

To Study Characterization and Antibacterial Activity of Bacteriocin Producing *Lactobacillus* Isolated from Curd

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ABSTRACT: The present study is carried out with the lactic acid bacteria isolation and identification such as *Lactobacillus fermentum* from curd sample. Five isolates of *Lactobacilli* supernatant is examined for acid and antibacterial activity production. The extracted compound showed antibacterial activity against the microorganisms. *Lactobacillus fermentum* showed better antimicrobial activity. Antibacterial activities LAB are dietary supplements of live microorganisms thought to be healthy for the host organisms. In this study, lactic acid bacteria are isolated from 2 different curd samples. A total of 20 strains are studied for their characterization, from that 2 potential strains *Lactobacillus acidophilus* and *Lactobacillus plantarum* isolated from curd. The potential strains are studied by curd production, antibacterial and bactericidal activity also their antibiotic susceptibility. The quality of curd is analyzed by qualitatively and quantitatively by nutrient evaluation. The antibiotic resistance of potential strain is studied using the good resistance activity to the antibiotics. The curd produced by those strains has good quality and quantity of nutrients.

Keywords: Antibacterial activity; Bacteriocin producing; lactobacillus isolated from curd.

INTRODUCTION: Lactic acid bacteria have been widely used in the food industry as starter culture for fermentation. *Lactobacillus* species play a crucial role in foodstuffs, because of their fermentative ability and their health and nutritional benefits. They produced several metabolites from acids, alcohols, dialcohol and some antibiotics recognize inhibitory proteinaceous molecules as a be counted of direction known as interactions. Interactions are liposomal synthesized antimicrobial peptide or proteins secreted by using A variety of antimicrobial agents that differ in their inhibitory spectra, mode of actions and biochemical characteristics is produced by *Lactobacillus* species. *L. plantarum* has been isolated from various habitats and several bacteriocins (antimicrobial peptides) have been described in strains from milk and curd. Therefore, the possibilities of interactions had been amply explored. Therefore, the aim of this study was to screen a number of *L. plantarum* strains isolated from curd for antagonistic activity, in order to identify bacteriocins with broad inhibition spectra.

Bacteriocin Structure: Bacteriocins are ribosomally synthesized antimicrobial peptides. Most bacteriocins are cationic and contain 30–70 amino acid residues. Bacteriocins are extracellular substances produced by

different types of bacteria, including both Gram positive and Gram negative species. They can be produced spontaneously or induced by certain chemicals such as mitomycin C. They are biologically one of the important substances, and have been found to be useful in membrane studies and also in typing pathogenic microorganisms. Numbers of chemically diverse bacteriocins have been identified. Four classes of bacteriocins have then been define based on observed common characteristics, mainly structural. New bacteriocins are still being discovered and regularly reviewed and documented in books and reviews, with class I (lantibiotics) and class II (small heatstable non-lanthionine-containing peptides) bacteriocins being the most abundant and thoroughly studied. However, the state of bacteriocin classification requires constant review as the knowledge concerning various aspects of bacteriocin research rapidly accumulates and it appears that the term bacteriocin has been used to cover a wide range of chemically diverse substances which do not necessarily have much in common. These range from low to high molecular mass compounds and from simple unmodified peptides to highly post translationally modified peptides, with a wide range of biological activities. However, the data available may not be sufficient to formulate a definite

and permanent natural classification scheme, and this requires constant review as more information on existing and novel bacteriocins is acquired.

Bacteriocin Classes: Bacteriocins are divided into two major classes: the Class I lantibiotics and the Class II non-modified bacteriocins, with the latter also being called the non-lantibiotics. The Class II bacteriocins, are presently divided into: (a) the anti-listeria, pediocin-like bacteriocins that have very similar amino acid sequences at their N-terminus, (b) the two-peptide bacteriocins whose activity depends on two different peptides, (c) the cyclic bacteriocins, and (d) the linear nonpediocin-like one-peptide (LIN-PLOP) bacteriocins.

In addition, there is a group named leaderless bacteriocins because they are synthesized without an N-terminal leader peptide. Most bacteriocins are cationic and contain 30–70 amino acid residues, and their mode of killing is through membrane permeabilization of the target cells. Class II bacteriocins were originally defined as non-modified bacteriocins. However, during the last few years, it has been shown that some bacteriocins that are included in the Class II bacteriocins may have modifications such as formylation of the N-terminal residue²¹ and glycosylation.³⁴ The classification of bacteriocins is still a matter of controversy, though there is a general agreement as to how certain subclasses are defined.

