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Studies on solvent precipitation of levan synthesized using *Bacillus subtilis* MTCC 441

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ABSTRACT

Levan is a water soluble biopolymer widely used in food, pharma, personal care and aquaculture industries. In this work, levan was synthesized by *Bacillus subtilis* MTCC 441 using sucrose as a sole carbon source. Effects of pH, sucrose concentration, nitrogen source, nitrogen concentration, inoculum size and agitation speed on levan production were studied. Yeast extract (YE) was found to be the best nitrogen source. Sucrose concentration – 100 g/L, pH – 7, YE concentration – 2 g/L, inoculum size 10% (v/v) and RPM – 150 were found to be optimal values for levan production. Effects of precipitation pH (3–12), choice of solvent (ethanol, isopropanol, acetone, and methanol) and supernatant to solvent ratio (1:1 to 1:6) on levan yield were also studied. Isopropanol resulted in maximum levan recovery among the four solvents considered. Optimal pH and supernatant to solvent ratio for levan precipitation were found to be 11 and 1:5, respectively. Corresponding levan yield was 0.395 g/g of sucrose supplied. The product obtained was characterized using FTIR, ^{1}H-NMR, ^{13}C-NMR, and GPC. The cytotoxicity of the precipitated levan was studied on EA.hy926 cell line using MTT assay and the compound was proven to be non-toxic to the cells.

1. Introduction

Levan is one of the commercially important fructans that occur in a wide range of microorganisms and in a few plants as a non-structural carbohydrate reservoir (Han, 1990; Banguela et al., 2011). It is a water soluble, and eco-friendly biopolymer comprising of fructofuranosyl residues joined by β – (2, 6) and β – (2, 1) linkages (Poli et al., 2009; Sarilmiser and Oner, 2014; Silbir et al., 2014). In general, plants produce low molecular weight levans while microorganisms produce high molecular weight levans. Microbial levan has a broad range of applications compared to plant derived levan. Levan is used in personal care, medical, aquaculture and food applications (Oner et al., 2016). Natural Polymers Inc. (USA), Real Biotech Co., Ltd., (Korea) and Advance Co., Ltd., (Japan) are the major producers of levan at commercial scale. Two common examples of levan based commercial products available in the market are Proteolea® and Slimexir® (Oner et al., 2016).

Variety of microorganisms produce levan by transfructosylation reaction catalyzed by Levansucrase (β – 2, 6 fructan: D-glucose-fructosyl transferase) from a sucrose-based substrate (Ni et al., 2018). The gene responsible for levan production is found to be SacB which gets activated by the presence of sucrose (Porras-Dominguez et al., 2017). Microorganisms such as *Zymomonas mobilis* (Jang et al., 2001; Silbir et al., 2014), *Bacillus subtilis natto*(Shih et al., 2010a,b), *Bacillus licheniformis* (Kekez et al., 2015); (Xavier and Ramana, 2017), *Clostridium acetobutylicum* (Gao et al., 2017), *Brennia goodwinii* (Liu et al., 2017; Xu et al., 2017), *Acinetobacter nectaris* (Gonzalez-Garcinuno et al., 2017)., *Lactobacillus reuteri* (Ni et al., 2018), *Halomonas* sp., (Poli et al., 2009; Sarilmiser and Oner, 2014), *Gluconoacetobacter xylinus, Microbacterium laevaniformans, Rahnella aquatilis* (Yoo et al., 2004), *Xanthomonas* (Fuchs, 1956), *Saccharomyces* (Franken et al., 2013), *Pseudomonas* (Kasapis et al., 1994; Laue et al., 2006), *Streptococcus* (Newbrun and Baker, 1968; Simms et al., 1990) etc. have been reported to synthesize levan.

Previous literatures reveal that studies on levan production processes had been focused mainly on upstream processing such as i) microorganism type, ii) alternate or low cost substrates rich in sucrose, and iii) optimization of media and fermentation conditions. To the best of our knowledge, no report is found on downstream process optimization such as solvent precipitation for levan recovery. Majority of the previous

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Table 1
Experimental conditions and medium composition for optimization of fermentation conditions.*

Experiment	Sucrose concentration (g/L)	Initial pH	Nitrogen source	YE concentration (g/L)	Inoculum size (%)	Agitation speed RPM
Effect of sucrose concentration	20, 40, 60, 80, 100	7	YE	2	5	150
Effect of pH	100	4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0	YE	2	5	150
Effect of nitrogen source	100	7	Yeast extract, Beef extract, Malt extract	2	5	150
Effect of Concentration of nitrogen source (YE)	100	7	YE	1.0, 1.5, 2.0, 2.5, 3.0	5	150
Effect of Inoculum size	100	7	YE	2	2.5, 5.0, 7.5, 10.0	150
Effect RPM	100	7	YE	2	10	0, 100, 120, 150, 170 200

* For all the experiments above the basic medium also contains the following components: ammonium sulphate ($(NH_4)_2SO_4$) – 3 g/L, potassium dihydrogen phosphate (KH_2PO_4) – 1 g/L, magnesium sulphate ($MgSO_4.7H_2O$) – 0.6 g/L, manganese sulphate ($MnSO_4$) – 0.2 g/L.

reports found in literature only use ethanol as anti-solvent for levan precipitation (Poli et al., 2009). However, choice of solvent and solvent precipitation conditions like precipitation pH, (cell free) broth to solvent ratio, and time allowed for precipitation affect product yield and productivity significantly and therefore they need to be carefully considered and studied for each system in order to obtain maximum possible product recovery. Thus, this study mainly focuses on optimization of the parameters involved in solvent precipitation.

