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Culture of Five Commonly Used Acid-Producing Bacteria on Banana Pulp

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A significant fraction of the bananas and plantains produced in the humid tropics are wasted due to rapid spoilage. The use of acid-producing, anaerobic fermentation may be a safe and economical way to preserve the fruit in both large- and small-scale applications. The abilities of five commonly used, acid-producing bacteria to ferment media containing ripe or green bananas were examined. Three homofermentative bacteria, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Streptococcus faecalis*, typically reduced the pH of a puree of ripe bananas from 4.8 to about 3.5 to 3.6 in 7 days at 37°C. Action on green bananas was similar, but the drop in pH was less pronounced. Very little gas evolved. *Lactobacillus fermentum* (formerly *Lactobacillus fermenti*) and *Leuconostoc mesenteroides* showed active gas evolution and a significant drop in pH. About twice as much gas evolved from ripe banana medium as from green bananas. The fermented purees were spongy, soft solids floating in a clear liquor. The color was medium to light creamy brown, and the odor was pleasant. Fermented products stored at room temperature retained good appearance and aroma throughout a 1-month storage test.

World banana production for export for the past several years has been about 6.5×10^6 metric tons of fresh fruit annually (1). Since only 15 to 20% of the bananas produced enter the world trade, the total world production must be between 35×10^6 and 40×10^6 metric tons per year. Most of this fruit is harvested at a tree-ripe stage and consumed soon after harvest. However, since the preferred state of ripeness persists for a relatively short time, a significant amount of the fruit cannot be sold or consumed and is subsequently wasted.

Whole banana fruit is composed of a moist, starchy pulp enclosed in a thick, tough peel or skin. The weight ratio of pulp to peel varies as the banana ripens and in green fruit is about 1.2 to 1.6; this changes to 2.2 to 2.4 at ripeness and to 3 or more in very overripe fruit (4).

The composition of the banana pulp also varies considerably as the fruit ripens. Dry matter content of the pulp is about 22 to 25% of the fresh weight, and almost 90% of this is carbohydrate. The carbohydrate is present as starch when the fruit is green, but as the banana passes through the ripening process, the starch is hydrolyzed into simple, soluble sugars. Sucrose, glucose, and fructose are the main sugars present, and their relative levels vary as the fruit ripens. About 2 weeks after the green fruit is picked, it reaches an overripe condition where almost all of the carbohydrate is present as sugars, the peel has been desiccated and weakened, and the fruit is subject to rapid rotting and decay.

Since bananas are produced in the tropics on a year-round basis, the systematic preservation of the fruit is seldom practiced on a large scale. However, simple techniques for preserving the palatability and nutritional quality of the fruit could greatly increase its useful life and permit its greater use as a valuable human food.

Simmonds (2) cited four methods of banana pulp preservation: canning, drying, freezing, and acid fermentation. He describes the advantages, disadvantages, and some instances in which each of these techniques has been used. In only one instance did he cite the application of acid fermentation to preserve banana fruit. This occurred in Samoa, where peeled, ripe fruit was placed in a pit lined with leaves, inoculated with bananas from a previous culture, covered with leaves and soil, and allowed to ferment. The product, called "masi fa' i," was reported to last for several years.

Simmonds (2) also cited several instances of ethanolic fermentations, but concluded that the ethanol levels that were reached in banana media with no sugar supplementation were too low to preserve the cultures.

We felt that it would be instructive to investigate further the application of facultative, acid-producing bacteria to banana pulp with the objective of developing a simple and inexpensive food preservation technique. Acid fermentations are well known, easy to carry out with simple equipment and techniques, and adaptable to a large or small scale. The technique demands little energy, and control of the fermentation is usually achieved by simple means.

In this work we sought to learn whether some commonly used food-fermenting bacteria would ferment bananas and whether they would produce conditions sufficient to preserve the fermented fruit.

No attempt was made to keep the banana cultures absolutely pure since our interest was in possible applications in areas with limited technical skill and equipment. However, simple techniques were used to assure sanitary operation and to allow repeatable product quality.

MATERIALS AND METHODS

Design of experiment. Five commonly used acid-producing bacteria were tested for their ability to ferment media consisting of pulp from green or ripe bananas. The cultures were monitored for 7 days of incubation at 37°C. The pH of the cultures and the amount of gas evolved were measured daily for each culture, and the initial and final concentrations of glucose in the media were reported. Qualities of color, odor, and texture were described for the product stored for several weeks. Uninoculated controls were included for each medium.

