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Leuconostoc mesenteroides NRRL B-1149 as probiotic and its dextran with anticancer properties

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ABSTRACT

Leuconostoc mesenteroides NRRL B-1149 was evaluated for its probiotic properties. It displayed *in vitro* cell surface traits in terms of hydrophobicity, autoaggregation property and cell adhesive capacity on HeLa, murine macrophage and HT29 cell lines, which revealed its capability to adhere and colonize the intestine. The tolerance to various biological barriers such as lysozyme (100 µg/ml), gastric juice (pH 3.0) and bile salts (0.5%, w/v) with 92%, 61.6% and 219% growth, respectively, confirmed its ability to survive in extreme conditions of digestive tract. It showed bile salts hydrolase activity signifying its ability to deconjugate bile salts. The whole cells of *Leuconostoc mesenteroides* B-1149 showed β-galactosidase activity with 94 Miller units displaying its importance in lactose utilization. The effect of dextran from *Leuconostoc mesenteroides* B-1149 having α-(1,6) linear glycosidic linkages and α-(1,3) branching was evaluated against cervical cancer (HeLa) and colon cancer (HT29) cell lines. The dextran from *L. mesenteroides* B-1149 (100 µg/ml) showed 25% inhibition of HeLa cell lines and 1000 µg/ml dextran displayed 40% inhibition of HT29 cell lines. The effect of dextran on murine monocyte-macrophage line (J774A.1) showed increase in growth of macrophages confirming its biocompatible nature.

Key words: *Leuconostoc mesenteroides*, probiotic, anticancer, branched dextran

Introduction

The lactic acid bacteria (LAB), a group of Gram-positive, non-spore forming, non-motile microorganisms are well known for their probiotic properties. They can produce inhibitory compounds such as lactic acid, bacteriocin and hydrogen peroxide preventing the growth of harmful microbes (Ringø & Gatesoupe, 1998). Probiotics are live microbial feed supplements that positively affect the host animal by improving its intestinal microbial balance. In recent years, the research on probiotic bacteria has been increased due to their beneficial effects on human health. The bacteria have to fulfil some criteria to behave as probiotic such as adhesion to gastro intestinal tract, resistance to low pH, bile salts and lysozyme (Nikoskelainen et al., 2001).

Leuconostoc mesenteroides is a LAB occurs in several naturally fermented foods and known to produce biodegradable glucose polymer dextran having wide range of applications in food, cosmetics, pharmaceutical and oil industries (Aman et al., 2012). *L. mesenteroides* is known to produce both water soluble and insoluble dextran (Shukla et al., 2011) having varied properties (Dols et al., 2001). In last few decades the interest in dextran has arisen due to their biological activities such as immunomodulatory and antitumor effects (Patel et al., 2012). Dextran can also be serving as cryoprotective agent for human, plant and animal cells (Purama & Goyal, 2005). The earliest polysaccharide having antitumor activity was Shear's polysaccharide isolated from the bacterium *Serratia marcescens* (Whistler et al., 1976), later some other bacterial polysaccharides with similar property were also reported (Umezawa et al., 1983). The polysaccharides from *Strongylocentrotus nudus* eggs having α-(1,6) and α-(1,4) linkages serve as anti-cancer agents (Liu et al., 2007). To the best of our knowledge, the antitumor

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activity of dextran from *Leuconostoc* spp. is not yet reported.

Therefore, in present study, we report the probiotic properties of *Leuconostoc mesenteroides* NRRL B-1149 such as cell adhesion capacity, hydrophobicity, autoaggregation, resistance to biological barriers (gastric juice, lysozyme and bile salts) and deconjugation of bile salts. In our earlier study, we reported the production and structural characterization of insoluble branched dextran from *Leuconostoc mesenteroides* NRRL B-1149 (Shukla et al., 2011). In the present study the branched dextran was analyzed for its *in vitro* anti-cancer properties.

Materials and Methods

Maintenance of cultures

Leuconostoc mesenteroides NRRL B-1149 was propagated as stab in modified MRS agar medium (Goyal & Katiyar, 1996) at 30°C and stored at 4°C. The cervical cancer (HeLa) cell line, colon cancer (HT29) cell line and murine monocyte-macrophage (J774A.1) line were purchased from National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) containing 4.0 mM L-glutamine and 110 mg/L sodium pyruvate (Sigma, St Louis, MO, USA) supplemented with 10% (v/v) fetal bovine serum (Sigma, St Louis, MO, USA), 50 µg/ml streptomycin and 50 IU/ml penicillin (Himedia India Pvt. Ltd., India) at 37°C in 5% carbon dioxide atmosphere.

