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Transglucosidase Activity of Rumen Strains of *Streptococcus bovis*

2. ISOLATION AND PROPERTIES OF DEXTRANSUCRASE*

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Dextransucrase, the enzyme which converts sucrose into dextran and fructose, has been found in the cell-free culture fluids of various strains of *Leuconostoc mesenteroides* (Hehre, 1951; Tsuchiya *et al.* 1952). The properties of the isolated enzymes have been described in detail (Koepsell *et al.* 1953; Bailey, Barker, Bourne & Stacey, 1957*a*). A number of species of streptococci have also been shown to produce dextran from sucrose (Hehre, 1951). In these organisms, however, the enzyme responsible for dextran synthesis does not appear to have been isolated or examined. Recently it was shown that various strains of *Streptococcus bovis* produce dextran in sucrose cultures only in the presence of added carbon dioxide (Dain, Neal & Seely, 1956; Bailey & Oxford, 1958*a*, *b*). In *Leuconostoc* species, however, dextran production appears to be independent of added carbon dioxide (Bailey & Oxford, 1958*a*). In view of the lack of information on streptococcal dextransucrase and because of the interesting effect of carbon dioxide it was decided to isolate and examine *S. bovis* dextransucrase.

In isolating *Leuconostoc* dextransucrase the main problem has been to obtain a preparation of the enzyme entirely free from preformed dextran. Various authors (e.g. Tsuchiya *et al.* 1952; Neely, 1958) have used the cell-free culture fluid from a *L. mesenteroides* strain grown in 2% sucrose medium as a dextransucrase preparation. Bailey *et al.* (1957*a*) found that dextransucrase isolated from this type of culture contained much dextran and could not be freed from this dextran. A modified method (Bailey, Barker, Bourne & Stacey, 1957*b*) gave a dextransucrase containing much less dextran. In this case, however, the enzyme had only weak dextransucrase activity except in the presence of added acceptor sugar, when it produced oligosaccharides and very little dextran.

This paper describes the isolation and properties of *S. bovis* dextransucrase, including a comparatively dextran-free preparation which gave a good conversion of sucrose into dextran and fructose.

* Part 1: Bailey (1959).

EXPERIMENTAL

Organism. *S. bovis* (strain I; Bailey & Oxford, 1958*a*, *b*), isolated from the rumen of a cow feeding on red clover, was used.

Culture media. (1) Basal medium. The organism was normally grown in a Bacto Casitone–Bacto Yeast Extract medium (Bailey & Oxford, 1958*b*) containing either sucrose (A.R.; 4%, w/v) or glucose (A.R.; 2%, w/v). Cultures were maintained in both the sucrose and glucose media.

(2) Synthetic medium. Oxford (1958) developed a synthetic medium suitable for growth and dextran production with *S. bovis*. This medium contained the same concentration of sucrose or glucose as the basal medium. Both basal and synthetic media were adjusted to pH 7·0. All cultures were incubated at 37° in CO_2 unless stated otherwise.

Carbohydrates. Sucrose and glucose were A.R. grade. All sugars used were shown, by paper-chromatographic analysis, to be free from other sugars (less than 0·1% of impurity). Dextran was isolated by precipitation with ethanol (2 vol.) from the cell-free culture fluid of *S. bovis* (strain I) which had been grown on sucrose for 8 hr. (to pH 5·1). The dextran was purified by the usual methods, e.g. Bailey & Oxford (1958*b*). Isomaltose and isomaltotriose were isolated from a partial acid hydrolysate of the dextran by fractionation with aqueous ethanol after absorption on a charcoal–Celite column (Turvey & Whelan, 1957).

Paper chromatographic analyses. Solutions were analysed for sugars by paper chromatography with the solvents and sprays described in Part 1 (Bailey, 1959). An additional spray, naphthoresorcinol (Forsyth, 1948), was employed. Enzyme solutions were desalted by treatment with Dowex 2 and Dowex 50 ion-exchange resins.

