APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1991, p. 2529–2533
0099-2240/91/092529-05$02.00/0

Vol. 57, No. 9

Isolation and Characterization of Microorganisms Associated with the Traditional Sorghum Fermentation for Production of Sudanese Kisra†

SULMA I. MOHAMMED, LARRY R. STEENSON,* AND ALLEN W. KIRLEIS
Department of Food Science, Purdue University, West Lafayette, Indiana 47907

Received 28 February 1991/Accepted 17 June 1991

Sorghum flour obtained from Sudan was mixed with water in a 1:2 (wt/vol) ratio and fermented at 30°C for 24 h. The bacterial populations increased with fermentation time and reached a plateau at approximately 18 h. At the end of 24 h, sorghum batter pH had dropped from 5.95 to 3.95 and the batter had a lactic acid content of 0.80%. The microbial population during the 24 h of fermentation consisted of bacteria (*Pediococcus pentosaceus*, *Lactobacillus confusus*, *Lactobacillus brevis*, *Lactobacillus* sp., *Erwinia ananas*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*), yeasts (*Candida intermedia* and *Debaryomyces hansenii*), and molds (*Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and *Rhizopus* sp.). *P. pentosaceus* was the dominant microorganism at the end of the 24-h fermentation. When three consecutive fermentations using an inoculum from the previous fermentation were carried out, the bacterial population increase plateaued at 9 h. The microbial populations in these fermentations were dominated by *P. pentosaceus*.

Sorghum is widely grown in the semiarid tropical regions of Africa and Asia because of its drought tolerance (8). It is considered poor people's food and may constitute more than 70% of the food intake for people in these regions (19). Several studies involving human, animal, and in vitro digestibility showed that cooked sorghum has low protein digestibility compared with other cereals (3, 25, 28). Fermentation prior to cooking was found to improve the in vitro and in vivo digestibility of sorghum (3, 16, 25). Sorghum fermentation is commonly used in Sudan for making, in addition to beverages, a number of foods, such as kisra, nasha, and aceda. Kisra, a flat bread, constitutes a major part of the staple diet for the people in Sudan. Traditionally, the naturally occurring microorganisms in sorghum flour are utilized in these fermentations.

The commercial production of kisra in urban areas of Sudan relies on natural fermentation. In order to develop controlled fermentation technology, it is necessary to understand the fermentation process and to characterize the microorganisms involved. This study was undertaken to characterize the microorganisms responsible for sorghum fermentation and to provide the necessary information for development of starter cultures with predictable characteristics that could be used commercially for production of kisra.

MATERIALS AND METHODS

Fermentation of sorghum. Sorghum grain of the Dabar variety was purchased from grain markets in Khartoum, Sudan, and stone milled to fine flour. The flour was transported to the United States and stored at 25°C until used.

Sorghum fermentation was carried out in the traditional way (9). Initially, however, a natural fermentation was performed by the original microorganisms present in the flour. Sorghum flour was mixed with sterile deionized water in a 1:2 (wt/vol) ratio. This mixture was incubated at 30°C (temperature used in a traditional Sudanese kitchen) for 24 h in a sterile covered flask. Four consecutive fermentations were carried out using inocula from each previous fermentation in a 1:2:0.1 (flour-water-inoculum, wt/vol/vol) ratio to start each subsequent batch. Each fermentation was performed in triplicate and sampled every 3 h for 24 h.

Sampling. At each sampling time, a 50-g sample was placed in a sterile blender jar and blended for 2 min with 450 ml of sterile 0.1% peptone water (Difco Laboratories, Detroit, Mich.). After further serial dilutions in 0.1% peptone water, samples were plated in selective agar media (see below) using pour plate techniques (7) and incubated at 30°C for bacterial counts. Yeast cells and molds were enumerated after incubation at 22°C.

Media. Acetate medium (36) was used for enumeration of lactic acid bacteria. Cycloheximide (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 0.01% was added to prevent yeast and mold growth. The plates were incubated for 48 h under anaerobic conditions by using GasPak H_2 anaerobic systems (BBL Microbiology Systems, Div. Becton Dickinson and Co., Cockeysville, Md.). Colonies from enumerated plates were further examined by Gram stain. Plate count agar (Difco) was used for total plate count and incubated for 48 h. Total acid-producing bacteria were enumerated in Elliker broth (BBL) to which 15 g of agar per liter was added. The Elliker agar was modified by adding 5% calcium carbonate solution and 0.01% cycloheximide. Calcium carbonate solution was prepared by the method of Vogensen et al. (42) except that calcium carbonate replaced calcium citrate in the solution.

