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Anaerobic Conversion of Lactic Acid to Acetic Acid and 1,2-Propanediol by *Lactobacillus buchneri*

STEFANIE J. W. H. OUDE ELFERINK,[1]* JANNEKE KROONEMAN,[2] JAN C. GOTTSCHAL,[2] SIERK F. SPOELSTRA,[1] FOLKERT FABER,[2] AND FRANK DRIEHUIS[1]

ID TNO Animal Nutrition, Lelystad,[1] and Department of Microbiology, University of Groningen, Haren,[2] The Netherlands

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The degradation of lactic acid under anoxic conditions was studied in several strains of *Lactobacillus buchneri* and in close relatives such as *Lactobacillus parabuchneri*, *Lactobacillus kefir*, and *Lactobacillus hilgardii*. Of these lactobacilli, *L. buchneri* and *L. parabuchneri* were able to degrade lactic acid under anoxic conditions, without requiring an external electron acceptor. Each mole of lactic acid was converted into approximately 0.5 mol of acetic acid, 0.5 mol of 1,2-propanediol, and traces of ethanol. Based on stoichiometry studies and the high levels of NAD-linked 1,2-propanediol-dependent oxidoreductase (530 to 790 nmol min^{-1} mg of $protein^{-1}$), a novel pathway for anaerobic lactic acid degradation is proposed. The anaerobic degradation of lactic acid by *L. buchneri* does not support cell growth and is pH dependent. Acidic conditions are needed to induce the lactic-acid-degrading capacity of the cells and to maintain the lactic-acid-degrading activity. At a pH above 5.8 hardly any lactic acid degradation was observed. The exact function of anaerobic lactic acid degradation by *L. buchneri* is not certain, but some results indicate that it plays a role in maintaining cell viability.

Although lactic acid bacteria are named after their ability to form lactic acid, many are able to degrade lactic acid as well, especially if O_2 is available as electron acceptor (21, 23). Some lactic acid bacteria are also able to degrade lactic acid under anoxic conditions in the presence of alternative electron acceptors. For example, *Lactobacillus plantarum* and *L. pentosus* can use citrate as electron acceptor (11, 19). The products of this cofermentation of lactic acid and citrate are succinic acid, acetate, formate, and CO_2. Other lactic acid bacteria, such as *L. brevis* and *L. buchneri*, can degrade lactic acid by using glycerol as an electron acceptor, while producing acetate, 1,3-propanediol, and CO_2 (26). *L. bifermentans* is thus far the only species known to ferment lactic acid, i.e., without requiring an external electron acceptor. This bacterium can form acetic acid, ethanol, CO_2 and H_2 from lactic acid at a pH of >4.0 (16).

From silage studies we obtained indications that yet another pathway for the anaerobic lactic acid degradation by lactic acid bacteria existed. In studies in which *L. buchneri* was used as an inoculant for silage fermentation, we observed an anaerobic degradation of lactic acid, and no production of succinic acid, formic acid, or H_2 was observed (13). The aim of the present study was to determine whether *L. buchneri* could indeed degrade lactic acid to acetic acid under anoxic conditions and to obtain more information on the possible degradation pathway. Furthermore, this study aims at investigating whether besides *L. buchneri* other *Lactobacillus* species are able to carry out anaerobic lactic acid degradation and how this degradation is influenced by such environmental conditions as pH and temperature. It was found that *L. buchneri* and relatives such as *L. parabuchneri* are indeed able to degrade lactic acid to acetic acid with the concomitant production of 1,2-propanediol, as well as traces of ethanol, under anoxic conditions without requiring an external electron acceptor. Based on stoichiometry and the high levels of NAD-linked 1,2-propanediol-dependent oxidoreductase (530 to 790 nmol min^{-1} mg of $protein^{-1}$), a novel lactic acid fermentation pathway for *L. buchneri* is proposed. Furthermore, it is shown that the occurrence of lactic acid degradation and its rate depend on pH and temperature.

MATERIALS AND METHODS

Organisms and culture conditions. *L. buchneri* (LMG 6892^T), *L. bifermentans* (LMG 9845^T), *L. brevis* (LMG 7944^T), *L. hilgardii* (LMG 9895^T), *L. kefir* (LMG 9480^T), *L. parabuchneri* (LMG 11457^T), and *L. plantarum* (LMG 6907^T) were obtained from the culture collection of the Laboratorium voor Microbiologie (LMG), Gent, Belgium. *L. buchneri* strain PW01 (NCIMB 40788) and *L. buchneri* strain PW07 were both isolated from separate batches of maize silage and were from our own laboratory collection. Stock cultures of the bacteria were maintained in 20-ml culture tubes with loose plastic caps, containing 10 ml of MRS-Broth (Oxoid). To test anaerobic lactic acid utilization, the bacteria were cultured in 120-ml serum vials closed with butyl rubber stoppers and aluminum crimp seals and in 1.2-liter glass bottles closed with butyl rubber stoppers and aluminum caps. The vials contained 50 ml, and the bottles contained 500 ml of modified MRS-Broth (MRS-MOD medium) with the following composition (per liter): peptone (5.0 g), Lab-Lemco powder (Oxoid, 4.0 g), yeast extract (2.0 g), Tween 80 (0.5 ml), K_2HPO_4 (1.0 g), $NaH_2PO_4 \cdot H_2O$ (3.0 g), sodium acetate (0.6 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), and $MnSO_4 \cdot H_2O$ (0.04 g). The vials were made anoxic by flushing with nitrogen gas and were autoclaved for 15 min at 120°C. Lactic acid and glucose were added separately to the medium from filter-sterilized stock solutions (1 M). Fermentation of lactic acid (45 mM) was tested with or without the addition of a small amount of glucose (5 mM). If required, the pH was adjusted by adding HCl from a sterile stock solution (1 M). The inoculum size was 1%. Unless stated otherwise, all cultures were incubated at pH 3.8, at 30°C in the dark, in a rotary shaker (100 rpm). All batch experiments were done in duplicate.

