



Antagonistic effect of Lactobacilli of Camel Milk against *Aeromonas veronii* isolated from Pichola lake, Udaipur, Rajasthan, India

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Abstract

Water microbiology is concerned with the microorganisms that live in water. Water may possess many pathogenic bacteria, viruses, protozoa and parasites. They are responsible for the emerging most widespread infections which are leading cause of death worldwide. *Aeromonas veronii* is commonly found in freshwater systems. *Aeromonas* is gram negative, facultative anaerobic bacteria. It causes aeromoniasis in humans and is also pathogenic for aquatic and terrestrial animals. Pichola lake is one of the most beautiful lake of Rajasthan, India. It is an important source of potable water supply for the city. In the present study, an attempt has been made to isolate the lactobacilli from camel milk and detect the antibacterial activity of lactobacilli against previously isolated and identified *Aeromonas veronii* from lake Pichola. A total of 10 lactobacilli were isolated and subjected to agar well assay for detection of antibacterial activity. Out of them, 2 isolates showed demonstrable antibacterial activity against *Aeromonas veronii*. On the basis of biochemical and molecular characteristics these two isolates were identified as *Lactobacillus rhamnosus* CMU 14 and *Lactobacillus fermentum* CMU 17. Further investigation may reveal that these lactobacilli isolates could be used as potential probiotics for aquaculture and to manage aeromoniasis.

Keywords: *Aeromonas veronii*, Pichola lake, lactobacilli, antibacterial activity.

Introduction

Water is the vital source of our planet. The consumable water levels are up to 2.7% of the total water content reported by United nations (UN) in 1978¹. Water quality now is a concern for all countries in the world. Microbial contamination is the most important factor of water pollution especially with pathogenic organisms². The pathogenic organisms includes various types of bacteria, viruses and protozoans. They cause infection in humans as well as in aquatic animals. *Aeromonas* are commonly isolated from a variety of aquatic environments, including freshwater, estuarine, brackish, and salt waters. *Aeromonas veronii* is gram- negative, facultative anaerobic bacterium which causes disease in humans as well as aquatic animals³. Lake Pichola, situated in Udaipur city is an artificial fresh water lake. It is polluted due to the disposal of sewage directly into the lake water.

“Probiotics” defined as “mono or mixed cultures of live microorganisms which, when applied to animal, beneficially affect the host by improving the properties of the indigenous microflora”. The term “Probiotic” refers to gram-positive bacteria associated with the genus *Lactobacillus*⁴. One of the requirements needed for probiotic strains is that they should possess antibacterial activity against various pathogenic organisms. According to Yasuda and Taga⁵ LAB would be useful both as food and as biological control agents of disease and activators of the rate of nutrient regeneration in aquaculture.

Hence the present work was carried out to screen the antibacterial activity of lactobacilli isolated from camel milk against water borne pathogen *Aeromonas veronii*.

Material and Methods

Isolation and morphological characterization of lactobacilli: For isolation of lactobacilli, serial dilution technique was used. Serial dilution was done using the saline solution. Serially diluted samples were pour plated with the MRS medium. Isolated colonies were recovered from the plates after an incubation period of 48 h at 37^oC. The individual colonies were selected and transferred into sterile MRS broth medium and were further purified by successive streaking. Selected colonies were screened on the basis of gram reaction, morphology and catalase test.

Screening of lactobacilli for antibacterial activity: For screening of antibacterial activity against *Aeromonas veronii*, lactobacilli were inoculated to MRS broth and incubated at 37^oC for 24-48 h. The stationary cells were centrifuged at 3000 rpm for 20 min. The culture supernatant neutralized with and without 1N NaOH was tested for antibacterial activity by the agar well diffusion assay as described by Schillinger and Luke⁶.

Biochemical characterization of lactobacilli isolates: Biochemical identification was based on the phenotypic properties such as CO₂ production from glucose, hydrolysis of arginine, growth at different temperatures (15 and 45^oC). Fermentation of carbohydrate was determined using CHL medium (a basal media used for sugar fermentation test).

Molecular characterization of the lactobacilli Isolates:

The isolates were identified by 16S rDNA gene sequence analysis. The primers used for amplification of 16S rDNA region were Lb1 (5' AGAGTTTGATCATGGCTCAG- 3') and Lb2 (5'-CGGTATTAGCATCTGTTTCC-3') designed by Klijn et al⁷. Amplified PCR fragments were sequenced by Bangalore genei pvt. ltd. The basic local alignment search tool (BLAST) was carried out with NCBI genebank database.

Results and Discussion

Results: A total of 10 isolates were recovered on MRS agar medium from camel milk sample. These 10 isolates were characterized on the basis of morphology. Among 10 isolates only 2 isolates displayed the antibacterial activity against *Aeromonas veronii* figure-1 which was isolated from Pichola lake, Udaipur and was previously identified.

