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Adhesive properties of food and faecal potential probiotic lactobacilli

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Abstract: In the present investigation, total four isolates of *Lactobacillus* species i.e. *L. casei, L. helveticus, L. brevis* and *L. fermentum* were examined for the cell surface hydrophobicity by bacterial adherence to hydrocarbons assay in LAPTg broth and hydrophobicity was calculated as percentage decrease in Optical Density at 600 nm. The general range of hydrophobicity in Lactobacilli was found in between 6-73%. Remarkably, *L. helveticus* and *L. fermentum* showed 73% hydrophobicity in xylene. Higher value of hydrophobicity could point toward a better ability of lactobacilli to adhere to epithelium cells. The outcome of present study concludes that *L. helveticus* and *L. fermentum* have good adhesive properties which may help them to adhere to surface epithelium of host cell and further screening with other probiotic attributes could be designated as probiotics.

Keywords: Lactobacillus, Probiotic, Hydrophobicity, Optical density.

INTRODUCTION

Lactobacilli are considered potentially probiotic organism because of its ability to adhere host tissue and prevents the colonization of enteric pathogen. These are a part of the normal flora of human and animal oral cavity, vaginal and gastrointestinal tract. They are extensively used in the production of different kind of health promoting fermented food, beverage and vegetables. Due to their anticipated health promoting properties, *Lactobacillus* species are mostly used as a probiotic (Ouwehand *et al*., 2002 and Puniya *et al.*, 2008). Probiotic is a microbial feed supplement that exerts beneficial effects for the host in improvement of the microbiological balance in the intestine (Fuller, 1989). Development of a probiotic product is dependent on strains that fulfill the strict criteria of: human origin, possession of generally regarded as safe (GRAS) status, production of antibacterial factors against invasive gram negative pathogens, desirable metabolic activity, technological suitability, nonpathogenic, immune-stimulatory, anti-carcinogenic, anti-mutagenic etc. (Fuller and Gibson, 1997 and Drisko *et al*., 2003).

An important property supposed for a probiotic bacterium is the ability to adhere and colonize host tissues, which enhances reproduction and survival of bacteria in the host and inhibits colonization by pathogenic bacteria. Inhibition of the multiplication of pathogen can be through production of antimicrobial components such as organic acid-Lactic acid, acetyl, hydrogen peroxide and bacteriocins (Jin *et al*., 1996). The mechanism by which *Lactobacillus acidophilus* adheres to the human gastrointestinal tract has been partially elucidated (Coconnier *et al*., 1992; Reid *et al*., 1993; Aleljung *et al*., 1994). Beside this cell surface lectin like proteins have been found in many bacterial species and have been suggested to play an important role in the defense, adhesion, and recognition of bacterial cells (Isberg and Barnes, 2002). The presence of various lectins like substances in the outer cell layer of *L. acidophilus* thought to contribute to cell adhesion through their binding to carbohydrate portions of the colonic mucus layer (Satio, 2004). It has been suggested that lectin like substances in surface layered proteins (SLP) of lactobacilli play an important role in adhesion to receptors, such as sugar chains of glycolipids (Yamamoto *et al.*, 1996) or glycoprotein's (Gusils *et al.*, 1999; Matsumura *et al*.,1999 and Annuk *et al*., 2001), on the surfaces of intestinal epithelial cells. The present investigation is an analysis of cell surface hydrophobicity characteristics of four strains of *Lactobacillus* species, isolated from the indigenous and exogenous sources has been carried out.

MATERIALS AND METHOD

Source and maintenance of cultures : Lactobacilli strain used in this study were *L. casei, L. helveticus, L. brevis* and *L. fermentum.* These are of food (exogenous) and human faecal (indigenous) isolates. The standard culture of *Mycobacterium smegmatis* MTCC 6 was procured from Microbial Type Culture Collection, IMTech Chandigarh, India. *Lactobacillus acidophilus* NCDC 15 was procured from National Collection of Dairy Cultures NDRI Karnal Harayana, India. Before experiments, lactic cultures were sub-cultured at regular intervals in chalk

litmus milk and stored under refrigeration conditions. Before use the cultures were activated in de Mann Rogosa Sharpae (MRS) broth. The culture of *M. smegmatis* MTCC 6 was maintained at refrigeration temperature in Lowenstein-Jensen (L.J.) medium. Before use cultures were activated in their respective fresh medium and checked for purity by microscopic examinations.