The basic medium used for continuous cultivation in a chemostat (500 ml, working volume) was the same as described above. Lactic acid (25 mM) and glucose (25 mM) were added from filter-sterilized stock solutions (1 M). The chemostat culture was magnetically stirred and was kept anoxic by passing a 100% N_2 gas flow (20 ml h^{-1}) over the culture continuously. The dilution rate of the chemostat was 0.025 h^{-1}. Traces of oxygen had been removed from the N_2 gas by passing it over reduced copper curls, which were kept at a temperature of

* Corresponding author. Mailing address: ID TNO Animal Nutrition, P.O. Box 65, 8200 AB Lelystad, The Netherlands. Phone: 31-320-237-359. Fax: 31-320-237-320. E-mail: s.j.w.h.oudeelferink@idtno.nl.

150 to 180°C. The pH was maintained at the required set point by automatic titration with sterile solutions of 0.5 M HCl or 0.5 M NaOH.

Analytical and microbiological procedures. Organic acids, alcohols, and sugars were determined by high-performance liquid chromatography (HPLC) (Beckman Instruments B.V., Mijdrecht, The Netherlands), with a prepacked cation-exchange resin column (Polyspher OA HY, 300 by 6.5 mm; Merck, Darmstadt, Germany) at a temperature of 45°C and a flow rate of 0.5 ml min^{-1} with 2.5 mM H_2SO_4 as the eluent. The injection volume was 20 μl. Detection occurred on a refractometer. Volatile fatty acids and alcohols were additionally determined by gas chromatography (GC) with a Hewlett-Packard 5890 gas chromatograph equipped with a Chrompack CP-Sil-5CB column (25 m by 0.32 mm [inner diameter] by 5-μm film thickness; Chrompack, Middelburg, The Netherlands) and a flame ionization detector. The injection volume was 1 μl. The injector was held at 250°C, the detector was kept at 300°C, and the He inlet pressure was 50 kPa. The temperature program for the column was 35°C for 0.25 min, followed by a 5°C min^{-1} rise to 150°C, followed by a 10°C min^{-1} rise to 225°C, which was held for 14.3 min. 1,2-Propanediol eluted from the GC and HPLC column as one peak and was separate from controls such as 1,3-propanediol, 1-propanol, 2-propanol, and 1,2-butanediol. Hydrogen was determined by GC (13).

Cell densities were measured by detecting the culture turbidity using a spectrophotometer at a wavelength of 660 nm. Cell numbers were determined by plate counts using double-layered MRS-Agar pour plates (Oxoid, Basingstoke, United Kingdom), i.e., after the inoculated medium had solidified, a second layer of medium was added. Cell numbers were expressed as CFU per milliliter. Protein concentrations in cell extracts were determined according to Bradford method (5).

Resting-cell experiments. The rates of lactic acid metabolism by resting-cell suspensions of *L. buchneri* (LMG 6892^T) were determined by taking 100-ml aliquots of a glucose-limited chemostat culture and incubating them anoxically under an N_2 atmosphere in the dark at 30°C. Chloramphenicol (30 mg $liter^{-1}$) was added to prevent protein synthesis during the incubation period. Lactic acid was added to the cell suspensions to a final concentration of approximately 50 mM. From duplicate incubations the disappearance of the substrate was monitored over time by HPLC analyses.

Preparation cell extracts and enzyme measurements. Aliquots (100 ml) taken from a glucose-limited chemostat culture were centrifuged (10 min at 4°C and 11,000 × *g*), washed twice in 25 mM phosphate buffer (pH 5.8 for cells grown at pH 5.8 and pH 3.8 for cells grown at pH 3.8), and concentrated 25-fold. Crude cell extracts were obtained by using a French pressure cell (10 times at 6.9 MPa). Cell debris was removed by centrifugation (30 min, 11,000 × *g* at 4°C). The resulting cell extract was stored on ice. Reverse 1,2-propanediol-dependent NADH-linked oxidoreductase activities were measured in cell extracts at 30°C (26). The 1,2-propanediol-dependent formation of NADH was monitored in anaerobic cuvettes in an assay mixture containing 1 ml of 100 mM Tris-HCl buffer (pH 9.0) in which 1 mM $MnCl_2$, 10 mM 1,2-propanediol, 5 mM NAD, and 5 to 50 μl of cell extract were present. The forward reaction was measured by monitoring the disappearance of NADH in an assay mixture containing 50 mM Tris-HCl (pH 7.5), 0.2 mM NADH, and 5 to 50 μl of cell extract. The reaction was started by adding 2 mM lactaldehyde.

