Front. Biol. 2014, 9(3): 244–253
DOI 10.1007/s11515-014-1303-5

RESEARCH ARTICLE

Fermentative production of dextran using *Leuconostoc* spp. isolated from fermented food products

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Abstract ***Leuconostoc*** **spp. (LS1and LI1) isolated from sauerkraut and idli batter was selected for dextran production. To enhance the yield of dextran, effects of various parameters such as sucrose concentration, pH, temperature, incubation and inoculum percentage were analyzed. The optimum sucrose concentration for the *Leuconostoc* spp. (LS1 and LI1) was found to be 15% and 25% respectively. Isolates produced maximum dextran after 20 h of incubation at 29° C and the optimum pH was found between 8 and 8.5. The inoculum concentration of 7.5% was more favorable for the production of dextran by *Leuconostoc* spp. (LS1 and LI1). The growth kinetic parameters were studied and compared for the strains LS1 and LI1. Mass production of dextran was carried out using a stirred tank batch reactor. FTIR analysis was done to determine the functional groups of dextran. sephadex is prepared by cross linking dextran using epichlorohydrin and the functional groups are determined by FTIR analysis.**

Keywords dextran, *Leuconostoc* sp., FTIR, bio-polymer, sephadex, epichlorohydrin

Introduction

Dextrans are homopolysaccharides of D-glucose, synthesized by dextransucrase, an extracellular enzyme elaborated by lactic acid bacteria viz., *Leuconostoc, Lactobacillus* and *Streptococcus, Weisella* (Katina et al., 2009). The Gram-positive *Leuconostoc mesenteroides* NRRL B-512F, which synthesizes the extracellular homopolysaccharide dextran, is an extensively used organism for the industrial production of dextransucrase. Dextran gained importance owing to its applications in the pharmaceutical, food, photo film manufacturing and fine chemical industries. Dextrans are used in the manufacture of blood plasma expanders, heparin substitutes for anticoagulant therapy, cosmetics, and other products (Alsop, 1983; Kim and Day, 1994; Leathers et al., 1995; Sutherland, 1996). Another use of dextran is the manufacture of Sephadex gels or beads, which are widely used for industrial and laboratory protein separations (Sutherland, 1996). The primary aim of this project was to isolate and identify the strains of *Leuconostoc* spp. which produce more amount of dextran in comparison to the already identified strains. After isolation of dextran producing strains another important task was to optimize the process parameters which effect the production of dextran at a commercial scale. The important parameters were optimised and mass production studies were carried out using a stirred tank batch fermenter. The dextran produced was then cross linked to produce sephadex, a column packing material used in protein purification.

Received February 12, 2014; accepted April 2, 2014
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Materials and methods

Isolation of lactic acid bacteria from fermented food products

Lactic acid bacterial strains were isolated from two different fermented food products namely Sauerkraut and idli batter. Sauerkraut was prepared by adding 2.5% (w/w) of NaCl to thin, long cabbage shreds followed by a 15 d anaerobic fermentation (Halasz et al., 1999). 1g of sauerkraut and 1g of idli batter were serially diluted using 0.9% sterile saline and the dilutions were poured on MRS agar plates to isolate the organisms.

Screening for dextran producing *Leuconostoc* spp.

The isolates were streaked on the Sucrose agar medium with 0.005% of sodium azide and incubated for 24 h at 37°C (Sarwat et al., 2008). Colonies showing highly viscous slimy growth on sucrose agar plates were selected.

Strain selection and identification

Two Slimy bacterial colonies were selected for this study. Morphological and biochemical characterization was done for the isolates. Bacterial strain LS1 isolated from sauerkraut and LI1 isolated from idli batter were identified according to the Bergey's Manual of determinative bacteriology. Pure cultures of the identified two strains were maintained on MRS plates and stored at 4°C.

Microbial growth kinetics

Leuconostoc sp. (LS1) and *Leuconostoc* sp. (LI1) were transferred from MRS slants to 100 mL sterile MRS broth. The broth was incubated at room temperature overnight on a shaker at 120 r/min. The subsequent day a small inoculum was transferred from the MRS broth to 100 mL sterile dextran production broth and incubated overnight on a shaker at 120 r/min (Sarwat et al., 2008; Aman et al., 2011).

After incubation, 10 mL of inoculum was transferred to 90 mL sterile MRS and dextran production broth in side arm flasks (growth curve studies) and conical flasks (biomass studies). The growth curve and biomass study was done for 24 h with readings being taken at an interval of 1 h. While the growth curve was determined by measuring the OD value at 610 nm using a colorimeter. The growth curves were plotted and the curves were used to determine the generation time, growth rate constant and mean generation time of the three organisms.

Dextran production

Growing cultures of *Leuconostoc* spp. (LS1 and LI1) was transferred to 100 mL sterile dextran production medium. The broth cultures were monitored for an OD in the range of 0.2–0.3 at 610 nm. When the OD value of the broth cultures was in the desired range, 10 mL of the inoculum was transferred to 90 ml sterile dextran production broth and incubated at room temperature for 20 h in a shaker at 120 r/min (Holt, 1994).

