Original article

Screening of Probiotic potentiality of Lactobacilli Strains, isolated from Fermented Bamboo Shoots of Assam.

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Abstract

Lactic acid bacteria were isolated from collected samples of fermented bamboo shoot of two different species of bamboo from distinguished communities of Namrup, Dibrugarh district of Assam. Pure culture and biochemical characterization of the isolated LAB were done which were grown on De Man Rogosa and Sharpe Agar medium. Probiotic characteristics of isolated lactobacilli were tested by NaCl tolerance and in-vitro studies of gastro-intestinal performance by applying the acid tolerance (pH 3) and bile salt tolerance (0.3% ox gall) test, that was evaluated through observing the viable counts after grown on MRS Agar and measuring the absorbance spectrophotometrically at 600nm. A total of eight lactobacilli were primarily isolated based on their negative result of catalase test and absence of any spores. They were further investigated for the utilization of nine types of sugar, based on which isolates are biochemically screened out as Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus helveticus. This experimental result also facilitated with the test of API 50 CH (bioMerieux). In the study of in vitro gastrointestinal performance it shows that out of the 8 isolated LAB, Lactobacillus plantarum that we have screened shows good tolerance to pH 3 having survivability difference in comparison to control is less than 0.14 in log of CFU/ml, which also shows reduction in cell viability less than 0.14 log unit after growing in 0.3% bile containing MRS medium. The isolated all Lactobacillus brevis and Lactobacillus helveticus indicates poor growth in MRS medium of pH 3 but, all the three Lactobacillus brevis shows good tolerance to bile. The isolated Lactobacillus helveticus only shows the growth at the pH 4.5-6.5, which is also not tolerate 0.3% bile (ox gall) containing medium.

1. Introduction

Probiotics are defined as live microorganisms which, when administered in adequate amount confer health benefits to the host (FAO/WHO, 2002). Though the probiotic bacteria have been utilizing by people with their traditional or common foodstuff for ancient times, its benefits and brand values are become popular in the current trends of growing demand on probiotic functional food, beverage and dietary supplements due to rising level of health consciousness and consumer awareness. Lactic acid bacteria especially Lactobacilli are most commonly use as probiotics in commercial foods. As a potential probiotics for human digestive tract it should be compatible to different physiological and biochemical environment of human digestive system, this includes, tolerance to high acidic condition of stomach and intestine, resistance to bile and pancreatin, capability of adherence to intestinal epithelial cells, antimicrobial effect on certain pathogens etc [4].

Fermented bamboo shoot (Gaj tenga and khorisa tenga) is a popular ethnic food of Assam having its typical flavor and aroma. Many lactic acid bacteria are associated with the fermentation of this indigenous product. Bambusa balcoona (bholoka banh), Bambusa teres (kako banh), Teinostachyum dalloa (dolo banh), Bambusa pallid (bijuli banh) are some selected species of bamboo which are commonly used for fermentation purpose in our study area. Though bamboo have worldwide distribution of over 1250 species all over world [28], its predominance and utilization as consumable product is mostly seen in South East Asian Countries. It is consumed predominantly by different ethnic communities of Northeast India with current popularization about its
deliciousness to all other parts of India. Fermented bamboo shoot also commercially available as canned and packaged food and well known ingredient used in numerous Japanese, Chinese, Taiwanese and other South East Asian cuisine[28]. In Northeastern states it is commercially available randomly in local daily markets, road sides of tourist places, and in some commercial zones. Popularity known as Gaj tenga or khorisa tenga (in Assamese ‘tenga’ means taste is sour) among the ethnic communities of Assam viz. Ahom, Sonowal Kochari, Chutia, Moran, Motok, Mising etc., it is exclusively prepared from young tender shoot of different species of bamboo, but the demand of the product and its particular flavor and aroma is species specific and limited to certain species. Bamboo shoots are immature, expanding portions of new culms that develop from the rhizome of bamboo plants. Young bamboo culms with compressed internodes and including a culm neck are generally harvested for fermentation or in generally for all other edible means. The nutritional value of bamboo shoot is based on its dietary fiber, lipid, fatty acid, protein, amino acid and several types of vitamin contents[31]. Some researcher had reported that bamboo shoot contain varying amount of cyanogens glycosides called tasyphyllin and β glycosidase may act on it to produce harmful hydrogen cyanide[20], therefore exclusion of it through boiling in water is an essential step before all edible use, that reduce the content of cyanide. Harvesting of bamboo shoot for the preparation of fermented product is done during the month of May and June in Assam, when new shoots are growing out. In the traditional preparation process of it at first young and tender bamboo shoots are collected and inner soft portion was taken by removing the bark sheaths then it is grinded or sliced into small pieces and put in a conical bamboo basket with the inner wall lined with banana leaves, or put in a plastic or glass container. After three or more weeks of fermentation process it is get ready to consume.