RESULTS

Anaerobic lactic acid degradation by *L. buchneri* and relatives. To investigate whether the disappearance of lactic acid in silage inoculated with *L. buchneri* was due to the metabolic activity of this *Lactobacillus* strain, the conversion of lactic acid was monitored in pure cultures of *L. buchneri* (strain LMG 6892^T, PW01, and PW07), under acidic and anoxic conditions closely resembling those that prevail in silage. Furthermore, to investigate whether, besides *L. buchneri*, other related lactobacilli (15) are also able to degrade lactic acid under "silage conditions," lactic acid conversion was monitored in pure cultures of *L. parabuchneri*, *L. brevis*, *L. hilgardii*, *L. kefir*, and *L. plantarum*. *L. buchneri* and the other lactobacilli were grown in anoxic batch cultures, at pH 4, with 45 mM lactic acid as a substrate, with or without 5 mM glucose as a second substrate to enhance initial growth. The disappearance of lactic acid, product formation, the pH, and the optical density of each of the cultures were monitored over time. In the series with lactic acid with the additional 5 mM glucose, all strains except *L. kefir* degraded the glucose (i.e., within 50 to 100 h). However, only *L. buchneri* and *L. parabuchneri* were also able to slowly degrade lactic acid. During glucose degradation significant growth, measured as an increase in cell density, was observed. During the degradation of lactic acid only a slight increase in cell density was observed. This suggests that growth mainly occurred at the expense of glucose and possibly small amounts of other carbon compounds initially present in the medium and was not due or was only slightly due to the degradation of lactic acid.

In the series with lactic acid without additional glucose, only *L. buchneri* and *L. parabuchneri* showed a small but detectable increase in cell density and were able to degrade the lactic acid. For *L. buchneri* (LMG 6892^T), the dynamics of growth and the formation of fermentation products in basic MRS-MOD medium with or without added lactic acid are depicted in Fig. 1. During the degradation of lactic acid (from 43.5 to 27.0 mM), acetic acid increased from 8.0 to 15.5 mM, 1,2-propanediol increased from 0 to 7.5 mM, and ethanol increased from 0 to 0.7 mM. The 1:1 molar ratio of acetic acid and 1,2-propanediol production during lactic acid degradation suggests that their production is linked together. The stoichiometry of lactic acid degradation by *L. buchneri* (Fig. 1A) and *L. parabuchneri* (data not shown) was in good agreement with the stoichiometry of the complete degradation of lactic acid according to the following equation (CO_2 was calculated based on the C and O atom balance): 1 lactic acid → 0.48 acetic acid + 0.48 1,2-propanediol + 0.04 ethanol + 0.52 CO_2.

The *L. buchneri* culture showed a small increase in cell density during lactic acid degradation (Fig. 1A). However, during the first 3 days almost the same increase in cell density was observed in basic MRS-MOD medium without lactic acid (Fig. 1B). The protein concentration in the culture with lactic acid was 4.0 μg ml^{-1} after 3 days of incubation and increased to a maximum of 5.5 μg ml^{-1} after 17 days of incubation. In the culture without lactic acid the protein concentration was 3.7 μg ml^{-1} after 3 days of incubation and decreased to 2.1 μg ml^{-1} after 17 days of incubation. This suggests that the initial growth was probably mainly due to the degradation of carbon compounds initially present in the medium and not to the degradation of lactic acid. The MRS-MOD medium was buffered at pH 4.0, but the pH did rise to pH 4.4 at 55 days, probably due to the production of acetate, which has a pK_a value of 4.77, at the expense of lactic acid, which has a pK_a value of 3.86.

A second batch experiment was carried out to further investigate the role of lactic acid degradation on growth. *L. buchneri* LMG 6892^T was cultured in anoxic batch cultures at pH 4, with or without 45 mM lactic acid as a substrate, and the optical density, the protein concentration, and cell numbers were monitored over time. During the first 3 days of incubation the cell numbers in the cultures with or without lactic acid increased from 3.6×10^6 CFU ml^{-1} to 2.0×10^8 or 1.3×10^8 CFU ml^{-1}, respectively. From day 3 to day 14, the cell numbers in the cultures with lactic acid slightly decreased from 2.0×10^8 to 1.9×10^8 CFU ml^{-1}, while the protein concentration increased almost twofold from 7.1 to 11.9 μg ml^{-1}. In the cultures without lactic acid, the cell numbers decreased from day 3 to day 14, from 1.3×10^8 to 8.1×10^7 CFU ml^{-1},

