	+ve	+ve	+ve	+ve
<i>Bifidobacterium bifidum 235</i>	-ve	-ve	+ve	+ve	+ve
<i>Bifidobacterium adolescentis 236</i>	-ve	+ve	+ve	+ve	+ve

Note: -ve means no turbidity, +ve means turbidity present after incubation at 37°C for 48 h.

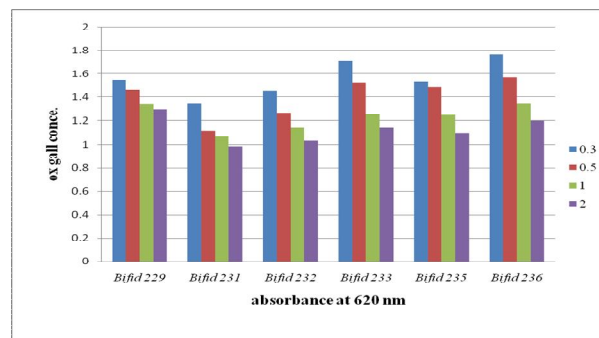


Fig 1 Graphical presentation of absorbance values in MRS broth inoculated with *Bifidobacterium bifidum* strains at different bile salt (Oxgall) concentration.

Antibiotic sensitivity test

All the six *Bifidobacterium bifidum* strains showed resistance to Nalidixic acid, Co-Trimoxazole and Gentamycin, whereas all the strains showed sensitivity to Tetracyclins and Ampicillins (Table 3) and these results are in accordance with the findings of Moubareck *et al.*, (2005). The resistance to aminoglycosidic group of antibiotics by *Bifidobacterium bifidum* strains may be due to the lack of cytochrome-

mediated drug transport among anaerobes as suggested by Bryan *et al.*, (1979).

Antibacterial activity

The six LAB strains used in the study have shown good antagonistic activity (Table 4) against different food borne pathogens with varying degree of zone of inhibition. Among six LAB's, *Bifidobacterium bifidum-232* and *Bifidobacterium bifidum-233* inhibited all the selected pathogenic bacteria *in vitro*. *Bifidobacterium bifidum-229* showed good antibacterial activity against the tested pathogens with the range of zone of inhibition 12 mm to 18 mm. *Bifidobacterium bifidum 231* inhibited the growth of all pathogens tested except *Pseudomonas*, and *Salmonella paratyphi*. *Bifidobacterium*

bifidum-235 inhibited the growth of *E.coli* ATCC, and *Pseudomonas aeruginosa*. *Bifidobacterium adolescentis* 236 has not inhibited the growth of tested pathogens in the study except *Salmonella paratyphi*.

The lactic acid bacterial strains used in the study have exhibited good antibacterial activity against the food borne pathogens tested and the results are in agreement with the findings of the Al-Allaf *et al.*, (2009). Antibacterial activity of LAB on enteric pathogenic bacteria viz, *S. aureus* and *E.coli* are in accordance with Sarkono *et al.*, (2010). According to (Saranya and Hemashenpagam, 2011) slight differences in antagonistic activity of LAB's on tested pathogenic organisms may due to production of antimicrobial compounds to varying degree. The antimicrobial effect of lactic acid is due to undissociated form of acid, which penetrates the membrane and liberates hydrogen ion in the neutral cytoplasm thus leading to inhibition of vital cell functions.

Bifidobacterium bifidum spp inhibited the growth of food borne pathogenic bacteria viz, *Salmonella spp*, *S.aureus* and *E.coli* effectively and similar results were also observed by (Goderska and Czarnecki, 2007).

Table 4 Antibacterial activity of *Bifidobacterium bifidum* strains against various pathogenic bacteria

Name of the probiotic organism	<i>S.aureus</i>	<i>Salmonella paratyphi</i>	<i>E.coli ATCC</i>	<i>Pseudomonas spp.</i>
<i>Bifidobacterium bifidum</i> -229	13mm	18mm	R	12mm
<i>Bifidobacterium bifidum</i> -231	10mm	R	17mm	R
<i>Bifidobacterium bifidum</i> -232	16mm	20mm	20mm	14mm
<i>Bifidobacterium bifidum</i> -233	14mm	22mm	20mm	16mm
<i>Bifidobacterium bifidum</i> -235	R	R	15mm	15mm
<i>Bifidobacterium adolescentis</i> 236	R	17mm	R	R

Note: R=Resistant

CONCLUSION

It may be concluded that among six strains of LAB's, two strains namely *Bifidobacterium bifidum*-231 and *Bifidobacterium bifidum*-233 have shown good probiotic characteristics which may be useful for commercialization and to safeguard the public health

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