Coliforms were enumerated on violet red bile agar (BBL) and incubated at 37°C. Counts were taken at 24 and 48 h. Ten representative colonies were inoculated into brilliant green bile broth (BBL) at 37°C (26). Tubes were examined at 24 and 48 h. Colonies producing gas in this broth were confirmed as coliforms. The number of coliforms per gram was determined by multiplying the percentage of tubes confirmed as positive by the original counts in violet red bile agar (26). Yeast cells and molds were enumerated on malt agar. Two

* Corresponding author.
† Journal Paper no. 12867 of the Purdue Agricultural Experiment Station.

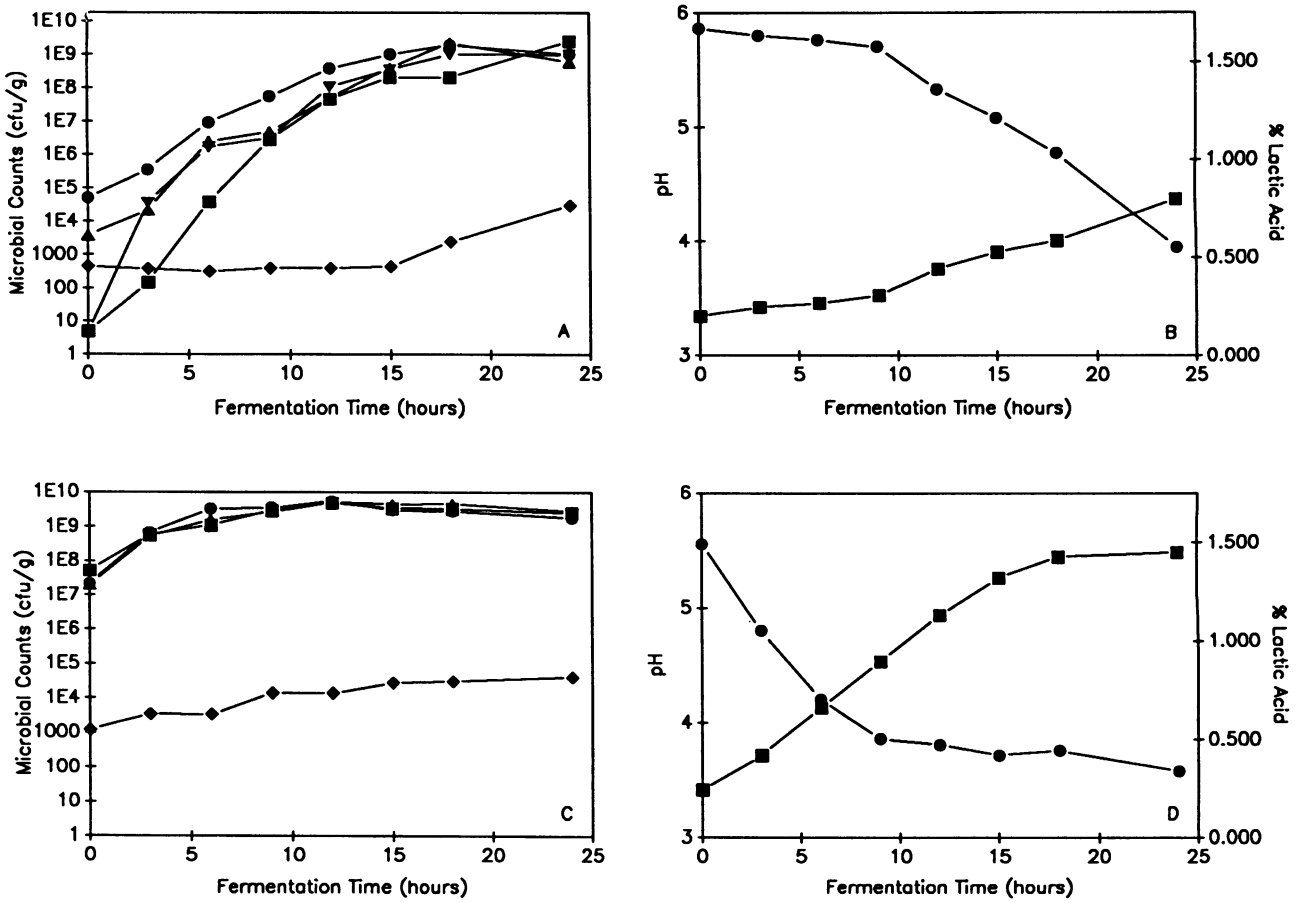

FIG. 1. Microbial counts, pH, and percent lactic acid during kisra fermentation. (A and B) 24 h of original fermentation (natural); (C and D) 24 h of first transfer (with inoculum). Symbols for panels A and C: ●, total plate count; ▲, total acid-producing bacteria; ■, lactic acid bacteria; ▼, coliforms; ◆, yeasts and molds. Symbols for panels B and D: ■, percent lactic acid; ●, pH.

milliliters of 1% antibiotic solution of chlorotetracycline HCl and chloramphenicol (23) was added per 100 ml of malt agar to prevent bacterial growth. Yeast and mold counts were taken after 2, 3, and 5 days of incubation.

Determination of pH and titratable acidity. The change in pH of fermenting dough was monitored initially and every 3 h for 24 h for each fermentation by using a pH meter (Brinkmann, Metrohm Herisau, Switzerland).

The titratable acidity, expressed as percent lactic acid, was determined by titrating 10 g of fermented dough against 0.1 N NaOH to pH 8.3 (15) with an automatic titrator (Brinkmann model E526). Triplicate determinations were made per sample.

Identification of isolates. Representative single colonies were picked from plates used for viable counts at each sampling time. Isolates were purified by repeated streaking on appropriate media. When pure cultures were obtained, gram-positive catalase-negative isolates were transferred to Elliker broth containing 20% glycerol. Gram-negative catalase-negative isolates were transferred to