International Journal of Research in Medical and Basic Sciences
Volume 7 Issue 1, January 2021 ISSN: 2455-2569 Impact Factor: 6.997
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

STUDIES ON *LACTOBACILLUS* BACTERIOCIN FOR PRODUCTION AND CHARACTERIZATION AGAINST SOME PATHOGENIC AND FOOD SPOILAGE BACTERIA

Mrs.Mamta Sahu[1], Dr.Mrs. Varun Dwivedi

[1,2] Department of Microbiology

[1,2] Dr. C. V. Raman University, Kargi Road, Kota Bilaspur (C.G.)

ABSTRACT

Bacteriocins produced by lactic acid bacteria (LAB) are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties inhibitory substances include bakery and dairy products, cereals, and bread and cheese. Nisin is a natural bacteriocin produced by Lactococcus lactis.It has a broad inhibitory effect against gram positive bacteria. It can be destroyed be proteolytic enzymes that exist in food systems. We found that, minimum inhibitory concentration of free nisin was higher than encapsulated nisin in both culture media and cheese for L.monocytogenes and S. aureus. E. coli resisted to any form of Nisin in culture media but not in cheese. Nisin A is being used at the concentrations of 100-200 ppm in the preservation of, dairy products such as cheeses and milk. In addition, encapsulation protected nisin against cheese fat and protease. Reuterin is a water – soluble non- proteinaceous product produced by Lactobacillus reuteri. It has been described to have antimicrobial effect against certain gram- negative and gram- positive bacteria, yeasts, fungi, and protozoa. It inhibits Salmonella, Shigella, clostridium, Staphylococcus, Listeria, and Trypanosoma. The aim of present work was to study the combination of Nisin and L. reuteri against eight bacterias (Staphylococcus aureus, Salmonella typhi, Escherchia coli, Klebsiella pneuoniae, Pseudomonas aeruginosa, Bacillus subtilus, Bacillus cereus, Streptococcus spp.)Reuterin was isolated from L. reuteri during the anaerobic fermentation of glycerol using Gas Pack EZ Anaerogas pack container system on MRS agar. Minimum inhibitory concentration of bacterioncins (Nisin, crude Lactobacillus reuteri, Nisin + crude Lactobacillus reuteri) was studied using broth micro dilution method. Bacteriocins reuterin showed best synergism for

International Journal of Research in Medical and Basic Sciences
Volume 7 Issue 1, January 2021 ISSN: 2455-2569 Impact Factor: 6.997
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

both gram positive and gram negative bacteria used in the study. MIC of Bacteriocin (Nisin) alone against tested strains was determined to be 0.017+_0.001 to 0.40+_0.002 mg/ml. crude Lactobacillus reuteri alone was determined to be 0.016+_0.001 to 0.033+_0.001mg/ml which is 3 or 2 fold higher for MIC of Bacteriocins(Nisin + crude Lactobacillus reuteri) in combination i.e. 0.010+_0.001to 0.029+_0.001mg/ml. The synergistic activity of Biopreservative i.e. bacteriocins (Nisin and crude lactobacillus reuteri) with chemical preservative (sorbic acid) for pathogenic bacteria was evaluated using well diffusion assay. The diameter of inhibition zones increased on combination. The highest zone increased was seen in case of Bacteriocins in combination i.e. 29+_0.06 AB-3 (E.coli)

Keywords - Antimicrobil activity, Bacteriocin, Food Preservation, Lactic Acid Bacteria.

I. INTRODUCTION

Lactic acid bacteria (LAB) are food- grade microorganisms used for the production of numerous fermented food products to improve their flavor, texture and shelf-life.LAB proudce antibacterial compounds that include organic acids, diacetyl, hydrogen peroxide and bacteriocins,which are known to reduce food spoilage and growth or proliferation of pathogenic bacteria. Use of these naturally produced compound as food bio-preservative agents has therefore gained increasing attention in the food industry and now represents a promising way to preserve food without chemical agents, especially in ready-to-use.Bacteriocins may also find use in the preparation of products that are not submitted to sufficient thermal sterilizaton during their production, since they represent a risk of contamination by pathogenic bacteria such as *Listeria monocytogenes*, known in the art to be responsible for numerous worldwide outbreaks.

Lactobacilli are important organisms recognized for their fermentative ability as well as their helth and nutritional benefits (Gilliland, 1990). They produce various compounds such as organic acids, diacetyl, hydrogen peroxide,and bacteriocin or bactericidal proteins during lactic fermentations (Lindgren and dobrogosz, 1990) the antimicrobial properties of *Lactobacilli* are of special interest in developing strongly competitive starter cultures for food fermentation. *Lactobacilli* exert strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens .Producation of the primary metabolite, lactic acid and the

resulting pH decrease is the main preserving factor in food fermentation. In addition, some strains may contribute to the preservation of fermented foods by producing other inhibitory substances, such as bacteriocins.

Control of both pathogenic and spoilage microbe in a variety of foods is important to guarantee food quality and safety. Recently, biopreservation has become a topic of interest [1]. This technique is used as an alternative to chemical additives for increasing self- life storage and enhancing safety of food by using natural microflora and their antimicrobial products [2]. Lactic acid bacteria are believed to be safe because they have been long established as the normal flora in fermented food; thus they have great potential for use in biopreservation. The preserving effects of lactic acid bacteria are due to the production of antimicrobial agents such as organic acids, hydrogen peroxide and bacteriocin or related substances [3,4].

Bacteriocins are proteinaceous compounds that mainly inhibit closely related species [5]. Some bacteriocins have been shown to possess the ability to inhibit the actions of unrelated genera such as *Clostridia, Listeria,* enteropathogenic bacteria and gram- negative bacteria. For these reasons bacteriocins are promising candidates for biopreservation of food [6]. Sereral *Lactobacillus* strains are an important dairy culture starter and used for the manufacture of fermented food [7,8].

The discovery of bacteroicins gave a new way for food development in better hygienic quality [7,8]. In recent years, there have been many reports on bacteriocins that are producted by lacis acid bacteria. However, most reports deal with bacteriocins that are produced by lactic acid bacteria. However, most reports deal with bacteriocins that are produeed by various *Lactococci Pediococci ,Leuconostoc, Enterococci and Lacctobacilli* (9-11), The search for new strains of lactic acid bacteria that produce antimicrobial substances is a universal objective for the creation of new cultures starter with a high biosafety for fermented food. The inhibition of pathogenic bacteria such as *Staphylococcus aureus* by lactic microflora was announced by Heikkila and Saris (12). The technological characterization of the lactic acid bacteria leads to the development of well defined bacterial strains with specific characters . The latter gradually replace the nondefinite mixtures starters traditionally used in dairy industry (13). ln order to avoid the side effect of chemical preservatives , these last years, the interest of the use of the bacteriocins or strains of lactic acid bacteria for applications as bio-preservative caused many

research tasks (4,14-18), several lactic acid bacteria bacteriocins offer potential applications in food preservation and the use of bacteriocins in the food industry can help to reduce the addition of chemical preservatives as well as the intensity of heat treatments ,resulting in foods which are more naturally preserved and richer in organoleptic and nutritional properties (19),

The aim of this work is isolatoion of lactic acid bacteria that produce antimicrobial substances belonging to bacteriocin type able to inhibit the bacteria which causes food poisoning.