Optimization of process parameters for dextran production

The production of dextran at small scale or at a commercial scale depends on several process parameters. These parameters vary for microbial strains being employed for the production and hence the process parameters like pH, temperature, time, sucrose concentration and inoculum percentage have been optimized to ensure maximum dextran production. The sucrose concentration was varied from 10% to 40% in the production medium, the effect of temperature was studied in the range of 4°C to 45°C, for temperature optimization 100 mL of fresh broth containing 15% sucrose was used, pH of the production medium was adjusted from 6.0 to 8.0. For all the studies, the flasks were incubated at 30°C for 20 h.

Fermentative production of dextran using the batch reactor

925 mL dextran production broth was prepared. Two separate batches were run for the two different strains of *Leuconostoc* spp. isolated (LS1 and LI1). The *Leuconostoc* spp. from sauerkraut (LS1) requires 15% sucrose concentration, pH of 6.5 while the other media components were kept constant. The room temperature was maintained and the fermentative production was carried out for 20 h following the addition of 7.5% inoculum with an OD of 0.24 (measured colorimetrically at 610 nm). The *Leuconostoc* sp. from idly batter (LI1) requires a sucrose concentration of 25% and a pH of 7.0. The fermentation batch was run for 20h at room temperature following the addition of 7.5% inoculum with an OD of 0.24 (measured colorimetrically at 610 nm).

Extraction and purification of dextran

The 20 h fermented broth cultures from conical flasks and bioreactor were centrifuged at 12000 r/min for 10min at 4°C. The supernatant was then transferred to sterile conical flask and twice the volume of absolute ethanol was added to it. The supernatant-ethanol mixture was then incubated overnight at 4°C to precipitate the slime. The mixture was then centrifuged at 12000 r/min for 10 min at 4°C to pellet out the slimy dextran. The dextran pellet was then washed with distilled water by centrifuging it at 12000 r/min for 10 min at 4°C thrice. The pellet was then dried at 80°C using a hot air oven and stored at 4°C.

Dextran characterization

Fourier Transform-Infra Red spectroscopy (FTIR) analysis

FTIR (IRAffinity-1, Schimadzu, Japan) analysis was done to determine the functional groups. The FTIR spectra of the produced dextran from two different *Leuconostoc* strains (LS1 and LI1) were obtained at a resolution of 4 cm^{-1}.The sample was incorporated into KBr (spectroscopic grade) and pressed into a 2 mm pellet. IR spectra were recorded in the transmittance mode from 4000 to 400 cm^{-1} (Kim et al., 2003; Moosavi-Nasab et al., 2010)

Preparation of sephadex and characterization

Sephadex is prepared by crosslinking dextran using agents like epichlorohydrin. The cross linking reaction is carried out in an alkaline environment at 25–50°C. The required alkaline environment was provided by 50 mL of 2 M NaOH. To this 250 mg of the dextran was added. Two separate crosslinking reactions were carried out in two separate flasks for two different dextran samples produced by the two *Leuconostoc* spp. (LS1 and LI1). After dissolving dextran in NaOH, 0.1mL of epichlorohydrin was added to the flasks and kept on a shaker at 100 r/min for 4 h at room temperature. After the 4h incubation period the flask contents were centrifuged at 6000 r/min for 15 min and the supernatant was discarded. The pellet was dried in a hot air oven at 40°C overnight (Ali Güner et al., 2001; Khan et al., 2010). Then the pellet was subjected to FTIR analysis to obtain the spectral peaks pertaining to the functional groups.

Results and discussion

Identification of organism

Morphological and biochemical assessment of the isolated two strains confirmed the characteristic properties of *Leuconostoc* spp. (Fig. 1 and Table 1). Also the results of the strain LS1 and LI1 are compared with the positive control organism *Leuconostoc mesenteroides* NCIM 2947 and identified as a member of genus *Leuconostoc.* Further the pure cultures (Fig. 2) were sent to 16S rRNA gene analysis for species identification.

Growth curve

The growth rate pattern of the isolates was studied in MRS and dextran production broth (Fig. 3).

