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Veerapagu M

Department of Biotechnology,
P.G. Extension Centre,
Bharathidasan University,
Kurumbalur, Perambalur, Tamil
Nadu, India

Jeya KR

Department of Biotechnology,
Bharathidasan University
Constituent Model College (W),
Veppur, Perambalur, Tamil
Nadu., India

Evaluation of probiotic characteristics of bacteria isolated from fermented food

Veerapagu M and Jeya KR

Abstract

Lactic acid bacteria are a major part of the probiotics, microflora of the intestine and of fermented dairy products, and are found in a variety of environments. In this study kambu koozh (pearl millet) was used as a source to isolate bacteria by serial dilution method. The isolate was identified as *Enterococcus* sp. by morphological, cultural and biochemical characteristics and evaluated for probiotic characteristics acid tolerance, bile tolerance and tolerance to digestive enzymes pepsin and trypsin. Acid tolerance of the *Enterococcus* sp ranges from 80% (pH5) to 69% (pH2) survivability for 1 hr and 68% (pH5) to 52.0% (pH2) survivability for 2hr. Bile salt tolerance of *Enterococcus* sp. was 69% survivability at (1%w/v) to 51.0% survivability (4%w/v) bile salt concentration for 24 hrs. Tolerance to digestive enzymes showed 78.6% to 35.7% survival (2g/L - 10g/L) pepsin and 68.9% to 13.8% survival (2g/L - 10g/L) trypsin for 2 hrs.

Keywords: Probiotic, *Enterococcus*, Koozh, acid tolerance, pepsin, trypsin

1. Introduction

Probiotics are defined as viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human organism through their effects in the intestinal tract^[1].

The physiological effects related to probiotic bacteria include the reduction of gut pH, production of some digestive enzymes and vitamins^[2], production of antibacterial substances, e.g., organic acids, bacteriocins, hydrogen peroxide, diacetyl, acetaldehyde, lactoperoxidase system, lactones^[3] and other unidentified substances^[4], reconstruction of normal intestinal microflora alter disorders caused by diarrhoea, antibiotic therapy and radiotherapy, reduction of cholesterol level in the blood, stimulation of immune functions, suppression of bacterial infections, removal of carcinogens, improvement of calcium absorption as well as the reduction of faecal enzyme activity^[5].

Lactic acid bacteria (LAB) are "milk souring" or "lactic acidproducing" bacteria. They are Gram-positive, catalase-negative bacteria which grow under microaerophilic to strictly anaerobic conditions, non-spore-forming, mainly nonmotile rods or cocci in shape^[6]. The important genera of LAB are *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Weissella*, *Carnobacterium*, *Tetragenococcus* and *Bifidobacterium*^[7-9]. Lactic acid bacteria are used as probiotics. This group of bacteria has a particular interest for food industries due to their technological properties, being often used as starter cultures to produce fermented products^[10]. Many reports have shown that traditional fermented foods are rich sources of LAB with probiotic characteristics^[11-13].

Koozh is a traditional fermented Porridge food made from millet in South India. Koozh is beverage either made from finger millet-*Eleusine coracana* (*Kezhvaragu*) or pearl millet-*Pennisetum glaucum* (*Kampu*) flour and broken rice^[14]. Traditionally, *koozh* is considered nutritious and health promoting, but there is little scientific documentation on its nutritive or microbial composition. The aim of this study was to isolate and evaluate the probiotic characteristics of lactic acid bacteria from South Indian fermented food koozh.

2. Materials and methods

2.1 preparation of south Indian fermented food

Koozh: About 300 gm of Kambu (pearl millet) flour was mixed with 250 ml of water and allowed to ferment at room temperature for two days.