II. RESEARCH ELABORATIONS

K. REVATHI ET AL (2016)

The present study was conducted to evaluate the efficiency of Panchagavya as probiotics by enumerating the total bacterial and lactic acid bacterial count of fermented Panchagavya and demonstrate the antibacterial activity of lactic acid bacterial isolates of Panchagavya against gram positive and gram negative organism.

AHMED ABDELRAZAK ET AL (2016)

The isolated strain was found to be able to produce bacteriocin with antimicrobial activity against a vast number of pathogens and food spoiling microbes. The activity and stability of the produced bacteriocin was studied at different pH , storage time and temperature via applying a Response Surface Methodology and the optimum activity was achieved at pH 4, after 2 days of incubation and 4^0 C. These results indicate that; the produced bacteriocin could be used as antimicrobial compound added to fresh food taking the advantage from protect fresh food from spoilage and disappear of the antimicrobial compound prior to consumption which is undesirable.

SARAH S SAMUEL ET AL (2016)

To study lactic acid bacteria occur naturally as indigenous micro flora. In fermented foods, starter culture of lactic acid bacteria is added for fermentation which results in desired changes in the food and dairy products. Isolation and identification of *Lactobacillus* spp. in various food products reveals the indigenous microflora of that region. Isolation of such regional strains helps in identification the best isolates which can be utilized for further study.

R S UPENDRA ET AL (2016)

In the present study attempt were made successfully in producing bacteriocine for indigenous cultures of LAB isolated from selected fermented foods samples. Lactic acid bacteria (LAB) are a group of Gram-positive; non-spore forming, non-motile, non-respiring bacteria produces variety of antimicrobial compounds such as lactic acid, acetic acid, ethanol, formic acid, fatty acids, hydrogen peroxide and bacteriocins.

INDIRA MIKKILI ET AL(2015)

Bacteriocins are proteinaceous antibacterial compounds, which constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides. The main aim of this study was to isolate the bacteriocin producing lactic acid bacteria and checking their antagonistic activity and potential as good probiotic charateristics. The isolates LABs producing bacteriocins has high antagonistic activity against *E.coli* and Staphylococcus aureus pathogens. These pathogens causing very severe infections in the intestine and urinary tracts and become as multidrug resistant pathogens.

THIRUNAVUK KARASU RAMASAMY AND KANNAN SUYAMBULINGAM (2015)

The present study has highlighted the antibacterial role of bacteriocins isolated from lactic and acid bacteria by indicating their potential to treat a variety of human and animal diseases. Bacteriocins extracted from lactic acid bacteria have the potential to inhibit the growth of many antibiotic resistant bacteria including Methicillin resistant *Staphylococcus aureus, E. coli* and *Staphylococcus aureus*. So we can use them as an alternative therapeutic agent with no risk of antibiotic resistance as well as their use in the form of probiotic can reduce the risk of different infections.

ATUL T. SIRSAT ET AL (2015)

These substances have increasing interest. Their proteinaceous nature implies their putative degradation in the gastro- intestinal tract of man and animals. The sole purpose of the project is to isolate lactic acid bacteria, mainly lactobacilli sepsis from curd and raw milk samples, followed by the identification of the isolated species by RAPD, Biochemical examination and microscopic examination then extraction of the Bacteriocin by ammonium precipitation method and test antibacterial activity against some test organisms.

MAHDIEH IRANMANESH ET AL (2015)

This study showed the presence of viable probiotic LAB micro flora in these products. The antagonistic activity possessed by these isolates might be used for the control of unwanted pathogens mainly in dairy products, and could be exploited further for use in fermented dairy products. The inhibitory activity of these lactic acid bacteria started in the early logarithmic phase and continued to the end of exponential phase. During ultrafiltration studies, bacteriocins produced by *Pediococcus acidilactici*, *Lactobacillus paracasei* were able to pass through the cellulose membranes with 10 and 30 KDa.

SHYAMAPADA MANDAL (2015)

Lactobacilli are used as probiotics, but scientific investigation of such strains is scanty. The current study aims to isolate and identify *Lactobacillus* strains from curd samples for probiotic characterization and antibiotic resistance determination.

SUKHVIR KAUR(2015)

Lactic acid bacteria (LAB) can produce antimicrobial substances with capacity to inhibit the growth of pathogenic and spoilage microorganisms in foods. The preservative ability of LAB in foods is attributed to the production of antimicrobial metabolites including organic acids and bacteriocins. Behavior of bacteriocin produced by isolated strains in the present investigation was considered as bacteriocidal.

KOMKHAE PILASOMBUT ET AL (2015)

The aim of this study was to screen and in vitro characterize the properties of bacteriocin produced by lactic acid bacteria isolated from Vietnamese fermented pork (Nem chua). One

hundred and fifty LAB were isolated from ten samples of Nem Chua and screened for bacteriocin- producing lactic acid bacteria. Antimicrobial activity of bacteriocin was carried out by spot on lawn method against both gram positive and gram negative bacteria.

SHARMILA P.S AND VIDYA A.K (2015)

This study presents bactetiocin (antimicrobial compound), produced from *Bacillus subtilis* isolated from dairy products. The strains from unpasteurized milk that showed the largest zone of growth inhibition against the indicator strain, Micrococcus luteus was selected for the study. The selected strain was identified as *Bacillus subtilis* based on its morphological, physiological and biochemical characteristics. The antibiotic susceptibility tests for *B. subtilis* against various antibiotics demonstarted high sensitivity to Tetracycline , Moderate and less sensitivity towards Streptomycin and Chloramphenicolm respectively while being resistant to penicillin.

S KARTHICK RAJA NAMASIVAYAM ET AL (2014)

The present study aimed to study the effect of media on bacteriocin production by *Lactobacillus brevis* isolated from curd, cabbage and meat and its antibacterial activity against common food spoiling bacteria . In this study, MRS medium with different carbon sources such as sucrose, lactose and maltose nitrogen sources such as tryptone, glycine, cysteine, histidine, ammonium nitrate, urea and soyabean meal.

M.KANNAHI AND N. VIJI (2014)

The present study was carried out with the lactic acid bacteria isolation and identification such as *Lactobacillus lactis, Lactobacillus bulgaricus , Lactobacillus plantarum, Lactobacillus brevis* and *Lactobacillus fermentum* from butter sample. Five isolates of Lactobacilli supernatant was examined for acid and bacteriocin production. The extracted compound showed antibacterial activity against the pathogenic microorganisms.

BAMGBOSE TIMOTHY AND SUMIT SHARMA (2014)

In this study, the isolated microbe showed bacteriocin production and its effect against different microbial species, these microbes have been isolated from different sources such as curd , milk and soil. The selected isolated strain producing bacteriocins have been further characterized.

DESHMUKH P.V AND THORAT P.R. (2014)

In antimicrobial substances is often important trait in the context of bacterial fitness but also in terms of probiotic efficacy. Several probiotic bacteria produce a variety of antimicrobial compounds viz. short chain fatty acids, Hydrogen peroxide, Nitric oxide, Bacteriocins etc. Bacteriocins are peptide or protein complexes showing antibacterial activity against closely related species.

SARAH SHARMILA SAMUEL AND SANGEETA SHUKLA (2014)

The aim of the investigation was to isolate and identify Lactic acid bacteria (LAB) from curd, and also to study the inhibitory activity of the bacteriocin against selected pathogens. Thus, the isolates of Lactobacillus spp. found effective against the test microorganisms can be further studied and their probiotic and antibacterial properties could be utilized in therapeutic and food applications .

