



Production and characterization of a high molecular weight levan and fructooligosaccharides from a rhizospheric isolate of *Bacillus aryabhatai*



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ABSTRACT

Bacillus aryabhatai GYC2-3 strain, capable of synthesizing EPS, was isolated from the rhizosphere of *Taraxacum* spp. plant. The EPS was identified as levan by ¹³C NMR spectroscopy and methylation analysis. The levan produced had a high weight average molecular weight of 5.317×10^7 Da and degree of branching 5.19%. Thin layer chromatography and high-performance anion-exchange chromatography revealed that it is capable of producing broad range of fructooligosaccharides (FOS) and higher levels of FOS synthesis were observed with increasing sucrose concentrations. The optimum temperature, initial pH and sucrose concentration for levan biosynthesis were studied to be 30 °C, 8.0 and 250 g/L, respectively. Levan production was increased by ~38% (26 g/L) when provided aeration as compare to static cultures. To the best of our knowledge, this is the first report on biosynthesis of levan and FOS by a *B. aryabhatai* strain.

1. Introduction

Levan is a fructose homopolysaccharide that is produced naturally by several microorganisms and few plant species (Öner, Hernandez, & Combie, 2016). Microbial exopolysaccharides (EPS) are of particular interest due to their diversity in chemical/structural composition, rheological and physical properties (Vu, Chen, Crawford, & Ivanova, 2009). Structurally, microbial levan is composed of D-fructofuranosyl groups linked to each other by β-(2, 6) bonds in the main chain and by β-(2, 1) bonds at the branches (Han, Xu, Gao, Liu, & Wu, 2016; Srikanth, Reddy, Siddhartha, Ramaiah, & Uppuluri, 2015). Structural diversity in levan from different sources arises from the number of D-fructofuranosyl units per chain i.e. degree of polymerization (DP) and branching pattern of the repeating units. The extracellular enzyme named as levansucrase (EC 2.4.1.10) is responsible for levan synthesis in microorganisms using sucrose as a substrate. Besides extensive studies on enzymatic levan synthesis, its production has also been reported by whole cells of several diverse types of bacteria such as *Lactobacillus reuteri* 121 (van Geel-Schutten, Flesch, Ten Brink, Smith, & Dijkhuizen,

1998), *Bacillus subtilis* (Shih, Yu, Shieh, & Hsieh, 2005), *Halomonas smyrnensis* (Erkorkmaz, Kirtel, Duru, & Öner, 2018) *Paenibacillus polymyxa* EJS-3 (Liu et al., 2010), *Lactobacillus gasseri* DSM 20077 (Anwar et al., 2010), *Brachy bacterium* sp. CH-KOV3 (Djurić et al., 2017) and *Bacillus licheniformis* BK AG21 (Wahyuningrum & Hertadi, 2015).

Like high DP levan polysaccharides, fructooligosaccharides (FOS) have also gained considerable interest during recent years because of their ability to modulate the activity of colonic microbiota (Li et al., 2014), being non-cariogenic, low caloric sweeteners (Wiebe et al., 2011) and ability to improve intestinal immune responses by inhibiting pathogen multiplication (Liu, Gibson, & Walton, 2016; Sivieri et al., 2014). Therefore, microorganisms that synthesize FOS in addition to other types of EPS could be of high commercial interest. *Bacillus* species have been known to produce a wide range of biologically significant exopolysaccharides e.g. levan (Zhang et al., 2014), curdlan (Gummadi & Kumar, 2005) and uncommon sugars (Kodali, Das, & Sen, 2009).

In this paper, a novel *Bacillus aryabhatai* GYC2-3 strain capable of synthesizing levan-type EPS was isolated from rhizosphere. The EPS was characterized and optimum conditions were worked out for its

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maximum yield by the growing cells of the isolate. To the best of our knowledge, this is the first report on production of levan and FOS by a *B. aryabhatai* GYC2-3 strain.

2. Materials and methods

2.1. Screening and identification of microorganisms

Rhizospheric soil of a wild *Taraxacum* spp. plant collected from uncultivated land in Faisalabad, Pakistan (31.3980° N, 73.0257° E) was suspended in sterilized saline water (0.9% w/v NaCl). Serial dilution and spread-plate methods were used for obtaining different microorganisms. The screening GYC medium contained (g/L): Glucose 50, yeast extract 10, calcium carbonate 5 and 2% agar supplemented with 20% sucrose (pH 6.8 ± 0.2). The plates were incubated at 37 °C for 16 h and screening was conducted based on slimy and mucoid appearance of colonies, indicating EPS production from sucrose. Genomic DNA of the isolate was extracted using Thermo Scientific GeneJET Genomic DNA Purification kit (#K0721). The 16S rRNA gene sequence was amplified using universal primers FD1 (5'-AGAGTTTGATCCTGGC TCAG-3') and rP1 (5'-ACGG(ACT)TACCTTGTACGACTT-3'), as reported previously (Akhtar, Ghauri, Iqbal, Anwar, & Akhtar, 2008). The fragment was cloned in pTZ57R/T vector using InsTAclone PCR cloning kit (Thermo Scientific). Cloned product was sequenced by Macrogen, Korea and analyzed using the BLASTn program (<http://www.ncbi.nlm.nih.gov/BLAST/>) to find the matching sequences.

