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Isolation of Lactic Acid Bacteria and Detection of their Antimicrobial Activity

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ABSTRACT: Lactic acid bacteria have been widely used in the preservation of variety foods. Such as dairy products, vegetables products, fish and meat. Metabolites produced from lactic acid bacteria such as acetic acid, hydrogen peroxide, lactic acid, bacteriocin and low molecular weight proteins have antimicrobial activities. Lactobacilli strains were isolated from home made curd by tenfold dilution plating method and plated on De Man Rogosa and Sharpe Medium. Identification of Lactic acid bacteria was done Gram's staining method and Biochemical test.

The aim of our study was to test the antimicrobial activity of the isolated Lactobacilli strains against bacterial and fungal organism filter paper using disc diffusion method and two fold serial dilution methods.

I. INTRODUCTION

The discovery of penicillin marked the beginning of the antibiotics era, the development of its fermentation has ushered in what might be called the golden era of industrial microbiology. The onset of this has resulted in the production of large number of plant or microbial primary and secondary metabolites that are of commercial importance. Whereas primary metabolism is universal among living system, secondary metabolism is carried out by plant and microbes and is usually strain specific.

The primary metabolites that are important in the bio-industry are the organic acid, Vitamins, Amino acid and purine nucleotides. However, of all the traditional products made by bioprocess, it is the secondary metabolites that are of great importance and value to the human health. Secondary metabolites, particularly from microbial sources, are selective in their action on pathogenic bacteria and fungi. The success rate was so importance that the pharmaceutical industry screened secondary metabolites almost exclusively for antibacterial, antifungal and antitumor chemotherapy as well as against diseases not caused by bacteria, fungi or tumors.

Crude lactic cultures are known to inhibit some psychrotrophs in milk and ground beef. A large number of lactic acid bacteria, singly or in combination, have been shown to display varying degrees of antimicrobial activity against pathogenic microorganisms. In addition, viable cultures or components of lactic acid bacteria are useful in the treatment of displaced endogenous intestinal micro flora, which are character tic of many intestinal disorders and enhanced gut permeability of the host. Such bacteria are able to survive gastric conditions to colonize the intestine, at least temporarily by adhering to the epithelium. They have been reported to improve the growth rate and feed utilization in pigs, chicken and calves and to improve their feed conversion ratio. There is significant decrease in the occurrence of diarrhea observed in Pigs and Calves fed with these beneficial bacteria such as *Clostridium perfringens* to reduction of bacterial urease activity to digestion.

Lactobacilli are one of the most important types of friendly bacteria found in the digestive tract. These bacteria get their name (lacto) because they are able to form lactic acid. They play a key role in producing fermented foods, fermented milk, yogurt, and cheeses. They are often referred to as "probiotic" since they are positive microorganisms.

Lactic acid bacteria (LAB) commonly used as starter cultures in food known to produce antimicrobial substances such as bacteriocin, having great potential as food biopreservative. Metabolites produced from LAB including lactic acid, acetic acid, hydrogen peroxide, bacteriocin and some low molecular weight compounds have antimicrobial activity.

Microbial peptides with pronounced antimicrobial activity are commonly isolated from animals, plants, microbes and in fermented food. They are small and cationic with molecular masses of between 3000 and 6000 DA. Post translational modification of precursor peptides has been shown to introduce intramolecular thio ether bridges to cationic peptides such as pep 5, nisin and subtilin.

Although these peptides offer an important potential safety advantage over chemically synthesized preservatives when incorporated into food, many

peptides are not suitable owing to the pathogenic capture of the producer strains. Bovine lactoferrin, an antimicrobial component of colostrums and milk, helps in the protection of infants from gastrointestinal infections porcine pepsin cleavage of native lactoferrin produces low molecular weight peptides inhibitory to some gram positive and gram negative bacteria. In addition, hydrolysis of native lactoferrin at pH 2 and 120°C produces active peptides which are bactericidal.

