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L-Lyxose Metabolism Employs the L-Rhamnose Pathway in Mutant Cells of *Escherichia coli* Adapted To Grow on L-Lyxose

JOSEFA BADIA,[1]† ROSA GIMENEZ,[2] LAURA BALDOMÁ,[2] ESTHER BARNES,[2] WOLF-DIETER FESSNER,[1] AND JUAN AGUILAR[2]*

*Department of Organic Chemistry and Biochemistry, University of Freiburg, D-7800 Freiburg, Federal Republic of Germany,[1] and Department of Biochemistry, School of Pharmacy, University of Barcelona (Pedralbes), Barcelona 08028, Spain[2]**

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***Escherichia coli* cannot grow on L-lyxose, a pentose analog of the 6-deoxyhexose L-rhamnose, which supports the growth of this and other enteric bacteria. L-Rhamnose is metabolized in *E. coli* by a system that consists of a rhamnose permease, rhamnose isomerase, rhamnulose kinase, and rhamnulose-1-phosphate aldolase, which yields the degradation products dihydroxyacetone phosphate and L-lactaldehyde. This aldehyde is oxidized to L-lactate by lactaldehyde dehydrogenase. All enzymes of the rhamnose system were found to be inducible not only by L-rhamnose but also by L-lyxose. L-Lyxose competed with L-rhamnose for the rhamnose transport system, and purified rhamnose isomerase catalyzed the conversion of L-lyxose into L-xylulose. However, rhamnulose kinase did not phosphorylate L-xylulose sufficiently to support the growth of wild-type *E. coli* on L-lyxose. Mutants able to grow on L-lyxose were analyzed and found to have a mutated rhamnulose kinase which phosphorylated L-xylulose as efficiently as the wild-type enzyme phosphorylated L-rhamnulose. Thus, the mutated kinase, mapped in the *rha* locus, enabled the growth of the mutant cells on L-lyxose. The glycolaldehyde generated in the cleavage of L-xylulose 1-phosphate by the rhamnulose-1-phosphate aldolase was oxidized by lactaldehyde dehydrogenase to glycolate, a compound normally utilized by *E. coli*.**

Escherichia coli does not grow on L-lyxose as the sole carbon and energy source, and so far the utilization and possible metabolism of this sugar by *E. coli* have not been described. A pathway for the L-lyxose metabolism leading to intermediates of the pentose phosphate route has been proposed for *Klebsiella pneumoniae* (1, 27, 28). However, as stated by Mortlock (27), the origin of the enzymes involved remains obscure.

L-lyxose, thought to be an uncommon sugar in nature, is structurally related to the 6-deoxyhexose L-rhamnose (see Fig. 1) or to the hexose L-mannose by the stereo configuration of the hydroxyls at carbons 2, 3, and 4 in the same way as the pentose D-arabinose is structurally related to the 6-deoxyhexose L-fucose or to the hexose L-galactose. It has been proved by several in vitro studies (9, 19, 23) that D-arabinose may be converted to glycolaldehyde and dihydroxyacetone phosphate by the enzymes of the L-fucose metabolism pathway, and Zhu and Lin (31, 32) have shown how L-galactose may be metabolized by the enzymes of the same pathway.

L-Fucose and L-rhamnose are metabolized in *E. coli* through parallel pathways formed by the sequential action of a concentrating process mediated by a permease, an aldose-ketose isomerization mediated by an isomerase, a phosphorylation mediated by a kinase, and an aldol cleavage mediated by an aldolase. The two homologous sets of inducible proteins are each specific for the metabolism of its corresponding sugar and are encoded by two different gene clusters located on the *E. coli* chromosome at 60.2 min for fucose and 88.2 min for rhamnose (3).

The two pathways converge after the corresponding aldolase action takes place, cleaving the six-carbon derivative of either 6-deoxyhexose into the same products: dihydroxyacetone phosphate and L-lactaldehyde. The aldehyde is oxidized in two steps to pyruvate by means of NAD-dependent lactaldehyde dehydrogenase (12) and flavin-linked lactate dehydrogenase (17), thus channeling all the carbons from fucose or rhamnose into central metabolic pathways.

In the present report we demonstrate how mutants of *E. coli* adapted to grow on L-lyxose utilize the enzymes of the L-rhamnose pathway (Fig. 1) to incorporate all carbons of the pentose through the generation of dihydroxyacetone phosphate and glycolaldehyde. This aldehyde is subsequently oxidized by the action of lactaldehyde dehydrogenase (10) to glycolate, a compound normally utilized by *E. coli*.