Since, levan is commonly used in wide range of medicinal and food related applications, cytotoxicity of the product obtained in this process was also studied. Cytotoxicity analysis using MTT assay done on cell line primarily aims to evaluate the suitability of the product to be used in drug delivery applications. Here, we have used endothelial cell line EA.hy926 which are very intact with the blood vessels and play major role in tumor progression (Rajabi and Mousa, 2017), cardiovascular diseases (Jay Widmer and Lerman, 2014) and inflammation (Pober and Sessa, 2007). These cells are very sensitive to the drugs. So, synthesized levan was checked for its non-toxicity on this cell line.

2. Materials & Methods

2.1. Media components

Sucrose was procured from HiMedia, India. Ethanol was purchased from MP Biomedicals, India; isopropanol, acetone was from SRL chemicals; methanol was procured from SDFCL, Mumbai. All other chemicals unless specified were obtained from Merck enterprises, India.

2.2. Microorganism and culture condition

Microorganism used in the study was obtained from Microbial Type Culture Collection, Chandigarh, India. Glycerol stocks were maintained at – 20 °C. The Luria Bertani broth was used for culturing *Bacillus subtilis* MTCC441. The well grown overnight culture maintained at 150 RPM, 37 °C was used for inoculation in the production medium throughout the study.

2.3. Effect of medium composition and fermentation conditions

The composition of basic medium used for levan production is: Sucrose- 100 g/L, Yeast Extract (YE) - 2 g/L, ammonium sulphate ($(NH_4)_2SO_4$) – 3 g/L, potassium dihydrogen phosphate (KH_2PO_4) – 1 g/L, magnesium sulphate ($MgSO_4.7H_2O$) – 0.6 g/L, manganese sulphate ($MnSO_4$) – 0.2 g/L (Laddha and Chitanand, 2017). The initial pH was adjusted to 7 before autoclaving. Levan production was carried out in 250 mL Erlenmeyer's flask with a culture volume of 50 mL. The fermentation temperature was maintained at 37 °C. The flasks were incubated in an orbital shaker at 150 RPM. Effect of fermentation time, carbon source concentration, fermentation pH, nitrogen source, concentration of nitrogen source, inoculum size, and agitation speed were examined by conventional one factor at a time method. The range and levels of variables investigated are indicated in Table 1. All experiments were run in triplicates.

For the recovery of Levan the culture was centrifuged at 6000 RPM for 20 min and pellet was dried at 60 °C for biomass estimation (Tabernero

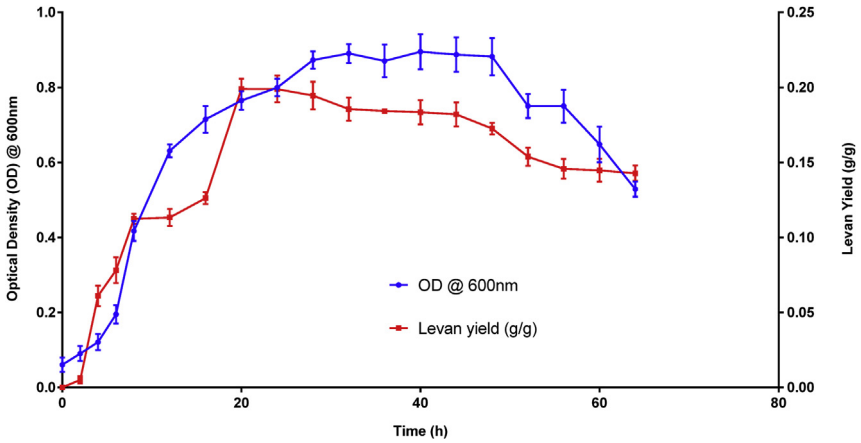


Fig. 1. Growth curve for levan production [Sucrose – 100 g/L, initial pH – 7, RPM – 150, incubation temperature – 37 °C].

