APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1990, p. 943–948
0099-2240/90/040943-06$02.00/0

Vol. 56, No. 4

Utilization of Glycerol as a Hydrogen Acceptor by *Lactobacillus reuteri*: Purification of 1,3-Propanediol:NAD^+ Oxidoreductase†

TODD L. TALARICO,[1] LARS T. AXELSSON,[2] JAMES NOVOTNY,[1] MITRA FIUZAT,[1] AND WALTER J. DOBROGOSZ[1]*

Department of Microbiology, North Carolina State University, Raleigh, North Carolina 27695,[1] and Department of Microbiology, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden[2]

Received 10 October 1989/Accepted 17 January 1990

***Lactobacillus reuteri* utilizes exogenously added glycerol as a hydrogen acceptor during carbohydrate fermentations, resulting in higher growth rates and cell yields than those obtained during growth on carbohydrates alone. Glycerol is first converted to 3-hydroxypropionaldehyde by a coenzyme B_{12}-dependent glycerol dehydratase and then reduced to 1,3-propanediol by an NAD^+-dependent oxidoreductase. The latter enzyme was purified and determined to have a molecular weight of 180,000; it is predicted to exist as a tetramer of identical 42,000-molecular-weight subunits.**

Lactobacillus reuteri has been shown to be unique among bacteria in its ability to produce and excrete a broad-spectrum antimicrobial agent during anaerobic dissimilation of glycerol (1, 4, 8). This agent, termed reuterin, is known to inhibit the growth of gram-positive bacteria, gram-negative bacteria, and lower eucaryotic organisms (1, 4, 8). Reuterin was isolated, purified, and identified as an equilibrium mixture of monomeric, hydrated monomeric, and cyclic dimeric forms of 3-hydroxypropionaldehyde (3-HPA) (25, 26). A coenzyme B_{12}-dependent glycerol dehydratase isolated and purified from *L. reuteri* was shown to catalyze the synthesis of reuterin from glycerol (27).

In this report, it is shown that reuterin synthesis is associated with the use of glycerol by *L. reuteri* as an alternate hydrogen acceptor. Inclusion of glycerol in the culture medium with a utilizable source of carbon and energy, such as glucose, results in increased growth rates, higher cell yields, and an altered end product profile. Methods for the purification and characterization of an oxidoreductase believed to be responsible for reduction of 3-HPA to 1,3-propanediol are also described.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A washed overnight culture of *L. reuteri* 1063 was adjusted to an optical density at 420 nm of 0.5 with 0.1 M potassium phosphate buffer (KPB) (pH 7.1) and diluted 1:100. This culture was used as an inoculum for growth studies with a modified *Lactobacillus* carrying medium (MLCM) containing glucose and various concentrations of glycerol. MLCM was the *Lactobacillus* carrying medium (LCM) described by Efthymiou and Hansen (9) without sodium acetate and ammonium citrate but containing 1.2 g of ammonium sulfate per liter of medium. Growth was monitored on the basis of absorbancy change (optical density at 600 nm), by using a Bausch & Lomb Spectronic 20, and on the basis of viability (in CFU per milliliter), by using *Lactobacillus* selection medium agar prepared as described elsewhere (22). Cell mass was calculated from a predetermined relationship between *L. reuteri* cell mass and absorbancy.

Biochemical analyses. Glucose utilization was measured enzymatically by using the glucose oxidase–peroxidase–*o*-dianisidine dihydrochloride method as described by the manufacturer (procedure no. 510; Sigma Chemical Co.). The appearance of reuterin was monitored by the method of Circle et al. (5) and by high-performance liquid chromatography (HPLC) as previously described (22). End product analysis of glucose and glycerol metabolism by *L. reuteri* was performed by the HPLC method of McFeeters and Armstrong (19) in combination with the procedure of Talarico et al. (25). Protein concentration was determined by the method of Bradford (3) with bovine serum albumin (Bio-Rad Laboratories) as the standard.