Trypticase soy broth (BBL) containing 20% glycerol and were stored at −20°C. Presumptive lactic acid bacteria picked from acetate medium plates were subjected to morphological and biochemical characterization and classified to genera according to Garvie (11). Morphology was determined by the Hucker and Conn (18) modification of Gram stain and a scanning electron microscope (JEOL JSM-840). The semisolid medium of Gibson and Abd-el Malek (14) was used to determine

TABLE 1. Morphological and biochemical characteristics of lactic acid bacteria isolated during kisra fermentation

Isolate (% of total)	Morphology[a]	Planes of division[a]	Growth in litmus milk	Gas from glucose[b]	Acid from glucose	Hydrolysis of arginine	Dextran from sucrose	Type of lactic acid
Lactobacillus sp. (24)	Rods to coccobacilli	1	Weak	+	+	+	+	DL
Pediococcus sp. (71)	Cocci	2	Weak	−	+	+	−	DL
Enterococcus sp. (5)	Cocci	1	Strong	−	+	+	−	D(−)

[a] Confirmed by scanning electron microscope.
[b] +, positive reaction; −, negative reaction.

TABLE 2. Carbohydrate reactions of *Lactobacillus* isolates from kisra fermentation[a]

Isolate (% of total)	Amygdalin	Arabinose	Cellobiose	Glucose	Melibiose	Melezitose	Mannose	Mannitol	Maltose	Ribose	Xylose	Sucrose	Raffinose	Trehalose	Salicin
L. confusus (51)	+	−	+	+	−	−	+	−	+	+	+	+	−	−	+
L. brevis (3)	+	+	−	+	+	−	+	−	+	−	−	+	−	−	−
Lactobacillus sp. (44)	+	+	+	+	−	−	+	−	+	−	−	+	−	−	+

[a] +, acid produced during 48-h incubation at 30°C; −, no acid produced.

the hetero- and homofermentative natures of lactic acid bacteria, which were also confirmed by gas chromatography (41) and testing for the presence or absence of carbon dioxide in Durham tubes. Lactic acid configuration was determined according to Noll (31). Each identified genus (*Pediococcus*, *Lactobacillus*, or *Enterococcus*) was further categorized according to the schemes outlined by Garvie (12), Kandler and Weiss (20), and Mundt (29), respectively. Plasmid profiles were done to differentiate strains of *Pediococcus pentosaceus*. DNA was isolated according to Klaenhammer (21) with modifications described by Sanders and Klaenhammer (37).

