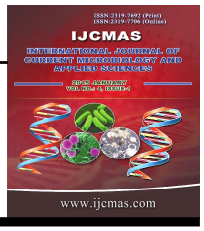
Int.J.Curr.Microbiol.App.Sci (2015) 4(1): 359-363

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 1 (2015) pp. 359-363**
http://www.ijcmas.com



Original Research Article

Effect of certain abiotic factors on growth and antibacterial activity of vaginal *Lactobacilli*

M.K.Bhattacharya[1]*, S.Das Purkayastha[1], H.Upadhyay[1], K.Pal[2] and S.Das Lala[3]

[1]Department of Botany and Biotechnology, Karimganj College, Karimganj- 788710, Assam, India
[2]Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela-769008, India
[3]Redcross Hospital, Karimganj-788710, Assam, India
**Corresponding author*

A B S T R A C T

In recent years, *Lactobacillus* species isolated from human vagina received attention due to their probiotic properties. The vagina of healthy women is a typical balanced ecosystem in which lactobacilli represent major microflora and confer protection of the host. This microbial flora plays an important role in regular vaginal function and support host defence from attack by pathogens. In the present experiment vaginal microflora were isolated and were identified on the basis of different phenotypic and biochemical characteristics, pure cultures of organisms obtained and of these *Lactobacillus spp were* subcultured and used for different experiments. The culture filtrates of *Lactobacillus* grown under different pH and temperature conditions were isolated. The antimicrobial activity of culture filtrate of lactobacilli was tested against *Staphylococcus aureus & E. coli* by standard methods. It has been found that lactic acid bacteria (LAB) cultures isolated from human vagina show good antagonistic activity against both Gram positive and Gram negative organisms. The highest growth of Vaginal *Lactobacilli* is observed at 35°C and highest antimicrobial activity at heatshock treatment.

Keywords

Lactobacillus, Antimicrobial, Potentially pathogenic microbes, Probiotics

Introduction

Lactobacillus and other Lactic Acid Bacteria (LAB) are known for producing bactericidal substance called bacteriocin. Bacteriocins are ribosomally synthesized antimicrobial peptides and bactericidal proteinaceous molecule produced by bacteria. Apart from it, the lactic acid produced by them also possesses antimicrobial property and it helps in maintaining the pH within 3.5–4.5 thereby not allowing a conducive environment for the growth of the pathogenic microbes. Production of H_2O_2 by *Lactobacillus spp*. may be a non-specific antimicrobial defense mechanism of the normal vaginal ecosystem. H_2O_2 inhibits both Gram-positive and Gram-negative

organisms. Instead of the normal predominance of *Lactobacillus* spp, increased numbers of organisms such as *Gardnerella vaginalis, E.coli,* and *Mycoplasma hominis* are found in the vagina of women with bacterial vaginosis (BV).

Materials and Methods

In the present paper, *Lactobacillus* species were isolated from vaginal swab of females of reproductive age. The antimicrobial activity of culture filtrate of *Lactobacilli* was tested against *Staphylococcus aureus & E. coli* by standard method. The culture filtrates of *Lactobacillus* grown under different pH and temperature conditions were isolated and the same was also tested against potential bacterial pathogens. In the present paper, the effect of culture filtrates of *Lactobacillus* which contained bacteriocin, organic acid and Hydrogen peroxide (H_2O_2) was compared with the effect of bacteriocin independent of other factors (H_2O_2 and lactic acid) as far as practicable. The paper simulates certain vaginal conditions for *in vitro* study of antimicrobial efficacy of *Lactobacillus* based suppository for vaginal application. For checking the viability, the lactobacilli loaded suppositories were incubated overnight in MRS media. The formulations were tested under *in vitro* conditions on *E. coli*, taken as a model causative organism.