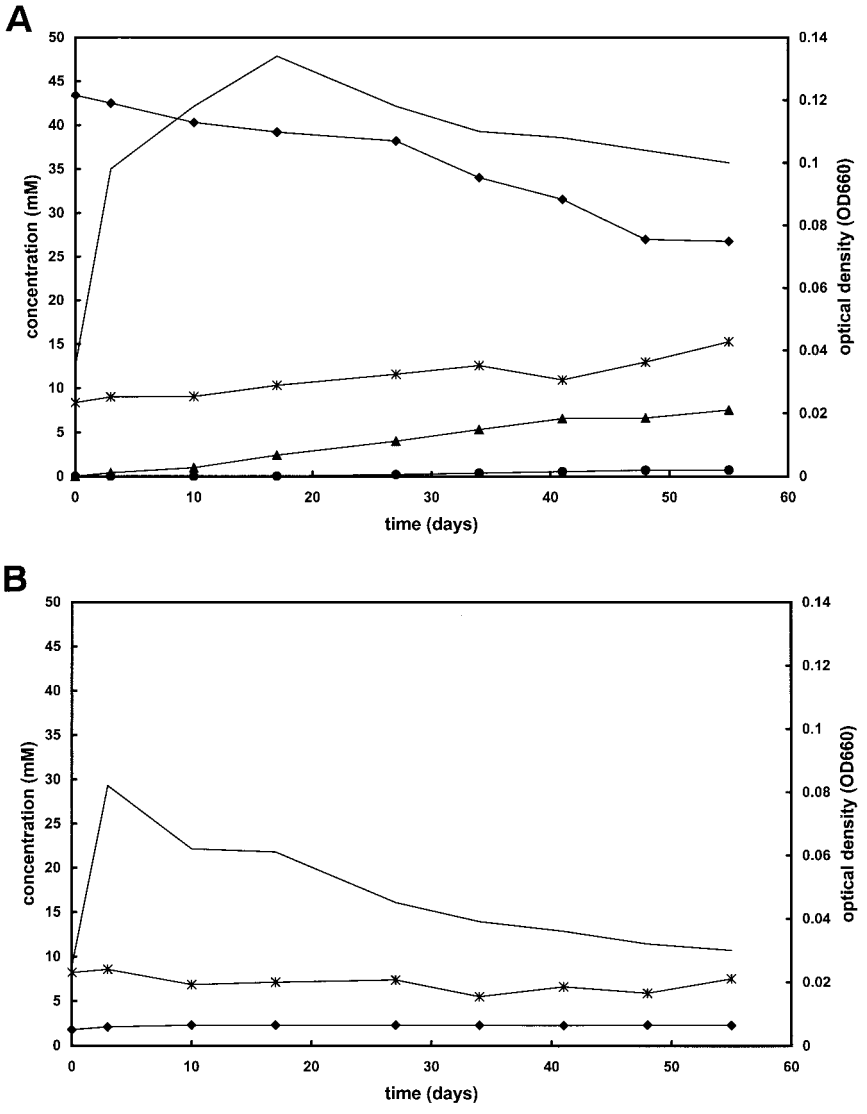

FIG. 1. Dynamics of growth and formation of fermentation products by *L. buchneri* LMG 6892^T cultured in basic MRS-MOD medium with (A) or without (B) added lactic acid. Lactic acid (◆) acetic acid (∗), 1,2-propanediol (▲), ethanol (●), and biomass (—) are shown. At t = 0 days the pH was set at 4.0, at t = 55 days the pH had increased to pH 4.4.

while the protein concentration decreased slightly from 5.1 to 4.9 μg ml^{-1}. These results indicate that cell growth, as represented by increasing cell numbers, indeed only occurs during the first few days of incubation. Lactic acid does not seem to support detectable growth.

Anaerobic lactic acid degradation at different temperatures. To investigate the effect of temperature on the degradation rate of lactic acid, *L. buchneri* (strains LMG 6892^T, PW01, and PW07) and *L. parabuchneri* were incubated for 50 days in basic MRS-MOD medium with lactic acid (45 mM), at 15, 20, 25, 30, and 37°C. The disappearance of lactic acid, product formation, the pH, and the optical density of the cultures were monitored over time. The protein concentrations of the cultures were estimated on the basis of a calibration curve of protein content versus optical density of a culture grown at 30°C (data not shown). The dynamics of growth and the stoichiometry of the lactic acid degradation for the different cultures were similar to those depicted for *L. buchneri* (LMG 6892^T) in Fig. 1. Between days 10 and 50 a linear decrease in lactic acid concentration in the culture medium could be observed, and the optical density during this period remained almost constant. The rate of lactic acid degradation was calculated for this period for all cultures. The rate of lactic acid degradation (in millimoles per day per gram of protein) varied between the different strains. Furthermore, the rate of lactic acid degradation also varied with temperature (Fig. 2). Lactic acid could be degraded at temperatures between 15 and 37°C by all strains. The optimum temperature for lactic acid conversion for the different strains appeared to be between 20 and 30°C (Fig. 2), except for *L. buchneri* strain (LMG 6892^T), which had the highest degradation rate at 15°C.

Anaerobic lactic acid conversion at different pH values. To investigate the influence of the pH on the lactic acid conversion and to obtain information on the moment at which the

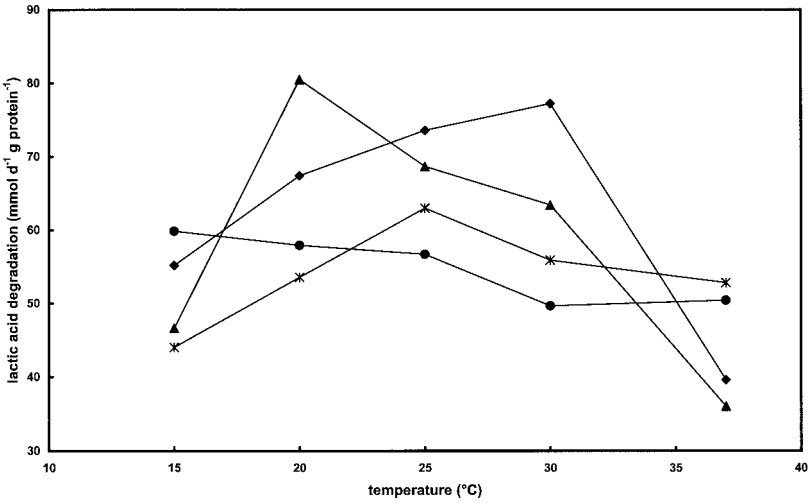

FIG. 2. Rate of lactic acid degradation by *L. buchneri* LMG 6892^T (●), PW01 (◆), and PW07 (*) and *L. parabuchneri* (▲) in MRS-MOD medium with 45 mM lactic acid at different temperatures.

