JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2008. Vol. **2**(1), p. 139-146

Antibiotic Sensitivity, Carbohydrate Fermentation Characteristics and Plasmid Profiles of Glucansucrase Producing four *Leuconostoc* Strains

Ravi Kiran Purama, Mayur Agrawal, Avishek Majumder, Shadab Ahmed and Arun Goyal*

Department of Biotechnology, Indian Institute of Technology, Guwahati - 781 039, India.

(Received: 18 February 2008; accepted: 25 March 2008)

Four glucansucrase producing *Leuconostoc* species *Leuconostoc mesenteroides* B-512F and B-640, *Leuconostoc citreum* B-742 and *Leuconostoc dextranicum* B-1146 were characterized for their antibiotic resistance, carbon source utilization, plasmid profiles and glucan synthesizing activities. All the four species were resistant to the antibiotics cotrimazine, norfloxacin and vancomycin. All four species were sensitive to amoxycillin, bacitracin, carbenicillin, cephalothin, cephatoxamine, chloramphenicol, clindamycin, linomycin, oxytetracyclin and tetracyclin. All the four species utilized glucose, fructose, mannose, melibiose, sucrose and trehalose and did not show any activity towards arabinose, lactose and mannitol. The *Leuconostoc* strain B-640 was unique in fermenting rhamnose. The *Leuconostoc* species B-640 and B-1146 possessed plasmids and both showed sucrose hydrolyzing activity by displaying the polysaccharide formation patterns in native polyacrylamide gels. These data will enable in finding similar glucansucrase producing *Leuconostoc* species which can prove better species used for industrial applications.

Key words: Glucansucrase, glucan, antibiotic, plasmid, *Leuconostoc*, carbohydrate.

Homo and Hetero fermentative lactic acid bacteria are used in various food fermentations. Growth associated decrease in pH of fermented products is due to the organic acid production is a characteristic feature of lactic acid bacteria. The important applications of lactic acid bacteria in the food industry are production of bacteriocins, carbohydrate fermentation, improved mineral availability by enzyme production and synthesis of polysaccharides and oligosaccharides as prebiotics[1-4]. Bioactive polysaccharide and oligosaccharides production by glucansucrases from lactic acid bacteria are recent interests in the last decade. Four different genera of lactic acid bacteria: *Streptococcus*, *Leuconostoc*, *Weissella* and *Lactobacillus* are major producers of glucansucrases[5-7]. Glucansucrases from *Leuconostoc* genus are inducible enzymes, which are induced in the presence of sucrose[8]. The fermentation temperature ranging from 20 to 30°C have been used for glucansucrase production from *Leuconostoc*[9]. Organisms of the *Leuconostoc* genus are considered micro-aerophilic. The extracellular glucansucrase is used

* To whom all correspondence should be addressed.
Tel.: 91-361-258 2208, Fax.: 91-361-258 2249
E-mail: arungoyl@iitg.ernet.in

for synthesis of dextran which has numerous applications in pharmaceutical, food and fine chemical industries[3,4,9,10]. Despite their commercial significance relatively little is known about their antibiotic resistance, physiology and genetics of these glucansucrase producing bacteria. Limited information is available on antibiotic susceptibilities and carbohydrate fermentation behavior of glucansucrase producing *Leuconostoc* species[11,12]. It was found that several dairy and wine *Leuconostoc* species were resistant to vancomycin[12-14]. The type of glucansucrase elaborated by a strain can be identified by the type of glucan produced depending upon the type of substrate utilized. The sucrose hydrolyzing activity of *Leuconostoc* species was analyzed by the synthesis of polysaccharide which was detected as activity bands within a polyacrylamide gel using a Periodic acid Schiff staining protocol[15]. There are few reports on the presence of plasmid DNA from *Leuconostoc* species[13,16]. The plasmids possessing genes for lactose utilization[17], citrate utilization[18] and for bacteriocin[19] have been identified. There are no reports available on the antibiotic resistance, carbohydrate fermentation behavior, plasmid content and polysaccharide synthesis activity of *Leuconostoc* species B-640 and B-1146. In the present study, four glucansucrase producing *Leuconostoc* species *viz. Leuconostoc mesenteroides* B-512F and B-640, *Leuconostoc citreum* B-742 and *Leuconostoc dextranicum* B-1146 were characterized for their antibiotic sensitivities, carbohydrate fermentation profiles, plasmid profiles and glucan synthesizing activities.

