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Isolation and Characterization of an Anaerobic Ruminal Bacterium Capable of Degrading Hydrolyzable Tannins

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An anaerobic diplococcoid bacterium able to degrade hydrolyzable tannins was isolated from the ruminal fluid of a goat fed desmodium (*Desmodium ovalifolium*), a tropical legume which contains levels as high as 17% condensed tannins. This strain grew under anaerobic conditions in the presence of up to 30 g of tannic acid per liter and tolerated a range of phenolic monomers, including gallic, ferulic, and *p*-coumaric acids. The predominant fermentation product from tannic acid breakdown was pyrogallol, as detected by high-performance liquid chromatography and mass spectrometry. Tannic acid degradation was dependent on the presence of a sugar such as glucose, fructose, arabinose, sucrose, galactose, cellobiose, or soluble starch as an added carbon and energy source. The strain also demonstrated resistance to condensed tannins up to a level of 4 g/liter.

Tannins are water-soluble polyphenolics which precipitate proteins from solution. Condensed tannins (proanthocyanidins), hydrolyzable tannins (gallotannins, ellagitannins, and taragallotannins), and phenolic acids are common components of forages and inhibit growth of many microorganisms. The condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) bind to cell coat polymers and inhibit cell-associated proteolytic activity in *Butyrivibrio fibrisolvens* and *Streptococcus bovis* by 48 and 92%, respectively, at 25 mg of the condensed tannin per liter (20). Activity of the extracellular endoglucanase of *Fibrobacter succinogenes* S85 is decreased by 25 mg of condensed tannins from bird's-foot trefoil (*Lotus corniculatus* L.) per liter. Total inhibition of both extracellular and cell-associated endoglucanase activity occurs at 400 mg/liter (2). Carob (*Ceratonia siliqua*) pod extract completely stops growth of *Cellvibrio fulvus* and *Clostridium cellulosolvens* at 15 mg/liter, *Sporocytophaga myxococcoides* at 45 mg/liter, and *Bacillus subtilis* at 75 mg/liter (18). The same bacteria are unable to grow in tannic acid at concentrations of 12, 10, 45, and 30 mg/liter (18). Ruminal bacteria are generally more sensitive to ferulic acid (8) and *p*-coumaric acid (4, 8, 16) than to the other phenolic monomers. The most important cellulolytic ruminal bacteria are unable to grow in concentrations of ferulic or *p*-coumaric acids exceeding 5 mM (16, 25). Glucose uptake by *Prevotella ruminicola* is most strongly inhibited by vanillin and *p*-coumaric acid (24). The relative abilities of various microorganisms to tolerate phenolic compounds differ (2, 20, 31).

Recent studies have focused on the degradation of tannin-protein complexes by microorganisms. A *Streptococcus* species capable of degrading tannic acid-protein complexes has been isolated from the cecum of koalas (28), and enterobacteria that degrade hydrolyzable tannin-protein complexes have been isolated from the koala alimentary tract (27). More recently, Brooker et al. (5) isolated *Streptococcus caprinus*, which gave zones of clearing on tannic acid-protein agar medium. This organism was isolated from the ruminal contents of feral goats browsing tannin-rich *Acacia* species.

In this communication, we report the isolation and characterization of a ruminal bacterium that can degrade hydrolyzable tannins, metabolize a range of phenolic monomers, and tolerate high levels of condensed tannins. Ruminal fluid was obtained from a goat fed a diet containing desmodium (*Desmodium ovalifolium*), a tropical legume which contains up to 17% condensed tannins as measured by the method of Hagerman (12, 15).

MATERIALS AND METHODS

Chemicals. Tannic acid (ACS reagent), *p*-coumaric acid, ferulic acid, pyrogallol, gallate, and phloroglucinol were purchased from Sigma Chemical Co., St. Louis, Mo. Spray-dried quebracho (*Loxopterygium* spp.) was purchased from Trask Chemical Co., Marietta, Ga. Reagents used, including acetic, propionic, and butyric acids, were analytical grade.

