Antibiotic Sensitivity Of Acid Stressed Probiotic Lactobacillus Acidophilus Ncdc 291

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Abstract

LAB (Lactic Acid Bacteria)-Lactobacillus acidophilus, a major inhabitant of gastrointestinal tract of human beings is used as a probiotic. Antibiotics taken during illness not only kill the disease causing microorganisms but also disrupt the normal microbial balance of the gut leading to a number of side effects and encouraging the patients to restore their natural gut microflora with the intake of probiotics. A well known probiotic Lactobacillus acidophilus NCDC 291 was used in the present study and its resistance towards antibiotics was determined at optimum pH and also by growing it under acid stressed conditions. Studies revealed that culture adapted to acid stressed conditions had increased resistance towards antibiotics than that grown at optimum pH.

INTRODUCTION

A well known proverb “health is wealth” has become the most important subject of concern with the modern world. Healthy lifestyle can be achieved with a nutritious diet, regular exercise etc. When it comes to health and diet, an increasing trend for food containing probiotics is in demand today. Probiotics are usually defined as microbial food supplements, that when administered in adequate amount exert beneficial effects on the host (Khalil et al., 2007).

LAB (Lactic Acid Bacteria)-Lactobacillus acidophilus is a major inhabitant of gastrointestinal tract of human beings. It is used as a probiotic because it has tendency to adhere to the intestinal epithelial cells thereby colonizing the gut. It can survive harsh conditions in the stomach and can also deconjugate bile salts released in the intestine. It lowers pH in the gut making the environment unfavourable for the growth of pathogenic bacteria.

Antibiotics taken during illness act as a double edged sword killing not only the disease causing microorganisms but also disrupting normal microbial balance of the gut. According to the latest statistics one in five persons taking antibiotics does not take full course of antibiotic therapy because of the side effects associated with it. Common antibiotic side effects include upset stomach, diarrhea, and vaginal infections etc which occur mainly because of the disturbed microbial load or normal bacterial flora of the patient. To overcome this problem supplementary diet including probiotics is a must. For this the antibiotic sensitivity of the probiotic microorganism holds foremost position. Lactobacilli show varied response towards different antibiotics. Increased resistance of the acid stressed culture provides us with a potent probiotic supplement that can withstand high acid conditions in the stomach and colonize the intestine further enhancing the immunity of the patient by discouraging the growth of pathogenic microorganisms. In the present study a well known probiotic i.e. Lactobacillus acidophilus NCDC 291 was taken and its potential to survive the antibiotic stress at optimum pH and of acid stressed culture was determined.

MATERIAL AND METHODS

Microbial culture of Lactobacillus acidophilus NCDC 291 was obtained from Dairy Microbiology division, National Dairy Research Institute, Karnal, India.

Initially it was cultured in 12% (v/v) skimmed milk autoclaved at 15 psi for 30 min and incubated for 24 h at 37 °C. Curdling of milk marked the growth of L. acidophilus. Subsequent culturing was carried out by inoculating in readymade MRS medium (HIMEDIA), pH 6.5 ± 0.2 autoclaved at 15 psi for 15 min. This was followed by incubation for 24 h at 37 °C.

EFFECT OF PH ON GROWTH:

Following the method of Joshi et al., (2006) pH of MRS medium was altered to 3.5, 4.5, 5.5, 6.5 and 7.5 using 0.1 N

NaOH/ 0.1 N HCl. It was then inoculated @ of 1% (v/v) of overnight grown culture and incubated for 24 h at 37 °C. Absorbance was taken every 6 h at 600 nm and SPC was also recorded.

ANTIBIOTIC SENSITIVITY:

Antibiotics namely Ampicillin, Erythromycin, Streptomycin, Vancomycin, Penicillin, Chloramphenicol and Tetracycline were chosen in the present study. Ampicillin, Vancomycin, Streptomycin and Penicillin function by inhibiting the cell wall synthesis whereas Chloramphenicol, Erythromycin and Tetracycline inhibit the protein synthesis thereby exerting the bactericidal effect.