Probiotic potential of Leuconostoc mesenteroides NRRL B-1149

Adhesive capacity and cell surface traits

Hydrophobicity

The hydrophobicity is the ability of microorganisms to adhere to hydrocarbons. The hydrophobic behavior of *Leuconostoc mesenteroides* NRRL B-1149 was determined by the method as described by Perez et al. (1998). The cells of *L. mesenteroides* NRRL B-1149 from overnight grown culture in 5 ml MRS medium at 28°C and 180 rpm were harvested by centrifugation at 12,000*g* for 5 min at 4°C. The cell pellet was washed twice by 5 ml 50 mM K_2HPO_4 buffer (pH 6.5) and finally resuspended in the same buffering agent. The absorbance of cell suspension was measured at 600 nm and adjusted to value of approximately 1.0 with the buffer. To the 3 ml of the bacterial suspension 0.6 ml of *n*-hexadecane was added and mixed well for 2 min. The two phases were allowed to separate at 37°C for 20 min from which the aqueous phase was removed cautiously and the absorbance was measured at 600 nm. The cell surface hydrophobicity (H%) was measured by observing the drop in absorbance of the aqueous phase using the formula:

$$\mathbf{H\% = [(A_0\text{-}A) / A_0] \times 100}$$

where A_0 and A are the absorbance before and after extraction with *n*-hexadecane, respectively.

Autoaggregation assay

The autoaggregation assay was performed by method as described by Del Re et al. (2000) with some modifications; the incubation time for autoaggregation was 5 h instead of 2 h. The culture (4 ml) of *Leuconostoc mesenteroides* NRRL B-1149 containing approximately $4x10^8$ cells was centrifuged at 12,000*g* and 4°C for 10 min. The cell pellet was resuspended in 4 ml of 150 mM phosphate buffer saline (PBS, pH 7.0) and allowed to aggregate by further incubating at 37°C for 5 h. Aliquots of 100 µl were withdrawn at regular interval of 1 h from upper suspension and mixed in 3.9 ml 150 mM PBS (pH 7.0) and absorbance was measured at 600 nm. The autoaggregation percentage (A_a%) was calculated using following equation (Del Re et al., 2000):

$$\mathbf{A_a\% = 1 - (A_t / A_0) \times 100}$$

where, A_t = absorbance at time t=1, 2, 3, 4 or 5 h and A_0 = absorbance at t=0 h.

The autoaggregation percentage was expressed as a function of time until it was constant.

In vitro cell adhesion assay

The adhesive capacity of *L. mesenteroides* NRRL B-1149 was studied using HeLa cell line, monocyte-macrophage line (J774A.1) and colon cancer (HT29) cell line by the method as described earlier (Chauviere et al., 1996). All three cell lines were grown in Dulbecco's Modified Eagle's medium (DMEM) containing 4.0 mM L-glutamine and 110 mg/l sodium pyruvate (Sigma, St Louis, MO, USA) supplemented with 10% (v/v) foetal bovine serum (Sigma, St Louis, MO, USA), 50 µg/ml streptomycin and 50 IU/ml penicillin (Himedia India Pvt. Ltd., India) and incubated at 37°C in 5% carbon dioxide atmosphere. The monolayers were prepared on glass coverslips in 6 well tissue culture plates. Each of HeLa, J774A.1 and HT29 cells were seeded at a concentration of 4×10^4 cells/cm^2 and were incubated at 37°C in a 5% CO_2 incubator. 1% (v/v) culture of *Leuconostoc mesenteroides* NRRL B-1149 was grown in MRS medium at 28°C and 180 rpm for 12 h. The cells from overnight grown

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cultures were counted and ~10^8 cells from each culture were added over the monolayer of both cell lines at post confluence. The plates were incubated at 37°C under 5% CO_2 atmosphere for 90 min. After incubation the monolayers were washed twice with phosphate buffer saline (PBS) to release unbound bacteria followed by fixing with methanol and finally stained with Giemsa solution. The adherent bacteria were counted microscopically under oil immersion of 20 random microscopic fields per each glass coverslip monolayer. The adhesive capacity of bacterium was calculated as described by Del Re et al. (2000). Based on the number of adherent bacteria, they were put under three categories: non-adhesive (< 5 bacteria adhered to 100 cells); adhesive (6-40 bacteria adhered to 100 cells); strongly adhesive (> 40 bacteria adhering to 100 cells) (Del Re et al., 2000).