MATERIAL AND METHODS

Bacterial strains and culture conditions

The four *Leuconostoc* species, *Leuconostoc mesenteroides* B-512F and B-640, *Leuconostoc citreum* B-742 and *Leuconostoc dextranicum* B-1146, were obtained from the Agriculture Research Service culture collection in Peoria, USA. The stock cultures of *Leuconostoc* species were maintained as MRS-agar stab[20] cultures at 4°C. All species were grown at 28°C for all experiments without shaking.

Antibiotic sensitivity

Leuconostoc species were tested for susceptibility to thirty antibiotics using agar disc diffusion test[21]. The antibiotic tests were performed using commercially available antibiotic octodiscs containing Amoxyclav (Ac), Cephalexin (Cp), Ciproflaxacin, (Cf), Clindamycin(Cd), Claxacillin (Cx), Erythromycin (E), Tetracyclin (T), Ampicillin (A), Carbenicilllin (Cb), Cephatoxamine (Ce), Chloramphenicol (C), Co-Trimazine (Cm), Gentamicin (G), Norflaxacin (Nx), Oxacillin (Ox), Amikacin (Ak), Amoxycillin (Am), Bacitracin (B), Cephalothin (Ch), Novobiocin (Nv), Oxytetracyclin (O), Vancomycin (V), Penicillin-G (P), Tobramycin (Tb), Cephaloridine (Cr), Kanamycin (K), Linomycin (L), Methicillin (M), Norfloxacin (Nf), Oleandomycin (Ol) from Hi-media Pvt. Ltd. India. MRS medium containing 2% glucose as carbohydrate source with 1.8 %, (w/v) agar and 0.8%, (w/v) agar were used. The petri-plates were first prepared with MRS medium containing 1.8%, (w/v) agar. The test strain was seeded in MRS-soft agar (0.8%, (w/v) agar) and overlaid in the Petri-plate having a bottom layer of above MRS agar (1.8%, w/v). The culture plate was allowed to dry for about 2 min. The octodiscs were gently placed over the surface of the seeded plate. The Petri plates were incubated in inverted position overnight in an incubator at 28°C and were observed next day for zone of inhibition around the discs.

Carbohydrate fermentation

The *Leuconostoc* species were tested for their ability to ferment various carbohydrates using the method of Kandler and Weiss (1986)[22]. From the overnight grown MRS broth culture containing 2% glucose as carbohydrate source, 50 ml was inoculated in 5.0 ml liquid MRS medium lacking glucose but containing Phenol red and other test carbohydrates to give a final inoculum to medium ratio of 1% (v/v). The test media were incubated for 2 days at 28°C with out shaking. The acid production was recorded between 24-48 h. The acid production was indicated by a change in colour of the phenol red indicator dye from red to yellow.

Plasmid isolation

The *Leuconostoc* species were grown in 5 ml liquid MRS medium for 24 h. The species were screened for the presence of plasmid DNA

