TEMPERATURE-SENSITIVE DEXTRANSUCRASE SYNTHESIS BY A LACTOBACILLUS

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Received for publication 26 June 1963

ABSTRACT

DUNICAN, L. K. (Cornell University, Ithaca, New York), AND H. W. SEELEY, JR. Temperature-sensitive dextransucrase synthesis by a lactobacillus. J. Bacteriol. **86**:1079–1083. 1963.—Dextran synthesis was found to be temperature-dependent in *Lactobacillus* strain RWM-13. Dextran was not formed above 37 C, although growth of cells occurred up to 42 C. Logarithmically growing cells transferred from 30 C to 40 C ceased producing dextran while growth decreased nominally. An examination of the extracts of cells broken by sonic treatment showed that as the temperature of growth was increased above 37 C the production of dextransucrase decreased. By use of an inhibitor of invertase, 10^{-4} M $AgNO_3$, it was shown that invertase replaced dextransucrase activity at temperatures above 37 C. In contrast to dextransucrase in *Leuconostoc mesenteroides*, the enzyme in *Lactobacillus* strain RWM-13 was constitutive and thus resembled that of *Streptococcus bovis*. Thermosensitivity of dextransucrase synthesis has not been observed in *Leuconostoc* or *Streptococcus*.

Unusual heat sensitivity of enzyme synthesis has been observed in many investigations and has been reviewed by Knox (1953). This review was concerned primarily with the decreased synthesis of certain inducible enzymes at elevated temperatures which, nevertheless, allowed growth of the organisms. *Salmonella paratyphi* B produced tetrathionase at 37 C but not at 40 C (Knox, 1950). Inducible urease production by *Proteus* spp. was likewise inhibited at temperatures above 37 C (Knox, 1951). Many psychrophiles also exhibited thermosensitivity of synthesis of several enzymes. The extracellular lipases of *Pseudomonas fragi* and *P. fluorescens* were not formed at temperatures above 30 C. These enzymes were produced maximally at 15 C, although they were most active at 40 C (Nashif and Nelson, 1953; Alford and Elliot, 1960). Upadhyay and Stokes (1963) described a psychrophilic bacterium which failed to produce formic hydrogenlyase above 20 C, although mesophilic bacteria were reported to produce the same enzyme at temperatures up to 45 C.

Many organisms have been described which produce enzymes whose synthesis is more heat-sensitive than that of enzymes produced in related organisms.

During an investigation of the synthesis of dextran by *Lactobacillus* strain RWM-13, it was found that the polysaccharide was not formed by cells grown at 42 C, although the organism grew readily at this temperature. The present report describes the heat sensitivity of dextransucrase in this strain of *Lactobacillus* and the resultant utilization of sucrose by a second enzyme system in cultures grown above 37 C. Other factors controlling the synthesis of dextran in lactobacilli will appear in a later report.

MATERIALS AND METHODS

The medium of deMan, Rogosa, and Sharpe (1960) was used with the modification that peptone was omitted. The carbohydrate content varied in the experiments and is recorded with the results. The organism, *Lactobacillus* strain RWM-13, was obtained as a slime-producing lactobacillus from the laboratory of T. Gibson, Edinburgh, and was originally isolated from silage. Cell dry weight and dextran production were measured gravimetrically by removing 40-ml portions from cultures and centrifuging them at 8000 × *g* for 30 min. The dextran in the supernatant liquid was then precipitated by adding 2 volumes of 95% ethanol. The precipitate was dried and weighed. The turbidity of the dextran in the cultures prevented the turbidimetric measurement of growth The pellet of cells in the centrifuge tube was washed into a tared weighing dish, dried, and weighed.

