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Journal of Hill Research
21(1) : 1-7, 2008

Microbiological Evaluation of *Maseura*, an Ethnic Fermented Legume-Based Condiment of Sikkim

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(Received 25 November 2007; revised version received 18 April 2008; accepted 22 May 2008)

ABSTRACT

Maseura, is a fermented legume-based condiment prepared and consumed by the Nepalis of the Himalayas. Microbiological studies of maseura were studied. Microbial load of lactic acid bacteria, yeast and spore-formers were found at a range between 10^4-10^7 cfu/g. Moulds were not detected. Lactic acid bacteria were identified as Lactobacillus fermentum, Lb. salivarius, Pediococcus pantosaceus *and* Enterococcus durans. *Spore-formers were identified as* Bacillus subtilis, B. mycoides, B. pumilus *and* B. laterosporous. *Yeasts were identified as* Saccharomyces cerevisiae, Pichia burtonii *and* Candida castellii.

INTRODUCTION

Ethnic fermented foods are important dietary components of the people in the Himalaya regions of Sikkim, the Darjeeling hills, Nepal and Bhutan (Tamang, 2005). *Maseura,* a cone-shaped hollow, brittle and friable product, is one of the lesser-known fermented legume foods consumed a condiment or an adjunct in cooking vegetable. It is an important food item of the Nepalis mostly Newar communities. During its production, seeds of black gram (*Vigna mungo*) or blacklentil (*Phaseolus mungo*) or rice-bean (*Phaseolus calcaratus*) are cleaned, washed and soaked overnight. Soaked seeds are dehulled by pressing through hands and the hulls are flown off, ground into thick paste using mortar and pestle. Water is carefully added while grinding, until paste becomes sticky, which is then made hand-moulded into small balls or cones. If rice- bean is used, then boiled potato or squash or yam is mixed with the paste to make it sticky. The mixture is placed on a bamboo mat and fermented in open kitchen for 2-3 days, and then sun-dried for 3-5 days depending upon the weather condition.

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Maseura can be stored in a dry container at room temperature for a year or more. It is usually fried in edible oil with vegetable to make curry or soup and served with rice. *Maseura* is similar to North Indian *wari* and South Indian *sandige* (Soni and Sandhu, 1990; Dahal *et al*., 2005). Biochemical and nutritional evaluation of *masuera* of Nepal was reported by Dahal *et al*. (2003). However, as per our literature search, there is no report of microbiological profile of *maseura*. The aim of this paper was to evaluate different microorganisms involved in production of *maseura.*

MATERIALS AND METHODS

Samplings

Samples of *maseura* were collected from different places of Sikkim. All samples were collected aseptically in sterile poly-bags, and transported to the laboratory for analysis.

Microbiological analysis

Ten grams of samples were mixed with 90 ml of 0.85 % (w/v) sterile physiological saline and homogenised in a Stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution in the same diluent was made. Lactic Acid Bacteria (LAB) were isolated on plates of MRS agar (M641, HiMedia) supplemented with 1 % $CaCO_3$ and incubated

at 30° C in an anaerobic gas-jar (LE002, HiMedia) for 48-72 h. Aerobic spore-forming bacteria were isolated on nutrient agar, after inactivation of vegetative cells by heating at 100° C for 2 min and were incubated at 37° C for 24 h (Tamang and Nikkuni, 1996). Aerobic mesophillic counts were determined on plate count agar (M091A, HiMedia) incubated at 30° C for 48 h. Colonies of moulds and yeasts were examined on potato dextrose agar (M096, HiMedia) and yeast-malt (YM) agar (M424, HiMedia), supplemented with penicillin, which were incubated aerobically at 28° C for 72 h. Identified isolates were preserved at -20 °C in respective media with 15 % (v/v) glycerol added.