Correspondence

Veerapagu M

Department of Biotechnology,
P.G. Extension Centre,
Bharathidasan University,
Kurumbalur, Perambalur, Tamil
Nadu, India

2.2. Isolation of probiotic bacteria

Probiotic bacteria were isolated from the south Indian fermented food Koozh. After incubation period the sample (Koozh) was serially diluted in normal saline. Then 0.1ml from the appropriate dilution was plated on MRS (de Mann Rogosa Sharpe) agar and incubated at 37 °C for 2-3 days. The isolated colonies were purified by repeated streaking on MRS agar. The selected isolates were grown on MRS broth and stored at 4 °C until further use.

2.3 Identification of the probiotic bacteria

The bacterial isolate was identified by morphological, cultural and biochemical characteristics upto the genera level. Morphological characterization shape, arrangement and gram's nature of the isolates were studied by gram's staining technique [15, 16]. Salt tolerance cultural characteristics and biochemical test such as Indole test, MRVP test, citrate test and catalase tests were performed for identification [17-19].

2.4 Evaluation of probiotic characteristics of the isolates

2.4.1 Low pH Tolerance

The probiotic bacterial isolate was grown in MRS broth for overnight. Five different 10ml of MRS broth was prepared and pH was adjusted to 2.0, 3.0, 4.0 and 5.0 with 1N HCL. To this 1%v/v of overnight culture was added to each medium separately. The cultures were incubated at 37 °C for two hours. The survival rate was calculated as the percentage of cells grown on MRS plate compared to the initial concentration.

% Survival = number of viable cells survived/ Number of initial viable cells × 100

2.4.2 Bile Salt Tolerance

The probiotic bacterial isolate was grown in MRS broth for overnight. Five different 10ml of MRS broth containing 1%, 2%, 3% and 4% w/v bile salt was prepared. To this 1%v/v of overnight culture was inoculated to each medium separately. The cultures were incubated at 37 °C for 24 hrs. The number of viable bacteria was counted after 0 h and 24 h incubation periods on a MRS agar plate. Triplicates of each sample were performed. The survival rate was calculated as the percentage of cells grown on MRS plate after 24 hrs compared to the 0 hrs of incubation.

2.4.3 Tolerance to digestive enzymes

The lactic acid bacteria of probiotic microorganisms and its ability to reach and survive in the environmental conditions are important. The bacterial suspension (10^6 cfu/mL of the isolate) was prepared with sterile PBS containing trypsin of 2, 4, 6, 8, 10 g/L at pH 6.8, respectively. Tolerance for pepsin juice was determined using a bacterial suspension (10^6 cfu/mL of the isolate) in sterile PBS containing pepsin of 2, 4, 6, 8, 10 g/L respectively at pH 3.0, respectively and incubated for 2hrs. After incubation each bacterial suspension was spreaded on the surface of the MRS agar and then viable cell was counted.

3. Results and discussion

India is traditionally rich in fermented foods. Pearl millet, a traditional south Indian food is also consumed in the fermented form as *koozh* in rural and urban households [20]. Koozh was prepared in the laboratory. It was serially diluted in normal saline after incubation. Probiotic bacteria were isolated from diluted sample on MRS agar plate.

3.1 Identification solation of bacterial isolates

The bacterial isolates were identified by morphological and biochemical characteristics. The isolates were gram positive cocci arranged in pairs. The Growth was observed on nutrient broth containing 6.5% NaCl. The results of the test are in the table 1. The isolate was identified to be *Enterococcus* sp.

3.2 Evaluation of probiotic characteristics of the isolates:

3.2.1 Low pH Tolerance

For the characterization of probiotic strains they should survive in conditions of the gastrointestinal tract, so the survival at the variable pH environment condition is necessary for the strains. The time from entrance to release from the stomach has been estimated to be approximately 90 min with further digestive processes requiring longer residence time [21]. Most of the bacteria do not survive well at low pH (fig.1&2). Acid tolerance of the *Enterococcus* sp. ranges from 80% (pH5) to 69% (pH2) survivability for 1 hr and 68% (pH5) to 52.0% (pH2) survivability for 2hr of incubation. The result indicated that pH value could have the key effect on the isolated strain and the viable counts of the isolate came down with pH value decreasing, but the isolate still could survive well in acid condition.