Enzyme assays. Oxidoreductase activity was assayed by monitoring the A_{340} at 37°C in a Perkin Elmer 552 spectrophotometer equipped with a Haake circulating water bath to control the sample cell temperature (Worthington Biochemical Corp., *Worthington Enzymes Manual*, p. 41–42, 1972). The reaction volume was 1 ml and contained 25 μl of the enzyme solution eluted from a Blue Sepharose CL-6B (Pharmacia Fine Chemicals) column as described below, containing 0.05 M KPB (pH 7.2), 7 mM NAD^+, and 0.5 M 1,2-propanediol (1,2-PD). One unit of activity was defined as the amount of enzyme needed to reduce 1 μmol of NAD^+ per min. The activity in polyacrylamide gels was visualized by immersion in a solution containing 66 mg of NAD^+, 35 mg of Nitro Blue Tetrazolium, 4 mg of phenazine methosulfate, and 1.5 g of 1,2-PD per 100 ml of 0.1 M KPB (pH 7.2) (7). Gels were incubated at 37°C for 10 to 30 min (or until the bands were visualized).

Enzyme purification. A 10% inoculum of an overnight culture of *L. reuteri* 1063 grown in LCM (19) containing 2% glucose was added to fresh LCM containing 2% glucose. The culture was grown at 37°C for 20 h, harvested, and washed twice with sterile deionized water. The washed pellet was suspended in 15 ml of 0.05 M KPB (pH 7.2) containing 0.15 M potassium chloride and 2% 1,2-PD.

For extraction of the enzyme, all manipulations were carried out at 4°C unless otherwise stated. Cells were disrupted by two passages through an ice-cold French pressure cell at 500 kg cm^{-2}. DNase (2 mg; Sigma) and 0.2 g of acid-washed activated charcoal (Sigma) were added to the disrupted cell suspension, which was then placed on ice for 20 min; the cell debris were removed by centrifugation at

* Corresponding author.

† Paper number 12872 of the Journal Series of The North Carolina Agricultural Service of Raleigh, NC 27695.

20,000 × *g* for 20 min. The supernatant was decanted and centrifuged at 40,000 × *g* for 20 min.

The cleared supernatant was applied to a column of DE53 DEAE-cellulose (2.4 by 50 cm; Whatman, Inc.) equilibrated with 0.05 M KPB (pH 7.2) containing 0.15 M KCl and 2% 1,2-PD. The enzyme was eluted by passage of 75 ml of the equilibration buffer followed by several hundred milliliters of 0.05 M KPB buffer (pH 7.2) containing 0.5 M KCl and 2% 1,2-PD. Protein was detected by measuring the optical density at 280 nm with a Waters 481 Lambda Max UV Spectrophotometer equipped with a semipreparative flow cell to allow low-pressure passage of the eluent. Fractions displaying oxidoreductase activity were pooled and concentrated to 2 ml on a stirred Amicon ultrafiltration cell with an Omega Series OM-0100 membrane (Pharmacia).

The concentrated enzyme fraction was applied in 1-ml portions to a diposable 10-ml column (Bio-Rad) packed with 3.5 ml of Blue Sepharose CL-6B equilibrated in 0.01 M KPB (pH 7.2) containing 2% 1,2-PD. The enzyme fraction was allowed to stand on the column for 1 h at room temperature. Elution was performed in a stepwise manner with steps consisting of 20 ml each of (i) equilibration buffer, (ii) 1 M KPB containing 2% 1,2-PD, and (iii) 1 M KPB containing 2% 1,2-PD and 10 mM NAD^+. The oxidoreductase activity eluted with the third solvent, and fractions containing activity were pooled and concentrated to 2 ml. The concentrated enzyme was washed with 50 ml of 0.1 M KPB to remove the 1,2-PD and NAD^+ present in the elution buffer.

Polyacrylamide gel electrophoresis. Electrophoresis was carried out by the method of Laemmli (16). Nondenaturing gels were composed of the same components as the denaturing gels less sodium dodedyl sulfate (SDS) for a given acrylamide concentration. All electrophoretic operations were carried out at 4°C, and the gels were stained with Coomassie brilliant blue R-250.

Molecular weight determinations. The molecular weight of the native enzyme was estimated by gel filtration on a Sepharose CL-6B column (1.6 by 100 cm). The enzyme was eluted with 0.05 M KPB (pH 7.2)–0.5 M KCl–2% 1,2-PD at a flow rate of 12 ml/h; total protein and enzyme activities were detected as described above. The column was calibrated with known molecular weight standards: thyroglobulin, 669,000; ferritin, 440,000; amylase, 200,000; alcohol dehydrogenase, 150,000; albumin, 66,000; carbonic anhydrase, 29,000; cytochrome *c*, 12,400; blue dextran, 2,000,000 (Sigma).