Vaginal samples were obtained by streaking vaginal swab on MRS agar. Women were premenopausal, non menstruating and of 25–40 year age group. Samples were taken from the posterior zone of the fornix of the vagina. After 48 hrs of growth on solid medium, the bacteria were then inoculated in MRS Broth. The isolates were identified on the basis of different phenotypic and biochemical characteristics pure cultures of organisms identified and of this *Lactobacillus spp* were subcultured and used for different experiments. The antimicrobial activity of culture filtrate of lactobacilli was tested against *Staphylococcus aureus & E. coli* by standard methods (Fig. 2 and Table 3). The culture filtrates of *Lactobacillus* grown under different pH and temperature (Table 1 & 2; Fig. 1) conditions were isolated and tested against potential bacterial pathogens stated above.

The isolates were first tested against one gram positive and one Gram negative organisms. The bacteria showing highest antibacterial activity was then inoculated in MRS Broth. After overnight growth, cells were removed by centrifuging at 10,000 g for 5 min. The supernatant was filtered through a sterile 0.22 μm syringe filter and 50 μl of the supernatant was used as bacteriocin. The effect of bacteriocin on growth of *E. coli* and *S. aureus* was tested on Luria-Bertani broth (LB) and Nutrient broth (NB) media respectively. Further, the effect of culture filtrates of *Lactobacillus* which contained bacteriocin, organic acid and Hydrogen peroxide (H_2O_2) was compared with the effect of cell-free supernatant adjusted to pH 6.0 using 1N NaOH and considered as crude bacteriocin (Ogunbanow *et al.*, 2003).

Results and Discussion

The cell free supernatant inhibited the growth of potentially pathogenic bacteria. It is possible that the lactic acid produced by *Lactobacillus* acts as a permeabilizer of the Gram-negative bacterial outer membrane, allowing other antimicrobial substances produced by the host to penetrate and thereby increasing the susceptibility of pathogens. The cell free supernatant showed high antimicrobial efficacy against *E. coli*. Recent reports have revealed that some vaginal *Lactobacilli* and *Bifidobacteria*

produce antimicrobial substances that are active against pathogens. The *in vitro* application of *Lactobacillus* against *E. coli* in Agar plate also indicated that the developed products from vaginal Lactobacilli may be used against Bacterial pathogens. The vagina, which has a mucosal lining, may serve as an excellent biological substrate for the probiotic microbe and the product developed on the basis of present work may be used successfully for delivering the bacteria into the vaginal lumen for a prolonged period.

It is suggested that the most possible mechanism for the control of growth of pathogenic bacterial populations by lactobacilli is thought to be the production of hydrogen peroxide, bacteriocin and lactic acid from glycogen in vagina of healthy women and the LAB may be introduced for colonization in the vagina of diseased women as a curative measure. Vaginal lactobacilli control growth of potentially pathogenic bacterial populations by production of hydrogen peroxide, bacteriocin and lactic acid from glycogen in vagina of healthy women. The highest growth of Vaginal *Lactobacillus* is observed at 35°C and highest antimicrobial activity at heatshock treatment. It is suggested that the LAB may be introduced for colonisation in the vagina of diseased women as a curative measure. The indigenous microflora has advantages in suppressing undesirable micro organisms. It has been found that lactic acid bacteria (LAB) cultures isolated from human vagina show good antagonistic activity against both Gram positive and Gram negative pathogens.

The pH of MRS broth was 6.68 which has been adjusted to, different pH (pH3, pH4, pH5, pH6, pH7 and pH8) and after that we inoculated the samples with LAB strains and incubated at 35±2°C and on the next day the absorbance and pH were measured. The initial pH and the final pH along with growth of isolates (OD value) are tabulated in Table 2 and it has been found that the highest bacterial growth takes place at pH 8 but after the growth of the isolates in given pH, the pH of the culture medium came in the range of 3.54–6.27.

