ELSEVIER

International Journal of
Food Microbiology 29 (1996) 49–58

International Journal
of Food Microbiology

Microbiology of mesu, a traditional fermented bamboo shoot product

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Received 21 September 1994; accepted 10 April 1995

Abstract

The use of mesu as a pickle and as the base of curry is a tradition in the Darjeeling hills and Sikkim of India. A total of 327 strains of lactic acid bacteria, representing *Lactobacillus plantarum*, *L. brevis* and *Pediococcus pentosaceus* were isolated from 30 samples of mesu. These species were present in all samples of raw bamboo shoots tested. Mesu was dominated by *L. plantarum* followed by *L. brevis*; *P. pentosaceus* was isolated less frequently and recovered from only 40–50% of the mesu samples. The fermentation was initiated by *P. pentosaceus*, followed by *L. brevis*, and finally succeeded by *L. plantarum* species. During fermentation, the titratable acidity increased from 0.04 to 0.95%, resulting in the decline in pH from 6.4 to 3.8.

Keywords: Mesu; Traditional fermented product; Pickled vegetable; Microbial succession; Young bamboo shoot

1. Introduction

Mesu, a non-salted fermented bamboo shoot product, has traditionally been consumed by the people in the bamboo-growing regions of the Darjeeling hills of West Bengal and Sikkim in India (Tamang et al., 1988). History of the art of pickling this vegetable is lost in antiquity. Mesu is prepared only during the months of June to September when bamboo shoots sprout. In the traditional method of its preparation, young edible shoots of bamboo (*Dendrocalamus hamiltonii* Nees and Arnott, *Bambusa tulda* Roxb. and *Dendrocalamus sikkimensis* Gamble, locally

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SSDI 0168-1605(95)00021-6

known as "choya bans", "karati bans" and "bhalu bans", respectively) are defoliated, chopped, pressed tightly into a hollow bamboo stem, covered tightly with leaves of bamboo or other wild plants and left at ambient temperature (20–25°C) to ferment for 7–15 days. Completion of fermentation is indicated by the typical mesu flavour and taste. Mesu is commonly used as a pickle by mixing it with salt, mustard oil and green chillies. It is also used for preparing curry by frying and mixing it with cooked meat. While fresh mesu has a shelf-life of only about a week, its pickle can be stored for a year or more.

To our knowledge, mesu has so far not been scientifically investigated. The present investigation examined the succession of microflora during fermentation of young bamboo shoots to produce mesu.

2. Materials and methods

2.1. Samples

Samples were collected aseptically in sterile glass bottles from different weekly markets of Kalimpong in the Darjeeling hills and Gangtok in Sikkim. The bottles were kept in an ice-box and transported immediately (within 24 h) to the laboratory for analyses. Mesu prepared in laboratory following the traditional method was also analyzed.

In order to study the microbial succession, mesu was prepared in the laboratory by fermenting pieces (1–1.5 cm × 0.3–0.7 cm × 0.3–0.7 cm) of young shoots of bamboo (*Dendrocalamus hamiltonii*) in a glass jar at 30°C for 10 days. Quintuplet bottles were removed as indicated for analyses.

2.2. Determination of pH and acidity

A 10 g sample was blended for 15 s with 20 ml deionized water in a homogenizer (Remi, Bombay, India), and pH of the slurry was determined using a pH meter (Type 335, Systronics, Naroda, India) calibrated with standard buffer solutions (Merck, Bombay, India). Titratable acidity was calculated by titrating the filtrate of the homogenate with 0.1 N sodium hydroxide to an end point of phenolphthalein (0.1% w/v in 95% ethanol (AOAC, 1990).

2.3. Isolation of micro-organisms

A 10 g sample was blended in 90 ml sterile diluent (0.85% w/v sodium chloride in water) by use of a Stomacher lab-blender 400 (Seward Medical, London, UK) for 2 min at "normal" speed, and 1 ml of the appropriate decimal dilutions were mixed with molten (45°C) medium and poured into plates. The nutrient agar (M087, HiMedia, Bombay, India), de Man, Rogosa and Sharpe (MRS) agar (HiMedia M641), all purpose Tween (APT) agar (HiMedia M226) and glucose-yeast extract-peptone [(GYP)-$CaCO_3$] agar (Okada et al., 1986) plates were incubated at 30°C in a candle jar for 48–72 h; and yeast extract-malt extract (YM) agar (HiMedia M424) and potato dextrose agar (PDA) (HiMedia M096), supplemented with benzylpenicillin (10 IU/ml) and streptomycin sulphate (12 μg/ml), plates were incubated at 28°C for 48 h. The colonies appeared were counted as colony forming units (cfu) per g wet weight of sample. The colonies were randomly picked from selected plates (having 50–300 colonies per plate) to obtain representative strains at regular intervals of fermentation time, and the isolates were purified by successive subculturing in the corresponding broth and streaked onto the agar surface. After microscopic examination, purified cultures were grown on slants of the same medium and stored at 5°C.