Methanol acetone extract of lyophilized fermented milk by lactic cultures have been reported to contain ninhydrin positive materials, these proteinaceous material do not loss antimicrobial activity when exposed to 100°C for 10 min, are active at pH 5.4 and not inactivated by pepsin treatment. Purified cationic, low molecular weight material isolated from *Steptococcus diacetylactis,* has been found to be heat stable and active towards several pseudomonas species Upto pH 6.0. Acidophilin, a low molecular weight (approx 700 Da) nitrogenous compound from *Streptococcus diacetylactis* and *Leuconostoc citrovorum* cultures, has potent antimicrobial activity. Antimicrobial compound(s) from *Streptococcus thermophilus* are likely to be amines of low molecular weight, which are heat stable. An antimicrobial substance from Lactobacilli sp. Strain GG is inhibitory to Gram-positive as well as Gram-negative bacteria but not lactobacilli. It is active between pH 3and 5, heat stable and resistant to proteinase K, α-chymotrypsin, trypsin and carboxypeptidase A. It resembles a microcin from some of the characteristics studied. The antimicrobial substance from Lb sp. Strain GG has a molecular weight of less than 1,000da and is soluble in acetone-water.

Microcin are low molecular weight amino acid-oligopeptide antibiotics, which are resistant to some protease, extreme ph values and heat, and soluble in methanol. In contrast, bacteriocins are larger proteins, which can be inactivated by protease. Microcin A15 (<500da) is a bacteriostatic compound with a methionine moiety. Microcin B 17, with a molecular weight of 40000 Da is sensitive to pronase, subtilisin, and thermolisin. Microcin C7, which is about 900 da, is sensitive to trypsin and subtilisin but loses little activity when exposed to 100^0C for 30 min. Microcin C7 is a linear structure consisting of acetyl-methionine, arginine, threonine, glycine, asparagines, alanine and a supposed ethanolamine entity at the carboxyl- terminal end. Microcin D15, D93 and D140 are highly hydrophilic, basic and small substance.

Bacteriocins are am extremely heterogeneous group of substances. of The original definition bacteriocins reffered to proteins of the colicin type produced by *Escheichia coli*. Bacteriocins are proteinaceous compounds usually with bactericidal activity against species closely related to the producer bacterium. These secondary metabolites also possess fungicidal, metal-chelating and immune-modulating properties. Most bacteriocins are produced *Staphylococcus aureus* and *Listeria monocytogenes,* produced by Gram positive and Gram negative bacteria; they are characterized by lethal biosynthesis, intraspecific activity, and adsorption too specific receptors. Bacteriocins are active macromolecules possessing narrow inhibitory spectrum of activity, protein in nature, plasmid encoded and without effect on producer cells, Reports have shown that most bacteriocin produced by Gran-negative bacteria act on closely related species. On the other hand, inhibition of Gram-negative bacteria has not been clearly demonstrated by purified bacteriocins from Gram-positive organism. In this regard, the presence of a lipoteichoic acid receptor for pediocin in the host cell wall suggested that the bacteriocons from Gram-positive organism. In this regard, the presence of a lipoteichoic acid receptor for pediocin in the host cell wall suggested that the bacteriocins from Gram-positive bacteria may not be inhibitory to Gram-negative organism since the latter do not possess cell wall teichoic acid.

Many stains of Gram-positive fermentation starter culture bacteria produce bacteriocins. These bacteriocins possess narrow or wide spectra of activity. However, it is those with wider range of activity that are desirable as food biopresevatives. Characterizations that define a baceriocin have been expanded to include their chemical nature, stability.

Lactic acid bacteria (LAB) are widely distributed in nature such as in dairy, fish as well as vegetable and grains. During the last few decades, fermented food can suppress the microbial spoilage and prolong shelf life in food preservation. (Ross et al., 2002) previous studies showed that metabolites produced from LAB have antimicrobial abilities because of organic acids (lactic acid, acetic acid), diacetyl, hydrogen peroxide and some low molecular weight compounds. (Gilliland and Speck, 1975; Barnby-Smith et al., 1989 Daeschel, 1989; Larsen et al., 1993)

Among the antimicrobial metabolites, bacteriocins are peptides or proteins with bacteriostatic action especially against closely-related species (Klaenhammer, 198; Garneau et sl., 2002) One of bacteriocin against Staphylococcus aureus is produced by *Lactobacillus plantarum* BS (Elegado et al., 2004) The bavaricin A, a bacteriocin produced by *Lactobacillus avarices* MI401 inhibits the growth of Listeria monocytogenes (Larsen et al ., 1993) Therefore the bacteriocins were attracted interest of researchers and food producers for the potential to be biopreservative additives (Ross et sl.,2002). Klaenhammer classified bacteriocin of LAB into four groups (I) lantibiotics, small (<5kDa), membrane-active peptides containing lanthionine, β -methyl lanthionine

containing membrane- active peptides; (III) LARGE (>30k Da), heat- stable proteins and (IV)complex bacteriocins composed of protein plus one or more chemical moieties aaaa9lipid of carbohydrate) required activity (Klaenhammer,1993)