using an alkaline lysis protocol. The cells were collected by centrifugation from 5 ml cultures and resuspended in 100 ml TGE buffer (25 mM Tris HCl, pH 8.0, 50 mM glucose and 10 mM EDTA). A lysozyme solution was added (40 ml of stock solution consisting of 50 mg/ml), and the mixture was incubated at 25°C for 60 min. Following incubation, 200 ml lysis solution (1%, w/v, sodium dodecyl sulphate in 0.2N NaOH) was added, mixed three times by inversion, and incubated on ice for 5 min. 150 ml of ice-cold 5M potassium acetate buffer pH 4.8 to the lysis mixture, mixed by vortex action for 10 s and incubated on ice for 5 min. The mixture was centrifuged at 16000*g* for 10 min in a microcentrifuge and the supernatant was transferred to a fresh tube. Supernatant was extracted with an equal volume of phenol/chloroform (50:50, v/v) and then with an equal volume of chloroform/isoamyl alcohol (24:1, v/v). The plasmid DNA was precipitated from the aqueous supernatant with two volumes of cold 100% ethanol, and the DNA pellet was collected by centrifugation at 16000*g* for 5 min a microcentrifuge. The pellet was washed twice with 500 ml 70% (v/v) ethanol and resuspended in 35 ml of 20 mM Tris-EDTA buffer (pH 8.0). The DNA samples were examined by using agarose gel (0.8%, w/v) electrophoresis and 1xTAE (Tris base, Acetic acid, EDTA; pH 8.0) buffer for preparing gel as well as for running buffer.

Detection of sucrose hydrolyzing activity

In-situ activity of glucansucrase was detected on a 7.5% acrylamide gels run under SDS-non-denaturing conditions using the protocol described by Holt *et al.* (2001)[12] with modification. The crude cell free extract samples from all four *Leuconostoc* species were loaded on duplicate 7.5% acrylamide gels under SDS-non-denaturing condition. After the run, SDS was removed by incubating the gel in sodium acetate buffer (20 mM sodium acetate, pH 5·4, 0·3 mM $CaCl_2$ and 0·1%, v/v Tween 80) at 4°C 30 min. Then the gel was incubated in sodium acetate buffer (20 mM sodium acetate, pH 5.4, 0.3 mM $CaCl_2$) supplemented with 5% sucrose for 48 h at 30°C. Following incubation, the gel was washed once with a solution of methanol:acetic acid (50:10, v/v) in water for 30 min, then with water for 30 min, and incubated in a periodic acid solution (1% w/v periodic acid and 3% v/v acetic acid) for 45 min at room temperature. After the periodic acid treatment, the gel was washed with water for 2 h with several changes. The gel was then stained with 15 ml Schiff reagent (0.5% w/v Fuchsin basic, 1% sodium bisulphite and 0·1 N HCl) until the discrete magenta bands within the gel matrix appeared, which confirmed glucansucrase activity. The other gel was stained with Coomassie Brilliant Blue for location of activity bands. Molecular mass marker proteins (Myosin from Rabbit Muscle, 205000; Phosphorylase b 97400; Bovine serum albumin, 66000; Ovalbumin, 43000; Carbonic anhydrase, 29000 Da) purchased from Genei, India, were used as standard for SDS-PAGE.

RESULTS

Antibiotic susceptibility of *Leuconostoc* strains

A standardized filter-paper disc-agar diffusion assay was used to determine the drug susceptibility of microorganisms. This method allows rapid determination of the efficacy of the drug by measuring the diameter of the zone of inhibition which results from diffusion of the agent into the medium surrounding the disc. In this procedure, the filter-paper discs of uniform size are impregnated with specified concentrations of different antibiotics and then placed on the surface of an agar plate that has been seeded with the organism to be tested. Following incubation, the plates are examined for the presence of growth inhibition, which is indicated by a clear zone surrounding each disc. The susceptibility of an organism to a drug is determined by the size of this zone, which itself is dependent on variables such as: the ability and rate of diffusion of the antibiotic into the medium and its interaction with the test organism; the growth rate of the organism; the degree of susceptibility of the organism to the antibiotic. A measurement of the diameter of the zone of inhibition is made and its size is compared to that contained in a standardized chart. Based on this comparison, the test organism is determined to be resistant, moderate or susceptible to the antibiotic.