Characterization and identification

Cell morphology of all bacterial isolates and their motility were determined using a phase contrast microscope (Olympus CH3-BH-PC, Japan). Bacterial isolates were tested for Gram-reaction and for catalase production by placing a drop of 10 % hydrogen peroxide solution on isolates, and were preliminarily identified on the basis of gas production from glucose, ammonia production from arginine, growth at different temperatures, pH and concentrations of sodium chloride (Dykes *et al*., 1994). Lactate isomer was determined enzymatically using D-lactate and L-lactate dehydrogenase test kits (Tamang *et al*., 2005). Sugar fermentation of LAB isolates were determined by the API 50 CHL test strips (bioMérieux, France) and the identifications were interpreted using APILAB PLUS software (bioMérieux, France). Taxonomical keys of Wood and Holzapfel (1995) were followed for identification of LAB isolates. Bacilli were identified as per description given by Slepecky and Hemphill (1992). Characterization and identification of yeasts were carried out as per the method of Kurtzman and Fell (1998).

RESULTS AND DISCUSSION

A total of 14 samples of *maseura* were microbiologically analysed. Average mean of aerobic mesophilic counts in samples of *maseura* was 10^8 cfu/g, out of which population of Lactic acid bacteria, bacilli and yeast were found at the level of 10^7 cfu/g and 10^4 cfu/g, respectively (Table 1). Filamentous moulds were not detected in any of the samples analysed. *Maseura* is slightly acidic with the pH ranging from 5.6 to 6.3 (Table 1). A total of 56 LAB strains, 23 strains of spore-former and 33 yeast strains were isolated from *maseura*. All bacterial isolates were considered LAB because they grew well in anaerobic agar and formed clear halo in $CaCO_3$ supplemented MRS agar plates, were Gram-positive, catalase-negative

Table 1. Microbial load of *maseura* of Sikkim

Place of collection*	Log cfu/g sample			
	LAB	Bacilli	Yeast	AMC
Singtam (pH 6.0 ± 0.1)	7.7 ± 0.1	<DL	4.5 ± 0.3	8.4 ± 0.3
Rongli (pH 6.1 ± 0.1)	7.8 ± 0.4	6.2 ± 0.6	5.7 ± 0.1	8.4 ± 0.1
Salangdang, (pH 6.3 ± 0.1)	6.5 ± 0.1	<DL	5.3 ± 0.1	8.5 ± 0.1
Namchi, (pH 5.8 ± 0.1)	7.1 ± 0.1	7.4 ± 0.1	5.8 ± 0.4	8.5 ± 0.1
Jorethang (pH 6.2 ± 0.1)	6.8 ± 0.1	4.6 ± 0.1	<DL	7.5 ± 0.1
Geyzing (pH 5.8 ± 0.1)	7.5 ± 0.1	7.7 ± 0.1	5.7 ± 0.1	8.0 ± 0.1
Gangtok (pH 5.6 ± 0.1)	7.7 ± 0.1	5.8 ± 0.1	4.4 ± 0.1	7.7 ± 0.3

*two samples from each place was collected,total samples was 14.

cfu/g, colony forming unit

Data represents the means (± SD) of number of samples. Mean pH (± SD) of each sample is shown in parenthesis.

DL, less than detection limit (10 cfu/g).

LAB, Lactic acid bacteria; AMC, aerobic mesophillic count.

Mould was not detected.