Resistance to biological barriers

Lysozyme resistance

The tolerance of *L. mesenteroides* NRRL B-1149 against lysozyme was determined by method of Zago et al. (2011). The culture was grown in 10 ml MRS medium at 25°C and 180 rpm for 10-12 h. The cells were centrifuged at 12,000*g* and 4°C for 10 min. The cell pellet was washed with 5 ml of 0.1 M potassium phosphate buffer (pH 7.0) two times and re-suspended in 2 ml of Ringer solution (Sigma Aldrich, USA). 0.5 ml suspension of B-1149 from above solution was inoculated in 5 ml sterile electrolyte solution (SES; 0.22 g/l $CaCl_2$, 6.2 g/l NaCl, 2.2 g/l KCl, 1.2 g/l $NaHCO_3$) containing 100 mg/l of lysozyme (Sigma Aldrich, USA) and incubated at 37°C for 2 h. The bacterial suspension in SES without lysozyme was taken as a control. The aliquot (10 µl) from each sample was spread on MRS agar plates and incubated at 25°C for 48 h. The microbial colonies grown on plates were counted and the survival rate was calculated as percent of CFU/ml (colony forming unit per ml) obtained from lysozyme treatment with respect to the control (without lysozyme treatment).

Tolerance to simulated gastric juice

The resistance of *L. mesenteroides* NRRL B-1149 against simulated gastric juice was determined by the method of Charteris et al. (1998). The simulated gastric juice was prepared by mixing pepsin (1000 U/mg, 3 mg/ml) and NaCl (0.5% w/v) in water and pH was adjusted to 2 and 3. 30 ml overnight grown culture of isolate was centrifuged at 6,000*g* and 4°C for 20 min. After removing the supernatant, the cells were washed twice with 10 ml 50 mM K_2HPO_4 (pH 6.5) and resuspended in 3 ml of the same solution. 1 ml of cell suspension was added to 9 ml of gastric solution of pH 2 and 3 and incubated at 37°C for 3 h. The total viable cells (CFU/ml) were counted, before and after incubation period and expressed as the difference in colony counts.

Bile salts resistance

The ability of the *L. mesenteroides* NRRL B-1149 to tolerate different concentrations bile was evaluated by method of Walker & Gilliland (1993) with some modifications; instead of MRS-THIO medium (containing 0.2% sodium thioglycollate), MRS medium was used. The overnight grown culture of *L. mesenteroides* NRRL B-1149 (2%, v/v) was inoculated into 10 ml MRS medium (pH 6.4) containing 0.3, 0.5 or 1% (w/v) of bile salts (Sigma, St Louis, MO, USA). The cultures were incubated at 25°C and after 24 h absorbance was measured at 600 nm and compared to a control culture (without bile salts). The results were expressed as the percent of growth (A_{600} nm) in the presence of bile salts with respect to the control.

Bile salts deconjugation

Bile salts hydrolase (BSH) activity of the isolate was determined according to the method of Taranto et al. (1995). The MRS medium (pH 6.4) plates were made supplemented with 1.7% (w/v) agar and 0.5% (w/v) sodium salt of taurodeoxycholic acid Sigma, St Louis, MO, USA). The medium was autoclaved (121°C, 15 min, 15 psi) and immediately used. 12 h old culture of *L. mesenteroides* NRRL B-1149 was streaked on petri plate and was incubated anaerobically in air-tight gas jar containing the gas-pak sachet at 37°C for 72 h.