The four *Leuconostoc* species were tested for susceptibility to thirty antibiotics representing the major antibiotics. All the four species were resistant to the antibiotics co-trimazine,

norflaxacin, norfloxacin and vancomycin (Table 1). All the four species were sensitive to amoxycillin, bacitracin, carbenicillin, cephalothin, cephatoxamine, chloramphenicol, clindamycin, linomycin, oxytetracyclin and tetracycline. The data is in agreement with the reports of Orberg and Sandine (1984)[13], Kelly *et al.* (1986)[11] and Ammor *et al.* (2007)[14] who also reported resistance of *Leuconostoc* species to vancomycin was a common characteristic. Antibiotic such as vancomycin may be used as selective marker during gene transfer with *Leuconostoc* involving conjugation with other microbes of other genus that are not resistant to Vancomycin. All the four species were moderately sensitive towards claxacillin, erythromycin, ampicillin, gentamycin and novobiocin. From Table 1 it can be seen that the antibiotic susceptibility pattern of strain B-512F is 90% similar to B-1146, 80% similar to B-640 and 77% similar to that of B-742. The species B-640 and B-1146 were 83% similar however B-640 and B-742 were only 67% similar, whereas the B-742 and B-1146 were 83 % similar.

Carbohydrate fermentation of *Leuconostoc* strains

The ability of all four species to degrade and ferment carbohydrates with the production

Table 1. Antibiogram of *Leuconostoc* species using antibiotic sensitive octodiscs on MRS agar.

S. No.	Antibiotic	Concentration (mg)	B-512F	B-640	B-742	B-1146
1.	Amoxyclav (Ac)	10	M	M	S	M
2.	Cephalexin (Cp)	10	M	R	S	M
3.	Ciproflaxacin (Cf)	10	M	R	M	M
4.	Clindamycin (Cd)	2	S	S	S	S
5.	Claxacillin (Cx)	1	M	M	M	M
6.	Erythromycin (E)	15	M	M	M	M
7.	Tetracycline (T)	30	S	S	S	S
8.	Ampicillin (A)	10	M	M	M	M
9.	Carbenicilllin (Cb)	100	S	S	S	S
10.	Cephatoxamine (Ce)	30	S	S	S	S
11.	Chloramphenicol (C)	30	S	S	S	S
12.	Co-Trimazine (Cm)	25	R	R	R	R
13.	Gentamicin (G)	10	M	M	R	M
14.	Norflaxacin (Nx)	10	R	R	R	R
15.	Oxacillin (Ox)	5	M	M	S	M
16.	Amikacin (Ak)	10	R	M	R	M
17.	Amoxycillin (Am)	10	S	S	S	S
18.	Bacitracin (B)	10 Units	S	S	S	S
19.	Cephalothin (Ch)	30	S	S	S	S
20.	Novobiocin (Nv)	30	M	M	M	M
21.	Oxytetracyclin (O)	30	S	S	S	S
22.	Vancomycin (V)	30	R	R	R	R
23.	Penicillin-G (P)	10 Units	S	S	S	S
24.	Tobramycin (Tb)	10	M	M	R	M
25.	Cephaloridine (Cr)	30	M	R	M	M
26.	Kanamycin (K)	30	M	S	R	R
27.	Linomycin (L)	2	S	S	S	S
28.	Methicillin (M)	5	S	M	S	S
29.	Norfloxacin (Nf)	10	R	R	R	R
30.	Oleandomycin (OI)	15	M	S	S	S

R- Resistant (0-2 mm[a]); M- Moderate (3-6 mm[a]); S- Sensitive (7-13 mm[a])

[a]Values in millimeters are the distance of zone of inhibition of growth of microorganism.

of an acid was tested. The *Leuconostoc* species were tested for their ability to ferment 15 carbohydrates. The critical nature of the fermentation reaction and the activity of the indicator make it imperative that all cultures should be observed with 48 h. Extended incubation may mask acid producing reactions by production of alkali because of enzymatic action on substrates other than the carbohydrate. There is single report on the carbohydrate utilization by species B-512F and B-742[12]. There are no reports on carbohydrate fermentation patterns for species B-640 and B-1146. All the four *Leuconostoc* species used in the present study utilized fructose, glucose, mannose, melibiose, sucrose and trehalose and did not show any activity towards arabinose, lactose, mannitol and rhamnose (Table 2). *Leuconostoc* species B-512F, B640 and B-1146 were able to weakly ferment galactose and xylose. Both *Leuconostoc* species B-640 and B-742 fermented cellobiose. And the raffinose was fermented by both B-512F and B-1146. B-640 was unique being the only one in fermenting rhamnose. *Leuconostoc* species B-512F, B-640 and B-1146 had nearly identical fermentation profiles, whereas, *Leuconostoc* strain B-742 showed quite different fermentation pattern. Most of these results of B-512F and B-742 are in agreement with those of Holt *et al.* (2001)[12] with a few exceptions. The major exception is that they reported all the species fermented arabinose whereas we did not observe this with any of the strain used. One possible reason could be the time period, as we reported the observation after 2 days whereas they reported after 7 days of incubation. As mentioned earlier that prolonged incubation might have caused production of alkali as a result of certain enzymatic action on substrates other than the carbohydrate.

