

Original Research Article

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Isolation and Characterization of *Lactobacillus casei* and in vitro inhibition of enteric pathogens by *Lactobacillus casei* strains

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A lab experiment was carried out in 2012-13 and the main objective of the investigation was, to study in vitro inhibition of enteric pathogens by *Lactobacillus casei* strains. The experiment was laid out in completely randomized block design with six treatments of test organism such as *E.coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and Control. Supernatant of *Lactobacilli* grown on Man, Rogosa, Sharpe (MRS) media adjusted pH-7 and unadjusted pH showed antagonistic response against these test organism. *Salmonella typhimurium* was significantly superior over rest of other treatments, indicating that supernatant of *lactobacilli* grown on MRS media and adjusted pH-7 showed good antagonistic response against *Salmonella typhimurium*. *Pseudomonas aeruginosa* unadjusted pH and *Streptococcus mutans* unadjusted pH showed equal antagonistic response. *E. coli* unadjusted pH and *Staphylococcus aureus* adjusted pH showed equal antagonistic response.

Introduction

Enteric pathogens are a risk factor for gastric adenocarcinoma and lymphoma in humans. In symptomatic patients, the antibiotic treatment has a high cost and is not 100% effective because of resistance to antibiotics and to moderate patient compliance. This review discusses the role of probiotics as alternative solutions to assist in the control of enteric pathogens colonization in at risk populations.

DPC16 cell-free supernatants (fresh and freeze-dried, designated as MRSc and FZMRSc) from anaerobic incubations in pre-reduced MRS broth, have shown

significant inhibitory effects against selected pathogens, including *Salmonella typhimurium*, *E. coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes* (Bian, 2008). Probiotics protect against gastrointestinal pathogenic infection via several mechanisms.

Probiotic bacteria have been documented as being effective in biotherapeutic applications against gastrointestinal pathogens, e.g. *Helicobacter pylori*, *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and rotaviruses (Phirabunyanon, 2010). Probiotic *lactobacillus rhamnosus* strain shows broad

spectrum activity against GIT pathogens and food spoilage organisms (Pithva *et al.*, 2011).

Materials and Methods

An experiment was undertaken at the Department of Post Harvest and Food Biotechnology of MGM College of Agriculture Biotechnology Aurangabad. There were six treatments, each replicated thrice.

The treatments comprised of 5 pathogenic bacteria (test organisms) e.g. *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus mutans* along with Control (without test organism).

Lactobacillus casei was isolated from the curd as described by (Bhardwaj *et al.*, 2012) and were activated by growing thrice on Man, Rogosa, Sharpe (MRS) broth (deMan *et al.*, 1960) at 37°C for 24 hrs. The test organisms were obtained from MGM medical College, Aurangabad and grown on nutrient agar under anaerobic condition for 72 hrs at 37°C.

For the experimental purpose, *Lactobacillus casei* was grown on MRS broth at 37°C for 24hrs. The supernatant was then obtained by centrifuging an aliquot at 4000 rpm for 15min at 4°C. The pH of supernatant was adjusted to 7.0 (T1-T6) with the use of 2M NaOH while another treatments (T7-T12) was unadjusted pH and it was filtered. Antibacterial activity of the supernatant was measured by food poison method (Amdekar *et al.*, 2010) and expressed as the diameter of inhibition zone.

Results and Discussion

In this experiment attempts were made to

see the antagonistic activity of *Lactobacilli* isolated from Curd. The results were shown in terms of zone of inhibition.

Among the five test organisms used *Lactobacilli* was found effective in inhibiting Adjusted pH-7 of *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and unadjusted pH of *E.coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Streptococcus mutans* which was indicated by zone of test organisms. But it was failed to destruct adjusted pH of *E.coli*, *Streptococcus mutans* and unadjusted pH of *Staphylococcus aureus* as there was no zone of inhibition in these test organisms.

Salmonella typhimurium (39.25) at pH-7 was significantly superior over rest of the other treatments, indicating that supernatant of *lactobacilli* grown on MRS media and unadjusted pH-7 showed good antagonistic activity against *Salmonella typhimurium* were unable show antagonistic response over rest of other test organism.

Pseudomonas aeruginosa (33.50) at pH-7 was at par with *Salmonella typhimurium* unadjusted pH and was significantly superior over T3, T7, T10 and T11.

Pseudomonas aeruginosa unadjusted pH was at par with *Streptococcus mutans* unadjusted pH and zone of inhibition was significantly superior over T3 and T7.

E.coli unadjusted pH and *Staphylococcus aureus* adjusted at pH-7 was at par with each other, while *lactobacillus* did not showed any antagonistic effect against T1, T5, T6 and T9. Where zone of inhibition observed was zero.

Table.1 Treatment Details and Zone of Inhibition of Lactobacilli in Mm against Test Organism

Treatment (Test organisms)	pH	Diameter of inhibition zone
T1 (<i>E. coli</i>)	pH-7	00.00
T2 (<i>Salmonella typhimurian</i>)	pH-7	39.25
T3 (<i>Staphylococcus aureous</i>)	pH-7	12.50
T4 (<i>Pseudomonas aeruginosa</i>)	pH-7	33.50
T5 (<i>Streptococcus mutants</i>)	pH-7	00.00
T6 (control) without lactobacillus	pH-7	00.00
T7 (<i>E.coli</i>)	Unadjusted	13.00
T8 (<i>Salmonella typhimurian</i>)	Unadjusted	30.50
T9 (<i>Staphylococcus aureous</i>)	Unadjusted	00.00
T10 (<i>Pseudomonas aeruginosa</i>)	Unadjusted	26.75
T11 (<i>Streptococcus mutants</i>)	Unadjusted	26.50
T12 (control) without lactobacillus	Unadjusted	00.00
Mean		15.16
S.E±		0.76
CD		2.19

Fig.1 *Ctobacilli* Showed Antagonistic Activity Against *Salmonella typhimurium*



Fig.2 *Lactobacilli* Showed Antagonistic Activity Against *Pseudomonas aeruginosa*



Lactobacilli cells were isolated from the Curd and identified, confirmed by using biochemical test and morphological characters. In this experiment the antagonistic activity of *lactobacilli* was checked against the enteric pathogens. The test organism such as *E.coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* were used in the experiment. It was found that *lactobacilli* showed significant inhibitory effect against *Salmonella typhimurium* (Fig.1). It also showed good effect against *Pseudomonas aeruginosa*, *E.coli* with unadjusted pH.

Thus we can say that *lactobacillus* can be used as a biocontrolling agent against the infection of enteric bacteria such as *E.coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Thus we can prevent the infections by avoiding the use of synthetic antibiotics.

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