Standard method for measuring dextransucrase activity. Experiments showed that *S. bovis* dextransucrase acted on sucrose in the same manner as the *Leuconostoc* enzyme. The method used for measuring the activity of *Leuconostoc* dextransucrase (Bailey *et al.* 1957*a*), which depends on the measurement of the liberated fructose in an enzyme–sucrose solution, was therefore used for measuring the activity of *S. bovis* dextransucrase. The only modification to the method was that the solutions for measurement of activity were incubated at 37°. The solutions (7 ml.) contained either freeze-dried enzyme (2–5 mg.) or cell-free culture fluid (1–2 ml.), sucrose (300 mg.) and acetate buffer (0·05M, pH 5·2; 5–7 ml.). Fructose was measured by the method of Shaffer & Hartmann (1921) after treatment with hypoiodite (Van der Plank, 1936). Controls showed that fructose was liberated only in the solutions containing both dextransucrase and sucrose. A unit of dextransucrase activity (*Leuconostoc* enzyme) has been defined as the level of enzyme activity which converts 1 mg. of sucrose into fructose and dextran in 1 hr. at a specified temperature and pH, provided that not more than half of the sucrose has been consumed (Koepsell & Tsuchiya, 1952). This definition was used in the present work. As with the *Leuconostoc* enzyme (Bailey *et al.* 1957*a*) high concentrations of fructose depressed the activity of *S. bovis* dextransucrase. However, the conditions used in measuring dextransucrase activity were such that the amount of fructose produced never approached the concentration at which significant depression of enzyme activity occurred. In measuring the activity of undialysed cell-free cultures a correction was made for any fructose already present. Minor modifications made to the standard method when measuring the effect of pH, temperature and added sugars on dextransucrase activity are described below.

Isolation of dextransucrase. S. bovis cultures grew very rapidly and soon reached the range pH 4·3–4·6, where dextransucrase is unstable. Experiments showed that a culture contained its maximum dextransucrase activity when the pH had fallen to between 5·8 and 5·2. With basal medium this generally required incubation for 7–9 hr. at 37°.

Basal medium (250–500 ml.; containing sucrose or glucose) was inoculated from the appropriate *S. bovis* culture and incubated until the pH had dropped to 5·8–5·2. Only small inocula (1–2 drops/250 ml. or one loopful/50 ml.) were used. The final culture was rapidly cooled to 0–2° and centrifuged (30 000 *g*) for 30 min. on a Spinco preparative ultracentrifuge. The enzyme was precipitated from the cell-free supernatant with ammonium sulphate (A.R.; 60 g./100 ml.) and the solution left overnight at 2°. The precipitate was collected by centrifuging (30 000 *g*) at 0–5° for 30 min., dissolved in citrate buffer (0·05M, pH 6·0; 50 ml.) and recentrifuged. The enzyme solution was dialysed for 48 hr. at 2° against three changes of citrate buffer (2 l.) in dialysis tubing which had been well washed with the citrate buffer. The final solution of enzyme in citrate buffer was recentrifuged and freeze-dried. The polysaccharide content of the freeze-dried dextransucrase preparations was measured by the anthrone method of Roe (1954) and expressed as dextran.

RESULTS

Dextransucrase production during growth of Streptococcus bovis

In order to establish the cultural conditions for maximum dextransucrase production the dextransucrase activity of a growing culture of *S. bovis* was examined at intervals. Basal sucrose medium was inoculated from a sucrose culture of *S. bovis* and incubated at 37°. At intervals portions (20 ml.) were removed and, after measurement of pH, centrifuged (30 000 *g*) at 0–5° for 30 min. Dextransucrase activity in the cell-free fluid was measured by the standard method. The results obtained are listed in Table 1.

The effect of cultural conditions on dextransucrase production by *S. bovis* was examined by measuring the dextransucrase activity in cultures in various media which had been grown to within the range pH 5·8–5·1. The results obtained are listed in Table 2. Cultures in the synthetic medium showed a rather variable lag phase (8–12 hr.) before any growth occurred. These cultures were first incubated overnight at 28°, during which time the pH dropped to 6·0–6·2, and then transferred to the incubator at 37°, when incubation for 2–4 hr. gave a culture within the desired pH range.

Dextransucrase preparations

Two main dextransucrase preparations were isolated and used in an examination of the properties of the enzyme. These were: (1) Dextransucrase I from basal sucrose media (250 ml.) inoculated from a sucrose culture. The culture, after incubation for 8 hr. to pH 5·5, yielded a cell-free culture fluid (200 ml.) containing 5·5 units of dextransucrase/ml. (1100 units). The dialysed

Table 1. *Dextransucrase production in a culture of* Streptococcus bovis

Organism (strain I) was inoculated into basal sucrose (4%, w/v) medium (200 ml.) and incubated at 37° in CO_2. Portions (20 ml.) were removed at intervals and centrifuged before measurement of dextransucrase activity.

