Partial characterization of a new acidic heteropolysaccharide produced by a native isolate of *Lactobacillus* sp. CFR-2180.

R. Mohan and S.V.N. Vijayendra*

Department of Food Microbiology,

Central Food Technological Research Institute,

Mysore - 570 020, India

Running head: New heteropolysaccharide of *Lactobacillus*

*** Corresponding author:**

Tel: +91-821-2517539; Fax: +91-821-2517233

***E-mail address*: svnvijayendra@yahoo.com (S.V.N.Vijayendra)**

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ABSTRACT

Polysaccharides are being used in food and allied industries as texture improving agents, stabilizers, for the preparation of the edible films etc. Plant and seaweed derived polysaccharides suffer lack of assured supply and variations in quality. Microbial exopolysaccharides (EPS) provide a valid alternative. An EPS producing lactic acid bacterium was isolated from cabbage and identified as *Lactobacillus* sp. CFR-2180. Production of 22 g/L of EPS in 24 h at 30°C was noticed. The EPS had 65% total carbohydrates, 0.7% protein, 10% uronic acid and 2.0% moisture. Analysis by gas chromatography revealed that the EPS is a heteropolysaccharide with the presence of mannose, galactose and glucose in a ratio of 1:7:5, respectively. Gel permeation chromatography and HPLC analysis of the EPS indicated presence of multiple peaks with molecular weight ranging from 1.8×10^4 to 2.5×10^6 Da., confirming the heterogeneity of the EPS. The results of the preliminary characterization of the EPS indicated that it is a new EPS that has not been reported earlier.

***Key words*:** Exopolysaccharide, fermentation, characterization, identification, heteropolysaccharides, uronic acid

INTRODUCTION

Microbial exopolysaccharides (EPS) are the biopolymers that are secreted into the environment in the form of slime. Lactic acid bacteria (LAB) are food-grade organisms and are having GRAS (Generally Recognized As Safe) status. Some of these are known to produce EPS. LAB are being used in food industry since long, because of their ability to preserve the foods for longer time, besides imparting aroma, flavour and texture to the fermented foods. In addition to these features, the safety of the fermented products is also enhanced by way of the production of lactic acid, hydrogen peroxide and bacteriocins by several LAB strains. Hence, the EPS of LAB can replace the stabilizers and thickeners currently produced by non food grade bacteria. The EPS of LAB are produced *in situ* and therefore are being considered as natural biothickeners. EPS of β-D-glucan type have been found to possess antithrombotic, antitumoral or immunomadulatory activities (Sutherland, 1998). Therefore, EPS from LAB have potential for development and exploitation as food additives or functional food ingredients with both health and economic benefits (De Vuyst et al., 2001). As a result, novel products which are safe, natural and healthy besides enhanced texture and improved stability can be developed.

Heteropolysaccharides (HePS) are composed of a backbone of repeated subunits that are branched or unbranched and consist of more than one type of monosaccharides. HePS producing *Lactobacillus* strains play an important role in the dairy industry, because of their contribution to the consistency and rheology of fermented milk products, such as yoghurt. EPS of lactobacilli have most valuable applications in the improvement of the rheology, texture and mouth- feel of the fermented milk products. Synthesis of HePS by several lactobacilli is currently being studied intensively (Lin and Chang Chein, 2007;

Vijayendra et al., 2009; Welman et al., 2003). EPS produced by different strains vary in sugar composition, chain length, degree of branching or sugar linkages. Many reports and reviews are available on the production and characterization of HePS of lactobacilli that differ considerably in their physico-chemical properties, composition and structure (Degeest et al., 2001; Lin and Chang Chein, 2007; Ruas-madiedo and de los Reyes-Gavilan, 2005; Torino et al., 2005, Vijayendra et al., 2009). However, reports on the production of polyanionic HePS, containing uronic acid or glucuronic acid residues, by lactobacilli are very limited (Cerning, 1990; Robjin et al., 1996). The properties of EPS determine its application. Hence, the knowledge on the structure-function relationship of these biopolymers is crucial in order to choose or design polymers for a specific technological application (De Vuyst et al., 2001). The composition of the EPS varies with the sugars present in the substrate (Grobben et al., 1995). Differences, not only in the molecular weight but also in the composition and viscosity of the EPS produced by the same *Lactobacillus* strain are reported (Grobben et al., 1997). LAB strains that are able to produce large quantities of the EPS can act as an alternate source for biopolymer production for food applications. However, many of the *Lactobacillus* species produced very low quantity (<2 g/L) of HePS (Dueñas et al., 2003; Lin and Chang Chein, 2007; Torino et al., 2005). At the same time, the yields of other polymers traditionally used such as xanthan or gellan, which are produced by non-food grade microorganisms are very high (10-25 g/L) (De Vuyst and Degeest, 1999). Although the amount of HePS being produced by LAB is enough for *in situ* applications, many more folds increase in the HePS production by the lactobacilli may be required for its use as a food additive. Therefore, the present investigation is aimed at the isolation of a native *Lactobacillus*