β-galactosidase activity

The β-galactosidase activity in bacterial cells was determined by the method described by Miller (1972) with some modifications. 5 ml of overnight grown culture of *L. mesenteroides* NRRL B-1149 was harvested in the stationary phase by centrifugation at 12000*g* and 4°C for 5 min. The cells were washed twice with 0.85% (w/v) NaCl solution and finally resuspended in 1 ml of the same solution. 1% (v/v) of each culture was inoculated in 5 ml MRS-lac broth and incubated at 25°C for 24 h. The cells were harvested and washed twice with Z-buffer (0.854%, w/v Na_2HPO_4; 0.55%, w/v $NaH_2PO_4.H_2O$; 0.075%, w/v KCl and 0.025%, w/v $MgSO_4.7H_2O$). The triplicate dilutions of 0.5 ml cells with 0.5 ml complete Z-buffer (Z-buffer containing 0.28%, v/v β-

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mercaptoethanol) were prepared in test tubes on ice (1.0 ml Z-buffer was taking as control). The cells were permeabilised by adding 1 drop of 0.1% SDS and 2 drops of chloroform using a Pasteur pipette and vortexed. The tubes were incubated in a 30°C water bath and allowed to equilibrate for about 2 min. 0.2 ml *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (4 mg/ml) was added to each tube and vortexed. When a yellow color appeared, the reaction was stopped by adding 0.5 ml of 1 M Na_2CO_3. The absorbance was measured at OD_{420} and OD_{550} for each tube. The absorbance of cell suspension at OD_{650} was also measured. The β-galactosidase activity was calculated in Miller Units (MU) using the equation shown below,

$$\text{B-galactosidase activity} = \frac{OD_{420} \times (1.75 - OD_{560})}{OD650 \times t \times v} \times \frac{1\ \text{nmol}}{0.0045\ \text{ml cm}} \times 1.7\ \text{ml}$$

where t = time of reaction in min; v = volume of cells added to the assay tubes; 0.0045 OD_{420}/nmol = e_{420} *o*-nitrophenol; 1.7 ml = total volume; cuvette = 1 cm path length; β-galactosidase activity = nmol / min / OD_{650} ml

One unit of β-galactosidase activity was defined as 1 µmol of *p*-nitrophenol released per minute under experimental conditions.

***In vitro anticancer activity assay of dextran from L. mesenteroides* NRRL B-1149**

The lyophilized powder of dextran from *L. mesenteroides* NRRL B-1149 (Shukla et al., 2011) was used for *in vitro* anticancer activity assay. The effects of dextran on HeLa, HT29 and murine J774A.1 cells were determined using the colorimetric 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) assay as described earlier (Mosmann, 1983). Each of HeLa, HT29 and murine J774A.1 cell lines at density approximately, 1.2 x 10^4 cells/well in a 100 µl medium in 96-well plates were seeded and allowed to attach for 12-16 h at 37°C in 5% CO_2 atmosphere. After incubation the medium was removed from all plates and the dextran at different concentrations (1-1000 µg/ml) was added in each well. The medium without dextran was used as negative control. All the plates were incubated at 37°C in 5% CO_2 atmosphere for 48 h. After 48 h the media were removed, 100 µl MTT (500 µg/ml) was added to each well and incubated at 37°C for 4 h. The supernatant was removed and 100 µl DMSO was added to each well. Absorbance was measured at 570 nm by a 96-well microplate reader (Tecan, Infinite 200 Pro).

Results

***Probiotic potential of L. mesenteroides* NRRL B-1149.** *Adhesive capacity and cell surface traits*

The hydrophobicity shown by *L. mesenteroides* NRRL B-1149 was 42.9% after 20 min, which was significantly higher than to other reported probiotic bacteria such as *Lactococcus aidophilus* (38.1%), *Lactococcus casei* (24.1%), *Lactococcus lactis* (31.3%) (Zago et al., 2011). The autoaggregation (%) of *L. mesenteroides* NRRL B-1149 increased with increasing time showing 55.2% auto-aggregation after 5 h displaying its ability to form clumps (Figure 1).

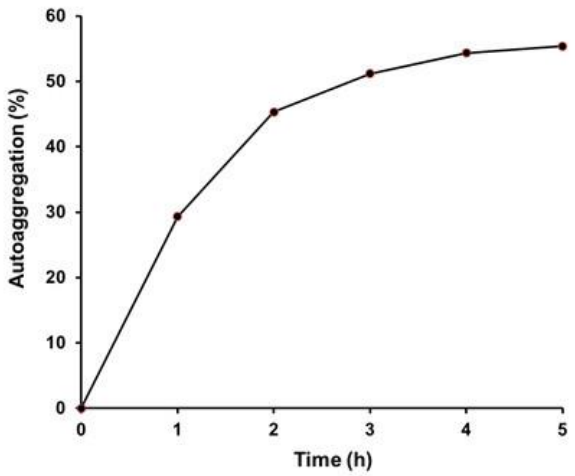

Figure 1. *Autoaggregation capacity shown by Leuconostoc mesenteroides NRRL B-1149 with time (h).*