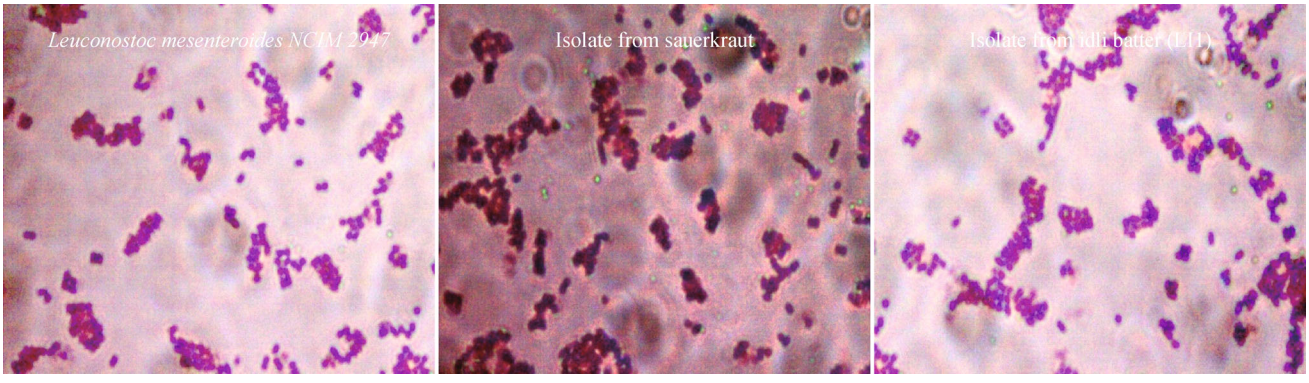

Figure 1 Gram staining 100× magnification.

Table 1 Morphological and biochemical characterization of *Leuconostoc* spp.

Sr. No.	Characterization	Positive control (*Leuconostoc mesenteroides* NCIM2947)	Isolate from sauerkraut (LS1)	Isolate from idli batter (LI1)
1	Grams staining	Purple chains of cocci	Purple chains of cocci	Purple chains of cocci
2	Blood agar	Non hemolytic	Non hemolytic	Non hemolytic
3	MRS agar	Gummy colonies	Gummy colonies	Gummy colonies
4	Indole test	Negative	Negative	Negative
5	MR test	Negative	Negative	Negative
6	VP test	Positive	Positive	Positive
7	Citrate utilization test	Negative	Negative	Negative
8	Eschulin hydrolysis test	Positive	Positive	Positive
9	Catalase test	Negative	Negative	Negative
10	Sucrose fermentation	Positive	Positive	Positive
11	Glucose fermentation	Positive	Positive	Positive
12	Lactose fermentation	Positive	Positive	Positive
13	Arabinose fermentation	Positive	Positive	Positive
14	Maltose fermentation	Positive	Positive	Positive
15	Mannitol fermentation	Negative	Negative	Negative

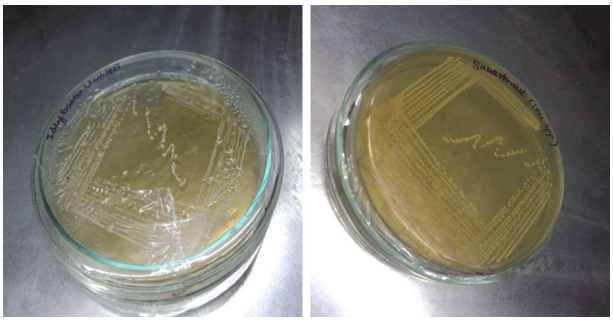

Figure 2 Pure cultures of *Leuconostoc* sp.(LS1) and *Leuconostoc* sp.*(*LI1).

Dextran production typically takes 20 h and the graphs plotted clearly indicate that the production of dextran happens at the late exponential phase. Growth curves of *Leuconostoc mesenteroides* subsp. *mesenteroides* MCRI and *Leuconostoc citreum* MCRI 4 in MRS broth has been studied to enter the stationary phase in the 11th hour (Hamasaki et al.,2003). while the *Leuconostoc mesenteroides* NCIM 2947 and the isolates, *Leuconostoc* spp. (LS1 and LI1) from sauerkraut and idli batter enter the stationary phase around the 12th hour. The stationary phase is of prime importance in dextran production as the production of dextran begins somewhere around the late stationary phase.The growth kinetics was also studied in dextran production broth and the growth curve patterns showed a great similarity to *Leuconostoc mesenteroides* CMG713. The graphs plotted were also used to calculate and determine the generation time, doubling time and growth rate constant. The growth kinetic parameters for the three organisms have been compared (Table 2).

It is clearly evident that the isolate LS1 and LI1 from sauerkraut and idly batter have a higher doubling rate than the positive control *Leuconostoc mesenteroides* NCIM 2947 in the dextran production broth, which in turn favors the fermentation process. The newly isolated dextran producing strain, *Leuconostoc mesenteroides* CMG713 (Holt, 1994) has also shown similar growth kinetic parameters owing to the similar growth curves.

Production, extraction and purification of dextran

In the current study the amount of dextran produced by the isolate *Leuconostoc* sp. (LI1) from idli batter was the highest (0.282 g/100 mL) however both the isolates showed better production than *Leuconostoc mesenteroides* NCIM 247. A comparison of the amount of dextran produced has been given in Table 3.

In another study, the amount of dextran produced by an isolate from grapes, *Leuconostoc mesenteroides* CMG713 has been observed to be around 0.480 g/100mL (Holt, 1994). Another strain *L. mesenteroides* AA27 isolated from peach has shown a yield of 0.255 g/100 mL (Aman et al.,2011).

