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Short Communication

Production of microbial levan from sucrose, sugarcane juice and beet molasses

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SUMMARY

Bacillus polymyxa (NRRL-18475) produced a levan-type fructan (B, 2⟶6 fructofuranoside) when grown on sucrose, sugarcane juice, and sugarbeet molasses. The organism converted about 46% of the fructose moiety of sucrose to levan when grown on sucrose medium, however, the yields of levan from sugarcane juice and beet molasses were much less than sucrose solution. Such sugarcane juice and beet molasses can be made a good substrate for levan production by various modifications. Adding peptone to sugarcane juice or passing beet molasses through a column of gel filtration media improved levan yield to a level almost comparable to that obtained from sucrose.

INTRODUCTION

Industry uses large quantities of natural polysaccharides, and new sources of polysaccharides are being sought. In recent years, attention has been directed toward producing extracellular polysaccharides by microbial fermentation. Examples are dextran and xanthan gum produced by *Leuconostoc mesenteroids* and *Xanthomonas campestris*, respectively [5,7,9,15]. Levans are natural polymers of sugar fructose and are found in many plants and microbial products. Levans and inulins are both fructan but have different chemical structures and properties: the former is B-(2⟶6) linked fructans whereas the latter is B-(1⟶6) linked fructans. A variety of microorganisms produce levan. Most common ones are *Bacillus subtilis* [2,17], *Aerobacter levanicum* [16] and *Streptococcus salivarius* [13]. We have isolated a strain of *B. polymyxa* which produced a large quantity of extracellular polysaccharide when grown on sucrose solution [6]. The polysaccharide was fructose polymer, linked by B-(2⟶6) fructofuranosyl linkage. ^{13}C-NMR and methylation analysis revealed the polymer to be a levan type fructan [8].

Microbial levan has been known for many years [11], but has not been commercially produced and utilized, mainly because a large quantity of low-cost source of levan was not available and its characteristics were unknown. Like dextran, it is a by-product of sugar processing and imparts deleterious effects [1,4]. On the other hand, levans, which can only be produced from sucrose, have potential in industrial applications, such as surface coating or emulsion stabilization [7]. Because of its low viscosity and high water solubility, levan may be a potential substitute for gum arabic.

Cane juice and beet molasses are relatively inexpensive and renewable materials that are widely used as a substitute for sucrose in various industrial fermentation media [10,12,18]. However, sugarcane juice and beet molasses in their native state are poor substrates for microbial levan production. In this study, *B. polymyxa* was grown on sugarcane juice and beet molasses, and its fermentation characteristics and levan yield were studied to assess the suitability of these materials as a substitute for sucrose in levan production.

MATERIALS AND METHODS

Microorganism and media. Bacillus polymyxa (NRRL B-18475) was grown and maintained on a sucrose medium consisting of (g): sucrose 150, $(NH_4)_2SO_4$ 2.0, peptone 2.0, yeast extract 2.0, $MgSO_4$ 0.3, in a liter of water.

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Sugarcane juice, a mixture of several varieties of sugarcane harvested in Louisiana in 1988, was obtained from the USDA Sugarcane Field Lab, in Houma, LA. The sugar juice contained total solid 21.8%, sucrose 18.4%, glucose 1.1%, fructose 0.9%, total nitrogen 0.1%, and total phosphorus 0.01%. Sugarbeet molasses (Island variety) were obtained from Westway Trading Corporation, New Orleans, LA. The beet molasses contained total solid 82.8%, sucrose 56.0%, glucose 0.4%, fructose 0.3%, total nitrogen 2.4%, and total phosphorus 0.02%.

After filtering through 6 layers of cheesecloth, sugarcane juice was used as a fermentation medium without further dilution because it contained a similar level of sucrose as sucrose medium. However, 0.2% levels of $(NH_4)_2SO_4$, peptone, and yeast extract were added to fortify the sugarcane juice. Beet molasses were diluted three times with water to make the final sucrose concentration of 15%. The diluted molasses were then passed through columns of gels (Sephadex G-25, fine grade, Pharmacia Fine Chemicals and Cellufine GH-25, Amicon Corp.) or anion exchange resin (Dowex 1-X8 200–400 mesh, chloride form). The column contained about 15 volumes of the gels or anion exchange resin. The fermentation was carried out in 2800 ml Fernback flasks at 30 °C on a rotary shaker at 150 rpm. The initial pH of the media was 7.0 for sucrose medium, 6.8 for sugarcane juice, and 7.0 for beet molasses. The media were inoculated with about 5% volume of actively growing culture.