Table-1
Characteristics of isolate CMU 14 and CMU 17

Charcterstics	<i>L. rhamnosus</i> CMU 14	<i>L. fermentum</i> CMU 17
Gram reaction	+	+
Colony morphology		
Size	Small	Small
Shape	Concave	Concave
Colour	Offwhite	Offwhite
Biochemical Characteristics		
Catalase	-	-
Litmus milk coagulation	48h	48h
Gas from glucose	-	+
Growth at 15 ⁰ C	+	+
Growth at 45 ⁰ C	+	-
Growth on BCP-MRS medium	Yellowish	yellowish
Nitrate reduction	-	-
Arginine hydrolysis	-	+
Esculin hydrolysis	+	-
Sugar fermentation		
Maltose	+	+
Fructose	+	+
Dextrose	+	+
Lactose	+	+
Sucrose	+	+
Rhamnose	+	-
Raffinose	-	+
Mellibiose	-	+

+ = Positive, - = Negative

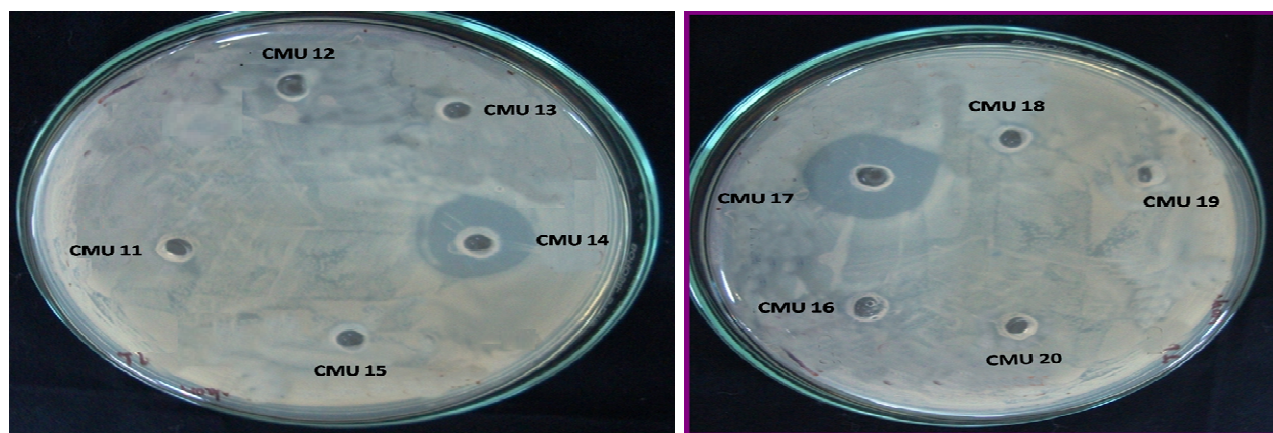


Figure-1
Antibacterial activity of isolates CMU 14 and CMU 17 against *Aeromonas veronii*

On the basis of biochemical characters and sugar fermentation test (table-1), the isolates were tentatively designated as *L. rhamnosus* CMU 14 and *L. fermentum* CMU 17.

The identification of CMU 14 and CMU 17 were also done by 16S rDNA sequence analysis. The DNA of isolate CMU 14 and CMU 17 were amplified by PCR using semi universal primers (Lb1 and Lb2). Both isolates gave specific band of 200bp which were further sequenced by Bangalore genei pvt. Ltd. The use of 16S rDNA sequence analysis allowed to identify CMU 14 strain as *L. rhamnosus* and CMU 17 strain as *L. fermentum*. The obtained sequences were registered in EMBL-EBI database system under accession numbers LM994031 and LM994033, respectively.

Discussion: The use of probiotics for removal of pathogenic bacteria in water is a research area of increasing interest. The World Health Organization – Food and Agriculture Organization defined probiotics as "live microorganisms" which when administered in sufficient amounts, gives a health benefit on the host. Probiotic bacteria have been potential to inhibit pathogenic organisms of water and overcome infections in humans, aquatic and terrestrial animals which are evaluated by several scientists. The production of inhibitory compounds toward known pathogens for the considered species has often been used in the selection of putative probiotic strains for aquaculture. Lactic acid bacteria are usually used as probiotic bacteria. Bacteriocins produced by lactic acid bacteria inhibit the growth of other microorganism⁸.

In the present study, 10 lactobacilli isolates were isolated from camel milk. These isolates were screened for antibacterial activity against *Aeromonas veronii*. Only 2 isolates gave the significant result. Similar work was carried out by Dhanasekaran *et. al.*¹ who have reported antibacterial activity of lactobacilli isolated from fish gut against *Aeromonas sp.* Vine *et. al.*⁹ also reported antibacterial activity of probiotic bacteria against *Aeromonas hydrophila* and *Vibrio alginolyticus*. The possible involvement of lactic acid bacteria in aquaculture were also reported by Ringo and Gatesoupe¹⁰.

Conclusion

The study concluded that these *Lactobacillus* isolates could be used as potential probiotics for aquaculture. The use of probiotics in aquaculture could offers higher quality livestock in terms of size, production time and health. It will be also helpful in the management of bacterial disease *Aeromonosis*.

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