Time of incubation (hr.)	pH of culture fluid	Dextransucrase activity of cell-free culture fluid (units/ml. at pH 5·2)
0	7·0	0
5·5	6·7	0
6·5	6·4	0·1
7·5	5·1	6·2
9·5	4·4	1·75

Table 2. *Dextransucrase activity of various cultures of* Streptococcus bovis

Cultures (50 ml.) containing glucose (2%, w/v) or sucrose (4%, w/v) were incubated at 37° in CO_2 until the pH fell to within the range 5·1–5·8 and were then centrifuged.

Culture medium		pH of culture fluid	Dextransucrase activity of cell-free culture fluid (units/ml. at pH 5·2)
Inoculation	Test		
Basal, sucrose	Basal, sucrose	5·3	5·1
Basal, sucrose	Basal, sucrose (no CO_2)	5·3	1·0
Basal, sucrose	Basal, glucose	5·1	3·7
Basal, glucose (sixth successive culture)	Basal, glucose	5·8	2·4
Synthetic, sucrose	Synthetic, sucrose	5·4	0·5
Synthetic, glucose (sixth successive culture)	Synthetic, glucose	5·4	0·2

solution of the ammonium sulphate precipitate contained 800 units and the final freeze-dried solid (0·7 g.) 790 units of dextransucrase activity. (2) Dextransucrase II from basal glucose medium (500 ml.) inoculated from a sucrose culture. The cell-free culture fluid (475 ml.) obtained from the culture after incubation for 8 hr. to pH 5·2 contained 3·15 units of dextransucrase activity/ml. (1500 units). The final dialysed solution contained 1300 units of activity and the freeze-dried solid (1·12 g.) 1240 units of activity.

Dextransucrase I contained 70% of polysaccharide (0·62 mg./unit of dextransucrase activity) and 1·79% of nitrogen. It gave in water a solution with the opalescence typical of a dextran solution. Dextransucrase II, however, contained only 4% of polysaccharide (0·036 mg./unit of dextransucrase activity). This enzyme preparation contained 9·06% of nitrogen. It gave a clear solution in water. Other preparations of dextransucrase isolated from glucose cultures contained similar low concentrations of polysaccharide.

Action of dextransucrase on sucrose and other carbohydrates

Both enzyme preparations were incubated with sucrose and tested for dextransucrase activity. Solutions were incubated for 3 and 24 hr. together with appropriate controls containing (*a*) dextransucrase but no sucrose, (*b*) boiled dextransucrase and sucrose and (*c*) sucrose but no enzyme. Paper chromatograms of the desalted solutions at the end of incubation showed no free sugars in the controls except for added sucrose. The solutions with enzyme plus sucrose, however, showed in addition to sucrose a component chromatographically identical with fructose; glucose was absent from the papers. The intensities of the fructose spots on the papers were proportional to the time of incubation. The two enzymes produced dextran, as shown by the development of opalescence. The enzyme preparations always produced fructose plus polysaccharide but no glucose from sucrose. No sign of invertase activity was detected in cell-free culture fluids from the various cultures, including those grown in sucrose medium in the absence of carbon dioxide.

Table 3. *Effect of sucrose concentration on dextransucrase activity*

Solutions (7 ml.) containing dextransucrase (2 mg.), acetate buffer (0·05M, pH 5·2; 5 ml.) and sucrose were incubated at 37° for 3 hr.

Sucrose (mg.)	Dextransucrase activity (units)	
	Dextransucrase I	Dextransucrase II
25	1·52	1·52
50	1·90	1·81
100	2·03	2·06
200	2·28	2·23
300	2·28	2·23

The effect of sucrose concentration on dextransucrase activity was examined by measuring the fructose liberated, after incubation for 3 hr., in solutions containing dextransucrase (2 mg.) and various amounts of sucrose. The results, converted into units of dextransucrase activity, for the two dextransucrase preparations are listed in Table 3. A similar effect of sucrose concentration on enzyme activity has been reported for *Leuconostoc* dextransucrase (Bailey *et al.* 1957*a*).

The effect of incubation time on dextransucrase activity was examined by incubating solutions containing dextransucrase (2 mg.) and sucrose (500 mg.) for various times and measuring the liberated fructose. The results obtained, for both enzyme preparations, are listed in Table 4. On prolonged incubation there is an apparent lowering of dextransucrase activity due, in part at least, to the depressing effect of high fructose concentrations on dextransucrase activity (see Table 6). In the standard method used for measuring dextransucrase activity, solutions are incubated for only 3 hr., when no decrease in activity occurs.