Hydrophobicity and auto-aggregation of microorganism are phenotypically related to its adhesion capacity (Del Re et al., 2000; Kos et al., 2003). It is the hydrophobic nature of outer layer of microbe which helps in attaching to the mammalian cell surface (Rosenberg et al., 1980; Zago et al., 2011). The adhesion property also supports a probiotic microbe to compete with other microorganisms in gastrointestinal tract (Naidu et al., 1999). The adhesion of *L. mesenteroides* NRRL B-1149 on HeLa, J774A.1 and HT29 cell lines is shown in Figure 2. The microscopic examination of adhesion between mammalian cell lines and the isolate showed good adhesion property of the isolate with all the cell lines (Figure 2A-D). The adherence of *L. mesenteroides* NRRL B-1149 to HeLa cell line, J774A.1 cell line and HT29 cell line is shown in Figure 2B, 2D and 2F, respectively. As shown in Figure 2B, 2D and 2F more than 40 cells adhered to cell lines indicating good adhesion property of the isolate.

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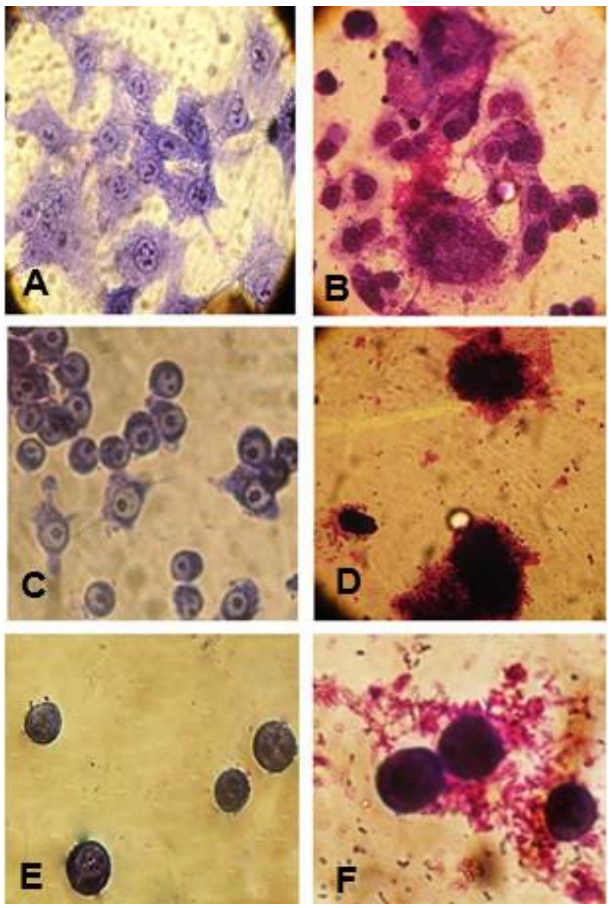

Figure 2. *Determination of adhesive capacity of Leuconostoc mesenteroides NRRL B-1149 on HeLa and J774A.1 cell lines. (A) Untreated HeLa cells (control); (B) Adherence of Leuconostoc mesenteroides NRRL B-1149 on HeLa cells; (C) Untreated J774A.1 cells (control); (D) Adherence of Leuconostoc mesenteroides NRRL B-1149 on J774A.1 cells; (E) Untreated HT29 cells (control); (F) Adherence of Leuconostoc mesenteroides NRRL B-1149 on HT29 cells.*

The adhesion capacity is a key property of probiotic through which it colonizes the intestine and competing with pathogenic bacteria. The characterization of many probiotic bacteria has been done on the basis of their adhesion capacity (Del Re et al., 2000). The bacteria having high autoaggregation capacity also show good adhesion capacity.

