Screening of *Lactobacillus rhamnosus* CM19 for probiotic attributes

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Abstract: - Importance of probiotics have been well set and their demand is increasing day by day. Lactobacilli offer two important applications biopreservation of foods and use as probiotic cultures. Large number of lactobacilli have known to show health benefits in human beings and are available commercially. In the present study isolated from camel milk was screened for probiotic attributes such as antibiotic resistance, bile tolerance and antibacterial activity. The strain showed resistance to various antibiotics, antibacterial activity against both gram-positive and gram-negative bacterial and showed survival upto 0.3% concentration of oxgall. All three properties confirm potential of *Lactobacillus rhamnosus* CM19 as an efficient probiotic candidate. It can be further explored for health benefits.

Key Words: —*Lactobacillus rhamnosus CM19, probiotic, antibiotic resistance, bile tolerance and antibacterial activity.*

I. Introduction

The major probiotic bacteria are strains belonging to the genera *Lactobacillus* and *Bifidobacterium*, although other representatives, such as *Bacillus* or *Escherichia coli* strains, have also been used. The probiotics belonging to different species included in the EFSA QPS list (EFSA, 2012) have excellent safety records, and detrimental effects produced as a consequence of their ingestion are very scare (Gouriet *et al*., 2012). Great interest to investigation whether these determinants can be transferred in the food and gut environment (Lahtinen, 2009) for their effective use as probiotics.

Antibiotic resistance, bile tolerance and antibacterial activity are three important probiotic characteristics which can be evaluated for a probiotic strain (Singhal *et al*., 2009; Tambekar and Bhutada, 2010). For various species such as *L. casei, L. acidophilus, L. reuteri, or L. rhamnosus*, among others or the yoghurt starter bacteria *L. delberuckii* chromosomal mutations leading to antibiotics resistance phenotypes have also been described (Ammor *et al*., 2008). The antagonistic action of lactobacilli is important probiotic property in the protection of the gut from infection by pathogenic bacteria.

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Antimicrobial compounds are produced by one bacterium that kill or inhibit the growth of other bacteria**.** Allaf *et al*., 2009 studied antimicrobial activity of *Lactobacillus* strains from minced beef meat against some gram-positive and gram-negative pathogenic bacteria. *Lactobacillus acidophilus* produces lactocidin substance, which have broad antibacterial spectrum (Vincent *et al*., 1959). Bile tolerance is one of the most crucial properties for probiotics bacteria, as it determines its ability to survive in the small intestine, and consequently its capacity to play its functional role as a probiotic. Active efflux of bile acid/salts (Pfeiler and Klanhammer, 2009; Ruiz *et al*., 2012) bile salts hydrolysis and changes in the architecture/ composition of cell membrane and cell wall appear to the most prevalent bile-specific mechanism mediating resistance in both genera (Gomez-Zavoglia, 2002).

The present research was carried out to screen *Lactobacillus rhamnosus* CM19 for antibiotic resistance, bile tolerance and antibacterial activity for its possible use as probiotic strain.

II. Materials And Methods

2.1 Culture, Source and Maintenance

Lactobacillus rhamnosus CM19 was isolated from camel milk. The culture was grown in MRS broth at 37°C for 24 hours culture was inoculated at a concentration of 1% in skim milk and incubated at 37°C for 24 hours.

2.2 Detection of Antibiotic Resistance

Antibiotic resistance was detected by disk diffusion method (Bauer *et al.,* 1966). MRS agar medium was prepared by dissolving 27.5 gm to 500 ml of distilled water, medium was sterilized by autoclaving. Media was melted cooled and poured in sterile petri plates and allowed to solidify. 100µl of probiotic *Lactobacillus rhamnosus* CM19 was spread on the solidified surface of media with the help of sterile L shape spreader. Antibiotic discs were placed in petri plates respectively with help of sterile forceps. Plates were sealed by using parafilm and incubated at 37ºC for 24-48 hours in upright position. A zone of clearance around disc will show that probiotic culture is sensitive to particular antibiotic. No zone of clearance will show that given culture *Lactobacillus rhamnosus* CM19 is resistant to that antibiotic. Zone of clearance was also measured.

2.3 Determination of Antibacterial Activity

Antibacterial activity was determined by agar well diffusion method (Schillinger and Lucke, 1989). Nutrient agar was autoclaved, cooled and poured into sterile petri-plates and allowed to solidified. A 100 µl of different bacterial cultures (*Micrococcus lutes, Serratia marcescens*, *Bacillus subtilis* and *Escherichia coli*) were spread on the solidified media with sterile L-Shaped spreader. Wells were punched with help of well borer and 80µl of supernatant *Lactobacillus rhamnosus* CM19 was filled in each well. Petri-plates were sealed and incubated at 37°c for 24hour in upright position. The diameter of inhibition zones was noted down**.**

2.4 Detection of Bile Tolerance

The bile salt tolerance of *Lactobacillus rhamnosus* CM19 was performed by streak plate method on MRS agar with and without bile salt (Sirilun *et al.,* 2010). An overnight MRS broth culture of *Lactobacillus rhamnosus* CM19was streaked on MRS agar plate without bile salt (control) and MRS agar plates containing 0.1%, 0.2%, 0.3%, 0.4% and 0.5% (w/v) bile salt namely ox-gall (Hi-media) respectively and were incubated at 37° C for 72 h. The growth on MRS plates were indicated as positive sign (+) whereas negative sign (-) indicates no growth.

