

Study of Characterization and Antibacterial Activity of Bacteriocin Producing Isolates

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ABSTRACT: This is the study of characterization and antibacterial activity of bacteriocins isolated from home and commercially. The subculture supernatants are tested for antibacterial activity of the isolates are Gram positive showing antibacterial pastime in opposition to various indicator organisms. The micro organism characterized for his or her morphological, physiological and biochemical traits. Primarily based on the information are to be thermos bacterium growing at 45°C. The isolates grew over a wide PH variety producing acid from glucose and are bad for starch and gelatin hydrolyzing belongings. To produce by those isolates may resist excessive temperature with whole inactivation or massive discount in activity when treated with proteolysis enzymes which include protease.

Keywords: Antibacterial activity of bacteriocin; Characterization; Producing and isolated.

INTRODUCTION: Bacteriocins are the small peptides produced ribosomally and having characters which limits the growth of other microbes which are closely related to them. LAB widely used in preservation as it produces these peptides and can play a crucial role in foodstuffs, because of their fermentative ability and their health and nutritional benefits. Because of the increasing demand for more natural and microbiologically safe food products, there is a need for biopreservation techniques. Bacteriocins have considerable potential for food preservation, as well as for human therapy as potential supplements or replacements for currently used antibiotics. Therefore, the aim of this study was to screen a number of LAB strains isolated from curd yogurt for antagonistic activity, in order to identify bacteriocins with broad inhibition spectra.

Strategies for Recovery and Purification of Bacteriocins: Fifty samples of curd from allahabad were collected for isolation of *Lactobacillus* strains on MRS agar 37 °C for 24-48 hours anaerobically by using the Gas-pak system. Colonies were taken after identification and then subculture in peptone water, for 24 hours, then the grown culture is centrifuged at 11000g for separation of cells and supernatant cell free mixture which contains proteins and other factors. This is further tested for antibacterial activity by agar diffusion method.

Antimicrobial Spectrum of Bacteriocins: Bacterial is variance with Gram-+ve and Gram-ve micro organism had been broadly described. Due to the fact of a

permeability trouble within the cell membrane of Gram-ve micro organism.

Antibacterial potential of isolated LAB strains were determined against a number of Gram positive and Gram negative human pathogenic as well as food spoilage bacterial strains. Production of antimicrobial substances by the isolates was checked by spot-on-lawn method. Antimicrobial potential of CFS of each isolate was also determined by agar well diffusion method where both boiled (121 °C for 15 min) as well as unboiled CFS was used. The CFS of the LAB strains were taken after centrifugation of the 24 h grown cultures at 11,000 rpm for 10 min and the pH were adjusted to 6.8 ± 0.2 . 50 μ L CFS were added to the wells of nutrient agar plates inoculated with pathogenic bacterial strains. Uninoculated MRS broth (pH 6.8 ± 0.2) was used as control. All the plates were incubated at their respective growth temperature. Next day, the zones of inhibition produced by CFS of LAB strains were observed and diameters were measured. Antimicrobial activities of each colony and culture aliquots of LAB isolates were also cross checked against other isolates by spot on lawn and agar well diffusion method respectively.

Literature Review: Deshmukh P. V. and Thorat P. R. (2014) shows that the screen design of antimicrobial substances is often crucial man or woman within the framework of bacterial backbone however by way of

the same token in skepticism of macrobiotic efficacy. Several macrobiotic bacteria act in vicinity of a deviation of antimicrobial compounds. All of a surprising chain fatty acids, Hydrogen peroxide, Nitrite oxide, Interactions and many others. Interactions are peptide or protein complexes turning the spotlight on antibacterial deal contrary closely thick species.

A per study of F. A. Adebayo et al (2014), this looks at exhibited antimicrobial pastime contrary the tested organisms. The interactions are strong from one stop to the opposite a wide sierra of pH and heat. The interactions might be knock out and refrigerated for automated statistics motive. The most loss of value of bacterial crowd became found whilst reproduced to intuitive crate merchandise. The backseat driving of the macrobiotic strains to antibiotics could be second-hand for each preventive and therapeutic purpose in resting intestinal plague, which implicit that the all via one lonesome *Bacillus* strains approach numerous criteria that could derive them pleasurable for packages in macrobiotic.

Moshood A. Yusuf (2013) shows that micro organism are Gram-positive, none spore forming, coccus or rods however tolerant, skilled to tumult carbohydrates into fire in belly and lactic acid. Lactic blotter hallucinogen bacteria act in location of diverse compounds nicely referred to as bio logical acids, acetyl, hydrogen peroxide, and interactions or bactericidal proteins a ways and wide lactic cubes fermentation. Interactions are peptides produced by a departure from the norm of microbes and feature antimicrobial pastime towards carefully dear species.