Microorganisms. Pure cultures of three homofermentative and two heterofermentative bacteria commonly used in acidic food fermentations were obtained from Robert Marshall (Department of Food Science and Nutrition, University of Missouri, Columbia). The homofermentative organisms used were *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Streptococcus faecalis*. Heterofermentative organisms used were *Lactobacillus fermentum* (formerly *Lactobacillus fermenti*) and *Leuconostoc mesenteroides*.

Cultures of all strains of *Lactobacillus* were maintained in 10-ml tube cultures of litmus milk medium (BBL Microbiology Systems, medium 11343) containing 5% (vol/vol) V-8 juice. The cultures were incubated for 2 to 3 days at 37°C, and growth was indicated by a pink color and curdling of the medium. *Leuconostoc* and *Streptococcus* strains were maintained in 10-ml tube cultures of liquid brain heart infusion medium (BBL Microbiology Systems, medium 11059). The *Streptococcus* organisms were incubated at 37°C and the *Leuconostoc* organisms were incubated at room temperature, about 22°C, for 2 to 3 days. Growth was indicated by cloudiness of the liquid media.

Inocula for the jar fermentors were obtained from tube cultures which were from 3 to 5 days old and had demonstrated growth. Each of the cultures could be stored at 4°C for several weeks with only a slight loss of activity.

Preparation of banana medium. Two separate bunches of bananas were used to prepare two media. The bananas were obtained at a local supermarket and were of the type normally imported into the United States: either Cavendish or Gros Michel. One bunch was predominantly green in color with firm, hard fruit. This bunch was used to prepare "green banana medium." The second bunch was yellow with large black splotches, and the fruit was quite soft. This bunch was used to prepare "ripe banana medium."

Intact, unpeeled bananas of the desired degree of ripeness were placed in a 5% (vol/vol) Chlorox solution for 10 min to cleanse and sanitize the exterior of the fruit. The fruit was then carefully peeled, avoiding contact with the pulp, and the pulp was placed in a sterile Waring blender jar and reduced to a puree. Sterile water was added in a water-to-puree ratio of 1:3 (vol/vol) to make a final puree which could be poured. If the puree was to be inoculated, the inoculum was added with thorough stirring to assure even distribution in the puree.

Preparation of jar fermentors. Fermentation vessels were 1-pint (ca. 0.473-liter) Mason jars. Each jar lid was modified by installing a 25-mm rubber stopper through the center part of the lid. The stopper had two glass tubes (outer diameter, 6 mm) passing into the gas headspace of the jar. One tube was connected to a short length of silicon rubber tubing which was sealed with a clamp at all times except when the headspace was being purged. The other tube connected the jar to a gas collector tube (100-ml volume) which was inverted in a beaker of water.

In operation, the lid of the mason jar was sealed after the medium had been added, and the headspace of the jar was vented only into the inverted, graduated gas collector tube where gas generated during the fermentation could be collected and measured.

Preparation of the apparatus for fermentation involved pouring 240 ml of control or inoculated banana puree into the jar. Inoculum volumes were 5 ml of the tube culture contents. No inoculum was added to the control jars. Each jar was sealed and purged for 10 min by flowing CO_2 gas into the short rubber tube and allowing it to bubble through the water in the beaker containing the gas collection tube. After 10 min, the short rubber tube was clamped, and the gas collection tube was filled completely with the CO_2-saturated water and inverted over the outlet of the fermentor vent tube. A water seal was maintained to keep water in the gas collection tube.

The jar and the gas collection apparatus were placed in an incubator and allowed to reach the desired incubation temperature. The gas that vented into the collection tube due to thermal expansion was removed to rezero the evolved gas volume at the operating temperature. All fermentations were performed at 37°C for 7 days.

Monitoring the fermentation. The volume of gas evolved and the pH were measured daily for each jar. The appearance and odor of each culture were also noted when the jar was opened for sampling. When samples were to be removed from a jar, the gas vent tube was clamped and the lid was removed from the vessel. CO_2 gas was bubbled through the water in the gas collection beaker to assure saturation, and a sterile pipette with an enlarged tip opening was used to remove a sample of the culture. The pH of the sample was measured with a combination electrode and Corning model 10 pH meter. The jar was resealed after the headspace had been purged with CO_2, and the gas vent tube was opened.