culture producing large quantity of the HePS and partial characterization of the new acidic HePS produced by the isolate was also carried out.

MATERIALS AND METHODS

Isolation and identification of the EPS producing LAB strain

Curd samples collected from different hotels and roadside fast food centers of Mysore and Coimbatore, India, along with vegetables such as cucumber, cabbage leaves, capsicum and bitter guard collected from local market were processed to isolate EPS producing lactic cultures. These samples were subjected to serial decimal dilutions in 0.85% (w/v) saline and suitable dilutions were spread plated on to deMan Rogosa Sharpe (MRS) agar added with 3% (w/v) sucrose. The plates were incubated at 37°C for 24-48 h and observed for the presence of mucoid colonies. The microscopic observation of these colonies was performed using phase-contrast microscope (Leitz, Switzerland) after subjecting the air-dried smears to Gram staining method. Various biochemical tests, as listed in results and discussion (section 3.1) were performed to identify the EPS producing LAB strain up to genus level (Sharpe, 1979).

Production of the EPS

Fermentation was carried out in a 2.2 l fermentor (Scigenics (India) Pvt. Ltd., India) with a working volume of 1.0 L of EPS production medium (Vijayendra et al., 2008) at 30°C. The pH was maintained at 6.5, using 10% concentrated sulphuric acid and 10% sodium hydroxide. The agitator speed was maintained at 300 rev/min. The culture medium was inoculated with 5% (v/v) of actively growing culture (12-15 h) and fermentation was allowed to proceed for 36 h. Samples were aseptically drawn after 12, 24 and 36 h and analyzed for dry biomass and EPS content.

Analysis of the fermented broth

Samples of fermented medium were centrifuged at 8000 g for 20 min (Remi Instruments, India) and cells were separated. The cell pellet was washed once with sterile saline and collected in pre-weighed aluminum foil cups and dried in a hot air oven at 90 ± 2 °C, till constant weight was obtained. Two volumes of ice-cold isopropyl alcohol (IPA) was added to one volume of the cell free supernatant of the fermented broth under continuous stirring and kept for precipitation in the refrigerator for overnight. Later the IPA was decanted and the mass was washed with acetone. The biomass and EPS were kept for drying at 90 ± 2°C, till constant weight was obtained and the dry cell weight and EPS content were expressed as g/L.

Characterization of the EPS

The EPS produced by the isolate in EPS Medium was dialyzed at 4°C using 10,000 cut off dialysis membrane (Sigma, USA) in distilled water for 24 h by changing the water thrice. The purified EPS was freeze-dried (Edwards, England) and used for characterization. The total carbohydrate and protein contents were estimated using phenol-sulphuric acid method (Mc Kelvy and Lee, 1969) and Bradford method (Bradford, 1976) using D-glucose and BSA, as standards, respectively. The amount of uronic acid was determined by carbazole method (Knutson and Jeans, 1968) with D-galacturonic acid as a standard. The moisture content was determined by drying the EPS to a constant weight at 90°C (Shivkumar and Vijayendra, 2006) and the result was expressed as g%.

To determine the monosaccharide composition, the purified EPS was hydrolyzed with sulphuric acid (Selvendran and O'Neil, 1988). The monosaccharides formed were derivatized into their alditol acetates (Sawardekar et al., 1965) and detected by gas chromatograph (Packard Model 437) equipped with flame ionization detector. The column (1/8"x 8 ft) used was OV-225, 3% on Chromosorb W (100-120 mesh) with an isothermal temperature of 190°C. Nitrogen was used as carrier gas at a flow rate of 20 mL/min. The temperature of injector and detector port was 220 and 230°C, respectively. Inositol hexaacetate (0.5 mg/mL) was used as an internal standard.