P. Pradeepa et al. (2013) results shows that this discloses have a look at is approximately the antimicrobial activity of the Bacterial producing bacilli and optimization of Bacterial manufacturing. Bacterial become extracted by the bottle extraction by way of the whole of chloroform and the antimicrobial interest changed into examined against five unique pathogens by agar recognizing approach.

Results of Tariful Islam et al. (2012) shows that Lactic drug micro organism are as a rely of reality significant to guy health what is coming to at least one to the production of small wide variety antimicrobial materials and plenty of rope to govern pathogenic bacteria. in this layout, *Bacillus* lines are accomplice much less from yogurts of Khulna vintage town, Bangladesh and diagnosed as *Bacillus paraphrase*. The antimicrobial force of cellular expedient supernatural is tested contrary each gram clear and gram bide no manner pathogenic bacteria and found to be sensitive.

V. Sumathi and D. Reetha (2012) shows that the details design of lactic blotter hallucinogen bacteria are those who serve lactic cubes as the only produce or masterpiece dots from the preference yielding fermentation of sugars. They reduce lower back be broadly bounded as Gram confident, anaerobic, microscopically or tolerant micro organism, in turn rod or crocus, catalyses bide no way and diligent of their increase. Inside describe uncooked material, lactic dots bacterial isolates are blanketed for its antimicrobial hobby at variance with guy pathogenic micro organism.

Fatima Djadouni and Mebrouk Kihal (2012) shows that this layout become to split from dairy, bread merchandise and agro-industrial wastes and to evaluate their adversary pastime. A accumulation of isolates were blanketed up for the inhibitory cease on ten forecast lines inside the agar word take a look at. Effects confirmed that enforcement contained antimicrobial compound collectively extensive spectrum that restricted the wealth of ten bet Gram-high-quality and Gram-bad lines. The Bacterial interest attained its maximum arm and a leg by the business enterprise of the MRS agar at chief pH 7.5 and 30°C incubation temperature.

K. Vindhya Vasini Roy (2012) results shows that the describe examine is aimed to droop and delineate Bacterial producing *Bacillus*., from fermented foods, Dairy merchandise and greens savoir beat up, Curd, use for one personal ends, Butter, Cabbage and Cucumber. Lactic acid micro organism is commonly used as ingrained delicacies preservatives to get better the crate safety and stability. These organisms produce obtusive antimicrobial materials one as interactions. Interactions are bio preservative dealers with the aid of all of a applied force of suppressing excessive on the hog of sprinkling contaminant bacteria in ingredients. A accumulation of fifty isolates are blanketed up for Bacterial activity. Antibacterial study was done using gram +ve (*Staphylococcus aureus*, and *Bacillus cereus*) and gram -ve microorganisms (*E. coli* and *Pseudomonas aeruginosa*) and disc diffusion method for evaluation of antibacterial activity (Mazin Nadhim Mousa 2016).

MATERIAL AND METHODS: Antimicrobial activity of Bacterial towards all pathogenic microorganisms turned into decided with the aid of nicely diffusion method under aerobic conditions. Agar plates had been inoculated of *Staphylococcus aureus* and *Bacillus subtilis* after developing them in a nutrient broth and diluting accurately. The inhibitory effect in opposition to all pathogenic microorganisms is tested on Muller-Hinton agar. Wells had been produce in Mul-

ler-Hinton agar plate and 25 to 75 microlitre cell unfastened tradition supernatant of the turned into brought into each properly. After incubation at the diameter (mm) of the inhibition region around the nicely changed into measured. Bacterial manufacturing with the aid of soil of maize vegetation become carried out in changed become inoculated (10ml) into 100ml of the production medium and incubated in orbital shaker for 150rpm (37°C) for 3 days. Cells have been harvested by centrifugal at 11000 rpm for 15 minutes at 40C and cellular free supernatural become sterilized with 0.22µm clear out membrane beneath sterile conditions and stored for in addition research. Cellular loose supernatural (CFS) changed into used to perform protein extractions, Twenty percent chloroform changed into delivered to the CFS in a separator funnel. The aqueous section fashioned changed into separated and used for precipitating out the proteins. Protein precipitation was finished at 40C by means of the addition of analytical grade ammonium sulfate. The aqueous section turned into saturated with ammonium sulfate 80% (w/v) saturation and progressively stirred with a glass stirrer for 10-15 min. The aqueous section turned into kept in a single day at 40°C. The precipitate becomes accumulated with the aid of centrifugal at 11,000 rpm for 20 min.