Production and purification of levan. The organism grown on a sucrose medium was successively transferred to larger volumes and the final culture was in 1500 ml medium in Fernbach flasks. The cultures were incubated at 30 °C on a rotary shaker (170 rpm) for 10 days. After growth, the culture was centrifuged at 27500 × *g* to remove bacterial cells, dialyzed through a membrane tubing (Spectrum Medical Industries, Inc., Los Angeles, CA) having a molecular weight cut off of 12000–14000 to remove unfermented sugars and fermentation products of small molecular weight, and finally the levan was precipitated by mixing the cultural broth with 1.5 volumes of ethanol or isopropanol. Repeated precipitation and dissolution in water was performed to purify the levan. The precipitate was finally collected and freeze- or vacuum-dried.

Analytical methods. Levan, sucrose, glucose and fructose were determined by high performance liquid chromatography (Beckman model 324 gradient liquid chromatograph) with an Altex refractive index detector and an Aminex HPX-87 column (Bio-rad Corp, Richmond, CA). Deionized water was used as a mobile phase. The levan produced by *B. polymyxa* grown on sucrose medium and purified by a series of centrifugation, dialysis and lyophilization was used as a standard. Gross estimation of levan was made by the weight of the precipitate formed by adding 1.5 volumes of ethanol to the culture filtrate. The precipitate was collected by siphoning off the supernatant, excess alcohol removed by blotting, and the sample vacuum dried and weighed. Total nitrogen was determined by the micro-Kjeldahl method of Perrin [14], and phosphorus was measured by the method of the Technicon Co. (Industrial Method No. 376-75W/B, Auto Analyzer II, Technicon Industrial Systems, Terrytown, NY). The number of viable cells was determined by plating a portion of the culture on sucrose medium solidified with agar. The pH of the medium was determined by a Corning 140 pH meter (Corning Scientific Product, Halstead, Essex, England).

RESULTS AND DISCUSSION

In a typical fermentation, *B. polymyxa* produced about 3.6 g of levan in 100 ml of a growth medium containing 15% sucrose (46% yield on an available fructose, where 7.89 g fructose are available from 15 g sucrose). However, the amount of levan produced from sugarcane juice (0.65% yield) and beet molasses (0.36% yield) was extremely low compared to that from sucrose, although sucrose content in the media were comparable (Table 1). Although sugarcane juice and beet molasses have been widely used as a substitute for sucrose in alcohol fermentation, levan production on these substrates was poor. This was probably due to the adverse effects of colloidal materials, high pH, and betain, the main nitrogen con-

TABLE 1

Effect of nutritional ingredient on levan production

Media	Levan (%)
Sucrose medium	3.60
Sugarcane juice	0.65
+ $(NH_4)_2SO_4$, 0.2%	0.61
+ Peptone, 0.2%	1.95
+ Yeast extract, 0.2%	0.96
+ All ingredients[a]	1.96
Beet molasses	0.36
+ $(NH_4)_2SO_4$, 0.2%	0.35
+ Peptone, 0.2%	0.53
+ Yeast extract, 0.2%	0.60
+ All ingredients[a]	0.83
passed through Sephadex G-25 gel	3.60
passed through Cellufine GH-25 gel	3.80
passed through Dowex 1-X8 anion exchange resin	1.40

[a] Combination of $(NH_4)_2SO_4$ 0.2%, peptone 0.2%, yeast extract 0.2%.

stituents of beet molasses, as suggested by Dhamija et al. [3]. The complexity and variability of cane juice and beet molasses made it difficult to establish their optimal fermentation conditions. However, adding 0.2% peptone in cane juice increased the levan yield from 0.65% to 1.95%, which is about half the amount obtained from sucrose medium. Addition of 0.2% $(NH_4)_2SO_4$ or 0.2% yeast extract had little effect. Desalting beet molasses through gel filtration media improved the levan yield to almost the same level as that from sucrose. However, addition of $(NH_4)_2SO_4$, peptone, and yeast extract had little effect. The texture and appearance of levan produced from beet molasses was different from that produced from sucrose. It was brownish and had a clay-like texture, devoid of the typical white, smooth, gummy properties. Desalting beet molasses remedied the problem. The levan produced from desalted beet molasses had almost the same texture and appearance as that from sucrose. Apparently, the excess minerals in beet molasses inhibited levan formation and produced a poor quality product.