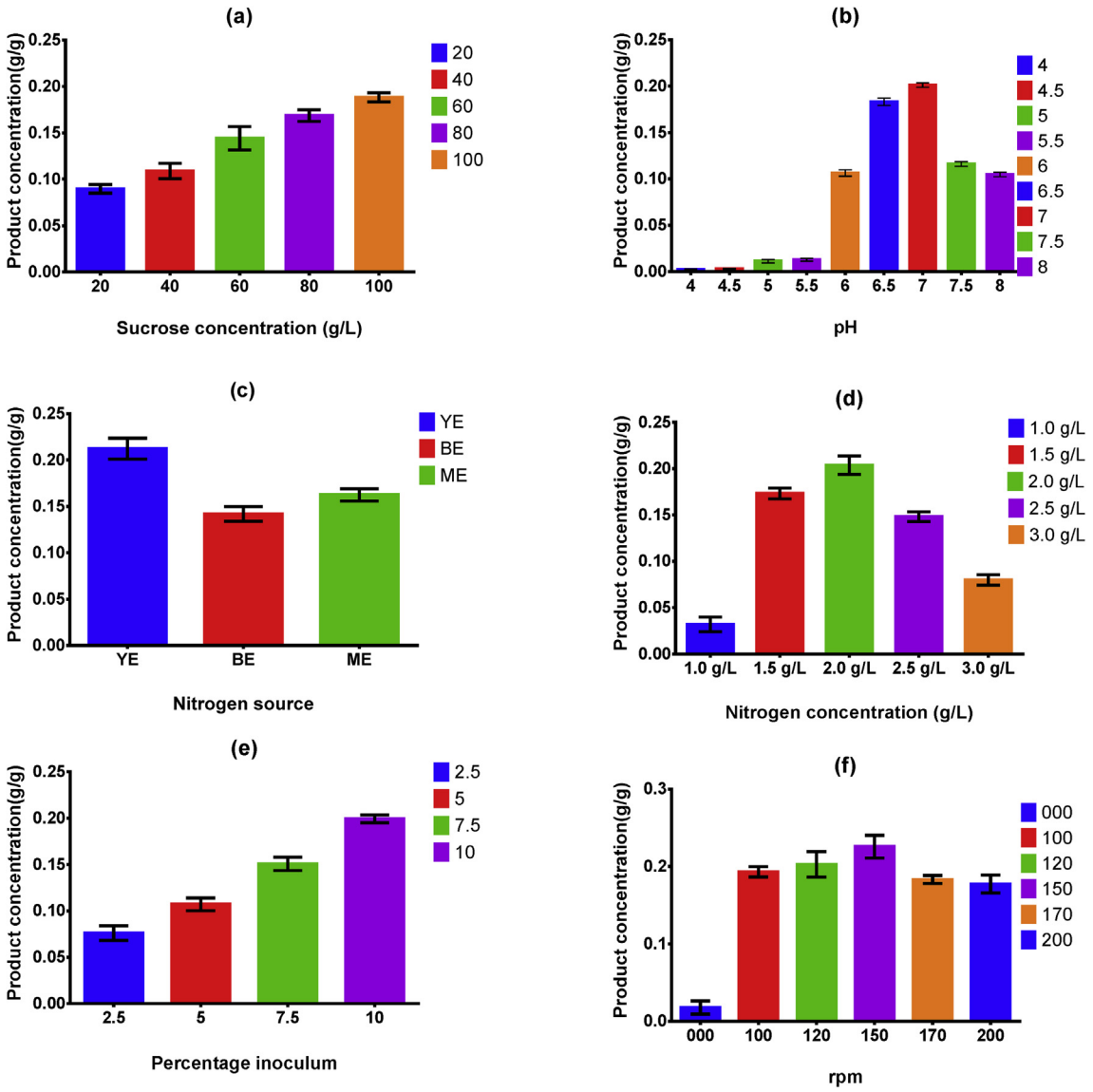


Fig. 2. Optimization of media composition and fermentation conditions for levan production. (a) Effect of sucrose concentration [20–100 g/L] (b) Effect of Initial pH [4 to 8] (c) Effect of Nitrogen source [Yeast Extract (YE), Beef Extract (BE), Malt Extract (ME)] (d) Effect of YE concentration [1–3 g/L] (e) Effect of percentage inoculum [2.5–10%] (f) Effect of RPM [0–200] Note 1. Levan precipitation conditions (Broth: Solvent ratio- 1(broth): 2 (ethanol), precipitation time – 24 h without pH adjustment).

et al., 2017). Twice the volume of absolute ethanol was added to the supernatant and the mixture was maintained at 10 °C for 24 h. At the end of 24 h, the mixture was centrifuged at 6000 RPM (1g = 1.417 RPM) at 4 °C for 20 minutes. The pellet was collected and it was washed twice with absolute ethanol, dried at room temperature overnight and weighed.

2.4. Optimization of levan recovery

Once the fermentation conditions for production of levan was optimized as mentioned above in section 2.3, optimization of downstream processing was carried out. Factors such as pH, choice of solvent, volume of solvent affecting levan yield were investigated in downstream processing study. Microorganism were cultured in a 2 L flask under optimal fermentation conditions (as identified in section 2.3) and the supernatant was collected and used for entire studies on evaluation of precipitation conditions (downstream processing study). To study the effect of pH on levan precipitation, ethanol was used at 1:2 supernatant to solvent ratio. Once the pH was optimized, the supernatant to solvent ratio on levan precipitation was performed at the optimized pH. Four solvents namely, ethanol, methanol, isopropanol and acetone were used at six different supernatant to solvent ratios 1:1, 1:2, 1:3, 1:4, 1:5, 1:6 at this pH.

2.5. Polymer identification

^{1}H – NMR analysis of the polymer were carried out in Bruker 500 MHz instrument at room temperature. ^{13}C – NMR analysis of the polymer were carried out in Bruker 300 MHz instrument. ^{1}H – NMR and ^{13}C – NMR samples were both prepared in D_2O. Total number of scans was 32 and 2048 for ^{1}H – NMR and ^{13}C – NMR, respectively. FTIR spectrum was recorded using Perkin-Elmer Spectrum One instrument, with a resolution of 5 cm^{-1}. Molecular weight was determined by GPC obtained using Waters HPLC system equipped with RI detector. Ultrahydrogel 1000 column was used with 0.6 mL/min 0.5 N sodium nitrite solution. Column and detector temperatures were maintained at 35 °C and 30 °C, respectively.

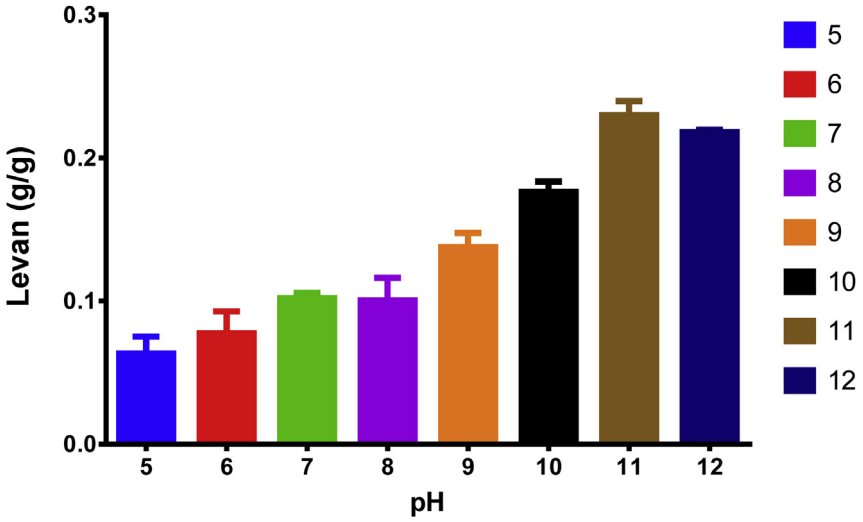


Fig. 3. Effect of pH on precipitation of levan. (Solvent – ethanol, Supernatant to Solvent ratio – 1: 2).