Plasmid profiles of *Leuconostoc* strains

The plasmid profiles of four glucansucrase producing *Leuconostoc* species were detrmined (Fig. 1). The *Leuconostoc* strain B-742 did not contain any plasmid and B-512F contained a single plasmid and showed a single band (data not shown) as reported earlier[12]. *Leuconostoc dextranicum* B-1146 contained at least 3 plasmids (Fig. 1A&1B, lane 2) that were estimated to be approximately, 1.5 kb (Fig. 1A, lane 2), 5.7 kb (Fig. 1A, lane 2 and also Fig. 1B, lane 2 when compared with lane 3 marker) and 23.5 kb (Fig. 1A&1B, lane 2) in size. (The size of the plasmid is just an approximation as they are compared with linear DNA ladder, however the size 5.7 kb was compared with a recombinant

Table 2. Carbohydrate fermentation characteristics of *Leuconostoc* species.

S. No.	Carbohydrate	B-512F	B-640	B-742	B-1146
1.	Arabinose	-	-	-	-
2.	Cellobiose	-	+++	++	-
3.	Dextrose	+++	+++	+++	+++
4.	Fructose	+++	+++	++	+++
5.	Galactose	+	+++	-	+
6.	Lactose	-	-	-	-
7.	Maltose	+++	+++	+++	-
8.	Mannitol	-	-	-	-
9.	Mannose	++	+++	++	+
10.	Melibiose	+++	+++	+	+++
11.	Raffinose	++	-	-	+++
12.	Rhamnose	-	++	-	-
13.	Sucrose	+++	+++	+++	+++
14.	Trehalose	+++	+++	++	+++
15.	Xylose	+	+	-	+

(+++) strongly positive; (++) fairly positive; (+) weakly positive; (-) negative

plasmid of the same size Fig. 1B, lanes 3). *Leuconostoc mesenteroides* B-640 also showed a single faint band showing that it contained a single plasmid (Fig. 1C, lane 2). No plasmid was observed for *Leuconostoc* species B-742 as also reported earlier[12]. The plasmids for *Leuconostoc* species have been reported earlier[13,23]. It has been reported that the plasmids are associated with cell metabolism[17,18] and with bacteriocin formation[19]. These plasmids may find applications in expression of genes within *Leuconostoc* species. Structural and functional study of the *Leuconostoc* plasmids must be undertaken to explore their potential use.