Fig. 1a shows the kinetics of levan formation by *B. polymyxa* grown on a sucrose medium and Fig. 1b fermentation characteristics of the organism grown on beet molasses. The organism converted sucrose into levan and accumulated glucose, as shown by a decrease in sucrose and an increase in levan and glucose in the growth menstruum. During fermentation, the sucrose level dropped and levan started to appear within 2 days; thereafter, the sucrose level rapidly decreased as levan increased. Levan level peaked at 5 days and about 3.6% levan was obtained from 15% sucrose medium (about 47% theoretical yield based on available fructose content). About 2–3% glucose was accumulated in the medium. A small amount of fructose and unidentified fermentation products, smaller in molecular weight, were also produced. Sucrose was almost completely diminished after 10-day fermentation. The levan production correlated to the cell growth, which reached the stationary phase within 3–4 days and the number of viable cells dropped quickly after 6 days of incubation. The pH of the growth medium fell from 7.0 to 4.7 due to acid formation. Maintaining the pH above 5.5 is important, because the optimum pH for levansucrase is 5.5–7.0 and levan may be hydrolyzed at low pH [1].

Levan was harvested by precipitation from the culture broth by adding ethanol or isopropanol. Acetone and methanol could also be used. Levan precipitated immediately upon addition of the solvents. The yield and consistency of the product varied depending on the amount of alcohol added. The levan precipitated at the medium-to-alcohol ratio of 1 : 1.2; any further increase in the alcohol hardened the levan texture. The product was purified by repeated precipitation and dissolution in water, followed by dialysis or ultrafiltration. The final product was an off-white, gummy material that could be freeze- or vacuum-dried.

Levans, natural polymers of fructose that are only made from sucrose, have never been commercially produced and used. Because of their low viscosity, high solubility and other characteristics, levan may find a unique use or replace existing polysaccharides in industry. Sugarcane juice and beet molasses, after proper modification, may be used as low-cost substitutes for sucrose in commercial production of levan. Although the need for proper modification of substrates was demonstrated, inexpensive practical methods must be devised before introducing industrial applications.