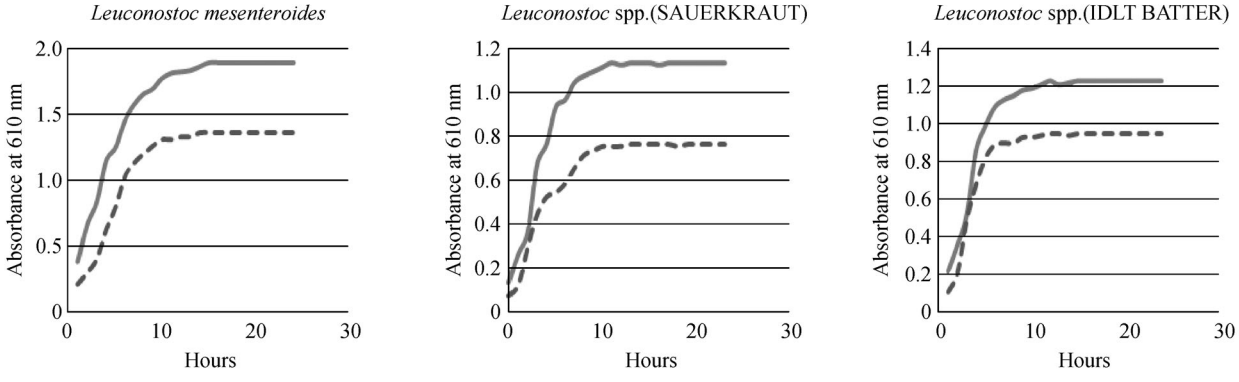

Figure 3 24 h growth curve study of *Leuconostoc mesenteroides* NCIM 2947; isolate from sauerkraut (LS1) and isolate from idli batter.

Table 2 Growth rate parameters in MRS and production medium

	Leuconostoc mesenteroides NCIM 2947	Isolate from sauerkraut (LS1)	Isolate form idli batter (LI1)
MRS Broth			
Generation time (min)	120	70	90
Growth rate constant (h^{-1})	0.2	0.3	0.3
Doubling time (h)	5	3.33	3.33
Production broth			
Generation time (min)	120	70	130
Growth rate constant (h^{-1})	0.2	0.171	0.185
Doubling time (h)	5	5.84	5.416

Optimization

Optimization of sucrose concentration

Sucrose concentration has been identified as an important parameter which affects dextran production. The effect of different concentration of sucrose was studied in the range from 10% to 40%. It was also observed that dextran production was effected by the different concentration of sucrose in the medium. For *Leuconostoc* sp. (LS1), the maximum dextran yield was obtained when 15% sucrose concentration was used in the production medium. But in case of *Leuconostoc* sp. (LI1),the maximum dextran yield was obtained when 25% of sucrose concentration was used in the medium. The effect of sucrose concentration on dextran production by the isolate LS1 and LI1 has been represented in the Fig. 4.

Perhaps higher concentration of sucrose in the medium had an inhibitory effect known as substrate inhibitory effect which decreased dextran production (Martinez-Espindola and Lopez-Manguia, 1985; Kim and Day, 1994). The other media components were kept constant while the fermentation was carried out at room temperature for 20 h following the addition of a 10% inoculum. The results suggest the optimum sucrose concentration for the *Leuconostoc* spp. isolated from sauerkraut and idly batter was 15% and 25% respectively in comparison to the *L. mesenteroides* CMG 713 at 15% (Holt, 1994) conventionally used.

Optimization of pH

Dextran production by *Leuconostoc* spp. (LS1, LI1) was observed between pH 8 to 8.5. The effect of pH on dextran production is represented in Fig. 5.

While the *Leuconostoc* spp. isolated from Sauerkraut favored dextran production at a pH of 8.5, the one isolated from Idli batter showed optimum production at a pH of 8.0. However production of dextran has been largely favored at pH of 6 to 8. It has been studied that extreme values of pH leads to the production of half the amount of dextran as would be produced at optimum pH (Holt, 1994).

Optimization of temperature

Dextran production was determined at different temperature from 4°C to 45°C and the maximum dextran production by *Leuconostoc* spp. (LS1 and LI1) was achieved at 29°C. Figure 6 represents the effect of temperature on dextran production.

It has been found that the production of dextran at a higher temperature like 37°C would be lesser than the dextran produced at 29°C due to the instability of the enzyme in fermentation broth 1(Kaboli and Reilly, 1980; Halasz et al., 1999). The production of dextran was optimum at 30°C as this temperature favored cell multiplication (Alsop, 1983) however, this experiment when carried out at room temperature (29°C) gave optimum production of dextran by the *Leuconostoc* spp. isolated from sauerkraut as well as idli batter.

