CCLI. INVESTIGATION OF A POLYSACCHARIDE PRODUCED FROM SUCROSE BY *BETA- BACTERIUM VERMIFORMÉ* (WARD-MEYER)

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WE have been able to examine a polysaccharide formed from sucrose by an organism (*Betabacterium vermiformé*) recently studied by Prof. A. J. Kluyver, to whom we are greatly indebted for a culture. The organism was isolated by Mr H. D. Meyer (private communication) in the course of an extensive and careful bacteriological examination of "Tibi-complex". The latter originates in Mexico where it occurs in the form of hard granules on the disk-shaped leaves of the *Opuntia* plant. It has long been used by the natives for the preparation of a beverage by fermentation and is analogous to the classic example, the Kephir yeast of the Arabs. As in the case of Kephir, Tibi fermentation is due to the symbiotic action of a yeast and a bacterium. The bacterium isolated by Meyer appeared to be identical with that isolated by Ward [1892] from the Ginger-beer plant.

On a sucrose-gelatin medium, the bacterium readily formed colonies surrounded by a highly viscous secretion and its growth on gelatin slopes closely resembled that of *Betacoccus arabinosaceus anhaemolyticus* (*Leuconostoc mesenterioides*). Meyer was able to show that the viscous secretion consisted mainly of a polyglucose with properties strikingly similar to those of the dextran synthesized by *B. arab. anhaemolyticus* [Tarr & Hibbert, 1931]. We have been able to confirm the finding that it is indeed a dextran, resembling closely in physical properties the dextrans from both *B. arab. haemolyticus* and *B. arab. anhaemolyticus*.

Our preliminary investigations were concerned with production of the polysaccharide in a liquid medium. The medium adopted for preparing the dextran from *B. arab. haemolyticus* [Stacey & Youd, 1938] was unsuitable for the growth of *Betabact. vermiformé* although when a satisfactory substrate for the latter was devised certain similarities in the growth of the two organisms were observed.

Both dextrans could be synthesized from sucrose only; moreover, in each case it was mainly the glucose constituent of the sucrose molecule which appeared to be utilized in the growth. For consistent dextran production both organisms required a source of available nitrogen which was best supplied by maple syrup to *B. arab. haemolyticus* and by yeast extract to *Betabact. vermiformé*. The polysaccharide could conveniently be prepared in yields amounting to 20% of the sucrose employed in a simple yeast extract-sucrose medium. The growth reached a maximum in about 10 days and was quite uniform even in large volumes of liquid—a property which facilitated the large-scale dextran production.

Separation of the polysaccharide was effected by alcoholic precipitation followed by washing and drying by the usual methods. The crude material contained approx. 0.2% N and was insoluble in water. This insolubility differentiates it from the *Leuconostoc* dextrans which are readily soluble in water.

During growth a large proportion of the dextran became insoluble in the metabolism solution and after isolation and drying it could not be dissolved even in boiling water. On dissolving this insoluble material in strong acid or alkali about 5% of bacterial "debris" separated and was removed in a centrifuge. Thereafter the dextran, precipitated by alcohol, was now water-soluble, N-free, showed $[\alpha]_D + 180^\circ$ in water and was indistinguishable in physical properties from the *Leuconostoc* dextrans.

From the products of acid hydrolysis, crystalline glucose (in 93% yield) was the only sugar isolated. Structural investigation has revealed that the main part of the molecule is constituted of α -glucose units mutually linked through the 1:6-positions, i.e. the linkage which obtains in gentiobiose [cf. Peat *et al.* 1938]. These and other investigations will be described later.

EXPERIMENTAL

Stock cultures of *Betabacterium vermiformé* received from Prof. A. J. Kluyver were maintained on gelatin slopes of the following composition: KCl, 0.5%; Na_2HPO_4 , 0.2%; sucrose, 10%; gelatin, 15%; yeast-water, 10%.

In a search for optimum conditions for dextran production in liquid media, the following main facts were established.

(1) The organism could synthesize the dextran from sucrose only, the yield increasing to a maximum at a sucrose concentration of 20%. Complete inhibition of growth occurred when the sucrose was replaced by glucose, fructose, maltose, lactose, glycerol, mannitol, sorbitol, inulin or laevan.

(2) No dextran formation took place in a sucrose-peptone medium in the absence of other sources of N the best of which was 10% yeast-water extract. Addition of maple syrup or beet sugar molasses gave unsatisfactory dextran production in the absence of an extra N supply, but in the presence of peptone (0.1%) or asparagine (0.1%) production was good.

(3) Tomato extract and a crude vitamin B concentrate were able also to provide the source of available N.