To know the homogeneity of the EPS, it was elucidated by gel permeation chromatography (94 x 2 cm column) (Sepharose CL-2B; bed volume-150 mL) using distilled water with a flow rate of 1 mL/min. All the fractions (3 mL) were analyzed for the presence of carbohydrate by Phenol-sulphuric acid method as described earlier. Calibration curve was prepared using (V_e, elution volume) standard dextrans of different molecular weights (T series dextrans, Sigma, USA) dissolved in triple distilled water. The void volume (V_o) was determined by using a pre-dialyzed blue dextran solution (10 mg/mL). From the plot of log Mw Vs V_e/V_o, the approximate molecular weight (Mw) of the fraction was computed. The aqueous solution of the dialyzed EPS was subjected to high-pressure liquid chromatography (LC-10A HPLC, Shimadzu, Japan) analysis using E-linear and E-1000 columns connected in series with RI detector. Degassed Milli-Q water was used as a mobile phase at a flow rate of 1 mL/min.

The EPS was analyzed by Fourier Transform Infra Red (FTIR) spectroscopy (NICOLET 5700 FTIR spectrometer) under dry air at room temperature, using a thin film

prepared with 4 mg of the EPS and 200 mg of KBr powder. Reproducibility of the spectra was verified by repeating the analysis thrice.

RESULTS AND DISCUSSION

Isolation and identification of EPS producing LAB strain

A total of three mucoid colonies, indicating the production of EPS, on MRS agar were picked-up and purified by streaking onto MRS agar plates. Based on the preliminary study carried out on the production of EPS among the three isolates, the culture isolated from cabbage waste, which was producing highest EPS content after 72 h of shake flask fermentation (data not shown) was selected for further studies. The cell morphology of the isolate was found to be slender, straight and short rods with average cell dimensions of 2.2 μ x 0.5μ. The isolate showed negative reaction to Voges-Proskauer, indole, methyl red, citrate utilization, nitrate reduction, gelatin hydrolysis, oxidase, catalase and arginine tests. With triple sugar iron agar, the isolate produced alkaline slant and butt with no gas and H_2S. It could breakdown glucose both in aerobic and anaerobic conditions (Sharpe, 1979). It produced only lactic acid indicating the homofermentative nature of the isolate. Based on the morphology and various biochemical tests results the isolate was identified as *Lactobacillus* sp. and designated as *Lactobacillus* sp. CFR-2180**.**

Production of EPS by Lactobacillus *sp. CFR-2180*

As shown in the Fig. 1, the amount of the EPS produced by *Lactobacillus* sp. CFR 2180 under controlled fermentation conditions increased from 12 to 24 h, reaching a maximum of 22 g/L in 24 h of fermentation. After which the EPS content decreased gradually, which might be due to the reduction in dissolved oxygen (DO) level, as the DO level could not be maintained constant (at 1 vvm) during fermentation. Reduction in

EPS yield with prolonged fermentation was recorded in earlier studies, which might be due to the production of hydrolyzing enzymes, such as glycohydrolase (Pham et al., 2000) and depends on the strain (Gancel and Novel, 1994) or culture conditions used, such as pH (Mozzi et al., 1996), temperature (Mozzi et al., 1995) and fermentation time (Lin and Chang Chien, 2007). The amount of EPS produced by our isolate in MRS broth (with 3% added sucrose) was only 12.7 g/L, after 72 h of shake flask fermentation at 30°C (data not shown), indicating that the composition of the medium and fermentation conditions such as pH, temperature and fermentation time play a major role in the production of EPS (Looijesteijn and Hugenholtz, 1999; Torino et al., 2005). However, this amount is far better than the yields reported for HePS production by any *Lactobacillus* species (Dueñas et al., 2003; Lin and Chang Chen, 2007; Torino et al., 2005). The highest EPS yield of only 0.73 g/L from *Lact. helveticus* BCRC14030 after prolonged fermentation (60 h) was reported (Lin and Chang Chien, 2007). Similarly, *Lact. helveticus* ATCC 15807 produced only 208 mg/L of exopolysaccharide (Torino et al., 2005). Thus the results obtained in the present study are encouraging to exploit this HePS as an alternate to plant based, seaweed based biopolymers or EPS of non food grade microorganisms for various food applications.