Table 4. *Effect of incubation time on dextransucrase activity*

Solutions (7 ml.) containing dextransucrase (2 mg.), sucrose (500 mg.) and acetate buffer (0·05M, pH 5·2; 5 ml.) were incubated at 37°.

Incubation time (hr.)	Dextransucrase I		Dextransucrase II	
	Fructose produced (mg.)	Dextransucrase activity (units)	Fructose produced (mg.)	Dextransucrase activity (units)
3	3·6	2·28	3·5	2·23
6	7·2	2·28	7·0	2·23
17	18·3	2·05	18·1	2·03
24	23·8	1·88	24·0	1·90
48	35·3	1·40	37·1	1·47

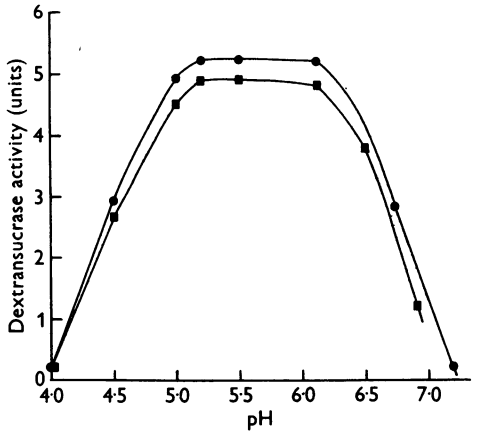

Fig. 1. Effect of pH on dextransucrase activity. Solutions containing dextransucrase (5 mg.) and sucrose (300 mg.) in acetate solution (0·05M; 5 ml.) were incubated for 3 hr. at 37°. ●, Dextransucrase I; ■, dextransucrase II.

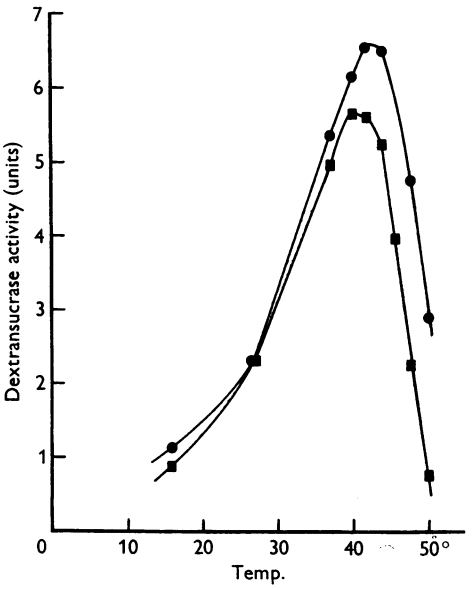

Fig. 2. Effect of temperature on dextransucrase activity. Solutions containing dextransucrase (5 mg.) and sucrose (300 mg.) in acetate buffer (0·05M, pH 5·2; 5 ml.) were incubated for 3 hr. ●, Dextransucrase I; ■, dextransucrase II.

Carbon dioxide undoubtedly stimulates dextran production in sucrose cultures of *S. bovis* (Bailey & Oxford, 1958*a*, *b*). To test the effect of carbon dioxide on the enzymes, solutions of dextransucrase I and II with sucrose were incubated in unstoppered flasks for 3 hr., in jars filled with either CO_2 or air. Measurement of the liberated fructose showed that the dextransucrase activity was the same in air or CO_2.

The action of the enzyme preparations on other sugars was also checked. Solutions were prepared each containing dextransucrase (I and II; 5 mg.) and fructose or glucose (10 mg.) but no sucrose. The reducing sugar content of each solution was measured both before and after incubation for 24 hr. under toluene. There was no loss of reducing sugar in any of the solutions. Other solutions containing dextransucrase (5 mg.) and various added sugars (20 mg.) but no sucrose were incubated for 24 hr. and the solutions examined by paper-chromatographic analysis. The results obtained showed that both enzyme preparations were without action on maltose, isomaltose, raffinose, melibiose, cellobiose, lactose, αα-trehalose, melezitose and starch.

Effect of pH *and temperature on dextransucrase activity*

The effect of pH on dextransucrase activity was obtained by measuring activity in solutions in which the pH of the added acetate solution (0·05M) ranged from 4·0 to 7·2. The results obtained with both enzyme preparations, after incubation for 3 hr., are shown in Fig. 1.

The effect of temperature on dextransucrase activity was obtained by measuring the activity in solutions of each enzyme after incubation for 3 hr. at various temperatures. The results obtained are shown in Fig. 2.