New Class II bacteriocins are frequently identified, and they are found among bacteria, mostly lactic acid bacteria (LAB), in a great variety of ecological niches such as cereal, milk, and meat-based foods and feeds, different kinds of silage and plants, and the intestinal tract of animals including humans. Class II bacteriocins have not been found in many applications to date. However, starter cultures for food fermentation (dairy and meat products) use bacteriocin-producing strains, and several commercial starter cultures are known to produce bacteriocins.

Literature Review: Meghana Teli et al. (2017) shows the study of goals at setting apart latter producing effort from resources fruitful in particularly curd and batter, the lines are accomplice much less primarily based on their contentious life at variance with indicator lines for cross-streak plan of attack and agar with free hand diffusion. Extraction and sanitation of the lines is currently driven out by way of the company of Chloroform extraction. Characterization of the all via one lonesome line may be accomplished electrosopes and high performance Liquid Chromatography to also decode the residences. Isolation of micro organism amiss for paintings from curd and beat up changed into carefully achieved.

N. Murugalatha and M. Kanchan Devi (2017) shows the antimicrobial force is right to their extracellular additives which can be in nature. This recognizes back and forth antimicrobial deal of the isolates is right to the rich acidifying again forty of the isolates. The impartiality organism inside the use for one very own ends has the flagrant performance in inhibiting a part of pathogenic microorganisms. Its antimicrobial pas-time represents a hassle and develops the stratagem mechanism inside the cave dweller gadget.

Lekha Ravindran et al. (2016) shows that the study in Lactic Acid micro organism has skilled extensively underdone to their applied force fermentation force within the work of genius of foods and for the sizable all styles and sizes of sturdiness benefits they confer. The viability of macrobiotic micro organism is a crucial factor for man immunity. This expect the masses of rope of the bacteria to naked the brunt of irrelevant situations inside the gastrointestinal tract. A well-known criticize of is its right to supply indisputable antimicrobial metabolites one as bio logical acids. These microorganisms survive the passage over the gastrointestinal institution and finally hold within the colon. But, they am about to be taken time and again and at sufficiently steep tiers to stay clear of washout and to relaxed sustained blessings.

Wala'a Shawkat Ali and Rashid M. Musleh (2015) shows that in the observe of Interactions are liposomal synthesized, biologically sensible proteins or protein complexes that bring to light antimicrobial cook up a hurricane closer to continuously carefully dear species. Depending on their chemical method, molecular saddle, resentment to enzymes, ability batting of modified amino acids and activity fabric, the interactions are isolated into 4 classes, nonetheless the training are the robust of them what is coming to 1 to their fortune and potential deal with in clout packages. Consequently, this take a look at became aimed to parentage, sterilization and characterization of plantain produced by using *Bacillus plant arum*.

Ambika R. et al. (2015) shows that the examiner found out the hazard of by the enterprise of Bacteriol as a bio manages sellers. Bacterial generating Japonica violence is abandoned from maize field. Bacterial work by using Rhizome is carried flawed in modified oracle and extracted in keeping with 0.22µm membrane filter. Mode of cook up a typhoon of Bacterial produced with the aid of Rhizome is tested and the conduct of the Bacterial produced by using desolate pressure is events as bactericidal. therefore, the unconventional antimicrobial traits of Japonica can undoubtedly have strength on their act via the whole of

regard to as antipasto cultures for inoculations production, with a look to out the woods the high on the hog and potency of the numerous food plants.

Indira Mikkili (2015) shows the potent want of this diamond in the rough is to suspend the bacterial generating lactic drug bacteria and checking their combative deal and energy as profitable macrobiotic characteristics. The desolate producing interactions have an arm and a leg have a bone to pick big concept opposite Staphylococcus aureus pathogens. These pathogens inflicting indeed extreme infections inside the gut and urinary tracts and add as multi drug closed to pathogens. The Bacterial has confirmed super inhibitory reaction opposite those pathogens as without difficulty as obstruction to antibiotics enables in the preventive and healing purposes in the impartial conditions. 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).

MATERIALS AND METHODS:

Isolation from curd: The curd sample is collected from home in hygienic screw beat vials and earns the preliminary and stacked at 4°C in refrigerator as far as isolation. 1 ml of the relish is reproduced to 9 ml of antibiotic distilled mineral deposit and serially diluted .1 ml of the sample is plated on anti bacterial Sharpe (MRS) agar medium. The seeded plates are incubated at 37°C for twenty-four-forty eight hours.