LACTOBACILLUS

Scientific classification

Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Lactobacillaceae
Genus: ***Lactobacillus***

Lactobacillus, also called Doderlein's bacillus, is a genus of Gram-positive facultative anaerobics or microaerophilic rod-shaped bacteria organotroph. They are usually straight, although they can form spiral coccobacillary forms under certain conditions. They are often found in pairs or chains of varying length. They are a major part of the lactic acid bacteria group, named as such because most of its members convert lactose and other sugars to lactic acid. In humans they are present in the vagina and the gastrointestinal tract, where the makeup small portion of the gut flora. They are usually benign, except in the mouth where they have been associated with cavities and tooth decay (dental caries). Many species are prominent in decaying plant material. The production of lactic acid makes its environment acidic, which inhibits the growth of some harmful bacteria. Several members of the genus have had their genome sequenced.

Lactobacilli are ubiquitous and widespread bacteria in the human and animal microflora. They are widely used by humans: as adjuvant against gastrointestinal disorders, as dietary supplements, and as biological food processors in view of their fermentative properties.

APPLICATIONS

IN PHARMACEUTICALS. L (+) Lactic acid is used in pharmaceutical industry as a very important ingredient in IV Fluids. Lactated Ringers solution and Sodium Lactate are used in IV Fluid therapy. They provide the energy and volume for blood besides regulation of pH Calcium, Sodium, Ferrous and other salts of lactic acid are used in the pharmaceutical industry in various formulation.

Lactate salts have better absorption metabolized resulting in , solubility and easily metabolized resulting in administration of some very important drugs like ciprofloxacin as a lactate salt, Lactic acid based formulations find use for their anti tumor activity.

IN COSMETICS. Natural L (+) Lactic acid is used in many applications in cosmetics. Lactic acid is an alpha hydroxyl acid (AHA) and is found in the skin. It is used as a skin rejuvenating agent, pH regulator. It is a common ingredient in Moisturizers, Skin whiteners, anti acne preparations, etc. Since L(+)- lactic acid is naturally present in the skin, Lactic acid and Sodium lactate are extensively used as moisturizing agents in many skin care products. Lactic acid is also used as a pH-regulator. It is one of the most effective AHAs and has the lowest irritation potential. Lactates are regarded as skin whitening agents that have been shown to produce a synergistic effect when combined with other skin whitening agents. Sodium Lactate is used in anti perspirant preparations. L (+)-Lactic acid is used as a pH-regulator in many types of hair care formulations.

IN FOOD INDUSTRY. Lactic acid occurs naturally in many food products. It has been in use as an acidulant, preservative and pH regulator for quite some time. Some of the important applications of lactic acid in the food industry are detailed below. There are many properties of lactic acid which make it a very versatile ingredient in the food industry. It has a pronounced preservative action, and it regulates the microflora. It has been found to very effective against certain type of micro organisms Sometimes combination of lactic acid acetic acid is used as it has a greater bactericidal activity. Because it occurs naturally in many food stuffs, it does not introduce a foreign element into the food.

Bear and wine. Lactic acid is a natural beer acid, and hence it is used for pH adjustments during the mashing process and in cooking. Lactic acid improves the microbial stability and also enhances the flavor. Almost all the breweries in India use the L (+) Lactic acid manufactured by Lactochem Limited.

Beverages. Lactic acid due to its mild is the acidulant of choice on delicately flavored soft drinks and fruit juices. It does cot mask not mask or over power the natural flavor. Its flavor enhancing property makes the beverage more palatable and leaves lingering taste. Lactic acid is preferred over citric acid of these reasons.

Olives, Pickles, Cabbage, Gherkins. Green olives, Gherkin sand others are often packed in a solution of salt, lactic acid and water. The lactic acid acts as a preservative and improves the clarity of the brine and flavor. A mixture of acetic acid and lactic acid in pickled products such as gherkins, silver skin onion etc imparts a milder taste and flavor, and improves microbial stability. Large quantity of gherkins in brine with Lactochems's L (+) Lactic acid is exported from India to many countries worldwide. Calcium lactate is reported to be used as firming salt, which have been used for canned fruits and vegetables.

