Lactic acid bacteria in Hamei and Marcha of North East India

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Abstract  Hamei and Marcha are mixed dough inocula used as starters for preparation of various indigenous alcoholic beverages in Manipur and Sikkim in India, respectively. These starters are traditionally prepared from rice with wild herbs and spices. Samples of Hamei and Marcha, collected from Manipur and Sikkim, respectively, were analysed for lactic acid bacterial composition. The population of lactic acid bacteria (LAB) was 6.9 and 7.1 Log cfu/g in Hamei and Marcha, respectively. On the basis of phenotypic and genotypic characters, LAB strains isolated from Hamei and Marcha were identified as Pediococcus pentosaceus, Lactobacillus plantarum and Lactobacillus brevis. Technological properties of LAB such as antimicrobial properties, effect on acidification, ability to produce biogenic amines and ethanol, degree of hydrophobicity and enzymatic activities were also performed. Pediococcus pentosaceus HS: B1, isolated from Hamei, was found to produce bacteriocin. None of the strains produced biogenic amines. LAB strains showed a strong acidifying ability and they also produced a wide spectrum of enzymes.

Keywords  LAB · Hamei · Marcha

Introduction

Use of mixed starters in the form of dry ball-like cakes containing amylolytic and alcohol-producing yeasts, starch-degrading moulds and lactic acid bacteria is common in many Asian countries¹. Hamei and Marcha (Murcha) are not foods but dry, round to flattened, solid ball-like mixed dough inocula used as starter cultures for preparation of various indigenous alcoholic beverages in North East India. Traditionally Hamei is prepared from crushed raw rice mixed with powdered bark of ‘yangi’ (Albizia myriophyl- la) and a pinch of previously prepared powdered Hamei. The dough is pressed into flat cakes and is kept over rice husk in a bamboo basket for 2–3 days at room temperature (20–30°C), and then sun dried for 2–3 days. Hamei is used to prepare a rice-based beverage locally called Atingba and a distilled clear liquor called Yu in Manipur.

During Marcha preparation, soaked glutinous rice is mixed with roots of ‘guliyo jara’ (Plumbago zeylanica), leaves of ‘bheemsen paate’ (Buddleja asiatica), flowers of ‘sengrekna’ (Vernonia cinerea), ginger, red dry chili and a pinch of powdered old Marcha. The mixture is then made into paste by adding water and kneaded into flat cakes of varying sizes and shapes, and placed individually on the ceiling floor made up of bamboo stripes above the kitchen, bedded with fresh fronds of ferns, locally called ‘pire uneu’ (Glyphylopteriolopsis erubescens), and covered with dry ferns and jute bags. These are left to ferment for 1–3 days, the longer period being used under the colder condition. Finally, cakes of Marcha are sun dried for 2–3 days². Marcha is used to prepare alcoholic beverages such as Kodo ko jaam (fermented finger millet beverage), Bhaati jaam (fermented rice beverages) and Raksi (distilled liquor) in
Sikkim and the Darjeeling hills. Rural women sell these starter-cakes in local markets in Manipur and Sikkim, respectively for their livelihood. Hamei and Marcha are similar to other Oriental starters such as Ragí of Indonesia, Nuruk of Korea, Babod of the Philippines, Loogpong of Thailand and Chinuyeh of China and Men of Vietnam.

Pediococcus pentosaceus was the only lactic acid bacterium along with yeasts and moulds reported from Marcha samples of Nepal, and from the Darjeeling hills and Sikkim in India. The identification of the reported Pediococcus pentosaceus was based on the limited phenotypic characteristics, without genotypic characterisation. To our best knowledge microbial composition of Hamei has not been reported yet. The aim of this paper was to identify lactic acid bacteria present in Hamei and Marcha based on both phenotypic and genotypic characteristics and also to study some technological properties of the LAB strains.

Materials and Methods

Collection of samples: Ten samples of Hamei were purchased from different shops in Ima market of Imphal in Manipur. Twelve samples of Marcha samples were collected directly from their places of preparation in different Marcha-making villages in Sikkim. All samples were collected aseptically in sterile containers, and transported to laboratory for analyses.

