Isolation and characterization of probiotic microorganism from fermented dairy products

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Abstract

Gut microflora is considered very important for maintenance of human health. The inherent growth of these microorganisms is also related to diet. Lactobacillus and bifidobacteria are of prime importance in this respect. Milk and fermented dairy products are considered as very good source of these microorganisms. Thus, an investigation is carried out to isolate and characterize a potential probiotic bacterium from some commonly consumed food materials. In the present study a bacterium was obtained from fermented dairy product was identified as *Lactobacillus casei*. The isolate showed potential as a probiotic owing to its antibiotic resistance, antimicrobial potential and ability to survive under acidic environment.

Key words: Dairy products, probiotics, antibiotic resistance, bioactive metabolites

Introduction

The fitness of food has been related to a nutritionally rich diet suggested by specialists and the role of it in totality has been emphasized instead of emphasizing on its components. During the past few decades, the lifestyle has been changing fast with respect to living standards, diet, hygiene and usage of antimicrobial molecules. The prevalence of chronic diseases like allergies and gut-associated disorders (e.g. Crohn’s disease, Ulcerative Colitis and Inflammatory Bowel Syndrome) are of rising importance in the world now days.

The microbial ecology in the gastrointestinal tract influences many functions in our body. These are digestion, absorption of nutrients, detoxification. It is finally affecting the functioning of immune system. Hence, the balance in microbiota of gut is focused to provide the colonization resistance against infectious agents and to promote anti-allergic processes and to reduce hypersensitivity. The oral administration of these bacteria (a probiotics approach) helps to maintain microbiota balance and also prevent the disease of gastrointestinal tract. To be considered a probiotic a strain should be able to colonize the gastrointestinal tract and promote host health through its metabolic activities. Specifically, probiotics should survive acidic conditions of the stomach and resist hive level of bile salts and adhere to gut epithelium. Gusils et al. found that probiotics can be...
administered to prevent infectious diseases, to strengthen the barrier function of the gut microflora and for a non-specific enhancement of the immune system. FAO/WHO (7) recommends testing of antibiotic resistance and bile salt conjugation ability property of the probiotic microorganism to be characterized. Similarly probiotic must confer a health benefit to the host and it is necessary to check its antimicrobial activity against human pathogens (8). A wide range of microorganisms have been used as probiotics. The most commonly used organisms in probiotic preparations are lactic acid producing bacteria such as lactobacilli, streptococci, Bifidobacteria, Bacillus spp. and fungi like Saccharomyces cerevisiae, Sacharomyces boulardii and Aspergillus oryzae (9; 10). However, lactic acid bacteria (LAB) have attained major attention for probiotic activity and have generally been considered as good probiotic organisms (11). Thus, considering the importance of fermented dairy products in human health and the role of Lactobacillus spp. to obtain their benefits the present study was undertaken to isolate and characterize probiotic microorganisms from food and dairy products.

Materials and Methods

Isolation of probiotic microorganism

Milk, fermented dairy products (curd, buttermilk, cheese) and vegetable pickle were used as a source to isolate probiotic microorganisms. The isolation was done by direct plating and enrichment techniques on MRS (deMan, Rogosa and Sharpe) agar and in MRS broth respectively. These samples were serially diluted up to $10^{-4}$ in 9 mL saline water (0.89% NaCl) and spreaded over petriplates containing MRS semi-solid media. The inoculated petriplates were incubated at 37 °C for 48h along with control (sterilized distilled water) to check the presence of zone of inhibition of indicator strain growth.

Results and Discussion

Isolation and identification of probiotic microorganism

The inoculated samples were appeared with microbial colonies on MRS agar plates. Their growth is envisaged as follows (Table 1).

The isolate of curd sample (Isolate A) was found Gram positive and did not show catalase activity. The isolate did not form endospore under unfavorable conditions but able to ferment Glucose and Mannitol without gas production. The isolate was identified as Lactobacillus casei on the basis of above morphological and biochemical features. The isolates of buttermilk sample (Isolate B) were found Gram positive, catalase negative and able to ferment Glucose. Cheese, Pickle, milk sample was found with Gram negative isolates (C, D, E respectively) and hence did not processed for further study. All milk samples were also observed with the presence of yeast cells. The presence of yeast is possibly a result of contamination from udder skin as previously mentioned by Hamed et al. (14). Among these, the isolate of curd sample i.e. isolate A was selected for antibiotic susceptibility, production of bioactive compounds and tolerance test to know its probiotic potential.

Antimicrobial production

The antimicrobial production of selected isolate was determined by following Bilkova et al. (13). E. coli was used as an indicator strain. The culture supernatant was harvested after time intervals 21h, 24h, 27h, 30h of inoculation. Above supernatant was centrifuged and digested with proteinase followed by heat treatment. 200µL of it was placed in to wells of plates containing E. coli lawn and were incubated at 37°C for 48h along with control (sterilized distilled water) to check the presence of zone of inhibition of indicator strain growth.