Dairy Product. Direct acidification with lactic acid, in dairy products such as cottage cheese, is preferred to fermentation as the risks of failure and contamination can be avoided. The processing time also can be saved. Lactic acid and calcium lactate are used extensively in the production of Channa and Pannier by direct

acidification. Lactic acid is also used as an acidulant in dairy products like cheese and yogurt powder.

Meat and Meat Products: Lactic acid is widely used in meat products as an anti microbial agent. Decontamination of beef, poultry and pork carcasses in slaughter house operations is practiced to reduce Salmonella infection. In sausages, sodium lactate is used to reduce water activity and achieve higher shelf-life. Recent research publication indicates the use of hot lactic acid spray on carcasses where reduction of over 99% of *E.Coli* has been observed.

INDUSTRIAL APPLICATIONS

Poly (lactic acid) or Lactic acid polymers are environmentally compatible because they degrade into natural, harmless products. The polymers degrade primarily by hydrolysis after exposure to moisture. These polymers find use in the pharmaceutical industry in dental, surgical implants, drug delivery systems and other applications.

II. MATERIAL AND METHOD

Chemicals	Manufacture
Beef Extract	Qualigens
Polysorbate 80 (Tween 80)	HEMEDIA
Dextrose	Qualigens
Magnesium sulphate heptahydrate	CDH(Central Drug House){P}Ltd.Mumbai
Magnesium sulphate tetrahydrate	Qualigens
Nutrient Agar	SRL (Sisco Research Laboratory)
Nutrient Broth	SRL (Sisco Research Laboratory)
Peptone	Qualigens
Potassium di Hydrogen Phosphate	SRL (Sisco Research Laboratory)
Sabouraud Dextrose Broth	SRL (Sisco Research Laboratory)
NaCl	SRL (Sisco Research Laboratory)
Yeast Extract	HEMEDIA

Instruments	Manufacture
Autoclave	Macro Scientific works 10A/UA, Jawahar Nagar , P.B.No- 2151 Delhi (tazer)
Centrifuge	Spinwin
Cooling Microfuge	REMI
Hot air oven	TANCO
Eppendroff tube	Tarson
Horizontal Laminar Air Flow	Macro scientific works 10A/UA, Jawahar Nagar , P.B.No- 2151 Delhi (MAC)
Incubator Shaker	Macro scientific works 10A/UA, Jawahar Nagar , P.B.No- 2151 Delhi(LARK)
Microwave Oven	KENSTAR
Incubator(37C)	MAC
Pipettes and other glassware	Borosil
Weighing Balance	Citizen ISO 9001:2000certified
µ pH System 362(pH meter)	Systronics (An ISO 9001:2000 Co.)(A Division of ASE Ltd.)

METHODOLOGY

ISOLATION OF LACTIC ACID PRODUCING BACTERIA FROM CURD

The bacterial strains were isolated from curd by 10 fold dilution plating method (Clark et.al.1988).One milliliter of curd was dissolved in a known quantity of water (9ml) and homogenized. One milliliter of above curd suspension was pipette out from the test tube and added to another test tube containing 9ml of water. The process was repeated to achieve a dilution 10 Dilution, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were placed on **de Man**, **Rogosa** and **sharpe** (MRS) medium plates (Composition of MRS medium is given below). The plates were incubated T 37^{0}C for 24 hrs. The bacterial strains were identified by the pink colour (Gram's staining method), morphology and biochemical test.

Composition of de Man, Rogosa and Sharpe (MRS) medium.

Components	Quantity g/l
Peptone	10.0
Meat extract	8.0
Yeast extract	4.0
Glucose	20.0
Sodium acetate trihydrate	5.0
Polysorbate 80(Tween 80)	1.0
Dipotassium hydrogen phosphate	2.0
Triammonium citrate	2.0
Magnesium sulphate heptahydrate	0.2
Magnesium sulphate tetrahydrate	0.05
Agar	15.0
pH	6.2

Composition of maintenance medium: Nutrient Agar.

Component	Quantity g/l
Peptone	5.0

NaCl	5.0
Yeast extract	2.0
Beef extract	1.0
Agar	15.0
pH	6.2

Sabouraud's dextrose agar.