Characterization of EPS produced by Lactobacillus *sp. CFR-2180*

The proximate composition of the dialyzed EPS of *Lactobacillus* sp. CFR-2180 was determined. It had a moisture content of 2% with negligible amount of protein (0.7%), as an impurity, which was also confirmed with the presence of a peak at 289 nm in UV spectrum (data not shown). The total carbohydrate and uronic acid contents of the sample were found to be 65 and 10%, respectively and the remaining might be the minerals or

other impurities. Similarly, presence of glucuronic acid in the EPS produced b by *Lact. acidophilus* LMG 9433 was reported (Robjin et al., 1996). The sugar composition of the dialyzed EPS was found to be unique with the presence of mannose, galactose and glucose in a ratio of 1:7:5, respectively. However, the EPS produced by *Lact. delbrueckii* NCFB 2772 had glucose, galactose and rhamnose in a ratio of 1: 6.8: 0.7 (Grobben et al., 1995) and the EPS produced by *Lact. rhamnosus* strains RW-9595M and R consisted of galactose, glucose and rhamnose in 1:1:4 ratio (van Calsteren et al., 2002). In addition to these differences, variations in the ratio of sugars were also noticed even among different strains of the same species of *Lactobacillus* (van Geel-Schutten et al., 1998) and in different fractions of the same polysaccharide (Grobben et al., 1997). The proportion of the monosaccharides varies as a function of the carbohydrate source. Difference in the sugar composition of galactose, glucose and rhamnose with different carbon sources was noticed and it was in the molar ratio of 5:1:1 with glucose and 11:1:0.4 with fructose (Grobben et al., 1997). It was noticed that the EPS produced by *Lact. delbrueckii* ssp. *bulgaricus* NCFB2074 had a heptasaccharide repeating unit with a 4:3 molar ratio of galactose to glucose (Harding et al., 2005). Although the yield of HePS was very low (10-166 mg/L), a large diversity in structure and molecular mass of the HePS ranging from 8 to >5000 kDa was noticed among several polymer producing LAB strains belonging to different genera and species (Mozzi et al., 2006). As referred above, the monomer composition of the HePS was found to vary from one culture to other with the presence of different sugar moieties such as galactose, glucose, galactosamine, glucosamine, rhamnose etc., in different ratios. This clearly indicated that there was a wide variation in the composition of EPS produced by different species of lactobacilli.

GPC profile of the HePS of *Lactobacillus* sp. CFR2180 had multiple peaks between fractions 35 and 60 (Fig. 2), indicating the heterogeneity of the compound, which was also confirmed by HPLC analysis, with peaks at 10.058, 11.950, 12.633 and 13.683 min (chromatogram not shown). The molecular weight of these fractions varied from 1.8 x 10^4 to 2.5 x 10^6 Da. Similarly, presence of several fractions differing in molecular weight was observed in the EPS produced by four different strains of *Lact. delbrueckii* ssp. *bulgaricus* (Petry et al., 2003). Substantial evidence for the synchronous production of EPSs having the same structure but different molecular masses was also available. The study conducted by Degeest & de Vuyst (1999) indicated the production of a high molecular mass (1.8 x 10^6 Da) and low molecular mass (4.1 x 10^5 Da) EPS by *Strep. thermophilus* LY 03 and the production of two different polysaccharides by *Lact. rhamnosus* has been reported (Pham et al., 2000). Low molecular mass material might have been generated by the glycosylhydrolase catalyzed hydrolysis of high molecular mass products (Pham et al., 2000).