process starts within treated silages, the effect of the pH on the process was studied in resting-cell suspensions of *L. buchneri* (LMG 6892^T) that were grown under glucose limitation conditions in continuous culture at three different pH values, i.e., 5.8, 4.3, and 3.8. From each steady state (pH 5.8, 4.3, and 3.8), triplicate samples were taken, and the lactic acid conversion rate was subsequently monitored over time under anoxic conditions at a pH equal to those in the continuous culture (Fig. 3). It appeared that cells grown at pH 5.8 hardly utilized any lactic acid during a time course of approximately 200 h. Cells grown at pH 4.3 showed an increased conversion rate of lactic acid, and even higher conversion rates were obtained with cells that were grown at pH 3.8 (Fig. 3). Both acetic acid and 1,2-propanediol accumulated in equimolar amounts (data not shown). In conclusion, an increasing lactic acid conversion rate was observed with decreasing pH.

Induction of the anaerobic lactic acid converting capacity. The fact that anoxically grown *L. buchneri* (LMG 6892^T) is able to utilize lactic acid only at pH values below approximately 5.8 raises the question as to whether induction of this lactic acid converting ability is also pH dependent. To answer this question cells were grown glucose limited in continuous culture at pH 3.8 and, once a steady state was obtained, triplicate samples were taken and anoxically incubated with lactic acid at pH 3.8 or 5.8, in the presence of the protein synthesis inhibitor chloramphenicol (Fig. 4A). In a second experiment, cells were taken from a glucose-limited chemostat grown at pH 5.8 and anoxically incubated with lactic acid at pH 5.8 and 3.8,

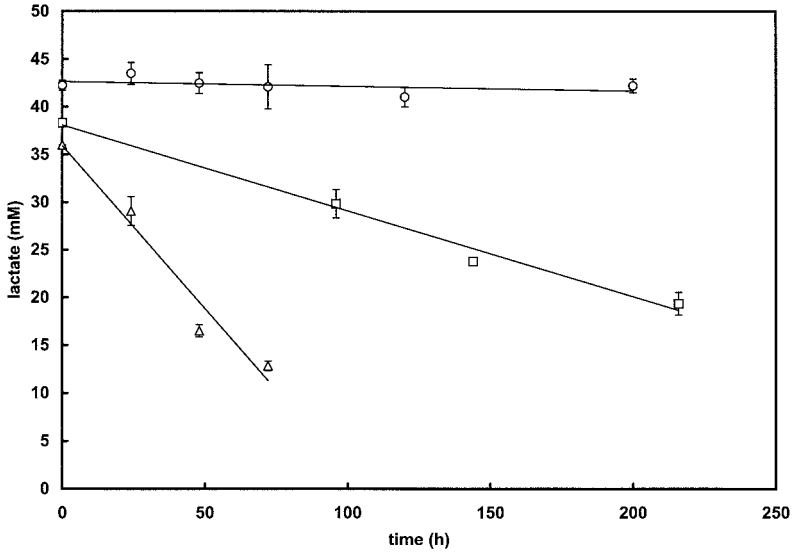

FIG. 3. Lactate utilization in time by anoxic resting-cell suspensions of *L. buchneri* pregrown in glucose-limited conditions at pH 5.8 (○), pH 4.3 (□), and pH 3.8 (△). The data shown data are mean values of triplicate incubations, and the standard errors of the means are shown as error bars.

