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PRELIMINARY REPORT

INITIAL PRODUCTS IN UTILIZATION OF SUCROSE BY *ACTINOMYCES VISCOSUS*[1]

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Strains of *Actinomyces* are generally found in the human mouth, and are isolated most frequently from the gingival crevice area and periodontal plaque (Howell et al., 1959, 1962). *Actinomyces viscosus* has the ability to form a gelatinous subgingival plaque, and to induce periodontal diseases and subgingival caries in experimental animals fed on a high sucrose diet (Jordan and Keyes, 1964, 1966; Howell et al., 1965; Jordan et al., 1965, 1969, 1972). Strain T6 also produces extracellular levan (fructan) when grown in the presence of sucrose (Howell and Jordan, 1967; Krichevsky et al., 1969). The present work was on the initial products formed on utilization of sucrose in relation to sucrose-hydrolyzing enzyme activities in the culture supernatant and whole cells of two strains of *A. viscosus*.

The organisms employed were *A. viscosus* ATCC 19246 and 15987. The latter strain was derived from *A. viscosus* T6, which causes periodontal diseases (Jordan and Keyes, 1964). Each strain was grown in Trypticase soy broth (BBL, Cockeysville, Md., U.S.A.) at 37 C for 48 hr. The resulting cultures were separated into the culture supernatant and whole cells by centrifugation at 6,000 $\times g$ for 10 min. The cells were thoroughly washed with 0.01 M phosphate buffer, pH 6.8, and resuspended in one-hundredth of the original culture volume of the same buffer. The enzyme proteins in the culture supernatant were precipitated by addition of 60% saturation of ammonium sulfate. The precipitate was collected by centrifugation, dissolved in 0.01 M phosphate buffer, pH 6.8, and dialyzed against the same buffer. This fraction contained sucrase activity and was used as the culture supernatant preparation. Utilization of sucrose was assayed using uniformally labeled ^{14}C-sucrose (Daiichi Pure Chemicals Co. Ltd., Tokyo) with a specific activity of 10 mCi per mmole. A typical incubation mixture contained in a total volume of 20 μliters, 0.5 μmoles of phosphate buffer, pH 6.8, 10 μg of NaF, 20 nmoles of ^{14}C-sucrose and 5 μliters of culture supernatant or cell preparation, and was incubated at 37 C for 10 min to 3 hr. At intervals, 5 μliters volumes of the reaction mixture were removed and spotted on Toyo filter paper, No. 51A (Toyo Roshi Co. Ltd., Osaka). The paper was developed by the ascending method with n-butanol: pyridine: water (6: 4: 3, by volume) for 3 to 4 hr in a thin layer tank, and then redeveloped three times in the same direction with the same solvent. The authentic ^{14}C-

1 A summary of this work was presented in the 16th Annual Meeting of the Japanese Association for Oral Biology in Sendai on September 25, 1974.

labeled standards, sucrose, glucose and fructose were used as controls and were chromatographed with the samples and located by autoradiography. After paper chromatography, a radioautogram was prepared. The areas of the paper corresponding to fructose, glucose and polysaccharide which remained at the origin on chromatography, were cut out and placed in vials containing 10 ml of scintillation fluid (Suginaka et al., 1975). Radioactivity was counted in a Packard scintillation spectrometer.

When ^{14}C-sucrose was incubated for 3 hr with the culture supernatant or whole cell suspension of *A. viscosus* ATCC 19246 or 15987, the radioactive sucrose was utilized, as shown in the Table 1. Consumption of the substrate was associated with liberation of glucose and fructose, and formation of a polysaccharide-product. The time courses of utilization of ^{14}C-sucrose by the culture supernatant and whole cell preparation of *A. viscosus* ATCC 15987 are shown in Fig. 1. When either the culture supernatant or the whole cell preparation was incubated with ^{14}C-sucrose, the sucrose decreased rapidly and had virtually disappeared after 3 hr. In parallel with the consumption of the substrate, glucose and fructose were released during incubation with the culture supernatant. The amounts of free glucose and fructose liberated were approximately equal. The amount of polysaccharide formed was neglegible compared with the amounts of the monosaccharides liberated. On the other hand, the whole cell suspension formed appreciable polysaccharide besides re-