The molecular weight of the native enzyme was estimated as described in *Sigma Technical Bulletin* no. MKR-137 by using polyacrylamide gel electrophoresis under nondenaturing conditions as described by Davis (6). Subunit size was estimated by removing a single band (corresponding to oxidoreductase activity) from an 8% native polyacrylamide gel containing the active protein fraction eluted from Blue Sepharose. This gel slice was boiled for 20 min in sample buffer containing 20% glycerol, 2% 2-mercaptoethanol, and 2% SDS, loaded into the well of a 10% denaturing gel, and subjected to electrophoresis with molecular weight standards (Bio-Rad).

Kinetic parameters. The apparent K_m values obtained with substrates and coenzymes were determined at 37°C and pH 7.2 by using Lineweaver-Burk plots. Coenzyme K_m values were determined in the presence of 500 mM substrate for 1,2-PD, glycerol, or 20 mM dihydroxyacetone (DHA). The K_m values for glycerol, DHA, and 3-HPA were determined in the presence of 8 mM NAD^+ or 0.1 mM NADH. The apparent K_m values for 3-HPA and DHA at pH 6.3 were also determined as described above.

TABLE 1. Effects of glycerol on growth rate and cell yield during the heterolactic fermentation of 20 mM glucose by *L. reuteri*

Concn of glycerol (mM)	Cell mass (10 h) (mg [dry wt]/ml)[a]	CFU/ml (10 h)[b]	Generation time (min)
0	0.3	1.7×10^8	66
20	1.14	4.5×10^8	42
40	1.32	5.5×10^8	42

[a] Measured as described in Materials and Methods.
[b] CFU determined as described in Materials and Methods.

Determination of the optimum pH and effect of cations. Assays to determine the optimum pH were performed with 0.5 M KPB adjusted to the appropriate values with 6 M KOH or 12 M HCl. DHA and 3-HPA were reduced in the presence of 0.1 mM NADH (20 mM DHA or 2 mM 3-HPA was used). 1,2-PD was oxidized in the presence of 8 mM NAD^+ (0.5 M 1,2-PD was used). The chloride salt of potassium, sodium, ammonium, magnesium, or lithium was included (100 mM) with 1,2-PD, NAD^+, and 0.05 M KPB (pH 7.2) to determine the effects of these cations on enzyme activity.

Analysis of oxidation products. The oxidation product of glycerol metabolism was amplified by coupling the reaction to the NADH-linked reduction of pyruvate by lactate dehydrogenase (Sigma) (13). This reaction mixture contained 0.2 M glycerol, 1 mM NAD^+, 8 mM pyruvate (potassium salt), 50 U of lactate dehydrogenase, and 100 μl of the enzyme solution eluted from Blue Sepharose CL-6B. The mixtures were held at 37°C for 1 h in a total volume of 1 ml of 0.05 M KPB (pH 7.2). Reaction products were determined by HPLC separation on an Aminex HPX-87H column (Bio-Rad) with an eluent composed of submicron-filtered 65% distilled deionized water, 35% acetonitrile, and 0.1% sulfuric acid. The separation was performed at ambient temperature with an injection size of 20 μl and a solvent flow rate of 0.4 ml/min; the elution pattern was monitored with a Waters 410 differential refractometer. Product identification was based on comparisons of retention times with those of standards prepared fresh in submicron-filtered distilled deionized water.

Production and measurement of 3-HPA. 3-HPA was produced by *L. reuteri* as previously described (25). Its concentration was determined by the method of Circle et al. (5) and by HPLC (25), and no attempt was made to purify it from other components (glycerol, 1,3-propanediol [1,3-PD], and 3-hydroxypropionic acid) present in the preparation.

RESULTS

Effect of glycerol on glucose metabolism. *L. reuteri* was determined to have a generation time of approximately 66 min when grown on glucose in MLCM (Table 1). Addition of 20 mM glycerol to this culture medium reduced the generation time to approximately 42 min and increased the final cell yield (measured by turbidity or CFU per milliliter) 2.6- to 3.3-fold. These measurements were made 10 h postinoculation, the time at which the glucose had been completely utilized. Doubling the glycerol concentration to 40 mM had no additional stimulatory effect on generation time but consistently increased the cell yield over that obtained in the presence of 20 mM glycerol. Glycerol addition also stimulated growth on galactose, lactose, maltose, and melibiose, but not on D-xylose, L-arabinose, or sucrose (data not

TABLE 2. Effect of glycerol on the heterolactic fermentation of 20 mM glucose by *L. reuteri*

Product[a]	Concn of product (mM) accumulated at 22 h in presence of:		
	No glycerol	20 mM glycerol	40 mM glycerol
Lactic acid	13.0	13.2	14.7
Acetic acid	2.5	7.7	9.2
Ethanol	14.7	7.9	9.0
1,3-PD	1.0[b]	16.7	25.0

[a] HPLC procedures for the determination of product concentrations are described in Materials and Methods.

