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Improvement in bioactivity of *Lactobacillus* isolates by encapsulation in sodium alginate beads: *In vitro*

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ABSTRACT

Probiotics are rapidly becoming popular and important tool for preserving our natural health. Probiotics usually comprise of lactic acid producing bacteria. The objective of the present study was to evaluate the antioxidant and anticholesterolemic activity of two probiotic isolates and further its improvement through sonication and encapsulation in sodium alginate beads in vitro. Two isolates (LB7 and LB9) were selected and tested for LPO, CAT and SOD activity. Result showed that LB9 strain exhibit more antioxidant activity compared to LB7. Both the isolates were subjected to anticholestrolemic activity evaluation in two forms (Encapsulated and Non Encapsulated). The immobilized cells exhibit better anticholesterolemic activity. Immobilization increased the antioxidant activity as well as the ability of Lactobacillus strains for cholesterol reduction in samples. It is concluded that the Lactobacillus isolated are antioxidants and anticholestrolemic agents and their bioactivity is improved by encapsulation.

Keywords: Probiotic, encapsulation, cholesterol, antioxidant.

INTRODUCTION

Probiotics have been associated historically with milks and dairy products, from which there is substantial evidence for positive effects on human health and general well-being [1]. Providing probiotic living cells with a physical barrier against adverse environmental conditions is an approach currently receiving considerable interest. Encapsulation is the good technique for the viability of probiotics in food products and gastrointestinal tract [2]. Oral microencapsulated probiotic cell formulation is a functional nutritional alternative for managing excessive serum cholesterol and triglyceride levels [3]. Probiotic yoghurt supplemented diet has been found to reduce serum total cholesterol [4]. Every one percent reduction in serum cholesterol concentration is associated with an estimated two to three percent reductions in risk for coronary heart disease. The present study was designed to find out antioxidant and anticholesterolemic activity of *Lactobacillus* isolates.

MATERIALS AND METHODS

Microbial cultures: *Lactobacillus* isolates (LB7, LB9) isolated from buffalo milk curd were maintained in our laboratory on MRS broth at 37°C.

Growth pattern of *Lactobacillus* isolates: Autoclaved MRS Broth pH 6.5±0.2 was taken and was inoculated 1% v/v culture and incubated at 37°C along with control (Uninoculated MRS Broth). 2ml sample was taken out after every 3hr upto 36hr and absorbance at 600nm was measured.

Preparation of sodium alginate beads: Sodium alginate solution (3%) and calcium chloride solution (0.075 mM) were autoclaved at 121°C for 15 minutes. Sodium alginate was filled in syringe. Beads were formed by drop wise addition of sodium alginate into gently stirred chilled $CaCl_2$ solution with the help of magnetic stirrer. After membrane formation beads were removed, washed with distilled water and transferred into hardening solution (100 Mm $CaCl_2$) and stored for further studies.

Preparation of intact cells, encapsulated cells and intracellular cell free extracts: Starter cultures of isolates were grown in MRS Broth (pH 6.5±0.2 for 24hr) at 37°C. Inoculums from these were transferred to MRS medium and incubated for 18 hours at 37°C. The culture suspension was centrifuged at 4400rpm for 10min to get the biomass. Washing was done thrice with 0.1 M PBS buffer (pH 7.2). After washing cells were suspended in 5ml of 0.1M PBS buffer (pH 7.2). The suspension was divided into three parts:

Intact cells: Culture broth was centrifuged at 4000 rpm and cell number was adjusted to 10^9 cells/ml.

Sonicated cells: Culture broth was sonicated for one minute interval at 20-10 pulse rate (on/off cycle) in ice bath. Resultant supernatant contained intracellular cell free extract after centrifugation at 7800rpm for 10min.

Encapsulation: 2% *Lactobacillus* cultures were mixed with autoclaved 4% sodium alginate solution and beads were formed by the method described earlier.