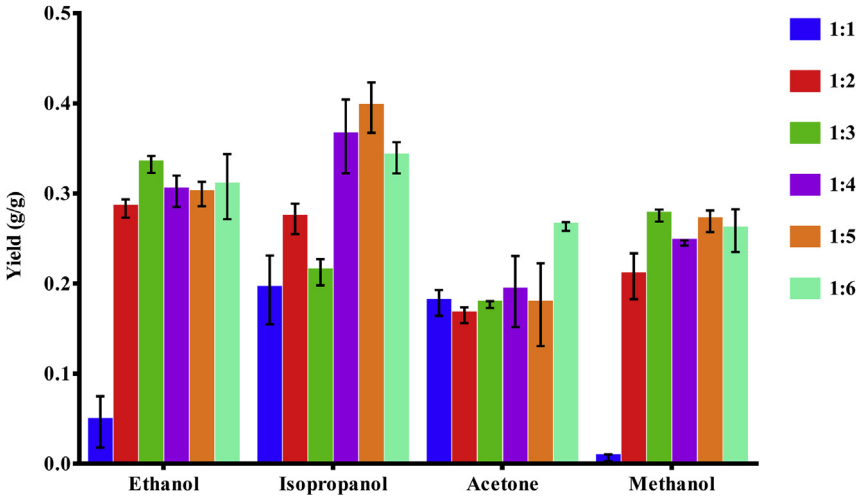


Fig. 4. Effect of various solvents and its ratio on precipitation of levan.

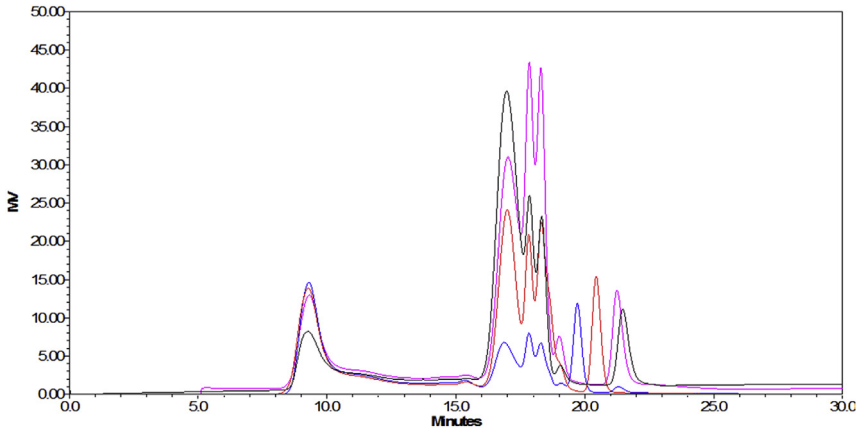


Fig. 5. Gel Permeation Chromatographs of levan obtained from different solvents (—Acetone, —Ethanol,—Methanol, —Isopropanol).

2.6. Cell culture and maintenance

The immortalized endothelial hybrid cell line EA.hy926 (kind gift from Dr. C.J.S. Edgell, University of North Carolina, Chapel Hill), cells were cultured with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), and 1 % penicillin/streptomycin antibiotics (w/v). The cells were maintained at 37 °C in a humidified atmosphere of 5 % CO_2.

Table 2
Comparison of molecular weight distribution of levan samples.

Solvent used for precipitation	Molecular weight of levan sample, kDa			
	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Ethanol	2887.7	10.14	5.50	3.85
Isopropanol	3008.6	10.32	5.40	3.82
Methanol	2841.5	11.07	5.48	3.90
Acetone	2790.0	9.96	5.42	3.91

2.7. Cytotoxicity analysis

Cytotoxicity of isolated levan compound was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Equal number of cells (1×10^5 cells/cm^2) was seeded in a 96-well plate. After 24 h incubation period, the cells were treated with different concentrations (C1 – 0.057 g/L, C2 – 0.142 g/L, C3 – 0.283 g/L, C4 – 0.425 g/L and C5 – 0.566 g/L) of levan compound for 24 h. N-acetylcysteine (NAC - 1000 μM) was used as positive control. After 24 h incubation, 1 mg/mL of MTT was added and further incubated for 3 h at 37 °C and DMSO was added to dissolve the formazan crystals. Absorbance was then measured at a wavelength of 570 nm. Microplate reader spectrophotometer (Synergy H1) was used to measure the absorbance.

3. Results and discussion

3.1. Growth curve

The growth pattern of *B. subtilis* was recorded along with levan production, and the trends are shown in Fig. 1. Exponential phase was observed between 6 h and 16 h. Production of levan steadily increased during exponential phase and reached a maximum during the stationary phase after 20 h. The yield of levan was found to decrease slowly after this. A similar trend was observed by Shih et al. (2010a,b) while studying levan production from *Bacillus subtilis*. They have reported that levan production increased gradually during exponential phase and reached a maximum after attaining stationary phase. Maximum levan produced under optimal conditions was reported to be 56 g/L (0.22 g levan/g of available sucrose). Srikanth et al. (2015) had reported a similar increasing trend during stationary phase while producing levan with *A. xylinum*. However, Srikanth et al. (2015) had reported a longer stationary phase (122 h). Typically maximum levan production is reported between 16 h and 60 h of fermentation depending on the organism used (Oner et al., 2016).