Condensed-tannin purification. Purification of the condensed tannin was based on the method of Asquith and Butler (1), modified by Hagerman and Butler (15). A 1-g portion of quebracho was suspended in 10 ml of 80% ethanol and applied to a Sephadex LH-20 column equilibrated with 80% ethanol. The column was washed with 95% ethanol until the eluate was clear and the A_{280} approached zero. Condensed tannins were eluted with 50% aqueous acetone, and the acetone was then evaporated. The aqueous sample then was extracted three times with an equal volume of ethyl acetate, the upper phase was discarded, and traces of ethyl acetate were subsequently removed by rotary evaporation. The aqueous fraction was lyophilized and stored in a desiccator in the dark at −20°C. The yield of condensed tannins from this purification was approximately 5 to 6% of the initial dry matter.

Culture technique and media. The anaerobic culture techniques of Hungate (19) with modifications (6) were used for all incubations. Rumen fluid medium (7), which was used for most incubations, had either 0.2% glucose or 0.3% cellobiose added as the carbon and energy source. After the medium had been autoclaved, filter-sterilized carbohydrates were added. Ruminal fluid for the medium was from a nonlactating cow fed medium-quality grass hay. The ruminal fluid was clarified aerobically by centrifugation at 20,000 × *g* for 20 min at 4°C.

Rum10 medium (23), which was used for initial transfers of the goat ruminal fluid, contained (per 100 ml) 0.3 g of Trypticase, 0.2 g of yeast extract, 0.1 ml of 0.1% aqueous resazurin, 7.5 ml of mineral solution 1 (6 g of K_2HPO_4 per liter), 7.5 ml of mineral solution 2 [6 g of KH_2PO_4 per liter, 12 g of $(NH_4)_2SO_4$ per liter, 12 g of NaCl per liter, 2.5 g of $MgSO_4$ per liter, 1.2 g of $CaCl_2$ per liter], 0.31 ml of volatile fatty acid mixture (17 ml of acetic acid, 6 ml of propionic acid, 4 ml of *n*-butyric acid, 1 ml each of isobutyric, *n*-valeric, and isovaleric acids, 1 g of phenylacetic acid), 1 ml of $FeSO_4 \cdot 7H_2O$ solution (0.01%), 1 ml of $CoCl_2 \cdot 6H_2O$ (0.01%), 0.025 g of cysteine hydrochloride, 0.025 g of sodium sulfide, and 5 ml of Na_2CO_3 solution (8%). Cellobiose (0.3%, wt/vol) was included as the energy source.

Basal medium (30), which was used to measure cell growth with ammonium as

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the only added nitrogen source, consisted of 4 ml of mineral solution 1, 4 ml of mineral solution 2, 1 ml of vitamin solution (0.2 g of pyridoxamine HCl per liter, 0.2 g of riboflavin per liter, 0.2 g of thiamine HCl per liter, 0.2 g of nicotinamide per liter, 0.2 g of Ca-D-pantothenate per liter, 0.1 g of lipoic acid per liter, 0.01 g of *p*-aminobenzoic acid per liter, 0.005 g of folic acid per liter, 0.005 g of biotin per liter, 0.005 g of cobalamin per liter) 1 ml of Pfennig's micromineral solution (32), 0.3 g of glucose, 87 ml of distilled water, 0.025 g of cysteine hydrochloride, and 0.025 g of sodium sulfide.

Medium was transferred in 8.7-ml quantities to butyl rubber-stoppered roll tubes purged with oxygen-free CO_2. The tubes were sterilized by autoclaving at 120°C for 15 min. The pH after autoclaving was between 6.5 and 6.7. Incubations were performed anaerobically in batch culture at 39°C.

When tannic acid or quebracho was included in the medium, it was added after autoclaving as a prereduced, filter-sterilized solution. Unpurified quebracho was used in medium for the maintenance of cultures, but purified quebracho was used to evaluate the ability of the bacterium to degrade or tolerate condensed tannins.

Microorganism isolation. As a source of inoculum, ruminal fluid was obtained from a fistulated goat located at Centro Internacional de Agricultura Tropical, Cali, Colombia, and fed forages in addition to significant amounts of desmodium. To meet U.S. importation requirements, the organisms from the ruminal fluid were passed through two successive transfers in Rum10 medium without tannins before being inoculated into rumen fluid medium with 0.3% (wt/vol) cellobiose as the energy source and either tannic acid or quebracho was added at a concentration of 1 g/liter. Samples were hand carried to Cornell University and arrived within 24 h of shipment.

