ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1989, p. 674–679
0066-4804/89/050674-06$02.00/0

Vol. 33, No. 5

Chemical Characterization of an Antimicrobial Substance Produced by *Lactobacillus reuteri*†

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Received 31 October 1988/Accepted 9 February 1989

Lactobacillus reuteri **converts glycerol into a potent cell growth inhibitor. This substance, termed reuterin, inhibits the growth of gram-positive and gram-negative bacteria as well as yeasts, fungi, and protozoa. Semipreparative chromatography was used to purify reuterin, and Fourier transform infrared spectroscopy and liquid chromatography-mass spectrometry were used to establish the molecular weight as well as the molecular functionality of the reuterin molecule. Nuclear magnetic resonance studies of purified reuterin carried out with deuterium oxide confirmed the presence of two three-carbon compounds, β-hydroxypropionaldehyde and the corresponding hydrated acetal, and a six-carbon cyclic dimer of the aldehyde. Further nuclear magnetic resonance studies with deuterated methanol revealed that in this solvent the compound existed as a three-carbon compound in a methoxy form. Trimethylsilyl derivatives of reuterin were analyzed by gas chromatography-mass spectrometry, and a molecule was identified which had a molecular weight corresponding to a disilylated dimeric structure. On the basis of the above information, reuterin was determined to be an equilibrium mixture of monomeric, hydrated monomeric, and cyclic dimeric forms of β-hydroxypropionaldehyde. This was subsequently confirmed by chemical synthesis.**

Lactobacillus reuteri is an obligately heterofermentative resident in the gastrointestinal tracts of humans and most other animals examined to date (6, 7). It is an efficient colonizer (19), appears to be the dominant heterofermentative species in these ecosystems (14), and may be unique among lactobacilli in its ability to convert glycerol into a potent, broad-spectrum antimicrobial agent (18; L. Axelsson, T. C. Chung, W. J. Dobrogosz, and S. Lindgren, Microb. Ecol. Health Dis., in press; T. C. Chung, L. Axelsson, S. E. Lindgren, and W. J. Dobrogosz, Microb. Ecol. Health Dis., in press; W. J. Dobrogosz, I. A. Casas, G. A. Pagano, T. L. Talarico, and B. M. Sjoberg, *in* T. Midvedt and D. C. Savage, ed., *Fifth B. E. Gustafsson Symposium, Wenner-Gren International Symposium*, in press). This substance, termed reuterin, is produced and excreted by resting cells under conditions of pH, temperature, and anerobiosis similar to those believed to exist in the gastrointestinal tract (Chung et al., in press; Dobrogosz et al., in press). The biological activity of reuterin was tested by using a MIC assay (Chung et al., in press); it was found that 2 to 5 units of reuterin per ml inhibited the growth of all bacteria tested except lactic acid bacteria, which required four- to fivefold higher concentrations. It has been postulated that the *L. reuteri*-reuterin system may play a symbiotic role in this complex ecosystem (Chung et al., in press; Dobrogosz et al., in press).

A fermentation system has been developed which allows *L. reuteri* to synthesize relatively large quantities of reuterin, which may then be purified by high-pressure liquid chromatography (HPLC) (18). Pure reuterin has been analyzed by Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), and mass spectrometery (MS). Its chemical structure was then deduced and subsequently confirmed by applying the same analytical techniques to chemically synthesized reuterin. The results of these investigations are presented in this report.

MATERIALS AND METHODS

Culture and culture conditions. A 1% inoculum of an overnight culture of *L. reuteri* 1063 (1) was grown for 24 h in modified lactobacillus carrying medium with glucose (LCMG), consisting of 5 g of yeast extract, 10 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 3 g of tryptose, 3 g of potassium phosphate (monobasic), 3 g of potassium phosphate (dibasic), 2 g of ammonium citrate, 1.15 g of sodium acetate, 5 mg of $MgSO_4 \cdot 7H_2O$, 0.31 mg of manganous sulfate, 0.2 mg of $FeSO_4 \cdot 7H_2O$, and 0.5 mg of L-ascorbic acid per liter. This medium was autoclaved, and 10 ml of filter-sterilized 2 M glucose was added to 1 liter of cooled medium. Cells of *L. reuteri* were harvested by centrifugation at 4,000 × *g* for 10 min and washed twice with 50 mM sodium phosphate buffer (pH 7.5).