Table 3 Dextran production

	Leuconostoc mesenteroides NCIM 2947	*Leuconostoc* sp. (LS1)from sauerkraut	*Leuconostoc* sp. *(LI1)* form idli batter
Amount of dextran produced (g/100 mL)	0.186	0.206	0.282

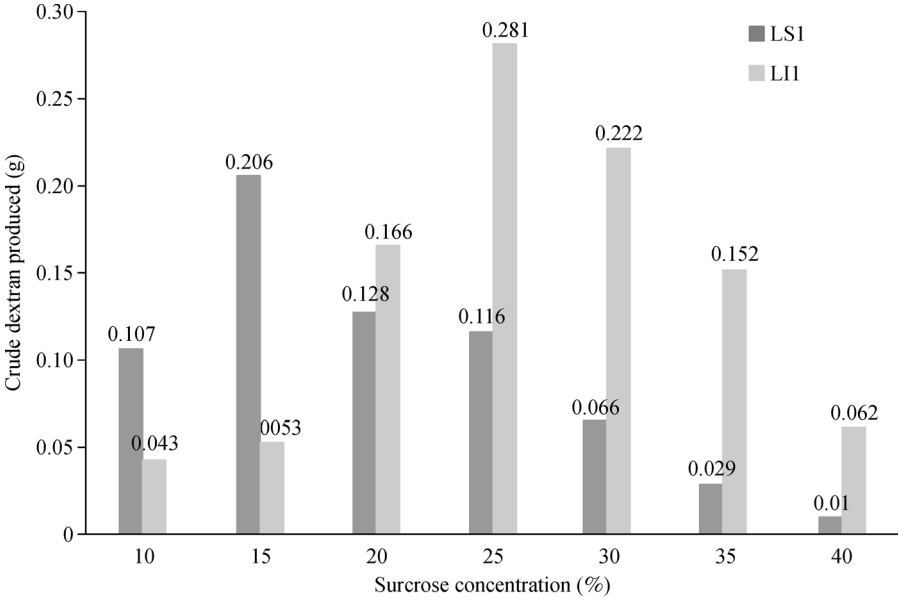

Figure 4 Optimization of sucrose concentration.

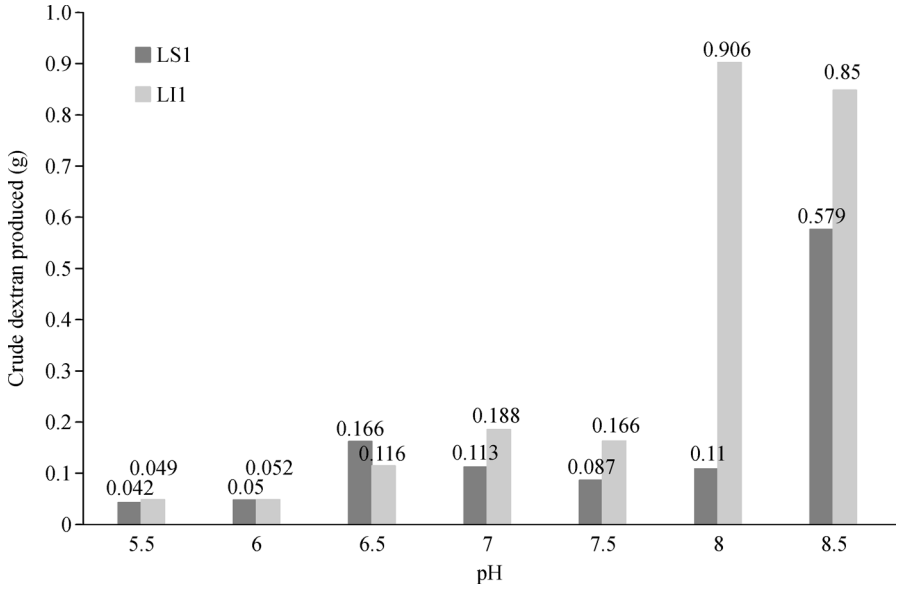

Figure 5 Optimization of pH.

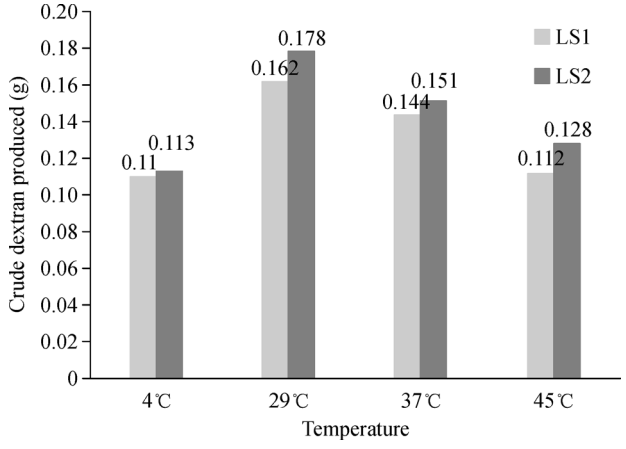

Figure 6 Optimization of temperature.

