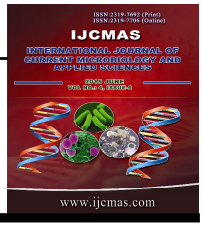
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 6 (2015) pp. 36-40**
http://www.ijcmas.com



Original Research Article

A Study showing antagonistic effect of *Lactobacilli casei* and *Lactobacilli sporogenesis* against some common pathogens- *in vitro*

Pragti Shukla and Jagriti Sharma*

Department of Microbiology, School of Life Sciences, Dr. B.R. Ambedkar University Khandari Campus, Agra-282 002, U.P. (India)

**Corresponding author*

A B S T R A C T

Keywords

Probiotics, *L. casei*, *L. sporogenesis*, Antagonistic effect, *Staphylococcus*, *Pseudomonas and Escherichia*

Lactobacillus is the most popular probiotic microorganism which commonly occupies the human intestine and put numerous beneficial effects on the host. This study was attempted to evaluate its antagonistic effect against the common pathogens. Two common strains of Lactobacilli, *L. casei* and *L. sporogenesis* were used to evaluate their antimicrobial effect against common pathogens, *Staphylococcus*, *Pseudomonas* and *Escherichia* by using the disc diffusion method. Maximum antagonistic activity was shown by the probiotic suspension of turbidity equal to the M.F.S. #1.0 while least or nil activity was shown by the M.F.S.#1/100. Thus the antagonistic potential of probiotic strains must be further evaluated to combat the diseases caused by the pathogens especially in case of drug resistance pathogens.

Introduction

Microorganisms that beneficially affect the host are called probiotics. *Lactobacilli* are the most widely distributed probotic strains within the Lactic acid bacteria (LAB) group. *Lactobacillus* sp. are Gram positive bacilli that are catalase negative and believed to be safe because they have been established as the normal flora in fermented food for a long time, so they have great potential to be used as a bio preservative and these preserving effects of lactic acid bacteria are due to the production of antimicrobial agents such as such as diacetyl, hydrogen peroxide, bacteriocins lactic and other organic acids and other related substances (Desmazeaud and Cogan, 1996; Cocolin *et al.*, 2007; Quwehand and Vesterlund, 2004). Several *Lactobacillus* strains are in important dairy culture starter and used for the manufacture of fermented food (Fitzsimmons *et al.*, 1999; Badis *et al.*, 2004). The antimicrobial effects of *Lactobacilli* lead to inhibition of the growth of potential pathogens through a variety of mechanisms including their capacity to decrease luminal pH and secrete bactericidal proteins and inhibit bacterial adhesion to epithelial cells. *Lactobacillus casei* is a species of genus *Lactobacilli* found in the human intestine and mouth.

This particular species of *Lactobacillus* is documented to have a wide pH and temperature range, and complements the growth of *L. acidophillus* a producer of the enzyme amylase. *L. sporogenesis* is a universally occurring beneficial bacterium. It is a gram-positive, spore-forming, lactic acid producing probiotic microorganism. Probiotics, such as *Lactobacillus sporogenesis*, support the growth of friendly bacteria and help to maintain a healthy balance of micro flora in the intestinal environment. Lactobacillus has shown to produce the antimicrobial activity against *P. aeruginosa, E. coli, S. aureus* and *Salmonella* (Gopal Pramod 2001 *et al.*, Karska –Wysocki *et al.*, 2010; Makras *et al.*, 2006; Saxena and Dutta, 2011). Intestinal Lactic acid bacterial species with alleged health beneficial properties have been introduced as probiotics, including *L. rhamnous, L. casei* and *L. johnsonii* (Elazab *et al.*, 2013). This study was carried out to evaluate the antimicrobial properties of *L. casei* and *L. sporogenesis* against the standard isolates of *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus in vitro* condition.

Materials and Methods

Isolation and cultivation of probiotic strains

Probiotic strains *Lactobacillus casei* and *Lactobacillus sporogenesis* were isolated from the commercial probiotic products "Yakult" and "Sporolac". To isolate the *L.casei* from yakult, a loopful of yakult suspension was inoculated on MRS agar media and kept at 37ºC in an anaerobic chamber. After 48 hrs, the pale whitish colonies appeared on the plates which were subcultured to get the pure colonies. To isolate the *L. sporogenesis* a pinch of sporlac, powder was dissolved in 2 ml of normal saline and the suspension was inoculated on the MRS agar medium and kept at 37ºC for 24 hr. After incubation the colonies were subculture to get the pure colonies. Pure colonies were stored at 4ºC in the butt slant tubes of nutrient agar.

Test pathogens

The standard strains of the pathogens, *Pseudomonas aeruginosa* MTCC-103, *Escherichia coli* MTCC-1652, *Staphylococcus aureus* MTCC-740, were obtained from IMTECH, Chandigarh India and stored at 4ºC.

Antibiogram of probiotic strains

To detect the antibiotic susceptibilities of *L. casei* and *L. sporogenesis* their suspensions of the turbidity M.F.S. # 0.5 were swabbed on the Muller-Hintom Media plate. Now the antibiotic discs were placed on the MHA surface and incubated at 37ºC for 24 hrs.