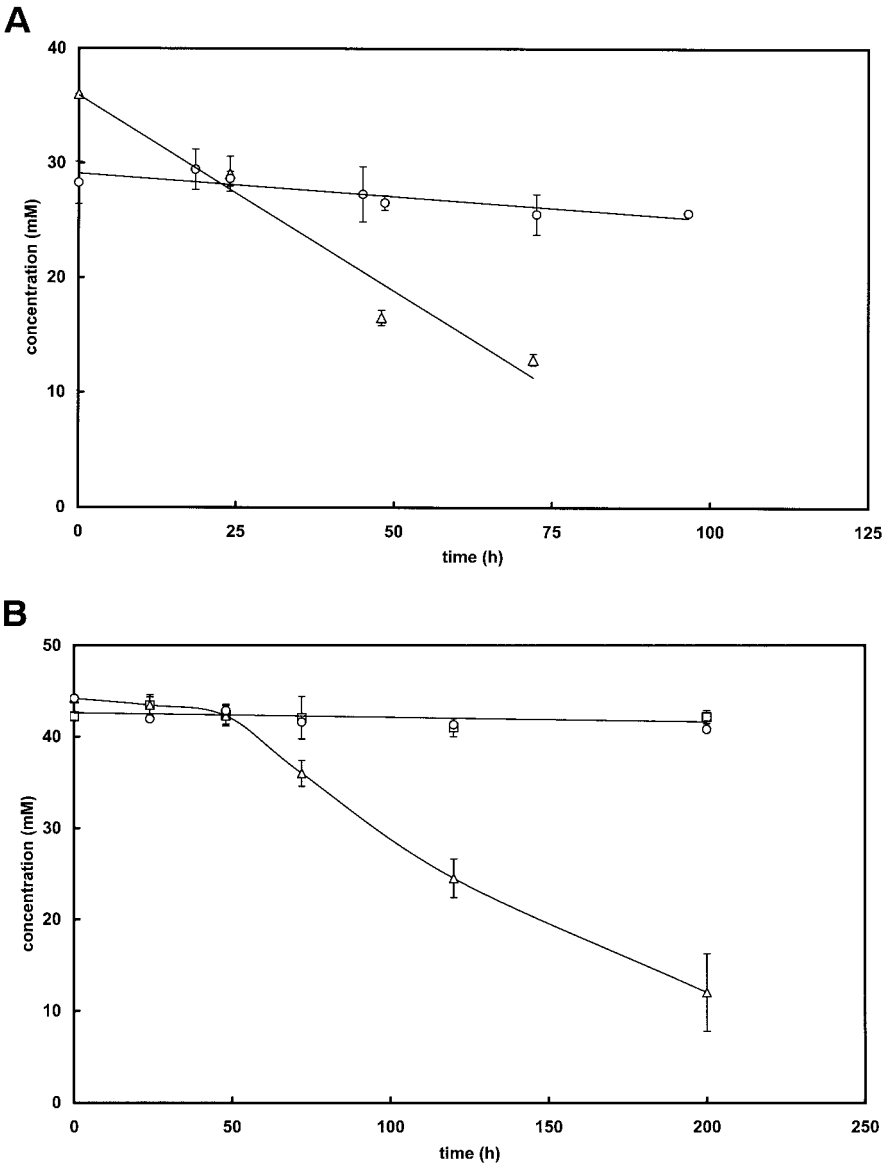

FIG. 4. (A) Anaerobic lactic acid conversion at pH 3.8 (△) and pH 5.8 (○), in the presence of the protein synthesis inhibitor chloramphenicol, by resting cells of *L. buchneri* that were pregrown in glucose-limited conditions at pH 3.8. (B) Anaerobic lactic acid conversion at pH 5.8 (○) and pH 3.8 (□) in the presence of chloramphenicol and at pH 3.8 in the absence of chloramphenicol (△) by resting cells of *L. buchneri* that were pregrown in glucose-limited conditions at pH 5.8. The data shown are mean values of triplicate incubations, with the standard errors of the means shown as error bars.

both in the absence and in the presence of chloramphenicol (Fig. 4B). *L. buchneri* cells pregrown at pH 3.8 (Fig. 4A) showed a rapid lactic acid utilization at pH 3.8 and a much lower rate of conversion at pH 5.8. In accordance with the experiments described above, the conversion of lactic acid yielded equimolar amounts of acetic acid and 1,2-propanediol (data not shown). In the presence of the protein synthesis inhibitor chloramphenicol, *L. buchneri* cells pregrown at pH 5.8 (Fig. 4B) showed no lactic acid utilization in cell suspensions maintained at pH 5.8 or in cell suspensions incubated at pH 3.8. However, in the absence of chloramphenicol lactic acid utilization commenced after approximately 50 h in those suspensions which were kept at pH 3.8 (Fig. 4B). These results show that the ability to convert lactic acid anoxically into acetic acid and 1,2-propanediol is only expressed at pH values well below 5.8.

Propanediol-dependent NADH-linked oxidoreductase activities in cells of *L. buchneri*. If the anaerobic degradation of lactic acid to acetic acid is indeed coupled to the reduction of lactic acid into 1,2-propanediol as presented above, this reduction could proceed via lactaldehyde as an intermediate. To support this hypothesis, the activities of an NADH-linked oxidoreductase that catalyzes the conversion of lactaldehyde into 1,2-propanediol were measured. *L. buchneri* (LMG 6892^T) cells pregrown under glucose-limited conditions in the presence of lactate at pH 5.8 indeed possessed high activities of this

enzyme of 534 nmol min^{-1} mg of $protein^{-1}$. Cells pregrown at pH 3.8 possessed even higher activities of 788 nmol min^{-1} mg of $protein^{-1}$.

DISCUSSION

Bacterial inoculants are a popular means to increase the quality of preserved plant materials and to enhance the aerobic stability of silages. Thus far, most commercial silage inoculants contain homofermentative or facultatively heterofermentative lactic acid bacteria (e.g., *Enterococcus* spp., *Pediococcus* spp., and *Lactobacillus plantarum*). These lactic acid bacteria have a positive effect on the extent and rate of lactic acid production in the silage, thus stimulating a rapid drop in silage pH and suppressing the growth of clostridia and other undesired anaerobic organisms in silage. However, these lactic acid bacteria sometimes impair the silage aerobic stability (27). This is probably due to the fact that yeasts, which generally cause the onset of aerobic silage spoilage, are inhibited more by acetic and propionic acid than by lactic acid (20, 27).