Isolates picked from plate count agar and modified Elliker agar were identified according to Benson (6). The oxidase-negative gram-negative rods and the coliforms confirmed by counts in violet red bile agar were characterized by Enterotubes II (Roche Diagnostics, Div. Hoffmann-La Roche, Inc., Montclair, N.J.). The gram-positive catalase-negative isolates were characterized as described above. For confirmation of the identity of microorganisms, representative isolates were characterized by Microbial ID (Newark, Del.).

Yeast cells isolated during the fermentations were tested for 40 characteristics (24) as described below. Cultural characteristics on solid medium and broth were examined in malt extract agar and malt extract broth, respectively. Cell morphology and sporulation were assessed with morphology agar (BBL) and examined for 4 weeks. Presence of pseudomycelia and mycelia was tested by the Dalmau plate technique (24). Fermentation of glucose, galactose, sucrose, maltose, lactose, and raffinose were screened for the production of carbon dioxide by using Durham tubes. The ability to assimilate 18 carbohydrates was also assessed. Nitrogen assimilation medium was used for assessing the utilization of nitrate, nitrite, L-lysine, and ethylamine hydrochloride. Vitamin-free medium was examined after 3 weeks of incubation. Ability to split arbutin and growth in 50% glucose were also assessed. Identification of yeast cells was according to Barnett et al. (5) and Kreger-van Rij (24).

Molds were identified by using 8-day-old cultures on potato dextrose agar (BBL). Cultural and microscopic characteristics were observed. Microscopic examination of molds was performed by staining the molds with lactophenol-cotton blue and examining them at ×40 magnification under a light microscope (17). Molds were classified according to Frazier and Westhoff (10) and Barnett and Hunter (4).

RESULTS

Enumeration of microorganisms. Figure 1A shows that in sorghum fermented naturally for 24 h at 30°C under aseptic conditions, the bacterial population increased with increasing fermentation time and reached a plateau at 18 h. The microflora of fermented sorghum consisted of lactic acid bacteria, coliforms, other acid-producing bacteria, yeasts, and molds. The lactic acid content increased with fermentation time. At the end of the fermentation, it was 0.80%, which resulted in a pH drop from 5.95 to 3.95 (Fig. 1B).

In sorghum fermented by adding an inoculum from a previously fermented sorghum (traditional fermentation), the bacterial populations also increased with fermentation time; however, they reached a plateau by 9 h. The bacterial population was predominantly lactic acid bacteria. The yeast and mold counts increased with fermentation time but remained low (Fig. 1C). Coliforms were not detected in subsequent fermentations. Lactic acid content and pH were 0.90% and 3.86, respectively, by 9 h and 1.45% and 3.58 by 24 h (Fig. 1D).

When three consecutive fermentations were carried out, each using an inoculum from the previous fermentation, data similar to those shown in Fig. 1C were obtained. The changes in pH and titratable acidity during the three consecutive fermentations were similar to data shown in Fig. 1D.

Identification of microorganisms. One hundred twenty presumptive lactic acid bacterial isolates from the initial fermentation and the third and fourth transfers were further characterized. The isolates were separated into three groups according to morphology and biochemical characteristics (Table 1). *Lactobacillus* isolates were characterized by their abilities to produce acid from various carbohydrates (Table 2). Two species of *Lactobacillus*, *L. confusus* and *L. brevis*, were identified. The species of one *Lactobacillus* isolate could not be determined (Table 2). *Enterococcus* isolates were characterized by using several biochemical tests as well as growth characteristics (Table 3). The isolates were identified as *Enterococcus faecium* (38) (formerly *Streptococcus faecium*). The *Pediococcus* isolates were identified

TABLE 3. Properties of *Pediococcus* sp. and *Enterococcus* sp. isolated during kisra fermentation[a]

Isolate	Growth					Hydrolysis of aesculin	Acid from:							
	pH 9.6	6.5% NaCl	40% bile	10°C	45°C		L-arabinose	Ribose	Maltose	Sorbose	Melibiose	Melezitose	Trehalose	Sorbitol
P. pentosaceus[b]	+	+	+	+	+	+	+	+	−	−	−	−	+	−
E. faecium	+	+	+	+	+	+	+	+	+	+	+	−	ND	−

[a] +, positive reaction; −, negative reaction; ND, not determined.
[b] The isolate did not produce acid from xylose, methyglucoside, arbutin, mannitol, glycerol, or lactose.