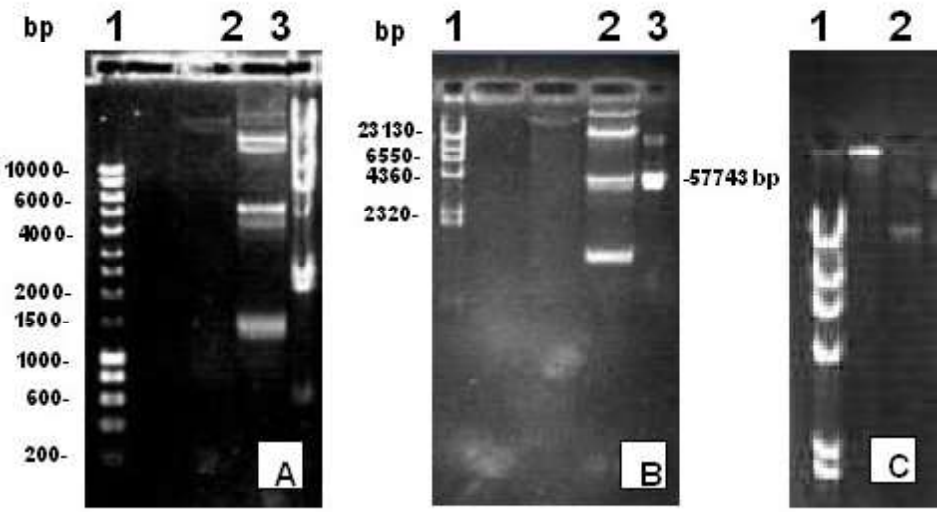

Fig. 1. Plasmid profile of *Leuconostoc* species examined by 0.8% agarose gel electrophoresis. A) Lane 1: DNA ladder (10,000 bp - 200bp); lane 2: *Leuconostoc* strain B-1146 and lane 3: lDNA Hind III digest B) Lane 1: lDNA Hind III digest; lane 2: *Leuconostoc* strain B-1146 and Lane 3: a recombinant plasmid of 5.7 kbp size C) Lane 1: lDNA Hind III digest; lane 2: B-640.

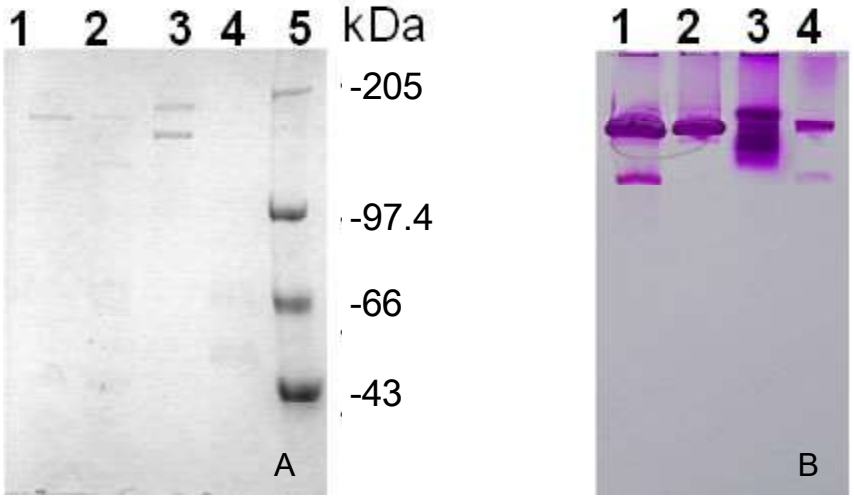

Fig. 2. Glucansucrase activity patterns by glucan producing *Leuconostoc* species; A) Coomassie Brilliant Blue staining B) Activitiy staining using periodic acid Schiff protocol. Lanes: (1) B-512F; (2) B-640; (3) B-742 and (4) B-1146.

Glucan synthesizing activity of *Leuconostoc* strains

Polyacrylamide gel electrophoresis was used for *in situ* detection of enzyme activity to characterize sucrase production by glucan-producing *Leuconostoc* species[15]. This study, however, was carried out to see if all the *Leuconostoc* species produce a similar or different glucan pattern that could be used to distinguish among the glucansucrase producing species. All four *Leuconostoc* species showed very faint bands on Coomassie Brillinat Blue staining (Fig. 2A) except the strain B-1146 showed extremely faint band that could be detected only by activity staining. All four *Leuconostoc* species tested produced at least one detectable glucan-synthesizing activity bands (Fig. 2B). *Leuconostoc* strain B-1146 was the only strain to produce single faint activity band (Fig. 2 lane 4). Three *Leuconostoc* species B-512F, B640 and B-1146 showed a common single band of 180 kDa, however, B-512F showed an extra faint band not shown by B-640 or B-1146 (Fig. 2B). *Leuconostoc* strain B-742 displayed the multiple activity bands compared with the other species tested (Fig. 2B, lane 3). *Leuconostoc* strain B-742 produced three close activity bands, and two were prominent and one band was very faint (Fig. 2). The results of B-742 are somewhat similar to those reported earlier[12] except that we found more intensity of the two bands. However, for strain B-512F our results were different from earlier reported[12]. We found that B-512F shows two bands corresponding to 188 kDa and 146 kDa sizes, whereas they reported two bands of 146 kDa and 118 kDa sizes. In our earlier report we have shown that the purified dextransucrase from B-512F resulted molecular size of 188 kDa (24).