ASHOK V. GOMASHE ET AL (2014)

This study were to isolate and identify bacteriocin producing LAB from various food samples such as curd, milk and probiotics and to evaluate their antimicrobial effects on selected spoilage and pathogenic microorganisms *in vitro*. Bacteriocins are ribosomally synthesized antimicrobial peptides that are active against other bacteria either of the same species (narrow spectrun), or across genera (broad spectrum). The biopreservation of foods using bacteriocinogenic lactic acid bacteria (LAB) isolated directly from foods was considered as an innovative approach.

YALIAN SUN ET AL (2014)

In the study Ninety-six strains of bacteria were isolated from newborn infants feces. After Antimicrobial activity was screened, these strains showed a broad inhibition spectrum. . Antibacterial activity, indicating that the proteinaceous nature of the antimicrobial compounds were produced by these strains . These strains could be introduced as preservatives in the food industry.

AMIT BHARAL AND VIPAN KUMAR SOHPAL (2013)

In this paper , the ability of *Lactobacillus acidophilus* strains isolated from human feces studied. *Lactobacillus acidophilus* strain is one of the stable and acid-resistant , which can use for preserving food and antimicrobial activity against some human pathogenic microbes.

K. SUGANYA ET AL (2013)

In this study, lactic acid bacteria were isolated from 2 different curd and milk sample. A total of 20 strains were studied for their characterization , from that 2 potential strains *Lactobacillus acidophilus* and *Lactobacillus casei* were identified . The potential strains were studied by curd production, antibacterial and bacteriocidal activity also their antibiotic susceptibility.

MOSHOOD A. YUSUF (2013)

These antimicrobial agents are gaining more and more attention as an alternative therapeutics not only in pharmaceutical but also as a preservative in food industries. The main aim of this review is to highlight lactic acid bacteria and its bacteriocins . The absence of this analysis could be probably due to the rapid degradation of these proteinaceous compounds in the digestive tract of mammals , In most cases, bacteriocin production and activity has been demonstrated only in the laboratory.

N. RAVI SANKAR ET AL (2012)

The study revealed the possibility of using bacteriocin as a food preservative and the *L. plantarum* as probiotic , Bacteriocin producing Lactobacillus plantarum stain isolated from raw cow's milk samples, showed broad range of antibacterial activity against food borne pathogens. The bacteriocin was purified in two step procedure involving ammonium sulfate precipitation and gel filtration (Sephadex G-100 column).

BALJINDER KAUR ET AL (2012)

Therapeutic application of this probiotic strain to protect against gastrointestinal infections may be of great importance for future medicinal use. Bacteriocin producing strains of lactic acid bacteria were isolated from vaginal swabs of healthy and fecund females and evaluated for their antimicrobial activity against pathogens causing important human diseases such as gastrointestinal infections, nosocomial and skin diseases.

SOUMYA .T.V ET AL (2012)

Lactic acid bacteria (LAB) play an important role in food fermentation and preservation either as natural microflora or as starter cultures. LAB displays numerous antimicrobial activities . This is mainly due to the production of organic acids , but also of other compounds , such as bacteriocins and antifungal pepides. Bacteriocin sensitivity to physical conditions and chemical substances were also evaluated. The test bacteriocin was found to be a sensitive to chloroform and resistant to catalase treatment.

K. VINDHYA VASINI ROY (2012)

The present study was aimed to isolate and characterize bacteriocin producing *Lactobacillus* sp., from fermented foods , Dairy products and vegetables like Dosa batter, Idli batter, curd, cheese , milk, butter, cabbage and cucumber. A total of 50 isolates were screened for bacteriocin activity. Lactic acid bacteria are commonly used as natural food preservatives to improve the food safety and stability. These organisms produce certain antimicrobial substances such as bacteriocins. Bacteriocins are biopresevative agents with a potential of suppressing growth of some contaminant bacteria in foods.

V. SUMATHI AND D. REETHA (2012)

The antimicrobial activity of LAB was estimated by Agar spot method, well diffusion assay and disc diffusion method. The extracts of ten-isolated LAB gave zones of inhibition against the indicator food pathogenic stains such as *Escherichia coli, Salmonella typhi, Shigella, Staphylococcus aureus, Enterobacter* and *Listeria monocytogenes.*

TARIFUL ISLAM ET AL (2012)

The antimicrobial activity of cell free supernatant was tested against both gram positive and gram negative pathogenic bacteria and found to be sensitive. The supernatant exhibited tolerance to extreme pH and was heat stable as well as sensitive to proteolytic treatment. The latter property could be attributed to a bacteriocin like inhibitory substane (BLIS) secreted from *L. paracasei* ssp. *paracasei*-1. In addition, the isolate showed good survival in presence of a number of bile salts indicating its potential application as probiotic supplement.

AROKIYAMARY AND P. K. SIVA KUMAR (2011)

The present study was focused on isolation and characterization of bacteriocin producing *Lactobacillus* sp., from a traditional milk product such as Curd, Cheese, Butter, Milkpeda and Ghee. The isolates were identified , based on characteristics of the strains of Lactobacillus sp., as present in Bergey's manual of determinative bacteriology, the metabolite bacteriocin was extracted from the isolated *Lactobacillus* LAB and the antibacterial activity was evaluated against bacterial pathogens.

A. MOHANKUMAR AND N. MURUGALATHA (2011)

The intent of the study is to determine the antimicrobial activity of *Lactobacillus* producing bacteriocin isolated from raw milk of cattle' s like cow, buffalo and goat and to characterize the bacteriocin. Hundered *Lactobacillus* isolates (50 isolates from cow , 22 isolates from buffalo and 28 isolates from goat) based upon the distinct morphology were isolated from the samples and identified as *Lactobacilli* according to phenotypic characteristics.

MARIA TUFAIL ET AL (2011)

The present investigation showed that, some *L. bulgaricus* strains which were isolated form yoghurts had antibacterial potential against some food borne pathogen and spoilage microoganisms especially V. cholera and E. coli, because of significant characteristic of bactericin production. Investigation revealed that incubation period (48h) results in maximum production of bacteriocin So, in yogurt manufacturing, there is need for considering different culturing parameters for best bacteriocin yield, there is need for more research on antibiotic resistance profiles of yoghurt bacteria.

G. RAJARAM ET AL (2010)

The study revealed the possibility of using bacteriocin as a food preservative and the *L. lactis* strain as probiotic. Bacteriocin producing *Lactobacillus lactis* strain isolated form marine environment, showed broad range of antibacterial activity against some major food borne pathogens. Maximum bacteriocin production was obsereved at 30^{o}C , pH 6.0 and 1.5% sodium chloride solution. In addition of enzymes, "-amylase , DNase , RNase and lipase were slightly positive effect bacteriocin production.

ADRIANO BRANDELLI (2010)

Lactic acid bacteria (LAB) were isolated from ovine milk and cheeses manufactured in the South Region of Brazil . Among 112 bacterial isolates investigated , 59 were chosen through a screening for LAB . Among these 59 strains of LAB, 21% showed antimicrobial, proteolytic and lipolytic activities.

V.KARTHIKEYAN AND S. W. SANTOSH (2009)

The study revealed the possibility of using bacteriocin as a food preservative and the *L. plantarum* strain as probiotic. Bacteriocin producing *Lactobacillus plantarum* strain isolated form marine shrimp (Penaeus monodon) gut, showed broad range of antibacterial activity against some major food born pathogens. Maximum bacteriocin producion was observed at 50^{0} C, pH 4 and 0.9% sodium chloride solution.