A similar study was reported by Handa and Sharma [22], who studied the effects of low pH (1, 2 and 3) on the viability of the *L. plantarum* F22 showed remarkable survival of 90.4% after 180 min at pH 1.0, whereas, at pH 2.0 and 3.0 it showed survival of 97.2% and 99.4%. Todorov *et al.* [23] also reported good growth of *L. lactis* sp. *Lactis* HV219 in MRS broth with initial pH ranging from 6.0 to 11.0 over the first 10 h of incubation, compared to slow growth at pH 3.0–5.0. Acidity is believed to be the most detrimental factor affecting growth and viability of *lactobacilli*, because their growth was down significantly below pH 4.5.

3.2.2 Tolerance to Bile

Tolerance to bile is important for the probiotic strains to grow and survive in the digestive tract. Colon region harbours around 500 species of bacteria that are involved in fermenting the foods and in releasing bioactive compounds. There are several reports that suggest that bile tolerance is one of the important parameters to consider any lactic acid bacteria as probiotic and the tolerance to bile allows lactic acid bacteria to survive in the small intestine. When bacteria was supplemented with bile the cellular homeostasis disruptions causes the dissociation of lipid bilayer and integral protein of their cell membranes, resulting in leakage of bacterial content and ultimately cell death [24]. In this present study the bile tolerance of the selective bacteria was performed. The probiotic bacteria *Enterococcus* sp. was able to tolerate bile salts up to 4% for twenty four hours (Fig. 3). Bile salt tolerance of *Enterococcus* sp. was 69% survivability at (1%w/v) to 51.0% survivability (4%w/v) bile salt concentration. Similarly Srinu *et al.* [25] reported that six lactic acid bacteria showed good survivability at high bile salt concentration (0.3 to 2.0%). Boke *et al.* [26] had shown bile salt tolerance at 0.3 % bile concentration of two strains of *L. delbrueckii* subsp. *bulgaricus*. *L. delbrueckii* subsp. *bulgaricus* (B3 and G12) strains showed survival of 36% and 33%, respectively.

3.2.3 Tolerance to digestive enzymes

The survival of the bacterial isolate in pepsin and trypsin was evaluated by incubating it at different concentration of 2, 4, 6,

8,10 g/L for two hours. The number of viable bacteria could maintain at 10^6 cfu/ml. Different pepsin concentrations had an effect on the survival of the bacteria. When the pepsin concentration was 10 g/L, the viable counts of the isolate decreased to 2.5×10^6 cfu/ml, compared to the control 7×10^6 cfu/ml (Fig 4) and showed 78.6% survival at 2g/L pepsin to 35. 7% survival at 10g/L pepsin. Similarly when the trypsin concentration was 10 g/L, the viable counts of the isolate decreased to 4.0×10^6 cfu/ml, compared to the control 29×10^6 cfu/ml (Fig 5) and showed 68.9%, survival at 2g/L trypsin to 13.8% survival at 10g/L trypsin. Gajbhiye [27]. Exposed lactic acid bacteria isolated from aerial surfaces of pomegranate to digestive enzymes pepsin and trypsin showed showed 60-70% survival in the presence of trypsin whereas in presence of pepsin the survival decreased in between 50 and 60%.

4. Conclusion

From the present study it could be concluded that *Enterococcus* sp isolated from South Indian fermented food koozh exhibited probiotic characteristics such as of acid tolerance, bile tolerance and tolerance to digestive enzymes and could be used as probiotics in the food and dairy industry for commercial use.

