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Research Article

Isolation and Identification of Probiotic Bacteria to Improve Animal Health and Production

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Abstract

The objective of present research was to isolate the Lactic acid bacteria as potential probiotic from animal gut. A total 10 bacterial strains were isolated from animal gut. These are mostly, non-motile, gram positive, catalase negative. All strains showed good antimicrobial activity. *Enterococcus Faecalis* (NMCC-7) bacterial strains showed best probiotic potential than any other strain. Our results demonstrated that the *Enterococcus Faecalis* (NMCC-7) is a unique bacterium for animal feed supplements.

Keywords: Probiotics; *Enterococcus Faecalis*

Introduction

Probiotics are living microorganisms such as bacteria which can provide very useful health promoting effects for the host in which they live. The most common and useful probiotics are Lactic acid bacteria and bifidobacterial which are isolated from the fermented products, human breast milk, and digestive systems. For further work many researchers have started their work on buffalo's milk to look at other potential sources of probiotics for health promoting effects. There are many useful nutritional benefits from some species of lactic acid bacteria. These improved nutritional values of food, control of intestinal infections, control of cancer, and control of serum cholesterol level can also produce antimicrobial products. LAB also used to ferment or culture foods for at least 4000 years. LAB used for fermentation of milk products including yoghurt, koumiss, cheese and buttermilk. In Pakistan according to 1994 report the production of milk for human use is approximately 71% from buffaloes, 24% from cows and 5% from camel and other species. Buffalo milk plays a very significant role in all over the world for satisfaction of nutritional demands of peoples in all over the world specially in developed countries because it contain Lactic acid producing bacteria like *lactobacilli bacteria's* such as *L. acidophilus, L. delbrueckii* and *L. helveticus bulgaricus, Lactococcus lactis* ssp. *cremoris, L. Lactis* ssp. *lactis*, and *Streptococcus thermophilus*. LAB grew more rapidly in buffalo's milk than others because buffalo's milk supported to its growth more than any other animal. Lactic acid bacteria were isolated from the buffalo's milk

by culturing its milk on a specific supported media by this way we obtained pure culture. Purification is confirmed by gram staining and after purification it is identified by different biochemical tests. Ability of each strain was tested for conversion of lactose which is found in the milk into lactic acid. 66% lactose was converted by *S. lactis* 20 and 74% lactose from the *L. acidophilus* into lactic acid respectively. But freeze drying slightly decreased cell count. Those strains which are isolated from the buffalo milk are used to prepared starter culture. By using Starter culture camel and buffalo cheese were prepared. The strains isolated from buffalo milk were best for acid production and coagulated the milk in less time. It is concluded that we can obtained lactic acid from the lactose of buffalo milk and cheese can be prepared successfully from buffalo milk and better results can be obtained by coagulating milk with starter culture.

Materials and Methods

Sample collection and processing

For the collection of fecal sample, fecal was collected in sterile bowls and stored in ice until delivered to the laboratory. A fecal sample from different buffaloes was further named as bowl 1-20 respectively. The samples were brought to the laboratory after collection and used for the isolation of probiotic bacteria.

Isolation of pure bacterial colonies from fecal sample

For the isolation of bacteria first of all MRS media was prepared and autoclaved. In the next step make the serial dilution with the help of PBS buffer. After pouring the media spreading of milk sample was done on the media plates and incubate the plates at 37C for 48 hours in incubator. After the incubation period, the growth appeared on the plates were purified. Pick the single colony from the isolates and streak out on freshly prepared plates of media with the help of platinum red hot inoculating loop.

Morphological characteristics bacteria from fecal sample

For gram staining one drop of distilled water was taken on a glass slide and with the help of red hot platinum loop picks a single colony from the Petri dish and makes a smear. After making the smearing air dry the slide and further use for staining.

Four important dyes of gram staining are

- Crystal violet for 1 minutes and then wash the slide with distilled water
- Gram iodine for 1 minutes and then wash the slide with distilled water
- Gram decolorizer for few seconds approximately 1-5 seconds and wash with distilled water
- Safranin for 30 seconds and then wash it with distilled water.
- After air drying observe the slides for microscopy
- For microscopy use one drop of emulsion oil on a glass slide and then visualize the morphology of the bacteria

Biochemical characterization of bacteria from fecal sample

Catalase test

- Transfer small quantity of culture from the plates on glass slide, add one drop of 3% H2O2 and observe bubble formation.
- Bubble formation indicated catalyze positive.
- No bubble formation indicated catalase negative.