E.D. SIMOVA ET AL (2009)

To isolate bacteriocin-producing lactic acid bacteria LAB with high wide spectrum antibacterial activity and to characterize their inhibitory peptides. The two bacteriocins are potential antimicrobial agents and, in conjunction with their producers may have use in applications to contribute a positive effect on the balance of intestinal microflora. Furthermore, *bulgaricin* BB18 strongly inhibits Helicobacter pylori.

III. MATERIAL AND METHODS

Bacterial Strains and Growth Conditions : The species of *Lactobacillus* were obtained from the collection of the Laboratory of Applied Micrbiology , Department of Biology , Faculty of Science , DR. C.V. Raman University Kota Bilaspur . The three pathogenic bacteria responsible of food toxi –infections (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25921 and *Bacillus* sp) were obtained from the collection of Medical Analysis Laboratory of the University –Hospital centre of Bilaspur.

The species of *Lactobacilli* were cultivated in liquid or solid MRS with ph 5, 4 then incubated at 30^0c during 48^{th} (20). The selective enumeration of *Staphylococcus aureus* is carried out on Chapman medium at 37 . The other bacteria, *Escherichia coli* and *Bacillus* sp. were grown on Muller-Hinton medium and incubated at 37^0C. The media used during this work were eirther of liquid solid (1.5% agar p/v) or the soft agar medium (0.7% agar). The skimmed milk medium (11% p/v) was sterilized at 110 for 10 min and all other media were sterilized at 121^0C during 20 min [21]. The isolated Lactobacillus strains were selected as bacteriocin producers because of their broad antimicrobial activity and subjected to phenotypic identification. Cell morphology and gram –staining reaction were examined by light microscopy . Test for catalase activity and fermentation of different sugars were also tested as described by Badis et al. [8].

Isolation of Microbes from Food Sample

Lactobacillus selection Agar

Sr.No	Compound	Amount for			
		100ml	250 ml	500 ml	1000 ml
1	Pancreatic Digest of Casein	1.0g	2.5 g	5.0g	10.0g
2	Sodium Acetate Hydrate	2.5 g	6.25g	12.5g	25.0g
3	Yeast Extract	0.5g	1.25 g	2.5 g	5.0 g
4	Acetic Acid	0.13 ml	0.33 ml	0.65ml	1.3 ml
5	Potassium Dihydrogen phosphate	0.6 g	1.5 g	3.0g	6.0 g
6	Ammonium citrate	0.2 g	0.5 g	1.0 g	2.0 g
7	Glucose	2.0 g	5.0 g	10.0 g	20.0 g
8	Magnesium Sulfate	58.0 mg	0.14 g	0.28 g	0.575 g
9	Manganese Sulfate	12.0 mg	30.0 mg	60.0 mg	0.12 g
10	Ferrous Sulfate	3.4 mg	8.5 mg	17.0 mg	34.0 mg
11	Polysorbate 80	0.1 g	0.25 g	0.5 g	1.0 g
12	Agar	1.5 mg	3.75 g	7.5 g	15.0 g
pH 5.5 +/- 0.2					

Isolation of Pure Culture

- Three sets of Lactobacillus selection agar plate of desired medium for each of the samples to be tested were prepared and labeled according to their dilutions (10^{-2}, 10^{-3}, 10^{-4}).
- 100 mg of each sample and 900 ul of sterile water was added to create the 10^{-1}, 10^{-2},10^{-3},10^{-4} dilutions at different concentration.
- Aseptically 100ul of different dilutions were spread in the respective plate.
- All plates were incubated in an inverted position for 24 to 48 hours at 37^{o}C.
- Pure distinct culture colonies were picked and streaked into new agar plate and incubated further in an inverted position for 24 to 48 hours at 37^{o}C.

Gram Staining

Microbial culture were heat- fixed on the slide and stained for 1 minute with crystal violet (2% Crystal violet, 0.8% Ammonium Oxalate in 50% ethanol) staining reagent. Slides were washed

with water and flooded with the mordant (Gram's iodine, 3% Iodine/Iodide Mixture in water). After 1 minute. Slides were washed with water and treated with decolorizing agent (95% ethanol). After decolorization slides were counterstained with safranin (0.25% in ethanol) for 30 seconds to 1 minute. Slides were then washed with water until no color appears in the effluent and then blot dry with absorbent paper. Slides were then observed under oil immersion using a Brightfield microscope (100X) and images were captured using Nikon Optiphot microscope equipped with Amscope MU1000 Camera.

DNA Sequencing and Phylogenetic Analysis Service

DNA Isolation

1. Centrifuge 1 ml of the overnight grown culture at 8000 rpm for 5 min ,or until a compact pellet forms . Discard the supernatant .
2. Resuspend pellet in 567 ul TE buffer by repeated pipetting . Add 10 ul Lysozyme (10mg/ml) and incubate for 30 mins at 37^0C.
3. Add 30 ul 10% SDS and 5 ul of RNAse (10 mg/ml). Mix thoroughly and incubate 1 hr at 37^0C .
4. Now add 3ul proteinase K (10mg/ml) and mix thoroughly and incubate 1 hr at 37^0C.
5. Add an equal volume of phenol/chloroform/isoamyl alcohol, extract thoroughly , and centrifuge at 8000rpm for 5 min.
6. Transfer the supernatant to a fresh tube . Repeat the step if necessary.
7. Add 70ul of 3M Sodium Acetate and add 1.2 ml of chilled ethanol to precipitate the nucleic acids . Shake the tube back and forth until a stringy white DNA precipitate becomes clearly visible .
8. Centrifuge at 8000rpm for 5 min to pellet down the precipitated DNA.
9. Wash the DNA with 70% ethanol and centrifuge at 8000rpm for 5 min at room temperature to repellet it. Repeat this step.
10. Carefully remove the supernatant and briefly air dry the pellet in laminar flow hood.
11. Redissolve the pellet in 50-200 ul nuclease free water.

PCR Conditions

International Journal of Research in Medical and Basic Sciences
Volume 7 Issue 1, January 2021 ISSN: 2455-2569 Impact Factor: 6.997
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

Reaction Mixture (50ul)		Cycling Conditions		
Template DNA	100 mg	Initial Denaturation	2 minuts at 95°C	
Forward Primer	0.3 uM	Denaturation	30 seconds at 95°C	35 Cycles
Reverse Primer	0.3 uM	Annealing	30 seconds at 52°C	
Master Mix	25 ul	Extension	2 minutes at 72°C	
Nuclease Free Water	Valume makeup 50ul	Final Extension	15 minutes at 72°C	

Primer Details :

No.	Oligo Name	Sequence (5'-3')	Tm(°C)	CG –Content
1	27F	AGAGTTTGATCMTGGCTCAG	56.3	47.5%
2	1492R	CGGTTACCTTGTTACGACTT	55.3	45%

Primer Details (For Sequencing):

No.	Oligo Name	Sequence (5'-3')	Tm(°C)	GC –Content
1	785F	GGATTAGATACCCTGGTA	56.3	47.5%
2	907R	CCGTCAATTCMTTTRAGTTT	55.3	45%