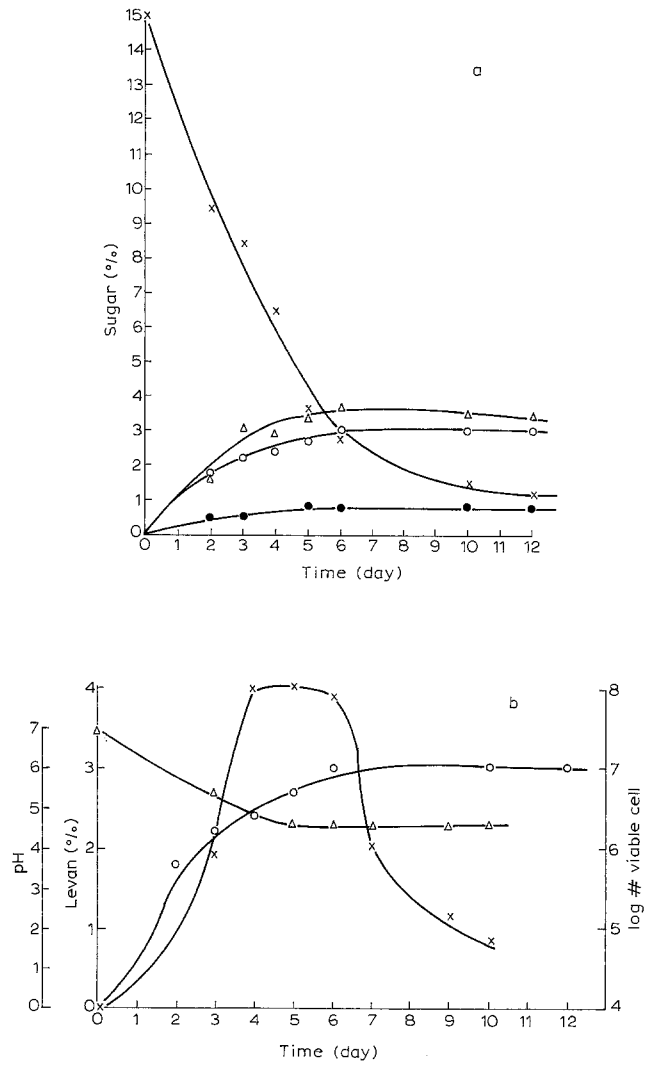

Fig. 1. (a) Kinetics of levan production by *B. polymyxa* grown on sucrose medium: —×— sucrose; —△— levan; —○— glucose; —●— fructose. (b) Fermentation characteristics of *B. polymyxa* grown on beet molasses: —×— viable cell count; —○— levan; —△— pH.

REFERENCES

1 Avigad, G. 1968. Levan. In: Encyl. Polym. Sci. Technol., Vol. 8. 711–718.

2 Dedonder, R. 1966. Levansucrase from *Bacillus subtilis*. Methods Enzymol. 8: 500–505.

3 Dhamija, S.S., D.S. Dahiya and M.C. Bardiya. 1980. Yeast screening and fermentation studies on beet molasses. Proc. Sugar Technol. Assoc. (India): 65–73.

4 Fuchs, A. 1956. Synthesis of levan by *Pseudomonads*. Nature 178: 921.

5 Glicksman, M. and R.E. Sand. 1973. Gum arabic. In: Industrial Gums. 198–263. Academic Press, NY.

6 Han, Y.W. 1989. Levan production by *Bacillus polymyxa*. J. Indust. Microbiol. 4(6): 447–451.

7 Han, Y.W. 1990. Microbial levan. Adv. Appl. Microbiol. 35: 171–194.

8 Han, Y.W. and M.A. Clarke. 1989. Production and characterization of microbial levan. J. Agric. Food Chem. 38(2): 393–396.

9 Kang, K.S. and I.W. Cottrell. 1979. Polysaccharides. In: Microbial Technology (2nd Edn). (Peppler, H.J. and D.I. Perlman, eds.) 417–481.

10 Letourneau, F. and P. Villa. 1987. *Saccharomyces* yeast growth on beet molasses: effects of substrate concentration on alcohol toxicity. Biotechnol. Lett. 9(1): 53–58.

11 Lippmann, E.O. 1881. Ueber das Lavulan, eine neue, in der Melasse der Rubenzuckerfabriken vorkommende Gummiart. Chem Ber. 14: 1509.

12 Lyness, E. and H.W. Doelle. 1981. Ethanol production from cane juice by *Zymomonas mobilis*. Biotechnol. Lett. 3(5): 257–260.

13 Lyness, E.W. and H.W. Doelle. 1983. Levansucrase EC-2.4.1.10 from *Zymomonas mobilis*. Biotechnol. Lett. 5(5): 345–350.

14 Perrin, C.G. 1953. Rapid modified procedure for the determination of Kjeldahl nitrogen. Anal. Chem. 25: 968–971.

15 Sanford, P.A. 1982. Potentially important microbial gums. In: Food Hydrocolloids. 167–207. CRC Press, Beca Raton, FL.

16 Takeshita, M. 1973. Translucent colony form of the Gram-negative levan-producing bacteria, *Aerobacter levanicum*. J. Bacteriol. 116: 503–506.

17 Tanaka, T., O. Susumu and Y. Yamamoto. 1979. Synthesis of levan by levansucrase. Some factors affecting the rate of synthesis and degree of polymerization of levan. J. Biochem. 85: 287–293.

18 Zayed, G.Z.A. and J. Foley 1987. The influence of fermentation condition on alcohol yield from sugar beet molasses and fodder beet juice using *Saccharomyces cerevisiae* strain. Irish J. Food Sci. Technol. 11: 119–133.