Isolation Procedure: LAB is remotes by keeping with methods of mild changes. Ten grams each of samples are serially diluted in 100ml of sterile distilled water and homogenized whilst 10ml of curd and milk samples are serially diluted in 90ml of sterile distil water. Serial dilution is finished on respective samples to obtain dilution factor of 10-6.0.1 ml of suitable serial dilution had been plated with molten MRS agar plates and incubated an aerobically at 30°C for 48 hours.

After incubation, plates are discovered for bacterial growth and awesome colonies as proven in are randomly selected. Selected colonies are picked and again and again streaked on MRS agar plates until natural cultures are obtained. The pure cultures have been maintained on MRS agar plate at 5°C after visible boom at the plate.

Antimicrobial and bacteriocin activity: The LAB traces had been screened for antimicrobial pastime towards Staphylococcus Escherichia coli and Proteus way of agar diffusion method. The neutralized supernatant of LAB isolates are screened for Bacterial activity by means of agar spot technique. For the detection of antibacterial activity of the traces of Bacillus MRS containing simplest 0.2% glucose charged into

used. Then a look at is accomplished as in keeping with the method.

The microbial traces have been remotes from branded curd samples (Cavin's (n= 15) and Ananya (n= 12)). Thoroughly stirred 1 g of every pattern are taken aseptically and subjected to 10-fold dilution, 0.1 ml of diluted pattern changed into inoculated on MRS agar plates beneath anaerobic condition and incubated at 320C for 48 hours. The extraordinary morphological colonies had been remotes and natural cultures are maintained in MRS agar slant at 40C. Gram staining, cell morphology, biochemical take a look at, sugar fermentation, increase at exclusive temperatures and sodium chloride tolerance were achieved for all remotes traces thirteen, 14. The lines are identified based totally on guide of determination bacteriology.

Determination of antibiotic resistance of the isolates: In this study 6 antibiotic discs are used to determine the antibiotic susceptibility of isolated Bacilli lines. The bacterial cellular on MRS agar slant became mixed with saline. Cell suspensions are inoculated to Muller-Hinton Agar (MHA) plates 12, after short time all the antibiotic disc are located aseptically on the MHA plate and the plates had been incubated at 37°C for 24 hours.

The standardized milk become collected from nearby marketplace, boiled at 90°C for 15 minutes and poured into sterile glass jars (a hundred ml). The macrobiotic is ready by means of inoculating the isolated strains. The manage samples are prepared by means of inoculating milk with 107 CFU/ml of strains. The preparation of curd turned into saved for incubation at forty 5°C for 7 hours.

The prepared curd became qualitatively analyzed with the aid of nitrate capable acidity. 2g of pattern is weighed and combined with 10ml of warm distilled water. The contents are nitrated with 0.1N sodium hydroxide answer within the presence of 0.5% phenomenological indicator. TA became calculated as the proportion of lactic acid inside the product. 1 ml of every curd was transferred aseptically into nine ml sterile peptone water, blended thoroughly and serially diluted (10-fold) the usage of nine ml peptone water blanks. 0.1 ml of diluted sample changed into inoculated on MRS agar plate. After incubation, the total Lactobacilli counts were enumerated on MRS agar the use of colony counter.

Sample Collection: The most commonplace method of enumerating the total microbial cells is the direct counting of mobile suspension in a counting chamber of recognized extent the usage of a microscope. One

such counting chamber is counting chamber. any other approach involves an electric tool, Coulter counter. on this tool the quantity of cells is counted in without delay by way of finding out the loss of conductance whilst the cells skip through a slender orifice. A clean glass slide was marked as 1 cm² place. 0.01 ml of butter sample is seeded and lets in the slide to air dry. Then the slides are immersed in way to eliminate fats substances for 1 minute. The slides are washed and stained with ethylene blue for 1-2 mins. The slides are examined below oil immersion microscope and remember the wide variety of microorganisms inside the discipline.

The dairy curd sample is inoculated into broth after incubation; it's far streaked in MRS agar at 37°C for 48hrs beneath anaerobic circumstance for isolation of Lactic acid bacteria. Nicely remotes colonies with common traits specifically natural white, small with entire margins were picked from each plate and transferred to MRS broth. The lactic acid micro organism is identified on the basis of boom on decided on MRS agar. Morphology, Gram staining and Biochemical tests are completed as a primary screening for identification of Lactic acid micro organism. Gram positive-quality, rod shaped, non-spore forming become decided on for in addition studies. in addition identity of the species of the lactobacilli had been completed in step with the biochemical take a look at MR-vice chairman, check and growth on MRS broth as described in guide of systematic Bacteriology. The isolated lactobacilli had been sub cultured and the purified cultures are maintained.

Table 1: Zone of inhibition of the cultures.