Stability of dextransucrase at 37°. The stability of dextransucrase at 37° was examined by incubating dextransucrase II (30 mg.) in acetate buffer (0·05M, 16 ml.) at pH 4·5 and 5·2, under toluene, in

Table 5. *Stability of dextransucrase solutions at* 37°

Solutions containing dextransucrase II (30 mg.) in acetate buffer (0·05M, 16 ml.) were stored at 37° under toluene in the presence and absence of dextran. Dextransucrase activities were measured by the standard method at pH 5·2 in 1 ml. portions of the solutions.

pH	Added dextran (mg.)	Dextransucrase activity after storage for 0 hr.	24 hr.	48 hr.	72 hr.
5·2	—	1·96	1·88	1·83	1·80
5·2	100	2·00	2·00	2·00	2·00
4·5	—	2·00	0·14	0	—
4·5	100	2·10	0·59	0·31	—

the absence and presence of dextran. At daily intervals portions (1 ml.) were withdrawn for measurement of dextransucrase activity at pH 5·2. The results, which show the effect of pH and dextran on dextransucrase stability, are given in Table 5.

Effect of added sugars on dextransucrase activity

Koepsell *et al.* (1953) showed that many sugars, in the presence of both *Leuconostoc* dextransucrase and sucrose, either stimulated or depressed dextransucrase activity and, at the same time, acted as alternative glucosyl acceptors to produce oligosaccharides. The effect of added sugars on the activity of *S. bovis* dextransucrase was also examined by the modified standard method for measuring dextransucrase activity as described by Bailey *et al.* (1957*a*). The solutions, which contained enzyme (2 mg.), sucrose (500 mg.) and added sugar (25–300 mg.), were incubated for 24 hr. Appropriate controls were run. The effect of each sugar on dextransucrase activity was expressed as the percentage change in the amounts of fructose liberated relative to the fructose produced in the standard dextransucrase–sucrose solution during incubation for 24 hr. Results from the study of the effect of incubation time on dextransucrase activity (Table 4) show an apparent loss of activity during incubation for 24 hr. In measuring the effect of added sugars on dextransucrase activity the long incubation time is necessary in order to obtain a suitable ratio, for the fructose measurement, of fructose produced to sugar added (Bailey *et al.* 1957*a*). As the results obtained were comparative in nature the falling off in dextransucrase activity during incubation was not considered serious. The results for those sugars (100 mg. dry wt.) which affected dextransucrase activity are listed in Table 6. Mannose, sorbose, raffinose, melezitose, αα-trehalose and dextran (all 100 mg.) had no effect on dextransucrase activity.

Of the readily available sugars, maltose had the most pronounced effect on the activity of *S. bovis* dextransucrase, as was the case with *Leuconostoc* dextransucrase (Koepsell *et al.* 1953; Bailey *et al.* 1957*a*). Dextransucrase activity was measured in

Table 6. *Effect of added sugars on dextransucrase activity*

Solutions containing dextransucrase (2 mg.), sucrose (500 mg.) and added sugar (100 mg.) in acetate buffer (0·05M, pH 5·2; 7 ml.) were incubated at 37° for 24 hr.

Added sugar	Effect on dextransucrase activity (% change in fructose production) Dextransucrase I	Dextransucrase II
Maltose	+174	+120
Isomaltose	—	+110
Isomaltotriose	—	+55
Glucose	+60	+46
Galactose	+12	+40
D-Arabinose	+12	+33
Methyl α-glucoside	+22	+33
Lactose	+7	+20
Cellobiose	0	+9·2
Melibiose	0	+20
Fructose	0	−29

Table 7. *Effect of added maltose on dextransucrase activity*

Solutions containing dextransucrase (2 mg.), sucrose (500 mg.) and added maltose (anhydrous) in acetate buffer (0·05M, pH 5·2; 7 ml.) were incubated at 37° for 24 hr.

Maltose (mg.)	Increase in fructose production (%) Dextransucrase I	Dextransucrase II
50	80	60
100	174	120
150	190	143
200	203	174
250	207	188

solutions containing various amounts of added maltose. The results obtained are listed in Table 7.

After desalting with Dowex 2 and Dowex 50 ion-exchange resins the final enzyme solutions and controls were examined by paper chromatography. Prominent series of oligosaccharide spots were present on paper chromatograms of the enzyme solutions containing added maltose, isomaltose and isomaltotriose. Much weaker oligosaccharide spots were present on paper chromatograms of the enzyme solutions containing added glucose, lactose,

cellobiose, fructose, galactose and methyl α-glucoside. None of the other enzyme solutions or any of the control solutions contained any detectable sugar except sucrose, fructose and added sugar.