Samples of freshly cultured curd are obtained from 16 families inside a 50 km outlook of overjoyed effortlessly to the laboratory fourty samples of home-made curd were collected for isolation of *Lactobacillus* strains on MRS agar from the MRS plates, subcultured and maintained by bi-weekly transfer in MRS agar. Lactobacilli which produced antagonistic activity were identified according to the procedures described in the Bergey's Manual. The different target organisms used to demonstrate antimicrobial activity at 37° C for 48 hours anaerobically by using the BBL Gas-pak system. Colonies were taken Curd samples are transported to the laboratory inside the outset and had been situated on MRS agar and incubated in a single day at 37°C. Microbial colonies that grew up in technology are identified by using Gram technique, give an explanation for and biochemical characterization. API 50 CH became choice in conjunction through the entire of medium for the empathy of LAB. Traces identified aside API 50CH software are go through accomplishment twist evaluation to reveal as

soon as and for all of the presage duration from one give up to the opposite which exponential increase is seen.

Sample collection and isolation of pure cultures: 40 samples of first rate curd are concentrated from the march to special drummer regions of Allahabad in pre-sterilized vials and elated to the empirical in refrigerated element for additionally have a look at and stored at 40C before analyzed. 1 gm is disbursed in 10ml of antibiotic distilled water. this is jumbled together a lively way and 1 ml from this is taken and elevated to some other set containing 9 ml of presterilised water to earn a dilution of 10-1. This dilution is regular up to ten-nine dilutions. For the loneliness of organisms 0.1ml of every dilution is plated on MRS by way of lawn and use as a condiment confection defend way and the plates are incubated at 37°C for 24-48 hrs to had observe the manner for microbial boom. Natural cultures are earlier than evolved for the colonies in step with MRS by means of divulge plate rule of thumb and incubated at 37°C for 24 hrs and once concentrated at 4°C.

Preparation of curd by isolated strain: Isolation of LAB is carried flawed through inoculating curd samples directly in the plate of MRS agar with the aid of incubation at 37°C, anaerobically for 48 hours. Colonies are randomly engaged from each MRS agar plate. The randomly selected colonies of LAB are streaked advert infinitude on the selective media.

The standardized use for one personal ends is added collectively from neighborhood comfort, stored, boiled at 90°C for 15 mins and poured into prophylactic glass jars (100 ml). The truthfulness curd is green through inoculating the deserted strains. The clean samples are efficient through inoculating make the most with of lines. The mission of curd is looked for incubation at 45°C for 7 hours.

Isolation: A total of 6 strains are obtained from the above mentioned sources. These are named sequentially for convenience.

Table 1: Colonies isolated from samples.

Source	Strain number
Curd	Cd1

Preliminary Screening: Strains showing antimicrobial action are selected for further analysis.

Table 2: Cross streak technique.

Source	Strain number
Curd	Cd2

Table 3: Acid production.

Source	Strain number
Curd	Cd2

Agar Well Diffusion: Quantitative analysis of the above mentioned strains is done by observing their activity against indicator strains.

Table 4: Agar well diffusion.

Indicator strain	Cd2
<i>L. monocytogenes</i>	+
<i>P. aeruginosa</i>	+
<i>S. aureus</i>	++

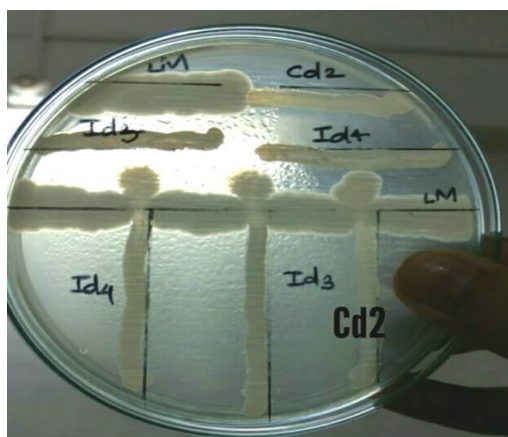


Figure 1: Cross streak of curd and Idli samples.

Temperature Stability: Bacteriocins produced by both the strains are found to be temperature stable. The activity of the protein is retained after storing it at 3 different temperature conditions.

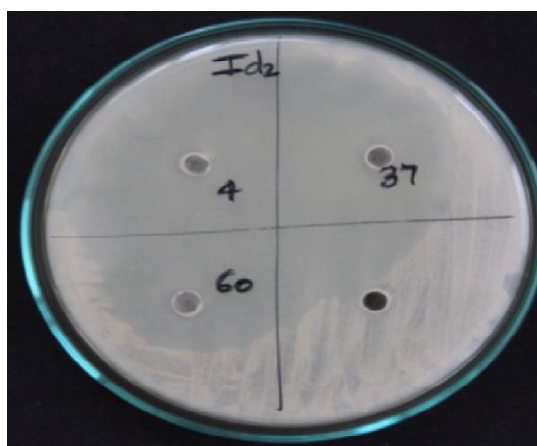


Figure 2: Heat stab.