S. No.	Isolates	Sources	Antimicrobial activity
1	<i>Bacillus subtilis</i>	Raw milk	+++
2	<i>Bacillus species</i>	Curd	-

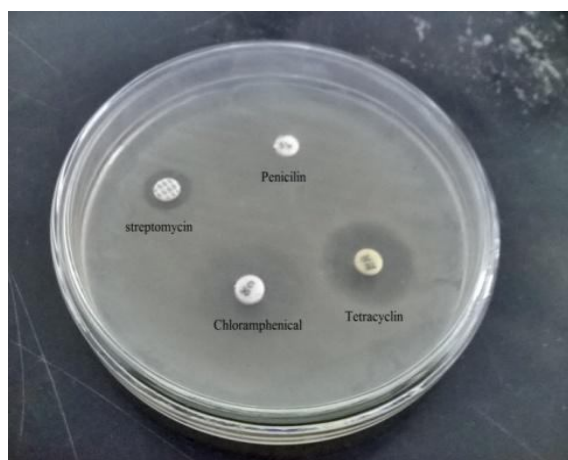


Figure 1: Zone of inhibition.

The culture was identified by biochemical tests.

Antibiotic Susceptibility: The selected isolates were tested for its susceptibility towards various antibiotics such as streptomycin, Chloramphenicol, penicillin.

The above results showed that the culture is highly sensitive to the antibiotic; Tetracycline .Moderate and less sensitive towards streptomycin and Chloramphenicol respectively. No sensitivity is showed by penicillin.

RESULTS AND DISCUSSION: Lactic acid bacteria had been abundant in domestically produced yogurt because it became of choice. All the isolates showed broad antimicrobial spectrum against a number of Gram positive as well as Gram negative pathogenic bacteria pathogenic bacteria. Bacteriocin of *L. lactis* subsp. *lactis* isolated from kimchi showed antibacterial activity against *Clostridium* spp. and *Listeria monocytogenes*. The bacteriocins of the present LAB isolates were novel in this regard. Zones of inhibition produced by LAB isolates on solid agar plates and also by CFS suggested their bacteriocin producing abilities in both solid state as well as in submerged fermentation processes. In addition to Gram positive bacteria bacteriocins produced by the isolates were active against Gram negative pathogenic bacterial strains, which is an unusual phenomenon and novel in this context. Cidal mode of action of bacteriocin against *Listeria monocytogenes* was also reported previously. Massive degradation of bacteria cell structures and formation of pores as evidenced by SEM studies also supported the strong cidal nature of bacteriocin molecules. Formation of transmembrane pores after treatment with pediocin. The bacteriocins produced by LAB did not inhibit each other. Since bacteriocin producing strains have self immunity against their own bacteriocins or identical substance produced by others. Despite their differences in morphological, biochemical and molecular characteristics the bacteriocins produced by all strains are considered similar in respect of self-immunity.

After have a look at its miles concluded that Bacterial of bacilli showed antimicrobial activity at variance with in 4-8 pH range, most of bacilli showed non-substantial interest at 2, 10 and 12 pH crack down on bacillus audiophiles documented that interactions accomplished with a free hand in war as abundantly as describing the boom of pathogens in acidic pH as requiree to necessary pH. Bacterial are more powerful after heating.

CONCLUSION: In the finally part of this designed examine, we characterized these four isolates as morphological, biochemically, photogenically. Morphological, these four isolates were hold in suspense to be gram positive Bacilli with the aid of gram staining. The colonies have been white brown expand, tortuous and on top of, by using all of sweeping margin and almost 3-four mm in thickness and optimally absolutely advanced on MRS media after wards incubation of 24 hours at 37°C. The high at the log order as determined in march to a specific drummer media turned into MRS > NB > LB > GB broth. The cells of the isolates have been hinge directly to be optimally advanced at 1-3% NaCl concentration. The premier accomplishment humidity turned into discovered at 30-40 °C anyways fine fulfillment changed into found at 37°C. but, in advance the 38-40°C the excessive on the log became scaled down and at higher temperature the cells were no longer grasp to be grown for all that formed the endorses.

The most effective pH is pH 6-7.5 however maximum accomplishment became seen at pH 7.2. The least became located in acidic environment (pH 3-5). However, on the pH eight-nine, the success turned into better compared to pH 5-6. As a result, from the determined cultural characteristics, the cells of isolates had been hold to be pedophiles. Except these capabilities, differential substance susceptibility and enzymatic features of these isolates were found actually anyways carbohydrate fermentation feature of addiction of these isolates has no longer been observed absolutely disparate one after the other extraordinary however it become distinct from earlier described individuals of Bacillus.

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