Production of reuterin. After being washed, *L. reuteri* cells were suspended to a concentration of 10 mg (dry weight) of cells per ml of deionized water. Sterile glycerol was added to 250 mM, and this suspension was incubated in a screw-top tube at 37°C for 2 h to produce and accumulate reuterin. Cells were pelleted at 4,000 × *g* for 10 min and discarded. The supernatant fluid was filtered through a filter (pore size, 0.45 μm; Acrodisc; Gelman Sciences, Inc., Ann Arbor, Mich.) to remove any remaining cells. The presence of reuterin was assayed by using HPLC and the MIC assay as previously described (18; Chung et al., in press).

Concentration of reuterin. Filtered supernatant fluid was frozen in a round-bottom flask by immersion in 2-propanol at −40°C. These preparations were lyophilized to either a reduced volume or dryness on a (no. 10-030; The Virtis Co., Inc., Gardiner, N.Y.) lyophilizer at 30×10^{-3} mm Hg (4 Pa) and stored at 0°C until the time of use. Concentration resulted in substantial yellowing of the preparations. The yellow substance was removed prior to purification by batch sorption on water-washed 400-mesh resin (no. AG50-W-8X; Bio-Rad Laboratories, Richmond, Calif.). Removal of the

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† Paper no. 11872 of the Journal Series of the North Carolina Agricultural Service of Raleigh.

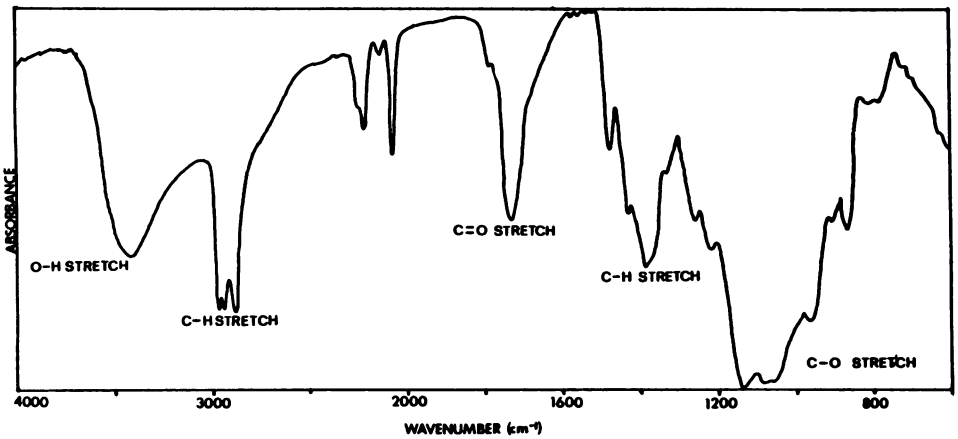

FIG. 1. FTIR analysis of reuterin. The major functional group absorbances of reuterin are noted. The sample was purified by semipreparative chromatography, and its purity was assayed by analytical HPLC before FTIR analysis.

color was tracked by monitoring A_{420}. MICs of reuterin preparations were unchanged after extraction.

Purification. Reuterin was purified by using a glass column (1 by 30 cm) packed with 8% cross-linked, 400-mesh resin (no. AG50-W; Bio-Rad). A solvent composed of 60% acetonitrile and 40% distilled-deionized water containing 1.1 g of trifluoroacetic acid (Fisher Scientific Co., Pittsburgh, Pa.) per liter was delivered via an HPLC pump (no. 110-A; Beckman Instruments, Inc., Fullerton, Calif.). The solvent flow rate was 1.5 ml/min, and reuterin was detected with a differential refractometer (no. 410; Waters Associates, Inc., Milford, Mass.), using a sensitivity of 2× and a scale factor of 5. A 400-μl sample of concentrated supernatant fluid was injected by using an injector (no. 210; Altex) with a 500-μl sample loop, and fractions were collected manually. Fractions were then evaporated under aspiration at ambient temperature to remove acetonitrile and lyophilized with a lyophilizer (no. 10-030; VirTis). Purity was assessed by passing portions of the fractions through an analytical column (Aminex HPX-87H; Bio-Rad) (18).

Trimethylsilylation. Trimethylsilylation was carried out with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Pierce Chemical Co., Rockford, Ill). Crude reuterin extract (2 ml) was purified by semipreparative chromatography as described above. BSTFA (1 ml) was added to the lyophilized reuterin, and silanization was carried out at ambient temperature. The sample was shaken gently by hand for 5 min until a white precipitate was detected, and enough acetonitrile was then added to dissolve the precipitate. The sample was then sparged with nitrogen, sealed in a screw-top vial, and examined by gas chromatography (GC)-MS.