Antagonistic activity of probiotic strains

Antagonastic activities of *L. casei* and *L. sporogenesis* were studied by disc diffusion method according to National Committee for Clinical Lab Studies (NCCLS) guidelines (Kirby *et al.*, 1966). For this the petriplates of diameter of 90 mm were poured 20 ml of Muller Hinton media, swabbed with the suspensions of turbity M.F.S. # 0.5 for *E. coli* MTCC-1652, *P. aeruginosa* MTCC-103 and *S. auresus* MTCC-740 and incubated at 37ºC for 15 minutes. Sterile blotting paper disc of 6mm diameter were soaked with the 20 µl of probiotic suspension of turbidity equal to M.F.S. ≠ 1.0 (3×10^8 cfu/ml) and the serial suspension of 1/10 ($3x10^7$ cfu/ml) and 1/100 ($3x10^6$ cfu/ml) so the disc now contained 6 x 10^6 cfu/disc (for M.F.S. ≠ 1.0), 6 x 10^5 cfu/disc (M.F.S. ≠ 1/10) and $6x10^4$ cfu/disc (M.F.S. ≠ 1/100).

Table.1 Antagonistic activity of *Lactobacillus sporogenesis* and *Lactobacillus casei* against *Pseudomonas aeruginosa MTCC-103, E. coli MTCC-1652 and Staphylococcus aureus* MTCC-740

S. No.	Antibiotic/ Probiotic disc	Diameter of the zones of inhibition (mm)					
		Pseudomonas		*E. coli*		*Staphylococcus*	
		L. spor.	*L.cas.*	*L. spor*	*L.cas.*	*L. spor*	*L.cas.*
1.	antibiotic	17	17	24	20	25	19
2.	1	15	14	17	13	15	12
3.	1/10	9	13	15	11	8	9
4.	1/100	0	0	0	0	0	0
5.	Distilled water	0	0	0	0	0	0

Fig.1 Antagonistic effect of *L. casei* against (a) *P. aeruginosa* MTCC-103, (b) *E. coli* MTCC-1652(c) *S. aureus* MTCC-740 and antagonistic effect of *L. sporogenesis* against (d) *P. aeruginosa* MTCC-103, (e) *E. coli* MTCC-1652 (f) *S. aureus* MTCC-740

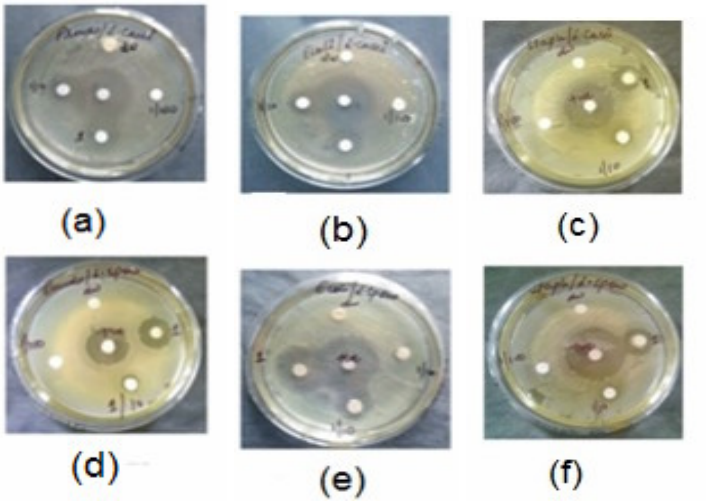
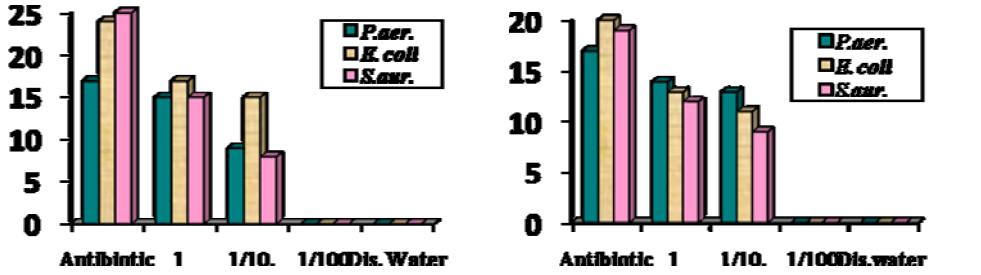


Fig.2 Showing the Zone of inhibition for (A) *Lactobacillus casei* (B) *Lactobacillus sporogenesis* against the different pathogens



(A) **(B)**

Readymade antibiotic disc Streptomycin was taken as a positive control and sterile distilled water as negative control. These discs were placed on Muller Hinton media and kept at 4ºC for 1 hr for proper diffusion. Now the plates were kept at 37ºC for 24 hours and zones were measured. All the tests were done twice in triplet and best was used for readings.

Both the strains were confirmed by colony morphology as appeared as round, small creamish colonies on MRS agar. Both Strains appeared as gram positive bacilli in microscopy.