(4) The yield of dextran from a 10% yeast-water-sucrose medium was slightly increased by the presence of urea (0.1%) or succinamide (0.1%) or creatinine (0.1%). Glycine, *l*-leucine, malonamide and *d*-alanine appeared to act as growth inhibitors.

(5) During growth the pH of the metabolism solution was unchanged so that addition of CaCO_3 offered no advantage.

(6) No dextran was produced when the organism was grown in the medium which had proved satisfactory for the production of dextran from *Leuconostoc dextranicum*.

(7) The volume of metabolism solution did not materially affect the rate or amount of dextran formed in a suitable medium.

Large-scale production

Satisfactory results were obtained with the following medium: yeast-water, 10%; KCl, 0.5%; Na_2HPO_4 , 0.2%; sucrose, 20%. The medium was distributed in 600 ml. quantities in 1-litre conical flasks and steamed on three successive days. The flasks were inoculated, using small sterile pipettes, from a viscous 48 hr.-old culture of *Betabact. vermiformé* growing in test tubes in an identical medium. After incubation at 30° for several days the medium became very viscous and a dense gummy layer was formed at the bottom of each flask. (In preliminary experiments this material was isolated separately by decantation and was shown to consist of crude dextran.) After 10 days' incubation the

contents of the flasks were well shaken and run through cotton wool into 2 vol. of alcohol.

The crude dextran which was thrown down was washed repeatedly with alcohol, ground to a fine powder in a mortar and dried in a vacuum. The average yield amounted to 20% of the sucrose used. A considerable amount of fructose was isolated from the mother liquors [cf. Carruthers & Cooper, 1936] indicating that the dextran was probably synthesized from the glucose moiety of the sucrose molecule.

Purification and properties

The crude dextran (N 0.2%) was completely insoluble in water and did not swell in boiling water. It dissolved readily in 5*N* NaOH and in 10*N* HCl and showed optical properties as follows: $[\alpha]_D^{20} + 177^\circ$ in 10*N* HCl (*c.* 0.68); $[\alpha]_D + 179^\circ$ in 5*N* NaOH (*c.* 0.90). It was rendered soluble in water by the following method suggested by Dr F. Smith: dextran (3 g.) was stirred vigorously in cold conc. HCl (20 ml.) and when evenly suspended was centrifuged. A small amount of amorphous material was thrown down and the clear supernatant liquid was poured into excess of ethyl alcohol. The dextran was precipitated in the form of a fine white powder which was washed free from acid by alcohol and ether and dried in a vacuum. Yield, 2.8 g.

The product was now readily soluble in water. It had no reducing action on Fehling's solution, contained no N and its specific rotation was unaltered by the acid treatment. $[\alpha]_D^{20} + 175^\circ$ in 10*N* HCl (*c.* 1.0); $[\alpha]_D^{20} + 177^\circ$ in water (*c.* 1.3); $[\alpha]_D + 179^\circ$ in 5*N* NaOH (*c.* 0.95).

The insoluble gummy layer usually found at the bottom of the metabolism solution and mentioned previously, was treated with conc. HCl in the manner described and yielded a product, $[\alpha]_D^{20} + 179^\circ$ in 5*N* NaOH (*c.* 1.3), identical with that in the metabolism solution. An average sample of dextran had the following properties: $[\alpha]_D^{20} + 180^\circ$ (*c.* 1.3); ash, 0.23%; i.v. 4.7. On hydrolysis with *N* H₂SO₄ at 100° the following polarimetric changes were observed: $[\alpha]_D^{20} + 183^\circ$ (0 min.), $+148^\circ$ (45 min.), $+142^\circ$ (80 min.), $+132^\circ$ (115 min.), $+117^\circ$ (190 min.), $+98^\circ$ (310 min.), $+85^\circ$ (370 min.), $+79^\circ$ (430 min.), $+66^\circ$ (490 min.), $+54^\circ$ (925 min., equilibrium value).

From the hydrolysate crystalline *D*-glucose, $[\alpha]_D + 52.4^\circ$, was isolated in 93% yield. The chemical investigation of the dextran will be reported at a later date.

SUMMARY

A practicable method is described for the preparation of a new polysaccharide from sucrose by means of an organism *Betabacterium vermiforme* (Ward-Meyer). The polysaccharide shows a close resemblance in properties to the dextrans synthesized by the *Leuconostoc* species.

The authors are indebted to Prof. A. J. Kluyver for suggesting this investigation and for providing cultures, and they also wish to express their thanks to Prof. W. N. Haworth and to Dr Stanley Peat for their interest in the work.

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