LC-MS. Liquid chromatography (LC) separation was carried out on an Aminex HPX-87H analytical column at a flow rate of 0.8 ml of 65% distilled-deionized water–35% acetonitrile (containing 1.0 g of concentrated sulfuric acid per liter) per min. The solvent stream was then mixed with 0.3 M ammonium acetate and introduced via a Vestec interface into an HPLC-MS system (no. 4500; Finnigan MAT, San Jose, Calif.). Positive-ion detection was used, with a vaporizer temperature of 210°C and a source temperature of 250°C. The electron energy of the source was 1,000 eV.

GC-MS. A GC-MS (no. 5985B; Hewlett-Packard Co., Palo Alto, Calif.) was used in studies on silylated reuterin derivatives. GC conditions were a flow rate of 1.1 ml/min and an injection temperature of 280°C. The program used for analysis consisted of an initial hold period for 3 min at 40°C with a ramp to 260°C at 6°C/min. The column was a 15-m DB5 fused silica capilary column (J&W Scientific, Folsom, Calif.). A mass range of 40 to 400, an ion source temperature of 200°C, electron energy of 70 eV, and a split time of 0.8 min were used in the analyses.

NMR. Proton and carbon NMR studies were carried out with both deuterium oxide and deuterated methanol (Aldrich Chemical Co., Inc., Milwaukee, Wis). Proton NMR analyses (including decoupling studies) were conducted on an FTNMR (no. WM 250; Bruker Instruments Inc., Billerica, Mass.) operated at 250 MHz. Carbon-13 spectra were generated on an FTNMR (NR-100 AF; IBM) operated at 25 MHz with a superconducting magnet.

FTIR analysis. FTIR analysis was carried out on purified reuterin. Reuterin was dissolved in HPLC-grade methanol and evaporated onto sodium chloride absorbtion cells (Sargent-Welch Scientific Co., Skokie, Ill.). Analysis was done on an FTIR spectrometer (no. 1550; The Perkin-Elmer Corp., Norwalk, Conn.), and data were manipulated on a data station (no. 7500; Perkin-Elmer). Samples were scanned

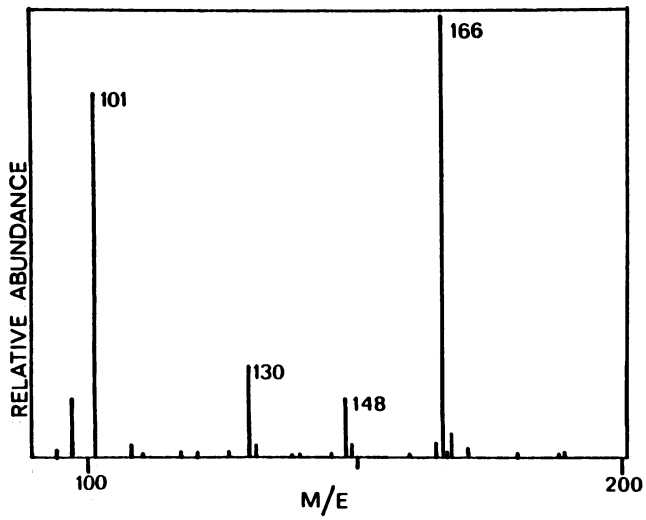

FIG. 2. LC-MS analysis of reuterin. The sample matrix contained reuterin, glycerol, trimethylene glycol, and β-hydroxypropionic acid. These components were separated by HPLC on an Aminex HPX-87H column. The spectrum of reuterin contained three major ions of *m/e* 166, 148, and 130, as noted above.

