JOURNAL OF BACTERIOLOGY, May 1988, p. 2159–2162
0021-9193/88/052159-04$02.00/0
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Vol. 170, No. 5

Anaerobic Metabolism of the L-Rhamnose Fermentation Product 1,2-Propanediol in *Salmonella typhimurium*

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Received 13 November 1987/Accepted 13 February 1988

When grown anaerobically on L-rhamnose, *Salmonella typhimurium* excreted 1,2-propanediol as a fermentation product. Upon exhaustion of the methyl pentose, 1,2-propanediol was recaptured and further metabolized, provided the culture was kept under anaerobic conditions. *n*-Propanol and propionate were found in the medium as end products of this process at concentrations one-half that of 1,2-propanediol. As in *Klebsiella pneumoniae* (T. Toraya, S. Honda, and S. Fukui, J. Bacteriol. 139:39–47, 1979), a diol dehydratase which transforms 1,2-propanediol to propionaldehyde and the enzymes involved in a dismutation that converts propionaldehyde to *n*-propanol and propionate were induced in *S. typhimurium* cultures able to transform 1,2-propanediol anaerobically.

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In *Escherichia coli*, L-rhamnose is metabolized by the sequential action of a rhamnose permease that transports the sugar across the membrane, a rhamnose isomerase (20) that converts it to L-rhamnulose, a rhamnulose kinase (21) that phosphorylates the L-rhamnulose to L-rhamnulose 1-phosphate, and an aldolase (5) that cleaves the L-rhamnulose 1-phosphate into dihydroxyacetone phosphate and L-lactaldehyde. Although no detailed description of the enzymes has been reported, the same pathway has been described in *Salmonella typhosa* (7) and *Salmonella typhimurium* (1).

Under anaerobic conditions, *E. coli* induces an NADH-dependent oxidoreductase that reduces L-lactaldehyde to 1,2-propanediol, which is excreted into the medium (4). On the basis of propanediol oxidoreductase activity induction and propanediol excretion, this fermentation mechanism has also been proposed for *S. typhimurium* (3). However, 1,2-propanediol, which is not further metabolized in anaerobic *E. coli* cultures, gradually disappears from the medium in *S. typhimurium* cultures maintained under similar anaerobic conditions (J. Badía, unpublished observation).

Aerobic metabolism of 1,2-propanediol has been described for several species (9, 13) and for mutant cells of *E. coli* (18) able to grow on the diol. In all cases the first step is the oxidation to lactaldehyde through the action of an oxidoreductase. Lactaldehyde is subsequently metabolized to lactate and pyruvate (6, 17). In contrast, anaerobic metabolism of 1,2-propanediol in *Klebsiella pneumoniae* (22) or *Clostridium glycolicum* (8) has been reported to be mediated by a coenzyme B_{12}-dependent diol dehydratase that yields propionaldehyde, which is immediately metabolized by a dismutation to *n*-propanol and propionate.

In this report we characterize the 1,2-propanediol transformation that occurs in *S. typhimurium* cultures and establish the metabolic pathway involved.

MATERIALS AND METHODS

Chemicals. DL-1,2-propanediol was purchased from E. Merck AG, Darmstadt, Federal Republic of Germany, and was purified by distillation. Acetaldehyde, propionaldehyde, potassium acetate, hydroxylamine, and succinate were also purchased from Merck. 3-Methyl-2-benzothiazolinone hydrazone was purchased from Carlo Erba, Milan, Italy. Acetyl phosphate (lithium potassium salt), L-rhamnose, ATP, coenzyme B_{12}, acetohydroxamic acid, coenzyme A, D-threonine, and ninhydrin were purchased from Sigma Chemical Co., St. Louis, Mo. Casamino Acids were obtained from Difco Laboratories, Detroit, Mich.

Bacteria and cell cultures. *S. typhimurium* LT2 was obtained from the American Type Culture Collection, Rockville, Md. (ATCC 23564), and *E. coli* K-12 strain ECL1, also known as E15 (2), was kindly provided by E. C. C. Lin, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass.

Carbon sources were added to a basal inorganic medium (4) as follows: for anaerobic growth, hexose at 0.02 M; for aerobic growth, hexose at 0.01 M, succinate at 0.015 M, DL-1,2-propanediol at 0.04 M, and casein acid hydrolysate at 0.5%. Aerobic growth was carried out at 37°C in 500-ml Erlenmeyer flasks partially filled (100 ml each) and vigorously swirled in a rotary shaker. Anaerobic growth was carried out at the same temperature in flasks completely filled and gently stirred by a magnet. Growth was monitored at 420 nm.