Optimization of inoculum percentage

The maximum dextran production was observed when 7.5% inoculum was used in the production medium. The effect of inoculum percentage has been represented in Fig. 7.

The lower inoculum percentages have been known to delay the dextran production (Shah Ali et al., 2005). The production rate gradually increases with the increase in inoculum percentage. However after a certain optimum percentage, the addition of further inoculum affects fermentation negatively by decreasing the dilution rate. The inoculum concentration of 7.5% was more favorable than the generally employed 10% (Santos et al., 2000) for the production of dextran by the *Leuconostoc* spp. isolated from sauerkraut and idli batter.

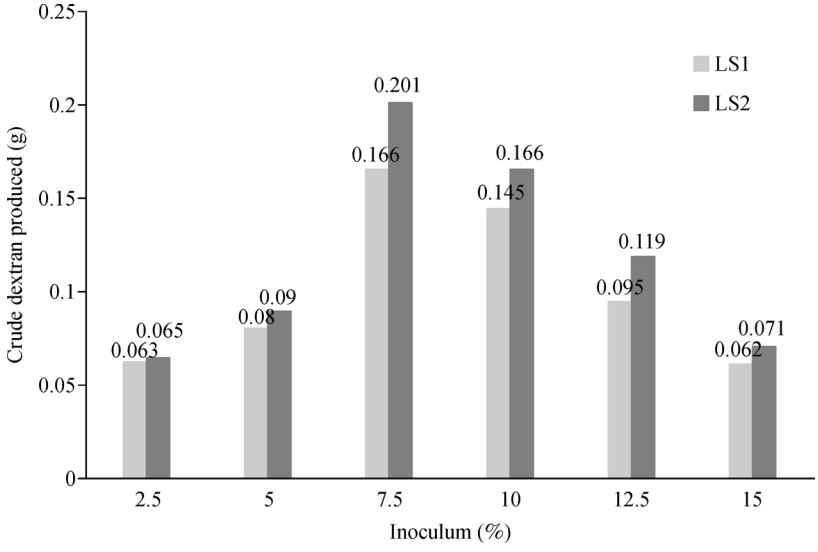

Figure 7 Optimization of inoculums percentage.

Optimization of incubation time

Results have suggested that the maximum dextran production was achieved at 20 h for both the strains LS1 and LI1. Figure 8 represents the effect of time on dextran production.

The production of dextran increased from the 16th to the 20th hour, the production declined in the 24th and 28th hour. Similar pattern of increase and subsequent decrease in the production of dextran was observed for the *Leuconostoc spp.* isolated from sauerkraut and idli batter.

Fermentative production of dextran

Experimental results on the batch fermentation of *Leuconostoc* spp. shown maximum dextran yield at optimized parameters. After optimizing the process parameters, the mass production was carried out using a stirred tank batch reactor with a working volume of 2 L. 3.15 g/L of dextran was produced by the *Leuconostoc* sp. (LS1) isolated from sauerkraut and 4.08 g/L by *Leuconostoc* sp. (LI1) isolated from idli batter. The amount of dextran produced by an isolate from grapes, *Leuconostoc mesenteroides* CMG713 has been observed to be around 0.455 g/100mL (Holt, 1994) Another strain *L. mesenteroides* AA27 has shown a yield of 0.255 g/100 mL (Aman et al.,2011). The strains isolated from sauerkraut and idlibatter (LS1 and LI1) shown maximum dextran production in bioreactor than several established commercial strains which have been isolated from grapes, peaches and tomatoes like *Leuconostoc mesentroides* AA27.

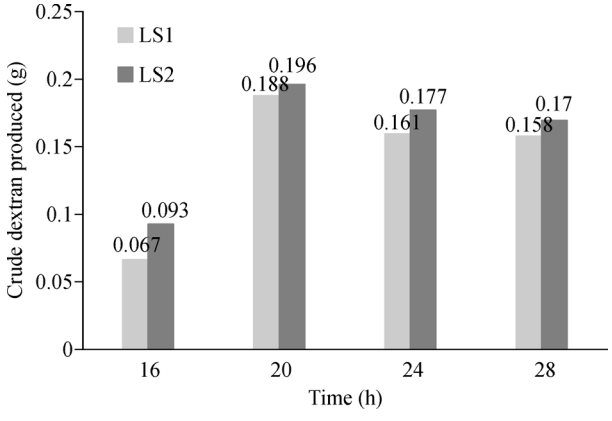

Figure 8 Optimization of incubation time.

Dextran characterization by FTIR analysis

The qualitative studies were done by FTIR analysis to determine the structure of the polysaccharide, dextran obtained from the *Leuconostoc* spp. (LS1 and LI1) isolated from sauerkraut and idly batter. Figures 9, 10 and 11 show the FTIR spectra of the produced dextran by different strains.