In Conclusion, Vaginal lactobacilli control growth of potentially pathogenic bacterial populations by production of antimicrobial substances in vagina of healthy women. The highest growth of Vaginal *Lactobacillus* is observed at 35°C and highest antimicrobial activity under heatshock treatment. It is suggested that the LAB may be introduced for colonization in the vagina of diseased women as a curative measure.

Table.1 Growth of isolates under different temperature treatment as depicted by OD value

Sl No	Sample No	OD for 25°C	OD for 35°C	OD for 45°C	OD for Heat Shock growth
1	L1	0.835	1.223	0.367	0.660
2	L2	0.731	0.838	0.081	0.555
3	L3	0.723	0.679	0.079	0.680
4	L4	0.887	0.839	0.511	0.610
5	L5	0.167	0.446	0.159	0.431

Table.2 Effect of bacterial growth (five bacterial isolates collected from vaginal swab) on pH of the medium before and after 24h

Sl No	Sample No	Zone of inhibition (in mm) (5µl of culture filtrate)							
		E Coli				*Staphylococcus aureus*			
		25°C	35°C	45°C	H	25°C	35°C	45°C	H
1	L1	5	5	4.5	5.5	4.5	5	4.3	5.5
2	L2	5	5	4.5	5.5	4.5.	5	4.5	5.2
3	L3	5	5	4.5	5	4.5	5	4.3	5.5
4	L4	5	5	4.5	5.5	4.5	5	4.5	5.5
5	L5	5	5	4.5	5.5	4.5	5	4.5	5.6

Table.3 Antimicrobial activity of the isolates against Gram positive and Gram negative bacteria as depicted by zone of inhibition after growing on different edaphic factor

S N		L1	L2	L3	L4	L5
1	Initial pH	3	3	3	3	3
	Final pH (after 24 hrs)	3.54	3.53	3.55	3.52	5.54
2	Initial pH	4	4	4	4	4
	Final pH (after 24 hrs)	4.43	4.51	4.47	4.48	4.49
3	Initial pH	5	5	5	5	5
	Final pH (after 24 hrs)	5.26	5.27	5.29	5.28	5.01
4	Initial pH	6	6	6	6	6
	Final pH (after 24 hrs)	5.35	5.36	5.32	5.26	5.16
5	Initial pH	7	7	7	7	7
	Final pH (after 24 hrs)	5.14	5.13	5.12	5.14	5.23
6	Initial pH	8	8	8	8	8
	Final pH (after 24 hrs)	5.08	5.08	5.09	5.06	6.27

Fig.1 OD values representing growth of bacterial isolates under different temperature treatment