2.2. Production and purification of exopolysaccharide

The EPS was produced by growing the isolate on GYC broth (supplemented with 20% sucrose) in an incubator at 37 °C for 120 h. The culture was centrifuged at 6000 × g for 10 min and supernatant was separated. The proteins in the polysaccharide were precipitated by adding trichloroacetic acid (TCA) at a final concentration of 14% (w/v) to the supernatant followed by shaking incubation (50 rpm at 23 °C for 40 min) and centrifugation at 10,000 rpm for 10 min at 4 °C (Abid et al., 2019). Subsequently, EPS was purified by ethanol precipitation according to the procedure reported earlier (Anwar et al., 2010).

2.3. Identification and characterization of the product

2.3.1. Thin layer chromatography (TLC)

Supernatant (2 µL) of the bacterial culture incubated for 120 h at 37 °C was analyzed by TLC plates (Silica gel 60 F₂₅₄; Merck) and run for about 6 h in a mobile phase consisting of butanol/ethanol/water (5:5:3). To detect the fructose containing compounds, TLC plate was air-dried and sprayed with a urea developing solution (100 mL water saturated butanol, 3.0 g urea, 5.9 mL phosphoric acid, 5 mL ethanol) followed by heating at 120 °C for 15 min (Trujillo Toledo et al., 2004). Fructan, FOS and fructose spots were detected under visible light.

2.3.2. ¹³C nuclear magnetic resonance (NMR) spectroscopy

Purified polymer was dissolved in dimethyl sulfoxide (DMSO) and ¹³C NMR spectra were recorded at 75 MHz on a 300 MHz NMR spectrometer (Avance AV-III). Chemical shifts of ¹³C in ppm were determined relative to DMSO peak at 39.51 ppm. Carbon spectra were recorded in 38 K datasets, with a spectral width of 18 kHz.

2.3.3. Methylation analysis

Fructan sample was derivatized to its partially methylated alditol acetates (PMAA) form following the method described by Pettolino and coworkers with modifications (Pettolino, Walsh, Fincher, & Bacic, 2012). Briefly, 0.8 mg of freeze-dried sample was dissolved in methanol and dried under nitrogen purging to remove moisture. Methylation was performed in 75 µL of NaOH/dimethylsulfoxide (120 mg/mL) solution by sequentially adding 70 µL of CH₃I and sonicating for 40 min. The

permethylated sample was extracted using 500 µL of CH₂Cl₂, washed with 1000 µL of dI water for three times, and dried under nitrogen purging. Hydrolysis of the methylated fructan was carried out using 100 µL of 2M trifluoroacetic acid at 121 °C for 90 min. The hydrolyzed residues were reduced using 50 µL of 1M NaBD₄ in 2M ammonium hydroxide solution. Acetylation of the partially methylated alditols was done by adding 250 µL of acetic anhydride and incubation at 100 °C for 2.5 h. The PMAA residues were extracted by 1000 µL of CH₂Cl₂, washed with 2000 µL of dI water for three times, and dried under nitrogen purging. The derivatized fructan residues were dissolved in 500 µL of acetone and analyzed by a GC-FID-MS (7890A and 5975C inert MSD with Triple-Axis detector, Agilent Technologies, Inc., Santa Clara, CA, USA) that was coupled with a Supelco capillary column (SP-2330, Sigma-Aldrich Inc., St. Louis, MO, USA).

2.3.4. High-pressure anion-exchange (HPAE) chromatography

Bacterial strain was cultured in GYC medium and incubated at 37 °C for 120 h, as described above. Oligosaccharides produced were separated and analyzed by HPAE chromatography with integrated pulsed amperometric detection (HPAEC-IPAD) as described by (Vergauwen, Van den Ende, & Van Laere, 2000). Fructose, glucose, sucrose, 1-kestose, 6-kestose, neokestose and nystose were used as standards.

2.3.5. Molecular weight analysis

Molecular weight of levan sample was determined by multi-angle laser light scattering-gel permeation chromatography (MALLS-GPC) according to the method described in previous published literature (Erkorkmaz et al., 2018).

2.4. Optimal conditions of levan biosynthesis

A step by step optimization process concerning the effect of important parameters (temperature, pH, substrate concentrations and aeration) on levan production by the isolate was carried out under static culture conditions. Variations in different parameters of the medium were also included accordingly and each test was performed in duplicate.