Antioxidant Activity

Determination of *in vitro* inhibition of lipid peroxidation (LPO): This is based on monitoring of inhibition of linoleic acid (source of unsaturated fatty acids) peroxidation by microbial cells. *In vitro* LPO of three different strains of *Lactobacillus* and ascorbic acid (standard) was determined by lipid peroxide formed, which was measured by employing modified method of Ohkawa *et al.*, 1979 [5]. Linoleic acid emulsion 1ml was added to solution containing 0.4 ml of 2×10^9 cells. 0.2ml of 0.01% $FeSO_4$ and 0.2ml of 0.56mM H_2O_2 was added to above solution. The reaction mixture was mixed thoroughly and incubated for 12hr at 37°C. After 12hr 0.2 ml TCA (4%) was added to 2 ml of the reaction mixture. To the above mixture 2ml TBA and 0.2ml BHT (0.4%) was added and incubated it in boiling water bath for 30min. Reaction mixture was allowed to cool and added 2ml chloroform for extraction. O. D. at 630nm was taken with phosphate buffer saline as blank.

$$\% \text{ inhibition of linoleic acid peroxidation} = \frac{\text{O.D. of control} - \text{O.D. of sample}}{\text{O.D. of control}}$$

Preparation of erythrocyte lysate for evaluation of CAT and SOD activity: Blood sample 0.5ml was taken in anticoagulant (Alsever reagent) in the ratio of 1:2 and centrifuged at 2000 rpm for 15 min. Supernatant was discarded and 3 times excess of PBS (0.1 M) was added to the pellet and twice centrifuged at 2000 rpm for 15 min. Clear supernatant was discarded and pellet was used for further experiment. **Preparation of packed cell volume** (5%): 50 µl of the pellet obtained was added to 950 µl of PBS thereby making a total volume to 1ml. 0.8 ml distilled water was added to 0.2 ml of the PCV so obtained in the previous step to constitute the erythrocyte lysate.

Determination of catalase (CAT) activity: Different dilutions of microbial cultures (10^6 and 10^9 cells/ml) and ascorbic acid (standard) were tested for their catalase activity measured by the method of Aebi *et al.*, 1993 [6]. The different dilutions of microbial cultures were incubated with erythrocyte lysate (5%) at 4°C for 0, 24, 48, 72 hr. Prepared buffered H_2O_2 by diluting 0.34ml of 30mM H_2O_2 with phosphate buffer to make the volume 100ml. Phosphate buffer 2ml was added to the cuvette containing 20µl of erythrocyte lysate. 1ml of H_2O_2 was added and mixed well. Checked the decrease in O.D. at 240nm after every 15s upto 2min.

$$\text{X micromoles/min/ml} = \frac{\text{Difference in absorbance x 106 x volume of reaction mixture}}{\text{Molar extinction coefficient x 1000 x volume of enzyme taken}}$$

Determination of superoxide dismutase (SOD) activity: Different dilutions of microbial cultures (10^6 and 10^9 cells/ml) and ascorbic acid (standard) were tested for their Superoxide Dismutase (SOD) activity [7]. To these above said dilutions were incubated with erythrocyte lysate (5%) at 4°C for 0, 24, 48, 72 hr. Untreated erythrocyte lysate was taken as blank. 1.5ml of Tris buffer (100mM) was added to 0.5ml of EDTA (6 mM). To this mixture 20µl of erythrocyte lysate was added. 1ml of 0.7mM pyrogallol is added and increase in O.D. was checked at 420nm at time interval of 15s for 2min .

$$X = \frac{\text{Final absorbance} - \text{Initial absorbance x 100}}{\text{Final absorbance}}$$

1 unit = 50% inhibition of autooxidation of pyrogallol

Determination of anticholesterolemic activity: For the evaluation of cholesterol reduction activity microbial culture was incubated with cholesterol 100mg/ml at 37°C. The activity of strains was checked at different time intervals (0, 6, 12, 18 hr) for cholesterol reduction. The cell number was maintained $1x10^9$ cells/ml and was tested for their anticholesterolemic activity by using CHOD- POD / Phosphotungstate method of cholesterol kit (Medsource Pvt. Ltd.).

RESULTS AND DISCUSSION

Growth profile of *Lactobacillus* isolates: Growth profile Growth profile of both LB7 and LB9 was measured by taking O.D. at 600nm of culture broth for a period of 0-36 hr at the interval of 3 hr as given in Fig. 1. Maximum growth of LB7 was observed at 24 hr and LB9 at 27hr.