Bacteria were subcultured every 2 days for 6 days in rumen fluid medium containing either tannic acid or unpurified quebracho (1 g/liter) and then plated on rumen fluid medium with agar added at 3% (wt/vol) of the total volume and overlaid with 10% (wt/vol) tannic acid or purified quebracho. Plating was done in an anaerobic chamber (Coy, Ann Arbor, Mich.) under 94% CO_2–6% H_2. Isolated colonies were selected and inoculated into rumen fluid medium with added tannic acid or unpurified quebracho. Cultures were checked routinely for purity by microscopic examination. Weekly transfers were necessary for survival of cultures, and, for long-term storage, cultures were frozen in 20% glycerol and stored at −80°C.

Growth studies. For all growth studies, 0.3 ml of mid-exponential-phase cultures grown in rumen fluid medium was inoculated into 9.7 ml of the appropriate medium. Gram stains and sodium monensin susceptibility were used to determine the type of cell wall of the isolated strain. For the sodium monensin trial, the bacteria were grown in 0, 1, 5, or 10 μM sodium monensin in rumen fluid medium (30). To study the effects of different phenolics on the metabolism of the isolated strain, cultures were inoculated into rumen fluid medium minus glucose (negative control), rumen fluid medium with glucose (positive control), and rumen fluid medium with glucose and the compound of interest (phenolic monomer, tannic acid, or purified quebracho). Phenolic monomers to be tested were each dissolved in phosphate buffer (pH 6.8) (13), filter sterilized, and added to the medium to yield final concentrations of 1, 5, 10, 20, 30, 40, or 50 mM in the medium. Tannic acid and purified and unpurified quebracho were added at 1, 2, 3, 4, or 8 g/liter. In addition, the ability of the bacterium to grow in medium containing 15, 20, 30, 40, 50, 60, or 70 g of tannic acid per liter was evaluated. The pH was not altered by addition of these compounds. The effect on growth rate was monitored by measuring changes in optical density caused by bacterial growth or by measuring gas production (29). The gas production method was used to quantify bacterial growth, because the presence of condensed tannins interfered with optical density measurements. Modifications to the published method included the use of 20-ml serum bottles with a final liquid volume of 10 ml. Cell growth in tubes was monitored by observing the increase in optical density at 600 nm against a blank of uninoculated medium. Readings were taken in a Spectronic 601 spectrophotometer (Milton Roy, Rochester, N.Y.). Generation times were calculated from the slope of the linear portion of the growth curve in semilogarithmic plots, and the lag was calculated by the logistic model (33, 35). Optical density and gas production measurements were made in triplicate.

Phase-contrast microscopy was used to monitor changes in cellular morphology as tannin concentrations were increased.

The isolated strain was tested for its ability to utilize different carbohydrates, phenolic monomers, tannic acid, and purified quebracho as sole energy sources. Rumen fluid medium without glucose (negative control) and rumen fluid medium plus 5 mM *p*-coumaric acid, ferulic acid, gallic acid, pyrogallol, or phloroglucinol, 1 g of tannic acid or purified quebracho per liter, or 0.3% (wt/vol) different carbohydrates were inoculated with mid-exponential-phase cultures of the isolate. The isolate also was grown in basal medium to test its ability to use ammonium ions as the sole nitrogen source. The inoculum used in these experiments was from cultures grown on rumen fluid medium with 0.3% glucose and without added tannins or phenolic monomers.

To determine the effects of pH on growth, the pH of the rumen fluid medium was adjusted after autoclaving by adding concentrated HCl or NaOH to tubes. Tested pH values were between 4.0 and 8.0. Tubes were subsequently inoculated with 0.3 ml of mid-log-phase cells, and increases in optical density were monitored.