Resistance to biological barriers

Resistance to lysozyme

The overall resistance of *L. mesenteroides* NRRL B-1149 to lysozyme, bile salts and simulated gastric juice was expressed in terms of percent survival. It showed resistance to 100 µg/ml of lysozyme with 92% (1.02 x 10^6 CFU/ml) survival after 2 h as compared to control having 1.11 x 10^6 CFU/ml (Figure 3). It was in good agreement with previously reported lysozyme resistance data of other probiotic bacteria such as *Lactobacillus plantarum* Lp793 (87.85%), *L. plantarum* Lp800 (84.69%) and *L. plantarum* Lp813 (74.04%) (Zago et al., 2011). The swallowed probiotic microorganisms generally encounter first biological barrier of lysozyme, which is present in saliva of mouth. The next barrier is gastric juice in stomach, where the pH is between 1.5 and 3.0 and the upper part of small intestine, which contains bile (Corzo & Gilliland, 1999).

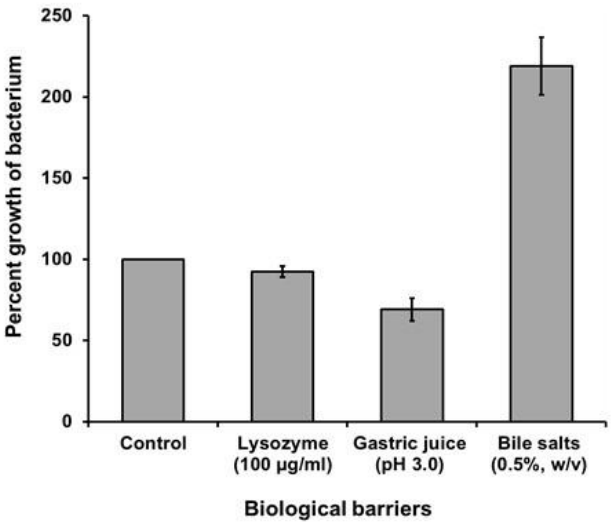

Figure 3. *Resistance of Leuconostoc mesenteroides NRRL B-1149 against biological barriers. Percent growth of the bacterium in presence of 100 µg/ml lysozyme after 2 h, simulated gastric juice (pH 3.0) and 0.5% (w/v) bile salts.*

Resistance to gastric juice

The resistance profile of B-1149 towards gastric juice is shown in Figure 3. The number of CFU/ml before incubation with gastric juice of pH 2 and pH 3 were 4.4 x 10^8 and 23 x 10^8, respectively. It showed resistance to simulated gastric juice of pH 3.0 with 61±6.02% survival (14 x 10^8 CFU/ml) but no survival was observed in pH 2.0. A lactic acid bacterium, *Leuconostoc mesenteroides* isolated from intestine of snakehead fish (*Channa striatus*) showing growth at pH 3 to 7 has also been considered as probiotic (Allameh et al., 2012). According to Erkkilä & Petäjä (2000) the strains of *Pediococcus acidilactici* (P2), *Lactobacillus curvatus* (RM10), and *P. pentosaceus* (FF) showed tolerance to acid

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(pH 3.0). The survival of *Leuconostoc mesenteroides* NRRL B-1149 at pH 3.0 indicated that it could transit through the stomach.

Resistance to bile salts

Amongst three different concentrations of bile salts (0.3%, 0.5% and 1.0%), *L. mesenteroides* NRRL B-1149 not only showed the survival, but also enhanced growth in presence of bile salts (Figure 3). In presence of 0.5% (w/v) bile salts, the growth of cells was 219±17.7%. In contrast to *Leuconostoc* spp. from intestine of snakehead fish, which resisted to 0.3% bile salts (Allameh et al., 2012), *L. mesenteroides* NRRL B-1149 served as a better candidate since it resisted up to 1% (w/v) bile salts. The resistance of bacterium in presence of bile salts could be due to the stress adaptation mechanism similar to that observed by Sahadeva et al. (2011). *Lactobacillus acidophilus* resisted high concentration (2%) of bile, which could be due to its adaptation to the low pH environment and hence decreasing the toxicity in the intestine (Begley et al., 2005). However, the enhanced growth in presence of bile salts might be due to the presence of bile salts hydrolase activity in *L. mesenteroides* NRRL B-1149, which helps in detoxifying bile salts and thereby increasing its survival and growth. The similar finding of enhanced growth of *Lactococcus lactis* strain A2 in presence of bile salts has also been reported earlier (Shitandi et al., 2007). The human body contains bile salts in large intestine and the relevant physiological concentrations of human bile range from 0.3% to 0.5% (Zavaglia et al., 1998). Thus, it is generally considered necessary to evaluate the ability of potentially probiotic bacteria to resist the effects of bile acids (Collins et al., 1998).