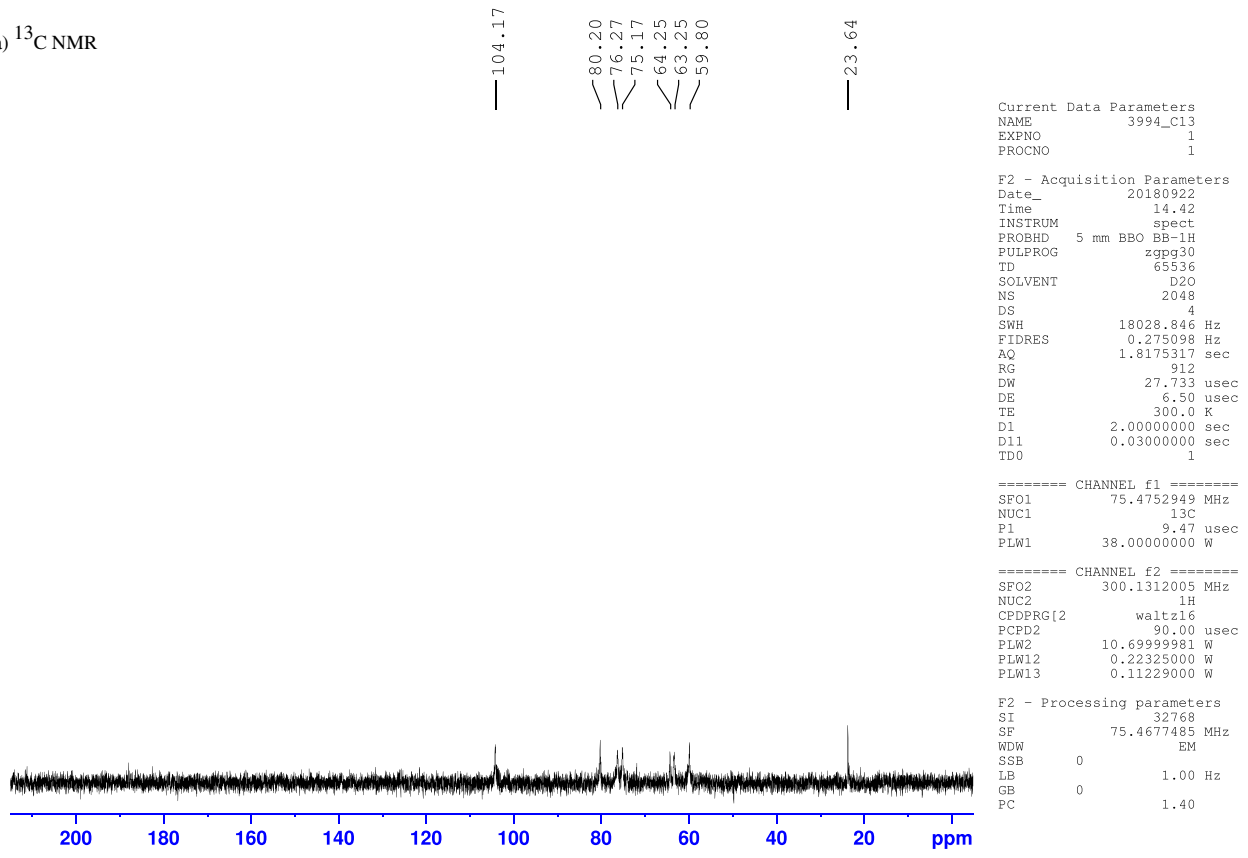
3.2. Effect of media composition and fermentation conditions on levan production

Sucrose is the main carbon source which plays a key role in the biosynthesis of levan and therefore, it is essential for the levan production. Effects of sucrose concentration, initial pH, nitrogen source and concentration of nitrogen source on levan production were studied by varying one factor at a time and the results are shown in Fig. 2. Steady increase in levan production was observed with increase in initial sucrose concentration from 20 g/L to 100 g/L (Fig. 2(a)). Thus, for further studies 100 g/L sucrose was used. Levan yield increased with increase in initial solution pH till yield reached a maximum at pH 7 and then decreased (Fig. 2(b)). The result was consistent with the previous literature

Table 3
Comparison of levan yield from literature.