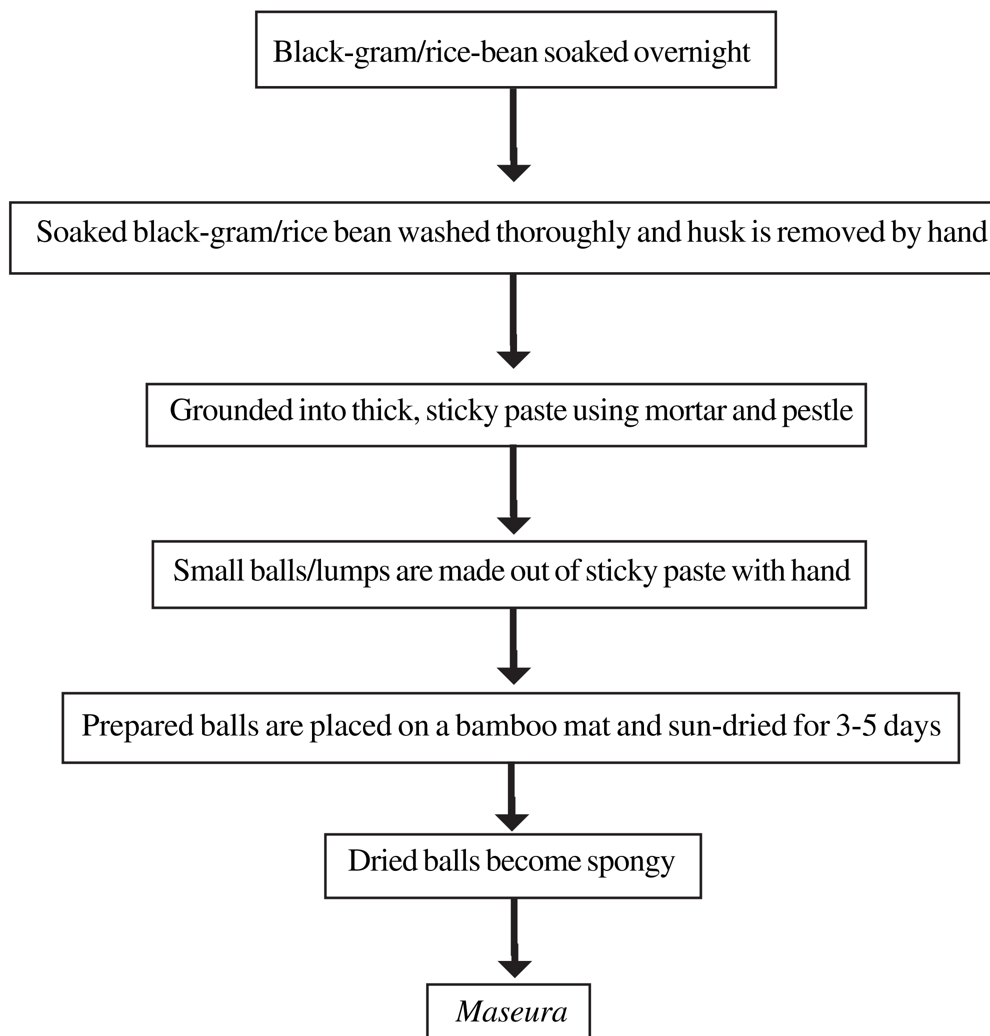

Fig 1. Flow sheet of preparation of *maseura* in East Sikkim

bacteria, non-motile and non spore-formers. The strains were selected randomly from each grouped strains having similar morphology, ability to produce gas from glucose and hydrolyse arginine, and were phenotypically characterised including determination of the sugar fermentation pattern by API system, lactic acid configuration, growth in different temperatures, pH and NaCl. The nine tetrad-forming cocci grew well at 15° C, 45° C, and in 6.5 % and 10 % NaCl (Table 2). Seven tetrad-forming cocci were identified as *Pediococcus acidilactici*, two tetrad-forming cocci were identified as *Pediococcus pentosaceus* (Table 2) based on the taxonomical keys of Simpson and Taguchi (1995). A coccus strain MS:B2 which grew well at 10° C and 45° C, in 6.5% NaCl and at pH 9.6, was tentatively identified as *Enterococcus*. Sugar fermentation pattern using API system confirmed its identity as *Enterococcus durans.*

