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METABOLISM OF PENTOSES AND PENTITOLS BY *AEROBACTER AEROGENES*

I. DEMONSTRATION OF PENTOSE ISOMERASE, PENTULOKINASE, AND PENTITOL DEHYDROGENASE ENZYME FAMILIES[1]

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ABSTRACT

MORTLOCK, R. P. (Michigan State University, East Lansing) AND W. A. WOOD. Metabolism of pentoses and pentitols by *Aerobacter aerogenes*. I. Demonstration of pentose isomerase, pentulokinase, and pentitol dehydrogenase enzyme families. J. Bacteriol. 88:838-844. 1964.—*Aerobacter aerogenes* PRL-R3 is capable of utilizing as sole substrates for energy and growth seven of the eight aldopentoses and all of the four pentitols. Growth upon media containing either D-xylose, L-arabinose, D-ribose, D-arabitol, or ribitol occurred within 24 hr at 26 C. When D-arabinose or L-arabitol were used as growth substrates, growth was complete within 2 days; 4 days were required for growth on D-lyxose or xylitol, and 3 to 4 weeks for growth upon L-xylose. The versatility of this strain of *A. aerogenes* is due to an ability to synthesize in the presence of appropriate carbohydrates (inducers) families of enzymes which catalyze the metabolism of the carbohydrates (i.e., families of pentitol dehydrogenases, aldopentose isomerases, and pentulokinases). The specificity of induction for members of the enzyme families was found to vary, and cross induction of enzyme activity was common, especially among the pentitol dehydrogenases. Ribitol dehydrogenase activity was detected in extracts of cells grown on all of the above carbohydrates with the exception of D-xylose, L-arabinose, and D-ribose. The ribitol dehydrogenase activity of xylitol-grown cell extracts was fivefold higher than the activity in extracts of ribitol-grown cells.

Aerobacter aerogenes, PRL-R3, is unusual among bacteria in that it can grow on all eight of the aldopentoses (Simpson, *unpublished data*) and all four of the pentitols. This versatility is more striking because many of these compounds rarely, if ever, occur in nature. Fermentation studies with specifically labeled pentoses showed that several of the pentoses yield the same fermentation products and with identical labeling patterns (Neish and Simpson, 1954; Altermatt, Simpson, and Neish, 1955). Thus, the same general metabolic routes must be involved for the fermentation of these compounds. The strategy for pentose utilization as elucidated in many organisms involves isomerization of an aldopentose to (one of the four) ketopentoses, phosphorylation of the ketopentoses, and epimerization of ketopentose-5-phosphates to D-xylulose-5-phosphate. Pentitols are utilized by dehydrogenation to the ketopentose, but the remaining steps are identical.

For *A. aerogenes*, these processes accommodate the whole range of C_5 structures. Current information on these pathways is summarized in Fig. 1. The individual reactions in the utilization of L-arabinose, L-xylose, L-lyxose, and the pentitols were intensively studied in this strain. As shown by Simpson, Wolin, and Wood [(1958); see also Burma and Horecker (1958) for similar processes in *Lactobacillus plantarum*], L-arabinose is isomerized to L-ribulose which is then phosphorylated to L-ribulose-5-phosphate. This phosphate ester is then epimerized at carbon four to form D-xylulose-5-phosphate, the substrate of transketolase. Similarly, L-xylose and L-lyxose are isomerized to L-xylulose, which is then phosphorylated to L-xylulose-5-phosphate. By epimerizations at carbons three and four, this ester is also converted to D-xylulose-5-phosphate (Anderson and Wood, 1962*a*). Thus, all of these isomers are converted to D-xylulose-5-phosphate by epimerases.

The oxidation of ribitol to D-ribulose and of

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D-arabitol to D-xylulose by specific nicotinamide adenine dinucleotide (NAD)-linked dehydrogenases has been documented (Fromm, 1958; Wood, McDonough, and Jacobs, 1961; Lin, 1961), as have the oxidations of L-arabitol to L-xylulose and xylitol to D-xylulose (Fossitt et al., 1964). For D-ribose, a difference in detail exists, in that phosphorylation precedes isomerization.

Growth of *A. aerogenes* on all of these five-carbon structures implies an unusual ability to synthesize several families of enzymes, with the members of each family catalyzing the same reaction, but each having a different specificity. Because of the value of these families in studying determinants of specificity, a detailed analysis of this phenomenon has been undertaken.