Component	Quantity g/l
Peptone	10.0
Dextrose	20.0
Agar	2.0
pH	6.2

Biochemical test for the identification of Lactobacilli

Many biochemical tests were performed for the identification of Lactobacillus isolated in MRS medium. Following biochemical test were done with 11 strains isolated on MRS medium:

Sugar fermentation: This test is done to determine whether the organism ferment the given sugar in the medium and produce gas or not. The isolated organism were inoculated in different sugar media each containing **Andrades Indicator** (Acid fuschin). A small inverted tube (Durham's tube) completely filled with medium is inserted in an inverted position. Each culture is incubated at 37^0C for 48 hrs. The gas and acid production was detected visually. The presence of empty space in the Durham's tube indicated the presence of gas and pink colour of broth indicated acid production. The result are shown in the table 1.

Catalase Test: This test is done to confirm the production of Catalase enzyme. This enzyme catalysis the conversion of H_2O_2 into O_2 and H_2O.Each isolated strain was inoculated on Nutrient broth and incubated at 37^0C for 24 hrs. After incubation 3% H_2O_2 was added to the culture. The presence of Catalase was detected by the evolution of bubbles which shows the production of O_2.Result are shown in table 2.

Gram's staining: All the isolates were subjected to Gram's staining reaction. A thin smear of the *Lactobaclli* was prepared and stained with **crystal violet.** After one minute the smear was washed with tap water and treated with **gram's iodine** for 15-20 second and washed with alcohol and again washed with water. Counter staining was done with **saffranin.** Microscopic observation revealed that out of eleven isolates seven were found gram positive cocci or bacilli (retained violet colour). Results are shown in table 2.

Table 1.

Sugar	Strain no1	Strain no3	Strain no3	Strain no4	Strain no5	Strain no6	Strain no7	Strain no8	Strain no9	Strain no10	Strain no11
Glucose	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	-	+	-	+	-	+	+	+	+
Lactose	-	+	-	+	-	+	-	+	+	+	+
Sorbitol	-	+	-	+	-	+	-	+	+	+	+
Fructose	-	+	-	+	-	+	-	+	+	+	+
Xylose	-	+	-	+	-	+	-	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+

Table 2: Result for Catalase test and gram's staining.

Test	**Strain no1**	**Strain no2**	**Strain no3**	**Strain no4**	**Strain no5**	**Strain no6**	**Strain no7**	**Strain no8**	**Strain no9**	**Strain no10**	**Strain no11**
Catalase test	+	-	+	-	+	-	+	-	-	-	-
Gram's staining	-	+	-	+	-	+	-	+	+	+	+

The composition of various media used for preparation of inoculums for the production of antimetabolite, for the preparation of different pathogens used in the testing (Nutrient broth and Sabouraud's dextrose broth) of antimetabolite produced by *Lactobacilli* curd isolates is given below:

The bacteria Lactobacilli **were** transferred in the nutrient broth and incubated at 37^0C and 100rpm. After 48 hrs of growth samples of fermented broth were taken and centrifuged at 5000 rpm for 5 min in spin with centrifuge. The supernatant was tested against 5

bacterial pathogens *Escherichia coli, Bacillus subtilis, Stphyllococcus aures, Salmonella typhii Pseudomonas* species and two fungal pathogens, *Cryptococcusn neoformes* and *Candida albicans.*

Antimicrobial assay. Screening of *Lactobacilli* isolates for antimicrobial activity:

The seven Lactobacilli isolates numbering 2,4,6,8,9,10 and 11 were tested for antimicrobial activity against five bacterial , *Escherichia coli, Pseudomonas* species and two yeast strains *Cryptococcusn neoformes* and *Candida albicans* by following methods:

Rapid screening method. As the name indicates this method is used for rapid screening of metabolite produced by culture organism. A straight line of the culture is streaked on nutrient agar plate and incubated at 37^0C. After 24hrs of growth test organism are streaked across the culture and again incubated at 37^0C. The zone of inhibition is measured in millimeters in order to determine the antimicrobial activity of the culture.

Disc diffusion method: Agar diffusion method (filter paper disc) is used to determine antimicrobial activity of cell free supernatant (CFS's). Filter paper disc are dipped in CFS's and placed on bacterial (test organism) lawn (agar) and incubated at 37^0C for 24 to 48 hrs. Zone of inhibition is measured in millimeter around each disc.