III. Results And Discussion

3.1 Antibiotic resistance of Lactobacillus rhamnosus CM19

Antibiotics resistance pattern of *Lactobacillus rhamnosus* CM19 was evaluated against 12 different antibiotics. The isolate showed resistance against to 6 antibiotics (namely cefixime, streptomycin, chloramphenicol, trimethoprin, ciprofloxacin, tetracycline) and was found sensitive to 6 antibiotics (namely ampicillin, streptomycin, rifampicin, erythromycin, vancomycin, gentamycin), Antibiotic resistance pattern has been presented in the table 1. For *Lactobacillus rhamnosus* CM19 the diameter of zone of inhibition against the different antibiotics was ranged from 6mm to 29 mm. streptomycin (15 mm), Rifampicin (25 mm), 29 mm (erythromycin), 12 mm (vancomycin), 6 mm (ampicillin), 27 mm (gentamycin).

Anisimova *et al.* (2019) reported the antibiotic resistance is a strain dependent property in various *Lactobacillus* species. According to previous reports resistance against streptomycin and tetracycline has been observed more frequently among lactobacilli (Zhou *et al*., 2005; Korhonen *et. al.,* 2007). The findings of present study are in accordance with above mentioned reports.

3.2 Antibacterial activity of Lactobacillus rhamnosus CM19

Lactobacillus fermentum CM19 isolate showed antibacterial activity in cell free supernatant without neutralization against all 4 test organisms. A clear zone of inhibition was observed for *Micrococcus luteus, Serratia marcescens*, *Bacillus subtilis* and *Escherichia coli.* The inhibition zone was seen around the well filled with supernatant of *Lactobacillus fermentum* CM19 which confirms the antibacterial activity of the isolate. The data for the same has been presented and shown in table 2. Diameter of inhibition zone including well diameter (6mm) against *Micrococcus luteus* was 3.4 cm, *Bacillus subtilis* was 2.9 cm, *Serratia marcescens* was 4 cm and against *E. coli* it was 3.1 cm.

Very recently it was reported that *Lactobacillus* strains isolated from fermented vegetables showed tremendous antibacterial along with other probiotic characteristics (Chen *et al*., 2022). The results of present study showed stronger agreement with the reports that revealed that *Lactobacillus* strains showed antibacterial activities against both gram-positive and negative bacteria (Voravuthikunchai *et al*., 2006; Djadouni and Kihal, 2012).

3.3 Bile tolerance of Lactobacillus rhamnosus CM19

Lactobacillus rhamnosus CM19 showed a varied degree of growth on MRS agar supplemented with different concentrations of bile salt. *Lactobacillus fermentum* CM19 was able to grow up to 0.3% ox-gall while no growth was observed on MRS agar containing 0.4% and 0.5% ox-gall after 72 h of incubation. Heavy growth was observed in control and 0.1%

while moderate growth was seen in MRS agar supplemented with 0.2% and 0.3% ox-gall. Data for the same has been presented and shown in the table 3.

The normal level of bile salt in the intestine is around 0.3% (Mourad and Nour- Eddine, 2006). The probiotic organisms could tolerate this concentration of bile to survive in the gut. *Lactobacillus fermentum* CM19 was able to grow up to 0.3% ox-gall which confirmed its ability to survive in stressful condition of intestine.

Table.1. Antibiotic resistance pattern of *Lactobacillus rhamnosus* CM19

Symbol	Antibiotic	Including disc (cm)	Excluding disc (cm)	Diameter of inhibition zone (mm)
S - 25	Streptomycin	2.1	1.5	15
RIF - 30	Rifampicin	3.1	2.5	25
E - 15	Erythromycin	3.5	2.9	29
VA - 30	Vancomycin	1.8	1.2	12
Amp - 10	Ampicillin	1.2	0.6	6
GE - 30	Gentamycin	3.3	2.7	27

Table.2. Antibacterial activity of *Lactobacillus rhamnosus* CM19 against different pathogenic bacteria

Bacteria	**Diameter of clear zone (cm) including well diameter**
Micrococcus luteus	3.4
Serratia marcescens	4.0
Bacillus subtilis	2.9
Escherichia coli	3.1

Table.3. Bile tolerance of *Lactobacillus rhamnosus* CM19 at different concentration of ox-gall

Conc. Of oxgall (W/V) in MRS agar	**Growth of *Lactobacillus rhamnosus* CM19**
MRS without oxgall (control)	++
0.1%	++
0.2%	+
0.3%	+
0.4%	-
0.5%	-

(+) positive growth, (-) no growth.

IV. Conclusion

In this study *Lactobacillus rhamnosus* CM19 showed good results when three different properties (antibiotic resistance, antibacterial activity and bile tolerance) were tested to confirm its probiotic attributes. It showed demonstrable antibacterial activity against all four test organisms namely M*icrococcus lutes, Serratia marcesens, E. coli* and *Bacillus subtilis* used in the study. It can be concluded that *Lactobacillus rhamnosus* CM19 has good probiotic potential and could be explored further for its use as probiotic strain.

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