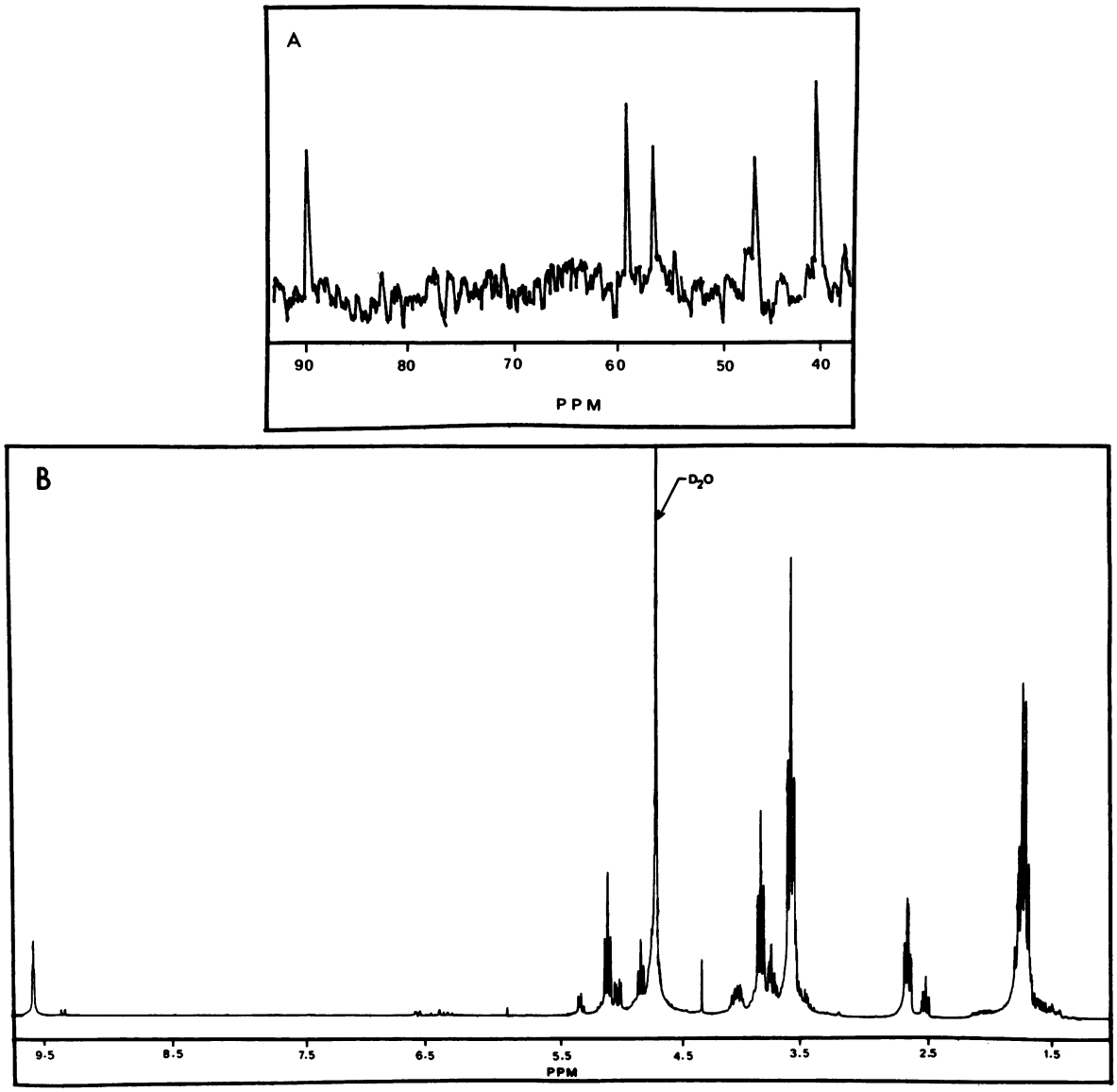

FIG. 3. Carbon-13 (A) and proton (B) NMR spectra of reuterin in deuterium oxide.

from 600 to 4,000 cm^{-1}, and the curves were smoothed by using the auto program available with the Perkin-Elmer software.

Synthesis of β-hydroxypropionaldehyde. Fresh acrolein (Aldrich) was used as the starting material for preparation of monomeric and dimeric β-hydroxypropionaldehyde in the hydration reaction as reported by Hall and Stern (4). The sample was concentrated on a rotavaporator at 2 mm Hg (266 Pa). The presence of β-hydroxypropionaldehyde was confirmed by FTIR and NMR analyses, as well as by derivation of the sample with 2,4-dinitrophenylhydrazine and analysis of the products by thin-layer chromatography on silica gel (4).

RESULTS

Reuterin purification. The supernatant fraction from resting cultures of *L. reuteri* fermenting glycerol contained unused glycerol, β-hydroxypropionic acid, 1,3-propanediol, and reuterin (18). Semipreparative HPLC was used to purify reuterin from the other components as described in Materials and Methods. Two fractions were detected; the first eluted at 15 min and contained reuterin and a leading shoulder of β-hydroxypropionic acid, and the second eluted at approximately 19 min and contained glycerol and 1,3-propanediol. This second peak was discarded, and only the middle and later portions of the first peak were collected. They were dried by evaporation under reduced pressure and then lyophilized. This process produced a viscous liquid, which, when rechromatographed under analytical conditions on an Aminex HPX-87H column, produced a single peak (18), which contained bactericidal activity as determined by the MIC assay (Chung et al., in press). No other collected fractions possessed bactericidal activity, and no protein could not be detected in the active fraction by the Bio-Rad dye-binding protein assay.