[b] This value represents a background level of 1,3-PD or another substance present in the culture medium which coeluted with 1,3-PD.

shown). Addition of glycerol to culture media lacking a carbohydrate source resulted in no more growth than did the use of the basal medium alone.

L. reuteri is a heterofermentative species (2, 15). As expected, the fermentation of glucose by *L. reuteri* 1063 resulted in the production of lactate, ethanol, and small amounts of acetate (CO_2 production was not measured) (Table 2). When glycerol was included in the culture medium, lactate production remained constant but a significant increase in acetate production and a decrease in ethanol production were evident. Also evident was the accumulation of 1,3-PD, the product of glycerol dehydration and reduction. Glycerol at both concentrations was completely utilized within 22 h of growth but could not be totally accounted for by the appearance of 1,3-PD.

These findings indicate that glycerol competes successfully with acetyl phosphate as a major hydrogen acceptor during the fermentation of glucose, resulting in the accumulation of 1,3-PD. There is a concomitant increase in conversion of glucose to acetate and decrease in ethanol accumulation, thus allowing acetyl phosphate to be channeled into energy-producing reactions leading to increased cell yields and growth rates. Surprisingly, these fermentations carried out with a molar glycerol/glucose ratio of 2:1 (or greater) did not completely eliminate ethanol production. This may reflect a competition between 3-HPA and acetyl phosphate as hydrogen acceptors in the *L. reuteri* system.

Oxidoreductase purification and characterization. The oxidoreductase believed to be responsible for the reduction of 3-HPA to 1,3-PD was eluted as a single peak from DEAE-cellulose with 0.05 M KPB–0.5 M KCl–2% 1,2-PD. Subsequent affinity chromatography on Blue Sepharose CL-6B, an affinity matrix useful for retention of NAD^+-binding proteins (29), retained this activity until elution with 1 M KPB containing 10 mM NAD^+. A summary of the purification protocol is presented in Table 3. The purified fraction contained one major band possessing oxidoreductase activity and a faint band of presumably lower-molecular-weight material as indicated by gel electrophoresis under nondenaturing conditions (Fig. 1).

TABLE 3. Purification protocol for 1,3-PD:NAD^+ oxidoreductase for *L. reuteri*

Fraction	Amt of protein (mg)	Total activity (U)	Sp act (U/mg of protein)	Purification (fold)	Yield (%)
Crude	306	11	0.04	1	100
DEAE-cellulose	47	32	0.68	19	290
Blue Sepharose	0.9	6.6	7.3	200	60

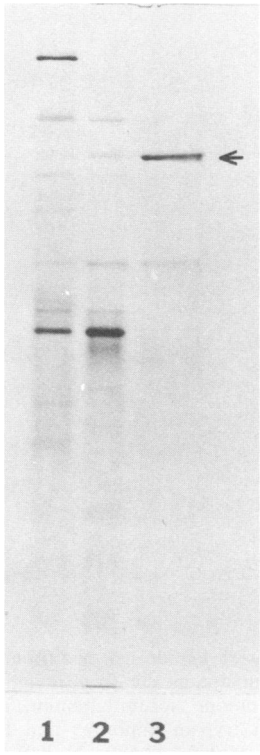

FIG. 1. Native 8% polyacrylamide gel electrophoretic pattern of *L. reuteri* extracts containing oxidoreductase activity. Lanes: 1: crude extract; 2: DEAE-cellulose; 3: Blue Sepharose CL-6B. The arrow indicates activity as determined by assay in the gel (see Materials and Methods).

The rate of oxidation of 1,2-PD and the rates of reduction of DHA and 3-HPA were determined for the purified oxidoreductase at various pH values. Two distinct maxima were observed: the oxidation reaction proceeded most rapidly at pH 7, whereas the reduction reactions proceeded most rapidly at pH 6.2. Glycerol dehydrogenase isolated from *Klebsiella pneumoniae* also produced distinct pH optima for oxidation (pH 8 to 9) and reduction (pH 4 to 8) (20). The optimum pH for 1,3-PD NAD^+:oxidoreductase isolated from *K. pneumoniae* was not reported, although oxidation activity assays on this enzyme were carried out at pH 9 (14).