Sl. No	Microorganism	Carbon source	Broth to solvent (Ethanol) ratio	Precipitation conditions (Temperature in °C, time in h)	Levan Yield	Reference
1	*Lactobacillus reuteri* LTH5448.	500 g/L Sucrose	1:3	4 °C, 10 h	(0.366 g/g) 183 g/L	Ni et al., 2018
2	*Saccharomyces cerevisiae*	Glucose + Sucrose	NA	-20 °C, 10 h		Franken et al., 2013
3	*Acinetobacter nectaris*, *Bacillus atrophaeus*	120 g/L, 180 g/L Sucrose	1:3	-20 °C, 25 h	0.025 g/g (3 g/L), 0.019 g/g (3.5 g/L)	Gonzalez-Garcinuno et al., 2017
4	*Bacillus subtilis* NATTO	400 g/L Sucrose	1:3	5 °C	0.279 g/g (111.6 g/L)	Dos santos et al., 2013
5	*Brenneria goodwinii*	Sucrose (50% w/v)	1:3	4 °C, overnight	0.37 g/g (185 g/L)	Liu et al., 2017
6	*Bacillus subtilis natto*	350 g/L sucrose	1:3	12 h	0.181 g/g (63.6 g/L)	Bersaneti et al., 2018
7	*Paenibacillus bovis sp.* nov BD3526	200 g/L	1:3	4 °C	0.181 g/g (36.25 g/L)	Xu et al., 2016
8	*Bacillus subtilis*	100 g/L	1:4	4 °C, 24 h	0.306 g/g (30.6 g/L)	Laddha and Chitanand, 2017
9	*Bacillus subtilis* (Natto) Takahashi	20 % sucrose	1:4		0.247 g/g (49.4 g/L)	Shih et al., 2005
10	*Bacillus lentus* V8 Strain	250 g/L of sucrose	1:5	1 h	0.23 g/g (57.95 g/L)	Abou-taleb et al., 2015
11	*Bacillus subtilis* NRC 108	50 g/L sucrose	1:4		0.21 g/g (10.5 g/L)	Ghoneim et al., 2016
12	*Bacillus subtilis* (natto) Takahashi	250 g/L of sucrose	1:4		0.224 g/g (56 g/L)	Shih et al., 2010a
13	*Bacillus subtilis* Natto CCT 7712	300 g/L of sucrose	1:3	4 °C, 12 h	0.65 g/g (195.51 g/L)	Goncalves et al., 2013
15	*Bacillus subtilis* (natto) Takahashi	200 g/L of sucrose	1:4		0.445 g/g (89 g/L)	Shih et al., 2005
16	Immobilized *Bacillus subtilis* natto on Ca-alginate gel	200 g/L sucrose	NA		0.353 g/g (70.6 g/L)	Shih et al., 2010b
17	*Halomonas sp.* AAD6	50 g/L sucrose	1:1	-18 °C, overnight	0.036 g/g (1.844 g/L)	Poli et al., 2009
18	*Zymomonas mobilis* strain ZAG-12	250 g/L sucrose	NA		0.0586 g/g (14.67 g/L)	Melo et al., 2007
19	*Bacillus subtilis* MTCC 441	100 g/L sucrose	1:5		0.30 g/g	Present study

(a) ^{13}C NMR



(b) ^{1}H NMR

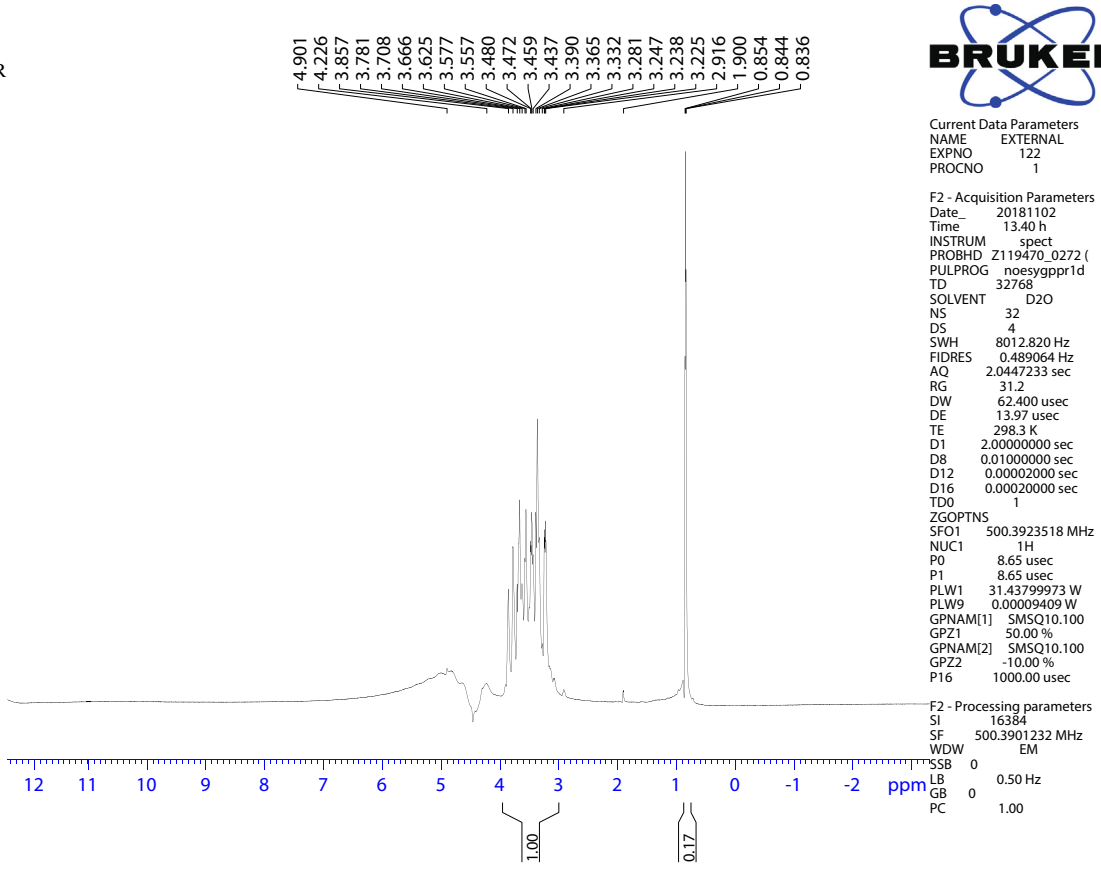


Fig. 6. (a) ^{13}C-NMR (b) ^{1}H-NMR of levan synthesized using *B. subtilis* MTCC 441.