To determine the effect of incubation temperature on levan production, GYC medium supplemented with 20% sucrose unless otherwise specified (pH 6.8 ± 0.2) was incubated at 20 °C, 25 °C, 30 °C, 37 °C and 45 °C for 120 h. The effect of pH on levan production was studied over a pH range of 4.0, 5.0, 6.0, 7.0 and 8.0 at 30 °C for 120 h. For this purpose, pH of the medium in each Erlenmeyer flask was adjusted to the desired value by adding acid or base (2M) before sterilization. To determine the effect of sucrose concentration on levan production, medium was supplemented with 10%, 15%, 20%, 25% and 30% (w/v) sucrose. To find out the effect of aeration rate on polymer production, the experiment was set up with two different approaches i.e. incubation in a shaker at 150 rpm or in a still incubator. Each flask was inoculated with 5% (v/v) inoculum containing 1 × 10⁶ CFU/mL and incubated at 30 °C for 120 h unless otherwise specified.

After 120 h of incubation, cell density in each flask was determined by measuring OD at 600 nm. The cultures were centrifuged to remove the cells and the supernatant was treated with TCA as described above to remove proteins. Finally, the polymer was ethanol precipitated, freeze-dried and weighed.

3. Results and discussion

3.1. Isolation and identification of bacterial strain

Rhizospheric soil sample was screened for the acquisition of EPS synthesizing bacteria. Among the isolates obtained this way, GYC2-3 was selected for further studies. Colonies of the isolate GYC2-3 exhibited slimy appearance on semi-solid GYC-sucrose-agar plates due to EPS synthesis. The slimy texture is attributed to the production of levan,

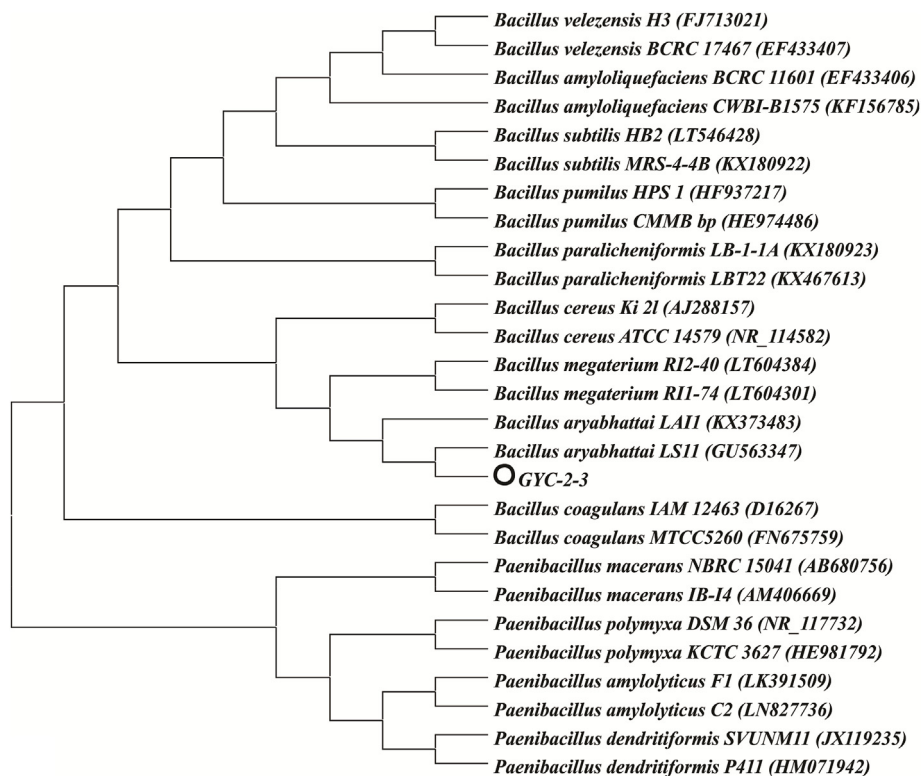


Fig. 1. Phylogenetic tree obtained on the basis of 16S rRNA gene sequence of the isolate GYC 2–3 with the other members of related bacteria.

a product of extracellular levansucrase which synthesizes large amount of levan using sucrose as a substrate (Belghith, Dahech, Belghith, & Mejdoub, 2012). Microscopic examination of the cells exhibited that these were non-motile rods. Molecular identification through BLASTn search revealed that isolate GYC2-3 had 100% homology with *B. aryabhatai* and partial 16S rRNA gene sequence of GYC2-3 was deposited to GenBank with the Accession Number KX180925. The phylogenetic tree analysis also showed that the isolate GYC2-3 closely clusters with *B. aryabhatai* strains (Fig. 1) and was therefore confirmed as *B. aryabhatai*. Though, levan synthesis has been reported in several *Bacillus* species (Abid et al., 2019; Belghith et al., 2012; Bersaneti, Pan, Baldo, & Celligoi, 2017; M.; Li et al., 2015), we hereby report first time its production by a *B. aryabhatai* strain.

3.2. Identification and characterization of the fructan products

The fructan polymer synthesized by GYC2-3 was analyzed by ^{13}C NMR spectroscopy at 150 MHz. The ^{13}C NMR spectrum (Fig. 2) of the purified product showed six broad resonance signals at 106.89 (C2), 82.98 (C5), 79.03 (C3), 77.91 (C4), 66.07 (C6), 62.65 (C1) ppm. The carbon chemical shifts were characteristic of β -configured fructofuranose units, as found in comparison with carbon chemical shifts of the standard methyl glycoside (Bock & Pedersen, 1983). These values were in accordance with the previous literature which confirmed the product as being levan (Poli et al., 2009; Xavier & Ramana, 2017).