Bile salts hydrolase activity

The appearance of halos (nearly 6 mm) around colonies after growth in MRS-TDCA medium validated the ability of *L. mesenteroides* NRRL B-1149 to hydrolyze sodium salt of taurodeoxycholate (data not shown). The inhibition of common intestinal bacteria had been related to the presence of free (deconjugated) bile acids rather than conjugated ones (De Smet et al., 1995; Grill et al., 2000). The ability to hydrolyze bile salts could help the microorganism in sustaining the balance of the gut microflora (Rosenberg et al., 1980; Taranto et al., 1995). It has also been suggested that bile salt hydrolase (BSH) enzyme might be a detergent shock protein that enables LAB to survive the intestinal bile stress (De Smet et al., 1995).

β-galactosidase activity

The β-galactosidase activity was determined by measuring the hydrolysis of chromogenic substrate, *o*-nitrophenyl-β-D-galactopyranoside (Miller, 1972). The colorless *o*-nitrophenyl-β-D-galactopyranoside turns to yellow in presence of β-galactosidase leaving colorless galactose. *L. mesenteroides* NRRL B-1149 showed β-galactosidase activity with 94.24±3.9 Miller units more than other probiotic bacteria such as *L. plantarum* Lp1017 (82.72 MU) and *L. plantarum* Lp814 (82.69 MU) (Zago et al., 2011). Most of the probiotic bacteria possess this property. De Vrese et al. (2001) reported that the person deficient of β-galactosidase enzyme shows intolerance to lactose because lactose is not utilized in small intestine and as a consequence it is used by the indigenous bacteria (De Vrese et al., 2001).

***In vitro anticancer activity of dextran from L. mesenteroides* NRRL B-1149**

The dextran from *L. mesenteroides* NRRL B-1149 is a branched α-glucan having α-(1,6) linked linear chain with α-(1,3) branched linkages (Shukla et al., 2011). The cytotoxic effect of dextran on HeLa and HT29 cell lines showed reduced viability of both cell lines as displayed in Figure 4. The dextran showed maximum inhibition of 25% of HeLa cells at concentration 100 µg/ml. No change in inhibition was observed at concentration of dextran above 100 µg/ml. However, the inhibition ratio of HT29 cells was 39.8±3.4% at 1000 µg/ml of dextran (Figure 4). The similar results were observed with APS-1d polysaccharide from the roots of *Angelica sinensis*, where APS-1d in the concentration range of 3 to 100 µg/ml significantly inhibited the proliferation of HeLa cells with maximum inhibition ratio of 23.0±5.5% at 100 µg/ml concentration (Cao et al., 2006). The anticancer effects of polysaccharides are due to stimulation of cell mediated immune response (Ooi & Liu, 2000), however the direct *in vitro* inhibition of cell proliferation by polysaccharides from *Phellinus linteus* (Li et al., 2004) and *Cordyceps sinensis* (Chen et al., 1997) has also been observed. As compared to β-glucans, the biological activity of α-glucans has been scarcely investigated. An α-glucan from a lichen *Ramalina celastri* showed cytotoxic effect against HeLa cells (Carneiro-Leao et al., 1997). The low molecular-weight α-glucan from edible mushroom *Pleurotus ostreatus* showed promising antitumorigenic properties against HT-29 colon cancer cell growth (Lavia et al., 2006). An antitumor polysaccharide SPR-901 from lactic acid bacteria has also been isolated from rice bran (Kado et al.,

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1991). It has been reported that some biological polysaccharides are involved in activation of granulocytes, monocytes, macrophages and NK-cells and trigger the secretion of IFN-γ, IL-6, IL-8 and IL-12 from macrophages, neutrophils and NK-cells (Ladanyi et al., 1993). Many of those natural polysaccharides also showed antibacterial and antitumor activities (Leung et al., 1977). Possibly, the antitumor effect of such polymers could be due to activation of macrophages.