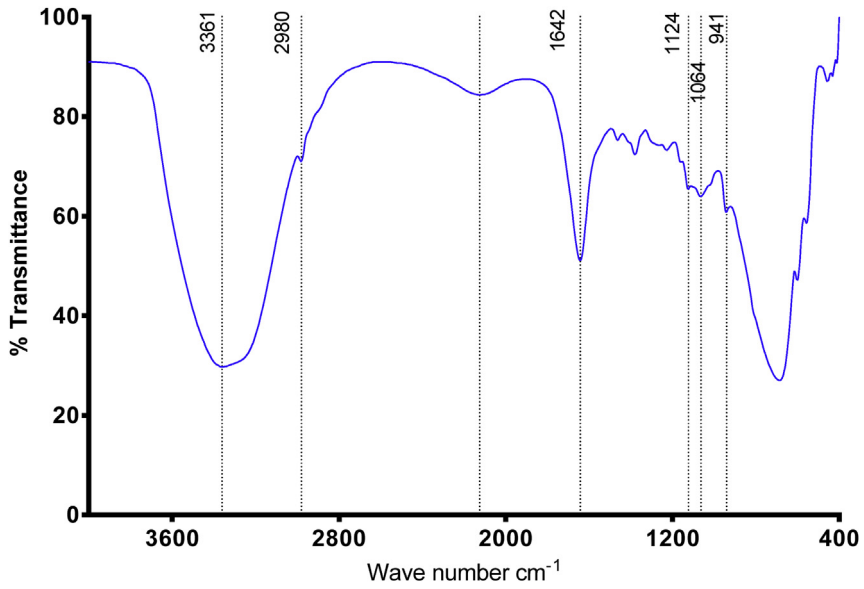


Fig. 7. FT-IR spectrum of the levan sample obtained from *B. subtilis* MTCC 441.

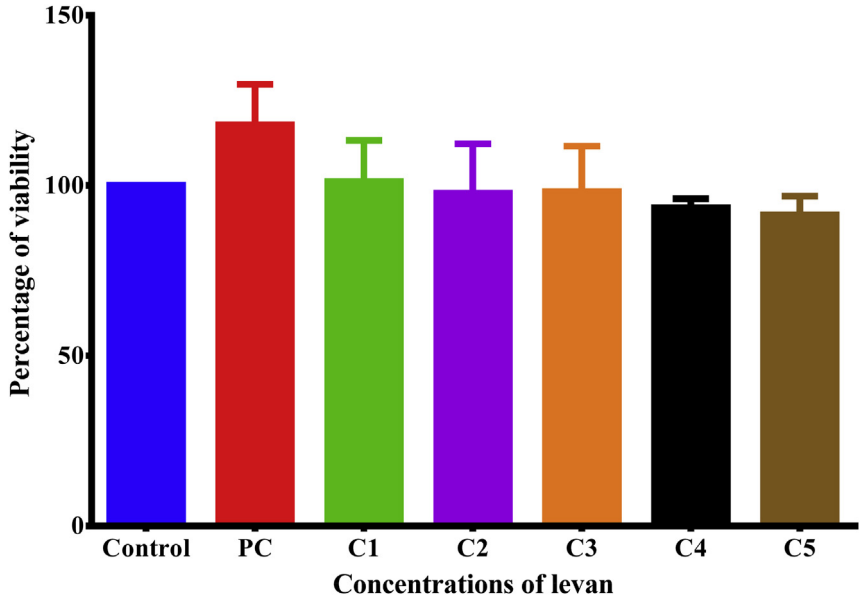


Fig. 8. Levan effect on cell viability: The cytotoxic effect of levan at different concentrations of levan (C1 – 0.057 g/L, C2 – 0.142 g/L, C3 – 0.283 g/L, C4 – 0.425 g/L and C5 – 0.566 g/L) on endothelial cells was measured by MTT assay. After 24 hour of treatment, cell viability was performed by using MTT. The experiment was repeated three times and the values are represented as mean ± SEM. N-AcetylCysteine (NAC) was used as a positive control (PC).

(Abou-taleb et al., 2015). Levansucrase, an extracellular enzyme, is responsible for levan synthesis. Levansucrase breaks down sucrose and polymerize fructose simultaneously to synthesize levan. Levansucrase exhibits hydrolytic activity along with transfructosylation activity. Typically in the pH range of 5–7, transfructosylation activity is dominant and levan accumulation reaches a maximum level. Beyond this pH range hydrolytic activity is predominant. Below pH 4 and above pH 9 transfrutosylation activity is suppressed almost completely (Liu et al., 2017). Among the three nitrogen sources, yeast extract was found to be the best supporter for levan production (Fig. 2(c)). Similar observations were reported by previous researchers. Silbir et al. (2014) compared various nitrogen sources yeast extract, corn steep liquor, peptone, tryptone, malt sprouts, and urea and reported that yeast extract was the best among them yielding maximum levan compared to other nitrogen sources. Once YE was chosen among the three nitrogen sources YE, BE and ME, concentration of YE was varied to determine the optimum YE concentration. The optimum YE concentration was found to be 2 g/L (Fig. 2(d)). Levan yield increased with inoculum size (Fig. 2(e)) and best results were obtained with 10% inoculum size. Abou-taleb et al. (2015) also reported that 10% inoculum size resulted in maximum levan yield using *Bacillus lentus* V8 strian. Agitation speed (RPM) did not affect levan yield significantly (Fig. 2(f)). Under optimum fermentation conditions, sucrose – 100 g/L, fermentation time – 20 h, Yeast extract – 2 g/L, inoculum size – 10 % (v/v), initial pH – 7 and orbital shaker speed – 150 RPM levan yield of about 0.22 g/g was obtained by ethanol precipitation. For subsequent optimization of downstream processing (solvent precipitation) these fermentation conditions were used.