MATERIALS AND METHODS

Bacteriological. A. aerogenes, PRL-R3, was grown aerobically at 25 C on a minimal medium (Anderson and Wood, 1962*a*) supplemented with 0.5% carbohydrate. The carbohydrate and $MgSO_4$ were autoclaved separately and added after cooling. For some experiments, the carbohydrate was sterilized by filtration through a type HA Millipore filter. The viable cell determinations were obtained by plating on the above medium supplemented with 1.5% Nobel Agar (Difco). Total cell counts were made with a Petroff-Hausser counting chamber. Turbidity determinations were made in a Bausch & Lomb Spectronic-20 colorimeter, and were converted to dry weight of cell material with a standard curve.

For standardized growth experiments, tubes containing 5.5 ml of medium were inoculated to give 2×10^6 cells per ml, inclined to 45°, and incubated at 26 C on a reciprocating shaker. The inoculum was washed twice with sterile salts solution prior to use. Cultures were considered grown when the turbidity at 620 mμ reached an optical density of 1.0.

Analytical. Kinase activity was estimated by use of the following coupled reaction sequence described by Anderson and Wood (1962*b*):

(a) pentulose + adenosine triphosphate (ATP) $\xrightarrow{\text{kinase}}$ pentulose-5-phosphate + adenosine diphosphate (ADP)

(b) ADP + phosphoenolpyruvate $\xrightarrow{\text{pyruvate kinase}}$ pyruvate + ATP

(c) pyruvate + reduced nicotinamide adenine dinucleotide (NADH) $\xrightarrow{\text{lactic dehydrogenase}}$ lactate + NAD

For determination of pentitol dehydrogenase activity, the same reaction mixture was used, except that ATP was omitted and the rate of pentulose reduction (NADH oxidation) at 340 mμ was determined. To measure kinase activity in the presence of large amounts of pentitol dehydrogenase activity, reduced nicotinamide adenine dinucleotide phosphate (NADPH) was substituted for NADH. NADPH serves as an efficient reductant for pyruvate in the presence of excess lactic dehydrogenase, but is not utilized by NADH oxidase or the NADH-specific pentitol dehydrogenases, thereby greatly decreasing the blank rate. A unit of kinase or pentitol dehydrogenase activity was defined as the amount of enzyme giving an absorbancy change of 1.0 per min at 340 mμ in a reaction volume of 0.15 ml and with a light path of 1 cm.

Isomerase activity was measured from the rate of pentulose formation by the method of Anderson and Wood (1962*a*); 1 unit of isomerase in 2.0 ml catalyzed the formation of 1 μmole of pentulose per hr at 37 C.

Oxygen utilization was determined by standard manometric methods. Protein was estimated by the method of Lowry et al. (1951). Aldopentoses were determined by the orcinol test (Mejbaum, 1939), with a 40-min heating time. Ketopentose was measured by the cysteine-carbazole test of Dische and Borenfreund (1951).

Chemical. Ribitol, D-arabitol, xylitol, D-xylose, and D-ribose were purchased from Nutritional

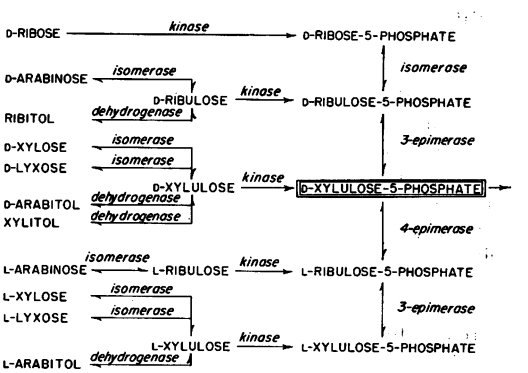

FIG. 1. *Aldopentose and pentitol metabolism by Aerobacter aerogenes.*

Biochemicals Corp., Cleveland, Ohio. L-Arabinose and D-arabinose were obtained from Pfanstiehl Laboratories, Inc., Waukegan, Ill.; L-arabitol was purchased from Mann Research Laboratory, New York, N.Y.; D-lyxose was purchased from General Biochemicals, Chagrin Falls, Ohio; L-xylose, L-lyxose, and L-xylulose were prepared as described by Anderson and Wood (1962*a*); and L-xylose was also purchased from General Biochemicals. D-Ribulose was prepared from the *o*-nitrophenylhydrazone derivative as described by Müller, Montigel, and Reichstein (1937). Recrystallization of aldopentoses and pentitols was carried out in absolute ethanol.