Enzymically synthesized dextran

Solutions containing dextransucrase (10–15 mg.), sucrose (1 g.) and acetate buffer (0·05 M, pH 5·2; 20 ml.) were incubated for 24 hr. at 37° under toluene. Dextran was precipitated from the opalescent solutions with ethanol (2 vol.), purified and freeze-dried. Three specimens of dextran were synthesized with (1) dextransucrase I (15 mg.), (2) dextransucrase II (15 mg.) and (3) dextransucrase (10 mg.) isolated from a glucose–basal medium culture which had been inoculated with a glucose culture of *S. bovis* (see Table 2). The yields of dextran were 160, 164 and 108 mg. respectively (85–90 % of theoretical). The dextrans did not stain with iodine and dissolved in water to give opalescent solutions. Partial and total acid hydrolysates of each dextran were prepared (Bailey, 1959) and examined by paper chromatography. Total acid hydrolysates of each dextran showed a single component chromatographically identical with glucose. The three partial acid hydrolysates showed components corresponding to glucose and an apparently homologous series of oligosaccharides. The first two members of the series were chromatographically identical with isomaltose and isomaltotriose respectively. The three dextrans had $[\alpha]_D^{20}$ (*c*, 0·15 in N-NaOH) +190°, +191° and +185° respectively.

A small amount of polysaccharide (20 mg.) was synthesized with dialysed cell-free culture fluid from a *S. bovis* culture in synthetic glucose medium (see Table 2). A mixture of cell-free fluid (10 ml.; 2 units of dextransucrase activity) and sucrose (300 mg.) was incubated at 37° for 24 hr. under toluene. The solution became opalescent and polysaccharide was isolated. A partial acid hydrolysate of the polysaccharide showed on paper chromatograms components which were chromatographically identical with glucose, isomaltose and isomaltotriose. A total acid hydrolysate showed a single component chromatographically identical with glucose.

DISCUSSION

S. bovis produces an extracellular enzyme which converts sucrose into a polysaccharide and fructose. The polysaccharide has the properties of dextran and the enzyme is therefore designated a dextransucrase. The action of the enzyme on sucrose, both alone and in the presence of added acceptor sugars, and the effect of sucrose concentration, incubation time and pH on its activity, are similar to the properties of *Leuconostoc* dextransucrase (Bailey *et al.* 1957*a*). Although *S. bovis* produces good yields of dextran only in the presence of carbon dioxide (Bailey & Oxford, 1958*a*, *b*), the isolated dextransucrase is active in its absence. It seems likely therefore that carbon dioxide stimulates either the actual formation of dextransucrase or its release into solution.

The production of dextransucrase in the absence of added sucrose was unexpected and suggests that *S. bovis* dextransucrase might be a constitutive rather than an adaptive enzyme. There are several other possible explanations, including: (1) the glucose media may have contained a trace contamination of sucrose; (2) the organism may secrete traces of intracellular sucrose which are sufficient to stimulate dextransucrase production; (3) *S. bovis* may only gradually cease to produce dextransucrase after it has been deprived of sucrose. In connexion with the first possibility, no sucrose could be detected in a concentrated solution of the yeast extract used in the media or in the glucose medium itself. In a synthetic glucose medium dextransucrase could also be detected although at a much lower level of activity. Good yields of enzyme would not be expected in this glucose medium as results reported by Oxford (1958) suggest that even under optimum conditions of pH, dextransucrase is unstable unless large molecules, either preformed dextran or added protein, are present.

From cell-free culture fluid obtained from a sucrose (2 %, w/v) culture of a *Leuconostoc* species Bailey *et al.* (1957*a*) isolated dextransucrase (containing 70–80 % of dextran) by ethanol precipitation at 0°. When the sucrose cultures also contained maltose (10 %, w/v) (Bailey *et al.* 1957*b*), and produced oligosaccharides rather than dextran, the dextransucrase (containing 7 % of dextran) could be precipitated with ethanol only at −20°. This latter dextransucrase exhibited good activity only in the presence of maltose, when it produced oligosaccharides containing α-1:6-linkages, e.g. panose, and very little dextran. Dextransucrase could not be precipitated with ammonium sulphate from either type of culture. In contrast with these results *S. bovis* dextransucrase is easily precipitated with ammonium sulphate from both sucrose and glucose cultures. Whereas dextransucrase precipitated from sucrose cultures of *S. bovis* contained 70 % of dextran the enzyme from glucose cultures was low in dextran (4 %). This latter enzyme preparation contained only 0·036 mg. of polysaccharide/unit of dextransucrase activity, compared with 1·01 mg. of polysaccharide/unit of dextransucrase activity for the *Leuconostoc* dextransucrase from the sucrose–maltose culture (Bailey *et al.* 1957*b*). Moreover the glucose-culture dextransucrase produced good yields of dextran (90 % of

