ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol.3, Issue, 6, pp.355-357, June, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PRELIMINARY SCREENING AND CHARACTERIZATION OF BIFIDOBACTERIUM FROM FAECAL SAMPLES

P. Bhuvaneswari[1]* and S. Ahmed John[2]

[1]Department of Microbiology, A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai
[2]PG and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirapalli

ARTICLE INFO

Article History:
Received 17th April, 2011
Received in revised form
19th May, 2011
Accepted 15th June, 2011
Published online 26nd June 2011

Key words:
Bifid bacterium,
Bifidus shunt, Acid bile resistance.

ABSTRACT

Human faecal samples were used as a source for *Bifidobacterium*, Gram positive bacteria found in normal microbiota of human beings. There are thirty faecal samples were collected from the new born infants (2- 4 months). Bifidobacterium strains were isolated (MRSc medium), Out of sixty three isolates twenty six showed positivity for bifidus shunt and its species identified based on the biochemical profile. According to the bile and acid tolerance only seven strains were isolated, among this S2 (Bifidobacterium bifidum) showed more resistant capacity against both pH – 2.5 and bile – 0.5% of oxgall (at 4 hrs of treatment).

© Copy Right, IJCR, 2011, Academic Journals. All rights reserved

INTRODUCTION

Bifidobacteria play an important role in the microbial ecology of the human and animal gut. They have health promoting properties by maintaining an improved intestinal bacterial composition (Bezkorovainy, 2001), stimulation of the immune response, possible anticarcinogenic activity and protection against infections (Saavedra *et al.,* 1994). The gastrointestinal microflora is influenced by diet, age, environmental conditions and by the host genotype. Tissier (1906) showed that *Bifidobacterium* species were the predominant microflora in breast fed infants and speculated that infant diarrhoea could be treated by giving large dose of bifidobacteria orally. *Bifidobacterium* is the predominant species of human colonic and faecal microbiota. It has been extensively introduced as probiotics in industry and pharmaceutical application (Guarner and Malagelada, 2003). Human faecal samples were used as a source of *Bifidobacterium* strains which are resistant to both acid and bile (Chung *et al.,* 1999). The objective of this study was to isolate and identify the *Bifidobacterium* sp., from the infant faecal material. The effective strains were identified based on acid and bile tolerance test. Probiotics are defined as live microbial feed supplement that beneficially affects the host by improving its intestinal balance (Fuller, 1989). Most probiotic microorganisms are lactic acid bacteria such as *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus* and *Streptococcus lactis* (Sindhu and Khetarpaul, 2001).

***Corresponding author:** bhuvanak.micro@gmail.com

MATERIALS AND METHODS

Collection of Sample

Thirty fresh faecal samples were collected from the new born infants (aged 2 to 4 months). They were carefully transferred to the laboratory by using saline water containing sterilized screw capped bottles.

Isolation of bacteria

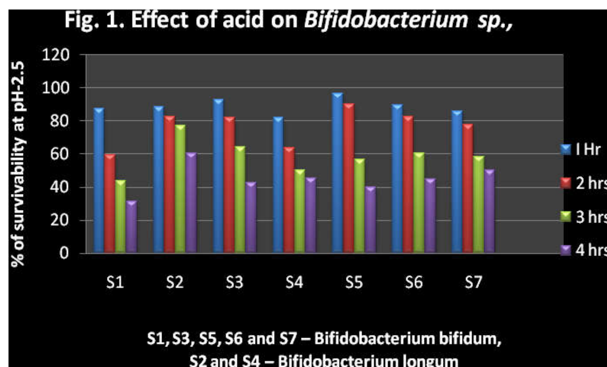
Isolation of strains: One gram of fresh feces was transferred in to the flask containing 9 ml of 0.2 % cysteine-Hcl and the suspension was homogenized for 2 minutes. Serial dilutions were made and using spread plate technique, the suspension was inoculated in MRSc agar, Bifidobacterium agar and TP agar. Then the plates were incubated at 37°C for 48 hours in the anaerobic chamber. Colony with distinct morphology was selected for further analysis. Morphological (Gram staining) and their metabolic properties were analyzed with selected colonies.