Well Diffusion method was followed. Stock solutions of antibiotics were prepared in sterile distilled water. 100 µl of filter sterilized (using syringe filter of pore size 0.22µ) antibiotic solution with final concentration of 1 mg/ml was added into the wells. Control well contained sterile distilled water. Diameter of zone of inhibition was measured with antibiotic zone scale.

ANTIBIOTIC SENSITIVITY OF ACID STRESSED CULTURE:

As per method of Ehab Essa Kheadr (2006), the culture was sensitized to acidity by repeated subculturing in medium, the pH of which was altered using 1M glacial acetic acid to 4.0. It was subcultured every 24 h for 5 days. Subsequent procedure followed to study the effect of antibiotics is mentioned above.

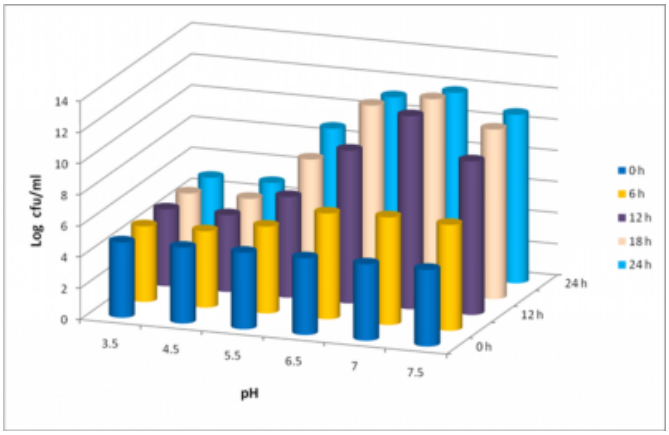
RESULTS

EFFECT OF PH:

Growth of L. acidophilus NCDC 291 varied with the change in pH (Graph 1). At 3.5 and 4.5 pH though the microorganism was able to survive but the growth was severely retarded with an increase of only 0.1 log cfu/ml from 0 h to 24 h at both pH values. An increase of 3.91 log cycles was observed at pH 5.5 with continuous growth till 24 h. The rise in log cfu/ml at pH 6.5 was 6.28 log cfu/ml, at pH 7.0 was 6.96 log cfu/ml and at pH 7.5 was 5.90 log cfu/ml indicating maximum increase in colony forming units at neutral or near neutral pH.

Figure 1

Graph 1: Effect of pH on growth of NCDC 291.



ANTIBIOTIC SENSITIVITY:

Growth inhibiting effects of Ampicillin, Erythromycin, Streptomycin, Vancomycin, Penicillin, Chloramphenicol and Tetracycline were studied and L. acidophilus NCDC 291 was found to be sensitive to all of them except Erythromycin (Table 1). Maximum zone of inhibition, 38.5 mm was obtained with Vancomycin and minimum i.e. 11.5 mm with Streptomycin. In Ampicillin and Tetracycline zone of 33.0 mm and 31.0 mm was recorded respectively. Zone observed in Chloramphenicol was 29.0 mm.

Acid stressed culture on the other hand became resistant to all the antibiotics except Chloramphenicol and Tetracycline where a clear zone of 11.0 mm was observed in both (Table 1). 19 mm and 20 mm decrease in zone diameter in case of Chloramphenicol and Tetracycline respectively, indicates that the microorganism became less sensitive towards these antibiotics as well.

Figure 2

Table 1: Antibiotic sensitivity of optimally grown and acid stressed NCDC 291

Sr. No.	ANTIBIOTICS	ZONE OF INHIBITION (mm)	ZONE OF INHIBITION of Acid Stressed Culture (mm)
1	Erythromycin	--	--
2	Streptomycin	11.5	--
3	Chloramphenicol	29.0	11.0
4	Penicillin	30.0	--
5	Tetracycline	31.0	11.0
6	Ampicillin	33.0	--
7	Vancomycin	38.5	--