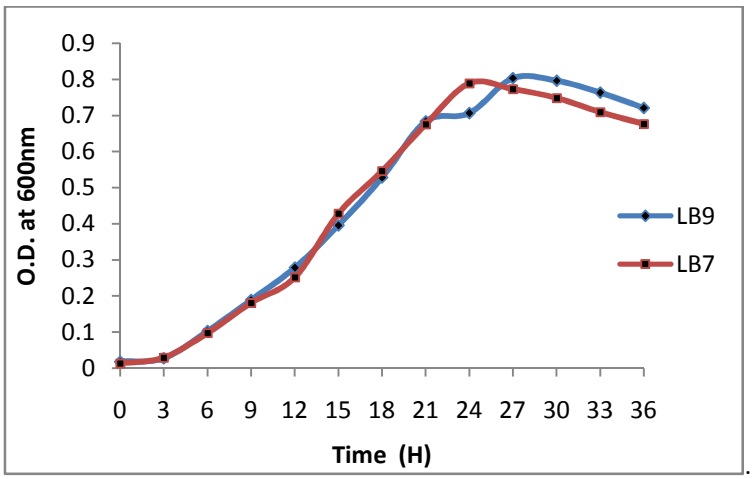

Figure 1 Growth profile of LB7 and LB9 grown in MRS Broth pH 6.5±0.2. Data are the mean of duplicate samples, which did not differ by more than 5%.

Evaluation of Antioxidant Potential

Effect of sonication and encapsulation of bacteria on LPO inhibition: Both LB7 and LB9 were analyzed for lipid peroxidation inhibition in different forms as shown in Fig. 2 and Table 1. It was observed that sonication and encapsulation increases the LPO inhibition. Percent LPO inhibition by sonicated and encapsulated bacterial strains in decreasing order was

Sonicated strains > Encapsulated > Non-sonicated

Results showed that LB9 strain exhibited more LPO inhibition than LB7.

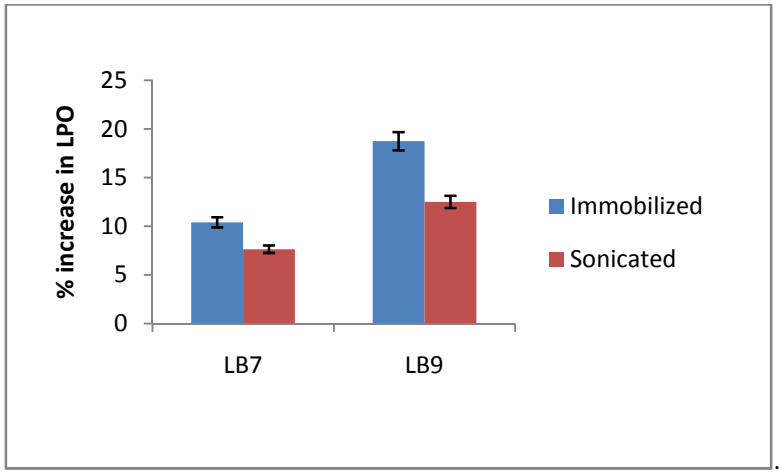

Figure 2 Effect of Sonication and Encapsulation on LPO inhibition. Data are the mean of duplicate samples, which did not differ by more than 5%.

Table 1 Inhibition of lipid peroxidation (LPO) by bacterial isolates. Readings are expressed as %mean ± SD.

Bacterial isolate	% increase in LPO by Sonication	% increase in LPO by Encapsulation
LB7	10.41 ± 0.89	7.64 ± 0.40
LB9	18.75 ± 0.76	12.5 ± 0.88

***Ex vivo* determination of Catalase activity (CAT) of bacterial strains:** The effect of the different concentration of cells, sonication and encapsulation of both the strains was evaluated for decrease in catalase activity at interval of 24 hr upto 72 hr. Results showed that out of these two LB9 strain exhibited more CAT activity i.e. L-ascorbic acid > LB9 > LB7**.** Best catalase activity was exhibited by sonicated strain with cell concentration of 10^9 cells/ml. The final reading taken at 72 hr revealed that highest decrease in CAT activity was in non-sonicated strains and lowest decrease was in sonicated strains and intermediate between the two was the encapsulated strain. Results for decrease in CAT activity are shown in Table 2 and Fig. 3. Decrease in CAT activity in decreasing order was **Non-sonicated strain > Encapsulated strain > Sonicated strain**