With respect to cation effects, the purified enzyme from *L. reuteri* exhibited the highest levels of activity (measured as the oxidation of 1,2-PD) in the presence of 100 mM K^+. Inclusion of 100 mM Na^+, Li^+, Mg^{2+}, or NH_4^+ with K^+ resulted in a 13 to 23% decrease in activity of the enzyme.

The purified oxidoreductase was estimated to have a molecular weight of approximately 180,000 based on elution from a calibrated column of Sepharose CL-6B. A second estimation of molecular weight was made by using native gel polyacrylamide electrophoresis. The estimated molecular weight of the enzyme based on this method was also approximately 180,000. SDS-polyacrylamide gel electrophoretic analysis of the denatured enzyme exhibited a single band with a molecular weight of 41,000 (Fig. 2). The native enzyme is therefore predicted to exist as a tetramer of this subunit.

Oxidation of glycerol (or 1,2-PD) by the oxidoreductase was linked to the reduction of pyruvate by lactic dehydro-

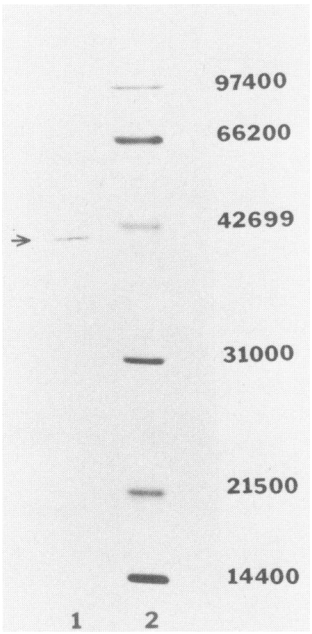

FIG. 2. SDS-polyacrylamide gel electrophoresis on a 10% gel. Lanes: 1, oxidoreductase as cut from a native gel; 2, standards (phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme [molecular weights of 97,400, 66,200, 42,699, 31,000, 21,500, and 14,400, respectively]). The arrow indicates the protein band.

genase to accumulate sufficient amounts of product for identification by HPLC. In this manner it was shown that the oxidoreductase produced DHA and hydroxyacetone as the sole oxidation product of glycerol and 1,2-PD, respectively (data not shown). The accumulation of lactate, the product of pyruvate reduction by lactate dehydrogenase, was also observed in these experiments. Oxidation of these diols yielded the corresponding ketone rather than the aldehyde product.

Substrate specificity studies showed that the oxidoreductase was capable of catalyzing a number of oxidation and reduction reactions. The highest activities were found for substrates which had adjacent hydroxyl functionality. Glycerol and 1,2-PD were oxidized at equivalent rates (Table 4); the oxidation of glycerol occurred at approximately 50% of the maximal rate when $NADP^+$ was substituted for NAD^+ in the reaction mixture. The reverse reaction, i.e., the reduction of the corresponding aldehydes, DHA, and Acetol, also yielded similar rates. Acetone was reduced at approximately half the maximal rate observed for DHA. Of particular significance was the observation that 3-HPA was rapidly reduced by this enzyme. This enzyme is believed to be responsible for the reduction of 3-HPA to 1,3-PD during growth in the presence of glycerol.

The apparent K_m values determined at pH 7.3 for the various reactions catalyzed by this enzyme were calculated from standard Lineweaver-Burk plots and are summarized in Table 5. The K_m values for NAD^+ were approximately equivalent in the presence of either substrate. At this pH, the K_m values for DHA and 3-HPA also were equivalent. The reduction of 3-HPA and DHA occurred more rapidly at pH 6.2, and therefore the K_m values of these substrates were also measured at this pH. The apparent K_m value for DHA at this pH decreased approximately 70-fold, while the apparent K_m value of 3-HPA decreased only 4-fold.

TABLE 4. Effect of different substrates on oxidoreductase activity

Substrate	Relative activity (%)[a]
Reduction by NADH	
3-HPA	100
Acetol	100
DHA	100
Acetone	50
Glyceraldehyde	5
Propionaldehyde	2
Acrolein	0
Oxidation by NAD^+	
1,2-PD	100
Glycerol	100
2,3-Butanediol	14
n-Propanol	11
Ethylene glycol	7
1,3-PD	5
Ethanol	1

[a] Relative activities for oxidation and reduction of substrates were determined as described in Materials and Methods.