TABLE 4. Morphological and biochemical properties of yeast cells isolated during kisra fermentation

Isolate	Colony morphology	Cell shape	Pseudomycelium	Sporulation	Acid from [a]:						Assimilation of [b]:		
					Glucose	Galactose	Maltose	Sucrose	Lactose	Raffinose	Nitrate	Nitrite	Vitamin-free medium
C. intermedia	Cream, rough, dull	Long (chains)	Candida type	None	+	+	+	+	−	−	−	−	+
D. hansenii	Cream, smooth	Round (clumps)	None	Ascospore	+ (very weak)	+ (very weak)	+ (very weak)	+ (very weak)	−	−	−	−	−

[a] +, positive reaction; −, negative reaction.
[b] Both isolates were able to assimilate glucose, galactose, L-sorbose, D-ribose, D-xylose, L-arabinose, sucrose, maltose, trehalose, cellobiose, melibiose, lactose, raffinose, melezitose, mannitol, and dulcitol.

as *P. pentosaceus* (Table 3). Plasmid profiles of *P. pentosaceus* showed that all isolates belonged to one strain (data not shown). *L. confusus*, *L. brevis*, *Lactobacillus* sp., and *E. faecium* were found throughout the fermentations, while *P. pentosaceus* maintained a high population and dominated the fermentations. The gram-negative oxidase-negative isolates were characterized as *Erwinia ananas*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*; the coliforms were identified as *Enterobacter cloacae* and *K. pneumoniae* (data not shown). Fatty acid analysis confirmed the identification of the lactic acid bacteria and members of the family *Enterobacteriaceae* (data not shown). The yeasts were identified as *Candida intermedia* and *Debaryomyces hansenii* (Table 4), and the molds were identified as *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., and *Rhizopus* sp. (data not shown).

DISCUSSION

The natural flora in sorghum flour prior to fermentation consisted of *Enterobacteriaceae*, lactic acid bacteria, and the molds *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and *Rhizopus* sp. Lactic acid bacteria and coliforms constituted very low proportions of the population. However, as the fermentation progressed, the total plate count and the numbers of lactic acid bacteria and coliforms increased and reached a plateau by 18 h. The total acid-producing bacteria attained their highest counts by 18 h, and their numbers declined as the pH of the batter decreased. Similar results were reported in soda cracker fermentation (27). *Enterobacteriaceae* were inhibited by the low pH at 24 h and were not isolated from successive fermentations. The reason for this may be that lactic acid bacteria outnumbered the *Enterobacteriaceae*, dominated the fermentation, and resulted in faster acid production. Therefore, in contrast to findings for other indigenous fermented foods (1, 2), *Enterobacteriaceae* in the flour do not appear to be important in kisra fermentation.

As the population of lactic acid bacteria increased from initial low numbers and dominated the fermentation, the amount of acid produced increased with the concomitant drop in the pH. However, when an inoculum was added, acid was produced at a higher rate and the pH dropped faster, i.e., from 5.95 to 3.86 in 9 h, than in the original fermentation, which reached a pH of 3.95 by 24 h. This difference was due to the large numbers of lactic acid bacteria in the inoculum.

A succession in the growth of lactic acid bacteria was observed. In the first 24 h of natural fermentation, *L. confusus*, *L. brevis*, *Lactobacilli* sp., *P. pentosaceus*, and *E. faecium* were found. However, *P. pentosaceus* maintained a large population and dominated until the end of the fermentation and also during consecutive fermentations. Similar successions of microorganisms have been seen during the fermentation of corn, tef, cassava, and soda crackers (1, 13, 35, 39). Although the genera identified in those studies were identical to the genera found in our study, species were different.

Yeasts and molds continued to increase until the end of the original fermentation (24 h) and maintained the same pattern of growth during the three successive fermentations. The yeasts *D. hansenii* and *C. intermedia* isolated during kisra fermentation have been encountered in many other indigenous foods (32, 35). It is possible that, in addition to lactic acid bacteria, yeasts were important in sorghum fermentation for producing kisra, as has been reported for other indigenous food (22, 30, 32–35, 39, 40).