End product analysis. Metabolism of phenolic monomers and tannic acid was measured by high-pressure liquid chromatography (HPLC), gas chromatography-mass spectrometry, and thin-layer chromatography. After sample centrifugation at 6,000 × *g* for 10 min, 1 ml of the supernatant was collected and frozen at −20°C until HPLC analysis. HPLC was performed on a modified Pico-Tag system (Waters, Milford, Mass.) equipped with dual pumps, a variable-wavelength UV detector, a system interface module controller, and a computer with Maxima 3.3 software. Separations were carried out on a column (15 cm by 4.6 mm: 5-μm particles packed with Supelcosil LC-8-DB; Supelco, Bellefonte, Pa.). The linear gradient of glacial acetic acid-water (25:975 [vol/vol]; solvent A) and methanol (solvent B) (26) was pumped at a rate of 1 ml/min at 40°C. Sample components were detected at 280 nm, and injection volumes were 5 μl. Chromatographic peaks were identified by comparison with authentic samples.

Isolated end products and nonvolatile components were prepared by extracting the supernatant of cells grown with the respective compounds with an equal volume of ethyl acetate and drying the extracts under CO_2. For gas chromatography analysis, trimethylsilyl derivatives were prepared and chromatographed on a 5890 series 2 gas chromatograph (Hewlett Packard Co., Wilmington, Del.). Separations were carried out on an HP-5 column cross-linked with 5% phenyl methyl siloxane (30 m long by 0.25 mm internal diameter, and 0.25 μm film thickness; Hewlett-Packard Co.). The temperature gradient was set from 40 to 250°C. Chromatographic peaks were identified by comparison with the gas chromatography-mass spectrometry internal library or with authentic samples.

Thin-layer chromatography was used to demonstrate disappearance of tannic acid from the culture medium over time. Samples were taken hourly and prepared by centrifugation at 6,000 × *g* for 10 min. Silica gel plates were run by a modification of the method of Ferry and Larson (9). Our solvent was pentane-saturated acetonitrile and toluene (2:1, vol/vol) made up to 75 ml with 1.25 ml of formic acid to reduce trailing. Culture aliquots (10 μl) were applied, and the chromatogram was developed to a solvent front of approximately 10.5 cm. Spots were observed with short-wavelength UV, and chromatograms were also stained with iodine vapor to visualize pyrogallol better. Retention times were compared with those of authentic standards.

For volatile fatty acid and lactate profiles from cultures grown with tannic acid, 1-ml aliquots of culture medium were taken during the early, mid-, and late logarithmic phases of the fermentation and mixed with 50 mg of polyvinylpyrrolidone to remove residual tannic acid. Samples were vortexed and allowed to stand at room temperature for 60 min, after which they were centrifuged at 6,000 × *g* for 10 min. Aliquots of 40 μl were analyzed on a Rainin HPLC (Macrode, Woburn, Mass.) with a Knauer differential refractometer, a Varian model 9176 recorder, and a Bio-Rad Aminex HPX87H fast acid column (10 cm by 7.8 mm). The solvent was 1.5 mM H_2SO_4 at a flow rate of 2.4 ml/min. A mixture of acetic, propionic, isobutyric, butyric, fumaric, and lactic acids was included as a calibration standard in all analyses. Glucose was used as a separate standard for calibration.

Carbon dioxide was determined with a model 550 thermal conductivity gas chromatograph (Gow Mac Instrument Co., Bound Brook, N.J.), operated under the following conditions: carrier gas, He; flow rate, 50 ml/min; column, copper (1 m by 2 mm [inner diameter]) packed with 120/140 Carbosieve S (Supelco); column temperature, 100°C.

L-Lactate determination. The supernatant of cells grown in the absence of tannins was used to determine which lactate isomer was present at the end of the fermentation. Aliquots (50 μl) of the culture medium were mixed with NAD^+ (10 mmol) in 3 ml of glycine-hydrazine buffer (pH 9.5). The difference in the A_{340} of the mixture with either L-lactate dehydrogenase or D-lactate dehydrogenase was measured (14). Tannin-free medium was used to avoid interferences of tannic acid and its metabolites with lactic acid dehydrogenase in the enzymatic assay. Tannic acid metabolites also interfered with the UV A_{340}.

Statistical analysis. Differences in lag time, glucose disappearance, and lactate production as a result of levels of tannin added to the growth medium were determined by one-way analysis of variance (SigmaStat; Jandel Scientific, San Rafael, Calif.). Data are presented as the means ± standard deviations. If the overall analysis of variance indicated that there were significant differences among treatments ($P < 0.05$), the Student-Newman-Keuls test was used to determine differences among the means.