DISCUSSION

In a previous report a method for purification and characterization of a coenzyme B_{12}-dependent glycerol dehydratase from *L. reuteri* was described (27). This enzyme catalyzes the conversion of glycerol to 3-HPA, a transient intermediate which functions as a hydrogen acceptor in *Lactobacillus*, *Klebsiella*, and *Citrobacter* species (10–12, 24). A second enzyme from *L. reuteri*, which has activity as both a glycerol dehydrogenase and 1,3-PD:NAD^+ oxidoreductase, has been isolated. The enzyme is capable of oxidizing glycerol to DHA and reducing DHA to glycerol. It also catalyzes the reduction of 3-HPA to 1,3-PD, with little or no ability to catalyze the reverse reaction. Given the observation that *L. reuteri* is unable to grow on glycerol as the sole carbon and energy source, it appears that the physiological role of this oxidoreductase may be the reduction of 3-HPA to 1,3-PD, thus providing *L. reuteri* with a highly effective alternate hydrogen acceptor system which can be utilized during fermentation of various carbohydrates, resulting in increased growth rates and cell yields.

K. pneumoniae, which can grow on glycerol as the sole carbon and energy source, possesses an active glycerol dehydrogenase, a coenzyme B_{12}-dependent glycerol dehydratase, and a 1,3-PD:NAD^+ oxidoreductase (10, 12, 14). This species utilizes the glycerol dehydrogenase for the production of DHA, which is subsequently phosphorylated and enters the glycolytic pathway (11, 12). Glycerol dehy-

TABLE 5. Determination of K_m values for substrates and coenzymes of the oxidoreductase at pH 7.3

Substrate or coenzyme tested[a]	Other reactant	K_m (mM)
1,2-PD	8 mM NAD^+	0.2
NAD^+	0.5 M 1,2-PD	0.15
Glycerol	8 mM NAD^+	12
NAD^+	0.5 M glycerol	0.1
DHA	0.1 M NADH	7.2
NADH	20 mM DHA	0.03
3-HPA	0.1 M NADH	7.8

[a] K_m values were determined as described in Materials and Methods.

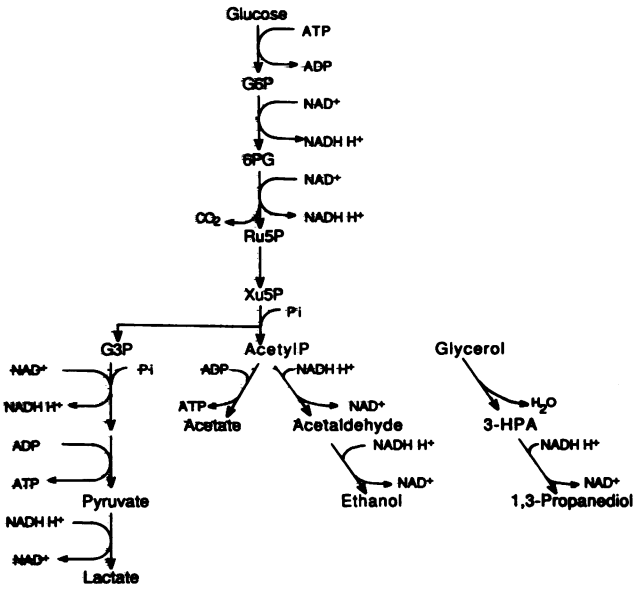

FIG. 3. General scheme of glucose and glycerol utilization by *L. reuteri*. Abbreviations: G6P, glucose 6-phosphate; 6GP, 6-phosphogluconate; Ru5P, ribulose 5-phosphate; Xu5P, xylulose 5-phosphate; G3P, glyceraldehyde 3-phosphate; AcetylP, acetyl phosphate.

dratase and 1,3-PD:NAD^+ oxidoreductase are responsible for the formation and reduction of 3-HPA, respectively, and thereby also for regeneration of NAD^+ required for the continued production of DHA (11, 12). *L. reuteri*, on the other hand, cannot grow on glycerol alone; it utilizes glycerol only as an alternate hydrogen acceptor during growth on available carbohydrates.