theoretical) from sucrose. In the dextran specimens prepared with this enzyme only 0·4% of the synthesized dextran was due to the preformed polysaccharide present in the enzyme.

The properties of dextransucrase preparations I and II are in sufficient agreement to suggest that they are the same enzyme. The main difference from *Leuconostoc* dextransucrase is in the effect of temperature on dextransucrase activity. *S. bovis* dextransucrase has optimum activity in the temperature range 37–44° whereas the *Leuconostoc* enzyme has optimum activity between 25° and 29° and rapidly loses activity above 35°. A higher temperature optimum for *S. bovis* dextransucrase is not unexpected, as optimum temperature for growth of the organism is in the range 37–42°. A second possible difference from *Leuconostoc* dextransucrase is the absence of enzymes producing branching of the dextran molecule. Many, but not all, strains of *L. mesenteroides* produce dextrans which are highly branched. The strain of *L. mesenteroides* used by Bailey *et al.* (1957*a*, *b*) produced a dextran containing 10–15% of branch (α-1:3)-linkages (Barker, Bourne, Bruce, Neely & Stacey, 1954). Dextransucrase isolated from cultures of this organism contained, in addition to the enzyme synthesizing the main chain of α-1:6-linked anhydroglucose units, a second, more labile, enzyme which synthesized the branch linkages in the dextran molecule (Bailey *et al.* 1957*a*). Dextran produced by *S. bovis* (strain I) has been shown to be entirely unbranched (Bailey, 1959). The dextransucrase produced by this organism would therefore be expected to be free from branching enzymes and in fact specimens of dextran synthesized with the enzyme have $[\alpha]_D$ values expected for an unbranched dextran.

It is evident that, in addition to any stabilizing effects due to co-precipitated protein or dextran, pH is an important factor in determining the stability of *S. bovis* dextransucrase (see Table 5). The activity of *S. bovis* dextransucrase is stimulated by the same sugars which stimulate the activity of *Leuconostoc* dextransucrase (Bailey *et al.* 1957*a*) with the addition of D-arabinose. As with the *Leuconostoc* enzyme, fructose at high concentrations depresses the activity of *S. bovis* dextransucrase. Except for melibiose and arabinose all of the sugars which stimulate activity act as alternative glucosyl acceptors to produce oligosaccharides. Fructose, although it depresses activity, also gives rise to oligosaccharides; the same effect has been reported by Koepsell *et al.* (1953) for *Leuconostoc* dextransucrase. The main oligosaccharide produced by this enzyme in the presence of fructose and sucrose is a disaccharide leucrose (Stodola, Sharpe & Koepsell, 1956). Large amounts (representing up to 12% of the available sucrose) of a sugar apparently identical with leucrose have been observed in dextran-producing cultures of *S. bovis* strain I (Bailey & Oxford, 1958*a*).

All of the evidence available at present seems to indicate that the mechanism of dextran synthesis is the same for both *S. bovis* and *Leuconostoc* dextransucrase. The most interesting property of *S. bovis* dextransucrase is its occurrence in glucose cultures containing no added sucrose. No matter what explanation is offered for its presence in these cultures, they do provide a ready source of comparatively dextran-free dextransucrase.

SUMMARY

1. Dextransucrase has been isolated by ammonium sulphate precipitation from cell-free culture fluids of *Streptococcus bovis* grown on sucrose or glucose in the presence of carbon dioxide.

2. The dextransucrase isolated from the sucrose cultures contained 70% of polysaccharide and 1·79% of nitrogen, whereas the enzyme from the glucose cultures contained 4% of polysaccharide and 9·06% of nitrogen.

3. Both dextransucrase preparations had maximum activity in the range pH 5·0–6·5 and the temperature range 37–44°.

4. At pH 4·5 the enzyme was very unstable.

5. Maltose, isomaltose, isomaltotriose, glucose, galactose, methyl α-glucoside, lactose and cellobiose stimulated dextransucrase activity and acted as alternative glucosyl acceptors to produce oligosaccharides.