Preparation of cell extracts. Cells were harvested from exponentially growing cultures, washed once with 0.05 M potassium phosphate (pH 8.0), and suspended in a quantity of the same buffer four times their wet weight. The dispersed cells in the tube were disrupted (for 30 s/ml of suspension) in a model 150 W ultrasonic disintegrator (MSE) while being chilled in a −10°C bath. Except when indicated, the resultant mixture was centrifuged at 20,000 × *g* for 30 min at 4°C, and the supernatant fraction was used for enzyme assays.

Enzyme assays. Propanediol dehydratase (diol dehydratase or DL1,2-propanediol hydro-lyase [EC 4.2.1.28]) was assayed by the 3-methyl-2-benzothiazolinone hydrazone method as described by Toraya et al. (23). This assay was performed with the crude extract before it was centrifuged at 20,000 × *g*. The alcohol dehydrogenase activity (alcohol:NAD^+ oxidoreductase [EC 1.1.1.1]) of the forward reaction was determined spectrophotometrically by measuring the rate of increase in the A_{340} in a reaction mixture containing 0.1 M propanol, 1 mM NAD, and 0.048 M potassium phosphate buffer (pH 8.0) in a final volume of 1 ml. The activities of coenzyme A-dependent acetaldehyde dehydrogenase (acetylating) (acetaldehyde:NAD^+ oxidoreductase [coenzyme A acetylating] [EC 1.2.1.10]), phosphate

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acetyltransferase (phosphotransacetylase or acetyl coenzyme A:orthophosphate acetyltransferase [EC 2.3.1.8]), and acetate kinase (ATP:acetate phosphotransferase [EC 2.7.2.1]) were assayed by the procedures of Rudolph et al. (16), Stadtman (19), and Rose (15), respectively. In all assays the substrate was omitted from the blank mixture, and reactions were started by the addition of the enzyme. One unit of enzymatic activity was defined as the amount of enzyme that transformed 1 μmol of substrate per min.

The concentration of protein in the cell extracts was determined by the method of Lowry et al. (12) with bovine serum albumin as a standard.

Analysis of fermented products. 1,2-Propanediol was measured by the colorimetric method of Jones and Riddick (11), and propionaldehyde was measured by the semicarbazone formation method of Olson (14). *n*-Propanol was estimated by gas-liquid chromatography with a model HP 5890 gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) with nitrogen as the carrier gas and a Supelcowax 10:2-4079 column at 80°C. Monitoring was performed with a flame ionization detector. Propionic acid was determined by high-pressure liquid chromatography with a model HP1090 chromatograph (Hewlett-Packard) with a spherical c18 Resolve column (Waters Associates, Inc., Milford, Mass.) at 40°C and a flow rate of 0.5 ml/min for 5% (vol/vol) acetonitrile in 95% (vol/vol) phosphoric acid (pH 2.3). Sample components were detected with a UV detector at a wavelength of 210 nm. *n*-Propanol and propionic acid analyses were performed by the Laboratori Agrari de la Generalitat de Catalunya, Cabrils, Barcelona, Spain.

RESULTS

Disappearance of excreted 1,2-propanediol. When grown anaerobically on L-rhamnose, *S. typhimurium* excreted into the medium 1 mol of 1,2-propanediol per mol of sugar. After exhaustion of the sugar, the diol concentration reached a maximum and gradually disappeared when the culture was kept under the same anaerobic conditions (Fig. 1). Disappearance of the diol did not occur when the cells were removed from the medium by centrifugation after L-rhamnose fermentation (Fig. 1). With *E. coli*, which also excretes 1,2-propanediol when fermenting L-rhamnose, no further metabolism of the diol was detected, even after 80 h (data not shown).

Effect of oxygen and cell growth conditions on propanediol metabolism capacity. Oxygen prevented the further metabolism of 1,2-propanediol excreted as an L-rhamnose fermentation product by *S. typhimurium*. Cells from cultures grown anaerobically on L-rhamnose metabolized added 1,2-propanediol under anaerobic but not aerobic conditions (Fig. 2). Cells from cultures grown anaerobically on glucose also caused 1,2-propanediol to disappear, although they were less active than L-rhamnose-grown cells. As with L-rhamnose-grown cells, this metabolism occurred only under anaerobic conditions (Fig. 2). Similar results were obtained when the cultures were grown in the presence of oxygen (data not shown).