To identify the glycosidic linkages of fructan, the PMAA derivatives were analyzed by GC-FID-MS. Identification was performed by comparing MS fragments, published literatures (Dong et al., 2015) and database offered by the Complex Carbohydrate Research Center (www.ccrcc.uga.edu). As shown in Fig. 3, mass spectra obtained from the breakdown of gas chromatogram peaks (Supplementary Fig. S1) showed the presence of three fructofuranosyl residues. Mass spectrum obtained with the lowest retention time (Fig. 3A) showed the presence of terminal (non-reducing) Fruf units, resulted from 2,5-di-O-acetyl-1,3,4,6-tetra-O-methyl-D-mannitol/glucitol which are representatives

of fructose at the extreme of fructan chains. GC-MS analysis confirmed the presence of > 80% 2,5,6-tri-O-acetyl-1,3,4-tri-O-methyl-D-mannitol/glucitol derivatives (Fig. 3B), which represents 6-Fruf linkages indicating the primary linkage backbone of fructan chains. Derivatives from 6-Fruf linkages yielded ions of m/z 162 and m/z 189 typical of levan-type fructan. Furthermore, the fragmentation pattern obtained from the mass spectrum of Fig. 3C correspond to the derivative of 1,2,5,6-tetra-O-acetyl-3,4-di-O-methyl-D-mannitol/glucitol, which confirms the presence of 1,6-Fruf linkages. Presence of 1,6-Fruf linkages represent fructose at the branching points of fructan chains. The reduction at C-2 of fructose was performed by using NaBD_4 which allowed the discrimination between 6-Fruf and 1-Fruf (Poli et al., 2009). Molar ratios based on the abundance were calculated for t-Fruf (12.53%), 6-Fruf (82.28%) and 1,6-Fruf (5.19%). Taken altogether, PMAAs indicate 6-Fruf linked backbone with minor 1,6-Fruf linkage at branching points, thereby confirming a levan-type fructan. These outcomes are similar to the mass spectra and fragmentation patterns from a levan-type molecule isolated previously from *Curcuma kwangsiensis* (Dong et al., 2015).

Thin layer chromatographic and HPAEC-PAD analysis was carried out to detect various oligosaccharides synthesized by GYC2-3 at different sucrose concentrations. In this regard, cell-free culture broth samples taken at different growth time intervals were run on TLC plate and the component spots were visualized by urea development solution, which is specific for fructose containing carbohydrates (Trujillo Toledo et al., 2004). The chromatogram developed with this solution clearly showed the presence of fructan and a wide range of FOS in the culture broth of the isolate (Fig. 4). The identity of FOS was further confirmed by HPAEC-PAD analysis (Fig. 5). Apparently, the production of these oligosaccharides increased with increasing sucrose concentration from 1% to 25% and incubation time from 24 h to 120 h (Supplementary Fig. S2). The fructan products from this isolate also showed a unique FOS pattern on TLC plate (Fig. 4). Normally, distinct equidistant spots are obtained on TLC plates for FOS differing in one fructosyl unit such as those observed for inulin synthesizing *L. gasseri* DSMZ 20604 strain

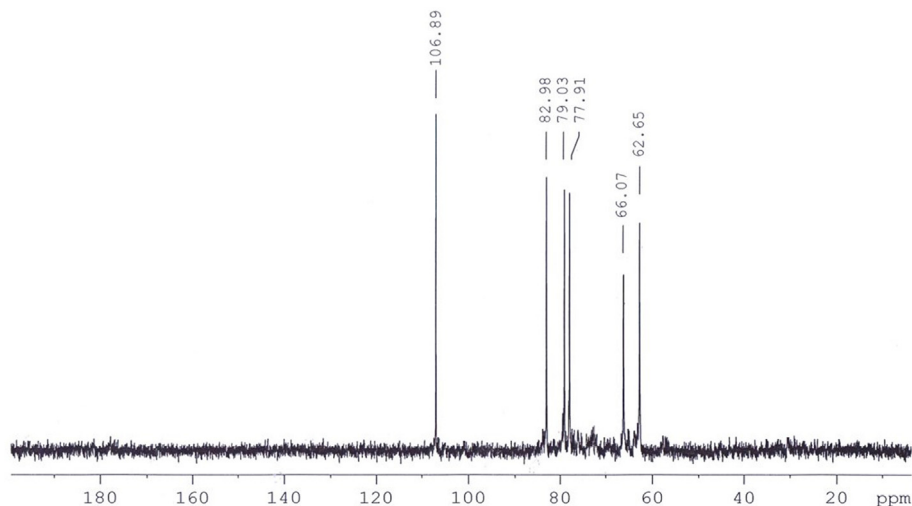


Fig. 2. The ^{13}C NMR spectrum of levan synthesized by the isolate GYC 2-3 as recorded in DMSO. Chemical shift values are given in parts per million (ppm) relative to the signal ($\delta = 39.51$) of DMSO.

