Microbiological profile of dried fish products of Assam

NAMRATA THAPA, JOYDEB PAL AND JYOTI PRAKASH TAMANG*

Department of Zoology, Sikkim Government College, Gangtok 737102, Sikkim
*Department of Zoology, North Bengal University, Siliguri 734013, West Bengal

ABSTRACT

Karati, bordia and lashim are dried fish products of Assam. A total of 30 samples of karati (12), bordia (10) and lashim (8) were collected from Guawahati markets and analysed for aerobic mesophilic bacteria, lactic acid bacteria, aerobic mesophilic spore-formers, yeasts and filamentous moulds. Population of lactic acid bacteria (LAB) ranged from 4- 6.2 log cfu/g, spore-former ranged from 1.4-3.8 log cfu/g, yeasts ranged from 1.8-3.3 log cfu/g, and aerobic mesophilic counts ranged from 4.2-6.3 log cfu/g, respectively in fish products. Mould was not recovered in any sample. Microorganisms isolated and identified from karati, bordia and lashim were Lactococcus lactis subsp. cremoris, Leuconostoc mesenteroides, Lactobacillus plantarum, Bacillus subtilis, Bacillus pumilus and yeasts Candida. Selected isolates were tested for proteolytic and amylolytic activities. Some of them showed high degree of hydrophobicity. None of the strains produced biogenic amines in the method applied.
Samples of karati, bordia and lashim were purchased from Guwahati markets and collected aseptically in sterile containers, and transported to laboratory for analyses. Ten gram of sample were homogenised with 90 ml of 0.85 % (w/v) sterile physiological saline in a stomacher lab-blender (400, Seward, London, UK) for 1 min and serially diluted in the same diluent. One ml each of these dilutions were pour-plated in the respective media for enumeration of lactic acid bacteria (LAB), aerobic mesophilic bacteria, yeasts and moulds. LAB were enumerated on MRS agar (M641, HiMedia, Mumbai, India) plates supplemented with 1 %CaCO3, after incubation under anaerobic conditions in an Anaerobic Gas-Pack system (LE002, HiMedia, Mumbai, India) at 30° C for 48-72 h (Tamang et al., 2005). Aerobic mesophilic counts were determined using plate count agar (M091A, HiMedia, Mumbai, India) incubated aerobically at 30° C for 48-72 h (Thapa et al., 2004). Aerobic spore-forming bacteria were isolated on nutrient agar, after inactivation of vegetable cells by heating at 100° C for 2 min and were incubated at 37° C for 24 h (Tamang and Nikkuni, 1996). Moulds and yeasts were isolated on potato dextrose agar (M096, HiMedia, Mumbai, India) and yeast extract-malt extract agar (M424, HiMedia, Mumbai, India), supplemented with 10IU/ml benzylpenicillin and 12 mg/ml streptomycin sulphate, respectively and incubated aerobically at 28° C for 72 h (Thapa, 2002). On the basis of methods described by Han et al. (2001), samples were tested for enumeration of Bacillus cereus using selective Bacillus cereus agar base (M833, HiMedia, Mumbai, India), Staphylococcus aureus using Baird Parker agar base (M043, HiMedia, Mumbai, India) and enterobacteriaceae using Violet Red Bile Glucose agar (M581, HiMedia, Mumbai, India).

Phenotypic characters of LAB were performed according to Schillinger and Lücke (1987) and identified following the taxonomical keys of Wood and Holzapfel (1995). The configuration of lactic acid produced from glucose was determined enzymatically using D-lactate and L-lactate dehydrogenase test 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 (Tamang et al., 2005). Carbohydrate fermentation patterns of LAB were determined using API 50 CHL (bioMérieux, France). The API LAB PLUS database identification software (bioMérieux, France) was used to interpret the results. Spore-forming bacteria were identified according to the taxonomical keys of Claus and Berkeley (1986). Identification of yeasts was carried out following the method of Kurtzman and Fell (1998).

Biochemical properties of selected LAB strains were tested. Surface-dried plates of milk agar and starch agar (Claus and Berkeley, 1986) were streaked with 24 h-old cultures, after incubation at 30° C for 4 days. The milk agar plates were examined for any clearing of casein around and underneath the growth for assessment of proteolytic activity. The starch plates were flooded with iodine solution for 15-30 min and examined the clear zone underneath for amylolytic activity. Protease and α-amylose activities were determined (Thapa et al., 2004). The ability of isolates to produce biogenic amines was determined qualitatively on an improved screening medium as described by Bover-Cid and Holzapfel (1999). The degree of hydrophobicity of the isolates was determined following the method as described.
by Thapa et al. (2004).

A total of 30 samples of karati (12), bordia (10) and lashim (8) were analysed for microbial load (Table 1). In all samples of dried fish products, the population of LAB and aerobic mesophilic counts ranged from 10^4 cfu/g to 10^6 cfu/g, respectively. Bacterial spores were detected at the level of <10^3 cfu/g. The load of Bacillus cereus, Staphylococcus aureus and enterobacteriaceae was less than 10^2 cfu/g and 10^3 cfu/g, respectively. Yeasts were detected at the level of <10^3 cfu/g. Mould was not recovered in any sample analysed.