2.4. Characterization of isolates

Colonies of young cultures were Gram stained (Bartholomew, 1962). General morphology and catalase activity were determined by the methods described by Norris et al. (1981). Cell dimension was measured with a standardized ocular micrometer. Motility of the isolates was checked using a phase-contrast microscope. Growth of the isolates in sodium chloride was observed in tubes of tryptone-glucose-yeast extract (TGYE) broth (HiMedia M952) (5 ml/tube), containing sodium chloride (4, 6.5 or 18% w/v), for 7 days at 30°C. The production of acid and gas from sugar was observed using 10 ml sugar basal broth (Garvie, 1984) containing 2% w/v sugar and inverted Durham tubes for 10 days at 30°C (Okada et al., 1986). Production of indole was tested by inoculating the isolates in 10 ml modified Davis and Mingioli's broth (Davis and Mingioli, 1950), prepared by replacing ammonium sulphate with L-tryptophan (0.1% w/v) and supplementing with yeast extract (0.2% w/v). Ehrlich Böhme reagent (1–2 ml) was layered on 3, 5 and 7 days-old broth cultures and observed for the formation of red ring at the culture-reagent interface (Iswaran, 1980). Hydrolysis of gelatin was tested by streaking MRS agar-gelatin (1% w/v) plates, incubating for 3 and 5 days at 30°C and then flooding the plates with 10 ml 1 N sulphuric acid saturated with ammonium sulphate (Sneath and Collins, 1974). Arginine hydrolysis test medium (Thornley, 1960) was inoculated by stabbing, and the method of Lelliott et al. (1966) was followed to determine its hydrolysis. Hydrolysis of fat was tested by streaking the surface-dried plates of tributyrin agar (HiMedia M157) and incubating for 4 days at 30°C. Slants of esculin hydrolysis test medium (g/l distilled water: esculin, 1.0; $FeCl_3$, 0.5; peptone, 5.0; yeast extract, 1.0; agar, 20.0) were inoculated and incubated at 30°C for 7 days and observed for any blackening of the medium. Reduction of nitrate was tested in nitrate broth (HiMedia M439) after 3, 7 and 14 days of incubation at 30°C using nitrate reduction test reagent (Norris et al., 1981). Growth at different pH values was studied by adjusting the pH of MRS broth to different levels using 1 N hydrochloric acid or 10% w/v sodium hydroxide. The tubes were inoculated and incubated at 30°C for 24 h (Hesseltine and Ray, 1988). Growth at different temperatures was tested by inoculating and incubating the isolates in TGYE agar slants immersed in water bath for a maximum period of 21 days.

2.5. *Statistical analysis*

The data obtained were analyzed statistically by determining analysis of variance (Snedecor and Cochran, 1989).

3. Results

Pure culture colonies developed well on MRS agar, APT agar and GYP-$CaCO_3$ agar, and poorly on nutrient agar. But, there was no growth on YM agar and antibiotics-supplemented PDA. A total of 327 representative and dominating strains of bacteria, isolated from 30 samples of mesu were grouped on the basis of Gram reaction, catalase activity, cell shape, gas from glucose, ammonia from arginine, and acid from a range of sugars (Table 1). Twelve representative strains, one from each group, were selected randomly for the purpose of identification.

All the isolates were non-motile, non-sporeforming, microaerophilic, catalase-negative and Gram-positive. The isolates having rod shaped cells were assigned to the genus *Lactobacillus*, whereas the spherical cells in tetrads belonged to the genus *Pediococcus*, according to the taxonomic keys of Kandler and Weiss (1986) and Garvie (1986), respectively. The detailed characteristics of the representative strains of *Lactobacillus* are shown in Table 2. The strains KM-R1, KM-R32, GM-R1, LM-R1 and LM-R14 were referred to as *Lactobacillus plantarum* Orla-Jensen, and the strains KM-SR1, GM-SR1 and LM-SR1 were referred to as *L. brevis* Orla-Jensen.