***Ex vivo* determination of Superoxide Dismutase Activity (SOD) of bacterial strains (Table 2, Figure 3).** Results showed that out of the two strains of *Lactobacillus* LB9 exhibited more SOD activity than LB7 i.e. L-ascorbic acid > LB9 > LB7. The effect of the different concentration and sonication and encapsulation of both the strains was evaluated for increase in SOD activity at interval of 24hr upto 72hr. Best SOD activity was exhibited by sonicated strain with cell concentration of 10^9 cells/ml. At 72hr revealed that highest increase in SOD activity was in sonicated strains and lowest increase was in encapsulated strain and intermediate between the two was the non-sonicated strains. Increase in SOD activity in decreasing order was

Sonicated strain > Non sonicated strain > Encapsulated strain

However the increase in activity was cell concentration dependent i.e. higher in suspension of 10^9 cells/ml as compared to 10^6 cells/ml.

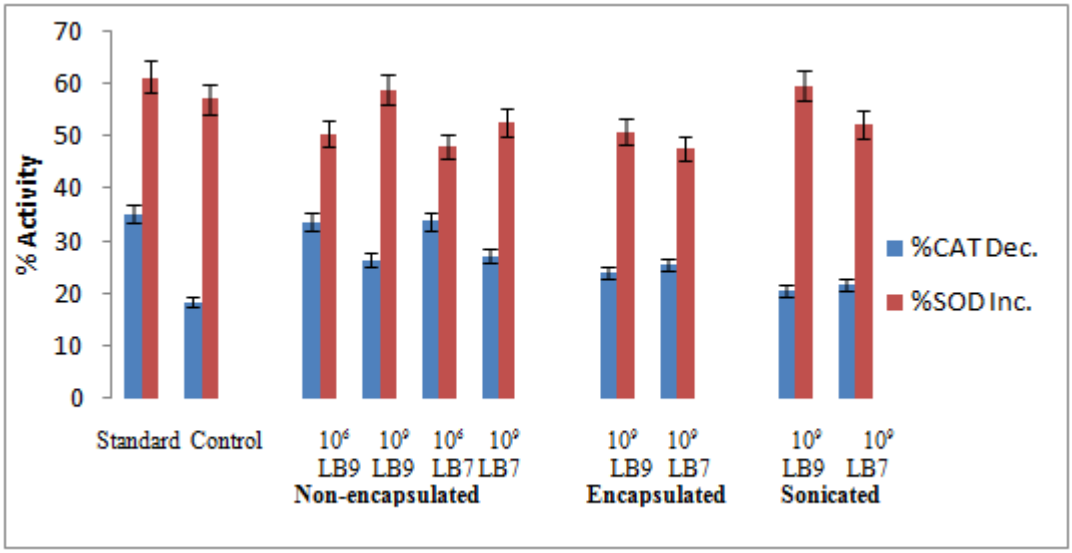

Figure 3 Effect of Sonication and Encapsulation on CAT activity (1 unit = X micromoles/min/ml) and SOD activity (1 unit = 50% inhibition of autooxidation of pyrogallol). Data are the mean of duplicate samples, which did not differ by more than 5%.

ANTICHOLESTEROLEMIC ACTIVITY

The effect of both *Lactobacillus* isolates LB9 and LB7 (unencapsulated and encapsulated) was checked for anticholesterolemic activity at an interval of 6hr upto 18hr. LB9 strain showed more cholesterol reduction (Table 3, Fig. 4). There was a gradual decrease in cholesterol level from 0-18hr. After 18 hr the highest increase in anticholesterolemic activity was in immobilized strains. Reduction in cholesterol was more in encapsulated strain than non-encapsulated strain (Fig. 5).

Table 2 *Ex vivo* determination of CAT and SOD activity. Readings are expressed as %mean ± SD.