Initial and final contents of glucose in the culture were measured by a Yellow Springs model 23A glucose analyzer. For this measurement, the sample was diluted with distilled water to permit the removal of solids by filtration and to insure that the glucose concentration was within the range of the instrument.

When gas evolution ceased, the product was removed from the jar. One half was kept sealed in a sample jar at room temperature, and the other half was frozen. The quality of these samples was later compared.

RESULTS AND DISCUSSION

If it is assumed that 22% of fresh, moist banana pulp is carbohydrate, the concentration of carbohydrate in the banana medium was approximately 165 g/liter. The progression of the hydrolysis of starch to sugar in ripening banana pulp was measured by Stratton and Von Loesecke (3) and is shown in Fig. 1. The approximate degrees of ripeness of the bananas used in the green and ripe banana media are also indicated. The level of glucose before inoculation was 39.6 g/liter for the ripe banana medium and 16.3 g/liter for the green banana medium. This would indicate that about 25% of the carbohydrate of the ripe medium and 10% of the carbohydrate of the green medium were present as glucose.

The time course of gas evolution and pH change for each culture is shown in Fig. 2. Gas evolution is reported as the cumulative gas volume evolved per volume of initial culture.

Gas evolution in the culture with *L. bulgaricus* showed a 2-day lag time for both green and ripe media, and pH change did not begin until day 3. By day 6, the pH of the green and ripe media had dropped from initial values of 4.4 and 4.8 to final values of 3.5 and 3.4, respectively. The decrease in pH of the ripe banana medium was greater than that for the green banana medium. Although gas evolution for both cultures was quite low, slightly more gas was evolved from the ripe banana medium. Apparently, pH change and gas evolution had ceased by day 7 of incubation.

The pH values and gas evolution rates of *S. thermophilus* and *S. faecalis*, the two remaining homofermentative organisms, behaved very similarly to those of the *L. bulgaricus* culture. All cultures demonstrated a lag time of several days for gas evolution, and pH change lagged the onset of gas evolution by about 1 day. Very little gas evolved from these cultures, but a pH drop was pronounced in all but the *S. thermophilus* culture in green bananas. Indeed, in every case the cultures showed a greater pH drop and higher gas evolution for the ripe medium than for the green medium. This probably denoted a higher degree of acid production and greater utilization of the carbohydrate substrate in ripe bananas.

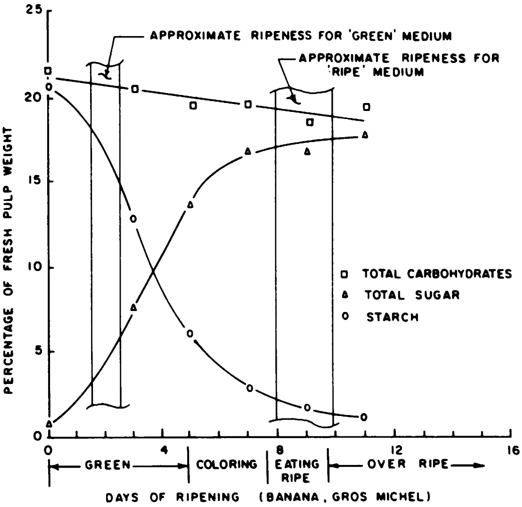

FIG. 1. *Content of total carbohydrate, sugar, and starch as a percentage of the fresh weight of banana pulp (Gros Michel). Compiled from data given by Stratton and Von Loesecke (3).*

The heterofermentative *L. fermentum* showed rapid gas evolution after day 1 and finally produced about 1.2 volumes of gas from the ripe medium and 0.5 volume of gas from the green medium after 7 days. The period of the most rapid gas evolution was between days 2 and 5, and gas production had essentially ceased after day 6. The pH of both media decreased at a rather constant rate to final values of 3.5 and 3.6 for the ripe and green media, respectively.

Fermentations by *L. mesenteroides* were similar to those with *L. fermentum* except that gas evolution started almost immediately, and the volume evolved was slightly higher in the green medium. Changes in pH were similar to those developed by the *L. fermentum* cultures.