Isolation of lactic acid bacteria: Ten gram of sample were mixed with 90 ml of 0.85 % sterile physiological saline in a stomacher lab-blender (400, Seward, UK) for 1 min and were serially diluted in the same diluent. Lactic acid bacteria (LAB) were isolated on MRS agar (M641, HiMedia) supplemented with 1 % CaCO₃ and incubated at 30°C in an Anaerobic Gas-Pack system (LE002, HiMedia) for 48–72 h. Purity of the isolates was checked by streaking again and sub-culturing on fresh MRS agar plates, followed by microscopic examinations. Identified strains of LAB were preserved in MRS broth (M369, HiMedia) using 15 % (v/v) glycerol at –20°C.

Phenotypic characterization: The cell morphology and motility of LAB strains were observed in a phase contrast microscope (CH3-BH-PC, Olympus, Japan). Isolates were Gram-stained and tested for catalase production. Isolates were identified by testing for phenotypic properties such as gas production from glucose, ammonia production from arginin, growth at different temperatures (10, 15 and 45°C) and at different pH (3.9 and 9.6), as well as the ability to grow in different concentrations of NaCl (6.5, 10 and 18 %) in MRS broth¹. The configuration of lactic acid produced was determined enzymatically using D-lactate and L-lactate dehydrogenase kits (Roche Diagnostic, France). The presence of meso-diaminopimelic acid (DAP) in the cell walls of LAB was determined using thin-chromatography on cellulose plates. Sugar fermentation patterns of LAB were determined using API 50 CHL test strips (bioMérieux, France) and the result was obtained using the APILAB PLUS database identification software (bioMérieux, France).

Genotypic characterisation: The DNA was extracted from overnight grown cultures in MRS broth. The primer M13 (5’-GAG GGT GGC GGT TCT-3’) was used for RAPD-PCR. The PCR amplification was conducted with a Primus 96 Plus thermal cycler (MWG Biotech, Ebersberg, Germany). For identification of Lactobacillus brevis, a species-specific PCR was applied using the oligonucleotide primer 5’-CTT GCC ATG ATT TTA ACA-3’ and 5’-GGG CGG TGT GTA CAA GGC-3’ as forward and reverse primers, respectively. To verify the identity of the PCR product, amplified fragments were digested with PstI in a 1 μl reaction mixture containing 11.5 μl of the PCR product, 1.5 μl incubation buffer and 10 U PstI (1 h, 37°C). For identification of Lactobacillus plantarum, rep PCR with the primer GTG₃ (5’-GTG GTG GTG G1G G1G-3’) was carried out using reaction and amplification conditions. Amplification products were subjected to electrophoresis in 1.8 % agarose gels in TBE buffer⁴.

Antagonism and bacteriocin activity: Isolates were screened for antagonistic activity by the agar spot method⁵. The indicator strains used for antagonisms were Listeria innocua DSM 20649, Listeria monocytogenes DSM 20600, Bacillus cereus CCM 2010 and Staphylococcus aureus S1. Cell-free neutralized supernatants of LAB isolates were screened for bacteriocin production by the agar spot test method⁶. Bacteriocin activity was quantified by two-fold serial dilutions of the neutralized supernatants, expressed as the reciprocal of the highest dilution exhibiting a zone of inhibition and were reported in activity unit (AU) per ml⁷.

Acidification and coagulation: The acidification and coagulating abilities of the LAB strains were assayed by inoculating 10 % skim milk (RM1254, Hi Media) at 1 % level and incubation at 30°C. Observation was made of the commencement of clotting and the pH was measured⁸.

Amylolytic activity: Surface-dried plates of starch agar were streaked with 24 h-old cultures, incubated at 30°C for 48 h. After incubation, the plates were flooded with iodine solution for 15-30 min and examined the clear zone underneath (after the growth was scrapped off) for amylolytic activity.

Enzymatic profile by API-zym system: The enzymatic profile of lactic acid bacteria were assayed using commercial API-zym (bioMérieux, France) galleries by testing for the activity of the following 19 enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine-, valine- and cystine-, arylamidase, trypsin,