The data indicate that certain microbial and biochemical changes occur during traditional fermentation of kisra. Knowledge gained about these changes during the fermentation process is necessary when commercial production of a fermented product with consistent characteristics and anticipated qualities is being considered. Studies on combining these organisms in different combinations and proportions for the development of starter cultures for commercial production of kisra are in progress.

ACKNOWLEDGMENTS

We thank Cynthia Koh-Knox and Becky Fickle for their help.
This work was supported by the International Sorghum and Millet Collaborative Support Program.

REFERENCES

1. **Adegoke, G. O., and A. K. Babalola.** 1988. Characteristic of microorganisms of importance in the fermentation of fufu and ogi—two Nigerian foods. J. Appl. Bacteriol. **65**:449–453.
2. **Akinrele, I. A.** 1964. Fermentation of cassava. J. Sci. Food Agric. **15**:589–594.
3. **Axtell, J. D., A. W. Kirleis, M. M. Hassen, N. DeCroz-Mason, E. T. Mertz, and L. Munk.** 1982. Digestibility of sorghum proteins. Proc. Natl. Acad. Sci. USA **78**:1333–1335.
4. **Barnett, H. L., and B. B. Hunter.** 1972. Illustrated genera of imperfect fungi, 3rd ed., p. 53–180. Burgess Publishing Co., Minneapolis.
5. **Barnett, J. A., R. W. Payne, and P. Yarrow.** 1983. Yeast: characteristics and identification. Cambridge University Press, New York.
6. **Benson, H. J.** 1985. Microbiological applications, a laboratory manual in general microbiology, 4th ed., p. 127–138. William C. Brown, Publishers, Dubuque, Iowa.
7. **Busta, F. F., E. H. Peterson, D. M. Adams, and M. G. Johnson.** 1984. Colony count methods, p. 63–83. *In* M. L. Speck (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
8. **Dogett, H.** 1988. Sorghum, p. 5–10. John Wiley & Sons, Inc., New York.