(Anwar et al., 2010). However, in the present study a complex TLC pattern was observed with several unidentified pairs of adjacent spots. Further confirmation by HPAEC-PAD analysis showed peaks eluting at the same retention times as 1-kestose, 6-kestose, neokestose and nystose references peaks (Fig. 5). Pairs of adjacent peaks can also be observed in the chromatogram possibly corresponding to the pairs of adjacent spots on TLC plate. Based on the relative location of the 6-kestose and nystose peaks, these pairs of peaks could most likely be assigned to longer DP FOS and their corresponding neo-series analogues. FOS have gained remarkable importance due to their influential prebiotic role associated to the positive and beneficial effect on gut microbiota, reducing the incidence of gastrointestinal tract infections and possessing bifidogenic effects. Due to their health beneficial roles, a plethora of studies have been conducted for their synthesis using whole cells of bacteria and

fungi or their enzymes (Picazo et al., 2019; Öner et al., 2016). Here, we report the synthesis of a wide range of FOS by the growing cells of *B. aryabhattai* GYC2-3. The longer DP FOS produced by this isolate are comparable to those reported for *Bacillus amyloliquefacians* and named as oligolevan by the authors (M. Li et al., 2015). It is likely that synthesis of wide range of FOS by GYC2-3 follows the non-processive mechanism, in which the products are released from the enzyme after each fructosyltransferase, resulting in the accumulation of intermediates in the medium (Kralj, Buchholz, Dijkhuizen, & Seibel, 2008; Ua-Arak, Jakob, & Vogel, 2017). It has also been reported that the fructan product size distribution could be modulated by the fermentation conditions (Ua-Arak et al., 2017).

The value of average molecular weight of GYC2-3 was determined by MALLS-GPC technique. The weight-average molecular weight (M_w)

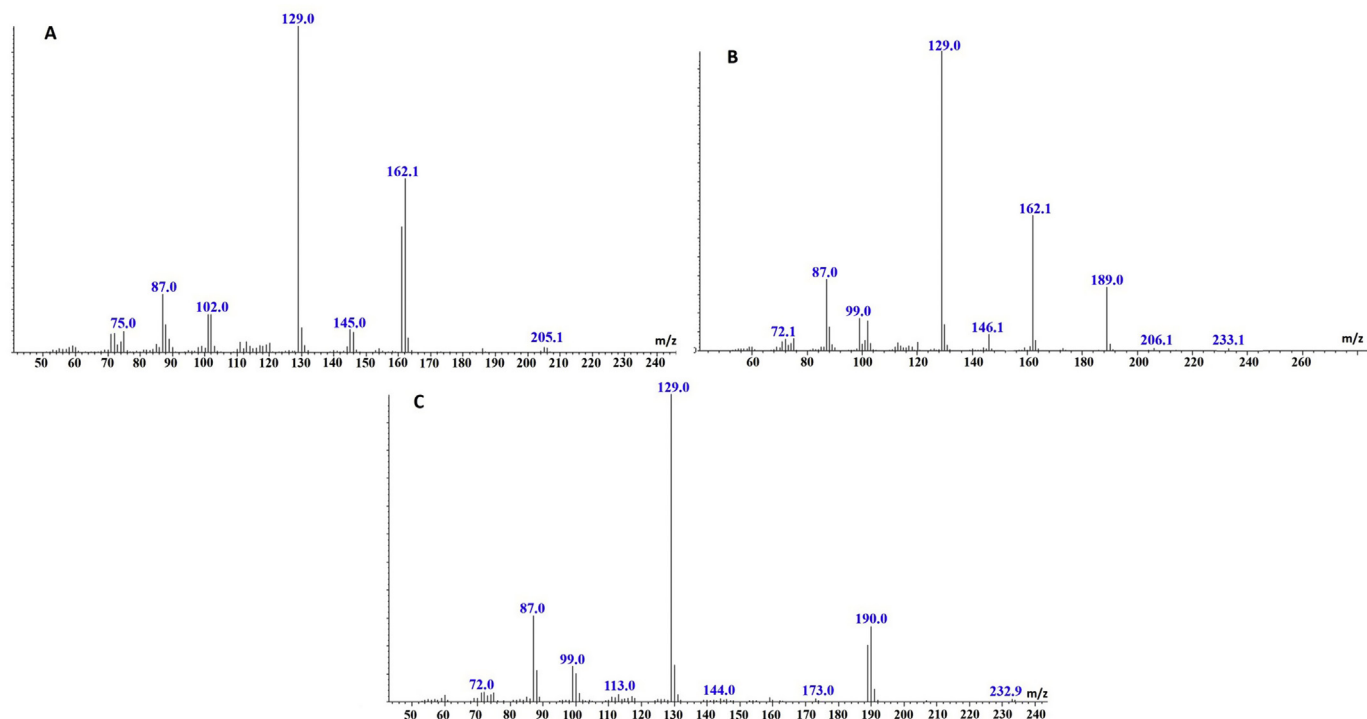


Fig. 3. Mass spectra of partially methylated anhydroalditol acetates. (A) 2,5-di-O-acetyl-1,3,4,6-tetra-O-methyl-D-mannitol/glucitol; (B) 2,5,6-tri-O-acetyl-1,3,4-tri-O-methyl-D-mannitol/glucitol; (C) 1,2,5,6-tetra-O-acetyl-3,4-di-O-methyl-D-mannitol/glucitol.