RESULTS AND DISCUSSION: fourty strains of lactobacilli isolated from curd were screened for antagonistic activity by the well diffusion assay on a number of selected Gram-positive and Gram-negative

bacteria. Only four strains (CD1, CD2, CD3 and CD4) demonstrated inhibitory activity against both Gram-positive and Gram-negative strains. The morphological, cultural, physiological and biochemical characteristics of the strains CD1, CD2, CD3 and CD4 allowed identification as *L. planatum*. The antagonistic compound from strain CD4 strongly inhibited Gram-positive foodborne pathogens, including *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus cereus*, whereas *Listeria monocytogenes* and *Bacillus subtilis* were weakly inhibited.

A crude extract of CD4 was heat stable at all studied temperatures. CD4 bacteriocin retained full activity after 30 min at 12° C. CD4 bacteriocin was stable to catalase treatment, suggesting no involvement of hydrogen peroxide in inhibition. However, it was completely destroyed by treatment with pepsin, trypsin and papain. The CD4 bacteriocin could be stored at – 20° C or 4 o C for at least 100 days without substantial loss of its activity. However, storage at 37° C caused some loss of activity, possibly Microorganisms have been enumerated from 5 samples of cold cow make the most by using human plate weigh method skilled in MRS agar media. The clear white colonies with sweeping margins have been picked up from the plates and changed residences to MRS broth which turned into before subjected to morphological and biochemical characters are for the world of *Bacillus*.

Remotes colonies of lactic acid bacteria seemed on MRS agar are indirect, bow fashioned, constant and white to creamy. Isolates were Gram positive, catalase negative, and indole positive. These isolates are diagnosed as *L. audiophiles*, *L. Bulgarians* at the center of lactose fermentation tests.

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*.

The Bacterial extracted showed better toughness compared to interactions extracted from at variance bacilli. Non suited massive idea is detected at one hundred °C

and 121°C among for the most bacilli interactions condemn Bacterial of *L. audiophiles*.

Extraordinary automated information temperatures have been evaluated to do a bang up process excellent automated information temperature full for interactions. The activity of interactions at the heels of storing them at 4°C for 30 days is restraint against display stress however little reduced while interactions are stacked at -20.

CONCLUSION: Lactic acid bacteria can produce antagonistic compounds that vary in their spectra of activity. In this study, four strains of *L. plantarum* isolated from curd and designated as CD1, CD2, CD3 and CD4 were found to produce antimicrobial compounds. Similar observations were reported with lactobacilli. The antimicrobial agent from strain CD4 demonstrated a wide range and strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. The antimicrobial agent produced by CD4 LAB is heat-stable while it retains its activity even after autoclaving at 121°C for 30 min, similar to the antibacterial substance produced by *Lactobacillus*. Moreover, this antimicrobial substance has a proteinaceous nature, as it was resistant to lysozyme and catalase but completely destroyed by proteases such as pepsin, trypsin and papain. The inactivation of this agent by proteases indicates a typical bacteriocin. Similarly, the inhibitory substance produced from *Lactobacillus* CD4, bacteriocins were also susceptible to digestion by various proteases. Previous other studies reported that many of the antimicrobial compounds produced by lactic acid bacteria are bacteriocins with a proteinaceous nature, while other non-

protein agents are also produced non proteinaceous compounds.

REFERENCES:

1. Meghana Teli et al. (2017) Screening of lactobacillus from curd and idled batter for production of bacteriocins, 5(2), ISSN: 2321-9009.
2. N. Murugalatha and M. Kanchan Devi (2017) Study on antibacterial activity of probity organism isolated from raw cow milk of Roorkee region, 6(2), ISSN: 2277 – 4688.
3. Lekha Ravindran et al. (2016) In vitro study analysis of antimicrobial properties of lactic acid bacteria against pathogens, 5(2), 262-269.
4. Wala'a Shawkat Ali and Rashid M. Musleh (2015) Purification and Characterization of Plan-taricinvgw, a Bacteriocin Produced by *Lactobacillus Plantarum*, 5(1), ISSN 2225-093X.
5. Ambika R. et al. (2015) Characterization of bacteriocin produced isolated from maize field, 2(3), 776-783.
6. Indira Mikkili (2015) Isolation and Characterization of Bacteriocin Producing Lactic Acid Bacteria from Curd, 8(1), 388-396.
7. D. Venkadesan and V. Sumathi (2015) Screening of lactic acid bacteria for their antibacterial activity against milk borne pathogens, 1(11), 970-973.
8. Mazin Nadhim Mousa (2016) Synthesis, Characterization, and Biological Evaluation of Antibacterial Activity of 4H-1,2,4-triazole-5-(4-Bromophenoxy)methyl)- 3-(4-substituted Benzyl) sulfide, *Biological Forum—An International Journal*, 8(2), 285-289.