The uninoculated controls evolved an insignificant amount of gas through the 7 days of incubation and showed only minor pH fluctuations. A black and a white mold developed on the surface of each of the control cultures by day 7, and the odor was very offensive by that time.

The presence of a surface mold indicated a lack of sufficient oxygen exclusion from the uninoculated controls. However, no mold development was noted on any inoculated medium, despite sampling by the same techniques as the controls.

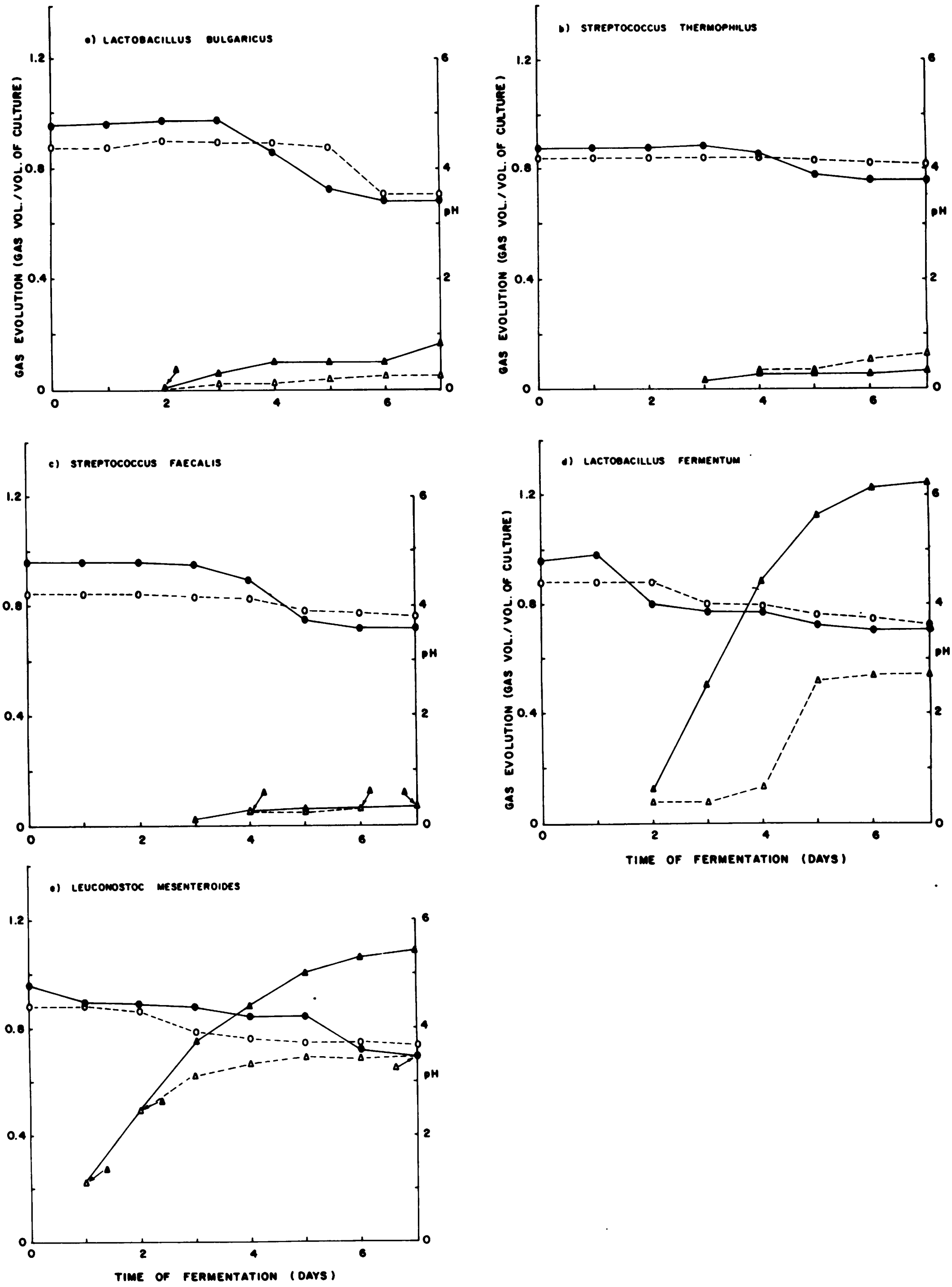

FIG. 2. *Course of gas evolution and pH change in ripe and green banana media incubated at 37°C with cultures of: (a) L. bulgaricus, (b) S. thermophilus, (c) S. faecalis, (d) L. fermentum, and (e) L. mesenteroides. Symbols: pH (●, ○) and evolved gas (▲, △) in green banana medium (---) and ripe banana medium (——).*