Enzymatic. To prepare cell-free extracts for enzyme assays, the cells were harvested by centrifugation, washed once in 0.3 volume of water, and broken with a 10-kc oscillator for 6 min. Whole cells and cell fragments were removed by centrifugation.

TABLE 1. *Growth of Aerobacter aerogenes PRL-R3 on the various pentoses and pentitols**

Growth substrate	Substrate used for growth of inoculum					
	D-Glucose	D-Arabinose	L-Arabitol	D-Lyxose	L-Xylose	Xylitol
D-Glucose	0.5†	0.5	0.5	0.5	0.5	0.5
D-Xylose	0.5	0.5	0.5	0.5	0.5	0.5
L-Xylose	7	25	22	27	2	5
D-Arabinose	2	1	4	2	1.5	1
L-Arabinose	0.5	0.5	1	1	1	1
D-Ribose	1	1	1	1	1	1
D-Lyxose	4	4	4	1	4	4
D-Arabitol	0.5	0.5	1	1	0.5	1
L-Arabitol	2	2	1	2	1	1
Ribitol	1	1	1	1	1	1
Xylitol	4	4	4	4	4	2

* The incubation was carried out in screw-cap tubes containing 5.5 ml of the salts solution supplemented with 0.5% of the indicated carbohydrate. The inoculum consisted of 2×10^6 cells per ml, and was prepared from a culture grown on the indicated carbohydrate, harvested by centrifugation, and washed twice with sterile salts solution. The tubes were slanted in test tube racks and shaken at 26 C. Turbidity determinations were made at time intervals, and were corrected for carbohydrate blanks.

† Figures indicate number of days required to reach a turbidity reading of 1.0.

RESULTS

Growth characteristics. A. aerogenes, PRL-R3, utilized as a sole source of carbon and energy for growth seven of the eight aldopentoses and all of the pentitols. The times required to reach a prescribed growth level on these substrates are shown in Table 1. With the use of a standard inoculum of D-glucose–grown cells, growth on D-glucose, D-xylose, D-ribose, L-arabinose, ribitol, and D-arabitol was complete within 1 day. A longer incubation time was required for the other substrates as follows: D-arabinose and L-arabitol, 2 days; D-lyxose and xylitol, 4 days; and L-xylose, from 3 to 4 weeks. The longer growth period on these substrates was due to a lag of approximately 15 hr with D-arabinose and L-arabitol, and 3 days with D-lyxose or xylitol. When L-xylose was used as substrate, the lag prior to growth varied from 25 to 35 days. Growth on L-xylose, once initiated, was complete within 2 to 3 days. Varying the substrate concentrations did not significantly alter the time required for growth, but growth of the inoculum on a pentose or pentitol which afforded slow growth reduced the subsequent growth time on the same substrate. Cross adaptation was noticed only in the case of L-xylose, xylitol, and L-arabitol; i.e., L-xylose– or xylitol-grown inocula grew on L-arabitol within 1 day. Extensive recrystallization of L-xylose, xylitol, D-lyxose, and D-arabinose from absolute ethanol did not decrease the time required for growth.

In a single experiment with L-lyxose, with the use of the standard inoculum of D-glucose–grown cells, growth was complete within 2 days. L-Lyxose was not available in sufficient quantity for further studies. Attempts to obtain growth with L-ribose as the substrate were unsuccessful.