MATERIALS AND METHODS

Chemicals. L-Rhamnose, L-lyxose, L-xylulose, and ethyl methanesulfonate (EMS) were from Sigma Chemical; casein acid hydrolysate and Bacto-Agar were from Difco. L-[^{14}C]rhamnose (210 mCi/mmol) was from Commissariat a l'Energie Atomique, Gif sur Yvette, France. Polymin P was from Aldrich. DEAE-Sepharose and Sephadex G-150 were from Pharmacia Fine Chemicals, and gel electrophoresis materials were from Bio-Rad. L-Rhamnulose was prepared by incubation of strain ECL714 in minimal medium (6) containing 1% casein acid hydrolysate and 20 mM L-rhamnose. After removal of the cells, the ketose was purified from the supernatant as described by Fan et al. (20). L-Rhamnulose 1-phosphate was also obtained as described by Fan et al. (20), using a cell extract of strain RhaD62 to phosphorylate L-rhamnulose.

Bacteria and mutant selection. The parental strain used was an *E. coli* K-12 strain, known as E-15 (2) and here referred to as ECL1 (24). Strain ECL714 is a rhamnulose

* Corresponding author.
† Present address: Department of Biochemistry, School of Pharmacy, University of Barcelona (Pedralbes), Barcelona 08028, Spain.

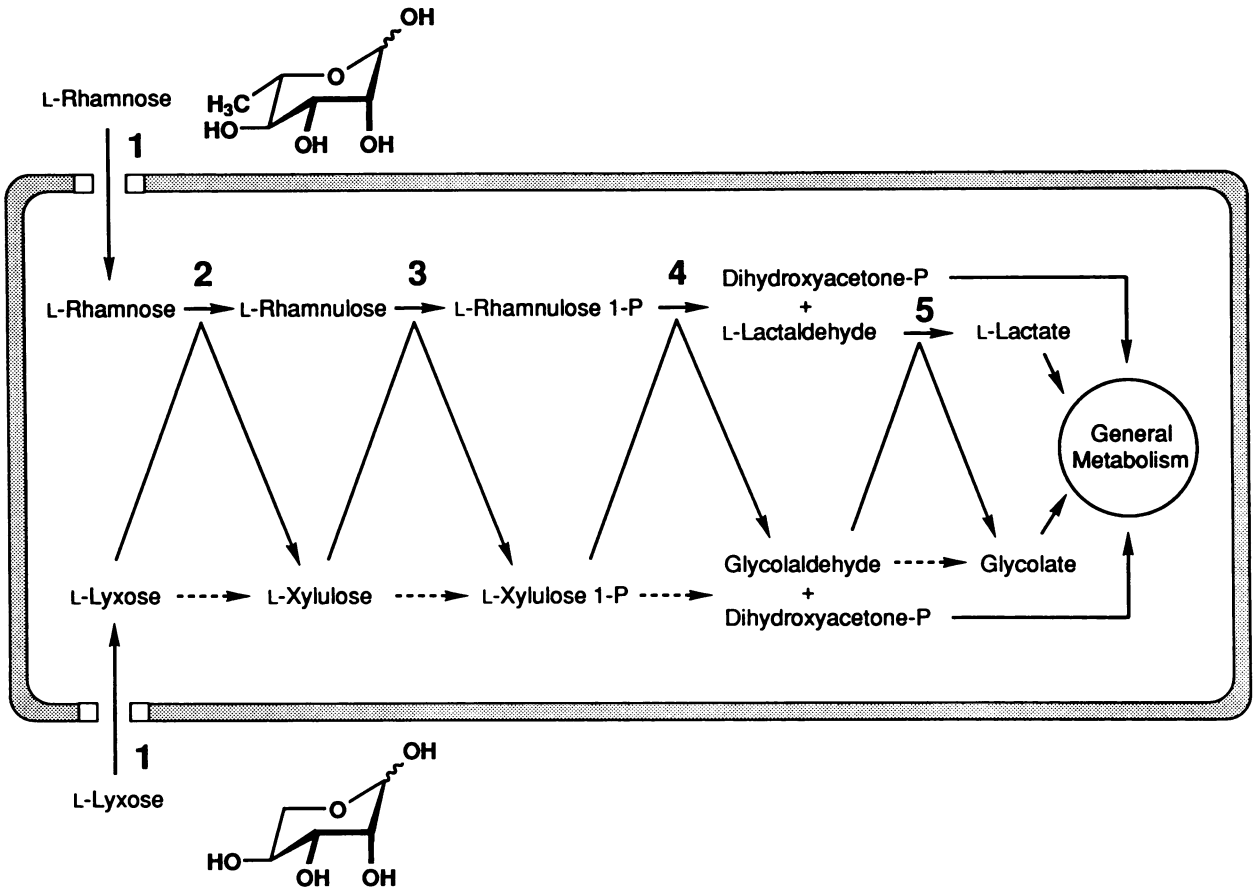

FIG. 1. Pathway for the degradation of L-rhamnose and L-lyxose. The pathway for L-rhamnose in wild-type cells consists of rhamnose permease (step 1), rhamnose isomerase (step 2), rhamnulose kinase (step 3), rhamnulose-1-phosphate aldolase (step 4), and lactaldehyde dehydrogenase (step 5). The pathway indicated for L-lyxose was found in mutant strain JA125 adapted to grow on this pentose.

kinase-negative mutant derived from strain ECL1 (11), and strain ECL493 (*ald-1*, *trg*::Tn*10*) is a lactaldehyde dehydrogenase-negative mutant with a closely linked Tn*10* insertion (12). Strain RhaD62 is a rhamnulose-1-phosphate aldolase-negative mutant isolated by Power (29). All these strains were kindly provided by E. C. C. Lin, Harvard Medical School, Boston. Strain DF903 [Δ(*rha-pfkA*) *pfkB1*] containing a large deletion encompassing the *rha* locus was kindly provided by D. Fraenkel, Harvard Medical School. Strain JA121 is a rhamnose isomerase overproducer obtained in our laboratory by transforming strain JA120 with plasmid pJB4.3 (4) from which rhamnose isomerase is expressed constitutively. Strain JA125 was obtained from ECL1 after mutagenesis with EMS and selected by its ability to grow on L-lyxose. Strains JA126 to JA132 are rhamnose-negative mutants of strain JA125 isolated after mutagenesis with EMS, growth on glucose and replica plating on L-rhamnose. Strain JA133 was an L-lyxose-positive, lactaldehyde dehydrogenase-deficient transductant obtained as follows. Strain JA125 was infected with P1 phage grown on strain ECL493, and transductants were selected for tetracycline resistance. The transductants were then tested for simultaneous loss of lactaldehyde dehydrogenase activity by growth on L-rhamnose and in vitro assay of the enzyme and for the maintenance of L-lyxose utilization. Transduction experiments were performed as indicated by Miller (26).

Cell growth and preparation of cell extracts. Cells were grown and harvested as described previously (6). The yield of the cultures was estimated as the amount of cell protein per gram of substrate determined in cell extracts before centrifugation. The carbon sources L-rhamnose and glucose were added to a basal inorganic medium (6) at 10 mM, L-lyxose was added at 12 mM, and glycerol was added at 20 mM. Casein acid hydrolysate was used at 0.5%. For purification of rhamnose isomerase, cells of strain JA121 were grown overnight aerobically on 1% casein acid hydrolysate in the presence of 100 μg of ampicillin per ml. Thiamine (1.65 μg/ml) was added to cultures of strains ECL714 and JA121. Extracts were prepared as described previously (7) with 10 mM Tris-HCl (pH 7.3) buffer containing 10 mM β-mercaptoethanol. When the extracts were used for rhamnose isomerase purification, the buffer also contained 1 mM Mn^{2+}.

Transport and enzyme assays. L-Permease activity was determined from the rate of L-[^{14}C]rhamnose (210 mCi/mmol) uptake by whole cells, and the assay was performed as described by Hacking and Lin (21) for fucose transport, with a rhamnose concentration of 0.2 mM (0.8 mCi/mmol). Isomerase activity was determined as described by Takagi and Sawada (30), measuring ketose formation by the cysteine-carbazol method (18) with tagatose as a standard when L-rhamnose was the substrate and with L-xylulose when L-lyxose was the substrate. Assays were performed routinely at a substrate concentration of 10 mM instead of 2 mM. L-Rhamnulose kinase and L-xylulose kinase activities

were determined from the rate of NADH oxidation in the presence of L-rhamnulose or L-xylulose, ATP, a pyruvate kinase–L-lactate dehydrogenase mixture (8 μg/ml), and phosphoenolpyruvate (14). Rhamnulose-1-phosphate aldolase activity was measured by the rate of dihydroxyacetone phosphate formation from L-rhamnulose-1-phosphate. Dihydroxyacetone phosphate was determined with glycerol phosphate dehydrogenase in the presence of NADH (16). The aldehyde dehydrogenase activity was measured by monitoring the rate of increase in A_{340} (NADH formation) with glycolaldehyde as a substrate (5).

The K_m and V_{max} values were obtained by linear regression analysis of the data plotted by the method of Lineweaver and Burk.

The protein concentration in cell extracts and purified preparations was determined by the method of Lowry et al. (25) with bovine serum albumin as the standard.

Ketose determination. We determined ketose excretion in aliquots of the culture medium taken at the indicated times. After removal of the cells, the samples were processed by the method of Dische and Borenfreund (18), using as a blank the culture growing without sugar. The ketose concentration was estimated from a tagatose calibration curve.

Purification of rhamnose isomerase. Cells of strain JA121 were grown and harvested and the cell extract was prepared as described above. The enzyme was purified in a three-step procedure carried out at 2 to 4°C as follows.

(i) Polymin P precipitation. A 0.2-ml sample of an 18% solution of polymin P (pH 7.9) was added to 20 ml of the cell extract. After equilibration for 20 min, the precipitate was removed by centrifugation at 30,000 × *g* for 10 min. The supernatant was then dialyzed overnight against the same buffer.

(ii) DEAE-Sepharose chromatography. The dialyzed sample was applied to a column (10 by 1.5 cm) of DEAE-Sepharose previously equilibrated with the same buffer containing 0.15 M NaCl. After washing with 5 to 6 bed volumes of this buffer, elution was performed with a 500-ml linear gradient of NaCl (0.15 to 0.35 M) in the equilibration buffer. The enzyme eluted at 0.225 M NaCl. Active fractions were concentrated in an Amicon ultrafiltration cell by using a PM-10 membrane.

(iii) Sephadex G-150 gel filtration. The concentrated enzyme solution was applied to a column (94 by 2 cm) of Sephadex G-150 equilibrated with 10 mM Tris · HCl (pH 7.3), 10 mM β-mercaptoethanol, 1 mM $MnCl_2$, and 100 mM NaCl. Elution was performed at a constant flow rate of 7 ml/h with a peristaltic pump. Active fractions were pooled, concentrated in an Amicon ultrafiltration cell with a PM-10 membrane, and stored at −20°C in the presence of 1 mM dithiothreitol and 20% glycerol.

Gel electrophoresis. Polyacrylamide gel electrophoresis under dissociating conditions was performed by the method of Laemmli (22), and the gels were stained with Coomassie brilliant blue R-250. For molecular weight estimations, the following standards were used: trypsin inhibitor, 20,100; carbonic anhydrase B, 29,000; aldolase, 40,000; and catalase, 58,100.

RESULTS

Induction of L-rhamnose enzymes by L-lyxose. The four proteins involved in the trunk pathway of rhamnose metabolism were induced by the presence of L-lyxose in wild-type *E. coli*. For these induction experiments, and because of the inability of strain ECL1 to grow on L-lyxose, cells were grown on 0.5% casein acid hydrolysate plus 10 mM L-rhamnose or 12 mM L-lyxose, and rhamnose enzymes activities were assayed in each case by using L-rhamnose or the corresponding metabolites as the substrate. In this way, L-lyxose induced L-rhamnose permease to 60 to 70% of the rhamnose-induced level when assayed as L-[^{14}C]rhamnose uptake. Interestingly, rhamnose isomerase assayed on L-rhamnose, rhamnulose kinase assayed on L-rhamnulose, and rhamnulose-1-phosphate aldolase assayed on L-rhamnulose 1-phosphate showed a higher level induced by L-lyxose than induced by L-rhamnose, even if L-rhamnose was used as the only carbon source (Table 1).

TABLE 1. Activities of the rhamnose system in wild-type strain ECL1 and mutant strain JA125 under different inducing conditions

Strain	Carbon source	Enzyme activity			
		Permease[a]	Isomerase[b]	Kinase[b]	Aldolase[b]
ECL1	CAA[c]	ND[d]	ND	ND	ND
ECL1	CAA + L-rhamnose	5.4	180	112	40
ECL1	CAA + L-lyxose	3.5	260	220	88
ECL1	L-Rhamnose	5.8	220	145	47
JA125	L-Rhamnose	5.1	250	114	45
JA125	L-Lyxose	4.5	460	200	85

[a] Expressed as nanomoles per minute per 10^9 cells at 30°C.
[b] Expressed as milliunits per milligram at 37°C.
[c] CAA, casein acid hydrolysate.
[d] ND, not detectable.

L-Rhamnose- and L-lyxose-induced ketose excretion. A first hint of the utilization of L-lyxose by some early steps in the rhamnose metabolic pathway came after the observation that these cells excreted a ketose not only in the presence of L-rhamnose but also in the presence of L-lyxose. When L-rhamnose was given to wild-type *E. coli*, the ketose rhamnulose formed in the first step of the pathway was excreted, leading to a transient increase in the concentration of this compound in the culture medium during the first periods of growth. The ketose was subsequently utilized for growth, as indicated by its disappearance from the culture medium and by the yield of the culture. The maximum concentration in the transient increase of the ketose was 2 mM when 10 mM L-rhamnose was used (Fig. 2). Evidently this was also seen when a rhamnulose kinase mutant was incubated in L-rhamnose, although in this case the ketose was not utilized again and the culture functioned as a system of biotransformation of L-rhamnose into L-rhamnulose. This phenomenon of ketose excretion also appeared for the wild-type and mutant strains if L-lyxose instead of L-rhamnose was given to the cell suspension. In this case, too, the ketose excretion led to a ketose accumulation to a point at which all L-lyxose was transformed, since no growth of cells was detected.

L-Lyxose as a substrate of rhamnose permease. The affinity of L-rhamnose permease for L-lyxose was shown by experiments involving competition of L-lyxose for rhamnose transport. Activities of L-rhamnose permease were indeed inhibited by the presence of increasing concentrations of L-lyxose in the assay mixture. As shown in Fig. 3, the time course of rhamnose uptake into the cells displayed a 20% reduction when equal concentrations (0.2 mM) of radioactive L-rhamnose and nonradioactive L-lyxose were present and a reduction of up to 56% when 5 mM L-lyxose was used.

L-Lyxose as a substrate of rhamnose isomerase. To prove the isomerization of L-lyxose to L-xylulose by rhamnose

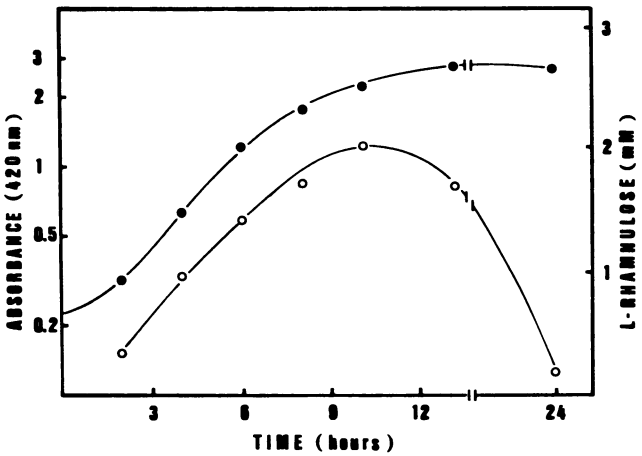

FIG. 2. Time course analysis of L-rhamnulose excretion during aerobic growth of strain ECL1 on L-rhamnose. Sample cultures were processed at the indicated times to measure the optical density (cell growth) (●) and extracellular L-rhamnulose concentration (○).

isomerase, isolation of this enzyme was required to avoid cross-reactions with other isomerases. To this end, the enzyme was purified as indicated in Materials and Methods. This procedure yielded a preparation of rhamnose isomerase with a specific activity of 6.2 U/mg, which corresponds to a fivefold purification of the activity in the crude extract (Table 2). It is of interest that the multicopy plasmid constitutively expressing the rhamnose isomerase in the cells used as the enzyme source highly enriched (six to eight times) the cell content in the rhamnose isomerase and that under the growth conditions used, a cell lacking the plasmid displayed no activity. The enzyme preparation yielded a single band of 47,000 Da when a sample of 20 μg was analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS).

The activity of this enzyme preparation was assayed with L-rhamnose and L-lyxose as substrates. Concentration kinetics for both substrates yielded a K_m of 2 mM for L-rhamnose

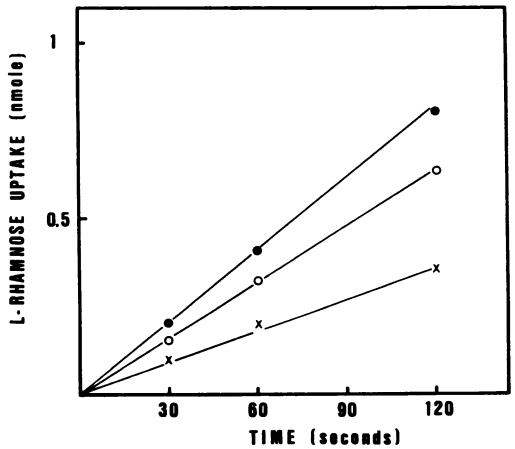

FIG. 3. Effect of L-lyxose on L-rhamnose uptake. Cells of strain ECL1 were grown on L-rhamnose and processed for rhamnose transport assay. Results for L-[^{14}C]rhamnose uptake by whole cells in the absence of L-lyxose (●) and in the presence of 0.2 mM (○) or 5 mM (×) L-lyxose in the assay mixture are presented.

TABLE 2. Purification of *E. coli* rhamnose isomerase

Purification step	Total protein (mg)	Total units (U)	Sp act (U/mg)	Purification (fold)	Yield (%)
Crude extract	204	251	1.25	1.0	100
Polymin treatment	184	246	1.34	1.1	98
DEAE-Sepharose	30	146	4.90	4.0	58
Sephadex G-150	17	105	6.20	5.0	42

and 5 mM for L-lyxose. The apparent V_{max} was 6.2 U/mg for L-rhamnose and 6.0 U/mg for L-lyxose.

Inability of rhamnulose kinase to phosphorylate L-xylulose. Since no phosphorylating activity toward L-xylulose, the product of the activity of L-rhamnose isomerase on L-lyxose, could be detected in crude extracts of *E. coli* grown in the presence of either L-rhamnose or L-lyxose (Table 3), no attempts were made to isolate the rhamnulose kinase to assay this activity on L-xylulose.

Selection of mutants able to use L-lyxose as the only carbon and energy source. Although L-lyxose is able to induce the complete rhamnose metabolic system and seems to be recognized by the rhamnose permease and transformed by rhamnose isomerase, L-lyxose cannot support the growth of *E. coli*. Mutant cells of *E. coli* able to grow on L-lyxose as the sole source of carbon and energy can easily be isolated by mutagenesis with EMS. About 10^8 cells of strain ECL1 were plated on 12 mM L-lyxose agar supplemented with 50 μg of casein acid hydrolysate per ml. A filter paper disk loaded with 50 μl of EMS was placed in the center of the plate for mutagenesis. After 7 days of incubation at 37°C, several colonies appeared around the disk. A large one, strain JA125, was purified on agar containing 12 mM L-lyxose as the sole carbon and energy source.

When tested for growth on L-rhamnose, strain JA125 displayed the same doubling time and yield as strain ECL1. In liquid medium, the mutant strain showed a doubling time of 200 min on L-lyxose and 100 min on L-rhamnose. Both cultures reached the same yield of 280 mg of protein per g of sugar.

Linkage of L-lyxose and L-rhamnose utilization traits in strain JA125. To isolate L-rhamnose-negative mutants derived from strain JA125, we mutagenized the cells with EMS as described by Miller (26); seven mutants were obtained by the selection procedure described above. Every isolate which lost the ability to grow on L-rhamnose also lost the ability to grow on L-lyxose. Further characterization of these mutants as described by Power (29) showed that the muta-

TABLE 3. Activity of rhamnulose kinase in wild-type strain ECL1 and mutant strain JA125 grown under different conditions[a]

Strain	Substrate	Activity under growth conditions:		
		CAA[b]	CAA + L-rhamnose	CAA + L-lyxose
ECL1	L-Xylulose	ND[c]	ND	ND
JA125	L-Rhamnulose	ND	114	200
JA125	L-Xylulose	ND	43	101

[a] Activities were determined by using L-rhamnulose or L-xylulose as the substrate in strain JA125 and L-xylulose in strain ECL1 and are expressed as milliunits per milligram at 37°C. The activity on L-rhamnulose in strain ECL1 is given in Table 1 for comparison.
[b] CAA, casein acid hydrolysate.
[c] ND, not detectable.

tions affected the metabolisms of L-rhamnose and L-lyxose in the same way. Two of them excreted a ketose when growing in the presence of either L-rhamnose or L-lyxose, indicating that the mutation affected the aldolase or the kinase. Another mutant, although unable to grow on 10 mM L-rhamnose or 12 mM L-lyxose, showed a positive phenotype at 50 mM sugar concentration, indicating that the mutation most probably affected the rhamnose permease.

The other four mutants should be affected in the isomerase or in the regulatory gene, since none of them grew at 50 mM L-rhamnose or L-lyxose or excreted a ketose when growing in the presence of these sugars.

Enzyme activities of the rhamnose system in cell extracts of strain JA125. Consistent with the maintenance of the ability to grow on L-rhamnose of this mutant was the observation that the rhamnose operon kept the double inducibility by L-lyxose and L-rhamnose. Furthermore, the possibility that the expression of the rhamnose system did not become constitutive was excluded by the absence of all activities found under noninducing conditions. Table 1 displays the induced activities of the rhamnose system after growth on L-rhamnose. These activities matched those found in wild-type cells. Interestingly, the induction was even higher, between 50 and 100%, when the cells were grown on L-lyxose.

Xylulose phosphorylation in cell extracts of strain JA125. As shown in Table 1, activities of the rhamnose system determined by using L-rhamnose or the corresponding derivatives as substrates were shown to be inducible in wild-type cells either by L-rhamnose or by L-lyxose. Rhamnose permease and rhamnose isomerase also acted on L-lyxose, transforming it into L-xylulose. However, although induced as indicated by the assay with L-rhamnulose, rhamnulose kinase was undetectable when L-xylulose was used as the substrate, indicating that this enzyme was unable to phosphorylate L-xylulose efficiently. The inability of rhamnulose kinase to phosphorylate L-xylulose was overcome in mutant strain JA125, in which the kinase not only was induced by L-rhamnose and L-lyxose but also was able to phosphorylate L-rhamnulose and L-xylulose (Table 3). The level of activity on L-xylulose was similar to that obtained on L-rhamnulose by wild-type cell extracts, thus permitting the growth of cells on L-lyxose. Multiple pieces of evidence indicated that kinase activity on L-xylulose was due to rhamnulose kinase: (i) xylulose kinase was induced by L-rhamnose; (ii) levels of activity with L-rhamnulose or L-xylulose displayed a striking parallelism; (iii) transduction of the L-lyxose-positive phenotype permitted us to confirm that the mutation allowing growth on L-lyxose is in the *rha* locus, and to this end P1 lysates were made from strain JA125 and then used to transduce the L-lyxose-positive phenotype into rhamnose-deleted strain DF903—all of the selected L-lyxose-positive transductants were able to grow on L-rhamnose; and (iv) the kinase activity tested on several of these transductants, chosen at random, was able to phosphorylate L-xylulose, indicating that the recovered kinase enzyme was the one belonging to the mutant strain JA125.

Oxidation of the glycolaldehyde generated from xylulose 1-phosphate. Cleavage of xylulose 1-phosphate by rhamnulose-1-phosphate aldolase yields dihydroxyacetone phosphate, an intermediate in the glycolytic pathway, and glycolaldehyde. This aldehyde can be oxidized by lactaldehyde dehydrogenase, yet another enzyme involved in the L-rhamnose metabolism.

The generation of glycolaldehyde as an intermediate in the metabolism of L-lyxose by mutant strain JA125 and its utilization by the action of lactaldehyde dehydrogenase were indicated by the yield of strains JA125 and JA133 on the pentose. Strain JA125 growing on L-lyxose presented a yield of 280 mg of protein per g of substrate, whereas strain JA133, a lactaldehyde dehydrogenase-deficient derivative of strain JA125 and hence unable to utilize L-lactaldehyde or glycolaldehyde, presented a lower yield (150 mg of protein per g of substrate). These results were very close to those obtained on L-rhamnose (278 and 160 mg of protein per g of substrate, respectively). The yield on L-rhamnose or L-lyxose of strain JA125 was equal to that of strain ECL1 on L-rhamnose, indicating that strain JA125 was utilizing all the carbons of the substrate.

TABLE 4. Activity of lactaldehyde dehydrogenase in wild-type strain ECL1 and mutant strain JA125 grown under different conditions

Strain	Activity under growth conditions[a]:		
	Glycerol[b]	L-Rhamnose	L-Lyxose
ECL1	54	250	NG[c]
JA125	62	237	320

[a] Activities were expressed as milliunits per milligram at 25°C.
[b] The basal activity level for both strains was determined by using crude extracts of cells grown on glycerol.
[c] NG, no growth.

Lactaldehyde dehydrogenase was indeed induced by L-lyxose in strain JA125 to levels even higher than those induced by L-rhamnose in both strains, JA125 and ECL1 (Table 4). The oxidation of glycolaldehyde by lactaldehyde dehydrogenase, the product of the gene *ald* (12), is well documented (10), and its product has been identified as glycolate (8), a compound that is able to support growth of strain JA125 and is normally utilized by *E. coli*.

DISCUSSION

Three different types of experiments suggest that the mutants able to grow on L-lyxose could also use the L-rhamnose metabolic pathway for growth on this pentose. First, the excretion of a ketose when L-lyxose was the growth substrate for wild-type cells, as occurred with L-rhamnose, indicated the possible entrance and isomerization of L-lyxose by, in principle, undetermined proteins.

This ketose excretion deserves some consideration. Interestingly, an intermediate in a pathway is excreted, presumably as a result of the saturation of the metabolic capacity of the later steps in the pathway; this compound is subsequently taken up again to be used when the flow through the pathway diminishes by exhaustion of the substrate being transformed. We have also observed this phenomenon for L-fucose (3a), and it is uncertain how general such an exchange of intermediate molecules with the environment could be. The isomerization as a first step, instead of other possible modifications of the molecule, causes the remarkably high motility of the intermediate. In any case, in an open system this would lead to diminished efficiency in the use of the substrate and might generate selective pressures for other modifications as first steps in metabolic pathways such as oxidations or phosphorylations hindering the exit of the intermediate.

A second type of experiment indicating the use of the L-rhamnose pathway for L-lyxose metabolism was the experiment measuring the capacity of the rhamnose permease and rhamnose isomerase to recognize and transform L-lyx-

ose. Thus, the undetermined permease and isomerase were most probably rhamnose permease and rhamnose isomerase.

Finally, it is highly significant to see how utilization of L-rhamnose and utilization of L-lyxose seem to be inseparable traits. Furthermore, the different L-rhamnose-negative mutants corresponding to each of the enzymes of the pathway have specific phenotypes (29), which may be revealed by using L-rhamnose or L-lyxose indistinctly. Hence, it seems reasonable from all this accumulated evidence to hypothesize that L-lyxose could be metabolized in mutants adapted to grow on this sugar by means of the L-rhamnose pathway. As shown in Table 1, the inability of *E. coli* to grow on L-lyxose was evidently not due to the absence of expression of the enzymes of the rhamnose operon, since all of them are normally induced by L-lyxose. Rather, the inability of the rhamnulose kinase to phosphorylate L-xylulose, the product of the action of rhamnose isomerase on L-lyxose, gives the clue to the nonutilization of L-lyxose by the cells. As indicated by Chiu and Feingold (15), purified rhamnulose kinase phosphorylates L-xylulose with 1/10 the efficiency found for L-rhamnulose, which in crude extracts of our wild-type cells becomes undetectable and seems to be insufficient to permit cell growth on L-lyxose. Consistently, the mutant strain JA125 displays a mutant kinase, with activity on L-xylulose reaching levels as high as those of wild-type enzyme on L-rhamnulose and therefore capable of supporting growth of the cells on L-lyxose. Mapping of this kinase mutation in the *rha* locus further supports the use of the rhamnose pathway for the L-lyxose metabolism.

Because of the nonavailability of L-xylulose 1-phosphate, there is no direct evidence of its catalytic cleavage by rhamnulose-1-phosphate aldolase. From the study of the culture yields of strains JA125 and JA133 on L-lyxose, it seems clear that one of the products generated in the pathway is metabolized by lactaldehyde dehydrogenase. According to the catalytic mechanism of the induced rhamnulose-1-phosphate aldolase (13, 16), this product should be glycolaldehyde, leaving one molecule of dihydroxyacetone phosphate, which would be introduced in the general metabolism. The metabolism of glycolaldehyde through glycolate and glyoxylate has already been established (8).

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