The lactobacilli are predominant type of bacteria that are used in different types of fermented foods as probiotics. These bacteria confers probiotic activity in the intestine of human, therefore presence of these microflora adds value in regular foodstuff that are beneficial for the health. Lactic acid bacteria are naturally occurring, anaerobic or facultative anaerobic gram positive micro flora that determines the flavour, texture and nutritive value of food and feed products with its fermentative properties[6]. The aim of the study was isolation and screening of potential probiotic lactobacilli from the collected samples, this connection isolated strains are tested for their survivability and growth responses to two stress factors that are low pH (pH 3) and 0.3% bile for the prediction of their basic probiotic properties. Following this, it may call on further rigorous works on targeted species, with emphasis of health benefits of their products and aggregated evaluation of quality of the samples and species specific lactobacilli.

2. Material & Methods

2.1. Sample

The present study was conducted by collecting fermented bamboo shoots of two different species of bamboo, Banbusa balooona and Bambusa teres (Bholoka and lako banh, respectively in Assamese). Both the samples belongs to Namrup area,(27.1836°N, 95.3469°E) Dibrugarh district of Assam, Northeast India. The associated data regarding the age of the sample, species of bamboo, preparation and preservation process etc. are gathered from the people of nearby communities of the study area, Namrup.

2.2. Culture condition & Isolation of Lactobacilli

Peptone water was used for harbouring the microflora from the sample. One gram of each sample was taken and fivefold serial dilution was done in peptone water and 0.1ml from each was taken and culture was done in Lactobacillus MRS Agar (Himedia). The culture plates were then incubated anaerobically in anaerobic jar with CO2 gas pack at 37°C for 48 hours. After 48 hours of incubation colonies were observed and randomly selected colonies were sub cultured in MRS agar plates by streak plate method. Pure culture of every isolates having the characteristics of gram positive, catalase negative & non-spore forming were done in separate plates.

All the isolates were tested for their acid production, acetonin production, and utilization of three sugar followed by the tests of MRVP & TSI Agar test. Temperature susceptibility of every isolates at different temperature (10°C, 15°C, 30°C, 45°C) were done by growing in MRS broth for 48 hours and growth was observed by turbidity measurement. Survivability in presence of 2%, 3%, 4% and 6.5% NaCl solution was tested by growing in MRS Broth for 48 hours in 37°C and observed by turbidity measurement. Effect of growth in different pH was observed by turbidity measurement, after culturing the every isolates at 37°C for 48 hours in MRS broth of different pH (3.5, 4.5, 6.5, 8.5 adjusted by 1N HCl).

For the test of utilization of different types of sugar, all the isolates were cultured in MRS broth (1% w/v) containing the following particular sugars L- arabinose, cellobiose, Mannitol, Mellobirose, Raffinose, Ribose, Sorbitol, Xyllose, Maltose, where devoid of glucose and beef extract with chlorophenol red as indicator. API 50 CH tests (bioMerieux) was also done for every isolates that concludes the overall presumptative in formations.