Carbohydrate oxidation. The ability of D-glucose–grown cells to acquire a system for oxidation of pentoses and pentitols was measured manometrically with washed-cell suspensions, in the presence of salts and inducers (substrates; Fig. 2). The lag in ability to oxidize the inducers was found to correlate with the time required to initiate growth on the same substrates. Relative to D-glucose, a lag of several hours was visible for L-arabinose, D-arabitol, D-ribose, and ribitol, presumably the time required to synthesize the necessary enzymes. A slightly longer lag was normally observed with D-xylose. At 32 and 37 C,

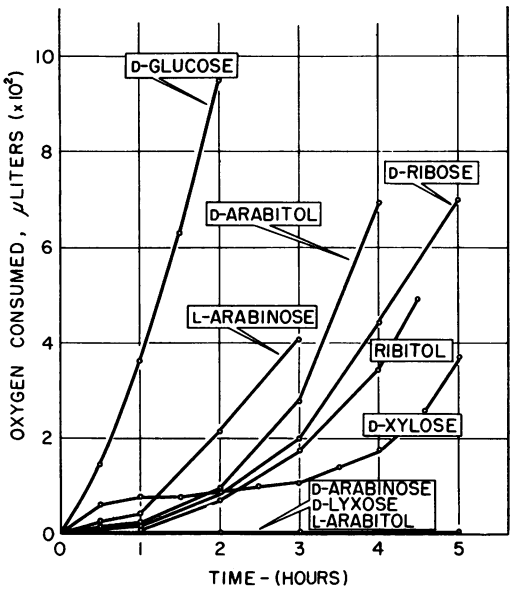

FIG. 2. *Oxidation of pentoses and pentitols by glucose-grown cell suspensions at 26 C. Each flask contained 2.6 ml of a cell suspension (3.6 mg, dry weight, per flask), 0.3 ml of 40% KOH in the center well, and 0.1 ml of 1 M substrate added from the side arm at 0 time. The cells were harvested by centrifugation, washed, and incubated in the salts solution for 3 days prior to use. All values are corrected for endogenous activity.*

the times for adaptation decreased but the results were qualitatively the same.

Specificity of induction—pentulokinases. The phosphorylation of D- and L-ribulose, D- and L-xylulose, and D-ribose is an integral part of pentose and pentitol utilization in *A. aerogenes*. The kinase activities for these substrates in extracts of cells grown on the various C_5 substrates were determined to establish the specificity for induction and to obtain evidence for the number of kinases involved. A tabulation of the specific activities found in extracts is shown in Table 2. In the assay used, NADH oxidation resulting from ADP formation was automatically plotted with high precision as a linear decrease in absorbancy vs. time (Wood and Gilford, 1961). Rates taken from slopes of these lines allowed discernment of activity at very low levels, but estimation of kinase activity was somewhat limited by the amounts of NADH oxidase and adenosine triphosphatase. A value of zero indicates that activity was not detectable or was no greater than 1.0 unit per mg of protein. When variable results were obtained from replicate extracts, the highest and lowest values are given.

The induction of L-ribulokinase and D-ribokinase proved to be highly specific. L-Xylulokinase activity was found in extracts prepared from cells grown on L-xylose and L-arabitol; occasionally, low activities were detected in extracts of xylitol-grown cells.

Of the three substrates whose dissimilation is known to involve D-xylulose as a common intermediate, D-xylose was the best inducer for D-xylulokinase, D-lyxose was the poorest, and D-arabitol was intermediate. Xylitol, also metabolized by oxidation to D-xylulose, induced D-xylulokinase to the same extent as did D-lyxose. Of the five kinases, D-ribulokinase showed the least specificity for induction. Although this enzyme is be-

TABLE 2. *Kinase activity of cells grown on various pentoses and pentitols**

Growth substrate	D-Ribulokinase	D-Xylulokinase	L-Ribulokinase	L-Xylulokinase	D-Ribokinase
D-Glucose	0†	0	0	0	0
D-Ribose	5.1–9.7	0	0	0	5.6–6.6
D-Arabinose	10–19.3	0–2.6	0	0	0
D-Xylose	0–2.0	15–50	0	0	0
D-Lyxose	0–1.0	1.4–3.3	0	0	0
L-Arabinose	1.2–4.5	0	1.2–8.8	0	0
L-Xylose	1.0–5.0	0	0	1.0–8.0	0
Ribitol	3.3–21.4	0	0	0	0
D-Arabitol	0	9.7–11.4	0	0	0
Xylitol	9.1–30	1.0–2.2	0	0–1.0	0
L-Arabitol	0–1.0	0	0	4.4–4.8	0

* Each cuvette contained 1.0 μmole of $MgCl_2$; 1.5 μmoles of glutathione; 8 μmoles of tris buffer (pH 7.5); 0.5 μmole of adenosine triphosphate; 0.25 μmole of phosphoenolpyruvate; 0.1 μmole of NADH or NADPH; 0.05 ml of lactic dehydrogenase containing pyruvate kinase (Worthington Biochemical Corp., Freehold, N.J.; muscle); 1.5 μmoles of pentulose; 3 to 8 units of enzyme; and water to a total volume of 0.15 ml. When a range of values was obtained with different preparations, the highest and lowest values are given. A value of 0 indicates that the specific activity was less than 1.0.

