



Evaluation of antibiotic resistance of *Lactobacillus rhamnosus* strains isolated from camel milk samples

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Abstract

The probiotic bacteria fulfil important functions in human gut contributing to good health. Antibiotic resistance is important feature found among probiotic bacteria for their survival in gut even after treatment of antibiotics. In present research evaluation of antibiotic resistance in *Lactobacillus rhamnosus* strains isolated from camel milk samples was done. A total of 4 samples were collected from Udaipur, Rajasthan (India). A total of 66 isolates were recovered on MRS agar at 37° C after 24h of incubation period. A total of 19 isolates out of 66 showed identification score 0.9965 for *Lactobacillus rhamnosus* and therefore they were identified as *Lactobacillus rhamnosus*. The isolates showed varied response to 7 different antibiotics used in the study. *Lactobacillus rhamnosus* CMU 50 showed complete antibiotic resistance to all 7 antibiotics tested. This isolate can be explored for further probiotic potential for its possible use in treating antibiotic associated diarrhoea.

Keywords: *Lactobacillus rhamnosus*, antibiotic resistance, probiotics, antibiotics

Introduction

Intake of antibiotics often disrupts the healthy balance of inhabited microflora in the host, causing intestinal disorders. The administration of antibiotic-resistant strains can help retain the normal bacterial ratio in the intestines, or its fast restoration if administered after the antibiotic treatment [1]. Therefore, antibiotic resistance is one of the criteria for selecting a good probiotic organism. There are three types of resistance found in lactobacilli: intrinsic or innate, acquired and mutational. Antibiotic resistance profiles have recently been reported for several lactobacilli [2-4].

Lactobacilli have been found susceptible to penicillins and ampicillin (cell wall synthesis inhibitor) [5, 6] in contrast to vancomycin. Most lactobacilli have been found to be resistant to glycopeptides types of antibiotics. However, the resistance towards vancomycin has been demonstrated being as intrinsic [7]. Lactobacilli are usually susceptible to chloramphenicol, erythromycin and clindamycin (protein synthesis inhibitors) [8]. In addition, resistance against trimethoprim (nucleic acid synthesis inhibitor), seems to be intrinsic [9]. Resistance to tetracycline has been observed more often among lactobacilli [10]. Resistance against neomycin, kanamycin, streptomycin and gentamycin (aminoglycosides) has been observed more frequently among lactobacilli [11]. An attempt has been made in present work to evaluate the antibiotic resistance in *Lactobacillus rhamnosus* strains isolated from camel milk samples.

Materials and Methods

Collection of Samples

The camel milk samples were collected from Udaipur, Rajasthan (India). These samples were collected in a pre-sterilized screw cap bottle.

Isolation of lactobacilli

The samples were first inoculated in MRS broth (de Man *et al.*) [12] and incubated at 37°C for 24 h. After enrichment process the isolation was done by pour plate method.

Identification of lactobacilli using PIB Bryant software

The test results obtained from biochemical tests were fed to the *Lactobacillus* identification matrices suggested by Maissin *et al.* 1987 [13] of PIBwin software and probabilistic identification score of the cultures was noted.

Evaluation of antibiotic resistance of lactobacilli

The antibiotic sensitivity of isolates against 7 antibiotics was evaluated using agar disc diffusion method [14]. The antibiotics discs (Hi-media) used were ampicillin (10 µg), tetracycline (30 µg), kanamycin (30 µg), streptomycin (25µg), penicillin G (10 Units), vancomycin (30 µg) and rifampicin (30 µg). The diameter of inhibition zone was carefully measured after incubation at 37°C for 24 h. Diameter of inhibition zones was

measured inclusive of the diameter of the disc. The clear zone of 1mm or more was considered as positive inhibition.

Results

Sample collection details

A total of 4 samples were collected from Udaipur, Rajasthan (India). These samples were collected in a presterilized screw cap bottle and used within 48h for isolation process.

Isolation of lactobacilli

A total of 66 isolates were recovered on MRS agar at 37° C after 24h of incubation period. The isolates showed white, pin pointed colonies with entire margin and convex elevation.

PIB Bryant software-based identification

The biochemical tests results were fed to *Lactobacillus* matrix of PIB Bryant software. A total of 19 isolates out of 66 showed identification score 0.9965 for *Lactobacillus rhamnosus* and therefore they were identified as *Lactobacillus rhamnosus*.

Antibiotic resistance of *Lactobacillus rhamnosus* strains

All *Lactobacillus rhamnosus* (n=19) isolates were found to be resistant to kanamycin and vancomycin. A total of 9 isolates out of 19 were sensitive to streptomycin. The remaining 10 isolates were resistant to streptomycin. All *Lactobacillus rhamnosus* isolates were sensitive against ampicillin, tetracycline, penicillin and rifampicin except isolate CMU 50. The isolate CMU 50 was resistant to all antibiotics which were used in this study (Fig.1). The inhibition zone of *Lactobacillus rhamnosus* against different antibiotics namely ampicillin, tetracycline, streptomycin, penicillin and rifampicin were ranged from 14 mm to 55mm. The data has been presented in Fig.2.