Cell surface hydrophobicity: The ability of organisms to adhere selected hydrocarbons was determined by the method of hydrophobicity assay (Rosenberg *et al.*, 1982) with some modifications**.** The test bacterium was grown in LAPTg broth using *M. smegmatis* as a positive control and *L. acidophilus* is a negative control, harvested after 24 hrs by centrifugation at 12000 rpm for 5 min at 5°C washed twice in 50 mM KH_2PO_4 (pH 6.5) buffer and finally suspended in same buffer. The cell suspension was adjusted to OD_{600} nm of approximately 1.0 with buffer and to 3 mL of bacterial suspension 1mL of test hydrocarbons (n-hexadecane, xylene and toluene) was added. The mixtures were vortexes for 90 sec. The tubes were allowed to stand for separation of two phases and OD_{600} nm of aqueous phase was measured. Hydrophobicity was calculated from percentage decrease in optical density of original bacterial suspension due to partitioning was calculated by equation:

Percent Hydrophobicity (H%) = ($O.D_{600}$ before mixing – $O.D._{600}$ after mixing)/ ($O.D._{600}$ before mixing) x 100.

RESULTS AND DISCUSSION

The adhering ability of lactobacilli studied *in vitro* by calculating the reduction in absorbance of buffer containing cellular suspension indicated that there was a vast difference in the hydrophobicity. *L. helveticus* of faecal origin revealed 73% hydrophobicity in xylene, 69% in toluene, and 37% in n-hexadecane, while *L. fermentum* showed 73% hydrophobicity in xylene, 68% in toluene, and 37% in n- hexadecane (Table 1). The isolates of food origin, *L. casei* and *L. brevis* showed in 21% to 52% value ranges of hydrophobicity. The higher value of cell surface hydrophobicity of *L. helveticus* and *L. fermentum* in three different hydrocarbons i.e. xylene, toluene and n-hexadecane were obtained.

Determination of microbial adhesion to hydrocarbons as a way to estimate the ability of strain to adhere to epithelial cells is a valid qualitative phenomenogical approach and considered one of the most important characteristics of probiotic lactic acid bacteria for their further probiotic action. Adhesion verifies the potential of the strain to inhibit the intestinal tract and to grow in intestinal conditions. Ly *et al*. (2008) confirmed that bacteria possess physicochemical surface properties such as hydrophobicity, Lewis acid/base and charge

Table 1. Hydrophobicity of lactobacillus species as determined in selected hydrocarbons.

Organism	Hydrophobicity in %[a]		
	n-Hexadecane	Xylene	Toluene
L. casei	21±4[b]	28±3	27±8
L. brevis	28±3	52±3	40±4
L. helveticus	37±10	73±3	69±4
L. fermentum	36±10	73±3	68±4
***M. smegmatis* MTCC 6.**[c]	70±3	86±4	78±3
***L. acidophilus* NCDC 15**[d]	06±3	12±4	07±4

[a] % Hydrophobicity = ($O.D.600$ before mixing – $O.D._{600}$ after mixing)/ ($O.D._{600}$ before mixing) x 100; [b] Mean ± S.D, (n=5); [c] Positive control; [d] Negative control

which are involved in physicochemical interactions between cells and interfaces. The mechanism of microbial adhesion to surfaces can be explained by two sequential step event (Perers *et al*., 1977; Handley *et al.*, 1987; Lindahl *et al.*, 1981 and Norde and Lyklemm, 1993) e.g. reversible adhesion due to long range forces (Derjaguin and Lanadau, 1941), and possibly subsequent interactions that mediate a direct contact between surfaces, such as hydrophobic interactions due to bacterial surface structures (Busscher and Weerkamp, 1987 and Gusils *et al.*, 1999).

In our study, *L. helveticus* and *L. fermentum* exhibited significantly higher cell surface hydrophobicity than *L. casei* and *L. brevis*. The high values of hydrophobicity could be a sign of a greater capability of bacteria to adhere the epithelial cells as indicated by Rosenberg *et al.* (1980). The strains e.g. *L. casei* and *L. brevis* showed low value of hydrophobicity designate a low ability of bacterial adhesion to host intestinal epithelium cells. The results obtained in the present study are in agreement with that of Vindderola (2003) who observed the hydrophobicity values for probiotic strains, found ranged from 38.1 to 67.8% (*L. acidophilus*) from 13.6 to 64.7% (Bifidobacteria) and from 10.9 to only 24.1% for the strains of *L. casei* and *L. rhamnosus.* In our work, the highest value of hydrophobicity was found for the *L. helveticus* ranged from 37 to 73%. It was interesting to see that hydrophobicity of *L. helveticus* and *L. fermentum* were slightly higher than reported by Vindderola (2003) for probiotic organisms. The findings of present study indicated that *L. helveticus* and *L. fermentum* have good adhesive properties which may help them to adhere to surface epithelium of host cell. These strains are being further investigated in our laboratory for other probiotic attributes. After screening, these cultures could be selected as probiotics and can be incorporated in commercially available dairy products to maximize health benefits.

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