† Figures indicate specific activity in units per milligram of protein.

lieved to be directly involved in the metabolism of only D-arabinose and ribitol, activity could readily be detected in extracts of D-ribose– and L-xylose–grown cells, and very high activity was always found in xylitol-grown cells.

Pentitol dehydrogenases. Table 3 demonstrates the NAD-linked, pentitol dehydrogenase activities in extracts. The data were calculated from rates of NADH oxidation under conditions of the kinase assays, but without ATP added and with the NADH oxidase rates subtracted. L-Arabitol (→ L-xylulose) dehydrogenase activity was normally detected only in L-arabitol–grown cells, although activity could be detected occasionally in cells grown on other substrates. The best inducer for D-arabitol (→ D-xylulose) dehydrogenase was D-arabitol. Smaller amounts for this enzyme were always obtained from growth on D-xylose, D-lyxose, and xylitol. Low levels of ribitol (→ D-ribulose) dehydrogenase could be detected in extracts prepared from L-xylose–, D-lyxose–, D-arabitol–, and L-arabitol–grown cells. High activity was present in D-arabinose– and ribitol–grown cells. NADH-linked, L-ribulose reductase activity was never observed.

TABLE 3. *Pentitol dehydrogenase activities of cells grown on various pentoses and pentitols**

Growth substrate	Dehydrogenase activities (units/mg of protein)			
	Ribitol dehydrogenase	D-Arabitol dehydrogenase	L-Arabitol dehydrogenase	L-Ribulose reductase
D-Glucose	0	0	0	0
D-Ribose	0	0	0	0
D-Arabinose	38–145	0–2.4	0	0
D-Xylose	0	1.0–4.1	0	0
D-Lyxose	1.1–5.1	3.1–17	0–1.7	0
L-Arabinose	0	0	0	0
L-Xylose	1.0–4.3	0	0	0
Ribitol	46–154	0–2.3	0–2.5	0
D-Arabitol	2.1–2.7	47–177	0	0
Xylitol	125–800	7.3–9.3	0–3.4	0
L-Arabitol	3.7–16	0–1.8	0–5.4	0

* The assay system was identical to that in Table 2 except that the adenosine triphosphate was omitted. The values given are for pentulose reduction. When different activities were obtained with different preparations, the highest and lowest values are given. A value of 0 indicates activity was not detectable (less than 1.0).

TABLE 4. *Isomerase activities of cells grown on various pentoses and pentitols**

Growth substrate	Specific activity (units per mg of protein)				
	D-Arabinose isomerase	D-Xylose isomerase	L-Xylose isomerase	L-Arabinose isomerase	D-Lyxose isomerase
D-Glucose	0	0	0	0	0
D-Ribose	1–2	0	0	0	0
D-Arabinose	17–39	0	0.5–1	0	0
D-Xylose	0	3–10	0	0	0
D-Lyxose	0	1–4	0	0	2–3
L-Arabinose	0	0	0	8–50	0
L-Xylose	239–584	0	8–15	0	0
Ribitol	0–1	0	0	0	0
D-Arabitol	0	0	0	0	0
Xylitol	0–0.2	0–0.5	0	0	0
L-Arabitol	0	0	0	0	0

* The assay system consisted of 130 μmoles of cacodylate buffer (pH 7.0), 10 μmoles of metal ($MnCl_2$, for D- and L-arabinose isomerases, $MgCl_2$ for D-xylose and D-lyxose isomerases, and $CoCl_2$ for L-xylose isomerase), 100 μmoles of substrate, and water to a total volume of 2.0 ml. Tubes were incubated at 37 C, and samples were removed at time intervals for analysis. A value of 0 indicates a specific activity of less than 0.2.