The odor and physical appearance of the inoculated purees were pleasant. All cultures were initially a uniform medium brown color. At the end of 7 days all of the cultures had developed a dark brown surface layer ranging in thickness from about 6 mm for *L. mesenteroides* to 12 to 25 mm for the other cultures. This layer developed in each culture before the onset of gas evolution and progressed no further after gas production started. The coloration was apparently an enzyme-catalyzed oxidation as typically caused by polyphenol oxidase. It is known that this enzyme is inactive at pH 3.0; thus, the lowered pH and the reduced oxygen levels in the subsurface layers of the cultures obviously prevented further discoloration.

Beneath the dark surface layer, all of the inoculated purees changed from the initial medium brown to a lighter brown color. Cultures of *L. mesenteroides* in both ripe and green media were closer to a light cream color. The cultures of *L. bulgaricus*, *S. thermophilus*, and *S. faecalis* exhibited a dilute, sweet-pickle odor. The *L. mesenteroides* and *L. fermentum* had much the same odor, except a stronger acetic acid odor was present. This would be expected in a heterofermentative culture. All of the odors were mildly astringent but not unpleasant, and the banana odor remained noticeable in all cultures to various degrees.

Another characteristic noted in the fermentations was separation of the liquid and the solids. The solid material was moist and spongy and filled with gas bubbles and tended to float on a clear, wheylike liquid for several days after the fermentation had ceased. Separation was more extensive with ripe bananas than with green bananas. This separation took place after several days of fermentation and did not occur in the uninoculated controls so was not merely caused by settling. The products were similar in texture, and the *L. mesenteroides* products had the most pronounced solidity and gas entrapment. Neither of the controls showed this aerated, spongy structure, and both turned a uniform dark brown color.

TABLE 1. *Initial and final glucose concentrations in cultures of five anaerobes on green and ripe banana media*

Organism	Medium[a]	Glucose concn (g/liter) Initial	Glucose concn (g/liter) Final
L. bulgaricus	Green	16.3	16.0
	Ripe	39.6	9.2
S. thermophilus	Green	16.3	12.2
	Ripe	39.6	15.2
S. faecalis	Green	16.3	14.1
	Ripe	39.6	16.0
L. fermentum	Green	16.3	17.7
	Ripe	39.6	10.9
L. mesenteroides	Green	16.3	8.3
	Ripe	39.6	10.3

[a] Denotes relative degree of ripeness of banana.

Initial and final glucose concentrations were measured for each inoculated culture. The glucose concentration dropped at least 60% in all fermentations with ripe banana medium (Table 1). This was expected since all of the organisms tested actively metabolize glucose. With green banana medium, however, a maximum decrease of 50% was observed with the *L. mesenteroides* culture, whereas only minimal decreases or even increases in the glucose level were observed with the other organisms. Although the cultures with green medium were apparently actively producing acid, the glucose levels did not reflect much change. Apparently, either the cultures produced amylases which produced glucose from starch at about the same rate as it was utilized, or the amylases present in the banana continued the ripening process in vitro. The decrease in the absolute amount of carbohydrate was not measured for any of the cultures.

The samples of the products which were placed in the freezer were compared (after 2 weeks for the ripe medium and after 4 weeks for the green medium) with the samples kept in the fermentation mother liquor at room temperature. The odor and appearance of each frozen sample after defrosting were nearly identical to those of the corresponding sample stored at room temperature, except the frozen samples were slightly darker in color for all products.

Samples of some cultures and uninoculated controls were stored for about 1 month at room temperature. Cultures from which the liquid had been drained, leaving the moist banana solids, developed vigorous growths of mold, as did the controls. However, those fermented products which remained undisturbed in the original liquid were preserved with no apparent change.

Preservative action is not assured at a pH as high as 3.5. Apparently, however, the combination of a low pH and preservation of limited oxygen access combine to offer good protection of the fermented products.

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