RESULTS

Characteristics of the new isolate. A bacterium that showed unusually high resistance to both condensed and hydrolyzable tannins was isolated. Microscopic examination of purified colonies showed homogeneous diplococci. Cells were 1.8 μm long by 0.8 μm wide. They stained gram variable but were unable to grow in the presence of 1 μM sodium monensin, suggesting that they have gram-positive cell walls (30). The isolate grew on glucose, cellobiose, lactose, fructose, sucrose, galactose, arabinose, and soluble starch. No growth occurred on cellulose or glycerol.

The isolate grew in medium that contained concentrations

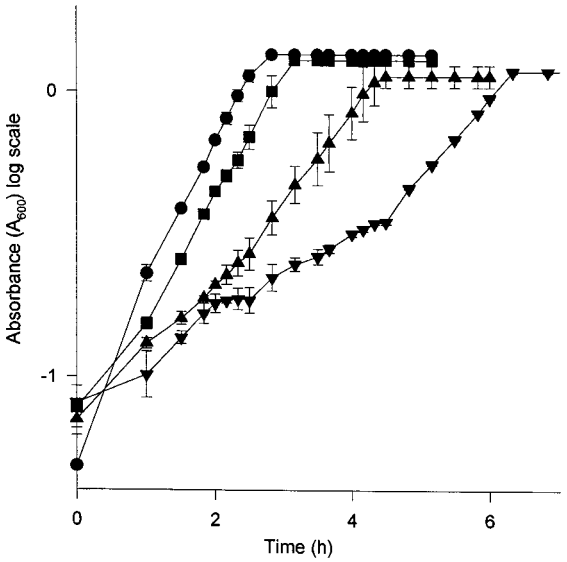

FIG. 1. Growth curves of our isolate in increasing concentrations of tannic acid. Symbols: ●, 0 g/liter; ■, 1 g/liter; ▲, 2 g/liter; ▼, 3 g/liter. Data are means of triplicate determinations.

of *p*-coumaric acid, ferulic acid, or gallic acid up to 50 mM, the highest concentration tested. The bacterium could tolerate concentrations of pyrogallol as high as 40 mM and phloroglucinol up to 30 mM. Also, the isolate grew in medium with tannic acid concentrations as high as 70 g/liter. However, when the tannic acid concentration exceeded 30 g/liter, not all of the tannic acid was soluble. Formation of protein-tannin complexes with ingredients in the rumen fluid medium did not decrease the biological activity of the tannins, because the bacterium was able to grow in the basal medium at concentrations up to 30 g/liter. The basal medium contained ammonium as the only nitrogen source and no yeast extract or Trypticase. Growth curves of pure cultures of the isolate in rumen fluid medium with four concentrations of tannic acid are shown in Fig. 1. Although inclusion of tannic acid in the medium did not affect the growth rate, the differences in lag time were significant ($P < 0.05$) as the concentration of tannic acid in the medium was increased. Average lag times were 82.4 ± 0.5, 105.4 ± 2.8, 159.9 ± 5.1, and 249.0 ± 0.5 min for 0, 1, 2, and 3 g/liter, respectively. The bacterium could not grow with tannic acid as the sole carbon source. However, cells did grow either with or without tannic acid when soluble sugars, such as sucrose, fructose, glucose, arabinose, galactose, soluble starch, or cellobiose, were added to the medium. The optimal pH for growth was 6.5 to 6.8, and the cells did not grow below pH 5.3. The isolate grew in basal medium in which ammonium was the only nitrogen source. Phenolic monomers, tannic acid, or purified quebracho was not utilized as the sole energy source for growth after 12-day incubations.

Identification of metabolites. HPLC analysis and UV spectrometry revealed that tannic acid was degraded anaerobically within 3 to 4 h at concentrations up to 3 g/liter (Fig. 2), the highest concentration monitored. There was also a proportional increase in levels of gas produced as concentrations of tannic acid were increased (Fig. 3). Carbon dioxide was the only gas produced. Lactic acid (as measured by HPLC) accumulated in all samples as degradation proceeded and was shown by an enzyme assay with either L- or D-lactate dehydrogenase in tannin-free samples to be L-lactate. Total L-lactate