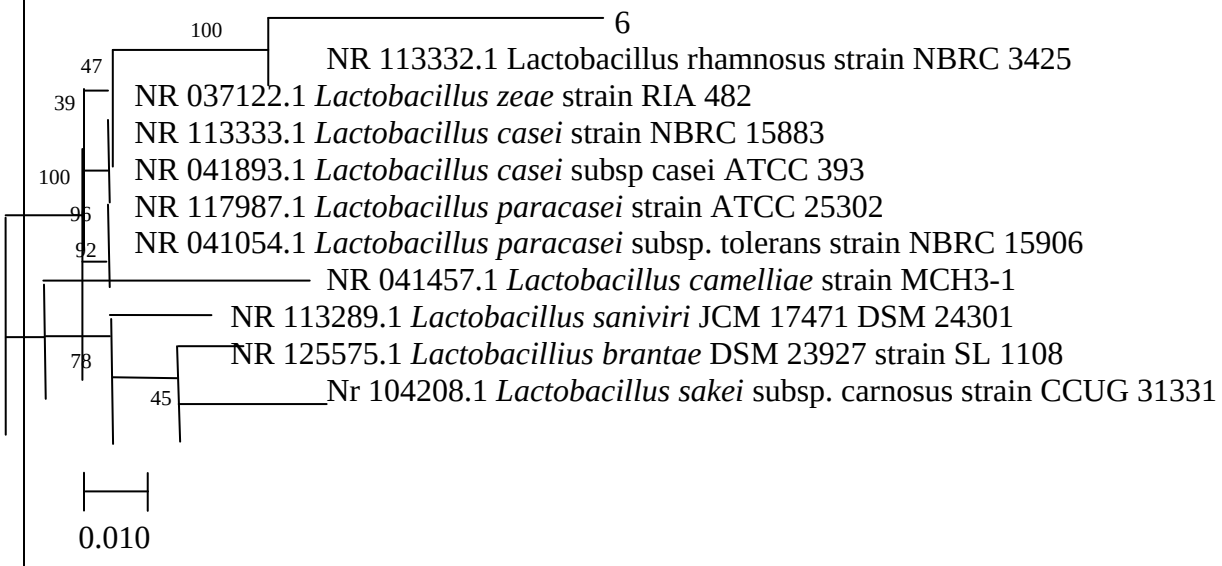
Sample ID	6
Sequence in FASTA Format	
AGTTGTATTGGTCGTATCTGTTACTAGGGAACCGCTTGAATCTTGATTTA ATTTTGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCT TAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAAATCCA AGAACCGCATGGCTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGG ATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGG CAATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGA GACACGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAAT GGACGCAAGTCTGATGGAGCAACGCCGCCGTGAGTGAAGAAGGCTTTCG GGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTT GTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAG CAGCCGCGGTAATACGTAGGTGGCAAGCGGTTATCCGGA	

International Journal of Research in Medical and Basic Sciences
Volume 7 Issue 1, January 2021 ISSN: 2455-2569 Impact Factor: 6.997
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

Alignment Table

s.no	Description	Max score	Total score	Query cover	E value	Ident	Accession
1	*Lactobacillus rhamnosus* strain NBRC 3425	900	900	93%	0	99.40%	NR_113332.1
2	*Lactobacillus zeae* strain RIA 482	867	867	93%	0	98.19%	NR_037122.1
3	*Lactobacillus casei* strain NBRC 15883	854	854	89%	0	99.16%	NR_113333.1
4	*Lactobacillus paracasei* strain ATCC25302	854	854	89%	0	99.16%	NR_117987.1
5	*Lactobacillus casei* subsp.casei ATCC 393	854	854	89%	0	99.16%	NR_041893.1
6	*Lactobacillus paracasai* subsp. tolerans strain NBRC 15906	848	848	89%	0	98.95%	NR_041054.1
7	*Lactobacillus saniviri* JCM 17471 DSM 24301	743	743	91%	0	94.25%	NR_113289.1
8	*Lactobacillus camelliae* strain MCH3-1	732	732	89%	0	94.75%	NR_041457.1
9	*Lactobacillus brantae* DSM 23927 strain SL1108	721	721	90%	0	93.96%	NR_125575.1
10	*Lactobacillus sakei* subsp. carnosus strain CCUG 31331	610	610	90%	0	89.83%	NR_104208.1
Tree File							

The evolutionary history was inferred using the Neighbor- Joining method [1]. The optimal tree with the sum of branch length= 0.20964036 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches[2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. This analysis involved 11 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). there were a total of 1578 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [4].



International Journal of Research in Medical and Basic Sciences
Volume 7 Issue 1, January 2021 ISSN: 2455-2569 Impact Factor: 6.997
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

Distance Matrix

The number of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Maximum Composite likelihood model [3]. This analysis involved 11 nucleotide sequences. All ambiguous positions were removed for each sequences pair (pairwise deletion option). There were a total of 1578 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [4].

		1	2	3	4	5	6	7	8	9	10	11
1	6		0.0093	0.0134	0.0150	0.0145	0.0154	0.0153	0.0235	0.0288	0.0282	0.0409
2	*Lactobacillus* rhamnosus strain NBRC 3425	0.0318		0.0028	0.0034	0.0035	0.0035	0.0035	0.0103	0.0126	0.0104	0.0156
3	*Lactobacillus* zeae strain RIA 482	0.0508	0.0088		0.0011	0.0024	0.0013	0.0026	0.0102	0.0132	0.0102	0.0159
4	*Lactobacillus casei* strain NBRC 15883	0.0575	0.0115	0.0020		0.0023	0.0000	0.0025	0.0104	0.0133	0.0104	0.0159
5	*Lactobacillus paracasei* strain ATCC 25302	0.0555	0.0113	0.0070	0.0070		0.0025	0.0006	0.0105	0.0144	0.0101	0.0158
6	*Lactobacillus casei subsp. casei ATCC 393*	0.0595	0.0122	0.0027	0.0000	0.0077		0.0026	0.0105	0.0133	0.0104	0.0160
7	*Lactobacillus paracasei* subsp. tolerans strain NBRC 15906	0.0597	0.0122	0.0081	0.0081	0.0007	0.0088		0.0104	0.0142	0.0100	0.0158
8	*Lactobacillus saniviri JCM 17471 DAM 24301*	0.0978	0.0469	0.0467	0.0476	0.0480	0.0484	0.0477		0.0136	0.0059	0.0155
9	*Lactobacillus camelliae* strain MCH 3-1	0.1230	0.0582	0.0602	0.0614	0.0663	0.0617	0.0661	0.0653		0.0126	0.0191
10	*Lactobacillus brantae* DSM 23927 strain SL1108	0.1204	0.0470	0.0468	0.0477	0.0457	0.0480	0.0455	0.0254	0.0602		0.0125
11	*Lactobacillus sakei subsp.* carnosus strain CCUG 31331	0.1771	0.0736	0.0753	0.0763	0.0751	0.0767	0.0755	0.0747	0.0894	0.0581	

Inference: *Lactobacillus rhamnosus* sp.

IV. RESULT AND DISCUSSION

In the last few decades, tremendous interest has swelled in the potential use of bacteriocins from lactic Acid Bacteria (LAB). The bacteriocins produced by this group of bacteria are considered potent bio- preservative agents and their application in food is currently the subject the subject of extensive research.

The present investigation highlights the isolation and characterization of bacteriocin producing *Lactobacillus* sp. isolated from Dosa (appam) batter, curd, sauces and cheese. Five Isolated bacteriocinogenic *Lactobaillus* sp. were characterized and identifed on the basis of their morphological, physiological and biochemical characteristics out of five, two were idenified as *L. plantarum* and coded as p1 and P2 due to their different potential in bacteriocin activity and other were identified as *L. casai, L. brevis, L. fermentum.*

Antimicrobial activity of the bacteriocins produced by the *Lactobacillus* sp. in this study was not due to hydrogen peroxide or acidity, as activity was not lost after treatment with catalase or peroxidase or adjustment of pH to 7.0.