The oxidoreductase purified from *L. reuteri* was found to have a molecular weight of 180,000 and to contain four subunits each with molecular weight of 41,000. The oxidoreductase purified from *K. pneumoniae* was reported to have a molecular weight of 328,000 and to be composed of identical subunits of molecular weight 45,000 (14). The glycerol dehydrogenase from *K. pneumoniae* has a molecular weight of 160,000 with subunits of molecular weight 40,000 (28). The enzyme purified from *L. reuteri* demonstrated strict specificity for glycerol or 1,2-PD as the substrate for oxidation. This specificity has been reported for glycerol dehydrogenases purified from a variety of sources (18). The corresponding reduction reactions with Acetol and DHA was also favored. However, to our knowledge the ability of glycerol dehydrogenase to reduce 3-HPA has not been reported previously in the literature, possibly owing to the instability and/or commercial unavailability of this substrate. The activity of 1,3-PD:NAD^+ oxidoreductase from *K. pneumoniae* was reported based on the oxidation of 1,3-PD and not on reduction of 3-HPA. Thus, it is difficult to make any comparison between this enzyme and the enzyme isolated from *L. reuteri* (14).

It seems clear that the availability of glycerol as an alternate hydrogen acceptor has a significant stimulatory effect on the ability of *L. reuteri* to grow on carbohydrates. Given the data provided in this report and by others (11, 12, 21), this stimulation involves the reactions shown in Fig. 3. The presence of an alternate hydrogen acceptor such as glycerol relieves the need for *L. reuteri* to utilize acetyl phosphate as a hydrogen acceptor, thereby sparing this high-energy metabolite for ATP synthesis via the acetate kinase reaction.

When glycerol is utilized as a hydrogen acceptor in other bacterial species, including other lactobacilli, most of the glycerol thus reduced can be accounted for as 1,3-PD. Schutz and Radler (21) recovered 79 to 94% of the glycerol utilized by growing cells of *L. brevis* and *L. buchneri* as 1,3-PD. However, Smiley and Sobolov (23) recovered only 16% of the glycerol utilized by *Lactobacillus* sp. strain 208-A as 1,3-PD. These studies indicate that production of 1,3-PD accounted for only 84 and 62% of the total glycerol utilized by growing cells of *L. reuteri* when glycerol/glucose ratios were 1:1 and 2:1, respectively (Table 2).

It appears that *L. reuteri* is unique in that it not only produces 3-HPA as a transient metabolic hydrogen acceptor, but also is able to excrete copious amounts of this substance into the surrounding medium, imparting to it strong antimicrobial properties (25). Although 3-HPA (reuterin) per se can be measured, it is a bioactive substance which undergoes rapid structural changes in a growth culture environment. At low concentrations it interacts with and inhibits the growth of heterologous cells in the culture medium, and at higher concentrations it inhibits the growth of the *L. reuteri* cells themselves (1). Future research will be directed at elucidating the nature of these interactions and thereby clarifying the mode of action of reuterin as an antimicrobial agent as well as the way *L. reuteri* cells are able to regulate production, excretion, and reduction of this substance.

ACKNOWLEDGMENTS

We thank Roger McFeeters, Food Science Department, North Carolina State University, for end product analysis of glucose and glycerol fermentations of *L. reuteri*. We also thank Thoyd Melton and Jerome Perry, the Microbiology Department, North Carolina State University, for providing the equipment used in enzyme purification.

LITERATURE CITED

1. **Axelsson, L. T., T. C. Chung, W. J. Dobrogosz, and S. E. Lindgren.** 1989. Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. Microb. Ecol. Health Dis. **2**:131–136.
2. **Axelsson, L. T., and S. E. Lindgren.** 1987. Characterization and DNA homology of *Lactobacillus* strains isolated from pig intestine. J. Appl. Bacteriol. **62**:433–440.
3. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
4. **Chung, T. C., L. Axelsson, S. E. Lindgren, and W. J. Dobrogosz.** 1989. In vitro studies on reuterin synthesis by *Lactobacillus reuteri*. Microb. Ecol. Health Dis. **2**:137–144.
5. **Circle, S. J., L. Stone, and C. S. Boruff.** 1945. Acrolein determination by means of tryptophane. A colorimetric micromethod. Ind. Eng. Chem. Anal. Ed. **17**:259–262.
6. **Davis, B. J.** 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**: 404–427.
7. **Deitz, A., and T. Lubrano.** 1967. Separation and quantitation of lactic dehydrogenase isozymes by disc gel electrophoresis. Anal. Biochem. **20**:246–257.
8. **Dobrogosz, W. J., I. A. Casas, G. A. Pagano, T. L. Talarico, B. M. Sjoberg, and M. Karlsson.** 1989. *Lactobacillus reuteri* and the enteric microbiota, p. 283–292. *In* R. Grubb, T. Midtvedt, and E. Norin (ed.), The regulatory and protective role of the normal flora. Wenner-Gren International Symposium. The Macmillan Press Ltd., London.
9. **Efthymiou, C., and C. A. Hansen.** 1962. An antigenic analysis of *Lactobacillus acidophilius*. J. Infect. Dis. **110**:258–267.
10. **Forage, R. G., and M. A. Foster.** 1979. Resolution of the

