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Abstract:-

Probiotic must be able to benefit on host through activity in human body. Lactobacillus organisms are normal inhabitants of the human intestine and vagina. They are the main ones that produce lactic acid in the digestive tract, which is important for overall health. For the prevention of intestinal disorders, probiotics can be rendered multidrug resistance to survive in the presence of antibiotics. Isolation and identification, detection and development of antibiotic resistecein this strains of lactobacillus species from commercially available probiotics and for this antibiotic susceptibility and Development of inducible drug resistance to amoxicillin and cephalexin were done and found that isolated probiotic strain was Lactobacillus rhamnosus. It is a Gram positive, nonmotile, non sporing, rod shaped bacteria. The size of colonies of Lactobacillus rhamnosus on MRS medium was of 1mm in diameter, circular, pale yellow, concave elevation, opaque and with a smooth surface and various sugar test also performed and from this result it is concluded A new strain of Lactobacillus rhamnosus that developed was resistant to amoxicillin and cephalexin at a concentration of 70µg/mL.

Keywords:

Amoxicillin And Cephalexin , Probiotic Strain , Lactobacillus .

DEVELOPMENT OF INDUCIBLE DRUG RESISTANCE TO AMOXICILLIN AND CEPHALEXIN IN A PROBIOTIC STRAIN OF LACTOBACILLI

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INTRODUCTION

Probiotics are the live microorganism which when administered in adequate amount confirms health benefit, to host and maintain intestinal flora. Infectious diseases are the biggest problem in human being and every year gastrointestinal infections are responsible for significant morbidity and mortality worldwide (Culligan *et al*, 2009). World Health Organization (WHO, 2004) estimates there to be more than four billion episodes of diarrhoeal disease annually, while there were 2.2 million deaths attributable to enteric infection, making it the fifth leading cause of death at all ages worldwide. Probiotics can be used in treatment and prevention of enteric infection and chronic inflammatory disorders of the gastrointestinal tract (Gill and Guarner, 2004). The following are microorganisms considered to be human Probiotics:
Lactobacillus species: *L. acidophilus, L. amylovorus, L. brevis, L. casei, L. casei subsp. Bifidobacterium species: B. adolescentis, B. bifidum, B. breve, B. infantis, B. lactis (B. animalis).*

Other lactic acid bacteria: *Enterococcus faecium, Lactococcus lactis, Leuconstoc mesenteroides,*

Nonlactic acid bacteria: Bacillus subtilis, Escherichia coli strain nissle, Saccharomyces boulardii,

Lactobacilli constitute a major part of the natural microflora of human intestine. These probiotic organisms when present in sufficient number can create a healthy equilibrium between beneficial and potentially harmful microflora in gut by creating unfavourable condition for the growth of commonly occurring intestinal pathogen (Tagg and McGiven, 1971; Salminen *et al.*, 1998).

Few *Lactobacillus* strains are able to inhibit the growth and adhesion of *Candida albicans* or other Candida species, and there is no solid evidence to indicate that intravaginal administration of lactobacilli can eradicate yeast infection. However, there is some evidence to suggest that lactobacilli ingestion and vaginal use can reduce the risk of recurrences (Hilton et al, 1992; 1995).

AIMS AND OBJECTIVES

For the prevention of intestinal disorders, probiotics can be rendered multidrug resistance to survive in the presence of antibiotics.Therefore the present study aims at,

- ❖ Isolation and identification of the strains of lactobacillus species from commercially available probiotics.
- ❖ Detection of the antibiotic sensitivity in the isolated lactobacillus strain.
- ❖ Development of antibiotic resistance in this strain by inducible drug resistance.

MATERIALS AND METHODS

Materials: The commertially available probiotic products like Ecobion, Vibact, Bioclin, etc were procured from the local medical stores.

Media (Hi media make)

1. Peptone physiological solution.
2. Phosphate buffer
3. De Man Rogosa and Sharp agar Composition
4. Antibiotics (Sigma) Amoxicillin, Cephalexin

A.Commercial probiotics and their contents.

1.Biocline -*Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum Lactobacillus casei*
2.Ecobion- *Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Bifidobacterium longum , Saccharomyce boulardii, Streptococcus thermophillus*
3.Vibact -*Streptococcus faecalis T-110JPC, Clostridium butyricum TO-A, Bacillus mesentricus TO-AJPC, Lactic acid bacteria (Lactobacillus sporogenes)*
5. Antibiotic discs (Hi media, Mumbai Ltd.)