Recently, silage studies with whole crop maize, using the obligately heterofermentative lactic acid bacterium *L. buchneri* as an inoculant, showed a 20-fold increase in the aerobic stability of the silage, which increased from approximately 40 h for nontreated silages to more than 790 h for the inoculated silages (13). Unexpectedly, it was demonstrated that in these treated silages the lactic acid concentration was significantly lower and the acetic acid concentration was significantly higher than with nontreated silage. It was suggested that this could be due to the capacity of *L. buchneri* to degrade lactic acid to acetic acid under anoxic conditions.

So far the anoxic degradation of lactic acid to acetic acid without an external electron acceptor has only been described for *L. bifermentans. L. bifermentans* produces hydrogen gas to get rid of its excess of reducing equivalents (16). However, the present study shows that *L. buchneri* and *L. parabuchneri* do not produce hydrogen gas during lactic acid degradation. Instead, they produce large quantities of 1,2-propanediol.

The formation of 1,3-propanediol under anoxic conditions by lactic acid bacteria has been shown before. Veiga da Cunha and Foster (26) reported the reduction of glycerol to 1,3-propanediol, coupled to the oxidation of lactate to acetate via pyruvate, by *L. brevis* B22 and *L. buchneri* B190. Anaerobic cultures of *Lactobacillus reuteri* grown on a mixture of maltose and glycerol were also shown to produce lactate, acetate, ethanol, and 1,3-propanediol (12).

However, production of 1,2-propanediol from sugars has only been found for some non-lactic acid bacteria such as *Escherichia coli* (6), *Clostridium sphenoides* DSM 614 (24), and *Thermoanaerobacterium thermosaccharolyticum* (7). Biesterveld and colleagues (2, 3) demonstrated the formation of traces of 1,2-propanediol by *Bacteroides xylanolyticus* X5-1 during growth on xylose, whereas significant amounts of 1,2-propanediol were only produced in the presence of acetol as an external electron acceptor during growth on xylose.

The pathway of lactic acid formation from 1,2-propanediol has been elucidated for *E. coli* (9). In this is process, 2 mol of NAD^+ is converted to NADH + H^+ per mol of lactic acid formed (9, 28). From the detailed pathways for mixed acid fermentation of sugars by lactic acid bacteria, it is expected that for each mole of lactic acid that is degraded to acetic acid, 2 mol of NAD^+ is converted to NADH + H^+ (8). Based on this knowledge and on the results we obtained in the present study, we propose a novel pathway of anoxic lactic acid degradation by *L. buchneri* and relatives (Fig. 5) in which 2 mol of lactic acid is degraded to 1 mol of acetic acid and 1 mol of 1,2-propanediol.

The presence of NAD-linked 1,2-propanediol-dependent oxidoreductase activity within cells of *L. buchneri* suggests that during the anaerobic conversion of two molecules of lactic acid, one lactic acid molecule is indeed reduced via lactaldehyde to 1,2-propanediol. Simultaneous anaerobic oxidation of another molecule of lactate into acetate could provide the required amount of hydrogen equivalents in the form of NADH for the reduction of lactate to one molecule of 1,2-propanediol. However, to conclude unambiguously that this pathway is used by lactic acid utilizing cells of *L. buchneri*, further evidence is needed based on the detection of intermediates and the activities of all enzymes involved in this pathway. Furthermore, it is not clear yet which enzymes, necessary for the anaerobic degradation of lactic acid, are lacking at pH 5.8 and are only induced at low pH. It appears not to be NADH-linked 1,2-propanediol-dependent oxidoreductase, because this enzyme is present in cells grown at pH 5.8 or 3.8, although cells grown at pH 5.8 possess a 30% lower enzyme activity than cells grown at pH 3.8.

In the proposed pathway, the degradation of 2 mol of lactic acid yields 1 mol of ATP. Yet, in batch culture studies the conversion of lactic acid did not result in significant growth of *L. buchneri*. This could point toward an alternative function of the lactic acid degradation process, especially in view of the facts that the ability of *L. buchneri* to degrade lactic acid is strongly influenced by the pH and that acidic conditions are needed to induce the lactic acid degradation. This response may in fact represent a protective mechanism against surrounding low pH. In acidic environments, many organic acids occur in undissociated form. It is known that undissociated organic acids readily penetrate cell membranes and then accumulate within the cytoplasm of the cells, thereby causing the loss of viability and cell destruction (4, 10, 17, 18). Therefore, degradation of lactic acid into an alcohol and a fatty acid with a higher pK_a may have survival value by decreasing the concentration of undissociated acids. Our observation in previous silage studies that the number of lactobacilli in silages inoculated with *L. buchneri* only decreased very slowly in contrast to uninoculated silages also indicates a positive effect of lactic acid degradation on cell viability (13). Even in maize silages that were stored for 2 years, relatively high numbers (6 log CFU g^{-1}) of lactobacilli were still present, while in the uninoculated silages no lactobacilli could be detected anymore (S. J. W. H. Oude Elferink, unpublished data).