In the FTIR spectrum of the sample (Fig. 3), the weak band around 2930 cm^{-1}, due to the -CH vibration, represented a good internal reference for comparison with other band absorbance. The adsorption peak of CH at 926 cm^{-1} is due to the presence of sugar derivatives suggesting that the polymer is a polysaccharide**.** Appearance of a strong and broad stretching band around 3390 cm^{-1} was indicative of the -OH and amine groups in abundance. A peak at 1630 cm^{-1} indicated the stretching of carboxyl groups. Absorbance at 1440 cm^{-1} was attributed to symmetric bending of CH_3 groups. A peak near 1350 cm^{-1} was indicative of the presence of carbonyl groups. This FTIR spectrum was not comparable to that of dextran nor to the spectrum of the HePS produced by *Leuconostoc*

sp. (Vijayendra et al., 2008) or *Lactobacillus* sp. CFR 2182 (Vijayendra et al., 2009). However, it has some similarities with that of sodium alginate, with peaks at 3391, 2933, 1628, 1417 and 1127 cm^{-1} (Yang et al., 2007). Presence of the peaks at 1120 and 1060 cm^{-1} ascertain presence of guluronic acid and mannuronic acid, which are the building blocks of alginic acid as confirmed by Coimbra and co workers (Coimbra et al., 1998).

CONCLUSION

In summary, *Lactobacillus* sp. CFR-2180 produced large quantity (22 g/L) of a new acidic HePS, which is being reported for the first time, which had mannose, galactose and glucose in 1:7:5 ratios. Optimization of the HePS production under defined fermentation conditions, using cheap carbon sources (byproducts of food industry) can further reduce its production cost. Upon the characterization of the functional properties of the HePS, its application in food and allied industries can be exploited.

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REFERENCES

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248-254.

Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria.

FEMS Microbiology Reviews, *87*, 113-130.

Coimbra**,** M.A., Barros, A., Barros, M., Rutledge, D.N. & Delgadillo, I. (1998) Multivariate analysis of uronic acid and neutral sugars in whole pectic samples by FT-IR spectroscopy. *Carbohydrate Polymers*, *37*, 241-248.

Degeest, B. & De Vuyst, L. (1999). Indication that the nitrogen source influences both amount and size of the exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *Journal of Applied Microbiology*, *84*, 1059-1068.

Degeest, B., Vaningelgem, F. & De Vuyst, L. (2001). Microbial physiology, fermentation kinetics and process engineering of heteropolysaccharide production by lactic acid bacteria. *International Dairy Journal*, *11*, 727-757.

De Vuyst L. & Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews*, *23*, 153-177.

De Vuyst, L, De Vin, F., Vaningelgem, F. & Degeest, B. (2001). Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *International Dairy Journal*, *11*, 687-708.

Dueñas, M., Munduate, A., Perea, A. & Irastorza, A. (2003). Exopolysaccharide production by *Pediococcus damnosus* 2.6 in a semi defined medium under different growth conditions. *International Journal of Food Microbiology, 87*, 113-120.

Gancel, F. & Novel, G. (1994). Exopolysaccharide production by *Streptococcus salivarius* ssp. *thermophilus* cultures. 2. Distinct modes of polymer production and degradation among clonal variants. *Journal of Dairy Science, 77*, 85-88.

Grobben, G.J., Sikkema, J., Smith, M.R. & de Bont, J.A.M. (1995). Production of extracellular polysaccharide by *Lactobacillus delbrueckii* ssp. *bulgaricus* NCFB 2772 grown in a chemically defined medium. *Journal of Applied Bacteriology*, *79*, 103-107.

Grobben, G.J., van Casteren, W.H.M., Schols, H.A., Oosterveld, A., Sala, G., Smith, M.R., Sikkema, J. & De Bont, J.A.M. (1997). Analysis of the exopolysaccharides produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in continuous culture on glucose and fructose. *Applied Microbiology and Biotechnology*, *48*, 516-521.

Harding**,** L. P., Marshall, V. M., Hernandez, Y., Yucheng-Gu., Maqsood, M., McLay N. & Laws, A. P. (2005). Structural characterization of a highly branched exopolysaccharide produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB2074. *Carbohydrate Research*, *340*, 1107-1111.

Knutson, C.A. & Jeanes, A. (1968). A new modification of the carbazole analysis: application to heteropolysaccharides. *Analytical Biochemistry*, *24*, 470-481.

Lin, T. Y. & Chang Chien, M. F. (2007). Exopolysaccharides production as affected by lactic acid bacteria and fermentation time. *Food Chemistry*, *100*, 1419-1423.