6. D-Arabinose and melibiose stimulated activity but did not act as glucosyl acceptors.

7. Fructose depressed dextransucrase activity but acted as an alternative glucosyl acceptor to produce oligosaccharides.

8. Dextransucrase preparations from both sucrose and glucose cultures gave good yields of dextran from sucrose.

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A Bacterial Dextranase

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Although several species of moulds, notably *Penicillium funiculosum* and *P. lilacinum* (Hulten & Nordström, 1949), have been shown to produce extracellular dextranases, members of only one bacterial genus (*Bacteroides*; Hehre & Sery, 1952) have been reported to produce this type of enzyme. In view of the possibility of dextran production by *Streptococcus bovis* in the rumen of cattle (Bailey & Oxford, 1958) we have been interested in the likelihood of rumen micro-organisms secreting dextranases. So far, two strains of an organism resembling *Lactobacillus bifidus* have been the only organisms possessing dextranase activity which one of us (R.T.J.C.) has isolated from the bovine rumen. As the end products of dextran hydrolysis by cell-free culture fluids of these organisms were rather unusual, it was decided to examine the properties of the dextranase. This paper reports the results obtained in a study of the dextranase preparations from cell-free culture fluids of these organisms and of one human strain of *L. bifidus*. For the purpose of this paper the two rumen organisms are referred to as strains of *L. bifidus*.

EXPERIMENTAL

Reducing sugars. Reducing sugars were measured in dextranase solutions, after neutralizing with 0·25 N-NaOH, by the cuprimetric method of Shaffer & Hartmann (1921). Preliminary experiments with isomaltodextrins showed that, with this copper reagent, a heating time of 15 min. was sufficient for full development of reducing power (cf. Turvey & Whelan, 1957). A standard curve was prepared with purified isomaltotriose, and all results were calculated in terms of this sugar. Although a mixture of oligosaccharides is produced by *L. bifidus* dextranase, only comparative reducing values were required and the triose standard was considered satisfactory.

Paper chromatographic analysis. Solutions were desalted with Dowex 50 and Dowex 2 ion-exchange resins. Chromatograms were developed with a solvent consisting of the top layer of ethyl acetate–water–pyridine (2:2:1, by vol.; Jermyn & Isherwood, 1949), and with a modified version of this solvent (Malpress & Hytten, 1958) which gave a better resolution of the higher oligosaccharides. Sugars were detected with silver nitrate (Trevelyan, Procter & Harrison, 1950) and aniline hydrogen phthalate (Partridge, 1949). Papers were also developed by the benzylamine–ninhydrin technique of Bayly & Bourne (1953).

Carbohydrates

Dextran. Dextran was isolated by ethanol precipitation (2 vol.) from a sucrose (4%, w/v) culture of *Streptococcus bovis* (strain I; Bailey & Oxford, 1958) which had been incubated for 48 hr. at 37° in an atmosphere of CO_2. The dextran was purified by the usual techniques and shown to be unbranched (Bailey, 1959). A standard solution of this dextran in water (10 mg./ml.) was prepared and, except where stated otherwise, used in all dextranase tests. In addition, a specimen of dextran produced from sucrose at 25° by *Betacoccus arabinosaceus* (*Leuconostoc mesenteroides*), Birmingham strain, was also used. It had been shown to consist of branched and unbranched chains (Bailey, unpublished work) and to contain 10–15% of branch (α-1:3-) linkages (Barker, Bourne, Bruce, Neely & Stacey, 1954).

Isomaltodextrins. *S. bovis* dextran (6 g.) was partially hydrolysed by heating at 100° in 0·33 N-H_2SO_4 (500 ml.) for 8 hr. After neutralizing ($BaCO_3$) and filtering, the hydrolysate was adsorbed on to a charcoal–Celite column (45 cm. × 3·5 cm.; Whistler & Durso, 1950). A batch of Darco charcoal (G60), which had been in store for several years and which had lost some of its absorption power, was used unwashed. After elution of glucose with water, ethanol–water mixtures eluted an apparently homologous series (by paper chromatography) of sugar fractions. The first two members of the series were chromatographically identical with authentic isomaltose and isomaltotriose (prepared from *Leuconostoc* dextran). As the *S. bovis* dextran was unbranched it was assumed that the other oligosaccharide fractions were members of the series isomaltotetraose–isomalto-octaose. Pure fractions were eluted with the following ethanol–water (v/v) mixtures: