JOURNAL OF BACTERIOLOGY, Apr. 1991, p. 2459–2464
0021-9193/91/082459-06$02.00/0

Vol. 173, No. 8

Isolation and Characterization of *Escherichia coli* Mutants Able To Utilize the Novel Pentose L-Ribose

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Received 29 March 1990/Accepted 29 January 1991

Wild-type strains of *Escherichia coli* were unable to utilize L-ribose for growth. However, L-ribose-positive mutants could be isolated from strains of *E. coli* K-12 which contained a ribitol operon. L-ribose-positive strains of *E. coli*, isolated after 15 to 20 days, had a growth rate of 0.22 generation per h on L-ribose. Growth on L-ribose was found to induce the enzymes of the L-arabinose and ribitol pathways, but only ribitol-negative mutants derived from strains originally L-ribose positive lost the ability to grow on L-ribose, showing that a functional ribitol pathway was required. One of the mutations permitting growth on L-ribose enabled the mutants to produce constitutively an NADPH-linked reductase which converted L-ribose to ribitol. L-ribose is not metabolized by an isomerization to L-ribulose, as would be predicted on the basis of other pentose pathways in enteric bacteria. Instead, L-ribose was metabolized by the reduction of L-ribose to ribitol, followed by the conversion to D-ribulose by enzymes of the ribitol pathway.

Several studies have been carried out with microorganisms to discover how organisms can evolve new metabolic pathways (8, 18, 23, 26). A general approach to studying evolution is to apply selective pressure, in the form of a novel substrate, to microorganisms to see whether they can develop new metabolic capabilities. We have selected strains of *Escherichia coli* which can degrade L-ribose to study how microorganisms can evolve new metabolic pathways.

L-ribose is a pentose which is not known to exist in nature. Therefore, organisms should not have evolved metabolic pathways to degrade L-ribose, and wild-type strains of *E. coli* and *Klebsiella pneumoniae* are in fact unable to grow using L-ribose. However, L-ribose-positive mutants of *K. pneumoniae* PRL-R3 have been isolated (20). We wanted to isolate L-ribose-positive strains of *E. coli* K-12 because the superior genetic tools available for *E. coli* would facilitate the study of the mutational events which allow growth on L-ribose.

E. coli K-12 differs from *K. pneumoniae* in its ability to degrade various pentoses and pentitols (22) and lacks the ability to degrade the common pentitols, ribitol and D-arabitol (27). The ribitol and D-arabitol operons are located together in a cluster which can be transduced in *E. coli* K-12, where they replace the galactitol operon (27). Initial attempts to select L-ribose-positive strains of *E. coli* K-12 were unsuccessful, but when we used strains of *E. coli* K-12 which contained the ribitol and D-arabitol operons, we were able to select L-ribose-utilizing strains.

In this study, we have shown that L-ribose-positive mutants can be isolated from strains of *E. coli* K-12 which contain a ribitol operon. L-ribose is not metabolized by isomerization to L-ribulose, as would be predicted on the basis of other pentose pathways in enteric bacteria. Instead, L-ribose is reduced to ribitol by a novel NADPH-linked oxidoreductase. The L-ribose pathway in *E. coli* is similar to the pathway found in *Mycobacterium smegmatis* (14).

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MATERIALS AND METHODS

Bacterial strains and culture conditions. Most of the bacterial strains used in this study (Table 1) are derived from *E. coli* K-20, an *E. coli* K-12 strain which has gained the ribitol–D-arabitol gene cluster by transduction (27). JM1000 is a *E. coli* K-12 strain which lacks the ribitol–D-arabitol genes (3). Cultures were grown in a mineral salts medium (7) supplemented with 0.4% carbohydrate or 0.8% casein hydrolysate. Thiamine was added at a concentration of 10 μg/ml. Amino acids were added at a concentration of 300 μg/ml to minimal salts medium for auxotrophic strains. Antibiotics were added at the following concentrations: tetracycline (25 μg/ml), kanamycin (50 μg/ml), chloramphenicol (25 μg/ml), rifampin (100 μg/ml), and streptomycin (100 μg/ml). Solid media were made by the addition of 0.75% Gelrite (Kelco) or 1.5% agar (Difco) to liquid media. All cultures were incubated at 30 or 37°C with shaking.

Isolation of L-ribose-positive strains. L-ribose-positive strains of *E. coli* were isolated by inoculating 0.1 ml of a culture grown in mineral salts medium and glucose overnight and then washed into 10 ml of 0.4% L-ribose minimal salts medium, which was then incubated at 37°C with shaking for 12 to 20 days. L-ribose-positive strains could also be isolated by plating 10^9 cells onto 0.4% L-ribose minimal salts plates and incubating at 37°C until growth occurred. L-ribose-positive cultures were streaked on L-ribose plates, and single colonies were isolated. Isolated cultures were maintained in 80% glycerol at −20°C.

Isolation of D-arabinose-positive mutants. D-arabinose-positive mutants were isolated by plating 10^9 cells onto plates containing 0.4% D-arabinose minimal salts agar and incubating at 37°C for 10 to 14 days. D-arabinose-positive colonies were picked and streaked onto D-arabinose plates. Isolated cultures were maintained in 80% glycerol at −20°C.

Isolation of L-arabinose- and ribitol-negative mutants. Strain TM2100 was mutagenized with ethyl methanesulfonate as described by Miller (21). After mutagenesis, carbohydrate-negative mutants were increased by using penicillin treatments (10,000 U of penicillin per ml) and the appropriate sugar (10). The cells were screened for mutants by plating ~200 cells per plate on eosin-methylene blue agar plates containing 1% L-arabinose or ribitol (17).

TABLE 1. Bacterial strains

Strain or phage	Genotype[a]	Source or reference
E. coli strains		
JM1000	*E. coli* K-12 *thi pro* Rif^r	3
K-20	*lacY rbt*$^+$ *atl*$^+$	27
TM2000	*E. coli* K-20 *lrb*$^+$	This work
TM2001	*E. coli* K-20 *lrb*$^+$	This work
TM2100	TM2000 D-Ara$^+$	This work
TM2120	TM2100 *rbtD*	This work
TM2121	TM2100 *rbtB*	This work
TM2050	TM2001 *zag*::Tn*10*	This work
AB1157N	*E. coli* K-12 *thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE2 rpsL31 tsx-33 sup-37 gyrA*	13
TM160	AB1157N *rtl*$^+$	This work
KL16	Hfr PO45 *thi-1 rel-1*	19
TM2500	KL16 *zag*::Tn*10*	This work
POl1734	MAL103 with Mu dI1734	4
SG404	MC4100 P1::Tn*9cIr*100	30
TM2321	TM2001 Mu dI1734 *lrb*	This work
TM2322	TM2001 Mu dI1734 *lrb*	This work
TM2326	TM2001 Mu dI1734 *lrb*	This work
TM2329	TM2001 Mu dI1734 *lrb*	This work
NK5525	W3110 *pro*[AB]::Tn*10*	N. Kleckner
Bacteriophages		
Mu dI1734	Mu dI1681 with Δ(Mu *A B*) *Hin*dIII	4
P1*cam*	P1::Tn*9cIr*100	30
λNK561	(λ *b221 c*I857*c*I::Tn*10 O29 P*80)	15

[a] rbt, ribitol; atl, D-arabitol.

Isolation of Tn*10* near L-ribose genes. TM2001 was mutagenized with λNK561 as described by Kleckner et al. (15). Mutagenized cells were pooled and a bacteriophage P1::Tn*9cIr*100 lysate of the pooled cells was prepared (28). This phage lysate was used to transduce *E. coli* K-20 so that it became L-ribose positive. The L-ribose-positive transductants were scored on L-agar plates containing tetracycline for cotransduction of the gene(s) conferring tetracycline resistance.

Mapping of the L-ribose genes. The approximate location of the L-ribose genes was determined by using the natural gradient of transfer which occurs in Hfr × F$^-$ matings. The Hfr donor strain TM2500 was mated with the F$^-$ recipient strain TM160 as described by Miller (21) for 90 min. TM2500 is a Hfr strain which transfers counterclockwise, starting from about 60 min on the *E. coli* chromosome. Various dilutions of the cells were spread onto plates containing streptomycin selecting for the following markers: galactose, proline, leucine, xylose, and resistance to tetracycline. The number of recombinants for each marker was determined and used to locate the position of the Tn*10* relative to the other known markers. Once the approximate map position of Tn*10* was determined, two-factor crosses using bacteriophage P1 transductions were used to locate the positions of the Tn*10* and L-ribose genes with respect to nearby markers. The cotransduction frequencies were converted into kilobases by using the formula of Wu (33).

Isolation of L-ribose-negative mutants. Cultures of L-ribose strains were mutagenized by using bacteriophage Mu dI1734 (4). Fresh Mu lysates from POI 1734 were made by the procedure described previously (4). L-ribose-positive strains were grown in L broth to mid-log phase, centrifuged, and resuspended in 10 mM $MgSO_4$–50 mM $CaCl_2$. Mu lysate was added and incubated at 37°C for 25 min. Then 40 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid] was added, and the culture was incubated for 1 h and plated onto L agar plates containing kanamycin. Kanamycin-resistant colonies were picked and transferred to L-ribose minimal plates. Any colonies unable to grow on L-ribose plates were then tested on glucose minimal and ribitol minimal plates. Those colonies which could grow on glucose and ribitol minimal plates but not on L-ribose plates were studied further.

Preparation of cell extracts. Cultures were grown to late log phase and then centrifuged at 12,000 × *g* for 10 min at 4°C. The cells were washed in mineral salts and then resuspended in 10 mM Tris-HCl buffer (pH 7.5) containing 2 mM β-mercaptoethanol or 5 mM dithiothreitol. The cells were broken by sonication with three 30-s bursts using a Braunsonic 1510 sonicator. Crude extract was prepared by centrifugation at 39,000 × *g* for 20 min at 4°C. Protein concentration was determined by the method of Bradford (5), with reagents from Bio-Rad Laboratories, using bovine serum albumin as the standard.

Enzyme assays. (i) L-ribose reductase. L-ribose reductase activity was measured spectrophotometrically by following the decrease in A_{340} due to the oxidation of NADPH. Cell extracts were added to a reaction mixture containing 50 mM Tris-HCl buffer (pH 7.5), 2 mM β-mercaptoethanol, 53 μM NADPH, and 100 mM L-ribose.

(ii) Ribitol dehydrogenase. Ribitol dehydrogenase (EC 1.1.1.56) was measured by following the oxidation of NADH in the presence of D-ribulose (32).

(iii) L-arabinose isomerase. L-arabinose isomerase (EC 5.3.1.4) activity was assayed by the rate of production of the keto sugar, L-ribulose, as described by Cribbs and Englesberg (9), in which L-ribulose is detected by the cysteine-carbazole test (11).

(iv) DL-ribulose kinase. Kinase activity was measured with a linked assay, using pyruvate kinase and lactic dehydrogenase to couple the phosphorylation of the keto sugar by ATP to the oxidation of NADH (1).

Thin-layer chromatography. Samples were spotted on cellulose thin-layer chromatography plates and chromatographed with a butanol-pyridine-water (2:2:1) solvent system. Chromatograms were developed with alkaline silver nitrate (31).

Electrophoresis. Nondenaturing electrophoresis was performed by using the modified Laemmli system as described by Doten and Mortlock (12). Gels were stained for protein with Coomassie blue or stained for ribitol oxidation by the method of Neuberger et al. (24) with the following modifications: NADP was used instead of NAD, ribitol was used at a concentration of 25 mM, and gels were allowed to develop for 20 to 60 min.

Chemicals and reagents. All sugars, amino acids, antibiotics, and ethyl methanesulfonate used in this study were purchased from Sigma Chemical Company. The chemicals used for electrophoresis were obtained from Bio-Rad Laboratories. L-ribulose was prepared by M. N. MBelle (20). D-ribulose was prepared by the method of Oliver et al. (25). Cellulose thin-layer chromatography plates were from Eastman Chemicals.

RESULTS

Growth characteristics of L-ribose-positive mutants. L-ribose-positive mutants could be isolated from *E. coli* K-20 after 12 to 20 days of incubation on 0.4% L-ribose minimal medium (Fig. 1). After isolation, an L-ribose-positive strain

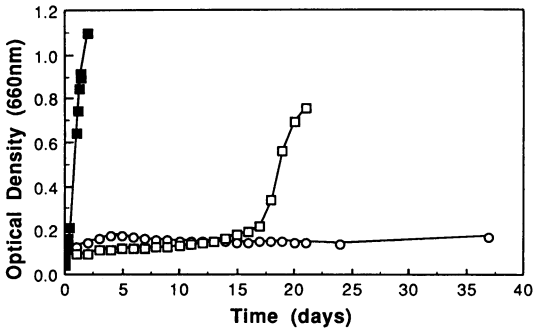

FIG. 1. Growth of *E. coli* on L-ribose minimal medium. Inocula were grown on glucose minimal salts medium, washed, and then put into 0.4% L-ribose minimal medium. Symbols: ○, JM1000; □, *E. coli* K-20; ■, TM2000.

named TM2000 showed complete growth on L-ribose in only 2 to 3 days. The same growth rate and lack of 10-day lag periods were still observed after several subculturings in glucose minimal salts medium, indicating that the ability to grow on L-ribose was due to a mutation and not from slow induction. Strain TM2000 had a K_s for L-ribose of 55 mM and had a maximum growth rate of 0.22 generation per h. The isolation of L-ribose-positive mutants on L-ribose plates indicated a mutational frequency of 10^{-8}. The colonies isolated from the L-ribose plates indicated that a second type of L-ribose-positive mutant was present (TM2001). L-ribose-positive strains could not be isolated from a similar strain of *E. coli* (JM1000) which lacked the ribitol and D-arabitol gene cluster.

Enzyme activities in L-ribose-positive mutants. The most likely routes for L-ribose metabolism involved the L-arabinose and ribitol pathways (Fig. 2). In order to determine how cells were metabolizing L-ribose, activities of key enzymes in these existing pathways which might be involved in L-ribose degradation were measured (Table 2). We measured the level of ribitol dehydrogenase and L-arabinose isomerase for wild-type (*E. coli* K-20) and L-ribose-positive strains (TM2000 and TM2001) grown without sugar (casein) or in the presence of sugar, L-arabinose, ribitol, or L-ribose. Both wild-type and L-ribose-positive mutant strains induced L-arabinose isomerase when grown with L-ribose, but the level of L-arabinose isomerase was only a third of the level induced by L-arabinose. Both strains also induced ribitol dehydrogenase when grown with L-ribose present in the medium. These data indicate that *E. coli* can induce both the ribitol and L-arabinose operons when exposed to L-ribose even if it cannot grow on L-ribose. The induction of both the

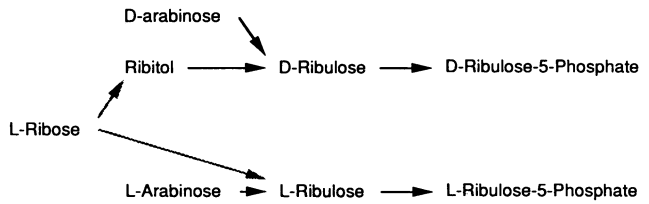

FIG. 2. Possible pathways for L-ribose metabolism. L-ribose can be converted into intermediates of the ribitol and L-arabinose pathways in one step or it can be reduced to ribitol or isomerized to L-ribulose.

TABLE 2. Comparison of enzyme activities in wild-type and L-ribose-positive strains of *E. coli*

E. coli strain	Relevant phenotype[a]	Medium supplement	Ribitol dehydrogenase activity[b]	L-arabinose isomerase activity[c]
K-20	Lrb⁻	Casein	0.074	7.51
		L-arabinose	0.019	106.32
		Ribitol	0.910	4.00
		Casein + L-ribose	1.989	25.35
TM2000	Lrb⁺	Casein	0.05	2.0
		L-arabinose	0.02	122.1
		Ribitol	1.16	4.1
		Casein + L-ribose	2.94	37.6
TM2001	Lrb⁺	Casein	0.05	6.74
		Casein + L-ribose	2.1	29.00

[a] Lrb, L-ribose.
[b] Enzyme activity in nanomoles of NADH oxidized per minute per milligram of protein.
[c] Enzyme activity in micromoles of L-ribulose formed per minute per milligram of protein.

L-arabinose and ribitol pathways indicated that these pathways might be involved in L-ribose metabolism.

Characterization of ribitol mutants. The L-ribose-positive strain TM2000 was used as the parent strain for the isolation of a D-arabinose-positive mutant (TM2100). This strain would convert D-arabinose to D-ribulose, the apparent inducer of the ribitol operon (Fig. 2) (16). The use of this strain to obtain ribitol-negative mutants permitted D-ribulose to be formed from D-arabinose in ribitol dehydrogenase-deficient mutants. Enzymes of the ribitol pathway were measured to determine the block in each mutant. Two types of ribitol-negative mutants were isolated, those which lacked ribitol dehydrogenase and those that lacked both ribitol dehydrogenase and D-ribulokinase (Table 3). The parent strain, TM2100, induced both ribitol dehydrogenase and D-ribulokinase greater than 10-fold over basal levels when grown with ribitol and about 5-fold when grown with D-arabinose. TM2120, a ribitol dehydrogenase-deficient mutant, showed no detectable dehydrogenase or kinase activity when grown with ribitol but could induce D-ribulokinase when grown with D-arabinose. TM2121, an apparent control negative mutant, showed no detectable enzyme activity above basal levels for either ribitol dehydrogenase or D-ribulokinase when grown with either ribitol or D-arabinose. All of the ribitol mutants isolated had also lost the ability to grow using L-ribose. Revertants of ribitol-negative mutants, obtained by selection on ribitol, simultaneously regained the ability to grow on L-ribose. The gene(s) conferring the L-ribose-positive phenotype could be transduced into *E. coli* K-20 (*rtl*$^+$) but not into *E. coli* K-12 (*rtl* mutant) by the P1 lysate from TM2001, indicating that the ribitol pathway is required for growth on L-ribose in TM2001.

Characterization of L-arabinose mutants. Mutants of the L-ribose-positive, D-arabinose-positive strain, TM2100, which had lost the ability to grow on L-arabinose were isolated. Cells were grown on casein hydrolysate supplemented with L-arabinose, and enzymes of the L-arabinose pathway were measured to determine the block in each mutant. L-arabinose mutants lacking either L-arabinose isomerase, L-ribulose kinase, or L-ribulose-5-phosphate-4-epimerase were isolated and characterized. These L-arabinose mutants were then tested to determine whether they retained the ability to

TABLE 3. Characterization of ribitol-negative mutants of *E. coli*

Strain	Relevant phenotype[a]	Medium supplement	Ribitol dehydrogenase activity[b]	D-ribulose kinase activity[c]
TM2100	Lrb$^+$ Rtl$^+$	Casein	0.03	0.07
		Casein + ribitol	0.93	0.67
		Casein + D-arabinose[d]	0.33	0.22
TM2120	Lrb$^-$ Rtl$^-$ (RDH$^-$)	Casein + ribitol	<0.01	<0.01
		Casein + D-arabinose	<0.01	0.42
TM2121	Lrb$^-$ Rtl$^-$ (RDH$^-$ DRK$^-$)	Casein + ribitol	<0.01	<0.01
		Casein + D-arabinose	<0.01	0.04

[a] Lrb, L-ribose; Rtl, ribitol; RDH, ribitol dehydrogenase; DRK, D-ribulokinase.
[b] Enzyme activity in micromoles of NADH oxidized per minute per milligram of protein.
[c] Enzyme activity in micromoles per minute per milligram of protein.
[d] D-arabinose is converted to D-ribulose, the apparent inducer of the ribitol pathway.

grow on L-ribose. Despite the enzyme deficiencies, all of the L-arabinose mutants retained the ability to grow on L-ribose. Therefore, the enzymes involved in L-arabinose catabolism could not be required for the L-ribose catabolic pathway.

Conversion of L-ribose to ribitol. One of the ribitol-negative strains obtained above which was L-ribose reductase positive and ribitol dehydrogenase negative, TM2120, was grown to late log phase and resuspended in 4 mM sodium carbonate (pH 8.8)–L-ribose. After incubation for 24 h, the cells were removed and the medium was assayed for sugars by thin-layer chromatography. Analysis of the medium showed that the cells had converted some of the L-ribose to ribitol. Added ribitol could also be converted to L-ribose by these cells (Fig. 3).

L-ribose reductase activity. The data indicated that L-ribose-positive strains were metabolizing L-ribose by reducing it to ribitol. Therefore, cell extracts were prepared from both wild-type (*E. coli* K-20) and L-ribose-positive mutant (TM2000 and TM2001) cells grown in the presence of L-ribose. An enzyme activity was found in extracts of TM2000 and TM2001 which could reduce L-ribose to ribitol when NADPH was present (Table 4). No activity could be detected when NADH was used instead of NADPH. This activity was found only in L-ribose-positive strains and appeared as a single band on a nondenaturing gel stained for ribitol oxidation (Fig. 4). The reductase activity was produced constitutively in all L-ribose-positive strains tested, but the enzyme was not detectable in wild-type strains under any growth conditions tested.

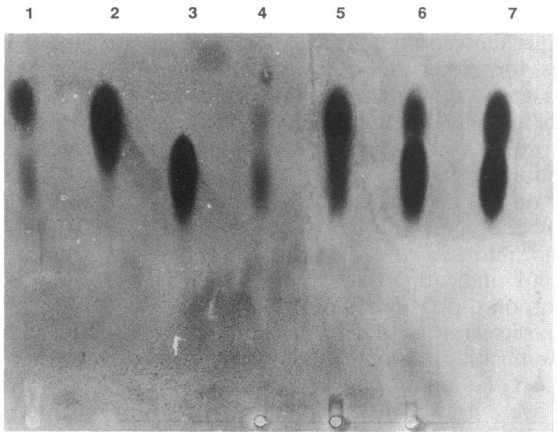

FIG. 3. Conversion of L-ribose to ribitol by a L-ribose-positive *E. coli* lacking ribitol dehydrogenase. Lane 1, D-ribulose standard; lane 2, L-ribose standard; lane 3, ribitol standard; lane 4, cell buffer (4 mM sodium carbonate [pH 8.8] plus washed cells); lane 5, cell buffer with 1% L-ribose; lane 6, cell buffer with 1% ribitol; lane 7, L-ribose and ribitol standards.

Transduction of L-ribose-positive phenotype. We wanted to characterize the nature of these mutations that allowed growth on L-ribose. P1 lysates were made from TM2000 and then used to transduce *E. coli* K-20 so that it became L-ribose positive. No L-ribose-positive transductants were found by using the P1 lysate from TM2000. The gene(s) conferring the L-ribose-positive phenotype could be transduced into *E. coli* K-20 by the P1 lysate from TM2001. TM2001 was an L-ribose-positive mutant isolated from *E. coli* K-20 by selection directly on L-ribose plates. TM2001 had a similar L-ribose reductase activity and growth rate on L-ribose to TM2000. The inability to obtain L-ribose-positive transductants from P1 lysate of TM2000 could indicate that more than one mutation had occurred to allow growth on L-ribose in TM2000. The ability to transduce the gene(s) responsible for the L-ribose phenotype from TM2001 indicated that the L-ribose-positive phenotype in TM2001 was due to a single mutation or two closely linked mutations.

Mapping of the L-ribose genes from TM2001. We wanted to map the L-ribose-positive mutation in TM2001. Since growth on L-ribose is slow, 3 to 4 days being required to form 3-mm-diameter colonies on L-ribose plates, we wanted to

TABLE 4. Comparison of L-ribose reductase activities in wild-type and L-ribose-positive strains of *E. coli*

E. coli strain	Relevant phenotype[a]	Medium Supplement	L-ribose reductase activity[b]
K-20	Lrb$^-$	Casein	<0.1
		L-arabinose	<0.1
		Ribitol	<0.1
		Casein + L-ribose	<0.1
TM2000	Lrb$^+$	Casein	2.8
		L-arabinose	0.9
		Ribitol	1.2
		Casein + L-ribose	2.1
TM2001	Lrb$^+$	Casein	8.3
		Casein + L-ribose	7.8

[a] Lrb, L-ribose.
[b] Enzyme activity in nanomoles of NADPH oxidized per minute per milligram of protein.

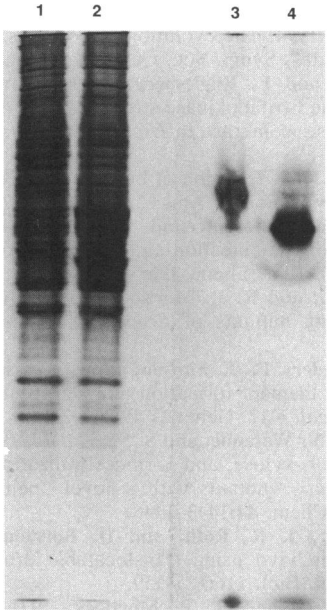

FIG. 4. Native gel of wild-type and L-ribose-positive strains of *E. coli*. Crude cell extracts of *E. coli* K-20 (lanes 1 and 3) and TM2000 (lanes 2 and 4) stained for protein (lanes 1 and 2) or ribitol oxidation (lanes 3 and 4).

place an easily selectable marker near the L-ribose locus to facilitate the mapping of the L-ribose genes. We were able to place a Tn*10* near the L-ribose locus which was 36% cotransduceable with the gene conferring the L-ribose-positive phenotype. The Tn*10* was moved into a Hfr strain so that we could use conjugation to map the location of the Tn*10*. Analysis of the gradient of transmission of markers into the recipient strain from mating with the Hfr strain showed that the Tn*10* was located near the *proAB* genes at 6 min on the *E. coli* chromosome.

Once we had determined the region of the chromosome where the L-ribose genes were located, P1 transduction with known markers in the region was used to locate more precisely the L-ribose genes. On the basis of these data, the Tn*10* insertion was mapped to 7 min on the *E. coli* chromosome and named *zag*::Tn*10*. The L-ribose gene(s) was mapped to 7.5 min (Fig. 5).

Characterization of L-ribose-negative mutants. We wanted to isolate L-ribose reductase-negative mutants so that the gene could be mapped. Bacteriophage Mu dI1734 was used to mutagenize L-ribose-positive strains TM2000 and TM2001.

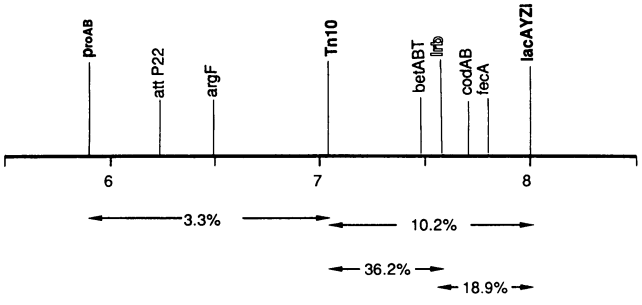

FIG. 5. Linkage map for *zag*::Tn*10* and *lrb*. Arrows show cotransduction with known markers, using bacteriophage P1. Locations of known markers are taken from *E. coli* linkage map (2).

We screened 3,302 Mu insertion mutants of TM2000 but could not find any L-ribose-specific mutants, although several ribitol-negative mutants which are also unable to grow on L-ribose were isolated. We were able to isolate four L-ribose-negative mutants from 3,547 Mu insertions in TM2001. Enzyme assays performed on the four L-ribose-negative mutants could not detect any L-ribose reductase activity in any of the mutants. All four of the mutations were cotransduceable with *zag*::Tn*10*.

DISCUSSION

E. coli was unable to grow using the novel pentose L-ribose, but L-ribose-positive mutants could be isolated at a frequency of 10^{-8} from *E. coli* strains containing the ribitol and D-arabitol catabolic genes. L-ribose-positive strains could only be isolated from strains which contained the ribitol catabolic genes, indicating that the ribitol pathway was involved in the degradation of L-ribose. Enzyme assays from L-ribose-positive strains showed that enzymes from both the L-arabinose and the ribitol catabolic pathways were induced during growth on L-ribose. Theoretically, L-ribose could be converted in one step to L-ribulose by isomerization and to ribitol by reduction. In order to determine the pathway used to metabolize L-ribose, mutants that were blocked at different steps of the L-arabinose and ribitol pathways were isolated. These mutations showed that none of the enzymes in the L-arabinose pathway were essential for growth on L-ribose. However, mutations which blocked the ribitol pathway also prevented growth on L-ribose. Mutants which did not grow on L-ribose were isolated. All of these mutants lacked detectable L-ribose reductase activity, indicating that the reductase was required for growth on L-ribose. These data indicated that the first step in L-ribose metabolism was its reduction to ribitol. In order to confirm this pathway, the ability to convert L-ribose to ribitol by cells was studied in a ribitol dehydrogenase-deficient mutant. The production and accumulation of ribitol from L-ribose in this strain further supported the proposed pathway for the degradation of L-ribose.

L-ribose-positive cells were found to contain an NADPH-linked enzyme which reduced L-ribose to ribitol. No activity was found when NADH was used instead of NADPH. This novel reductase was not detectable in wild-type cells under any conditions assayed. Since the reductase was constitutively produced in the L-ribose positive mutants, it is likely that at least one of the mutations allowing growth on L-ribose involved regulation of expression of the reductase. This mutation could be located in a regulatory gene or in the promoter for the reductase gene, although a mutation affecting the substrate specificity of the reductase could be possible.

The pathway for the degradation of L-ribose differs from that of the other pentoses in enteric bacteria. All the other pentoses are degraded by a mechanism that involves isomerization to a keto sugar. On the basis of studies of the metabolism of other uncommon pentoses (22) and the structure of L-ribose, we had predicted that L-ribose would be metabolized by "borrowing" L-arabinose isomerase to isomerize L-ribose to L-ribulose. Instead, L-ribose is metabolized by reduction to pentitol and then oxidation to the keto sugar. L-ribose-positive cells do induce the L-arabinose operon when grown on L-ribose, even though those enzymes are not involved in the L-ribose catabolic pathway. The induction of the L-arabinose operon by L-ribose-positive cells may be due to the ability of L-ribose to act as an analog of L-arabinose and bind to the *araC* protein. Mutants metabolizing L-ribose through L-ribulose could not be obtained. In

fact, L-ribose-positive strains of *E. coli* could not be isolated from strains lacking the ribitol operon. Therefore, it is possible that a single mutation in the L-arabinose isomerase will not permit it to convert L-ribose to L-ribulose at a sufficient level to allow growth. Although L-ribose and L-arabinose are structurally very similar, they do differ at carbon atom 2, which is the position of isomerization by L-arabinose isomerase.

Both the wild-type and L-ribose-positive strains induced ribitol dehydrogenase when grown in the presence of L-ribose. The L-ribose-positive strain produced a reductase which converted L-ribose to ribitol, which then is converted to D-ribulose, the apparent inducer of the ribitol operon. No reductase activity could be detected in the wild-type strain, but the ribitol enzymes were still induced. The wild-type strain may normally produce a low level of reductase, which could not be detected with our enzyme assay. This low level of reductase may produce enough ribitol from L-ribose to induce the ribitol genes but not allow detectable growth.

The origin of this novel L-ribose reductase is unknown. The fact that the enzyme uses the coenzyme NADPH instead of NADH suggests that it was originally a biosynthetic enzyme in the cell which has been recruited for the degradation of L-ribose. The same type of pathway has been found for the degradation of L-ribose in *M. smegmatis*, in which L-ribose is reduced by a novel pentose NADPH-linked reductase (14).

The L-ribose pathway in *E. coli* is similar to the type of pathway common in eucaryotes for the degradation of pentoses. In *Candida utilis*, D-xylose is first reduced to the pentitol xylitol by a NADPH-linked reductase and the pentitol is oxidized to D-xylulose by a NADH-linked dehydrogenase (29). A single-step isomerization pathway should be more efficient than the two-step reduction-oxidation pathway. Also, the use of NADPH as the cofactor in the reduction and NAD as the cofactor in the oxidation can cause problems for the cells in maintaining the balance of reduced nicotinamide cofactors. This can be seen in the inability of many yeasts to grow anaerobically on D-xylose (6). Further study of L-ribose metabolism in *E. coli* and identification of the origin of the L-ribose reductase may help us understand the evolution of metabolic pathways similar to the pentose catabolic pathways commonly found in yeast cells.

ACKNOWLEDGMENT

This material is based upon work supported by the National Science Foundation under grant PCM-8402573.

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