Production of bacteriocin was studied in both aerobic and anaerobic conditions. Anaerobic condition was found suitable for maximum production of bacteriocin by all isolates whereas the aerobic condition did not support growth of *Lactobacillus* sp. (as they are anaerobic or microaerophilic bacteria) as well as the production of bacteriocin.

Bacteriocin production by the test isolates displayed secondary metabolic kinetics because all bacteriocin were produced during the pre-and early exponential growth phases, reached a maximum level at late stationary phase. Some reports indicate that bacteriocins are produced throughout the experimental growth phase and not solely during late logarithmic or early stationary phase (Joerger and Klaenahammer, 1986 Piard et al, 1990).

Optimization of bacteriocin production process was carried out by taking different parameters such as different carbon and nitrogn sourcer, pH, Temperature, Salt concentration and optimized parameter was determined by Arbitrary unit.

Supplementation and/or replacement of carbon and nitrogen sources demonstrated that larger quantities of bacteriocin could be produced by addition of glucose (2.0%) while addition of other carbon sources had on effect or adverse effect on production and in case of nitrogen

source maximum production was achieved by addition of Tryptone , yeast extract and meat extract together in the medium..Maximal activity in composed medium was achieved at initial pH ranging from 6-8 while extreme alkaline and acidic pH did not support the bacteriocin production and optimized temperature was 30.c.

In the optimized conditions, the bacteriocin was produced at its maximum and the purifed bacteriocin could be directly used as bioprservative. Optimization of bacteriocin production will help to reduce their production cost and it could be available commercially (comparatively at low cost) to reduce or replace the addition of chemical preservatives.

All bacteriocin gave same results after treatment with enzymes. Complete inactivation of antimicrobial activity from all *Lactobacillus* sp. was observed after treatment of bacteriocin with proteinase k. trypsin and pepsin confirming its proteinaceous nature . Loss in antimicrobial activity by treatment with a-amylase suggesting that bacteriocin could be glycosylated. Lipase caused only a slight redution of bacteriocin activity, indicating that besides the proteinaceous subunit, some lipid components may also involve in antibacterial activity.

SDS PAGE of bacteriocins showed that the molecular weight of bacteriocin from *L. plantarum* P1and P2 were approximately same and other have comparatively high molecular weight. Complete inactivation by proteinases and some loss of bacteriocin activity with lipase and amylase showed that these molecular weight of protein contribute major part in the total molecular weight of bacteriocins with some contribution of carbohydrate and lipid moieties.

During the purification procedures, each step resulted in considerable loss of peotein concentration while specific activity increased. At 60% saturation with ammonium sulphate highest increase in activity was observed for *L. plantarum* P1, *L. casai* , *L. fermentum* . While in the case of *L. brevis* and *L. plantarum* P2 it was achieved at 80% saturation . This agreed with the findings of lvanova et al. (2000). The increase in activity could be due to release of active monomers from bacteriocin complexes. During salt precipitation various amount of the protein was fractionated as a surface pellicle, this might be due to the association of bacteriocin molecules with the hydrophobic globular micelle like structure in the supernatant fluid. Similar observations have also been recorded for lactocin S and lactacin F (Muriana and

klaenhammer,1991) The above fractions were subjected to ion exchange chromatography and production of active fraction of bacteriocin was achieved.

Thermal stability at 121.c for 20 min .was observed in case of bacteriocin produced by and it is important , if this bacteriocin is to be used as a food preservative, because many procedures of food preparation involve a heating step. The phenomenon of heat stability of LAB bacteriocins have been reported earlier for plantaricin A (Daeschel et al., 1990), Plantaricin C19 (Audisio, 1999), Plantaricin S (Jimenez-Diaz et al. 1990), Plantaricin 149 (kato et al., 1994), Plantaricin SA6 (Ralph et al., 1995), Plantaricin 423 (Van-Reenen,1998), pentocin TV35b (Okkers et al. ,1999), lactocin RN78 (Mojgani and Amirinia, 2007) and a bacteriocin produced by *L. brevis* 0G1 (Ogunbanwo et al. , 2003). The findings of this report are also in agreement with the above mentioned reports as we observed heat stability of *L. plantarum* P2 bacteriocin. The retention of activity by this bacteriocin after heating at 121^0 C for 60 min , place it within heat stable low molecular weight group of bacteriocins. This quality of the bacteriocin makes it superior in processed food stuffs where high heat is applied . While other bacteriocins were not heat stable. Andersson (1986) also reported loss of activity after heat treatment at 121^0 C for 15 min.

The activity of bacteriocin elaborated by the test isolates was also pH dependent. The bacteriocins produced by *L.brevis* and *L. plantarum* P2 were stable at acidic and alkaline pH as well as in high salt concentrations which make them an attractive applicant in food supplied i.e. they can be used in acidic foods like pickle, yogurt etc. while other showed stability only at acidic pH between 4-6. This was also shown by Reddy et. al., 1984; Abdel-Bar et. al., 1987 in two bacteriocins, namely bulgarican and lactobulgarican, isolated from *L. bulgaricus*, have, the highest activity and stability at pH 2.2 and 4.0 respectively, against a range of pathogenic and spoilage bacteria.

Increased antibacterial activity in the bacteriocin produced by *L. plantarum* P2 and L. brevis was observed in acidic pH specifically at pH 5. This may be due to the increase in net charge of bacteriocins at low pH might facilitate translocation of bacteriocin molecules through the cell wall. The solubility of bacteriocins may also increase at lower pH, facilitating diffusion of bacteriocin molecules.

Increased antimicrobial activity was also observed in low salt concentration in bacteriocins produced by *L. plantarum* P2 and *L. brevis* but others showed decreased bacteriocin activity this agreed with previous studies which have shown that the presence of NaCI enhanced the antimicrobial action of bacteriocins such as nisin, leucocin F10, enterocin AS-48 and others (Harris et. al., 1991; Thomas and wimpenny 1996; Mazzotta et al., 1997; Parenteet et.al., 1998; Ananou et al., 2004). However, nisin activity was also antagonized by low concentration of NaCI (Boutterfroy et al., 2000). sodium chloride also decreased the antilisterial activity of acidocin CH5 (at 1-2%; Chumchalova et al., 1998), lactocin 705 (at 5-7%; Vignolo et al., 1998), leucocins 4010 (at 2.5% NaCl; Hornbaek et al., 2004), pediocin (at 6.5% NaCl; Jydegaard et al., 2000), curvacin (Vaerluyten et al., 2002) and Carnobacterium piscicola A9b bacteriocin (at 2-4% NaCl; Himmelbloom et al., 2001), The protective effect of sodium chloride may due to interference with ionic interactions between bacteriocin molecules and charged groups involved in bacteriocin binding to target cells (Bhunia et al., 1991.) Sodium chloride may also induce conformational changes of bacteriocins (Lee et al., 1993) or changes in the cell envelope of the target organisms (Jydegaard et al., 2000).