Looijesteijn, P.J. & Hugenholtz, J. (1999). Uncoupling of growth and exopolysaccharide production by *Lactobacillus lactis* subsp. *cremoris* NIZO B40 and optimization of its synthesis. *Journal of Bioscience and Bioengineering*, *88*, 178-182.

Mc Kelvy, J.F. & Lee Y.C. (1969). Microheterogeniety of the carbohydrate group of *Aspergillus oryzae* α amylase. *Archives of Biochemistry and Biophysics*, *132*, 99-110.

Mozzi, F., Oliver, G., Savoy de Giori, G. & Font de Valdez G.F. (1995). Influence of temperature on the production of exopolysaccharides by thermophilic lactic acid bacteria. *Milchwissensaft, 50,* 80-82.

Mozzi, F., Savoy de Giori, G., Oliver, G. & Font de Valdez, G. (1996). Exopolysaccharide production by *Lactobacillus casei* under different growth conditions. *Milchwissensaft, 51,* 670-673.

Mozzi, F., Vaningelgem, F., Hébert, E.M., Van der Meulen, R., Moreno, M.R.F., Font de valdez, G. & De Vuyst, L. (2006). Diversity of heteropolysaccharide producing lactic acid bacterium strains and their biopolymers. *Applied and Environmental Microbiology, 72,* 4431-4435.

Petry, S., Furlan, S., Waghorne, E., Saulnier, L., Cerning, J. & Maguin, E. (2003). Comparison of the thickening properties of four *Lactobacillus delbrueckii* subsp. *bulgaricus* strains and physicochemical characterization of their exopolysaccharides. *FEMS Microbiology Letters*, *221,* 285-291.

Pham P.L., Dupont, I., Roy, D., Lapointe, G. & Cerning, J. (2000). Production of exopolysaccharide by *Lactobacillus rhamnosus* R and analysis of its enzymatic degradation during prolonged fermentation. *Applied and Environmental Microbiology, 66,* 2303-2310.

Robijn, G. W., Gallego, R. G., van den Berg, D. J. C., Haas, H., Kamerling, J. P. & Vliegenthart, J. F.G. (1996). Structural characterization of the exopolysaccharides produced by *Lactobacillus acidophilus* LMG 9433. *Carbohydrate Research, 288,* 203–218.

Ruas-Madiedo, P. & de los Reyes-Gavilán, C.G. (2005). Methods for the screening,

isolation and characterization of exopolysaccharides produced by lactic acid bacteria. *Journal of Dairy Science, 88,* 843-856.

Sawardekar**,** J.S., Slonekar, J.M. & Jeanes, A. (1965). Quantitative determination of monosaccharides as their alditol acetates by gas liquid chromatography. *Analytical Chemistry*, *37*, 1602-1604.

Schiraldi, C., Valli, V., Molinaro, A., Carteni, M. & de Rosa, M. (2006) Exopolysaccharides production in *Lactobacillus bulgaricus* and *Lactobacillus casei* exploiting microfiltration. *Journal of Industrial Microbiology and Biotechnology*, *33*, 384-390.

Selvendran, R.R. & O'Neil, M.A. (1988). Isolation and analysis of cell walls from plant material. *Methods in Biochemical Analysis*, *32*, 25-53.

Sharpe, M. E. (1979). Identification of the lactic acid bacteria. In: Skinner, F.A. & Lovelock, D.W., (Eds.), *Identification methods for microbiologists* (2nd Ed., pp 233-259). London, Academic Press.

Shivakumar, S. & Vijayendra, S.V.N. (2006). Production of exopolysaccharides by *Agrobacterium* sp. CFR- 24 using coconut water- a byproduct of food industry. *Letters in Applied Microbiology*, *42*, 477- 482.

Sutherland, I.W. (1998) Novel and established applications of microbial polysaccharides. *Trends in Biotechnology*, *16*, 41-46.