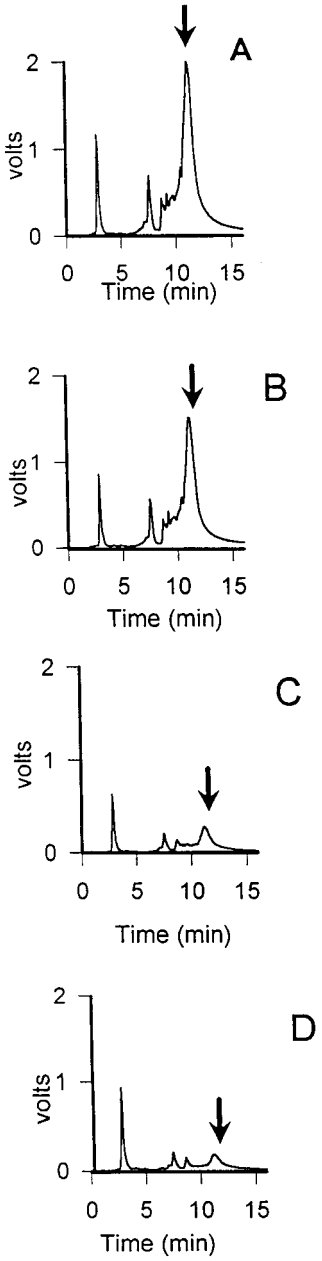

FIG. 2. HPLC chromatograms of tannic acid disappearance over time when present at an initial concentration of 2 g/liter. (A) 0.5 h; (B) 2 h; (C) 4 h; (D) 6 h. The large peak at 8.7 to 11.2 min represents tannic acid. The A_{280} of the samples was read; it does not show the appearance of the pyrogallol peak.

production did not change with increases in tannic acid concentration ($P = 0.43$). In the 1-g/liter tannic acid samples, the lactate concentration after 6 h of fermentation was 12.2 ± 0.58 mM compared with 12.1 ± 0.30 mM at the end of the fermentation in the 3-g/liter tannic acid samples and 12.7 ± 0.68 mM in the samples without added tannic acid. Glucose disappearance was similar for all treatments ($P = 0.66$), with 2.18, 2.06, and 2.12 mM glucose remaining at the end of the fermentation for the 0-, 1-, and 3-g/liter tannic acid treatments, respectively. Trace amounts of fumarate were found.

Analysis by gas chromatography-mass spectrometry confirmed the presence of pyrogallol as the predominant fermen-

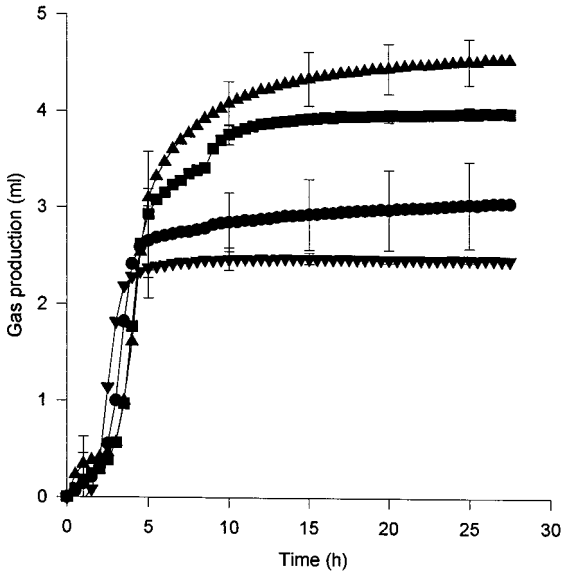

FIG. 3. Gas production by our isolate in the presence of increasing concentrations of tannic acid. Symbols: ●, 0 g/liter; ■, 1 g/liter; ▲, 2 g/liter; ▼, 3 g/liter. Data are means of triplicate determinations.

tation product of tannic acid. It is likely that the previously described increase in carbon dioxide production is associated with decarboxylation of gallate, which may be an intermediate in tannic acid breakdown to pyrogallol. The appearance of gallate was not detected by thin-layer chromatography (Fig. 4).

Changes in cellular morphology. Phase-contrast microscopic examination showed an increase in chain formation as the concentration of tannic acid in the medium was increased above 4 g/liter (Fig. 5). Clumping of cells was evident at and above 5 g/liter. Reduction in cell size became evident at 8 g/liter. Morphological changes were evident when the concentration of purified quebracho in the medium exceeded 1.5 g/liter and that of the unpurified quebracho was greater than 4 g/liter. Above those concentrations, clumping and chain formation were evident.