Minimum inhibitory concentration (MIC). Cell free extract is diluted half time in nutrient broth containing test organism and incubated at 37°C for 24hrs. The turbidity is compared with control and the antimicrobial activity of cell free extract is calculated µl/ml (MIC) against the test organisms.

Results

Antimicrobial activity of different isolates of Lactobacilli by filter paper disc method.

(Zone of inhibition in millimeter)

	Strain no. 2	Strain no. 4	Strain no. 6	Strain no. 8	Strain no. 9	Strain no. 10	Strain no. 11
Bacillus subtilis	-	16 mm	24 mm	25 mm	25 mm	20 mm	2o mm
Salmonella typhii	10 mm	15 mm	22 mm	18 mm	22 mm	12 mm	12 mm
Stphylloccus aures	-		21 mm	25 mm	22 mm	22 mm	21 mm
Esherichia coli	-	-	-	-	-	-	-
Pseudomonas sp.	-	-	13 mm	20 mm	15 mm	20 mm	15 mm
Cryptococcus neoformes	-	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-	-

Test organisms were placed on nutrient agar medium. Filter paper disc were dipped in the filtrates from the seven *Lactobacilli* culture and placed on the nutrient agar plates. The plates were incubated at 37^0C for 24 hrs.

Antimicrobial activity of different isolates of *Lactobacilli* by two folds dilution method (Minimum inhibitory concentration)

Test Organism	Strain no. 2	Strain no. 4	Strain no.6	Strain no.8	Strain no.9	Strain no.10	Strain no.11
Bacillus subtilis	-	25 mm	12.5 mm	12.5 mm	12.5 mm	25mm	25mm
Salmonella typhii	50 mm	25 mm	12.5 mm	25mm	12.5 mm	50mm	50mm
Stphylloccoccus aures	-		12.5 mm	12.5 mm	12.5 mm	12.5 mm	12.5 mm
Esherichia coli	-	-	-	-	-	-	-
Pseudomonas sp.	-	-	50mm	25mm	50mm	25mm	50mm

III. RESULT AND DISCUSSION

Out of eleven colonies isolated on MRS medium, seven were Gram positive, Catalase negative and fermented all the sugars tested. They showed potent antimicrobial activity against *S.typhii, S.aurcus, B.subtilis* and *Pseudomonas.* Not a single isolate found active against *E.coli.* All the eleven isolates were found inactive on the two dermatophytes, *C.albicans* and *C.neoformans.*

CONCLUSION

Culture numbers 6, 8, 9, 10 and 11 showed 11 broad spectrum activities. Maximum activity was showed by strain no.9against *S.typhii, S.aureus, B.subtilis* and *Pseudomonas* (22mm, 22mm, 25mm and 15mm respectively)

REFERENCES

[1]. Biswas, S.R., Ray, P., Johnson, M.C., and Ray, B. 1991. Influence of growth conditions on the production of a bacteriocin, pediocin AcH, By Pediococcus acidilactici H. *Appl. Environ. Microbial.* **57**; 1265-1267.

[2]. Barnby –Smith, F.M., Roller, S.D., Woods, L.F. J., Barker, M., Nightingale, M., Gibbs, P.A.1989. Production of antimicrobial compounds by lactic acid bacteria. The British Food Manufacturing Industries Research Association. Research Reports No.662. Leatherhead Food RA.

[3]. Chikindas, M.L., and T.J.Montville.2002.Perspectives for application of bacteriocin as food preservatives. Pages 303-321 in control of Foodborne Microorganisms. V.K Juneja and [4]. J.N Sofos, ed. Marcel Dekker Inc., New York, NY

Chen, X.; Fruehauf, J.; Goldsmith, J.D.; Xu, H.; Katchar , K.K.; Koon, H.W.; Zhao, D.;Kokkotou, E.G.et al. (2009). "Saccharomyces boulardii inhibits EGF Receptor Signaling and Intestinal Tumour growth in Apcmin Mice "Gastroenterology137(3):91492DOI:10.1053/j.gastro.2009.05 .050.PMC 2777664.PMID19482027

[5]. Cleveland, J., T. J. Montville, I. F.Nes, M. L. Chikindas.2001. Bacteriocins: Safe, natural antimicrobials for food preservation.Int. J. Food Microbial.71:1-20.