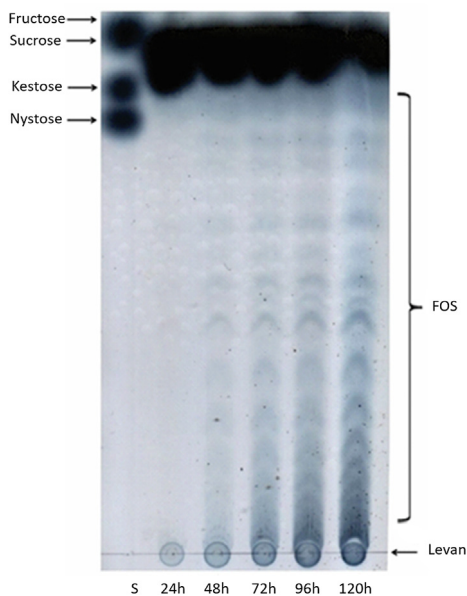


Fig. 4. Thin layer chromatographic analysis of the product synthesized by the isolate GYC 2-3. Abbreviation S stands for standards.

and the number-average molecular weight (M_n) were found to be 5.317×10^7 Da and 4.815×10^6 Da, respectively.

Levans from gram-positive bacterial genera exhibit molecular weights in the range of 10^4 to 10^7 Da e.g. levan from *B. licheniformis* 8-37-0-1 and *B. licheniformis* NS032, molecular weights of 2.826×10^4 Da and 5.82×10^6 Da were reported (Gojgic-Cvijovic et al., 2019; C.; Liu et al., 2010). Levans from some gram-negative bacteria are noticeable for their exceptionally high molecular weights e.g. levan from *Brenneria* sp. EniD312 was reported for a molecular weight of 1.41×10^8 Da (Xu et al., 2018) and *Kozakia baliensis* exhibited a molecular weight of 2.466×10^9 Da (Jakob et al., 2013).

The polydispersity (M_w/M_n) of levan from GYC2-3 was calculated to be high (11.041) indicating a wide distribution of molecules, which exhibited different molecular weights. Previously, high dispersity index of levan molecules was observed in *Zymomonas mobilis* strains ranging between 14 and 16.2 (Calazans, Lima, de França, & Lopes, 2000) and *B. licheniformis* NS032 (6.22) (Gojgic-Cvijovic et al., 2019).

3.3. Optimal conditions for levan biosynthesis

The economic and improved production of polymer is important for

inexpensive production and utilization at industrial scale. Therefore, some important process parameters such as aeration, initial pH, temperature and sucrose concentration were optimized to obtain maximum levan yield by the isolate GYC2-3 cells.

3.3.1. Optimum temperature

The effect of incubation temperature is a critical factor for levan biosynthesis by bacteria. In case of GYC2-3, it was found that when temperature varied over a wide range from 20 °C to 45 °C, levan production increases rapidly from 20 °C until optimum temperature was achieved at 30 °C and then decreased slowly from 30 °C to 45 °C. The microbial growth, determined as OD_{600nm} , also increased concomitantly indicating that the fructan production by GYC2-3 is growth associated. Both maximum levan yield of 4.6 g/L and cell mass was obtained at 30 °C after 120 h of incubation (Fig. 6A). The proper activation of levansucrase protein at 30 °C can be the cause of increased levan production (Youssef, Youssef, Talha, & El-Aassar, 2014). These results are in accordance with those reported by other investigators that the conversion of sucrose into levan by *Bacillus lentus* V8 and *B. subtilis* NRC33a was maximum at 30 °C (Abdel-Fattah, Mahmoud, & Esawy, 2005; Abou-Taleb, Abdel-Monem, Yassin, & Draz, 2015). The optimum temperature for levan production varies greatly among levansucrases from different types of bacteria. For instance, maximum levan yields were reported at 24 °C and 50 °C by *P. polymyxa* EJS-3 (Liu et al., 2009) and *Bacillus* sp. TH4-2 (Ammar et al., 2002) levansucrases, respectively.