Growth of cells on 1,2-propanediol. Anaerobic metabolism of 1,2-propanediol by *S. typhimurium* did not support growth under any of the conditions tested. Several concentrations of 1,2-propanediol were assayed at different cell concentrations in cultures started with inocula grown on L-rhamnose, casein hydrolysate, glycerol, or glucose, aerobically and anaerobically. When shifted to 1,2-propanediol, the cultures doubled in optical density and stopped growing or underwent lysis while the diol was being metabolized to exhaustion.

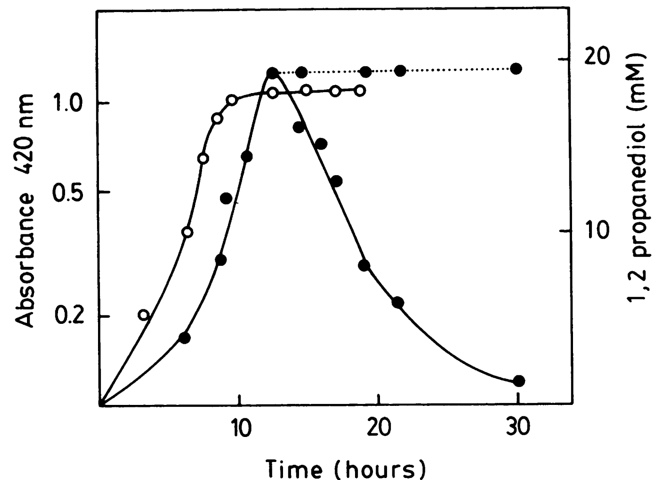

FIG. 1. Time course analysis of the extracellular 1,2-propanediol concentration in anaerobic cultures of *S. typhimurium*. Sample cultures were processed at the indicated times to measure the optical density (cell growth) (○) and 1,2-propanediol concentration (●). In parallel cultures, cells were removed by centrifugation at the end of the logarithmic phase, when extracellular 1,2-propanediol reached its highest concentration, and the 1,2-propanediol concentration was monitored (dotted line).

***n*-Propanol and propionate as propanediol fermentation products.** The anaerobic metabolism of 1,2-propanediol by *K. pneumoniae* yields *n*-propanol and propionate (22). Analysis of the culture medium from *S. typhimurium* also revealed the presence of both *n*-propanol and propionate. A time course analysis of the conversion of 1,2-propanediol into *n*-propanol and propionate revealed the accumulation of equimolar amounts of both products, each equivalent to one-half the concentration of 1,2-propanediol (Fig. 3). Propionaldehyde was only detected as an intermediate metabolite at the beginning of the process.

Enzymes involved in the anaerobic 1,2-propanediol metabolic pathway. To further confirm the dissimilation of 1,2-

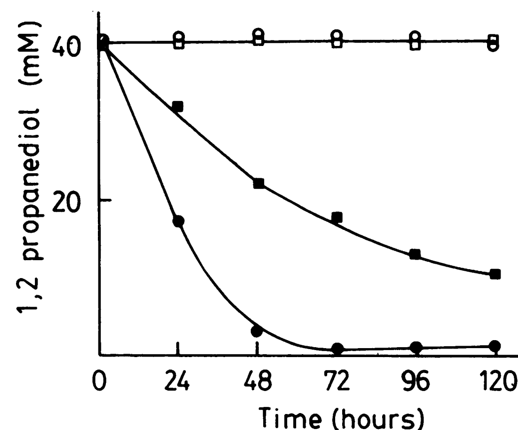

FIG. 2. Rate of 1,2-propanediol dissimilation by *S. typhimurium* grown under different conditions. The cells were grown on rhamnose (● and ○) or glucose (■ and □), centrifuged, and suspended in minimal medium containing 40 mM 1,2-propanediol at a cell density of 5×10^8 cells per ml. Aerobic (○ and □) or anaerobic (● and ■) incubation was maintained for 120 h, and at the indicated times samples were removed for 1,2-propanediol analysis.

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TABLE 1. Activities of 1,2-propanediol pathway enzymes in extracts of *S. typhimurium* as a function of growth conditions

Cell growth conditions		Sp act (mU/mg of protein) of:				
Carbon source	O_2	Diol dehydratase	Alcohol dehydrogenase	Acetaldehyde dehydrogenase	Phosphate acetyltransferase	Acetate kinase
Casein hydrolysate	+	0	120	<5	870	1,800
Succinate	+	0	70	<5	750	1,350
L-Rhamnose	+	350	120	20	610	1,030
L-Rhamnose	−	700	50	60	1,070	5,960
Glucose	+	0	60	20	1,110	1,920

propanediol through the pathway of dehydration to propionaldehyde and the subsequent dismutation to *n*-propanol and propionate, we analyzed the enzymatic activities involved in the pathway (Table 1).