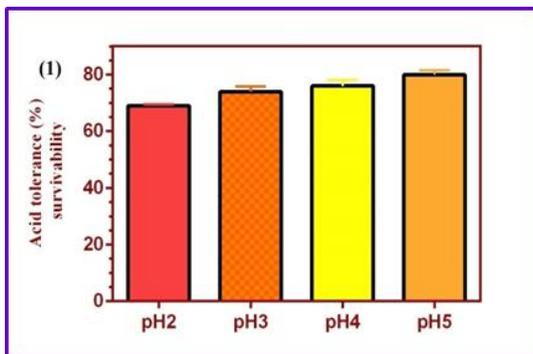


Fig 1: Acid tolerance of *Enterococcus* sp. 1 hour.

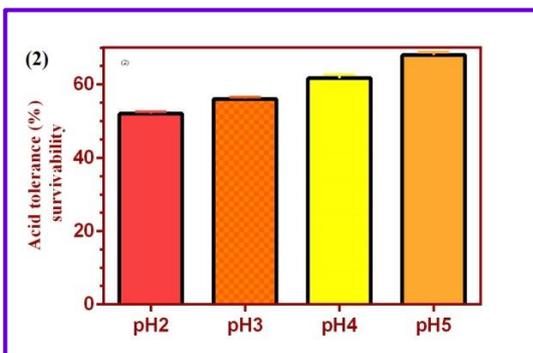


Fig 2: Acid tolerance of *Enterococcus* sp. 2 hour.

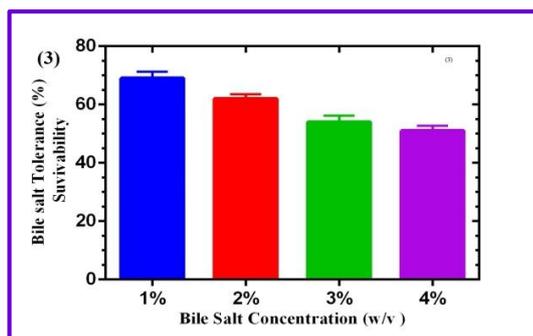


Fig 3: Bile salt tolerance of *Enterococcus* sp.

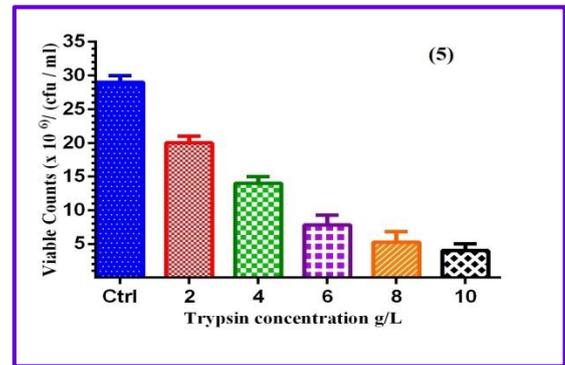


Fig 4: Tolerance to digestive enzyme pepsin of *Enterococcus* sp.

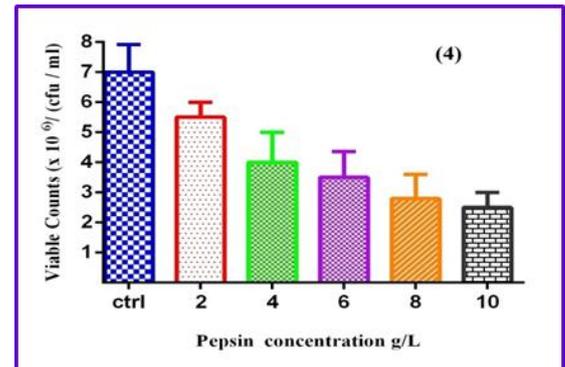


Fig 5: Tolerance to digestive enzyme trypsin of *Enterococcus* sp.

Table 1: Morphological and biochemical of characteristics of the isolate

Test	Isolate
Grams reaction	Gram Positive
Shape and arrangement	Cocci, arranged in pairs
Salt Tolerance	Growth
Indole	Positive
MR Test	Positive
VP Test	Negative
Citrate test	Positive
Catalase	Negative

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