Pentose isomerases. Table 4 demonstrates the specificity of induction of the aldopentose isomerases. High blank values in the cysteine-carbazole test, due to the acid-catalyzed conversion of residual aldopentose to ketopentose, the inherently low activity of the isomerases in crude extracts, and the possibility of further isomerization of the ketopentose to the epimeric aldopentose, decreased the sensitivity of the assay. The induction of L-arabinose (→ L-ribulose) isomerase and D-lyxose (→ D-xylulose) isomerase activities in detectable amounts occurred only with L-arabinose and D-lyxose as inducers, respectively. In contrast, D-xylose (→ D-xylulose) isomerase activity was detected in cells grown on either D-xylose or D-lyxose. Activity for D-arabinose isomerase and L-xylose (→ L-xylulose) isomerase was detected in cells grown on either D-arabinose or L-xylose. The isomerizations of D-arabinose and L-xylose are believed to be due to a single enzyme. Trace amounts of D-arabinose isomerase activity could also be detected in extracts of cells grown on D-ribose and ribitol.

DISCUSSION

The data indicate that the strain of *A. aerogenes* is capable of utilizing all of the eight aldopentoses except L-ribose and all four of the pentitols as substrates for growth. [The inability to observe growth on L-ribose as had been observed by Simpson (*unpublished data*) has not been resolved.] With D-lyxose, xylitol, and L-xylose, where a long lag occurs prior to growth, the possibility exists that mutation and selection rather than utilization of pre-existing genetic information is involved. Standard plating experiments have proved inconclusive in answering this question, because growth occurred on salt-agar plates in the absence of added carbohydrate. Growth on L-xylose is of special interest because of the very long lag. In a number of fluctuation tests performed with L-xylose, and with a population of 6.6×10^5 viable cells per tube, growth was not visable in any tube before 20 days. Thereafter, growth occurred in all tubes in the next 45 days or less. Thus, this experiment produced no definite evidence for spontaneous mutation to L-xylose utilization. However, L-xylose–grown cells retain the ability of rapid growth on L-xylose even after subsequent transfer on D-glucose, indicating a persistence of information and suggesting a mutation to L-xylose utilization. It should be noted that, with the possible exception of a specific permease and L-xylulose-5-phosphate 3-epimerase, all of the enzymes required for L-xylose metabolism can be induced within 2 days by growth on either L-arabitol or D-arabinose. That is, L-xylulokinase and L-ribulose-5-phosphate 4-epimerase were demonstrated in extracts of cells grown on L-arabitol (Fossitt et al., 1964), and L-xylose isomerase is believed to be identical with D-arabinose isomerase which is induced by D-arabinose (Anderson and Wood, 1962*a*).

With respect to the families of kinases, pentitol dehydrogenases, and isomerases, it is apparent that synthesis of at least four types each of pentulokinase, pentitol dehydrogenase, and pentose isomerase are possible. It is recognized, however, that purification and careful characterization are required before the final number can be established. Lack of specificity may reduce the number of distinct enzymes in each family; or, in contrast, more members of each family may exist since different proteins, with common enzymatic activities, may be synthesized in response to separate inducers, as in the case of the aspartokinases (Stadtman et al., 1961).

D-Ribulokinase and ribitol dehydrogenase appeared not to be under as strict control as were the other enzymes studied, since nonspecific induction of these enzymes was commonly observed. The D-ribulokinase activity observed in extracts of L-arabinose–grown cells may be explained by the nonspecificity of L-ribulokinase. This enzyme, purified from L-arabinose–grown cells, phosphorylates D-ribulose, L-arabitol, and ribitol (Simpson and Wood, 1958). The L-xylulokinase from L-xylose cells and the D-xylulokinase from D-xylose cells were purified and were shown to be specific (Anderson and Wood, 1962*b*; Bhuyan and Simpson, 1962). The ribitol dehydrogenase activities observed after growth on D-lyxose and D-arabitol might be explained by the formation of small amounts of D-ribulose in metabolism and the subsequent induction of low levels of ribitol dehydrogenase, as observed with *A. aerogenes*, 1033, by Hulley, Jorgensen, and Lin (1963). Perhaps the most interesting example of cross induction is the presence in xylitol-grown cells of higher levels of D-ribulokinase and ribitol dehydrogenase than were obtained by growth on any other inducer, including D-arabinose and ribitol.

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