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# Strain improvement of *Lactobacillus plantarum* for production of bacteriocin and their purification and characterization

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**Abstract**
The Five LAB was isolated and screened from 20 different sample sources. Among these two LAB found efficient producer of bacteriocin. Bacteriocin producing LAB was then screened on de Man Rogosa and Sharpe agar (MRS) medium. Morphological, biochemical, physiological characteristics and antimicrobial activity were preliminary examined. Antimicrobial activity of the 2 identified bacteriocin-producing LAB isolates were quantified by disc diffusion method against pathogens like *S. typhimurium*, *E.coli*, *Shigella flexneri*, *Staphylococcus aureus* and *Proteus vulgaris.* The efficient bacteriocin producer was identified as *Lactobacillus plantarum.* This was selected for the enhancement of activity by different factors like UV radiation, pH variation, temperature variation, Acridine orange and Ethidium bromide exposure. The decreasing order for the antimicrobial activity enhancer can be dictated as UV radiation, pH and temperature variation, Acridine orange and Ethidium bromide respectively. UV radiation found to be effective agents amongst others for enhancement the antimicrobial activity against various pathogens. Improved strain of *Lactobacillus plantarum* was then used for the production of bacteriocin and partial purification by salting out method.

**Keywords:** bacteriocin, LAB, antimicrobial activity, MRS medium

## Introduction
Lactic acid bacteria (LAB) play an important role in various food fermentations including meat, dairy, vegetable and fruit products. Antibacterial substances against food spoilage and food-borne pathogens such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl and bacteriocins can be produced from LAB. Bacteriocin is the proteinaceous antibacterial compounds and these are most efficient against bacteria closely related to the producer strain and/or Gram-positive bacteria. Bacteriocin producing strains are, therefore, practically used as food preservative cultures for ensuring the safety of fermented food product (Gorbach, 1960). Bacteriocin production could be considered as an advantage for food and feed producers since, in sufficient amounts, these peptides can kill or inhibit pathogenic bacteria that compete for the same ecological niche or nutrient pool. This role is supported by the fact that many bacteriocins have a narrow host range, and is likely to be most effective against related bacteria with nutritive demands for the same scarce resources (Deegan *et al*., 2006). Several types of bacteriocins from food-associated lactic acid bacteria have been identified and characterized. Because of the increasing demand for more natural and microbiologically safe food products, there is a need for bio preservation methods. Bacteriocins have considerable potential for food preservation, as well as for human therapy as potential supplements or replacements for currently used antibiotics. Therefore, in this project attempt of work was carried out antimicrobial properties of bacteriocin produced from *Lactobacillus plantarum*, isolated from curd which has been purified and characterized as well as mutation in the *Lactobacillus plantarum* for the enhancement of production of bacteriocin.

## Materials and Methods
**Collection of Sample:** A total of 20 samples, namely unpasteurized milk (5), curd (5), butter (5), and cheese (5) was collected from a local dairy farm in Aurangabad (M.S.), India, for the isolation of bacteriocin producing bacteria (*Lactobacillus* sp.).

**Isolation of Lactic acid bacteria:** The samples were diluted and plated onto de Man Rogosa Sharpe (MRS) agar plates and incubated at 37°C for 24-48 h. After incubation, typical colonies were isolated and purified. The isolates were differentiated on the basis of their morphological, cultural and physiological characteristics.

**Screening of LAB:** Screening of lactic acid bacteria from all isolates was done by observing the morphology of LAB on the MRS agar and grams staining and identification by biochemical tests.

**Production and extraction of bacteriocin:** Isolated strain was grown in MRS broth at 37°C for 24 hours for the production of bacteriocin. After incubation, the broth was centrifuged at 10000 rpm for 10 min and the cells were separated out. Supernatant was used as a crude bacteriocin. A sample of the culture of cell as sample of supernatant (after centrifugation) was made for assay of purification and bacteriocin activity (Joshi *et al.*, 2006).

**Bacteriocin assay/ Antibacterial activity of Bacteriocin:** The antibacterial activity of bacteriocin was determined by

using the disc diffusion method. The discs containing bacteriocin were placed in pathogen containing agar plates as previously seeded with the indicator bacteria. The plates were incubated at 37°C for 24 h. After incubation, the diameter of zone of growth inhibition was measured (Ivanova *et al.*, 2000).

**Results and Conclusion**

Bacteriocins of LAB are considered natural bio preservatives, as it is assumed that bacteriocins are degraded by the proteases of the gastrointestinal tract (Cintas 1995), and most of the LAB are considered as GRAS (Generally Recognized as Safe) microorganisms (Holzapfel *et al.* 1995).