coenzyme B_{12}-dependant dehydratases of *Klebsiella* sp. and *Citrobacter freundii*. Biochim. Biophys. Acta **569:**249–258.
11. **Forage, R. G., and M. A. Foster.** 1982. Glycerol fermentation in *Klebsiella pneumoniae*: functions of the coenzyme B_{12}-dependent glycerol and diol dehydratases. J. Bacteriol. **149:**413–419.
12. **Forage, R. G., and E. C. C. Lin.** 1982. dha system mediating aerobic and anaerobic dissimilation of glycerol in *Klebsiella pneumoniae* NCIB 418. J. Bacteriol. **151:**591–599.
13. **Gartner, G., and G. Kopperschlager.** 1984. Purification and properties of glycerol dehydrogenase from *Candida valida*. J. Gen. Microbiol. **130:**3225–3233.
14. **Johnson, E. A., and E. C. C. Lin.** 1987. *Klebsiella pneumoniae* 1,3-propanediol:NAD^+ oxidoreductase. J. Bacteriol. **169:**2050–2054.
15. **Kandler, O., K. O. Stetter, and R. Kohl.** 1980. *Lactobacillus reuteri* sp. nov., a new species of heterofermentative lactobacilli. Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt Orig. C **1:** 264–269.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
17. **Lee, H. A., and R. H. Abeles.** 1963. Purification and properties of dioldehydrase, an enzyme requiring a cobamide coenzyme. J. Biol. Chem. **238:**2367–2373.
18. **Marshall, J. H., J. W. May, and J. Sloan.** 1985. Purification and properties of glycerol:NAD^+ 2-oxidoreductase (glycerol dehydrogenase) from *Schizosaccharomyces pombe*. J. Gen. Microbiol. **131:**1581–1588.
19. **McFeeters, R. F., and S. A. Armstrong.** 1984. Measurement of pectin methylation in plant cell walls. Anal. Biochem. **139:** 212–217.
20. **McGregor, W. G., J. Phillips, and C. Suelter.** 1974. Purification and kinetic characterization of a monovalent cation-activated glycerol dehydrogenase from *Aerobacter aerogenes*. J. Biol. Chem. **249:**3132–3139.
21. **Schutz, H., and F. Radler.** 1984. Anaerobic reduction of glycerol to propanediol-1,3 by *Lactobacillus brevis* and *Lactobacillus buchneri*. Syst. Appl. Microbiol. **5:**169–178.
22. **Shrago, A. W., B. M. Chassy, and W. J. Dobrogosz.** 1986. Conjugal plasmid transfer (pAMB1) in *Lactobacillus plantarum*. Appl. Environ. Microbiol. **52:**574–576.
23. **Smiley, K. L., and M. Sobolov.** 1962. A cobamide-requiring glycerol dehydrase from an acrolein-forming *Lactobacillus*. Arch. Biochem. Biophys. **97:**538–543.
24. **Sobolov, M., and K. L. Smiley.** 1960. Metabolism of glycerol by an acrolein-forming *Lactobacillus*. J. Bacteriol. **79:**261–266.
25. **Talarico, T. L., I. A. Casas, T. C. Chung, and W. J. Dobrogosz.** 1988. Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. Antimicrob. Agents Chemother. **32:**1854–1858.
26. **Talarico, T. L., and W. J. Dobrogosz.** 1989. Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. Antimicrob. Agents Chemother. **33:**674–679.
27. **Talarico, T. L., and W. J. Dobrogosz.** 1990. Purification and characterization of glycerol dehydratase from *Lactobacillus reuteri*. Appl. Environ. Microbiol. **56:**1195–1197.
28. **Tang, C. T., F. E. Ruch, and E. C. C. Lin.** 1982. Immunochemical properties of NAD-linked glycerol dehydrogenases from *Escherichia coli* and *Klebsiella pneumoniae*. J. Bacteriol. **152:** 1169–1174.
29. **Thompson, S. T., K. H. Cass, and E. Stellwagen.** 1975. Blue dextran-Sepharose: an affinity column for the dinucleotide fold in proteins. Proc. Natl. Acad. Sci. USA **72:**669–672.