Sample	%CAT Dec.	%SOD Inc.
Standard	35.29±4.97	61.36±0.57
Control	18.36±4.11	57.23±0.86
Non-encapsulated		
LB9(10^6 cells/ml)	33.84±6.02	50.57±0.14
LB9 (10^9 cells/ml)	26.47±3.09	59.03±0.68
LB7 (10^6 cells/ml)	33.87±3.98	48.09±0.91
LB7 (10^9 cells/ml)	27.27±4.53	52.65±0.25
Encapsulated		
LB9 (10^9 CFU/bead)	23.99±1.08	50.87±0.84
LB7 (10^9 CFU/bead)	25.44±0.95	47.67±0.66
Sonicated		
LB9 (10^9 cells/ml)	20.51±1.25	59.76±0.29
LB7 (10^9 cells/ml)	21.76±0.67	52.46±0.26

Table 3 *In Vitro* determination of cholesterol reduction by bacterial strains. Readings are expressed as %mean ± SD.

% Cholesterol Dec.			
Sample	**6 hrs**	**12 hrs**	**18 hrs**
Non-encapsulated			
LB7 (10^6 cells/ml)	10.41±0.66	24.70±1.22	33.79±0.89
LB9 (10^6 cells/ml)	15.60±0.90	29.75±0.56	40.66±0.35
Encapsulated			
LB7 (10^6 cells/ml)	5.58±1.90	22.20±0.89	38.37±0.64
LB9 (10^6 cells/ml)	13.00±0.55	32.25±1.67	42.95±0.66

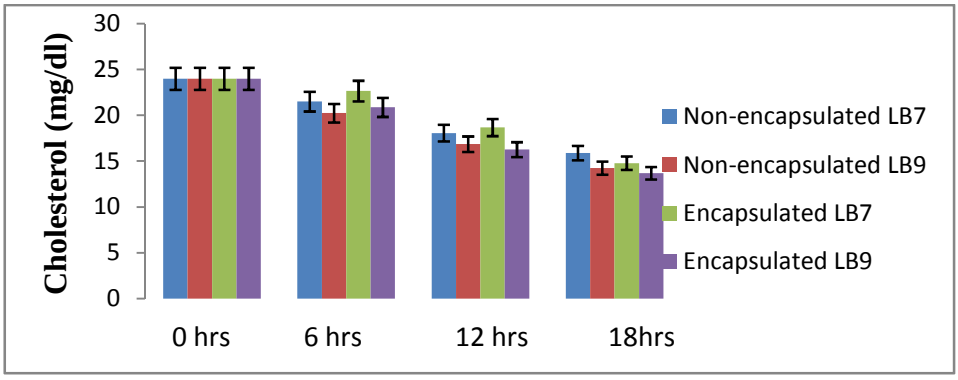

Figure 4 *In vitro* anticholesterolemic activity of Non encapsulated and Encapsulated microbial culture (1x10^9 cells/ml) in 100mg/ml Cholesterol containing MRS Broth. Anticholesterolemic activity was measured by using CHOD- POD / Phosphotungstate method of cholesterol kit. Data are the mean of duplicate samples, which did not differ by more than 5%.

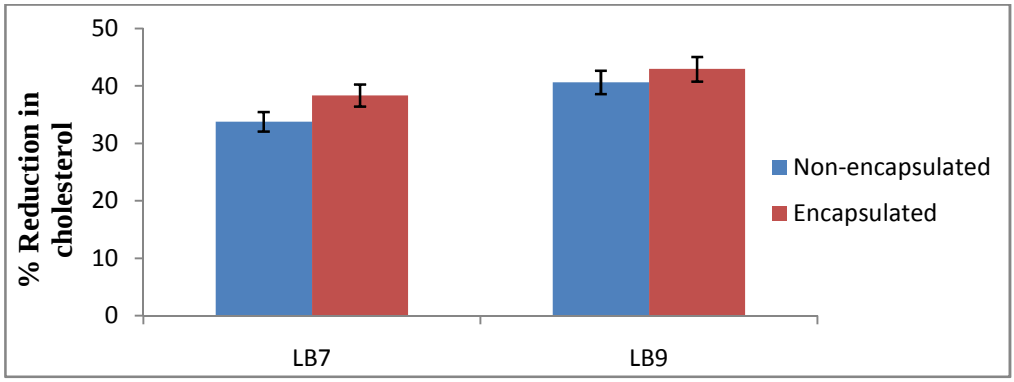

Figure 5 Effect of Encapsulation on anticholesterolemic activity after 18hr. Data are the mean of duplicate samples, which did not differ by more than 5%.