FTIR analysis. Spectra (Fig. 1) contained a large peak due

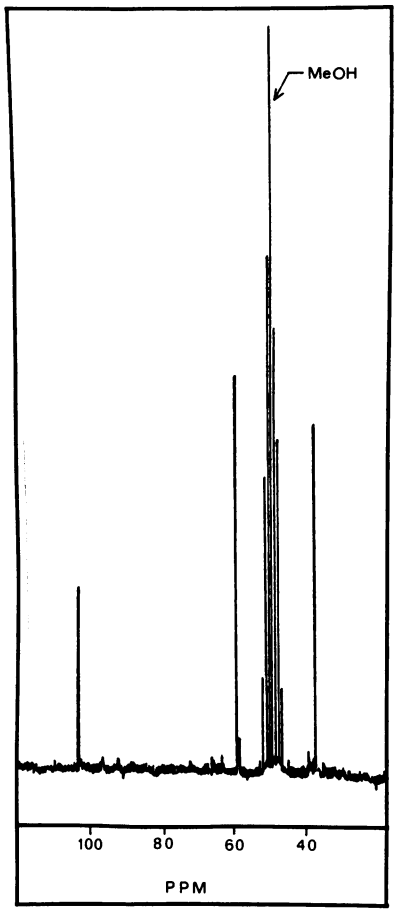

FIG. 4. Carbon-13 spectrum of reuterin in deuterated methanol (MeOH).

to C—O stretching at 1,050 to 1,150 cm^{-1} and a broad O—H stretch band at 3,450 cm^{-1}, indicating hydroxyl functionality. A C=O stretch typical of aldehydes was present at 1,730 cm^{-1}, along with C–H stretches at 2,880 and 1,380 cm^{-1} (11).

LC-MS analysis. LC-MS analyses were carried out on reuterin with postcolumn addition of ammonium acetate. The base peak occurred at *m/e* 166 (Fig. 2). This ion was interpreted to be the ammonium adduct of the molecular ion, and the molecular weight of reuterin was predicted to be 148. The signal at *m/e* 148 was predicted to be a loss of water from the adduct ion, and the signal at 130 represented the adduction with the loss of two molecules of water. The signal present at *m/e* 101 was believed to be the result of background solvent effects.

NMR in deuterium oxide. The carbon-13 NMR spectrum showed six signals, at chemical shifts of 40.1, 46.3, 56.2, 58.7, 89.7, and 207.7 ppm (Fig. 3A; signal at 207.7 ppm not shown). The signal at 207 ppm was interpreted as an aldehydic carbon; those at 89, 58, and 56 ppm were interpreted as carbon bound directly to oxygen; and those at 46 and 40 ppm were interpreted as aliphatic moieties. The sample was then subjected to proton NMR analysis, and the resulting spectrum is shown in Fig. 3B. Six major signal groups were also present in this spectrum, at 9.5, 5.0, 3.7, 3.5, 2.7, and 1.6 ppm. Homonuclear coupling experiments revealed that the signals at 9.5, 3.7, and 2.7 ppm were linked and that the signals at 5.0, 3.5, and 1.6 ppm were linked. Splitting patterns and signal area ratios together with the homonuclear coupling results indicated that two molecules were present in aqueous solutions: β-hydroxypropionaldehyde and a hydrated form of β-hydroxypropionaldehyde (see Fig. 7). Several weak signals were also present in the proton spectrum (Fig 3B), which represented a third molecule present in small quantities, a cyclic dimer of the aldehyde.

Confirmation of the molecular species present in aqueous solution was obtained by synthesizing β-hydroxypropionaldehyde as described by Hall and Stern (4) and analyzing the product by FTIR and NMR spectroscopy in D_2O. FTIR and proton NMR spectra of the synthesized molecule matched those obtained for the reuterin samples produced by *L. reuteri* (data not shown). Samples thus synthesized also possessed HPLC elution profiles and bactericidal activity (as measured by the MIC assay) (Chung et al., in press) equivalent to those produced by *L. reuteri*.

NMR in deuterated methanol. The carbon-13 signal pattern of reuterin in deuterated methanol (Fig. 4) was different from that observed in deuterium oxide. The spectrum contained only three sets of signals, at 36.8, 58.9, and 103.9 ppm. The signal at 103.9 ppm was interpreted as a carbon linked through two oxygen-containing bonds (5). The proton NMR spectrum also had three groups of signals, and homonuclear coupling, signal area ratio, and splitting results (data not shown) were interpreted to represent β-hydroxypropionaldehyde existing in a methoxy form (Fig. 5). The methoxy group signals are hidden by the signals as a result of methanol present in the solvent in the carbon-13 spectra (Fig. 4).