The effect of surfactants on bacteriocin activity was studied. Significantly enhanced bacteriocin activity was observed by adding EDTA and tween 80 against both gram positive as well as gram negative indicator bacteria. This increase might be due to the effect of surfactant on the permeability of the cell membrane (Graciella et. al., 1995). Chelating agents permeate the outer membrane (Graciella et, al 1995).chelating agents permeate the outer membrane (OM) of Gram- negative bacteria by extracting Ca2+ and Mg2+ cations that stabilize lipopolysaccharide of this structure, alllowing bacteriocins to reach the cytoplasmic membrane (Stevens et. al., 1991, Vaara 1992, Schved et. al., 1994, Helander et al., 1997). Brochrocin C and enterocin AS-48 also showed increased antimicrobial activity on EDTA- treated Gram-negative bacteira (Abriouel et al., 1998, Gao et al., 1999, Ananuou et al.,2005). Chelating agents can also enhance the activity of bacteriocins on Gram-positive bacteria. More recently, an increased activity of chrisin (a commercial nisin prepararion) in combination with EDTA has been reported against several gram-positive bacteria. (Gill and Holley, 2003). The combination of sodium tripolyphosphate and enterocin EJ97 showed increased activity against B. coagulans and B. macroides (Garcia et al., 2003, 2004). Whereas tween 20 had adverse effect on these bacteriocins and their activity was completely demolished after subjection to

this surfactant. However there were no significant effects of other surfactant on bacteriocin activity.

Extraction of bacteriocin in this study using organic solvents indicated that bacteriocin was removed from the aqueous phase and could be recovered from the organic phase. This suggests that at least part of the bacteriocin molecule has a hydrophobic character, and shares this property with most other bacteriocins. This was also showed be klaenhammer, 1993. Lactic acid bacteria synthesize bactericidal agents that vary in their spectra of activity . Broad-spectrum bacteriocins and narrow-spectrum bacteriocins. both have importance . On the basis of antibacterial study the, bacteriocin from *L. plantarum* was characterized as Broad-spectrum bacteriocins next followed by L. brevis , present potential wider uses. Earlier reports (Tagg et . al., 1976; Daeschel et al., 1985; Sanni et al., 1999) have shown that some bacteriocins produced by gram-positive bacteria have a broad spectrum of activity.

The most striking was that only bacteriocin from *L. fermentum* had narrow antibacterial spectrum . this agree with the *Lactobacillus fermentum* ME-3 which has also been found to have the capability to suppress mainly gram –negative bacteria. To a lesser extent, ME-3 has also been observed to be able to suppress *Enterococci* and *staphylococcus aureus*. The narrow antibacterial spectrum also reported fom some bacteriocins of some lactic acid bacteria, for example lactococcin a (Holo et al., 1991)and lactacin B (Barefoot and klaenhammer, 1983). Narrow-spectrum bacteriocins could be used more specifically to selectively inhibit certain high-risk bacteria in foods without affecting harmless microbiota.

V. CONCLUSION

the highly promising results of these studies underline the important role that functional, bacteriocinogenic *Lactobacillus* sp. may play in the food industry as starter cultures , co-cultures, or bioprotective cultures, to improve food quality and safety.

The characterization study of Bacteriocin from test isolates to exploit their potential make applicable them as suitable candidate for future application as a safe and efficacious biological preservative. The peculiar broad spectrum antibacterial characteristic, technological properties and especially heat and pH stability and salt tolerance capacity of *L. plantarum* , can positively has impact on their use as biopreservative, with a view to improving the hygiene and safety of the food products especially processed foods. However the pH stability and salt tolerance capacity of bacteriocin produced by *L. brevis* make it an attractive applicant in food supplies i.e. it can be used in acidic foods like pickle . The narrow-spectrum bacteriocins produced by *L. fermentum* could be used more specifically to selectively inhibit certain high-risk bacteria in foods without affecting harmless microbiota.

Bacteriocin producing lactobacilli with great potential could be directly used as starter culture or the concentrate from of these bacteriocins could also be used as biopreservative in the food industry and it can help to reduce the addition of chemical preservatives as well as the intensity of heat treatments, resulting in foods which are more naturally preserved and richer in organolptic and nutrional properties . This can be an alternative to satisfy the increasing consumers demands for safe, fresh-tasting, ready-to-eat, minimally-processed foods and also to develop ‘novel’ food products (e.g. less acidic, or with a lower salt content).

VI. APPENDIX

MEDIA COMPOSITON

DeMan, Rogosa and Sharp Medium per liter	20.0g
Dextrose	10.0g
Peptic Digest of Animal Tissue	10.0g
Beef Extract	5.0g
Yeast Extract	5.0g
Sodium Acetate	2.0g
Dipotassium Phosphate	2.0g

Ammonium Citrate	2.0g
Tween 80	1.0g
Magnesium Sulfate	0.1g
Manganese Sulfate	0.05g
Agar*	15.0g

Agar is added for MRS agar

Brain Heart Infusion Agar (Hi Media)

Approximate Formula* Per Liter	7.7g
Calf Brains, Infusion from 200	9.8g
Beef Heart, Infusion from 250g	10.0g
Proteose Peptone	2.0g
Dextrose	5.0g
Sodium Chloride	2.5g
Disodium Phosphate	15.0g
Agar	

Mueller Hinton Agar (M-H Agar Hi Media)

Ingredients Grams/Litre	4.0
Beef infusion solids	1.5
Starch	17.5
Casein hydrolysate	15.0
Agar	15.0

Final pH	7.4 +/-02 at 37 C

Mueller Hinton Agar

Carbohydrate Fermentation (g./l)

Peptone	10.0
Carbohydrate*	5.0
Sodium Chloride	15.0
Phenol Red	0.018
Phenol Red	1000
pH	7.3

Carbohydrate*- ribose, galactose, D- glucose, D-fructose,D- manose, ramnose, manitol, sorbitol, maltose, lactose , sucrose.

Enzymes (Genei)

Proteinase K

Lipase

Amylase

Trypsin

Pepsin

Reagent

Gram Staining Reagent (Hi Media)

Crystal Violet

Gram's Iodine

Alocohol

Saffranine

Buffer- Citrate phosphate buffer

For SDS PAGE

Stock Acrylamide solution

30% Acrylamide

0.8% Bis Acrylamide

2.5% Ammonium per sulphate

3.10% SDS

Seperating gel preparation

3.3ml of stock acrylamide soluction

2.5ml of separating gel buffer

3 ml of distutilled water

101 TEMED

200 SDS

Stacking gel preparation

1.5 ml of stock acrylamide soluction

1.0 ml of separating gel buffer

1 ml of distilled water

5 ul TEMED

100 SDS

Electrode buffer

0.05M tris	12g
0.192 M Glyin	28.8 g
0.1% SDS	2 g
water	2 litre

Chemicals

1. Surfactants
   EDTA
   Sodium Dodecyl sulphate
   Tween80
   Tween 20
   Deoxycholic Acid

2. Organic Solvents
   I-amylalcohol
   Chloroform
   n-Propanol
   Hexane
   Diethyl ether
3. Nacl
4. NaoH
5. HCl

VII. ACKNOWLEDGEMENTS

"Gratitude is the memory of the Heart"- J.B. Massiew''

Firstly I want to thank "**The GOD"** by whose graces. I am here to cherish my goal and giving me wonderful opportunity to serve the people in need in my coming here.

I express my deep gratefulness to the supervisor **Dr. Mrs. Varun Dwivedi** for his valuable suggestions and sincere guidance.

I am highly grateful to **vice chancellor of Dr. R.P. Dubey** and Ragistrar **Mr. Gaurav Shukla of Dr. C.V. Raman University,** kargi road kota Bilaspur (C.G.) for his kind permission to carry out the present study.

I would especially like to thanks to nice **brother**, Caring **sister** and my **friends, relatives** for their in completion of my project.

I am very thankful to my husband **Mr. Hemant Sahu** for her valuable support and encouragement.