2.3 Probiotic activity test

2.3.1. Bile tolerance

Bile tolerance test was performed according to Jacobson et al. with modifications[12]. 0.3% (ox gall) bile containing MRS broth and control without bile was inoculated (1%v/v) from the overnight culture of each isolates and incubated at 37°C. Bacterial growth of each isolates were monitored by measuring absorbance at 600nm with a spectrophotometer with interval of one hour time between each measurement for 4 hours. After that tenfold serial dilution of uptol 07 were prepared using phosphate buffer saline, then 100μl of 104 to 107 dilutions of each sample was spread plated on MRS agar and incubated at 37°C for 48 hours. After incubation viability of bacterial sample was calculated by colony counting and tolerance to 0.3% bile was evaluated by comparing between log of CFU/ml grown in MRS with bile and without bile (as control).

2.3.2. Acid Tolerance

The acid tolerance was tested according to Ehrmann at al. with
modifications[12]. 0.3% (ox gall) bile containing MRS broth and control without bile was inoculated (1%v/v) from the overnight culture of each isolates and incubated at 37°C. Bacterial growth of each isolates were monitored by measuring absorbance at 600nm with a spectrophotometer with interval of one hour time between each measurement for 4 hours. After that tenfold serial dilution of upto107 were prepared using phosphate buffer saline, then 100μl of 104 to 107 dilutions of each sample was spread plated on MRS agar and incubated at 37°C for 48 hours. After incubation viability of bacterial sample was calculated by colony counting and tolerance to 0.3% bile was evaluated by comparing between log of CFU/ml grown in MRS with bile and without bile (as control).

2.3.2. Acid Tolerance

The acid tolerance was tested according to Ehrmann at al with modifications[13]. Overnight culture of isolated strains were inoculated in (1%v/v) into sterile MRS broth of pH 3 (adjusted by 1N HCl) and normal pH 7.2 (control), the initial bacterial concentration was 106 CFU ml-1 and was checked by viable count determination on MRS broth. Bacterial growth of each isolates were also monitored by measuring absorbance at 600nm with a spectrophotometer with interval of one hour time for 4 hours. After that cells were serially diluted upto 10 fold, in phosphate buffer (0.1M, pH 6.2) in order to neutralize the medium acidity. The residual viable count was determined by spread plating 100μl from 104 to 107 dilutions on MRS agar after 48 hours of incubation at 37°C. The survival rate was calculated as the difference in log of CFU/ml between plate grown from normal pH 7.2 and pH3.

3. Result

Out of the total eight isolates, of lactobacilli, isolate no 1,3,4, and 7 are predicted as Lactobacillus plantarum, isolates no2 as Lactobacillus helveticus and 5,6,8 are Lactobacillus brevis. Table1 shows the differentiating characteristics of all the isolates and their utilization of different types of sugar based on which identification was predicted.[41]

### Table 1. Physiological and biochemical characteristics of the isolates with the utilization of different types of sugar.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>bacilli</td>
<td>bacilli</td>
<td>bacilli</td>
<td>bacilli</td>
<td>bacilli</td>
<td>bacilli</td>
<td>bacilli</td>
<td>bacilli</td>
</tr>
<tr>
<td>Gram staining</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid production from glucose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aerobic production</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>45°C</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2: Viability of Lactobacillus strain (log CFU/ml) after 4 hour exposure to pH 3 and pH 7.2 (control).