DISCUSSION

LAB Lactobacillus acidophilus is a well known probiotic and is commonly found in gastrointestinal tract of human beings. It exerts positive effect in the gut by helping in the

proper absorption of nutrients and by competing with pathogenic microorganisms that tend to colonize the gut. Penicillin was first antibiotic to be discovered in 1929 and since then many other antibiotics have been discovered and are used therapeutically. Nowadays with increased use of antibiotics in order to fight infections a major threat is posed to normal friendly gut microflora. They are used for about every disorder and disease. Frequent users of these "wonder drugs" face problems like overgrowth of yeast Candida albicans which thrives in a gut, low in friendly bacteria. The most common cure for yeast infections is recommendation of probiotics (Gionchetta and Campieri, 2002). In the present study the effect of acidic pH on growth of L. acidophilus NCDC 291 was observed. It was also subjected to acid stress and then tested for its antibiotic sensitivity.

Growth of L acidophilus NCDC 291 decreased with decreasing pH (3.5- 4.5). Maximum increase in cell count was observed at near neutral pH of 6.5 i.e. 6.28 log cfu/ml, 5.9 log cfu/ml at pH 7.5. An increase of 3.91 log cfu/ml was recorded at pH of 5.5. At lower pH of 3.5 and 4.5 the increase was only 0.1 log cfu/ml. Silvinia et al., (2003) reported pH 6.5 to 8.0, 37 °C to be the optimum range for growth of L. acidophilus. Llong and Shah (2005) studied effect of acid on different strains of lactobacilli and found all of them tolerant to pH 2.0 for 2 h despite variation in their degree of viability. Lactobacillus acidophilus ATCC 4962 was one of the most acid tolerant strains with more than 10^7 cfu/ml.

Our strain was found to be sensitive to all the antibiotics except Erythromycin. Delgado et al., (2005) reported that lactobacilli show species dependent resistance towards antibiotics. He observed L. acidophilus resistant to Erythromycin but sensitive to Chloramphenicol. Ocana et al., (2006) found strains of L. acidophilus sensitive to Vancomycin. Strains studied by Zhou et al., (2005) were sensitive to Erythromycin, Novobiocin, Tetracycline, Chloramphenicol and beta-lactam antibiotics-Penicillin, Ampicillin and Cephalothin. Therefore, different strains of L. acidophilus exhibit different response towards antibiotics.

On the other hand acid stressed culture of L. acidophilus exhibited resistance towards all the antibiotics (Ampicillin, Erythromycin, Streptomycin, Vancomycin and Penicillin,). Sensitivity towards Tetracycline and Chloramphenicol decreased to a lesser extent than other antibiotics. Ehab Essa Kheadr (2006) conducted a study on 13 Lactobacillus strains including 2 strains of L. acidophilus to determine their antibiotic susceptibility to evaluate the impact of gastrointestinal stressful conditions along with the acid adaptation on their antibiogram profiles. He adapted the strains to grow at pH 4.0 and found acid adapted Lactobacillus acidophilus R052 to be more resistant to antibiotics.

It can be inferred from the results that acid stressed probiotic Lactic acid bacteria come up as a savior for the individuals who have adverse reactions from these wonder drugs and also by those who suffer from yeast infections. Gut of the individuals harboring acid stressed probiotic microflora can help to improve health of that particular person by resisting the gastrointestinal aggression by disease causing microorganisms. Acid stressed probiotics being resistant to antibiotics can provide probiotic benefit during the course of treatment and alleviate the side effects of these antibiotics.

CONCLUSION

L. acidophilus NCDC 291 shows minimal growth at low pH and with increase in pH upto neutral, the growth increases. Maximum growth was observed at pH of 6.5. The antibiotic resistance of lactobacilli varies among different strains. Lactobacillus acidophilus NCDC 291 being a potential probiotic when adapted to low pH shows considerable resistance towards antibiotics like Ampicillin, Erythromycin, Streptomycin, Vancomycin, Penicillin, Chloramphenicol and Tetracycline. The acid stressed organisms are better probiotics than non-acid stressed ones. They can be an effective remedy for intestinal as well as vaginal infections.

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