The bands at 3444 and 3503 cm^{-1} are attributed to –OH stretching, the bands at 2931 and 2838 cm^{-1} indicates the presence of $–CH_2$ stretching, bands at 1647, 1641 and 1635 cm^{-1} are attributed to –C-C- stretching vibration, bands at 1396, 1427 and 1396 cm^{-1} for $–CH_2$ and –OH stretching and bands at 1082, 1085 cm^{-1} attributes to –C-O-C-. The peaks in the FTIR spectra are similar to the signature spectral peaks of standard dextran. The signature peaks have been reported as to be 3410 cm^{-1} for –OH; 2920 cm^{-1} for $–CH_2$; 1600 cm^{-1} for –C-C-; 1400 cm^{-1} for $–CH_2$ and –OH and 1100 cm^{-1} for –C-O-C- (Ali Güner et al., 2001). The spectral peaks obtained in the current research are in accordance to the already established signature peaks and hence the three samples have been identified to be dextran. The functional group analysis thus confirms the compound produced to be dextran. The results show that there is no significant difference between dextran produced by *Leuconostoc* spp. (LS1 and LI1).

Sephadex production and characterization

Dextran cross linking was carried out at alkaline environment at room temperature for 4h using epichlorohydrin. The strain LS1 produced 0.13 g/100 mL of sephadex and LI1 produced 0.122 g/100 mL. Dextran cross linked with epichlorohydrin in alkaline environment produced sephadex. The bands at

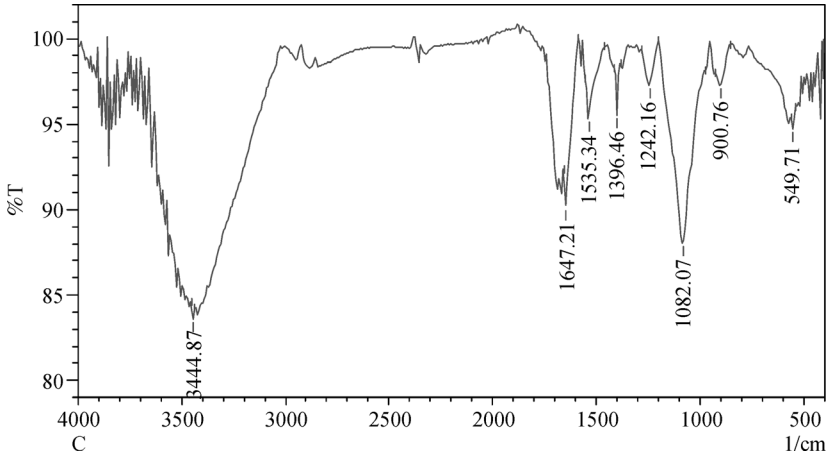

Figure 9 FTIR spectra of dextran produced by *Leuconostoc* spp. from sauerkraut.

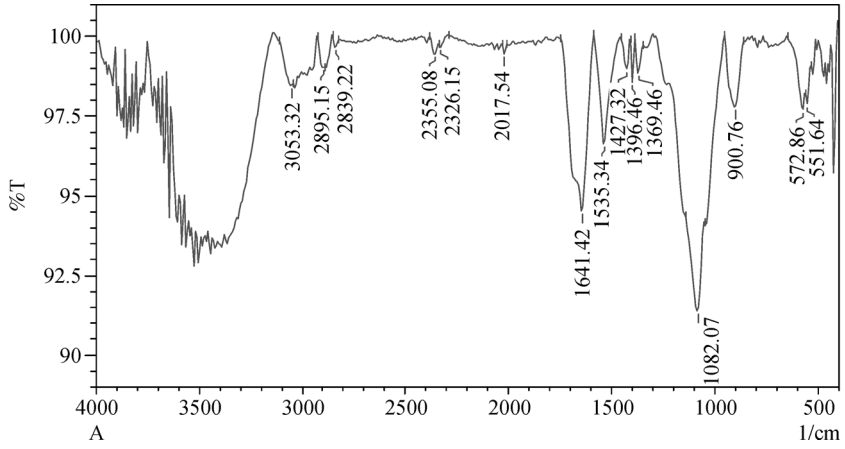

Figure 10 FTIR spectra of dextran produced by *Leuconostoc* spp. from idli batter.