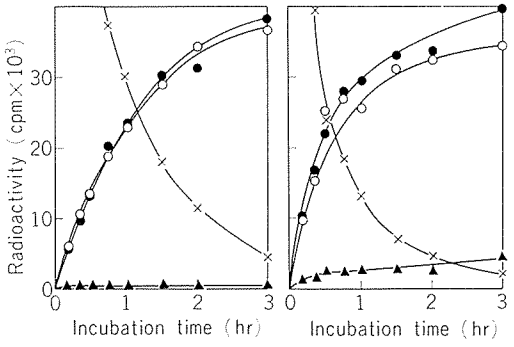

FIGURE 1. *Time course of utilization of ^{14}C-sucrose by the culture supernatant and whole cells of* A. viscosus *ATCC 15987. Assays were carried out as described in the text with the culture supernatant (left) or whole cells (right). Incubation was at 37 C for the times shown. Data are expressed as radioactivity (cpm) incorporated into the products. Symbols: ●, released glucose; ○, released fructose; ▲, polysaccharide formed; ×, residual sucrose.*

TABLE 1. *Distribution of radioactivity in products of ^{14}C-sucrose by the culture supernatant and whole cells of A. viscosus ATCC 19246 and 15987*[a]

Strains / Enzyme source	Distribution of radioactivity (cpm) (% of sucrose utilization)			
	Polysaccharide	Sucrose	Glucose	Fructose
ATCC 19246				
Culture supernatant	639 (0.9)	3,069	35,691 (51.8)	32,508 (47.3)
Whole cells	2,055 (3.0)	4,720	35,558 (52.3)	30,442 (44.7)
ATCC 15987				
Culture supernatant	297 (0.3)	4,626	38,251 (50.8)	36,633 (48.9)
Whole cells	4,696 (6.0)	2,233	39,500 (50.2)	34,477 (43.8)

[a] Assays were carried out as described in the text with the culture supernatant and whole cells of *A. viscosus* ATCC 19246 and 15987. Incubation was for 3 hr. Data are expressed as radioactivity (cpm) incorporated into the products.

leasing glucose and fructose, although the amounts of monosaccharides were greatly in excess of the amount of polysaccharide. This polysaccharide formation was accompanied by net release of more free glucose than fructose from the sucrose. The difference between the amounts of glucose and fructose liberated was approximately similar to the amount of polysaccharide formed. Thus the fructosyl moiety of sucrose was partially incorporated into the polysaccharide. This shows that the whole cell preparation produced fructan. This agrees with previous reports (Howell and Jordan, 1967; Krichevsky et al., 1969) that this organism synthesizes extracellular polysaccharide (fructan) from sucrose when grown on sucrose medium. This polysaccharide in particular may enable the organism to adhere to the tooth surface or dental plaque as in the case of *Streptococcus mutans* (Fitzgerald and Jordan, 1968; Gibbons, 1968; Guggenheim, 1970). On the other hand, both the culture supernatant and washed cells of this organism have an invertase-like activity which hydrolyses sucrose into glucose and fructose. These monosaccharides may be metabolized by the organism to acid products which demineralize the teeth. Recently, it was demonstrated that some strains of oral streptococci have this invertase-like activity (Gibbons, 1972; McCabe et al., 1973; Kuramitsu, 1973; Tanzer et al., 1973) besides the ability to form extracellular polysaccharides (glucan and/or fructan) (Fitzgerald and Jordan, 1968; Gibbons, 1968; Guggenheim, 1970) from sucrose. This result is also consistent with the recent report of Palenik and Miller (1975), that the culture supernatant of *A. viscosus* ATCC 15987 has invertase activity. Their report appeared after this work had been completed. The purification and characterization of this enzyme in the culture supernatant and whole cells of *A. viscosus* will be described in another paper (manuscript in preparation).

These results indicate that both the culture supernatant and whole cells contain an invertase-like activity, which directly hydrolyses sucrose releasing glucose and fructose and that the whole cells also have a fructosyltransferase which synthesizes fructan from sucrose.

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