<table>
<thead>
<tr>
<th>Lactobacillus strains</th>
<th>Cell viability log CFU/ml pH7.2</th>
<th>Cell viability log CFU/ml pH 3</th>
<th>Reduction in viability (log unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>7.56</td>
<td>7.42</td>
<td>0.14*</td>
</tr>
<tr>
<td>S2</td>
<td>7.99</td>
<td>7.84</td>
<td>0.15*</td>
</tr>
<tr>
<td>S3</td>
<td>7.60</td>
<td>7.48</td>
<td>0.12*</td>
</tr>
<tr>
<td>S4</td>
<td>8.07</td>
<td>7.78</td>
<td>0.29</td>
</tr>
<tr>
<td>S5</td>
<td>10.33</td>
<td>7.48</td>
<td>2.85</td>
</tr>
<tr>
<td>S6</td>
<td>7.91</td>
<td>7.64</td>
<td>0.34</td>
</tr>
<tr>
<td>S7</td>
<td>7.90</td>
<td>7.85</td>
<td>0.05*</td>
</tr>
<tr>
<td>S8</td>
<td>8.03</td>
<td>7.22</td>
<td>0.81</td>
</tr>
</tbody>
</table>
Spectrophotometric analysis

The graph of the fig.1 is depicting the growth responses of eight isolated lactobacilli strains at pH 3, as absorbance values were plotted against time of exposure to stress factor, that were taken at every interval of 1 hour for four hours. All the isolates are equally adopted to the stress of low pH in 1st hour of their growth but isolates no 2, 3 & 8 are unable to showing good responses of growth in the following hour of our observation time.

Figure 2: Growth responses of the isolates in 0.3% bile containing MRS medium.

3.1.2. Bile Salt tolerance

Viable Count

All the isolated lactobacilli shows good tolerance to 0.3% ox gall solution except strain no 2 Lactobacillus helveticus shows poor growth at 0.3% ox gall. Other isolates 1,3,4, and 7(Lactobacillus plantarum) and 5, 6, 8 (Lactobacillus brevis) shows reduction in viability in less than 0.14 log unit, that conclude to be susceptible to 0.3% ox gall.

Figure 2: Growth responses of the isolates in 0.3% bile containing MRS medium.

<table>
<thead>
<tr>
<th>Lactobacillus strains</th>
<th>Cell viability log CFU/ml</th>
<th>Viability in ox gall</th>
<th>Red (log unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>7.96</td>
<td>7.99</td>
<td>-0.03*</td>
</tr>
<tr>
<td>S2</td>
<td>8.17</td>
<td>7.70</td>
<td>0.47</td>
</tr>
<tr>
<td>S3</td>
<td>7.67</td>
<td>7.75</td>
<td>0.12*</td>
</tr>
<tr>
<td>S4</td>
<td>8.17</td>
<td>8.11</td>
<td>0.06*</td>
</tr>
<tr>
<td>S5</td>
<td>8.03</td>
<td>7.99</td>
<td>0.04*</td>
</tr>
<tr>
<td>S6</td>
<td>8.51</td>
<td>8.37</td>
<td>0.14*</td>
</tr>
<tr>
<td>S7</td>
<td>8.11</td>
<td>8.10</td>
<td>0.01*</td>
</tr>
<tr>
<td>S8</td>
<td>7.99</td>
<td>7.85</td>
<td>0.14*</td>
</tr>
</tbody>
</table>

Spectrophotometric analysis

The graph of the figure 2 is depicting the growth responses of eight isolates lactobacillus strains at 0.3% bile, that was taken at every interval of 1 hour time for four hours. S2 shows uniform growth throughout observation time.

4. Discussion

Fermented bamboo shoot may be consider as one of the rich source of potential probiotics. It is only traditionally produced by local communities, though more or less amount of packaged items as pickle and raw, unprocessed, fermented form, putting in container are seen in the feather regions of our study. This food items have enormous commercial possibilities in Northeastern region due to the growing popularity of its deliciousness. According to Tomar et al. noted lactobacilli available in fermented bamboo shoots are, Lactobacillus plantarum, L. brevis, L. Corniformis, L. fermentum, Leuconostoc fallax, Lactococcus lactis, L. mesenteroides, Enterococcus durans, Streptococcus lactis, L. Casei [35]. Dey and Halami in 2016, reported a dominant strains of lactobacillus plantarum in Mesu: a nonsalted fermented bamboo shoot product from Sikkim, India that have probiotic potentiality to becomes commercial application[36].