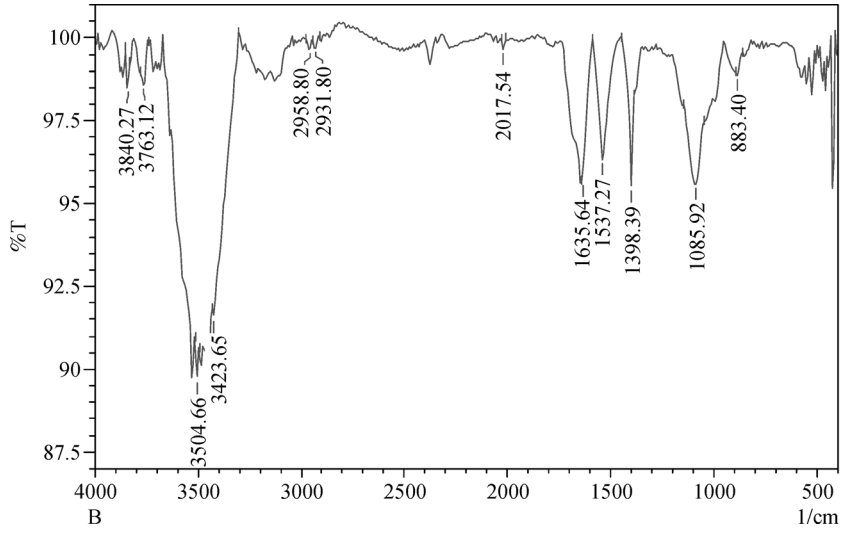

Figure 11 FTIR spectra of dextran produced by *Leuconostoc mesenteroides* NCIM 2947.

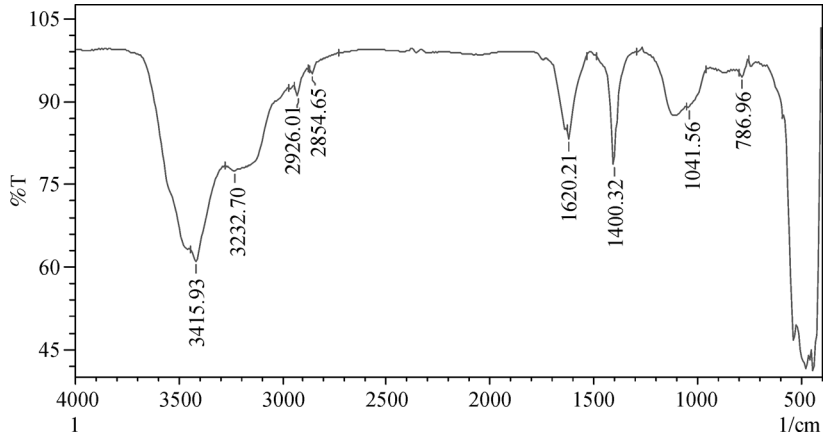

Figure 12 FTIR spectra of sephadex obtained by cross linking dextran obtained from idli batter.

3415 and 3419 cm^{-1} indicates the presence of epichlorohydrin cross linked – OH group of dextran and 1620 and 1737 cm^{-1} for C = O overlapping (Figs. 12 and 13).

Similar signature peaks of epichlorohydrin cross linked

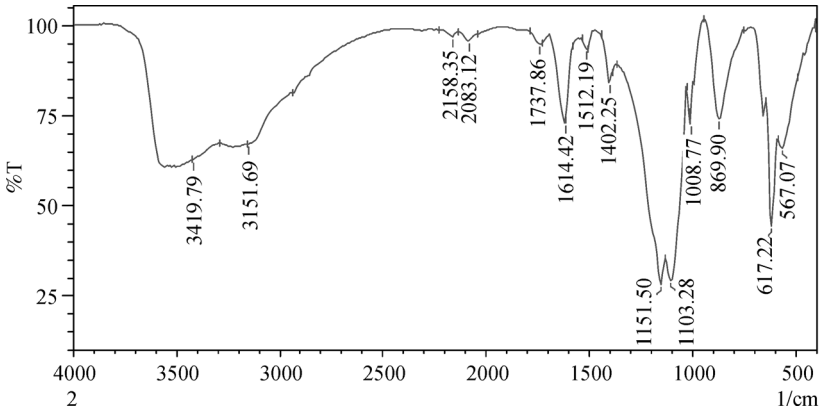

Figure 13 FTIR spectra of sephadex obtained by cross linking dextran obtained from sauerkraut.

dextran have been reported at 3500 cm^{-1} for epichlorohydrin cross linked – OH group of dextran and 1700 cm^{-1} for C = O overlapping (Khan et al., 2010).

Conclusions

The mass production studies with optimized parameters showed a significant and noteworthy increase in dextran production. These optimized conditions could be useful for the commercial production of dextran which has significant industrial perspectives. Dextran has been well known for its diverse applications and keeping the commercial value of dextran in view the current research also involved converting dextran into a cross linked polymer, sephadex. This form of dextran has been used in chromatographic columns and plays a vital role in gel permeation chromatography studies of larger molecules. Future studies includes the molecular characterization of the strain and molecular weight determination of the dextran produced by *Leuconostoc* spp. isolated from sauerkraut(LS1) and idli batter(LI1).

Acknowledgements

We are greatly indebted to Vellore Institute of Technology for the constant encouragement, help and support for extending necessary facilities.

Compliance with ethics guidelines

Subathra, Shantan, Mohanasrinivasan declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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