Acid sensitivity test

The ability of the isolated bacteria to grow in the acidic environment was checked by checking its growth at various pH in MRS broth. The OD values at 600nm were determined after 24h of incubation at 37°C at 150rpm. The acid sensitivity was determined by comparing the bacterial growth with a growth in MRS broth of pH 7.

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Table 1: Morphological and Biochemical Characterization of the Isolate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Isolate</th>
<th>Gram staining</th>
<th>Shape</th>
<th>Endospore</th>
<th>Catalase formation</th>
<th>Gas formation</th>
<th>Acid Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curd</td>
<td>A</td>
<td>+</td>
<td>Rods</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Buttermilk</td>
<td>B</td>
<td>+</td>
<td>Rods</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Cheese</td>
<td>C</td>
<td>_</td>
<td>Cocci</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Pickle</td>
<td>D</td>
<td>_</td>
<td>Cocci</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Milk</td>
<td>E</td>
<td>+</td>
<td>Cocci</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

N.A. means: The above identification tests were not performed.

Antibiotic susceptibility Test

The overnight grown culture of curd isolate (*Lactobacillus casei*) was inoculated in the presence of various antibiotics in a concentration 25, 50, 100, 200, 250, 500µg/mL in MRS agar medium along with control (MRS agar without antibiotic). Their growth was checked after 48h of incubation at 37°C. The bacteria showed following results (Table 2).

Table 2: Antibiotic susceptibility Test of the Isolate *Lactobacillus casei*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Control</th>
<th>Concentrations (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>+</td>
<td>25 50 100 200 250 500</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>+</td>
<td>25 50 100 200 250 500</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>+</td>
<td>25 50 100 200 250 500</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>+</td>
<td>25 50 100 200 250 500</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>+</td>
<td>25 50 100 200 250 500</td>
</tr>
</tbody>
</table>

+ means that the bacterial growth appeared on MRS agar plate.
- means that the bacterial growth was not observed on MRS agar plate.

The antibiotics used for this study were inhibitor of cell wall synthesis and protein synthesis. The isolate was insensitive to penicillin, streptomycin, gentamycin, vancomycin up to 500µg/mL concentration. The isolate showed resistance to tetracycline up to 100µg/mL concentration but higher dose of it imposes toxicity on the microorganism. The isolate was found resistant to cell wall synthesis inhibitor i.e Penicillin, Vancomycin and protein synthesis inhibitor Gentamicin, Streptomycin but sensitive to tetracycline at higher concentration 200µg/mL and above. The results of antibiotic susceptibility test with vancomycin and streptomycin were different from the observation made by Kim et al (15). Hoque et al (16) found that the *Lactobacillus* spp. are sensitive to gentamycin and resistant to tetracycline. It is possibly because of β lactamase presence in the isolate which is known to cause antibiotic resistance towards penicillin and other cell wall synthesis inhibitory compounds. Further it may be attributed that the presently studied strain is a mutant one or they might have used higher concentration of antibiotic. Similar results with tetracycline were also observed by Coppola et al. (17), Lira et al. (18), Caro et al. (19) and Hleba et al (20) where they examined antibiotic resistance of bacteria isolated from various food samples have argued that the results of antibiotic resistance vary from study to study.

Antimicrobial production test

Further the isolate was checked for the presence any bioactive molecule production. Initially the growth curve...
was prepared to know the stationary phase in its life cycle. The growth curve showed the bacterial stationary phase comes after 18h of inoculation. The *E.coli* plates showed different diameter of zones of clearance viz. 2mm after 21h, 5mm after 24h and 3mm after 27h around the isolate supernatant.

The control well did not show any kind of inhibition. The zone of clearance shows the inhibition of growth of indicator strain by the isolated *Lactobacillus casei*. It indicates the presence of bioactive molecules by the *Lactobacillus* culture. It has been attributed to production of organic acids or hydrogen peroxide or bacteriocins or any other inhibitory substances by bacterial cell as described in earlier report of Sieladie et al (21).

### Acidity sensitivity test

The growth of isolate in MRS broth of various pH showed following results (Table 3). The ability to grow at a pH 4 clearly says that the isolate is quite stable in acidic conditions. It has been attributed due to its ability to ferment lactose and production of lactic acid.

**Table 3: Growth of the isolate in MRS broth of various pH**

<table>
<thead>
<tr>
<th>pH</th>
<th>OD 600 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.891 ± 0.06</td>
</tr>
<tr>
<td>6</td>
<td>0.720 ± 0.06</td>
</tr>
<tr>
<td>5</td>
<td>0.650 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>0.564 ± 0.05</td>
</tr>
</tbody>
</table>

### Conclusion

The present study was aimed to isolate and characterize probiotic microorganism from fermented food samples. A bacteria from curd was identified as *Lactobacillus casei*, was selected for initially as a probiotic strain. The isolate showed antibiotic resistance towards very commonly used antibiotics. Moreover it is able to tolerate the acidic conditions of the environment and produces antimicrobial compounds. Thus the isolated bacteria can be exploited as a probiotic one after investigating its other health promoting features in details.

### References

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