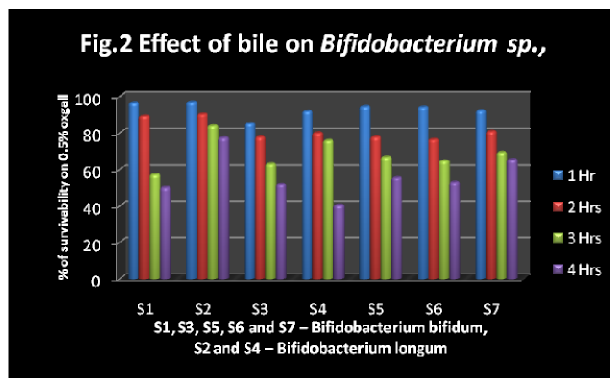
F-6-PPK Test: According to Scardovi (1986) method, cells were grown in 5 ml of MRSc broth at 37°C for 48 hrs under anaerobic condition and the cells were harvested by centrifugation at 5000g for 10 minutes. The pellet was twice washed with 5 ml of 0.5 g/l phosphate cysteine buffer. After centrifugation, the pellet was collected in 1 ml buffer and disrupted by the addition of 0.4 ml of cetridium bromide (0.4 mg CTAB in 1 ml of distilled water).

Table - 1 Characteristic feature of isolated strains

Biochemical tests	S1	S2	S3	S4	S5	S6	S7
Gram Staining	+ve, rod	+ve, rod	+ve, rod	+ve, rod	+ve, rod	+ve, rod	+ve, rod
Motility	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-
Carbohydrate fermentation							
Arabinose							
Cellobiose	-	+	-	+	-	-	-
Fructose	-	-	-	-	-	-	-
Galactose	+	+	+	+	+	+	+
Glucose	+	-	+	-	+	+	+
Inulin	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-
Maltose	+	+	+	+	+	+	+
Mannose	-	+	-	+	-	-	-
Melibiose	-	+	-	+	-	-	-
Raffinose	+	+	+	+	+	+	+
Rhamnose	-	+	-	+	-	-	-
Ribose	-	-	-	-	-	-	-
Salicin	-	+	-	+	-	-	-
Sorbitol	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-
Trehalose	+	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-
	-	+	-	+	-	-	-

S1, S3, S5, S6 and S7 – *Bifidobacterium bifidum,*; S2 and S4 – *Bifidobacterium longum*





Carbohydrate fermentation test: The carbohydrates fermentation was determined on TPY containing bromocresol purple (0.04g/l) as a pH indicator and supplemented with 1% of the following carbohydrates: Arabinose, Cellobiose, Fructose, Galactose, Glucose, Inulin, Lactose, Maltose, Mannose, Melibiose, Raffinose, Rhamnose, Ribose, Salicin, Sorbitol, Sucrose, Trehalose and Xylose To ensure the anaerobic condition, each tube was supplemented with two drops of sterile liquid paraffin after inoculation.

ACID TOLERANCE TEST

Acid tolerance capabilities were confirmed by viable count (Gilliland *et al.,* 1984). MRSc broth for tolerance test and MRSc agar for *Bifidobacterium* enumeration were used. One ml of the isolate grown in the MRS broth for three generations having an optimal density of 0.280 at 600 nm were inoculated in 9 ml of sterile MRSc broth whose pH was adjusted 2.5 with 0.5 N HCl. The inoculated broth was incubated at 37˚C for 4 hrs after inoculation. With different interval 1 hr, 2, 3, and 4 hrs, one ml of sample was taken and serially diluted with normal saline (0.84% sodium chloride) in order to neutralize the acidity of the medium. 100 µl of the specific dilution was inoculated onto MRSc agar plates. The agar plates were incubated at 37˚C for 24 – 48 hrs. The colonies were counted using colony counter. The reduction in log cycle after exposure to low pH for 4 hrs as compared to control was considered as the criteria for acid tolerance (Wright and Klaenhammer, 1983). Percentage of survivability of the strain to acidic pH was calculated using the formula.

$$\% \text{ survivability} = (\log \text{cfu } 4^{th} \text{ hour} / \log \text{cfu } 0^{th} \text{ hour}) \times 100$$

BILE TOLERANCE TEST

Bile salt tolerance capability of isolated strains was confirmed by viable count method. MRSc broth (pH) for the tolerance test and MRSc agar for the *Bifidobacterium* enumeration were used (Gilliland *et al.,* 1984). From MRSc broth culture having an optical density of 0.280 at 600 nm, 1 ml of the inoculums was inoculated in a 9 ml of sterile MRS broth enriched with oxgall (0.5 %) (w/v) and incubated at 37˚C for 4 hrs (reflecting the time spent by the food in the small intestine). With different time interval 0, 1, 2, 3 and 4 hrs, 1 ml of culture was taken and serially diluted with sterile saline solution (0.84%) and 100 µl of the specific dilution was inoculated on MRSc agar. The plates were incubated for 24 -48 hrs at 37˚C and the colonies were counted using colony counter. The reduction in the log values of survival after exposure to 0.5%

of bile salts for 4 hrs was compared with the values at 0 hr (as control) was considered as criteria for bile salt tolerance. The method was slightly modified as instead of 0.3% of sodium thioglycollate, 0.5 % of oxgall was used. Then the pH of the medium was maintained as alkali condition that reflects the pH of the small intestine. Percentage survivability of the strains to 0.5% oxgall was calculated using the formula.