Names of antibiotics	Concentrations
Amikacin	30 µg
Amoxycilin	10 µg
Ampicillin	10 µg
Ceftazidime	30 µg
Ceftriaxome	30 µg
Cephalexin	30 µg
Cephaxitin	30 µg
Chloramphenicol	30 µg
Cloxacillin	5 µg
Co-trimoxazole	25 µg
Erythromycin	15 µg
Gentamicin	10 µg
Penicillin	25 µg
Penicillin-v	3 µg
Piperacillin	100 µg
Tetracycline	10 µg

METHODS

1)Isolation of probiotic strain from commercial product

Commercially available powdered probiotic was inoculated in peptone physiological solution. 100mg sample was suspended in 100 mL of peptone physiological solution and incubated at 37°C for 24h. After incubation loopful culture from broth was inoculated on MRS agar medium and again incubated at 37°C for 24–48h. The colonies were identified by cultural and morphological characteristics and sugar fermentation tests.. Sugar fermentation test was done by using disc of sugars. Phenol red base broth was used in which the sugar disc were added and then the culture was inoculated into the broth. This was incubated at 37°C for 24h. Change in colour from red to yollow indicated sugar fermentation with formation of acid.

2)Antibiotic susceptibility test

Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method. The antibiotics to which the probiotic strain was highly sensitive were selected for further study.

3)Development of inducible drug resistance to amoxicillin and cephalexin.

a. Preparation of antibiotic solution

Pure antibiotic in powder form was procured from Sigma Company. Phosphate buffer of pH 6.0 was used as a solvent as well as dilutient for amoxicillin and cephalexin. Stock solution of antibiotics was prepaired by adding 500mg of antibiotic powder in 100mL of sterile phosphate buffer this was the stock solution and the concentration was 5mg/mL. Working solution was prepared by diluting 10 ml of stock solution in 100 mL of sterile phosphate buffer. This was first working solution and the concentration of this solution was 500µg/mL. From this first working solution 10 mL of solution was again diluted to 100 mL of phosphate buffer. This was second working solution and the concentration was 50µg/mL. Again from this solution 10 mL solution was taken to make the solution having concentration 5mg/mL. In similar manner dilution was carried out to get the final concentration of 5µg/mL. The prepaired antibiotic solution was added asceptically to sterile molten nutrient agar in increasing order and allowed to solidify.

b.Method for developing inducible drug resistance.

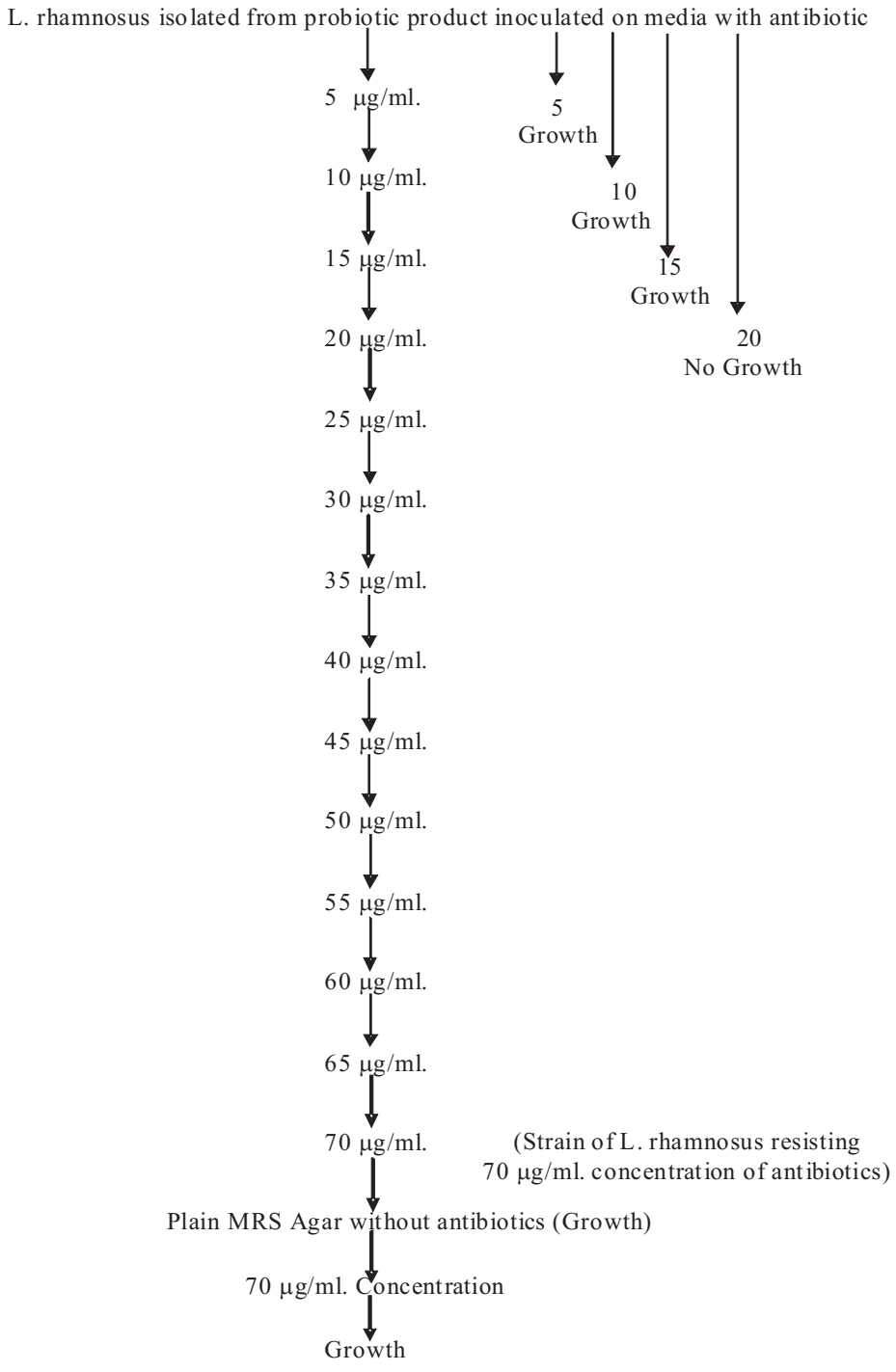
The isolated probiotic strain was inoculated on the nutrient agar plate containing 5µg/mL of antibiotic and incubated at 37°C for 24 h. after incubation growth observed was tranefered to nutrient agar containing 10µg/mL of antibiotic and again incubated. In such a way the growth was transferred to nutrient