Dextransucrase was measured by the method

of Hehre (1955). Reducing sugars in the assay mixtures were measured by the method of Somogyi (1945). Invertase activity in the extracts was determined by using replicate assays containing 10^{-4} M $AgNO_3$ (Myrbäck, 1926; Goodman, Weil, and Stern, 1955). The invertase activity was computed from the difference in reducing sugars produced in the presence and absence of the silver nitrate. Assays were performed at 30 C, and an incubation time of 6 hr was used. A unit of activity is defined as the amount of reducing sugars liberated after 6 hr of incubation per milligram of protein of the extract. Protein was measured by the biuret method of Gornall, Bardawill, and David (1949).

RESULTS

The effects of temperature on growth and dextran production in *Lactobacillus* strain RWM-13 are shown in Fig. 1. The results are notable for the wide range of temperature within which growth occurs, in contrast to the narrow range within which dextran production is optimal. The most striking effect of temperature on dextran synthesis was the significant drop in the quantity of dextran produced between 35 and 37 C.

Two possible events were considered to explain the rapid drop in dextran synthesis in the narrow temperature range observed in Figure 1. The first was the possible heat lability of the dextransucrase above 37 C. The second was the thermosensitivity of the synthesis of the enzyme at elevated temperatures. Experiments were designed to distinguish between these alternatives.

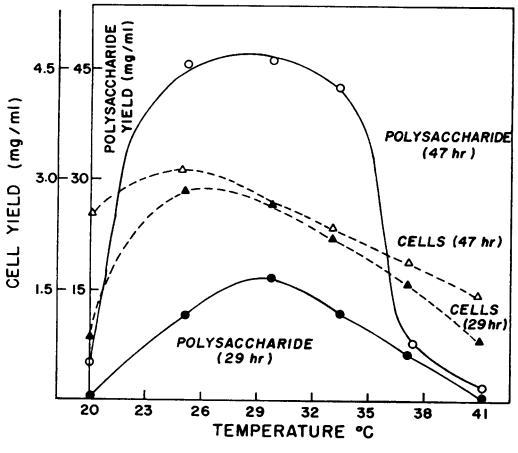

FIG. 1. *Effect of temperature on growth and polysaccharide production by Lactobacillus strain RWM-13. Substrate: 8% sucrose.*

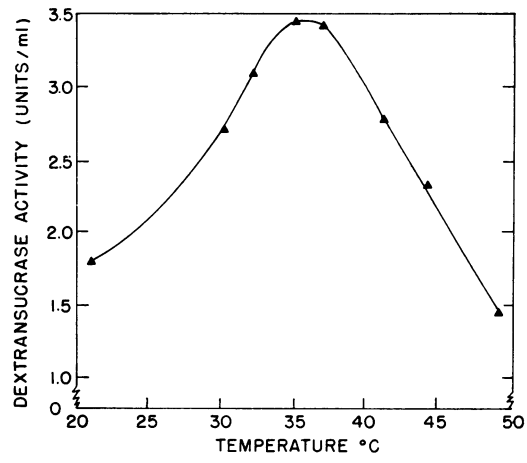

FIG. 2. *Effect of temperature on the activity of cell-free dextransucrase from Lactobacillus strain RWM-13. Assayed at 37 C.*

Figure 2 shows the effect of temperature on the activity of dextransucrase in extracts prepared by sonic treatment of *Lactobacillus* strain RWM-13. The optimal temperature for dextran synthesis was found to be 38 C; thus, the heat lability of dextransucrase was ruled out as the cause of the failure of the dextran synthesis above 36 C.

To explain the second possibility, growth experiments were used to show that active dextransucrase was not synthesized at the higher temperature. Cultures of cells actively growing at 30 C were transferred to 40 C, and cell dry weight and dextran production were measured at the two temperatures. Figure 3 shows the results obtained. After the transfer of the culture to the higher temperature, growth continued at a somewhat reduced rate, whereas dextran production ceased. The slight increase in the production of dextran after the change was attributed to the higher activity of the residual dextransucrase at 40 C.