"The guidelines for the evaluation of probiotics in food" (FAO/WHO, 2002) reports invitro tests for probiotic screening of lactobacilli includes, resistance to gastric acidity, bile acid resistance, adherence to mucus and/or human epithelial cells and cell lines, antimicrobial activity against potentially pathogenic bacteria, ability to reduce pathogen adhesion to surfaces and bile salt hydrolase activity[4]. pH 3 is the standard pH for Acid tolerance test of probiotics as reference of the result of the studies of researcher Prasad, Chan, Liang and Shah[38-40]. In our study all the isolated Lactobacillus plantarum shows good response in the in-vitro gastrointestinal performance test, except isolates No4 did not saw well growth at low pH and 0.3% ox gall is not suitable for the isolate No 2.Lactobacillus helveticus that shows much difference in viability in comparison to control medium. The isolated strain no 5, 6 and 8 Lactobacillus brevis also show well growth at 0.3% ox gall containing medium, but in the medium of pH 3, these three isolates shows drastic reduction in survivability. Therefore from our observations and based on the references and result of our study the Lactobacillus plantarum is only suitable species that may considered as probiotics related to those fermented bamboo shoot products samples that we have collected which are prepared from both the species of bamboo ie. Banbusa balcoona and Bambusa teres, but further investigation is required.
5. Conclusion

In Assam fermented bamboo shoot is popularly known as Gaj tenga, Banh tenga, and dhorisa (prepared with additives as pickle), in our study area which are mainly prepared from four species of bamboo that are Bambusa balcoona (bholoka banh), Bambusa teres (kako banh), Teinostachyum dalloa (doto banh), and Bambusa pallid (bijuli banh). Gaj tenga prepared from Bambusa tulda (jati banh), Bambusa nutans (Mokal banh) is not suitable due to its bitter taste, which may be due to the presence of high amount of cyanogens glycoside, tannins, and other inhibitors. Fermented Bamboo shoots is a high moisture (88.8%) containing product, having low fat and cholesterol (0.5%), high in carbohydrates(5.7%), Protein( 3.9%) and dietary fibers, in addition with mineral(1.1%), vitamin, and antioxidant such as flavones, phenols, steroids etc[28]. It was reported that bamboo shoots have the decreasing activity of total serum and serum LDL cholesterol in rat by 16.1mg/dl [28]. It contain 17 different types of amino acid, 10 different types of minerals viz. Co, Cr, Zn, Mn, Mg, Ni, Co, Cu. Presence of amino acid lysine in it induce the growth and development of children[30]. Germaclinian reported that shoot are also known to carry antiaging capacity[28].

The collected fermented bamboo shoots of both two species, Bambusa balcoona (bholuka banh) and Bambusa teres (kako banh) both have good sensory quality and taste, the Bambusa balcoona have a special flavor. The specific aroma of the product may be related to the specific biochemical composition of that particular species. The dominance in numbers of isolates also gives us satisfactory conclusion that our material of study i.e. both the samples of species of Bamboo, we have found variation in potentiality. There is no single isolates which may conclude to become fully tolerate to all other three types of isolates against both the stress factors. Its dominance in numbers of isolates also gives us satisfactory conclusion that our material of study i.e. both the samples of fermented bamboo shoot, one is prepared from Bambusa balcoona (bholoka banh) and another from Bambusa teres (kako banh) are rich in potential probiotics bacteria as Lactobacillus plantarum but its further in-details molecular characterization and more rigorous probiotic analysis need to be done.

Acknowledgement

The research was supported by DBT Government of India through Institutional Biotechnology Hub at Namrup College. The author gratefully acknowledges the corporation receives from the people of the different communities of Namrup, for providing the sample and associated data regarding, production, preparation, preservation of the samples, types of species, and views about taste and importance of particular sample.


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