Antibiotic susceptibilities of *L. casei* and *L. sporogenesis* were detected against 8 antibiotics Chloroamphenicol(C), Cefoxitin (CX), Azithromycin(AZM), Amoxyciilln (AMC), Ampicillin/Sulbactam(A/S), Meropenem (MRP), Ceftazidime(CAZ) and Levofloxacin (LE) by Placing them on the Muller Hinton agar plates already swabbed with their suspensions (M.F.S. ≠ 0.5) and kept at 37ºC for 24 hrs. *L. casei* and *L. sporogenesis* showed maximum sensitivities (11mm) towards chloramphenicol and Levofloxacin (LE) respectively while both were resistance towards Ceftazidime (CAZ).)

The maximum zone of inhibition was produced by *L. sporogenesis* for the serial suspension of M.F.S. ≠ 1.0 against *P. aeruginosa* MTCC-103, *E. coli* MTCC-1652 and *S. aureus* MTCC-740 (15, 17, 15 mm) and the medium zone of inhibition were observed in the serial suspension of M.F.S. ≠ 1/10 (9 mm to 15 mm). No zone was seen in the serial suspension of M.F.S. ≠ 1/100. In case of *L. casei*, the maximum zone of inhibition (14 mm) was produced against *P. aeruginosa* MTCC-103 followed by *E. coli* MTCC-1652(13) and *S. aureus* MTCC-740(12 mm) for M.F.S. ≠ 1.0. Although the medium zone of inhibition was observed by the serial suspension of M.F.S. ≠ 1/10 (9 mm to 13 mm) but no zone was seen in the serial suspension of M.F.S. ≠ 1/100(table-1, fig.1&2).

Both of the probotic strains *L. casei* and *L.sporogenesis* produced the zone of inhibition against all the microbial pathogens. *L. sporogenesis* produce better zone of inhibition than the *L. casei* against all the strains for M.F.S. ≠ 1.0. On the contrary *L. casei* gave better inhibition than *L. sporogenesis* against *P. aeruginosa* MTCC-103 and *S. aureus* for serial suspension of M.F.S. ≠ 1/10 portraying them as antimicrobial agents. Besides the antimicrobial potential, these strains also confer health benefits, when consumed. So, *L. casei* and *L. sporogenesis* as a drug or a food supplements can be used as a health boosters and also as a protection from the harmful pathogenic microorganisms.

Acknowledgement

I extend my sincere thanks to the department of Microbiology, Dr. B. R. Ambedkar University, Agra, for allowing me to carry out this piece of work.

References

Badis, A., Guetarni, D., Moussa-Boudjema, B., Henni, D.E., Kihal M. 2004. Identification and technological properties of lactic acid bacteria isolated from raw goats milk of furnace Algerian races. *Food Microbiol.,* 21(5): 579–588.

Cocolin, L., Foschino, R., Comi, G., Fortina, M. 2007. Description of the bacteriocins produced by two strains of Enterococcus faecium isolated from Italian goat milk. *Food Microbiol.,* 31: 753–758

Desmazeaud, M., Cogan, T. 1996. Role of cultures in cheese ripening. In: Dairy starter cultures. Cogan, T., Joined, J.P. (Eds.), VCH Publishers, Inc., New York. Pp. 207–231.

Elazab, N.A., Mendy, J., Gasana, E.R., Vieira, A., Quizon, Forno, E. 2013. Probiotic administration in early life, atopy, and asthma: a meta-analysis of clinical trials. *Pediatrics*, 132(3): 666–676.

Fitzsimmons, N., Cogan, T.M., Condon, S., Beresford, T. 1999. Phenotypic and genotypic characterization of not-choke lactic acid bacteria in mature cheddar cheese. *Appl. Environ. Microbiol.*, 65: 318–3426.

Gopal Pramod, K., Prasad, J., Smart, J., Gill Harsharanjit, S. 2001. In vitro adherence properties of Lactobacillus rhamnosus DR20and Bifidobacterium lactis DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. *Int. J. Food Microbiol.*, 67(3): 207–216.

Karska-Wysocki, B., Bazo, M., Smoragiewicz, W. 2010. Antibacterial activity of Lactobacillus acidophilus and Lactobacillus casei against methicillin-resistant Staphylococcus aureus (MRSA). *Microbiol. Res.*, 165(8): 674–86.

Kirby, W., Bauer, A.W., Sherris, J.C., Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 36: 493–496.

Makras, L., Triantafyllou, V., Fayol-Messaoudi, D., Adriany, T., Zoumpopoulou, G., Tsakalidou, E., *et al.* 2006. Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards *Salmonell aenterica* serovar *Typhimurium* reveals a role for lactic acid and other inhibitory compounds. *Res. Microbiol.*, 157(3): 241–7.

Quwehand, A.C., Vesterlund, S. 2004. Antimicrobial components from lactic acid bacteria. Lactic acid bacteria microbiological and functional aspects. Marcel Dekker Inc., New York, USA.

Saxena, S., Dutta, R. 2011. Antimicrobial resistance pattern of *Shigella* species over five years at a tertiary-care teaching hospital in north India. *J. Health Popul. Nutr.*, 29(3): 292–5.