Since the temperature sensitivity of dextransucrase synthesis was observed in growing cells, the possibility existed that the enzyme was formed at higher temperatures but was unable to leave the cell and come in contact with the substrate in the medium. This possibility was examined by measuring the dextransucrase activity of extracts of cultures of *Lactobacillus* strain RWM-13 which had been grown at different temperatures. Replicate assays were carried out in the presence of 10^{-4} M silver nitrate to measure the amount of invertase present in the extracts

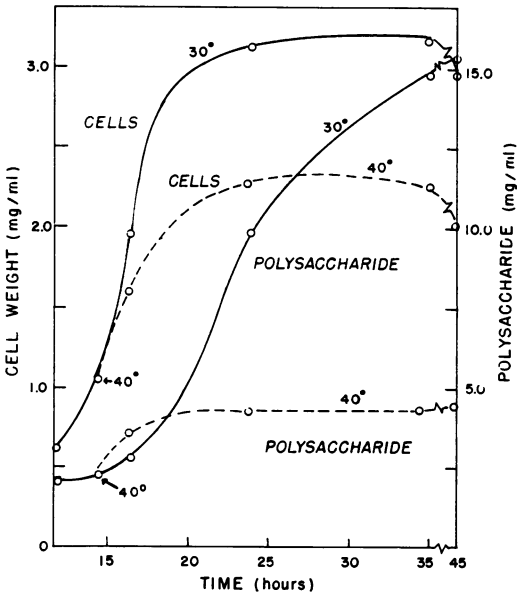

FIG. 3. *Effect of the change of temperature during incubation on cell growth and dextran production. Initial temperature of incubation, 30 C; transferred to 40 C at 15 hr. Substrate: 8% sucrose.*

TABLE 1. *Effect of silver nitrate on the liberation of reducing sugars from sucrose in extracts of Lactobacillus strain RWM-13*

Extract*	Reducing sugars in assay mixture		
	Control (0 hr)	Sample (6 hr)	Net increase (6 hr)
	mg	*mg*	*mg*
$AgNO_3$ absent....	2.38	6.3	3.92
$AgNO_3$ present...	2.06	5.7	3.64

* Conditions: temperature, 30 C; $AgNO_3$, 10^{-4} M; pH 5.6.

(Goodman et al., 1955). Table 1 shows that approximately 7% of reducing sugar production by the extracts of *Lactobacillus* strain RWM-13 was due to invertase, the remainder being due to dextransucrase.

Table 2 shows that the total activity of extracts of *Lactobacillus* strain RWM-13, grown at different temperatures, on sucrose was divided between dextransucrase and invertase activities. The extracts of cells grown at high or low temperatures utilized sucrose by an invertase hydrolysis, whereas extracts of cells grown at the intermediate temperatures possessed strong dextransucrase activity. The results of two individual experiments shown in Table 2 were not equivalent, although the same type of response was observed in both. This was owing to the different amounts of growth obtained at the different temperatures and to the absence of convenient methods of measuring cell growth in the cultures containing large amounts of dextran.

The reports in the literature on the thermosensitivity of enzyme synthesis have been confined

TABLE 2. *Dextransucrase activity of cell-free extracts of Lactobacillus strain RWM-13 grown at different temperatures*

Temp[a]	Reducing sugars from sucrose				
	Total[b]	Dextransucrase[c]		Invertase[d]	
		Amount	Per cent	Amount	Per cent
C	*mg*	*mg*		*mg*	
20	3.32	0.94	29.3	2.38	70.7
	13.60	2.25	16.5	11.35	83.5
30	2.66	1.98	76.0	0.68	24.0
	1.49	1.39	93.0	0.10	7.0
37	1.18	0.82	69.5	0.36	30.5
	0.28	0.26	92.0	0.02	8.0
42	2.72	0.64	23.5	2.08	76.5
	2.15	1.05	48.7	1.10	51.3

[a] Two separate experiments at each temperature.
[b] No $AgNO_3$.
[c] $AgNO_3$, 10^{-4} M.
[d] By difference.

