## Synthesis of Levan by Pseudomonads

THE property of forming levan from sucrose is rather common among *Bacillus* species[1] and is also found in *Streptococcus salivarius*[2] and *Aerobacter levanicum*[3,4]. Further, levan is produced by many phytopathogenic *Pseudomonas* and *Xanthomonas* species[5,6], and is said to be formed by *Pseudomonas betae-gelatae*[7], isolated from thawing sugar-beets. However, no mention has been made of levan-producing *Pseudomonas* species isolated from other than diseased or killed plant material.

In the course of an investigation on levan-synthesizing bacteria, colloidal solutions of levan in distilled water were regularly stored in the icebox at 2° C. Some of these solutions were found to have become infected by organisms, apparently capable of using levan as sole source of carbon. After isolation, however, the predominating organism proved also to be able to produce a polysaccharide on 4 per cent sucrose with 1½ per cent peptone agar. After cultivation at 25° C. for 2–3 days in liquid 4 per cent sucrose 1½ per cent peptone medium, the polysaccharide formed was precipitated by adding three parts of ethyl alcohol and identified as a levan according to the methods of Hestrin *et al.*[3]. Further examination showed the organism to be *Pseudomonas fluorescens*.

Since here a levan-synthesizing pseudomonad was found which did not originate from plant material, it seemed worth while to examine the possibility of a dependable enrichment culture for this organism. Sterile 1 per cent solutions in distilled water of levans of different origin were inoculated with garden soil, ditch water or tap water. After incubation for 2 days at 25° C. under aerobic conditions and subculturing once more in the same liquid medium, transfers were made on 4 per cent sucrose with 1½ per cent peptone agar. Several strains of the predominating, slime-producing organism were readily isolated, all of which have been proved to be levan-forming *Pseudomonas fluorescens* strains. Apparently, small amounts of impurities in the levan preparations and inoculation materials fulfil the mineral requirements of this organism. These simple growth conditions in the enrichment medium were found to be essential for its selectivity, since the mere addition of mineral salts caused a great variety of other organisms to come to the fore.

Furthermore, it has been found in this laboratory that *Pseudomonas aureofaciens* is a levan-forming bacterium, as already mentioned recently by Kluyver[8]. All strains examined possessed this property; they were isolated from mixtures of clay and kerosene (2 strains) and soil (1 strain).

On screening a number of *Pseudomonas* strains from the bacterial collection of our laboratory, four more strains, belonging to the species *Pseudomonas fluorescens* (3 of 8 strains) and *Pseudomonas chlororaphis* (1 strain), were found to be able to synthesize this polysaccharide. None originated from plant material.

No levan synthesis was found in about 80 strains of different *Pseudomonas* species, among which were: *Ps. putida*, *Ps. aeruginosa*, *Ps. fluorescens*, *Ps. aromatica* var. *quercito-pyrogallica*, *Ps. calco-acetica* and *Ps. punctata*.

Thus, in a total of three *Pseudomonas* species, levan-forming strains have been found, which did not originate from diseased or killed plant material. All these strains produce levan in considerable quantities; maximal production was shown by a strain of *Pseudomonas aureofaciens* which in one case produced 83 per cent of the theoretical yield on cultivation at 25° C. in 4 per cent sucrose with 1½ per cent peptone medium on a rotary shaker.

The greater part of the levan-synthesizing strains which have been identified as *Pseudomonas fluorescens* is able to use oxygen as well as nitrate as hydrogen acceptor. These nitrate-reducing strains appeared able to synthesize levan also in nitrate-containing sucrose media in total absence of air. Here, maximal production of levan was 49 per cent.

All the levan-producing *Pseudomonas* strains proved to have constitutive, endocellular levan-sucrases (= saccharose → levan-transfructosidase[9]); moreover, all these strains could be characterized on sucrose-containing agar media by colonies having a typical radial structure. In these respects, apparently all Gram-negative, levan-producing bacteria resemble each other closely since *Aerobacter levanicum* was found to show the same characteristics.

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[1] Forsyth, W. G. C., and Webley, D. M., *Biochem. J.*, **44**, 455 (1949).
[2] Niven, C. F., Smiley, K. L., and Sherman, J. M., *J. Biol. Chem.*, **140**, 105 (1941); *J. Bact.*, **41**, 479 (1941).
[3] Hestrin, S., Avineri-Shapiro, S., and Aschner, M., *Biochem. J.*, **37**, 450 (1943).
[4] Fuchs, A., *Tijdschr. Plantenziekten* (in the press).
[5] Cooper, E. A., and Preston, J. F., *Biochem. J.*, **29**, 2267 (1935).
[6] Erikson, D., *Ann. App. Biol.*, **32**, 44 (1945).
[7] Delaporte, B., and Belval, H., *C.R. Acad. Sci., Paris*, **22**, 1011 (1946); *Ann. Inst. Pasteur*, **73**, 862 (1947).
[8] Kluyver, A. J., *J. Bact.* (in the press).
[9] Hoffmann-Ostenhof, O., "Enzymologie" (Springer Verlag, Wien, 1954).

## Occurrence of a Malic Enzyme free of Oxalacetic Decarboxylase in Silkworm Hæmolymph

HÆMOLYMPH of the larval silkworm (*Bombyx mori* L.) contains a triphosphopyridine nucleotide-linked 'malic' enzyme that has been purified by ammonium sulphate fractionation followed by adsorption and elution from calcium phosphate gel[1,2]. It was of interest to measure the oxalacetic decarboxylase activity of the 'malic' enzyme from silkworm, since those of pigeon liver[3,4] and wheat germ[5,6] exhibit a constant ratio (approximately 1·2 : 1) between oxalacetic decarboxylase and 'malic' enzyme activities throughout all stages of their purification. Recently, a diphosphopyridine nucleotide-linked 'malic' enzyme free of oxalacetic decarboxylase was purified from homogenates of *Ascaris lumbricoides*[7]. 'Malic' enzyme activity has been reported in crude homogenates of larvæ from the blowfly[8]. These extracts also decarboxylate oxalacetate; but it has not yet been established whether both reactions are catalysed by the same enzyme.

The test system used for the spectrophotometric assay of the 'malic' enzyme of silkworm blood contained the following components (in μmoles/2·2 ml.): *tris* (hydroxymethyl) aminomethane buffer *p*H 8·5, 40; magnesium sulphate, 10; *l*-malate, 10; triphosphopyridine nucleotide, 0·5; and enzyme. The assay was carried out in quartz cells ($d = 1·0$ cm.) at 21° C. in a Beckman *DU* spectrophotometer. The control cell contained all the components except