All five required activities were present in cells grown on L-rhamnose either aerobically or anaerobically. Cells from cultures grown on casein acid hydrolysate or succinate lacked diol dehydratase and acetaldehyde dehydrogenase activities. Growth on glucose was also unable to induce diol dehydratase activity. Thus, these two activities are clearly induced by L-rhamnose, the induction being higher in anaerobic cultures than in aerobic ones. The presence of 1,2-propanediol also caused a slight induction of diol dehydratase activity. This was shown in a culture that was pregrown on glucose, centrifuged, and subsequently suspended in minimal medium containing 40 mM DL-1,2-propanediol. A diol dehydratase activity of 20 mU/mg was determined after approximately 20 h of anaerobic incubation.

DISCUSSION

Excretion of 1,2-propanediol as an L-rhamnose fermentation product is a common feature in enterobacteria such as *E. coli*, *K. pneumoniae*, and *S. typhimurium*. However, this product, while remaining stable in *E. coli* cultures maintained under anaerobic conditions, slowly disappears from *S. typhimurium* cultures. This result suggests a further propanediol metabolism in *S. typhimurium*.

The production of *n*-propanol and propionate at concentrations equal to one-half that of 1,2-propanediol concomitant with the disappearance of the diol and the presence of all enzymatic activities participating in the diol dehydratase metabolic pathway (22) permitted us to propose this pathway as the one operating in the case of *S. typhimurium*. The dismutation of propionaldehyde, formed from propanediol, into *n*-propanol and propionate, which are excreted into the medium, explains why the cells do not grow when they metabolize 1,2-propanediol anaerobically: none of the carbons from the substrate are incorporated to form cell materials. The yield of this anaerobic metabolism of 1,2-propanediol is limited to a gain of 1 mol of ATP per 2 mol of substrate consumed (22).

The pathway functions only under anaerobic conditions, although the enzymes involved can also be induced under aerobic conditions. Diol dehydratase, the key enzyme in the pathway, is inducible by L-rhamnose and partially inducible in the presence of 1,2-propanediol. This explains why cells pregrown on glucose, in which no diol dehydratase activity is detected, anaerobically metabolize the diol when confronted with this compound. The low amounts of dehydratase induced agree with the lower rate of 1,2-propanediol dissimilation.

At present, we do not know which factors regulate the initiation of the pathway promoting the recapture of the diol. The enzymes seem to be present in an active form from the very beginning of L-rhamnose metabolism. However, the 1,2-propanediol which is formed and which is the substrate of the proposed pathway is not metabolized but is excreted into the medium. Some unknown signal is required to start the metabolism of the diol when the methyl pentose has been exhausted.

The results presented above show how a fermentation product that has been excreted into the medium may be recaptured and further metabolized under the same anaerobic conditions. A similar phenomenon has been described for acetone-butanol fermentation in *Clostridium acetobutylicum*, in which the butyrate formed in the fermentation of glucose is recaptured and further metabolized to acetone and butanol (10). In the present case, the 1,2-propanediol formed in the fermentation of L-rhamnose is recaptured and further metabolized to *n*-propanol and propionate.

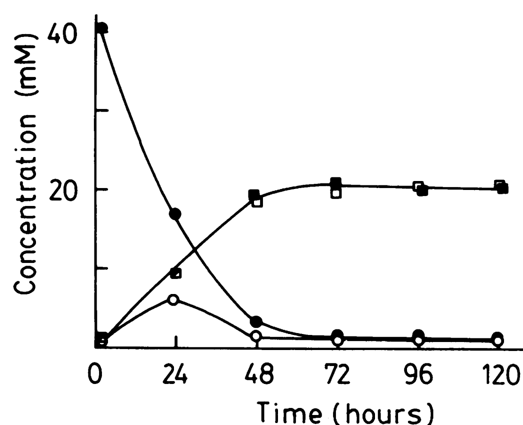

FIG. 3. Excretion of *n*-propanol and propionate as fermentation products of 1,2-propanediol in *S. typhimurium*. The cells were grown on rhamnose to the end of the logarithmic phase, centrifuged, and suspended in minimal medium containing 40 mM 1,2-propanediol at a cell density of 5×10^8 cells per ml. Samples were processed at the indicated times to measure 1,2-propanediol (●), propionaldehyde (○), *n*-propanol (■), and propionate (□).

ACKNOWLEDGMENTS

We thank J. Boatella for helpful advice in analytical determinations.

This work was supported by grant 1619/82 from the Comisión Asesora de Investigación Científica y Técnica, Spain.

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