GC-MS studies. Reuterin was found to be unstable at the high temperatures present in the GC injector. A stable reuterin derivative was produced upon silylinization with BSTFA at ambient temperatures. Chromatography of the derivatized sample produced a complex GC trace (data not shown), but a series of compounds with an apparent molecular weight of 292 (148 plus two trimethylsilyl groups) were found at retention times of between 11 and 14 min. One such compound, which eluted at 12 min, was identified as a disilylated dimer of β-hydroxypropionaldehyde, and its spectrum is shown in Fig. 6. The fragmentation pattern of this compound consists of major signals at *m/e* 219, 177, 147, and 73. If the molecular weight of the parent molecule was 292, the loss of one trimethylsilyl group would produce an ion of *m/e* 219. This ion was present and produced signals at *m/e* 220 and 221 due to the natural isotopes of silicon, carbon, and oxygen, respectively (10). The fragment of *m/e* 177 is due to a fragmentation of the two-carbon tail and contains a trimethylsilyl group as evidenced by the isotope pattern described above. The peak at *m/e* 147 is due to a

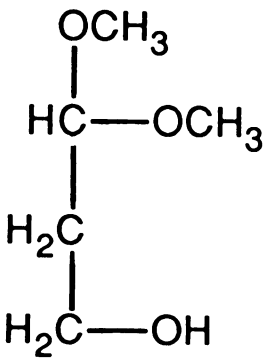

FIG. 5. Methoxy form of β-hydroxypropionaldehyde.

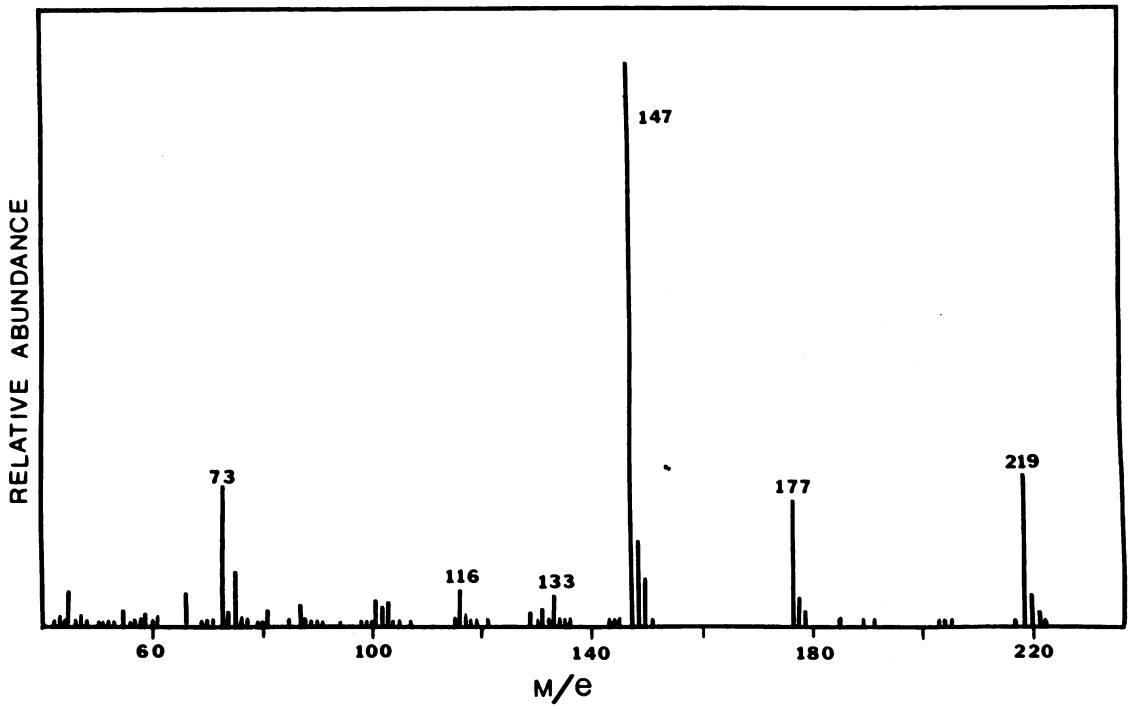

FIG. 6. Mass spectrum of a trimethylsilyl derivative of reuterin. The species of derivatized reuterin giving rise to this signal eluted from the GC at 12 min. The peak patterns around *m/e* 220, 177, and 147 are due to the natural isotopes of Si, C, and O, respectively.

fragmentation across the ring and would also contain a trimethylsilyl group. The signal at *m/e* 73 is due to the trimethylsilyl groups. These data confirm the molecule present in this GC fraction to be the cyclic dimer of β-hydroxypropionaldehyde.