Oxidase test

- Take an Oxidase disc in clean microscopic slide, pasted the culture on the Oxidase disc and observe for color changes.
- In oxidase test use NNNN-tetra methyl-p-phenylenediamine an Oxidase Reagent
- If no color appears it means it is oxidized and if purple color appears it means it is reduced.

Molecular identification of bacterial strains by 16S rRNA gene sequence

16S rRNA gene sequence was applied for the identification of the bacterial strains. The bacterial sequence was obtained from Macrogen Korea was identified through EXBioCloud service. The identified sequence was submitted to NCBI for accession numbers. The phylogenetic tree of the isolated strain was made through MEGA 7.

Results and Discussion

We have isolated 10 microbial strains and these strains shows from a morphological characteristics point of view that most of the strains are off white, creamy white, white creamy off-white color wise. And they have small round, puncti forms, round, small circular forms. There opacity was translucent, transparent and opaque. Elevation of the colonies was flat, slightly raise and elevated. There margins were entire and wavy. They all have smooth texture and no pigment was there. By the gram staining we found that all were gram positive and their shapes were rods and cocci. When catalase test was applied, they gave negative test. 6 out of 10 strains were antibiotic resistant (Figure 1; Table 1). As shown in Figure 1 the colonies of streaked bacteria are different. Some colonies are off-white, and some have creamy color. After fixing on glass slides, gram staining of slides was done, and these slides were examined under compound microscope. As it can be seen in Figure 2 all were gram positive and having rod shape. DNA of these strains was isolated, and PCR was done. Then identification with 16S RNA was done. Further analyses were done by preparing phylogenetic tree. The blastn search revealed that *Enterococcu faecalis* strain NMCC-7 (MK606082.1) had the highest sequence similarity with the *Enterococcu faecalis* strain NBRC 100480(NR 113901.1) [1-14].

Figure 1: Colony morphologies of bacterial isolates on MRS agar media.

Figure 3: Multiple sequence alignment using Clustal.

Figure 2: Microscopic observation of gram staining isolated bacterial stains.

Figure 4: Phylogenetic tree bacterial strain Bootsrap values can be seen at each node.

Lab ID	Colour	Form	Opacity	Elevation	Margins	Pigment	Texture	Gram staining	Catalase
1	White	Puncti form	Translucent	Flat	Entire	No	Smooth	Positive rods	-ve
2	Off white	Small round	Translucent	Flat	Entire	No	Smooth	Positive rods	-ve
3	Creamy white	Small round	Translucent	Slightly raised	Entire	No	Smooth	Positive rods	-ve
4	Off white	Small round	Translucent	Slightly raised	Entire	No	Smooth	Positive cocci	-ve
5	Creamy white	Round	Translucent	Slightly raised	Entire	No	Smooth	Positive rods	-ve
6	Off white	Small round	Opaque	Elevated	Entire	No	Smooth	Positive cocci	-ve
7	Creamy white	Small round	Opaque	Slightly raised	Entire	No	Smooth	Positive rods	-ve
8	Creamy off white	Small round	Translucent	Slightly raised	Entire	No	Smooth	Positive rods	-ve
9	Creamy white	Small round	Translucent	Slightly raised	Wavy	No	Smooth	Positive rods	-ve
10	Creamy white	Round	Translucent	Slightly raised	Entire	No	Smooth	Positive rods	-ve

Table 1: Morphological characterization of samples.

Conclusion

Identification of the probiotic bacterial strains is one of the circle steps for the preparation of novel probiotic product for animals and human use. Literature tells us that the latest molecular methods can provide the fast way for the identification of the bacteria from any source. The culture dependent (isolation, biochemical tests etc.) and culture independent methods (Direct DNA extortion from whole fecal sample) have been used for the identification of the best probiotic bacterial strains. In the present study, we used the molecular method 16s rRNA gene sequencing method from the bacterial isolation. We noted that the *Enterococcus faecalis* can be used a probiotic.

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