The four representative strains of *Pediococcus* differed only in the production of acid from D-xylose and lactose (Table 1). In every other respect tested, all those strains were identical. They were referred to as *Pediococcus pentosaceus*.

The average microbial loads studied in 10 samples of mesu collected from each of Kalimpong and Gangtok markets and laboratory-made as well as young shoots of bamboo are presented in Table 3. All the three species, including *L. plantarum*, *L. brevis* and *P. pentosaceus* were present in both the bamboo shoots and the fermented product. In the raw material *P. pentosaceus* was the most predominant species, whereas in mesu *L. plantarum* was the predominant organism. *P. pentosaceus* was only detected in 40–50% of the samples tested.

Fig. 1 shows the changes in microflora in young bamboo shoots during their fermentation to produce mesu. *Pediococcus pentosaceus* predominated in the early stages of fermentation. Its initial load increased significantly ($p < 0.05$) after 2 days, followed by a steady fall in the count. On each successive day, the decrease of this species was significant ($p < 0.05$). The load of *L. brevis* was maximum on the fourth day of fermentation, after which there was significant ($p < 0.05$) fall in

Table 1
Characteristics of representative strains of bacteria isolated from mesu samples [a]

Source	Number of strains [b] isolated	Cell shape	Gas from glucose	NH_3 from arginine	Acid produced from [c]							Grouped strains (No. of isolates)	Representative strains
					Ara	Xyl	Tre	Manl	Sorl	Cel	Lac		
Kalimpong market	109	Rod	–	–	+	–	+	+	+	+	+	52	KM-R1
		Rod	–	–	–	–	+	+	+	+	+	23	KM-R32
		Rod	+	+	+	–	–	–	–	–	–	19	KM-SR1
		Coccus	–	+	+	–	+	–	–	+	–	15	KM-T1
Gangtok market	103	Rod	–	–	+	+	+	+	+	+	+	49	GM – R1
		Rod	+	+	+	+	–	–	–	–	+	36	GM – SR1
		Coccus	–	+	+	+	+	–	–	+	+	18	GM-T1
Laboratory-made	115	Rod	–	–	–	–	+	+	+	+	+	38	LM-R1
		Rod	–	–	+	+	+	+	+	+	+	20	LM-R14
		Rod	+	+	+	–	–	–	–	–	+	30	LM-SR1
		Coccus	–	+	+	–	+	–	–	+	+	13	LM-T1
		Coccus	–	+	+	+	+	–	–	+	–	14	LM-T2

[a] Number of samples was 10 from each source.
[b] All the isolates were non-motile, non-sporeforming, Gram-positive and catalase-negative.
[c] Ara, L-arabinose; Xyl, D-xylose; Tre, trehalose; Manl, mannitol; Sorl, sorbitol; Cel, cellobiose; Lac, lactose.

Table 2
Characteristics [a] of representative strains of rod-shaped bacteria isolated from mesu

Parameters	KM-R1	KM-R32	GM-R1	LM-R1	LM-R14	KM-SR1	GM-SR1	LM-SR1
Cell width (μm)	0.7–0.8	0.7–0.8	0.7–0.9	0.7–0.8	0.7–0.9	0.6–0.7	0.6–0.7	0.6–0.7
Cell length (μm)	2.0–4.5	2.0–4.7	2.0–5.0	1.5–4.5	2.0–5.0	2.0–3.0	2.0–3.5	2.0–3.0
Hydrolysis of								
Arginine	–	–	–	–	–	+	+	+
Esculin	+	+	+	+	+	+	+	–
Growth at 45°C	–	+	+	+	–	–	–	+
Acid produced from								
L-Arabinose	+	–	+	–	+	+	+	+
D-Xylose	–	–	+	–	+	–	+	–
D-Galactose	+	+	+	+	+	+	+	–
Lactose	+	+	+	+	+	–	+	+
Cellobiose	+	+	+	+	+	–	–	–
Sucrose	+	+	+	+	+	+	–	–
Trehalose	+	+	+	+	+	–	–	–
Raffinose	+	–	–	–	+	–	+	–
Mannitol	+	+	+	+	+	–	–	–
Sorbitol	+	+	+	+	+	–	–	–
Gas from glucose	–	–	–	–	–	+	+	+

[a] Characters common to all strains: non-motile, microaerophilic, Gram-positive and catalase-negative rods in chains of 2–4 cells; fat and gelatin not hydrolysed; growth occurred at 15°C; optimum temperature of growth, 30°C; nitrate not reduced; indole not produced; acid produced from D-ribose, D-glucose, D-fructose, maltose and melibiose; acid not produced from D-mannose and starch.

the count. On the other hand, the population of *L. plantarum* increased continuously from the start of fermentation till the formation of mesu on the tenth day; again, every successive day, the increase was significant ($p < 0.05$) till the sixth day.