I am greatly indebted to all the lab staffs and lab assistance for their co-operation and inspiration.

And at last , but not least, I would always like to extend my greatest thanks my respected parents **Mr. Santosh Sahu and Mrs. Mathura Sahu** who provides me all needs, moral support, encouragement and valuable advise.

Mrs. MAMTA SAHU
PH.D SCHOLAR
MICROBIOLOGY
Dr. C.V. Raman University, Kargi road Kota Bilaspur (C.G.)

VIII. REFERENCES

(1) S. Akhtar,M.R. sarker, and A. Hossain, 'Microbiological food safety: a dilemma of developing societies' Critical reviews in microbiology, vol. 40, no.4, pp ,348-359,2013.

(2)Z. Muhammad, R. Ramzan, A. Abdelazez. et al., "Assessment of the antimicrobial potentiality and functionality of *Lactobacillus plantarum* strains isolated from the conventional inner mongolian fermented cheese against foodborne pathogens," Pathogens, vol. 8.no. 2,p.71, 2019.

(3)M. K. S. Yazdi. A. Davoodabadi, H. R. K. Zarin, M. T. Ebra- himi, and M. M. S. Dallal, "characterisation and probiotic potential of lactic acid bacteria isolated from Iranian tradi- tional yogurts", Italian Journal of Animal science, vol. 16, no. 2, pp. 185-188, 2017.

(4) L. G. Stoyanova, E. A. Ustyugova, and A. I. Netrusov, " Anti- bacterial metabolites of lactic acid bacteria : their diversity and properties," Applied Biochemistry and Microbiology, vol. 48, no. 3, pp. 229-243-2012.

(5) A. Bilkova, H. K. Sepova, F, Bilka, and A. Balazova, "Bacteriocins produced by lactic acid bacteria," Ceska a Slovenska fromacie vol. 60, no .2, pp . 65-72, 2011.

(6) M. P. Zacharof and R. W. Lovitt, "Baceriocins produced by lactic acid bacteria a review article," APCBEE Procedia vol. 2, pp. 50-56, 2012.

(7) M. P. Mokoena, " Lactic acid bacteria and their bacteriocins: classification, Biosynthesis and applications against uropathogens: a Mini- Review," molecules, vol. 22, no. 8, p. 1255, 2017.

(8) L. M. Ditu, M. Chifiriuc , D. Pelinescu, I. Avram, G. Pircalabioru, and G. Mihaescu," class I and II bacteriocins: structure, biosynthesis, and drug delivery systems for the improvement of their antimicrobial activity," Curr. Proteom, vol. 11, no. 2, pp. 121-127, 2014.

9. P.D. Cotter, R. P. Ross, and C. Hill," Bacteriocins- a viable alternative to antibiotics?" Nature Reviews. Microbiology, vol.11, no. 2, pp. 95-105, 2013.

10. H. Ghodhbane, S. Elaidi, J. M. Sabatier, S. Achour J. Benhmida, and I. Regaya, "Bacteriocins active against multi-resistant Gram Negative bacteria implicated in nosocomial infections," Infectious Disorders - drug Targets, vol. 15,no.1, pp. 2-12, 2015.

(11)M. Nishie, J .-I. Nagao, and K. Sonomoto, Antibacterial peptides “bacteriocins” an overview of their diverse characteristics and applications,” Biocontrol science, vol . 17,no.1,pp.1-16,2012.

(12)S.C.Yang, C. H. Lin, C.T. sung, and J.Y. Fang, “ Antibacterial activities of bacteriocins: application in foods and pharmaceuticals,” Frontiers in Microbiology, vol. 5,p. 241,2014.

(13)A. Nigam, D. Gupta, and A. Sharma, “treatment of infectious disease: beyond antibiotics,” Microbiological Research, vol. 169, no . 9-10. pp. 643-651, 2014.

(14) M. Gulluce, M. Karadayi, and O. Baris, “Bacteriocins: promising antimicrobials Microbial pathogens and strategies for combating them,” in science, Technology, and Education, Mendes- Vilas, ed., pp.1016-1027, A., FORMATEX, Madrid, Spain , 2013.

(15) U. Schillinger and F. K. Lucke, “Antibacterial activity of *Lactobacillus* sake isolated from meat,” Applied and Environmental Microbiology, vol. 55, no. 8, pp. 1901-1906, 1989.

(16) J. R. Tagg and A, R, McGiven, “ assay system for bacteriocins,” Applied Microbiology , vol. 21, no. 5, p. 943, 1971.

(17) J. W. Westley, R. H. Evans Jr., L. H. Sello, and N. Troupe , " lsolation and characterization of antibiotic, X-14547A, a novel monocarboxylic acid ionophore produced by Sreptomyces antibioticus NRRL 8167," Journal of Antibiotics (Tokyo), vol. 32, no. 2, pp. 100-107, 1979.

(18) A. E. Abo- Amer, "characterization of bacteriocin-like inhibitory substance 459 produced by *Lactobacillus plantarum* isolated from Egyptian home-made yogurt," Science Asia, vol. 33, no. pp.313-319, 2007.

(19) D. Fatima and K. Mebrouk, "characterization and determination of the factors affecting anti-listerial bacteriocins from *Lactobacillus plantarum* and *Pediococcus* pentosaceus isolated from dairy milk products," African Journal of food science, vol. 7, no. pp. 35-44, 2013.

(20) N. R. Sankar, V. D. Priyanka, P. S. Reddy, p. Rajanikanth, V. K. Kumar, and M. Indira, " purification and characterization of bacteriocin produced by *Lactobacillus plantarum* isolated from cow milk," International Journal of Microbiology Research, vol. 3, pp. 133.-137, 2012.

(21) M. C. Joerger and T. R. Klaenhammer, "Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481," Journal of Bacteriology, vol. 167, no. 2, pp. 439-446, 1986.

(22) M. Z. hoque, F. Akter, K , M, Hossain, M. S. M. Rahman, M. M. Billah, and K. M .D Islam, "Isolation, identify and analysis of probiotic properties of *Lactobacillus* spp. From selective regional yogurts," World Journal of Dairy and food sciences, vol. 5 , pp . 39- 46, 2010.

(23) M. Tokatli, G. Giilgor, S, Bagder Elmaci , N. Arslankoz lsleyen, and F. Ozcelik, " in vitro properties of potential probiotic indigenous lactic acid bacteria originating from traditional pickles," Biomed Research Intenational , vol. 2015, article ID 315819,8 pages, 2015.

(24) S. T. Ogunbanwo , A. I. Sanni, and A. A. Onilude, "characterization of Bacteriocin produced by *Lactobacillus plantarum* F1 *and Lactobacillus brevis* OGI," African Journal of Biotechnology, vol. 2, no. 477, pp. 219-227, 2003.

(25) S. D. Todorov, C. A. van Reenen and L. M. T. Dicks, "Optimization of bacteriocin production by *Lactobacillus plantarum* ST13BR, a strain isolated from barley beer," The Journal of General and Applied Microbiology, vol. 50. no. 3, pp. 149-157, 2004.

(26) A. Bonade, F, Murelli, M. Vescovo, and G. Scolari, "partial characterization of a bacteriocin produced by *Lactobacillus helveticus,"* Letters in Applied Microbiology, vol. 33, no. 2, pp. 153-158, 2001.

(27) Saitou N. and Nei M. (1987). The neighbor- joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

(28) Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

(29) Tamura K., Nei M., and kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor- joining method. Proceedings of the National Academy of Science (USA) 101:11030-11035.

(30) Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.