3.3. Optimization of precipitation condition

3.3.1. Effect of pH on levan precipitation

pH plays an important role in the precipitation of levan. Supernatant pH of the solution was adjusted to desired level by adding either acid or alkali. It is evident from Figs. 2 and 3 that the levan precipitatation was affected addition of acid/alkali to the broth. Levan precipitation increased with increase in pH and maximum levan precipitate (0.2298 g/g) was obtained at a pH of 11 (Fig. 3). Further increase in pH beyond 11 did not affect precipitation significantly and therefore for subsequent studies pH 11 was used.

Since there are no reports available on effect of solution pH levan precipitation, one to one comparison is not available. The trend obtained, after pH adjustment of the broth, may be attributed to the ionic strength in the solution and the surface charge of levan. However, this warrants more detailed investigation.

3.3.2. Screening of various solvents

Solvents such as methanol, ethanol, iso-propanol, and acetone were used for precipitation of levan from the fermentation broth. Desired solvent was added to the supernatant solution in the ratios of 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 (v/v) and the precipitation results are shown in Fig. 4. It can be seen from the figure that isopropanol was the best among the four solvents considered.

At a ratio of 1:5 (supernatant to isopropanol v/v ratio), maximum levan yield of 0.395 g/g was obtained equivalent to 79 % maximum theoretical yield based on available fructose. The amount of levan precipitated by isopropanol was approximately 20 % higher than the maximum obtained with ethanol. The moisture content of the sample was determined by drying the sample at 55 °C in a hot air oven until constant weight was obtained. The moisture content was found to be 6.36 $\pm$ 0.66%. Advantage of using isopropanol as an anti-solvent is that, it can be readily separated from the aqueous layer with addition of a suitable salt. Thus, recovery and reuse of the solvent is cost effective. In addition, there are stringent regulations with the supply and utilization of ethanol in few countries and the price is comparatively higher compared to isopropanol.

GPC results of the samples are shown in Fig. 5. Levan obtained by precipitation using different solvents had similar molecular weight distribution. All four samples contained had both low molecular weight and high molecular weight fractions as shown in Fig. 5. These results are consistent with the previous results (Shih et al., 2010a,b). A comparison given in Table 2 confirm that the molecular weight distribution of the products were identical. Molecular weight of the product was in the range of 2790–3010 kDa for the heavy fraction and 3.8 kDa–9.96 kDa for light fraction. A comparison of levan yield obtained from microbial fermentation with other solvents, mainly ethanol, is given Table 3.

3.4. Characterization of levan

^{13}C NMR peak shown in Fig. 6a confirms to the structure of levan. Six carbon shifts were obtained at 104.17 ppm, 80.20 ppm, 76.27 ppm, 75.17 ppm, 63.25 ppm, and 59.80 ppm. The results are consistent with the earlier reports. The signals observed at chemical shifts 104.17 ppm and 63.25 ppm are attributed the β - (2–6) linkages of levan (Zhang et al., 2014; Ni et al., 2018). ^{1}H-NMR peak (Fig. 6b) was observed in the chemical shift range of 3.225–3.857 ppm which was in accordance with the previous reports by Angeli et al. (2009) and Shih et al. (2005).

FT-IR spectra of levan (Fig. 7) showed O–H stretching at 3361 cm^{-1} and C–H stretching at 2980 cm^{-1} (Srikanth et al., 2015). Peak at 1642 cm^{-1} was due to C=O stretching (Singh & Kumar, 2013). The peaks observed at 1124 cm^{-1} and 1064 cm^{-1} represents the pyranose form of sugars. The region between 900 cm^{-1} and 1200 cm^{-1} corresponds to the fingerprint region are unique characteristic pattern for polysaccharide functional groups (Srikanth et al., 2015; Mamay et al., 2015).

3.5. Effect of levan on endothelial cells

The cytotoxic effect of levan compound on endothelial cell viability was assessed using MTT assay (Fig. 8). Statistical analysis on MTT assay results confirm that no significant cytotoxic effect was observed in the levan concentration range studied (0.057 g/L to 0.566 g/L) ($p < 0.05$).

4. Conclusion

Production of levan, a commercially important microbial polysachharide, by *Bacillus subtilis* MTCC 441 from sucrose was optimized. Optimum conditions for fermentation were found to be sucrose – 100 g/L, fermentation time – 20 h, amount of yeast extract – equivalent to 2 g of elemental nitrogen/L, inoculum size – 10 % (v/v), initial pH – 7, orbital shaker speed – 150 RPM. For the separation and purification of levan, solvent precipitation was employed. Factors affecting precipitation yield namely, precipitation pH choice of solvent and supernatant to solvent ratio were optimized to maximize levan recovery. Among four solvents considered, isopropanol was found to yield maximum recovery of the product. At a supernatant to solvent ratio of 1:5, maximum levan yield of 0.395 g/g of sucrose was obtained. This was about 1.2 times higher than the yield obtained with ethanol from the same fermentation broth. Structure of the product synthesized was confirmed with the help of ^{1}H-NMR and ^{13}C-NMR spectra. The cytotoxic effect of levan on endothelial cells was measured by MTT assay and no cytotoxic effect was observed upto a levan concentration of 0.566 g/L.

Declarations

Author contribution statement

Venkatachalam Ponnusami: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

C.A.C Jothi Sailaja: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

V Bhuvaneshwari: Performed the experiments; Analyzed and interpreted the data.

Kartik Kumar Sarwareddy: Performed the experiments.

Krishna Priya Mani: Conceived and designed the experiments; Analyzed and interpreted the data.

Saravanan Ramiah Shanmugam: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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