Torino, M.I., Mozzi, F. & Font de Valdez, G. (2005). Exopolysaccharide biosynthesis by *Lactobacillus helveticus* ATCC 15807. *Applied Microbiology and Biotechnology*, *68*, 259-265.

van Calsteren, M.R., Pau-Roblot, C., Begin, A. & Roy, D. (2002). Structure

determination of the exopolysaccharide produced by *Lactobacillus rhamnosus* strains RW-9595M and R. *Biochemical Journal*, *363*, 7-17.

van Geel-Schutten, G.H., Flesch, F., ten Brink, B., Smith, M.R. & Dijkhuizen, L. (1998). Screening and characterization of *Lactobacillus* strains producing large amounts of exopolysaccharides. *Applied Microbiology and Biotechnology*, *50*, 697-703.

Vijayendra, S.V.N., Palanivel, G., Mahadevamma, S. & Tharanathan, R.N. (2008). Physico-chemical characterization of an exopolysaccharide produced by a non-ropy strain of *Leuconostoc* sp. CFR 2181 isolated from *dahi*, an Indian traditional fermented milk product**.** *Carbohydrate Polymers*, *72*, 300-307.

Vijayendra, S.V.N., Palanivel, G., Mahadevamma, S. & Tharanathan, R.N. (2009). Physico-chemical characterization of a new heteropolysaccharide produced by a native isolate of heterofermentative *Lactobacillus* sp. CFR-2182. *Archives of Microbiology*, DOI 10.1007/s00203-008-0453-8.

Welman, A.D. & Maddox, I.S. (2003). Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends in Biotechnology*, *21*,269-274.

Yang, L., Zhang, B., Wen, L., Liang, Q. & Zhang, L.M. (2007). Amphiphilic cholesteryl grafted sodium alginate derivative: Synthesis and self-assembly in aqueous solution. *Carbohydrate Polymers*, *68*, 218-225.

Figure captions

Fig. 1 Production of exopolysaccharide by *Lactobacillus* sp. CFR 2180. Fermentation was carried out at a constant pH (6.5) and temperature (30 °C). Aeration: 1 vvm; (■) Biomass, (♦) EPS.

Fig. 2 Elution profile of the exopolysaccharide produced by *Lactobacillus* sp. CFR-2180 on Sepharose CL-2B column.

Fig. 3 FTIR spectrum of the exopolysaccharide produced by *Lactobacillus* sp. CFR-2180.

Fig.1.