Table 1: Microbiological profiles of dried fish products (karati, bordia and lashim) of Assam

<table>
<thead>
<tr>
<th></th>
<th>Karati (n=12)</th>
<th>Bordia (n=10)</th>
<th>Lashim (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid bacteria</td>
<td>4.2 (4.0-4.4)</td>
<td>5.3 (4.3-5.6)</td>
<td>5.8 (4.2-6.2)</td>
</tr>
<tr>
<td>Bacterial endospores</td>
<td>3.1 (2.8-3.3)</td>
<td>3.5 (2.9-3.8)</td>
<td>2.1 (1.4-2.6)</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>2.6 (2.2-2.8)</td>
<td>2.4 (1.8-2.8)</td>
<td>2.2 (1.8-2.5)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.8 (&lt;DL-2.0)</td>
<td>2.0 (0-2.2)</td>
<td>2.1 (&lt;DL-2.3)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>2.8 (2.0-3.0)</td>
<td>3.2 (2.2-3.5)</td>
<td>3.1 (2.6-3.3)</td>
</tr>
<tr>
<td>Yeast</td>
<td>3.1 (2.7-3.3)</td>
<td>2.2 (1.8-2.5)</td>
<td>3.0 (2.2-3.3)</td>
</tr>
<tr>
<td>Aerobic mesophilic count</td>
<td>5.1 (4.2-5.5)</td>
<td>5.6 (5.2-6.0)</td>
<td>6.0 (5.6-6.3)</td>
</tr>
</tbody>
</table>

Microbiological data were transformed into logarithms of the numbers of colony forming unit (cfu/g). Data represent the means of number of samples (n). Ranges are given in parentheses. DL, detection limit is 10 cfu/g.

Seventy three strains of bacteria isolated from samples of karati, bordia and lashim were considered to be lactic acid bacteria based on their positive Gram reactions, absence of motility, absence of spore formation and absence of catalase activity. Representative strains of LAB were selected randomly on the basis of cell morphology, gas production from glucose, hydrolysis of arginine and growth at different temperatures for further phenotypic characterisation including determination of the sugar fermentation pattern, lactate configuration and DAP. Based on phenotypic characterisation and interpretation of API-LAB PLUS database, strain KA1 (karati) was identified as Lactococcus lactis subsp. cremoris Schleifer et al. Lenticular-cocci strains BA4 and BA5 (bordia) were identified as Leuconostoc mesenteroides (Tsenkovskii) van Tieghem. Representative strain LG1 (lashim) was identified as Lactobacillus plantarum Orla-Jensen. The microbial load of dried fish products reveals that lactic cocci were predominant lactic flora. This may be due to gradations of concentration of salts used during processing, which control the bacterial flora (Tanasupawat et al., 1993). None of the LAB isolates obtained from the samples were halotolerant (i.e., 18% salt toler-
Identified as Bacillus pumilus Meyer and Gottheil. Thirteen strains of yeasts were selected on the basis of colony, cell morphology and type of mycelium. None of these strains produced asci and ascospores. Following the taxonomical keys of Kurtzman and Fell (1998), and also based on the result of sugar fermentation and assimilation tests, yeasts were grouped as Candida. Species could not be identified.

Isolates of LAB and bacilli were tested for proteolytic and amylolytic activities, respectively (Table 2). They showed low protease activity comparable to amylolytic activities which are essential in liquefaction during processing of fish products (Reddi et al., 1972). Isolates of LAB were screened for their ability to produce biogenic amines. None of them produced biogenic amines in the method applied. However, the inability of LAB strains isolated from dried fish products of Assam to produce biogenic amines is a good indication of their acceptability in the possible development of starter cultures. Two strains of Lactococcus lactis subsp. cremoris KA1 and Leuconostoc mesenteroides BA5 showed high degrees of hydrophobicity (Table 2). High degree of hydrophobicity by LAB probably indicates the potential of adhesion to gut epithelial cells of human intestine, suggesting a possible 'probiotic' character as reported earlier by Holzapfel et al. (1998).

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Han, B.Z., R.R. Beumer, F.M. Rombouts and M.J.R. Nout 2001. Microbiological safety of adhesion to gut epithelial cells of human intestine, suggesting a possible 'probiotic' character as reported earlier by Holzapfel et al. (1998).

TABLE 2: Enzymatic activity and hydrophobicity of the selected bacterial strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Protease (U/ml)</th>
<th>a-amylase (U/ml)</th>
<th>% Hydrophobicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcus lactis subsp. cremoris KA1</td>
<td>0.8</td>
<td>3.2</td>
<td>82.5 (++)</td>
</tr>
<tr>
<td>Leuconostoc mesenteroides BA4</td>
<td>0.8</td>
<td>4.2</td>
<td>70.3 (+)</td>
</tr>
<tr>
<td>Leuconostoc mesenteroides BA5</td>
<td>0.6</td>
<td>3.6</td>
<td>84.6 (++)</td>
</tr>
<tr>
<td>Lactobacillus planatarum LG1</td>
<td>1.2</td>
<td>3.1</td>
<td>46.1 (+)</td>
</tr>
<tr>
<td>Bacillus subtilis K1:S1</td>
<td>4.5</td>
<td>3.0</td>
<td>24.1</td>
</tr>
<tr>
<td>Bacillus subtilis BD:S1</td>
<td>4.3</td>
<td>3.1</td>
<td>25.3</td>
</tr>
<tr>
<td>Bacillus pumilus LD:S2</td>
<td>1.1</td>
<td>1.8</td>
<td>18.4</td>
</tr>
</tbody>
</table>

*a*Strains showing positive hydrolysis test (>2.0 mm) were assayed.

++ = hexadecane adherence >75% (hydrophobic); +, = hexadecane adherence 26-74% (intermediate).

Data represent the means of three sets.

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