DISCUSSION

Evidence is presented in this report that identifies reuterin as an equilibrium mixture of monomeric, hydrated monomeric, and cyclic dimeric forms of β-hydroxypropionaldehyde (Fig. 7). On the basis of MS data, reuterin was determined to have a molecular weight of 148. NMR studies of purified reuterin revealed that the compound existed as predominantly β-hydroxypropionaldehyde, a three-carbon compound (either hydrated or dehydrated), and, to a lesser extent, as a dimeric form of this aldehyde. The LC-MS-predicted molecular weight of 148 must therefore be for the cyclic dimeric structure, and the lack of resolution of the monomeric form could be due to background solvent effects masking detection of the lower-molecular-weight monomer. The molecular weight of the monomeric form is 74. These data fit well with previously reported results on the existence of β-hydroxypropionaldehyde in both monomeric and dimeric forms (4, 12). Further confirmation of the structure was provided by showing that chemically synthesized β-hydroxypropionaldehyde exhibited HPLC elution patterns, MIC biological activity, and spectroscopic profiles identical to those of reuterin which had been synthesized by *L. reuteri*.

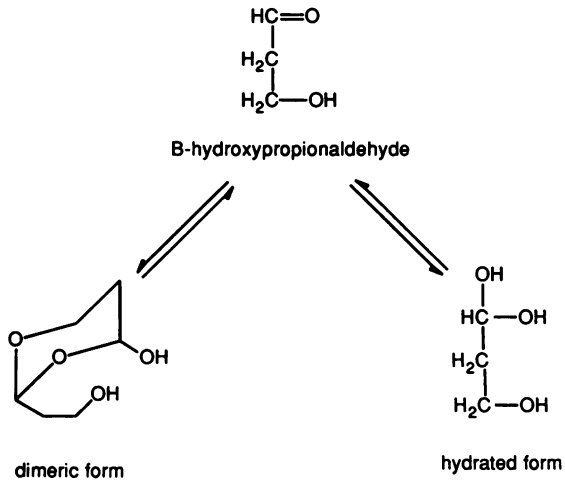

FIG. 7. The three forms of reuterin present in aqueous solution.

β-Hydroxypropionaldehyde has been found to be an intermediate in glycerol metabolism of *Clostridium*, *Klebsiella*, and *Lactobacillus* species (2, 3, 16). Glycerol and diol dehydrases from a number of these species have been purified and characterized (13, 15). These enzymes catalyze a coenzyme B-12-dependent dehydration of glycerol to yield β-hydroxypropionaldehyde (15). This compound does not accumulate but may undergo a dismutation, forming equimolar amounts of 1,3-propanediol (trimethylene glycol) and β-hydroxypropionic acid, or may function as a hydrogen acceptor, yielding only 1,3-propanediol (17). *L. reuteri* cultures appear to carry out a similar set of reactions, except for the accumulation of an additional product, reuterin, which exhibits potent antimicrobial activity. Although a number of lactic acid bacteria have been proposed to synthesize low-molecular-weight antimicrobial substances, to the best of our knowledge such substances remain to be purified and chemically characterized, exceptions being the well-known metabolic end products of the lactic acid fermentation,

acetic and lactic acids and hydrogen peroxide (8). On the other hand, a number of high-molecular-weight bacteriocin or bacteriocinlike substances are produced by various lactobacilli, lactococci, and pediococci (9). It appears that reuterin is the first low-molecular-weight antimicrobial substance produced by this group of bacteria to be chemically identified.

It is difficult at present to ascertain which combination of the various forms of β-hydroxypropionaldehyde is the biologically active entity. It is also difficult to ascertain changes that may occur in the relative concentration or biological efficacy of these entities, depending on water activity states. For example, is one form more capable than the others of traversing the cytoplasmic membrane or functioning within the cytosol? Preliminary studies conducted by Britt-Marie Sjoberg, Stockholm University, Stockholm, Sweden, show reuterin to effectively inhibit ribonucleotide reductase activity in vitro. These experiments were conducted with purified *Escherichia coli* ribonucleotide reductase (Dobrogosz et al., in press). Inasmuch as ribonucleotide reductase activity constitutes the first unique step in DNA synthesis, the broad-spectrum activity of reuterin could be explained on this basis. In these experiments it was also shown that reduced thioredoxin, another sulfhydryl enzyme, was inhibited by reuterin. The mechanism of action of reuterin may be directed toward sulfhydryl enzymes. This remains to be more fully explored.

The in vivo role of the *L. reuteri*-reuterin system also remains to be explored. Preliminary studies in our laboratory show that reuterin-producing *L. reuteri* cells are found at 10^3 to 10^5 CFU/cm^2 in all portions of the proximal gastrointestinal tract of piglets, i.e., the stomach, duodenum, jejunum, and ileum (Dobrogosz et al., in press). They are introduced into the piglets via suckling from the mother, and all of the above tissue segments appear well colonized by the first 2 days of life (G. A. Pagano, T. C. Chung, J. G. Lecce, and W. J. Dobrogosz, unpublished data). *L. reuteri* is thus a prominent member of the *Lactobacillus* population in this ecosystem, where it is believed to play a symbiotic role.