agar medium containing increasing concentration of antibiotics.

In a similar manner serial passage of the strain was carried out till 70µg/mL concentration of antibiotics. Simultaneously the initially isolated probiotic strain was also inoculated on the plate with increasing concentration of antibiotics to detect the development of drug resistance. The probiotic strain that resisted to 70µg/mL of antibiotic was again transferred to plane nutrient agar / MRS agar medium and then they again subcultured on nutrient agar with 70µg/mL of antibiotic to determine the stability of the acquired drug resistance.

RESULTS

From the three products different probiotic isolates were obtained. Among them one isolate was selected and identified by morphological, cultural characteristics and biochemical tests.The isolated probiotic strain was *Lactobacillus rhamnosus*. It is a Gram positive, nonmotile, non sporing, rod shaped bacteria. The size of colonies of *Lactobacillus rhamnosus* on MRS medium was of 1mm in diameter, circular, pale yellow, concave elevation, opaque and with a smooth surface. Sugar fermentation test was carried out to confirm the isolate. The isolated *Lactobacillus rhamnosus* was sensitive to all the antibiotics tested.



DISCUSSION

The present investigation was aimed at isolation, identification, antibiotic susceptibility testing and development of inducible antibiotic resistance in a strain of probiotic from a commercial product. Accordingly a strain of *Lactobacillus rhamnosus* was isolated from a commercially available probiotic powder. It was identified by cultural and morphological characteristics and confirmed by sugar fermentation test. Similar isolation and characterization of probiotic bacteria from commercial product was carried out by R Temmerman. They processed 55 europian probiotic products to identify and the antibiotic resistances of the bacterial isolates recover from this product and found that from 30 dried food supplement and 25 dairy products yielded a total 268 bacterial isolates.

Resistance to amoxicillin and cephalexin was induce in the strain of lactobacillus rhamnosus was developed that could resist the concentration of amoxicillin cephalexin upto 70µg/mL. If such probiotics strains are consumed probiotics as the pribiotic will not be destroyed by the antibiotics.

According to the study conducted by Behira Belkacem and Kihal Mebrouk, on antibiotic resistance of some lactobacilli isolated from the gut microflora of broiler such type of antibiotic resistance could also be induced in the gut when both the antibiotics and probiotics are consumed together.

CONCLUSION

In the present study *Lactobacillus rhamnosus* was isolated and identified from commercially available probiotic products. Its antibiotic susceptibility was determined to various antibiotics. It was found to be completely sensitive to all the antibiotic tested. While the resistant to amoxicillin and cephalexin was induced in *Lactobacillus rhamnosus*, it readily get resistance to the two drugs. It could resist upto 70µg /mL of the drug. This indicates that the bacteria can very easily acquired resistance to antibiotics. A new strain of Lactobacillus rhamnosus that developed was resistant to amoxicillin and cephalexin at a concentration of 70µg/mL. Thus it can be conclude from the study that such type of inducible drug resistance can be developed in probiotics so that it could be effectively administaded during the course of antibiotics and prevent the antibiotic induced diarrhoea.

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