20 samples were collected from dairy farms of Aurangabad (MS), India of different types i.e. 5 samples of cheese, 5 samples of curd, 5 samples of butter and 5 samples of unpasteurized milk. These samples were streaked on MRS agar. After incubation, number of colonies were observed onto the MRS agar. But from these 20 samples 9 Lactic acid bacteria were isolated and screened depending on the colony morphology and gram staining procedure.

These 5 LAB further screened by bacteriocin assay carried out to check the antibacterial activity. From these LABS the bacteriocin was produced by inoculatin it into the MRS broth followed by incubation of 72 hrs. After 3 days of incubation the bacteriocin was harvested by separating the cell debris from supernatant. This fractionated supernatant was then neutralized by 1N NaOH or 1N HCl followed by 0.2 u syringe filtration. This filtered bacteriocin was treated as a crude bacteriocin.

Crude bacteriocin was then used for the antibacterial assay against one pathogen as one of the screening method. When 9 different bacteriocin produced from 9 LAB was assayed against *S. typhimurium* it was observed that, Amongst these 9 isolates only two isolates can be selected for further study depending on the zone of inhibition that isolates named as 4b (18 mm zone), 3c (15 mm zone) (As per Table and Figure 1.)

**Table 1:** Screening of bacteriocin producing LAB from different samples

| S. No. | Samples | Isolate code | Bacteriocin assay (Zone of inhibition in mm) against *S. typhimurium* |
|---|---|---|---|
| 1 | Sample I (Curd) | 1a | 6 |
| 2 | Sample II (Milk) | 2a | 7 |
| 3 | Sample III (Butter) | 3a | 9 |
| 4 | Sample IV (Curd) | 4a | 12 |
| | | 4b | 18 |

The isolates were identified on the basis of their morphological and biochemical characters with the help of Bergye's Manual of Determinative Bacteriology. The table 2 shows that the isolate 1a was Gram positive rod, showing IMViC negative and able to ferment all sugars. On the basis of these characters it was probably identified as *Lactobacillus plantarum*. All these species were showing catalase, nitrate, oxidase and urease test negative.

The improvement of bacteriocin producing *Lactobacillus plantarum* has been carried out by mutagenesis and selection. The most employed technique has been by inducing mutations in parental strains using mutagens. Among physical mutagens, UV-radiation, pH and temperature variation have often used. To obtain hyper producer strains, frequently physical treatment could be combined with some chemical mutagens, e.g. Ethidium bromide and Acridine orange. To increase the production the microorganism was subjected to UV mutation. UV rays are important inducers of strain mutations. The pyrimidines (thymine and cytosine) are especially sensitive to modifications by UV rays absorption. This may result in the production of thymine dimers that distort the DNA helix and block future replications. In many cases, mutations by UV are harmful, but occasionally it may lead to a better adapted organism to its environment with improved bio catalytic performance. The potential of a microorganism to mutate is an important property conferred by DNA, since it creates new variations in the gene pool (Prabakaran, 2009). Likewise pH variation, temperature variation and chemical mutagens like Ethidium bromide and Acridine orange can be used to improve the strain quality in the sense of enhanced bacteriocin production.

*Lactobacillus plantarum* isolated from unpasturized milk was exposed to UV irradiation for 5, 10, 15, 20 min., Ethidium bromide and Acridine orange for interval of time 5, 10, 15 min and different pH and temperature exposure for 24 hrs. Then bacteriocin was produced from these mutated *L. plantarum* and bacteriocin assay was performed against *S. typhimurium.*

**Table 2:** Strain improvement of *L. plantarum* by UV mutation

| S. No. | Time of exposure (Mins.) | Zone of inhibition (mm) |
|---|---|---|
| 1 | 5 | 13 |
| 2 | 10 | 15 |
| 3 | 15 | 15 |
| 4 | 20 | 17 |
| 5 | 25 | 21 |
| 6 | 30 | 22 |

**Table 3:** Strain improvement of *L. plantarum* by temperature variation

| S. No. | Temperature | Zone of inhibition (mm) |
|---|---|---|
| 1 | $5^0$C | 15 |
| 2 | $25^0$C | 18 |
| 3 | $37^0$C | 20 |
| 4 | $45^0$C | 22 |
| 5 | $55^0$C | 23 |

**Table 4:** Strain improvement of *L. plantarum* by pH variation

| S. No. | pH | Zone of inhibition (mm) |
|---|---|---|
| 1 | 2 | 16 |
| 2 | 3 | 18 |
| 3 | 5 | 20 |
| 4 | 7 | 23 |
| 5 | 9 | 25 |