Three heterofermentative strains which grew at 45° C were further tested for sugar fermentation pattern using API system and were identified as *Lactobacillus fermentum* (Table 2). Arginine-negative homofermentative strain MS:B3 was identified as *Lactobacillus salivarius.* Though the dominant and major fermenting organisms in all samples of *maseura* were LAB, bacilli and yeast were also recovered. All spore-formers isolated from *maseura* were Gram-positive rods, catalase-positive, aerobic and motile. Following the taxonomical key of Slepecky and Hemphill (1992), spore-forming rods were identified as *Bacillus subtilis*, *B. mycoides, B. pumilus* and *B. laterosporus* (Table 3). Based on the identification

Table 2. Phenotypic characteristics of the LAB from *maseura*

Strain code	Cell Morphology	NH_3 from arginine	CO_2 from glucose	Growth in/at			Lactate isomer	
				$10\ ^0C$	$45\ ^0C$	pH 3.9		Arabinose
MS:B2	Coccus	+	–	+	+	–	L	–
MS:B3	Rod	–	–	+	+	+	L	–
MD:B1	Rod	–	+	+	–	–	DL	+
MN:B2	Coccus/tetrad	+	–	+	+	+	DL	–
ML:B2	Coccus/tetrad	+	–	+	+	+	DL	–
ML:B3	Rod	+	+	+	–	+	DL	+
ML:B7	Rod	+	+	+	–	+	DL	+
MN:B1	Coccus/tetrad	+	–	+	+	+	DL	–
MN:B2	Coccus/tetrad	+	–	+	+	+	DL	–
MA:B1	Coccus/tetrad	+	–	+	+	+	DL	–
MA:B2	Coccus/tetrad	+	–	+	+	+	DL	–
MA:B3	Coccus/tetrad	+	–	–	+	–	DL	–
ML:B1	Coccus/tetrad	+	–	+	+	+	DL	–
ML:B6	Coccus/tetrad	+	–	+	+	+	DL	+

All isolates were Gram-positive, catalase-negative and non-sporeformers.
% NaCl. All strains fermented Ribose, Esculin, Salicin, Cellobiose, Maltos

Table 3. Characteristic of *Bacillus* strains isolated from *maseura*

Strain code	Acid from glucose	Nitrate reduction	Growth at 7.0 % NaCl	Growth at 10 % NaCl	Anaerobic Growth	Growth at 5 ^{0}C	Growth at 50 ^{0}C	Growth at 65 ^{0}C	Starch hydrolysis	Casein hydrolysis	Voges-proskaeur reaction	pH in VP Broth	Acid from Arabinose	Acid from Mannitol	Acid from Xylose	Citrate test	Identification
MS:B1	+	–	+	+	–	–	–	–	–	+	+	5.1	–	+	+	+	*Bacillus pumilus*
MS:B2	+	+	+	+	–	–	+	–	–	+	+	5.0	–	+	+	+	*Bacillus subtilis*
MS:B3	+	+	+	+	–	–	–	–	–	+	+	5.2	–	+	+	+	*Bacillus subtilis*
MS:B4	–	+	–	–	+	–	–	–	–	–	–	5.2	–	+	–	–	*Bacillus laterosporus*
MS:B5	+	+	+	+	+	–	–	–	–	+	+	5.0	–	–	–	–	*Bacillus mycoides*
MS:B6	+	+	+	+	+	–	+	–	–	+	+	5.0	–	–	–	+	*Bacillus mycoides*
MS:B7	+	+	+	+	–	–	–	–	–	+	+	5.9	–	+	–	+	*Bacillus subtilis*
MS:B8	+	+	+	+	–	–	–	–	+	+	+	5.1	–	+	–	+	*Bacillus subtilis*
MS:B9	+	+	+	+	–	–	–	–	+	+	+	5.1	–	+	–	+	*Bacillus subtilis*
MS:B10	+	+	+	+	–	–	–	–	+	+	+	5.8	–	+	–	+	*Bacillus subtilis*

All spore-formers were rods;Gram-positive, catalase positive, hydrolysed Arginine and did not produce gas from glucose. All strains had elongated endospores with sub-terminal position and all grew at pH 6.8 and 40^0 C