Nutritional therapies such as probiotics have been suggested to manage elevated cholesterol. Many studies have shown the anticholesterolemic properties of *Lactobacilli* [8][9][10].The result of our study showed that encapsulation of *Lactobacillus* strains increases the cholesterol reduction *in vitro*.

Similar to our study Bhathena *et al.*, 2009[3] showed that treatment with microencapsulated *Lactobacillus fermentum* 11976 (LF11976) probiotic formulation produces significant reductions in serum total cholesterol, LDL cholesterol, and serum triglyceride levels in diet-induced hypercholesterolemic hamsters suggesting the potential of the oral microencapsulated probiotic cell formulation as a functional nutritional alternative for managing excessive serum cholesterol and triglyceride levels

In our study the antioxidant activity of *Lactobacilli* was found to be more in cell-free extract as compared to intact cells. This may be due to release of intracellular protein in cell free extract. It was also reported more antioxidant activity in the intracellular cell free extract of probiotic *yeast* [11]. The author used both intact cell and extract of them and demonstrated antioxidant activity ranged from 49% to 68%. In our study, out of the two strains of *Lactobacilli* the strain which showed best antioxidant activity also showed best anticholesterolemic activity.

Our results are quite similar to those by Jain *et al.* 2009 [12]. Authors in their study used three strains of lactic acid bacteria (LAB) viz. *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactococcus lactis* and milk fermented with these strains have been studied for antioxidant and cholesterol assimilation activities *in-vitro* and *in-vivo*, in addition to the effect on total *Lactobacilli*, *Lactococci* and coliform counts into the gut of mice fed with diets supplemented by fermented milk. The well known benefits of lactic acid bacteria for human health are stimulation of immune system and their anti-oxidative features [13][14]. The results in the present study corroborate the findings given earlier.

CONCLUSION

It is concluded that the *Lactobacillus* isolated are antioxidants and anticholestrolemic agents and their bioactivity is improved by encapsulation. Moreover in both cases the activity is cell concentration dependent.

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REFERENCES:

[1] G Reid, J A Hammond, *Can Fam Physician*, **2005**, 51, 1487-1493.
[2] A V Rao, N Shiwnarain, I Maharaj. *Can Inst Food Sci Technol J*, **1989**, 22, 345-349.
[3] J Bhathena, C Martoni, A Kulamarva, AM Urbanska, M Malhotra, S Prakash. *J Med Food*, **2009**, 12(2), 310-9.
[4] N H Mansoub, *Ann Biol Res*, **2011**, 2(3), 121-125.
[5] H Ohkawa, N Ohisi, K Yagi, *Ann Biochem*, **1979**, **95**, 351-358.
[6] HE Aebi. Catalase. In: Methods in Enzymatic Analysis, Bergmeyer, H.O., (ed.), Academic Press, New York, U.S.A., **1983**, 273-386.
[7] J M Cord, I Fridovich, *J Biol Chem*, **1969**, 244, 6049–6055.
[8] J A A afari, B Larijani, A H Majd, F Tahbaz, *Ann Nutr Metab*, **2009**, 54(1), 22-27.
[9] D K Walker, S E Gilliland, *J Dairy Sci*, **1993**, 76: 956-961.
[10] M M Brashears, S E Gilliland, L M Buck, *J Dairy Sci*, **1998**, 81, 2103-2110.
[11] L S Chen, Y Ma, L J Chen, Zhao, J L Maubois, T M Jiang, H M Li, S H He, *Int J food Sci Tec*, **2010**, 45, 555–561.
[12] S Jain, H Yadav, P R Sinha, *J Dairy Res*, **2009**, 76, 385–391.
[13] M Y Lin, F J Chang, *Dig Dis Sci*, **2000**, 45(8), 1617-1622.
[14] L K Hoolihan, *J Am Diet Assoc*, **2001**, 101(2), 229-238.