3.3.2. Optimum pH

The initial pH could also be considered as an effective factor controlling the levan yield as it may affect the polymer biosynthesis, overall structure and morphology of the cell and may also affect nutrients uptake by the cell. The effect of pH value on levan production was investigated at optimum temperature (30 °C) with varied pH values ranging from 5.0 to 8.0. The data showed that the cell biomass gradually increased with increasing pH, reaching maximum at pH 7.0 and then slightly decreased at pH 8.0. However, highest levan yield of 12 g/L was obtained at pH 8.0 (Fig. 6B). The above results indicate that although the GYC2-3 strain grows optimally at pH 7.0, its fructosyl-transferase has an optimum activity at pH 8.0. These results are significantly different from levan production by *B. lentus* (Abou-Taleb et al., 2015), where highest levan yield was obtained at initial pH of 6.5. However, these results are closely related to those obtained for levan synthesis by *Brachybacterium phenoliresistens*, where optimum initial pH was found to be 7.8 (Moussa, Al-Qaysi, Thabit, & Kadhem, 2017).

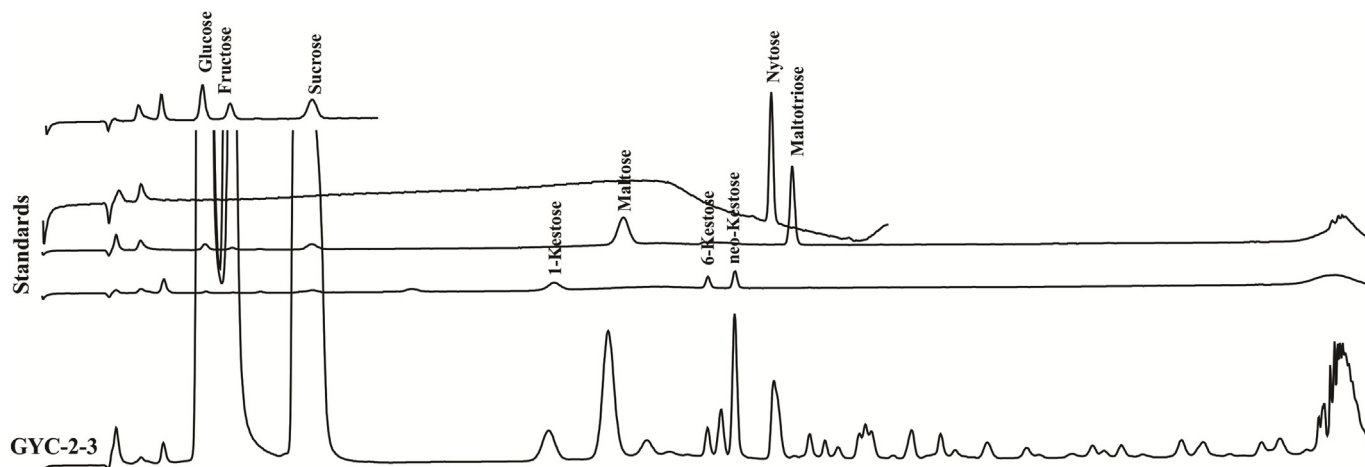


Fig. 5. HPAEC-PAD chromatogram of FOS synthesized by *B. aryabhattai* GYC 2-3 strain with 250 g/L sucrose after 120 h reaction at 30 °C.