DISCUSSION

The antibiotic resistance, carbohydrate fermentation profiles, sucrose hydrolyzing activity or polysaccharide synthesis activity along with plasmid profiles of the glucansucrase producing *Leuconostoc* species B-640 and B-1146 have never been reported earlier. The results of antibiotic resistance and carbohydrate utilization pattern will enhance understanding of these industrially significant species and will aid in distinguishing between physiologically similar *Leuconostoc* species. All the four species were resistant to the antibiotics cotrimazine, norflaxacin, norfloxacin and vancomycin. All four species were sensitive to amoxycillin, bacitracin, carbenicillin, cephalothin, cephatoxamine, chlorampenicol, clindamycin, linomycin, oxytetracyclin and tetracyclin. All the four species utilized glucose, fructose, mannose, melibiose, sucrose and trehalose and did not show any activity towards arabinose, lactose and mannitol. The *Leuconostoc* strain B-640 was unique in fermenting rhamnose. The *Leuconostoc* species B-640 and B-1146 possessed the plasmids and both showed sucrose hydrolyzing activity by displaying the polysaccharide formation patterns in native polyacrylamide gels.

Some of the metabolic functions such as lactose utilization[17] and citrate utilization[18] encoded by the plasmid bearing genes were identified in several *Leuconostoc* species. Plasmid coding for antibiotic resistance will be especially useful in the selective fermentation by specific species and for the development of cloning and shuttle vectors. The plasmid profiling of these four *Leuconostoc* species was done to relate the functional properties encoded by plasmids, such as antibiotic resistance and carbohydrate fermentation pattern of these *Leuconostoc* species. The recent reports to obtain sourdough rich in dextran using specific lactic acid bacteria producing high molecular weight dextrans in the bakery industry tells the importance of exploration of the new isolated species and with their novel characteristics[3]. This preliminary data about the carbohydrate fermentation, plasmid profile and glucansucrase production will enable in finding similar glucansucrase producing *Leuconostoc* species and their genetic studies, which can be used to identify better species for industrial applications.

ACKNOWLEDGMENTS

The research work in part and SA were supported by a grant from Council of Scientific and Industrial Research (CSIR), India to AG.