The changes in pH and acidity are shown in Fig. 2. During fermentation, the

Table 3
Microbial load [a] of bamboo shoots and mesu from different sources

Micro-organisms	x 10^6 cfu/g wet weight			
	Bamboo shoot	Mesu		
		Kalimpong market	Gangtok market	Laboratory-made
Lactobacillus plantarum	0.1 (0.006–0.2)	283.0 (227.0–331.0)	290.0 (234.0–395.0)	300.0 (240.0–390.0)
Lactobacillus brevis	0.08 (0.005–0.2)	0.004 (0.0008–0.008)	0.005 (0.0008–0.008)	0.004 (0.0008–0.009)
Pediococcus pentosaceus	0.2 (0.007–0.4)	0.00002 (< DL [b]-0.00004)	0.00003 (< DL-0.00006)	0.00002 (< DL-0.00004)

[a] Data represent the means of 10 samples. Ranges are given in parentheses.
[b] Less than detection limit (10 cfu/g).

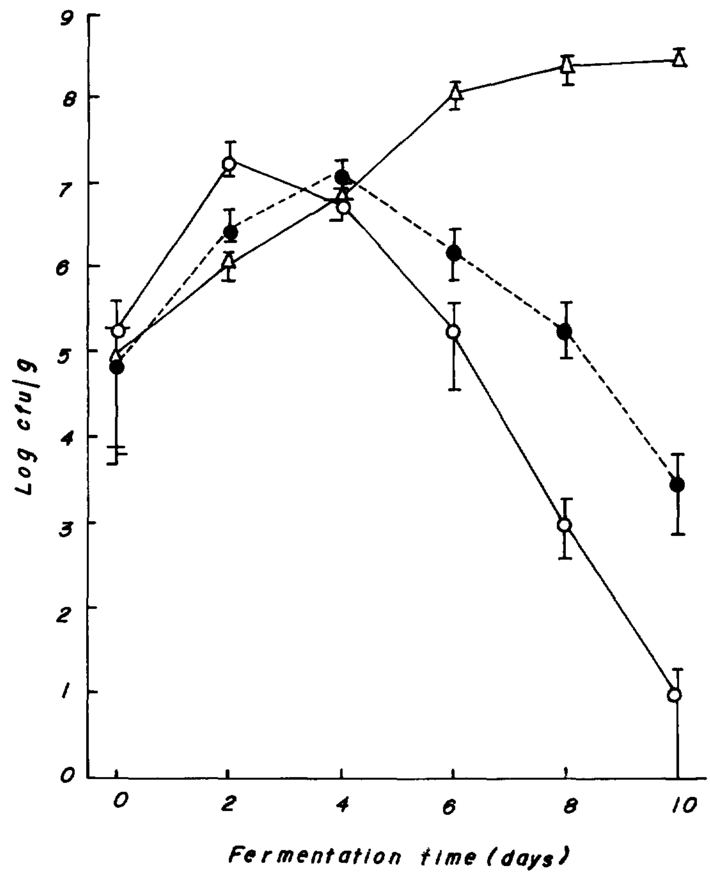

Fig. 1. Changes in microflora (○, *Pediococcus pentosaceus*; ●, *Lactobacillus brevis*; △, *Lactobacillus plantarum*) in bamboo shoots during the production of mesu. Values are the means of five batches of fermentation with ranges. Where ranges overlap, bars for the lowest mean are shown to the left; and where three ranges overlap, bars for the highest mean are shown to the right.

mean pH value decreased from 6.4 at 0 day to 3.8 at the end. The titratable acidity increased significantly ($p < 0.05$) on each day till the eighth day, after which the rise was not significant ($p < 0.05$).