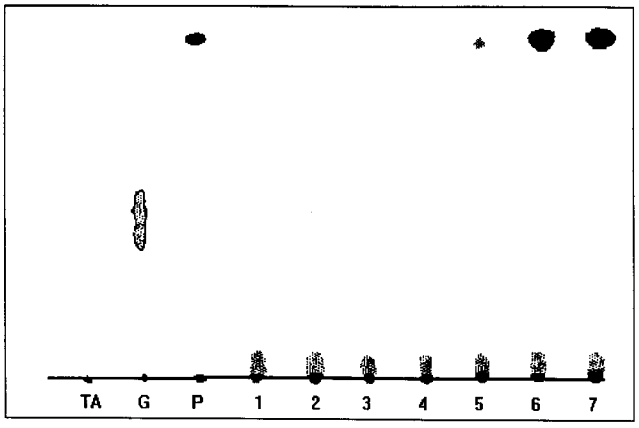

FIG. 4. Digitized copy of thin-layer chromatogram showing disappearance of tannic acid (initial concentration, 1 g/liter) and the appearance of pyrogallol over time for the 1-g/liter tannic acid sample. TA, tannic acid; G, gallate; P, pyrogallol; 1, tannic acid sample at 0 h; 2, tannic acid sample at 1 h; 3, tannic acid sample at 2 h; 4, tannic acid sample at 3 h; 5, tannic acid sample at 4 h; 6, tannic acid sample at 5 h; 7, tannic acid sample at 6 h.

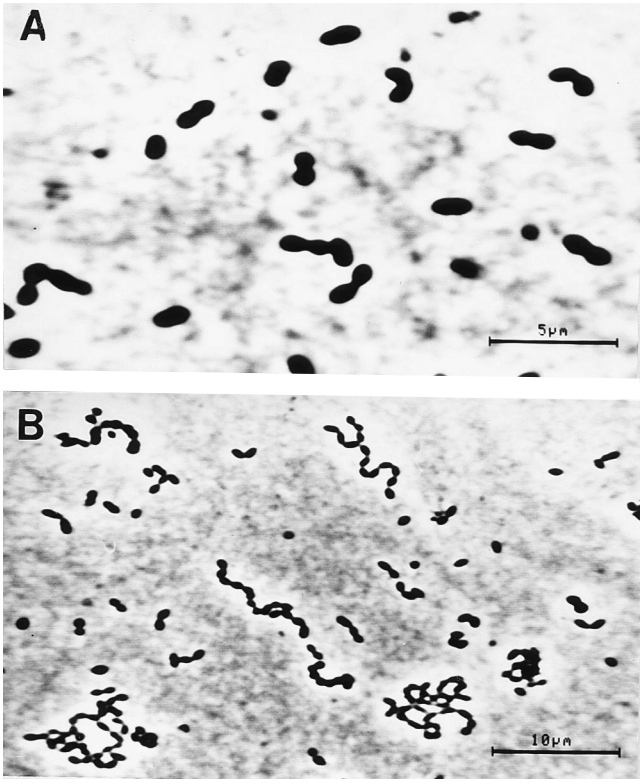

FIG. 5. Phase-contrast micrographs of tannic acid-degrading bacterium. (A) Cells in the absence of phenolic compounds. (B) Chain formation at a concentration of 8 g of tannic acid per liter.

DISCUSSION

The levels of phenolic monomers and hydrolyzable and condensed tannins used in these experiments are higher than those reported previously in the literature and are all higher than levels known to be toxic to bacteria such as *B. fibrisolvens*, *R. amylophilus*, *S. bovis* (20), and *F. succinogenes* (2). Although the specific concentrations of these phenolic compounds encountered in the rumen have not been documented, a concentration of 30 g/liter in the medium represents 15% (wt/vol) hydrolyzable tannins in the diet of a ruminant. Mirto (*Mirtus communis*), a forage plant considered to have high levels of hydrolyzable tannins, contains 4% hydrolyzable tannins on a dry-matter basis when measured as gallic acid equivalents (12). Thus, the isolate can tolerate almost four times the tannin concentration expected from a forage diet composed exclusively of high-tannin plants. The discovery of an anaerobic bacterium with the ability to degrade and/or modify monomers and hydrolyzable tannins within 3 to 4 h is novel. Tannic acid previously has been shown to be degraded by a consortium of anaerobic sludge bacteria (10).