% survivability = (log cfu 4th hour/ log cfu 0th hour) ×100

RESULTS AND DISCUSSION

Based on the cultural characteristics (creamy white colony, puncti form and 0.1-0.5 mm in diameter) morphology (Gram positive) the isolates were purified and presumed as lactic acid bacteria. Sixty three isolates were catalase, oxidase, and nitrate reduction negative. The presence of fructose 6 phosphate phosphoketolase enzyme was revealed by the appearance of reddish brown colour. This phenotypical characteristics and enzyme assay described by scardovi (1986) and Tamine *et al*., (1995) resulted the identification of *Bifidobacterium.* Among the sixty three isolates, twenty six isolates showed positive results for bifidus shunt.

According the method of Vlkova *et al*., (2002), modified F-6-PPK test was used for the identification of *Bifidobacterium.* Species level identification is accomplished based on the carbohydrate fermentation (Tab.1) (Miloud *et al*., 2005). Based on the acid and bile tolerance assay effective strain was identified (% of survivability). There are seven strains showed high % of survival rate that five isolates belongs to *Bifidobacterium bifidum* and two belongs to *Bifidobacterium longum* (Fig.1 and Fig, 2). Among the seven strains S2 (*Bifidobacterium bifidum*) showed maximum survivability against both acid and bile.

REFERENCES

Bezkorovainy, A., 2001. Probiotics determinants of survival and growth in the gut. *Am. J. Clin. Nutr.,* 73 (suppl): 3995-4055.

Chung, H.S., Kim, Y.B., Chun, S.L. and Ji, G.E., 1999. Screening and selection of acid and bile resistant bifidobacteria. *International journal of Food Microbiology.* 47: 25- 32.

Fuller, R., 1989. Probiotics in man and animals. Journal of Applied Bacteriology 66, 365 – 378.

Gilliland, S.E., Staley, T.E. and Bush, L.J., 1984. Importance of bile tolerance of *Lactobacillus* used as dietary adjunct. *J. Dairy Sci.,* 67 (12): 3045-3051.

Guarner, F. And Malagelada, J.R., 2003. Gut flora in health and disease. *Lancet.* 361: 512 – 519.

Miloud hadadji, Rabha benama, Noureddine Saidu, djamal Eddine Henni and Mebrauk., 2005. Identification of cultivable Bifidobacterium species isolated from breast fed infant faeces in West-Algeria. *African Journal of Biotechnolgy*. 4(5):422-430.

Saavedra, J.M., Bauman, N.A., Oung, I., Perman. J.A., and Yolken, R.H., 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospitals for prevention of diarrhoea and shedding rotavirus. *Lancet.*344: 1046-1049.

Scardovi, V. 1986. The genus *Bifidobacterium*. Bergeys manual of systematic bacteriology. J.G. holt ed. Willians and Wilkins Co., Baltimore.D. p. 1418 -1434.

Sindhu, S.C., and Khetarpaul, N., 2001. Probiotic fermentation of indigenous food mixture: effect on antinutrients and digestability of starch and protein. *Journal of Food composition and Analysis* 14: 601-609.

Tamine, A.Y., Marshall, V.M.E., and Robinson, E.K. 1995. Microbiological and technological aspects of milks fermented by bifidobacteria. *J. Dairy sci*. 62: 151-187.

Tissier, H., 1906. Traitement des infections intestinales par la method de la flore bacterienne de l'intestin. *C.R.Soc. Biol.* 60: 359-361.

Wright, C.T. and Klaenhammer, T.R., 1983. Survival of *Lactobacillus bulgaricus* during freezing and freeze drying after growth in the presence of calcium. *J.Food Sci.*48:773-777.http://prowl.rockefeller.edu/aainfo/ nuctrans.html.

Vlkova, E., Medokova, J., and Rada V., 2002. Comparison of four methods for identification of bifidobacteria to the genus level. *Czech. J. Food sci*. 20;171-174.

****