9. **El Tinay, A. H., A. M. Abd-el Gadir, and M. Elhidia.** 1979. Sorghum fermented kisra bread. 1. Nutritive value of kisra. J. Sci. Food Agric. **30**:859–863.
10. **Frazier, W. C., and D. C. Westhoff.** 1988. Food microbiology, 4th ed., p. 17–31. McGraw-Hill Book Co., New York.
11. **Garvie, E. I.** 1984. The separation of species of genus *Leuconostoc* and the differentiation of *Leuconostoc* from other lactic acid bacteria. Methods Microbiol. **16**:147–178.
12. **Garvie, E. I.** 1986. Genus *Pediococcus*, p. 1075–1079. *In* P. H. A. Sneath and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
13. **Gashe, B. A.** 1985. Involvement of lactic acid bacteria in the fermentation of tef (*Eragrostis tef*), an Ethiopian fermented food. J. Food Sci. **50**:800–801.
14. **Gibson, T., and Y. Abd-el-Malek.** 1945. The formation of carbon dioxide by lactic acid bacteria and *Bacillus licheniformis* and a cultural method of detecting the process. J. Dairy Res. **14**:35–44.
15. **Gilliland, S. E., W. E. Sandine, and E. R. Vedamuthu.** 1984. Acid-producing microorganisms, p. 184–195. *In* M. L. Speck (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C. A.
16. **Graham, G. G., W. C. Maclean, E. Morales, B. R. Hamaker, W. Kirleis, E. T. Mertz, and J. D. Axtell.** 1986. Digestibility and utilization of protein and energy from nasha, a traditional Sudanese fermented sorghum weaning food. J. Nutr. **116**:978–984.
17. **Harrigan, W. F., and M. E. McCance.** 1976. Laboratory methods in food and dairy microbiology, p. 106–108. Academic Press, Inc., New York.
18. **Hucker, G. L., and H. J. Conn.** 1923. Methods of gram staining. Technical bulletin no. 93. New York Agricultural Experimental Station, Geneva, N.Y.
19. **Hulse, J. H., E. M. Laing, and O. E. Pearson.** 1980. Sorghum and the millets: their composition and nutritive value, p. 1–32. Academic Press, Inc., New York.
20. **Kandler, O., and N. Weiss.** 1986. Genus *Lactobacillus*, p. 1208–1234. *In* P. H. A. Sneath and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
21. **Klaenhammer, T. R.** 1984. A general method for plasmid isolation in lactobacilli. Curr. Microbiol. **10**:23–28.
22. **Kline, L., and T. F. Sugihara.** 1971. Microorganisms of San Francisco sour dough bread process. II. Isolation and characterization of undesirable species responsible for the souring activity. Appl. Microbiol. **21**:459–465.
23. **Koburger, J. A., and E. H. Marth.** 1984. Yeasts and molds, p. 197–202. *In* M. L. Speck, (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
24. **Kreger-van Rij, N. J. W. (ed).** 1984. The yeasts: a taxonomic study, 3rd ed., p. 1–99. Elsevier Publishing Co., Amsterdam.
25. **MacLean, W. C., G. Lopez de Romana, R. P. Placko, and G. G. Graham.** 1981. Protein quality and digestibility of sorghum in preschool children: balance studies and plasma free amino acids. J. Nutr. **111**:1928–1936.
26. **Mehlman, I. J.** 1984. Coliforms, fecal coliforms, *Escherichia coli* and enteropathogenic *E. coli*, p. 265–285. *In* M. L. Speck, (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
27. **Mica, J.** 1955. Bacterial aspects of soda cracker fermentation. Cereal Chem. **32**:125–131.
28. **Mosha, A., B. O. Eggum, L. Hambraeus, S.-A. Leiden, B. L. Ljunggvist, O. Mellander, and U. Svanberg.** 1983. Influence of seed constituents and food preparation on nutrient bioavailability in sorghum food products. I. Proteins, abstr. X-23. Nutrition in Europe; food for thought for scientists. 4th Eur. Nutr. Conf.
29. **Mundt, J. O.** Enterococci, p. 1063–1065. *In* P. H. A. Sneath and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
30. **Ngaba, P. R., and J. S. Lee.** 1979. Fermentation of cassava. J. Food Sci. **44**:1570–1571.
31. **Noll, F.** 1984. L-(+)-Lactate, p. 582–588. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis, vol. VI, 3rd ed. Verlag Chemie, Deerfield Beach, Fla.
32. **Odunfa, S. A., and S. Adeyele.** 1985. Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. J. Cereal Sci. **3**:173–180.
33. **Okafor, N.** 1977. Microorganisms associated with cassava fermentation of "gari" production. J. Appl. Bacteriol. **42**:279–284.
34. **Okafor, N., B. Ijoma, and C. Oyolu.** 1984. Studies on the microbiology of a cassava retting for foo-foo production. J. Appl. Bacteriol. **56**:1–13.
35. **Oyewole, O. B., and S. A. Odunfa.** 1988. Microbiological studies on cassava fermentation for 'lafun' production. Food Microbiol. **5**:125–133.
36. **Rogosa, M., J. A. Mitchell, and R. F. Wiseman.** 1951. A selective medium for isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. **62**:132–133.
37. **Sanders, M. E., and T. R. Klaenhammer.** 1983. Characterization of phage-sensitive mutants from a phage-insensitive strain of *Streptococcus lactis*: evidence for plasmid determinant that prevents phage adsorption. Appl. Environ. Microbiol. **46**:1125–1133.
38. **Schleifer, K. H.** 1987. Recent changes in the taxonomy of lactic acid bacteria. FEMS Microbiol. Rev. **46**:201–203.
39. **Sugihara, T. F.** 1978. Microbiology of soda cracker process. 1. Isolation and identification of microflora. J. Food Prot. **41**:977–979.
40. **Sugihara, T. F., L. Kline, and M. W. Miller.** 1971. Microorganisms of the San Francisco sour dough bread process. 1. Yeasts responsible for the leavening action. Appl. Microbiol. **21**:456–458.
41. **Thornhill, P. J., and T. M. Cogan.** 1984. Use of gas-liquid chromatography to determine the end products of growth of lactic acid bacteria. Appl. Environ. Microbiol. **47**:1250–1254.
42. **Vogensen, F. K., T. Karst, J. J. Larsen, B. Kringelum, D. Ellekjaer, and E. W. Nielsen.** 1987. Improved direct differentiation between *Leuconostoc cremoris*, *Streptococcus lactis* subsp. *diacetylactis*, and *Streptococcus cremoris*/*Streptococcus lactis* on agar. Milchwissenschaft **42**:646–648.