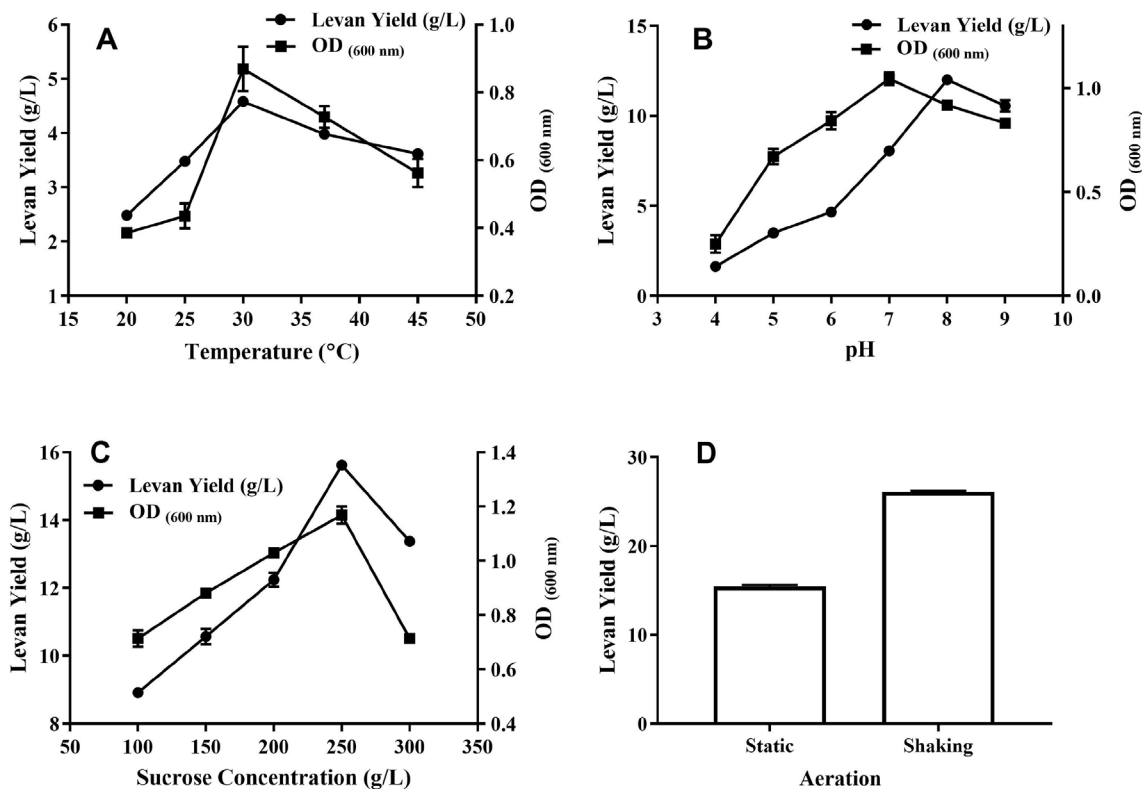


Fig. 6. Effect of environmental conditions on levan production by *B. aryabhatai* GYC 2-3 after 120 h of incubation. A: initial pH; B: temperature; C: sucrose concentration; D: aeration.

3.3.3. Optimum sucrose concentration

Besides other process parameters, sucrose concentration is also known to have important role in levan biosynthesis by bacteria. A high sucrose concentration favors transfructosylation reactions, which consequently increases levan production (W. Li et al., 2015). In the current study, sucrose concentration affecting the levan yield by GYC2-3 strain was investigated at pH 8.0 and incubation temperature of 30 °C. In a 120 h reaction period, about 9 g/L of the levan was produced in a medium having 100 g/L sucrose concentration. Increasing the sucrose concentration improved both the yield and cell growth, hence sucrose concentration is in direct relation to yield and cell growth. Optimum sucrose concentration for cell growth and levan synthesis (16 g/L) by the strain was found to be 250 g/L. Further increase in sucrose concentration of the medium declined the levan synthesis (Fig. 6C). Previous literature reports variability in optimum sucrose concentration for levan yield by different bacterial species. For instance, 300 g/L was optimum concentration of sucrose for *B. subtilis* NATTO (Dos Santos et al., 2013), while for *B. licheniformis* NS032 maximum levan productivity was observed at 200 g/L sucrose concentration (Kekez et al., 2015). Similarly, highest levan production (7.97 g/L) was observed by another rhizosphere isolate *B. phenoliresistens* at a sucrose concentration of 300 g/L (Moussa et al., 2017). Thus, our results are notably different from that reported earlier for levan synthesis by *B. licheniformis* NS032, though closer to the above-mentioned *B. subtilis* NATTO species and *B. phenoliresistens*.

3.3.4. Aeration conditions

The strain GYC2-3 yielded 26 g/L of levan under shaking conditions at 150 rpm, which is 38% higher than observed in static culture (Fig. 6D). This can be attributed to the aerobic mode of growth of the isolate. Using shaking or static conditions could be important to consider while upscaling the process of levan production for commercialization. To our knowledge, comparison of levan yield under these two conditions for aerobic bacteria has not been reported earlier, though

some investigators have compared levan yields at different shaking speeds. In those studies, shaking speed of 150 rpm has been found to be optimum for levan production (Ananthalakshmy & Gunasekaran, 1999; Shih et al., 2005).

4. Conclusion

Levan and FOS have emerged as important candidates in functional food markets for their prebiotic roles. In view of the great demand for levan and FOS as food ingredients, there is an urgent need to identify new bacterial strains that can be used for cheap and convenient production of novel types of prebiotics. Though several bacteria have been reported to produce levan, few levan producing bacteria are known to synthesize FOS. In this paper we report a novel strain of *B. aryabhatai*, which simultaneously produces levan and a broad range of FOS from sucrose. Future experiments will involve studying the impact of these carbohydrates on human gut microbiome. As the rhizosphere isolate reported here has very rare chance to be found within the gut microbiome, its exopolysaccharide and oligosaccharide products are unlikely to have a high flux through the human gut, which make them unique to the existing gut microbiota. Consequently, it is possible that these fibers will have an outsized impact upon the structure and function of the gut microbiome, increasing their potential as food additives.

CRedit authorship contribution statement

Anam Nasir: Investigation, Writing - original draft, Writing - review & editing. **Fazal Sattar:** Data curation, Writing - original draft, Writing - review & editing. **Iram Ashfaq:** Methodology. **Stephen R. Lindemann:** Resources. **Ming-Hsu Chen:** Formal analysis. **Wim Van den Ende:** Conceptualization, Resources, Writing - review & editing. **Ebru Toksoy Öner:** Resources, Writing - review & editing. **Onur Kirtel:** Formal analysis. **Shazia Khaliq:** Investigation. **M. Afzal Ghauri:** Supervision, Writing - review & editing. **Munir A. Anwar:**

Conceptualization, Supervision, Project administration, Resources, Funding acquisition, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2020.109093>.

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