ACKNOWLEDGMENTS

We thank Carol Ann Haney, North Carolina State University, for efforts spent in GC-MS analysis as well as in interpretation of data and review of the manuscript. LC-MS analysis was performed by Research Triangle Institute, Research Triangle Park, N.C., under the supervision of Robert Voyksner. Proton and carbon-13 NMR work was carried out by the North Carolina State University Chemistry Department under the supervision of Charles Moreland. We thank Anita Lewin, Research Triangle Institute, for time and effort spent helping with interpretation of NMR spectra. Additional help in interpretation of spectra, as well as synthesis of β-hydroxypropionaldehyde, was kindly supplied by Ramin Faghih and Greg Vite, both currently in positions in the laboratory of B. Fraiser-Reid, Duke University Chemistry Department. Special thanks go to J. R. Kirby, IBM, Research Triangle Park, N.C., for the use of the FTIR facilities.

LITERATURE CITED

1. **Axelsson, L., and S. E. Lindgren.** 1987. Characterization and DNA homology of *Lactobacillus* strains isolated from pig intestine. J. Appl. Bacteriol. **62**:433–440.
2. **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.
3. **Forsberg, C. W.** 1987. Production of 1,3-propanediol from glycerol by *Clostridium acetobutylicum* and other *Clostridium* species. Appl. Environ. Microbiol. **53**:639–643.
4. **Hall, R. H., and E. S. Stern.** 1950. Acid-catalysed hydration of acrylaldehyde. Kinetics of the reaction and isolation of β-hydroxypropionaldehyde. J. Chem. Soc. **1950**:490–498.
5. **Johnson, L. F., and W. C. Jankowski.** 1972. Carbon-13 NMR spectra. John Wiley & Sons, Inc., New York.
6. **Kandler, O., K. O. Stetter, and R. Kohl.** 1980. *Lactobacillus reuteri* sp. nov., a new species of heterofermentative lactobacilli. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. C **1**:264–269.
7. **Kandler, O., and N. Weiss.** 1986. Regular, nonsporing gram-positive rods, p. 1208–1234. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
8. **Klaenhammer, T. R.** 1982. Microbial consideration in selection and preparation of *Lactobacillus* strains for use in dietary adjuncts. J. Dairy Sci. **65**:1339–1349.
9. **Klaenhammer, T. R.** 1988. Bacteriocins of lactic acid bacteria. Biochimie **70**:337–349.
10. **McLafferty, F.** 1966. Interpretation of mass spectra, 4th ed. W. A. Benjamin, New York.
11. **Morrison, R. T., and R. N. Boyd.** 1979. Organic Chemistry, 3rd ed., p. 410–421. Allyn and Bacon, Inc., Boston.
12. **Nielsen, A. T., D. W. Moore, and B. D. Schuetz.** 1981. A ^{13}C and ^{1}H NMR study of formaldehyde reactions with acetaldehyde and acrolein. Synthesis of 2-(hydroxymethyl)-1,3-propanediol. Pol. J. Chem. **55**:1393–1403.
13. **Poznanskja, A. A., K. Tanizawa, K. Soda, T. Toraya, and S. Fukui.** 1979. Coenzyme B-12 dependent diol dehydrase: purification, subunit heterogeneity, and reversible association. Arch. Biochem. Biophys. **194**:379–386.
14. **Sarra, P. G., F. Dellaglio, and V. Bottazzi.** 1985. Taxonomy of lactobacilli isolated from the alimentary tract of chickens. Syst. Appl. Microbiol. **6**:86–89.
15. **Schneider, Z., E. G. Larsen, G. Jacobson, B. C. Johnson, and J. Pawelkiewicz.** 1970. Purification and properties of glycerol dehydrase. J. Biol. Chem. **245**:3388–3396.
16. **Smiley, K. L., and M. Sobolov.** 1962. A cobamide-requiring glycerol dehydrase from an acrolein forming *Lactobacillus*. Arch. Biochem. Biophys. **97**:538–543.
17. **Sobolov, M., and K. L. Smiley.** 1960. Metabolism of glycerol by an acrolein-forming lactobacillus. J. Bacteriol. **79**:261–266.
18. **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.
19. **Tannock, G. W., and R. D. Archibald.** 1984. The derivation and use of mice which do not harbor lactobacilli in the gastrointestinal tract. Can. J. Microbiol. **30**:843–853.