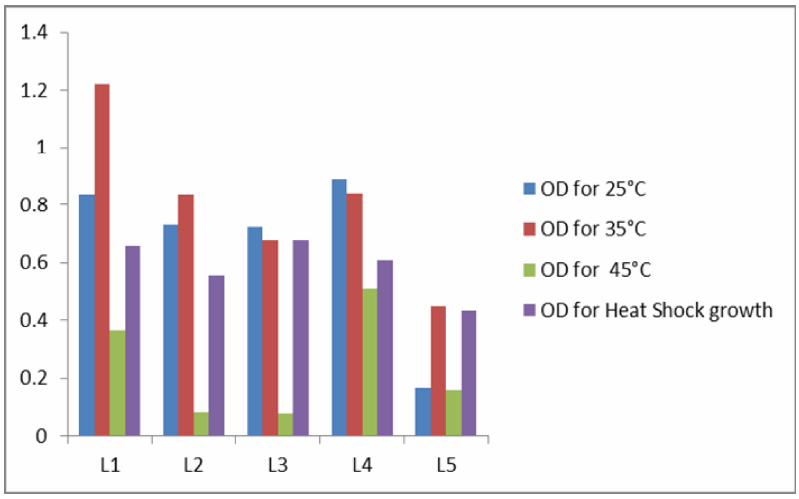
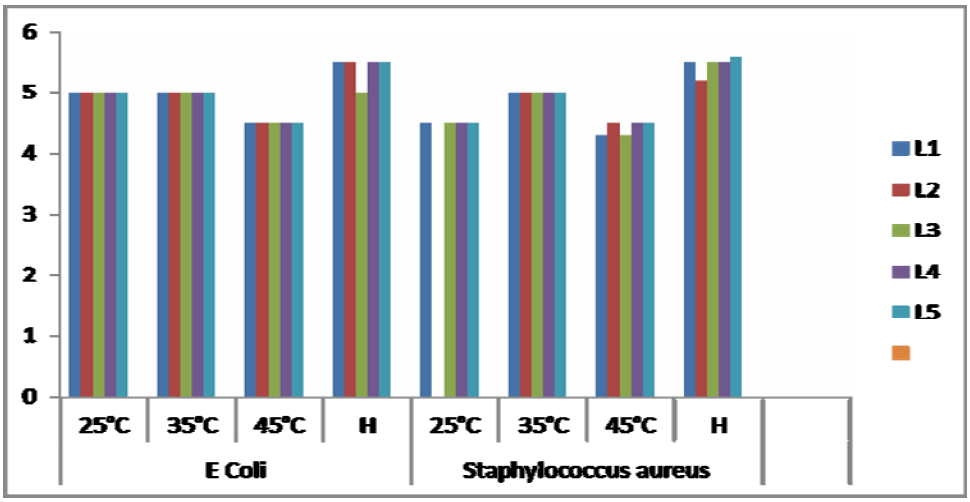


Fig.2 Inhibition zones representing antibacterial activity of the culture filtrates against *E.coli* and *Staphylococcus aureus*



Acknowledgement

The authors acknowledge financial assistance obtained from the NER twinning project (BT/220/NE/TBP/2011) sanctioned by the Department of Biotechnology, Govt. of India.

References

Anukam K. , Emmanuel O., Ijeoma A., Gregor R.: LactobacillusVaginal Microbiota of Women Attending a Reproductive Health Care Service in Benin City, Nigeria, Sexually Transmitted Diseases, Vol. 33, No. 1, p.59 – 62 (2006).

Garg K, Ganguli I,Das R.,& Talwar G: Spectrum of Lactobacillusspecies present in healthy vagina of Indian women. Indian J Med Res 129, pp 652-657 (2009).

Ogunbanow, S.T., Sanni, A.T., and Unilude, A.A, Characterization of bacteriocin produced by Lactobacillus plantarum F1 and Lactobacillus brevis OG1. African Journal of Biotechnology. 2(8), 219-227(2003).

Pavlova, S.I., Kilic, A.O., Kilic, S.S., So, J.S., Nader-Macias, M.E., Simoes, J.A., Tao, L.. Genetic diversity of vaginal lactobacilli from women in different countries based on 16S rRNA gene sequences. J Appl Microbiol. 2002;92(3): 451-9 (2002).

Rodr´ıguez C., Roberto R,Mario A, Ricardo F, Noem´I M, and Hernandez C.,: Vaginal Microbiota of Healthy Pregnant Mexican Women is Constituted by Four Lactobacillus Species and Several Vaginosis-Associated Bacteria. *Infectious Diseases in Obstetrics and Gynecology* (2011).

Tomas, M.S.J., Ocasna, V.S., wise, V., and Nader Macias, M.E., Growth and lactic acid production by vaginal Lactobacillus acidophilusCRL 1259 and inhibition of uropathogenic Escherichia coli. J. of Medical Microbiology 52(12), 1117-24 (2003).

Vásquez, A., Jakobsson, T., Ahrné, S., Forsum, U.,2 and Molin, G: Vaginal Lactobacillus flora of healthy Swedish women. J Clin Microbiol. Aug 2002; 40(8): 2746–2749 (2002).

Yura, T., and Nakahigashi, K Regulation of heat shock responses. Curr.opin. Microbiol.2:153-158 (1999).