**Table 5:** Strain improvement of *L. plantarum* by Ethidium bromide and Acridine orange (0.5 m M)

| S. No. | Time of Exposure | Ethidium bromide | Acridine orange |
|---|---|---|---|
| | | Zone of inhibition (mm) | Zone of inhibition (mm) |
| 1 | 5 min | 13 | 12 |
| 2 | 10 min | 14 | 14 |
| 3 | 15 min | 16 | 15 |

**Table 6:** Comparison of mutation for strain improvement.

| S. No. | Type of mutation | Larger zone of inhibition (mm) |
|---|---|---|
| 1 | UV mutation | 30 |
| 2 | Temperature variation | 25 |
| 3 | pH variation | 22 |
| 4 | Acridine orange mutation | 15 |
| 5 | Ethidium bromide mutation | 14 |

The above table represents the effect of mutation by UV radiation, treatment of Ethdium bromide and Acridine orange and pH and temperature variation on the bacteriocin production by the isolated potential isolates. After production, purification and bacteriocin assay, the *Lactobacillus plantarum* was showing 32 mm of larger zone of inhibition for UV mutation after 30 mins of exposure, 28 mm after temperature variation at $37^0$C, 28 mm at pH 7.0 for pH variation, 17 mm and 16 mm after 15 mins. exposure of acridine orange and ethidium bromide respectively.

It indicates that the UV mutation has improved isolated strain of *Lactobacillus plantarum* to enhance the production of bacteriocin to higher level than others. pH and temperature variation also have effect to certain extent to increase the production of bacteriocin. But chemical mutation with Acridine orange and Ethdium bromide did not impact at high level for hyperproduction of bacteriocin from *Lactobacillus plantarum.* The bacteriocin antimicrobial activity was checked against other 4 pathogens i.e. *E.coli, Shigella flexneri, Staphlochoccus aureus* and *Proteus vaginalis.* Antimicrobial activity was higher for UV mutated isolates against all these pathogenic bacteria. Hence, UV mutation was considered as an effective mutation for strain improvement of bacteriocin producer.

## Conclusion

This *Lactobacillus plantarum* was used for the production of bateriocin after mutation with various agents like UV, Temperature, pH, Ethidium bromide, and acridine orange. Then treated *Lactobacillus plantarum* was used for the production of bacteriocin and then it was purified by salting out combined with dialysis. After bacteriocin assay, the UV was found to be the most effective agent as compared to others to improve the strain for enhancement of antimicrobial activity.

## References


1. Ammor S, Tauveron G, Dufour E, Chevallier I. Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility: 1-Screening and characterization of the antibacterial compounds. Food Control. 2006; 6 (17):454-461.
2. Anderssen Erlend L, Diep Dzung Bao, Nes Ingolf F, Vincent Eijsink GH, Jon Nissen-Meyer. Antagonistic Activity of *Lactobacillus plantarum* C11: Two New Two-Peptide Bacteriocins, Plantaricins EF and JK, and theInduction Factor Plantaricin A. Appl. Environ. Microbiol. 1998; 64(6):2269.
3. Aronsson B, Barany P, Nord CE. *Clostridium difficle* associated diarrhoea in uremic patients. Eur. J Clin. Microbiol. 1987; 6:353-356.
4. Axelsson L. Lactic acid bacteria: classification and physiology, In: *Lactic acid bacteria*, S. Salminen, A. von Wright, A. Ouwehand (Eds), 3rd edn, New York: Marcel Dekker, Inc. 2004, 1-66.
5. Broadbent JR, Chou C, Guillies K, Kondo JK. Nisin inhibits several gram-positive, mastitis-causing pathogens. J Dairy Sci. 1989; 72:3342- 3345.
6. Brown AC, Valiere A. Probiotics and medical nutrition therapy. Nutr Clin Care. 2004; 7(2):56-68.
7. Bruno MEC, Montville TJ. Common mechanistic action of bacteriocins from lactic-acid bacteria. Appl. Environ. Microbiol. 1993; 59:3003-3010.
8. Caplice E, Fitzgerald GF. Food fermentation: role of microorganisms in food production and preservation. Int. J Food Microbiol. 1999; 50:131-149.

9. Caridi A. Selection of *Escherichia coli* inhibiting strains of *Lactobacillus paracasei subsp.* paracasei. J Ind. Microbiol. Biotechnol. 2002; 29:303-308.
10. Castellano P, Raya R, Vignolo G. Mode of action of lactocin 705, a two-component bacteriocin from *Lactobacillus casei* CRL705. Int. J Food Microbiol. 2003; 85:35-43.
11. Catanzaro J, Green L. Microbial ecology and probiotics in human medicine (part II). Altern Med Rev. 1997; 2(4):296 305.