The anaerobic degradation of tannic acid by this culture was rapid. Production of pyrogallol and not gallate (as detected by thin-layer chromatography) suggests that the intergallate depside linkages and the ester bond between gallate and glucose are both readily hydrolyzed and that gallate decarboxylation takes place rapidly (Fig. 6). Pyrogallol, the monomeric product of breakdown of tannic acid, is far less toxic than the intact hydrolyzable tannin (11); this may reflect the greater efficiency of protein binding and precipitation by the polymeric forms (17).

The isolate differs from *S. caprinus* (5) in its ability to hy-

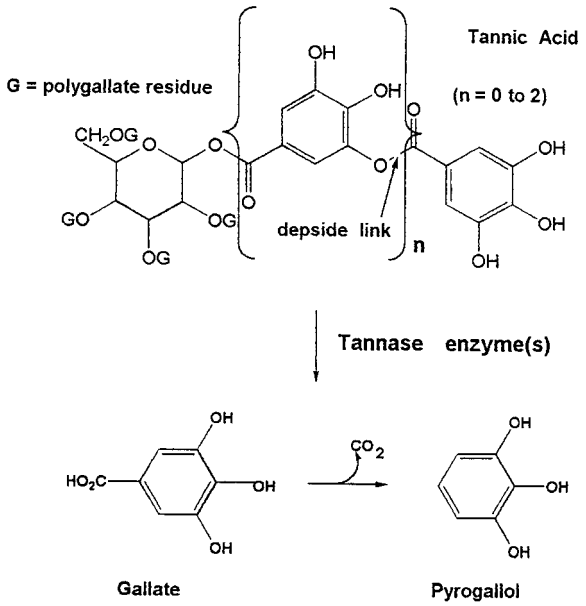

FIG. 6. Proposed pathway for hydrolyzable tannin degradation by our isolate.

drolyze tannic acid, its use of ammonium as the sole source of nitrogen, differences in fermentation end products, and the ability to tolerate high levels of tannic acid in the medium. Lactate production by this isolate remained constant when increasing amounts of tannic acid were included in the medium, whereas *S. caprinus* produced less lactate as the concentration of tannic acid in the medium increased. In addition to lactate, *S. caprinus* produced small amounts of acetate and ethanol, whereas our isolate produced lactic acid with a trace of fumarate. Both bacteria were able to grow in media containing 3% (wt/vol) tannic acid. Above this level, *S. caprinus* did not grow. Growth was evident in cultures of the new isolate at concentrations as high as 7% (wt/vol), although we must be cautious in interpreting these data, because there was some precipitation at concentrations above 3% in rumen fluid medium.

Several ruminal microorganisms with the ability to degrade phenolic monomers have been isolated. *Eubacterium oxidoreducens* is a strictly anaerobic ruminal bacterium that degrades gallate, phloroglucinol, and pyrogallol to acetate and butyrate in the presence of hydrogen and formic acid (22). *Syntrophococcus sucromutans* is another bacterium which demethoxylates various phenolic compounds (21). Tsai and Jones (34) also isolated *Streptococcus* strains and three *Coprococcus* strains from the bovine rumen that were capable of degrading up to 80% of phloroglucinol, present as the only added carbon source, within 2 days. It is clear from our data and those of Krumholtz and Bryant (22) that ruminal bacteria can detoxify hydrolyzable tannins and phenolic monomers. It is possible that these bacteria also derive energy from them through syntrophic associations in the rumen.

The degradation of tannic acid by our isolate indicates that tannin-protein complexes, once thought to be hydrolyzed only under the acidic conditions of the abomasum (3), may also be subject to microbial degradation in the rumen. Our isolate grows and alters tannic acid at pH levels higher than those required for acid hydrolysis. It is apparent that this strain, along with other recently isolated ruminal bacteria (21, 22), may play a role in the anaerobic detoxification of tannin-containing feeds in the rumen.

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