REFERENCES

1. Bergqvist, S.W., Sandberg, A.S., Carlsson, N.G., Andlid, T. Improved iron solubility in carrot juice fermented by homo- and hetero-fermentative lactic acid bacteria. *Food Microbiol.,* 2005; **22**: 53-61.
2. Herrerosa, M.A., Sandovalb, H., Gonzaleza, L., Castrob, J.M., Fresnoa, J.M., Tornadijoa, M.E. Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). *Food Microbiol.,* 2005; **22**: 455-59.
3. Lacaze, G., Wick, M., Cappelle, S. Emerging fermentation technologies: Development of novel sourdoughs. *Food Microbiol.,* 2007; **24**: 155–60.
4. Naessens, M., Cerdobbel, A., Soetaert, W., Vandamme, E.J. *Leuconostoc* dextransucrase and dextran: production, properties and applications. *J. Chem. Technol. Biotechnol.*, 2005; **80**: 845-60.
5. Monchois, V., Willemot, R.M., Monsan, P. Glucansucrases: mechanism of action and structure-function relationships. *FEMS Microbiol. Lett.,* 1999; **23**: 131-51.
6. Kralj, S., VanGeel-Schutten, G.H., Dondroff, M.G., Kirsanovs, S., VanDer Maarel, M.J.E.C., Dijkhuizen, L. Glucan synthesis in genus Lactobacillus: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different species. *Microbiol.,* 2004; **150**: 3681-90.
7. Tieking, M., Ganzle, M.G. Exopolysaccharides from cereal-associated lactobacilli. *Trends Food Sci. Technol.,* 2005; **16**: 79-84.
8. Neely, W.B., Nott, J. Dextransucrase, an induced enzyme from *Leuconostoc mesenteroides*. *Biochem.,* 1962; **1**: 1136-40.
9. Robyt, J.F.: Dextran. In: *Encyclopedia of Polymer Science and Technology* (Mark HF, Bikales NM, Overberger CG, Menges G, ed). New York: John Wiley and Sons, 1986; 752-67.
10. Purama, R.K., Goyal, A. Dextransucrase production by *Leuconostoc mesenteroides*. *Ind. J. Microbiol.,* 2005; **2**: 89-101.
11. Kelly, W.J., Asmundson, R.V., Harrison, G.L., Huang, C.M. Differentiation of dextran-producing *Leuconostoc* species from fermented rice cake (puto) using pulsed-field gel electrophoresis. *Int. J. Food Microbiol.,* 1995; **26**: 345-52.
12. Holt, S.M., Al-Sheikh, H., Shin, K.J. Charaterization of dextran producing *Leuconostoc* species. *Lett. Appl. Microbiol.,* 2001; **32**: 185-89.
13. Orberg, P.K., Sandine, W.E. Common occurrence of plasmid DNA and vancomycin resistance in *Leuconostoc* spp. *Appl. Environ. Microbiol.,* 1984; **48**: 1129-33.
14. Ammor, M.S., Florez, A.B., Mayo, B. Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. *Food Microbiol.,* 2007; **24**: 559-70.
15. Leathers, T.D., Ahlgren, J.A., Cote, G.L. Alternansucrase mutants of *Leuconostoc mesenteroides* strain NRRL B-21138. *J. Ind. Microbiol. Biotechnol.,* 1997; **18**: 278-83.
16. O'Sullivan, T., Daly, C. Plasmid DNA in *Leuconostoc* species. *Irish J. Food Sci. Technol.,* 1982; **6**: 206.
17. David, S., Stevens, H., vanRiel, M., Simons, G., DeVos, W.M. *Leuconostoc lactis* b-galactosidase is encoded by two overlapping genes. *J. Bacteriol.* 1992; **13**: 4475-81.
18. Vaughan, E.E., David, S., Harrington, A., Daly, C., Fitzgerald, G.F., De Vos, W.M. Characterization of plasmid-encoded citrate permease (citP) genes from *Leuconostoc* species reveals high sequence conservation with *Lactococcus lactis* citP gene. *Appl. Environ. Microbiol.* 1995; **61**: 3172-76.
19. Fremaux, C., Hechard, Y., Cenatiempo, Y. Mesentericin Y105 gene clusters in *Leuconostoc mesenteroides* Y105. *Microbiol.* 1995; **141**: 1637-45.
20. De Man, J.C., Rogosa, M., Sharpe, M.E. A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.,* 1960; **23**: 130-35.
21. Barry, A.L., Thornsberry, C.: Susceptibility testing: diffusion procedures. In: *Manual of Clinical Microbiology* (Lennette EH, Balows A, Hausler WJJr, Truant JP, ed). Washington, D.C: American Society for Microbiology, 1980; 463-74.
22. Kandler, O., Weiss, N.: Regular, nonsporing gram-positive rods. In: *Bergey's Manual of Systematic Bacteriology* (Sneath PHA, Mair NS, Sharpe ME, Holt JG, ed). Baltimore: Williams & Wilkins, 1986; 1208-19.
23. David, S., De Vos, W.M. Genetic characterization of *Leuconostoc spp*. from Dutch starter cultures. *FEMS Microbiol. Rev.,* 1987; **46**: 26.
24. Goyal, A., Katiyar, S.S. Fractionation of *Leuconostoc mesenteroides* NRRL B-512F dextran sucrase by polyethylene glycol: a simple and effective method of purification. *J. Microbiol. Method.* 1994; **20**: 225-31.