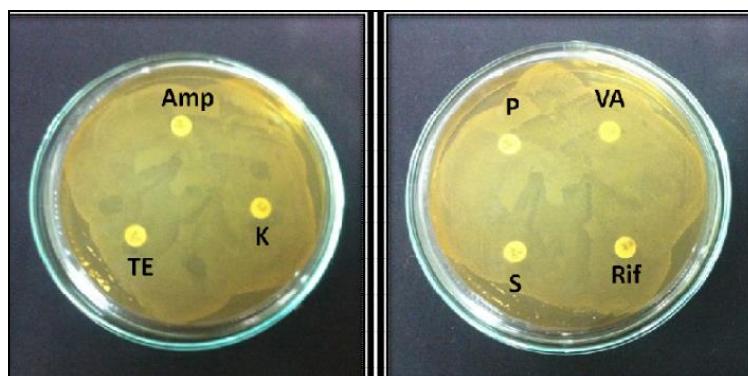


Fig 1: Antibiotic resistance pattern of *Lactobacillus rhamnosus* CMU 50 Amp = Ampicillin; TE = Tetracycline; K = Kanamycin; P = Penicillin; VA = Vancomycin; S = Streptomycin; Rif = Rifampicin

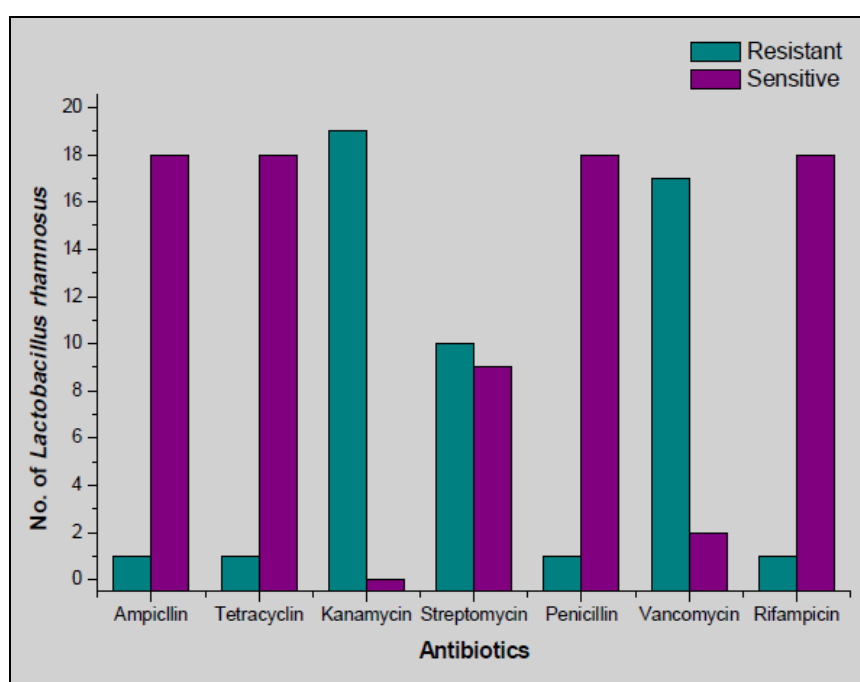


Fig 2: Antibiotic resistance pattern of *Lactobacillus rhamnosus* strains against various antibiotics

Discussion

The antibiotic susceptibility test should be incorporated for the safety assessment of the desired property of the promising probiotic strains. Almost all the *Lactobacillus* strains isolated from camel milk were found to be resistant to vancomycin. Resistance to vancomycin is an intrinsic property (chromosomally encoded and nontransmissible) of *Lactobacillus* strains [7]. All isolates were found to be resistant to kanamycin. In case of streptomycin, 10 isolates of *Lactobacillus rhamnosus* were found to be resistant. According to several workers [5, 6, 11], resistance against aminoglycosides (kanamycin and streptomycin) has been observed more frequently among lactobacilli. Coppola *et al.* [5] explained that this resistance is also due to the intrinsic property of lactobacilli. In our study, all strains were found to be sensitive to ampicillin and penicillin except one strain *Lactobacillus rhamnosus* CMU 50. The *Lactobacillus* species have been found susceptible to many cell wall synthesis inhibitors, like penicillins and ampicillin due to its intrinsic property [5, 6]. In our study, all *Lactobacillus* strains were found to be susceptible to tetracycline except *Lactobacillus rhamnosus* CMU 50. Korhonen *et al.* [15] reported that resistance to tetracycline has been observed more often among *Lactobacillus* species. The results of present study are contrary to this finding. According to Gfeller *et al.* [16], diverse level of susceptibility for tetracycline may be due to mutation and mobile genes which can be applicable to the findings of the present study. In our study, all *Lactobacillus* strains were found to be susceptible to rifampicin. Zhou *et al.* [5] also reported the *Lactobacillus* susceptibility to rifampicin which is similar to the findings of the present study. Anisimova *et al.* [17] reported the antibiotic resistance is a strain dependent property in various *Lactobacillus* species.

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