TABLE 3. *Constitutive nature of dextransucrase in Lactobacillus strain RWM-13*

Substrate for cell growth	Time*	Sucrose utilized	Sucrose utilized/mg of protein
	hr	*mg*	*mg*
Sucrose	0	0	—
	6	9.76	2.01
Glucose	0	0.21	—
	6	26.0	2.15

* Time 0 represents the reducing sugars present in the extract before the enzyme was allowed to act on the substrate. The activities, in column 4, are corrected for the presence of these reducing sugars. The temperature used in the assay was 37 C.

to inducible enzymes. Dextransucrase is inducible in *Leuconostoc mesenteroides* (Neely and Nott, 1962), whereas it has been reported to be constitutive in *Streptococcus bovis* (Bailey, 1959). Table 3 shows the content of dextransucrase in the extracts of *Lactobacillus* strain RWM-13 grown on glucose and sucrose. The activity was approximately the same in the two extracts, indicating that the enzyme was constitutive.

DISCUSSION

The effects of temperatures above the optimum for growth were studied in relation to the production of dextransucrase in a lactobacillus. The results show that, within a few degrees of temperature (about 34 to 37 C), dextran production virtually ceases, whereas cell growth decreases only a nominal amount. Experiments involving the relation of the isolated enzyme to temperature and experiments involving the drop of dextran production on transfer of the culture from 30 C to 40 C were interpreted to indicate that the synthesis of dextransucrase and not the enzyme itself was thermosensitive. In this respect, dextransucrase synthesis resembles tetrathionase synthesis by *S. paratyphi* B (Knox, 1950) and urease formation by *Proteus* spp. (Knox, 1951). However, it was demonstrated that the dextransucrase of lactobacillus was constitutive, and not inducible as in the case of the enzymes described by Knox.

At temperatures of 37 C and higher, growth was accompanied by little dextran production on a sucrose substrate. This suggested the presence of a second enzyme in *Lactobacillus* strain RWM-13 with activity on sucrose. Inhibitor studies indicated that this enzyme was invertase. This was confirmed by demonstrating an increase in glucose in the extracts as measured by the glucose oxidase method of Dahlqvist (1961). The data of Fig. 1 and Table 2 show that, in the absence of significant dextran production by whole cells grown above 37 C, there was appreciable invertase activity in the cell extracts. Under conditions of temperature which permitted dextran synthesis, there was extensive dextransucrase activity in the extracts.

Thermosensitive critical events in viral replication were reviewed by Lwoff (1962). Two events make a discussion of this work on poliovirus relevant to the thermosensitivity of dextransucrase synthesis: (i) the formation of poliovirus is optimal at 36 C and is completely inhibited at 40 C; (ii) a single event, *viz.*, the polymerization of monomers of the ribonucleic acid (RNA) polymerase, has been hypothesized to be the event that is thermolabile. Such events are significant in that the critical temperatures found in this investigation had the same parameters as those of dextransucrase, tetrathionase, and urease. In addition, the synthesis of a single enzyme has been implicated to account for the temperature sensitivity in each case.

The evidence presented by Lwoff (1962) suggests that the polymerization of monomers of the RNA polymerase is a prerequisite to enzymatic activity and that this polymerization is the thermolabile step in the enzyme synthesis. With the dextransucrase of *Lactobacillus* strain RWM-13, there was a decrease of production of this enzyme as the incubation temperature was increased. Paralleling this decrease was an increase of invertase activity. The explicit assertion that the effect of temperature on sucrose utilization is to be explained by invertase being a depolymerized form of dextransucrase cannot be made until purification of the latter enzyme can be achieved. A honey bee invertase with transglycosidase activity has been described (White and Mahler, 1953), the product of which was an oligosaccharide and not a dextran. These findings indicate that there may be a closer relationship between dextransucrase and invertase than has been generally recognized.

ACKNOWLEDGMENT

This work was supported in part by training grant 5 TI AI 144(01-04) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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