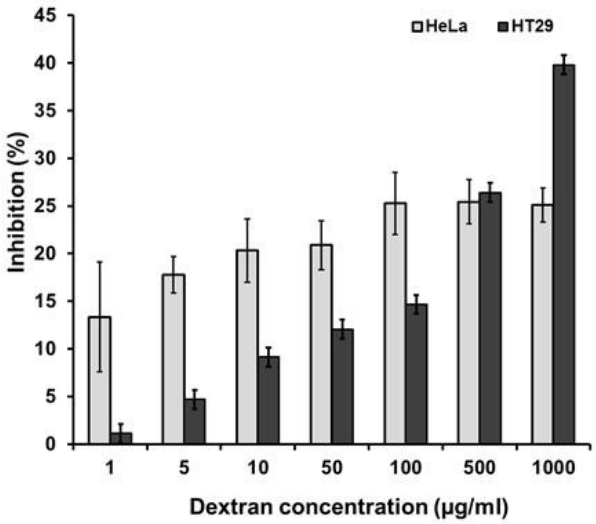

Figure 4. *Effect of various concentrations of dextran from Leuconostoc mesenteroides NRRL B-1149 on cervical cancer (HeLa) and colon cancer (HT29) cell lines.*

The effect of dextran (1-1000 µg/ml) on murine macrophage cell line was also analyzed and the increase in the percent survival of macrophages was observed with increase in dextran concentration (Figure 5). In the presence of dextran from *L. mesenteroides* NRRL B-1149 the maximum survival of macrophages was 167.6±6.6%, which indicated the non-toxic nature of polysaccharide on normal cell line. The enhanced cell viability might be due to the activation of macrophages. It has been reported that activated macrophages showed interference in MTT cell viability. The activated macrophages over-produce inducible nitric oxide synthase having NADPH diphorase activity, which reduces nitro-blue tetrazolium (NBT) salts to formazan blue resulting in overestimation of MTT assay (Pozzolini et al., 2003). It was concluded that the branched dextran from *L. mesenteroides* NRRL B-1149 reduced the cell viability of HeLa and HT29 cell lines, whereas, it enhanced the cell viability of murine macrophage cell line.

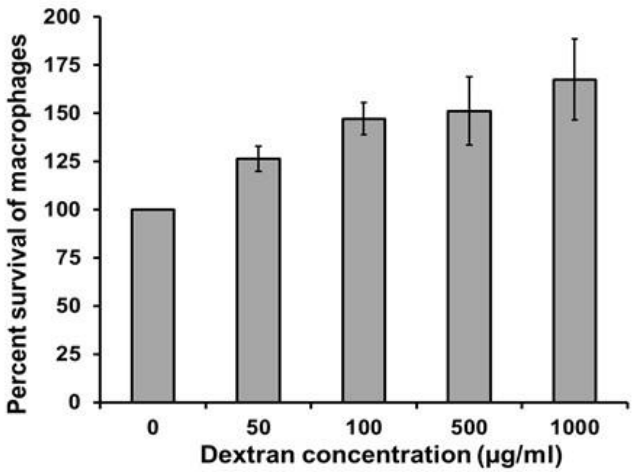

Figure 5. *Effect of different concentrations of dextran from Leuconostoc mesenteroides NRRL B-1149 on monocyte macrophages (J774A.1) cell line.*

Conclusion

Leuconostoc mesenteroides NRRL B-1149 proved to be a potential probiotic displaying cell adhesion property, hydrophobicity and autoaggregation properties. These properties indicated that it has the ability to adhere to the intestinal wall and to compete with undesirable microorganisms in the gut. It showed 92% survival in presence of lysozyme, the enzyme present in saliva. It displayed tolerance to simulated gastric juice (61.6% survival) and bile salts (219.0% growth), which further pronounced its probiotic nature. The presence of halo around colonies in MRS medium containing taurodeoxycholate showed its ability to hydrolyse bile salts. The cells of *Leuconostoc mesenteroides* NRRL B-1149 showed β-galactosidase activity with 94.24 Miller units, which signifies its importance in lactose digestion. Along with its role as a potential probiotic bacterium *Leuconostoc mesenteroides* B-1149 also produced branched dextran that displayed anticancer properties. The *in vitro* anticancer activity analysis of dextran from *L. mesenteroides* B-1149 against cervical cancer (HeLa) cell lines and colon cancer (HT29) cell lines showed that the viability of both cell lines was reduced. This may lead to new prospects of novel dextran as therapeutic in pharmaceutical industries. The dextran did not show any negative effect on macrophages displaying its biocompatible nature, however, the increased cell viability might be due to the activation of macrophages.

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