Daeschel, M.A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol.**43**: 164-167

[6]. Dicks, LMT; M. Silvester, PA Lawson, MD Collins (2000). "Lactobacillus fornicalis sp. Nov., isolated from the posterior fornix of the human vagina". *International Journal of Systematic and Evolutionary Microbiology* (Society for General Microbiology) 50 (3); 1253-8. DOI;10.1099/00207713-50-3-1253. ISSN 1466-5034. PMID 10843070.Retrieved February 14, 2010.

[7]. Elegado, F.B., Guerra, M.A.R.V., Macayan, R.A., Mendozab, H.A., Lirazan, M.B.2004. Spectrum of bacteriocin activity of Lactobacillus plantarum BS and fingerprinting by RAPD-PCR. Int.J.Food Microbiol.95:11-18

[8]. Eijsink, V. G. H., Skeie, M., Middelhoven, H., Brurberg, M.B. & Nes, I, F. (1998). Comparative studies of pediocin-like bacteriocins, *Appl Environ Microbiol* **64,** 3275-3281

[9]. Ennahar S, Sashihara T, Sonomoto K, Ishizaki A (2000). Class IIa bacteriocin biosynthesis, structure and activity. *FEMS Microbiol.Rev*. **24**; 85-106

[10]. Garneau, S., Martin, N. I., Vederas, J.C.2002. Two peptide bacteriocin produced by lactic acid bacteria. *Biochimie* **84**; 577-592

[11]. Hansen, J.N., 1993 Antibiotics synthesized by post translational modification. *Annu. Rev. Microbiol.***17**, 535-564

Joerger, R.D.and D.G. Hoover, 2000. (Ed.). Bacteriocins In: Encyclopedia of microbiology. Academic press.pp.383

[12]. Klaenhammer TR, (1993).Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev* **12**: 39-86

[13]. Klaenhammer TR, (1988).Bacteriocins of lactic acid bacteria, *Biochimie*, **70**: 337-349

[14]. McAuliffe, O., Ross, R. P. & Hill, C. (2001). Lantibiotics: structure, biosynthesis and mode of action. *FEMS Microbiol Rev* **25**, 285-308

[15]. Larsen, A. G., Vogensen, F.K., Josephsen.J.1993. Antimicrobial activity of lactic acid bacteria isolated from sour doughs: Purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* MI401.*J.Appl. Bacterio.***75**:113-122

[16]. Pascual, L.M.; Daniele, M, B.; Ruiz, F,; Giordano, W,; Pajaro, C.; Barberis, L. (2008). "Lactobacillus rhamnosus L 60, a potential probiotic isolated from the human vagina". *The journal of general and applied microbiology,* **54** (3): 141-148. PMID 18654035.

[17]. Prakash, S.et al. "The Gut Microbiota and Human Health with an Emphasis on the Use of Microencapsulated Bacterial Cells" Journal of Biomedicine and Biotechnology (2011)

[18]. Reid,G; Dols, J.; Mille, W. (2009). "Targeting the vaginal microbiota with probiotics as a means to counteract infectios" *Current Opinion in Clinical Nutrition and Metabolic Care* **12** (6)583-587.DOI;10.1097/MCO.obo13e328331b611 PMID 1974.1517

[19]. Ross R.P. Morgon, S., Hill, C.2002. Preservation and fermentation: past, presentand future. *Int. J. Food Microbiol*. **79**:3-16.

[20]. Sharpe,M.E.,1979, Identification of Latic Acid Bacteria. In Identification Methods for Microbiologist, Skinner, F.A, and. D.W. Lovelock (Eds). Academic Press, London, pp:233-259

[21]. TAGG, J.R., DAGSNI, A.S., and WANNAMAKER, L.W.(1976). Bacteriocins of gram positive bacteria. *Bacteriological reviews*, 4.0 722-756.

[22]. Tasli, L., C. Mat, C. De Simone, H. Yazici (September-October2006)."Lactobacilli lozenges in the management of oral ulcers of Behcet's syndrome "*Clinical and Experimental* Rheumatology **24**(5 Suppl 4.2): S83-6 ISSN 1593X.PMID 17067433.Retrieved February 14, 2010.

[23]. Vignolo, G.M., M.N. de Kairuz, A.A.P. de Ruiz Holgado and G.Oliver. 1995. Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by *Lactobacillus casei* CRL 705. *J. Appl Bacteriol***. 78**:5-10.