Table 4. Characteristic of yeasts isolated from *maseura*

Strain code	Colony morphology	Cell morphology	Mycelium	Growth at 37^0 C	Sugar Fermentation						Sugar Assimilation												Identification
					Glucose	Galactose	Maltose	Raffinose	Sucrose	Trehalose	Cellobiose	Galactose	Glycerol	Lactose	Maltose	Melibiose	Mannitol	Raffinose	Sucrose	Starch	Trehalose	xylose	
MS:Y1	Ss	O - E	pseudo	+	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+	+	–	+	*Pichia burtonii*
MS:Y2	Ss	O - E	pseudo	+	+	+	+	+	+	–	+	+	+	–	+	–	+	–	+	+	+	–	*Saccharomyces cerevisiae*
MB:Y1	Ss	O - E	pseudo	–	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	+	–	+	*Saccharomyces cerevisiae*
MB:Y2	Ss	O - E	pseudo	–	+	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	*Saccharomyces cerevisiae*
MD:Y1	Ss	O - E	pseudo	+	+	+	+	+	+	–	+	+	+	–	+	–	+	+	+	+	+	–	*Saccharomyces cerevisiae*
MD:Y2	Ds	O - Cy	true	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	*Saccharomyces cerevisiae*
MD:Y3	Ds	O - Cy	true	+	+	–	+	+	+	+	–	+	+	–	+	–	+	+	+	+	+	+	*Saccharomyces cerevisiae*
MD:Y4	Ds	O - Cy	true	+	+	+	+	+	+	–	+	+	+	–	+	–	+	+	+	+	+	–	*Saccharomyces cerevisiae*
MN:Y1	Ds	O - E	pseudo	+	+	+	+	+	+	–	+	+	+	–	+	–	+	+	+	+	+	–	*Saccharomyces cerevisiae*
MN:Y2	Ds	O - E	pseudo	+	+	+	+	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	*Saccharomyces cerevisiae*
ML:Y1	Ds	O - E	Absent	+	+	–	–	–	+	–	–	+	+	–	–	–	–	+	+	+	–	+	*Candida castellii*
ML:Y2	Ds	O - E	Absent	+	+	–	–	–	+	–	–	+	+	–	–	–	–	+	+	+	–	+	*Candida castellii*
ML:Y3	Ds	O - E	Absent	+	+	–	–	–	+	–	–	+	+	–	–	–	–	+	+	+	–	+	*Candida castellii*

All yeasts did not reduce nitrate. None of the strains fermented lactose and starch. Arabinose, inositol and rhamnose were not assimilated by any strain, O – E, oval to ellipsoidal; O – Cy, oval to cylindrical; Ss, smooth surface; Ds, dusty surface.

keys of Kurtzman and Fell (1998), yeast strains were identified as *Saccharomyces cerevisiae*, *Pichia burtonii* and *Candida castellii* (Table 4).

Bacteria, mostly lactic acid bacteria (LAB), yeasts and filamentous moulds constitute the microbiota of fermented foods and beverages, which are present in or on the ingredients, utensils, environment, and are selected through adaptation to the substrates (Tamang, 2007). The presence of high number of LAB among the microbial consortia in *maseura* fermentation may be due to predominance of LAB in dehulled black-grams, which have been reported earlier in similar black-gram fermented product *idli* (Mukherjee *et al.*, 1965). *Candida* spp. and *Saccharomyces cerevisiae* were reported in *wari* fermentation (Soni and Sandhu, 1990).

It was observed that production of *maseura* has drastically declined in many parts of Sikkim and the Darjeeling hills. Exact reasons could not be sorted out, but the unique indigenous method of *maseura* production is worth documenting as a low cost fermented legume condiment in rural areas, and also for microbial resources. Though the sizable number of yeast and spore-formers were recovered, the dominant microorganisms in *maseura* were lactic acid bacteria which probably play an important role in the traditional fermentation process.

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