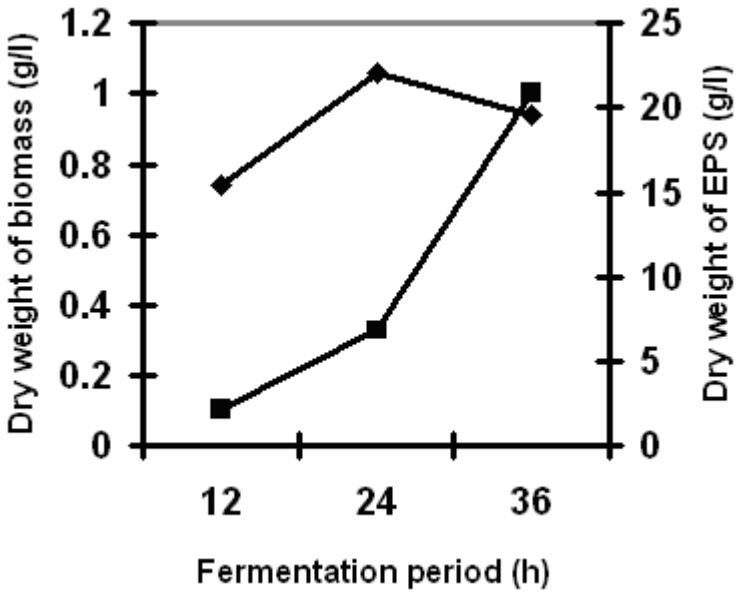
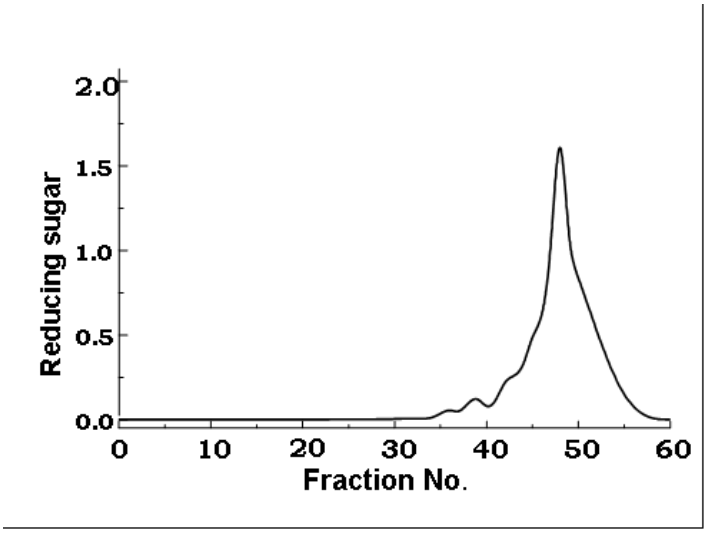
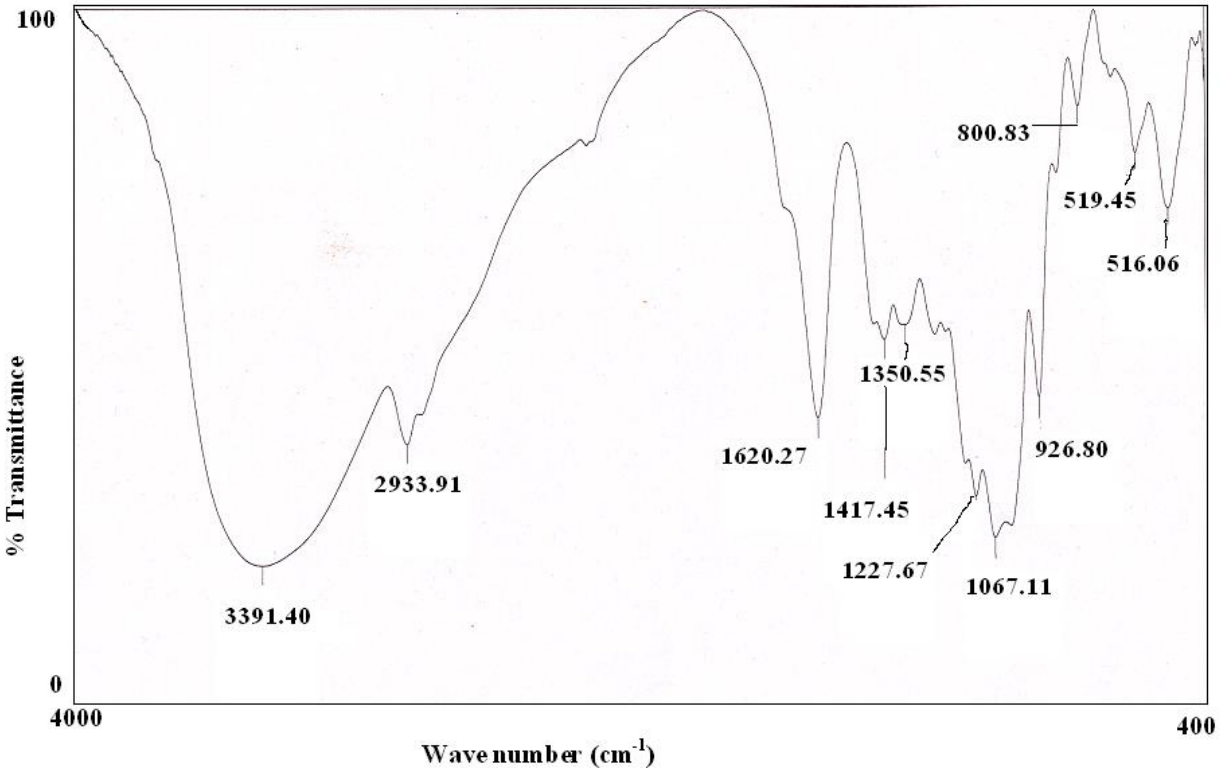

Dry weight of biomass (g/l)
Dry weight of EPS (g/l)
1.2
1
0.8
0.6
0.4
0.2
0
25
20
15
10
5
0
12
24
36
Fermentation period (h)

Fig.2


Reducing sugar
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
Fraction No.

1 Fig.3.


100
0
% Transmittance
4000
400
Wave number (cm-1)
3391.40
2933.91
1620.27
1417.45
1350.55
1227.67
1067.11
926.80
800.83
519.45
516.06

2