4. Discussion

Microbial analysis of young bamboo shoots demonstrated the presence of *L. plantarum*, *L. brevis* and *P. pentosaceus*. This finding is in agreement with the report of Garvie (1986) about the occurrence of pediococci and lactobacilli in plants. While *P. pentosaceus* was the most predominant micro-organism in the

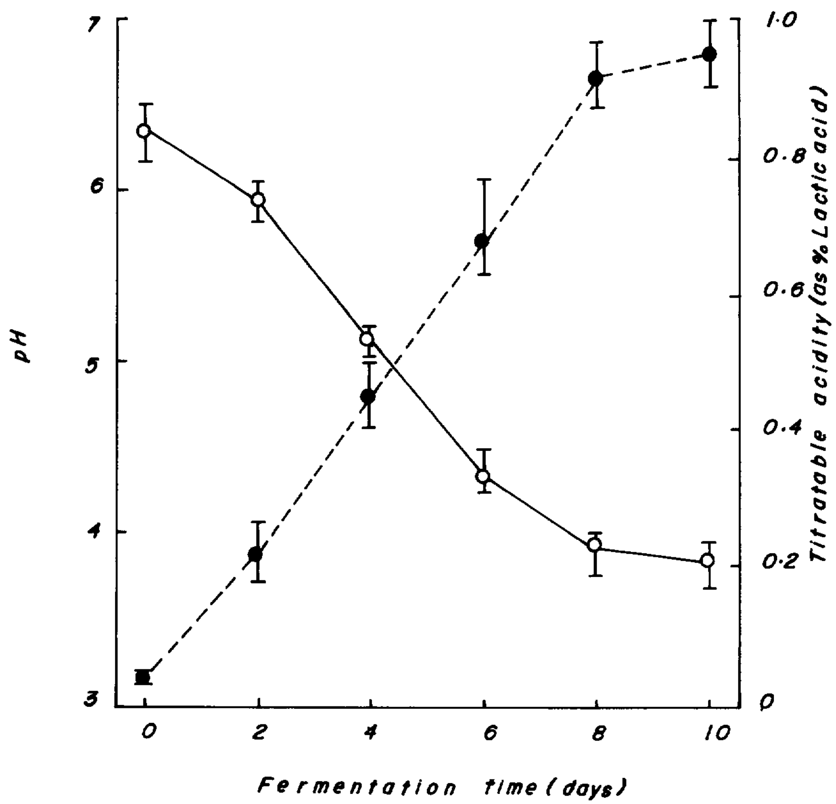

Fig. 2. Changes in pH (○) and titratable acidity (●) in bamboo shoots during the production of mesu. Values are the means of five batches of fermentation and bars indicate ranges.

substrate, *L. plantarum* predominated the final product. It seems that this fermentation is initiated by *P. pentosaceus*, but lastly dominated by *L. plantarum*.

Mesu is a naturally fermented product; no starter culture is added during its preparation. All the three bacterial species occurred in all of the samples of raw bamboo shoots. In the first two days of fermentation, *P. pentosaceus* predominated, reaching a maximum of about 10^7 cfu/g wet weight. The load of heterofermentative *L. brevis* reached its peak on the fourth day. Rapid growth, production of significant ($p < 0.05$) amount of acid by these lactics and the possible production of gas created an oxygen-depleting condition, suitable for the last successor, *L. plantarum*. After reaching their peaks, the counts of *P. pentosaceus* and *L. brevis* declined rapidly till the end of fermentation when there was predominance by *L. plantarum*. The increase in load of *L. plantarum* and the increase in acidity in the fermenting substrate were almost parallel. At the time of completion of fermentation, the total acidity was as high as 0.95% and had a pH value as low as 3.8.

This observation is in agreement with the report of Dhavises (1972). In naw-mai-dong, a similar Thai product, *P. cerevisiae* predominates during the initial stages of fermentation. In contrast to the succession of microflora in mesu, in naw-mai-dong the order is *Pediococcus*, *L. plantarum* and *L. brevis*. In sauerkraut fermentation it was observed that at a temperature above 18°C *L. brevis* and *L. plantarum* predominated, and at a temperature of 32°C or above the fermentation became essentially dominated by *L. plantarum* (Pederson and Albury, 1969). During the production of mesu at 30°C, the temperature of the fermenting mass rose above 30°C, and as such *L. plantarum* was finally selected.

Acknowledgements

This work was supported by grants from the University Grants Commission, New Delhi and the Sikkim Science Society, Gangtok.

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