Protective mechanisms against acidic environments with a fermentation product shift to less-acidic compounds have been implicated previously. Tsau et al. (25) reported that *L. plantarum* accumulated pyruvic acid at neutral pH values and that this strain shifted its fermentation at lower environmental pH values to the production of acetoin, a pH-neutral compound. A similar response was described for a *Clostridium acetobutylicum* (14). It was demonstrated that if this *Clostridium* sp. was grown in glucose-limited conditions at a neutral pH it pro-

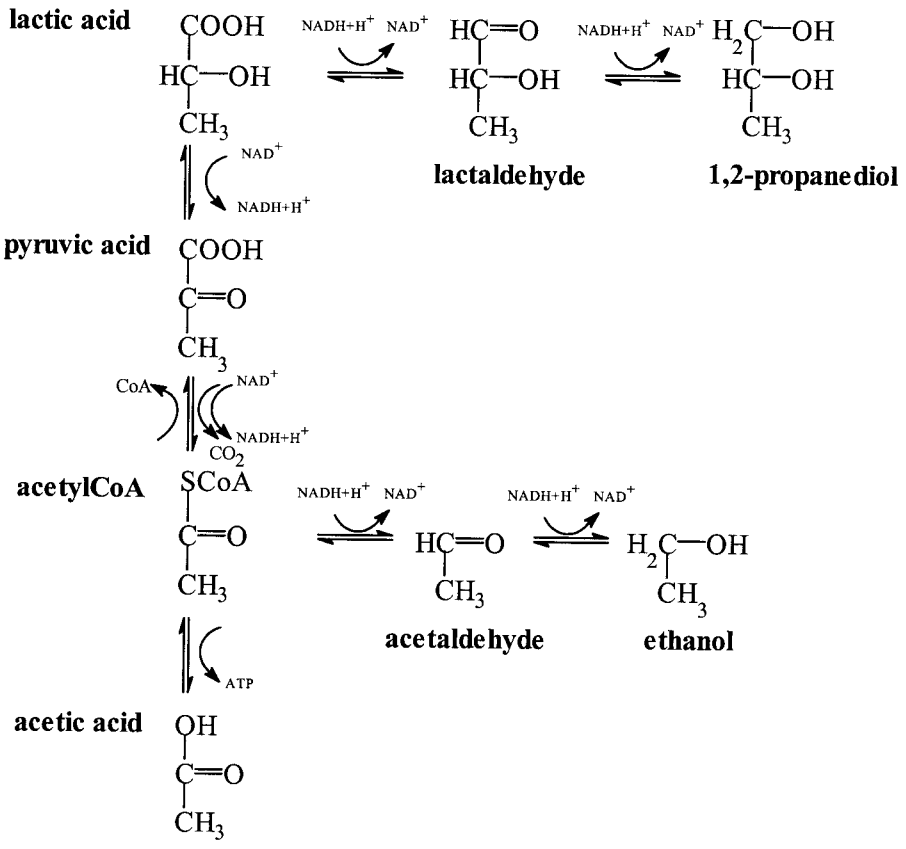

FIG. 5. Proposed pathway for anaerobic degradation of lactic acid by *L. buchneri* into equimolar amounts of 1,2-propanediol and acetic acid and trace amounts of ethanol.

duced acetate, butyrate, carbon dioxide, and hydrogen (1, 14). However, at pH values of <5.0, the organism produced the pH-neutral components acetone and butanol. Additions of butyric acid to the growth medium at pH 4.3 resulted in a shift from acid to solvent production, probably since butyric acid entered the cells by diffusion at the low pH values. In the case of *L. buchneri*, lactic acid may also freely enter the cells at low pH values. This results in a decrease of the internal pH. As a consequence, the bacteria probably need to expel protons by ATP hydrolysis in order to maintain a proton motive force as stated by Ten Brink and Konings (22). Maintaining a proton motive force at low pH is an energy-requiring process which may explain the "marginal growth" of *L. buchneri* with lactate as the substrate, as shown in our experiments.

In summary, we have shown that *L. buchneri* and some close relatives are capable of converting lactic acid into equimolar amounts of 1,2-propanediol and acetic acid and small amounts of ethanol under anoxic conditions. In addition, the lactate-converting ability is strongly influenced by the pH, and acidic conditions are even needed to induce this lactic-acid-converting capacity. Thus, the anaerobic conversion of lactic acid within silages that are inoculated with *L. buchneri* probably starts after initial fermentation of the water-soluble carbohydrates, which leads to the acidification of the environment. Once the environmental conditions are acidic, anaerobic lactic acid conversion by *L. buchneri* will start. Based on the batch culture studies presented here, it can be concluded that the lactic acid conversion rate will be influenced by the temperature of the silage, the number of *L. buchneri* or relatives present, and the strain used.

Surprisingly, 1-propanol and not 1,2-propanediol was measured as a dominant fermentation product in the *L. buchneri*-treated maize silages described by Driehuis et al. (13). This suggests that 1,2-propanediol is further degraded within silages. The fact that *L. buchneri* is unable to degrade 1,2-propanediol in pure cultures or in the heterogeneous silage habitat strongly suggests that other bacteria are involved in the further degradation of 1,2-propanediol. Recently, anaerobic 1,2-propanediol-degrading bacteria have been isolated from silages inoculated with *L. buchneri*. There are indications that 1,2-propanediol is indeed anaerobically degraded by anaerobic bacteria that are distinctly different from *L. buchneri*. These new isolates produce 1-propanol as a major fermentation product from 1,2-propanediol (F. Faber, unpublished data).

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