

Antibacterial Activity of Fused Zinc Oxide Nanoparticles with Bacteriocin Sub Class II B [Lactacin F] Against *Shigella Species* – A Synergistic Study

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ABSTRACT

Introduction: Antibacterial effect of zinc oxide nanoparticles (ZnO-NPs) has remarked a huge interest overall especially by the usage of nanotechnology to synthesis particles in the nanometer size. ROS has been a central point for a few systems including cell wall destruction because of ZnO confined communication, improved film porousness, the disguise of NPs because of loss of proton intention power and take-up of poisonous broke up zinc particles.

Objective: The antibacterial impact of zinc oxide (ZnO) nanoparticle and bacteriocin on *Shigella species*.

Methods: Preliminary tests have been done for the identification of *Lactobacillus acidophilus* strains producing bacteriocin. Chloroform–Methanol (2:1 v/v) was used for crude Bacteriocin extraction. However produced precipitate at Solvent-Aqueous interphase was collected aseptically, the solvent was evaporated and the precipitate was kept in a buffer which was used for the antimicrobial study. Sep-pack C18 cartridge and reverse-phase High-Performance Liquid Chromatography on a Nucleosil column were used for purification of lactation F. In this study Agar well diffusion and Broth dilution techniques were used to obtain antimicrobial activity of Lactation F. Synergistic antibacterial activity was done with 1: 1 combination of lactation f and ZnO nanoparticle against.

Results: Lactacin F was purified and the antimicrobial testing was done with and without zinc oxide nanoparticle, which shows that Lactation F with zinc oxide nanoparticle had expanded zone of restraint to *Shigella species* when contrasted with Lactacin F alone.

Conclusion: Invitro antibacterial activity of the synergistic compound was more effective than the crude extract of bacteriocin against *Shigella species*.

Key Words: Bacteriocin, Lactacin F, *Shigella species*, Synergy, Zinc oxide

INTRODUCTION

Bacteriocins have been grouped into three classes based on the different criteria such as producer organisms, molecular sizes, physical properties, chemical structures and mode of actions. In this present study, we isolated subclass II [b] bacteriocins.^{1,2} This subclass includes heterodimeric bacteriocins which consist of two peptides. Members of this subclass of the bacteriocins meet three criteria: (i) full antimicrobial activity needs both peptides and the individual peptides show little or no activity, (ii) one immunity protein is sufficient to get immunity, and (iii) the genetic organization of the bacte-

riocin system includes two sequential bacteriocin structural genes encoding the individual peptides and a single immunity gene. Lactococcin G is the first discovered bacteriocin of this group and its antimicrobial activity depends on both α - and β -peptides.³ Plantaricin and lactacin F are also other important representative examples for two-peptide bacteriocins. Their mechanism of action involves the dissipation of membrane potential and a decrease in the intracellular Adenosine Tri Phosphate concentration. Although the presence of both peptides is required to obtain full antimicrobial activity, the individual peptides can act as residual antimicrobials with a modest effect in some cases.

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Having a satisfactory measure of Zn in the body is essential for the protein and DNA combination, ideal working of insusceptible reaction like immune response and metalloenzyme work. Antibacterial effect of zinc oxide nanoparticles (ZnO-NPs) has gotten the huge interest of nanotechnology to integrate particles in the nanometer locale. Specific underline was given to bactericidal and bacteriostatic systems with centre around the age of receptive oxygen species (ROS) including hydrogen peroxide (H₂O₂), OH-(hydroxyl extremists), and O₂-2 (peroxide).⁴ ROS has been a main consideration for a few components including cell divider harm because of ZnO restricted connection, improved film penetrability, the disguise of NPs because of loss of proton intention power and take-up of poisonous disintegrated zinc particles. These have prompted mitochondria shortcoming, intracellular outpouring, and delivery in the quality articulation of oxidative pressure which caused possible cell development restraint and cell death.⁵

Thus, in synergy, zinc oxide nanoparticles and lactacin D were used against *Shigella* species in this study.

MATERIALS AND METHODS

This is the Cross-sectional study done for a period of one year from June 2018- May 2019 at Saveetha Medical College and Hospital, Thandalam.

Isolation and Identification of different probiotics strains⁶

Two grams of curd sample was transferred in a flask containing MRS Broth as enrichment media and added distilled water to 100 ml and incubated in 37°C. After 24 h, 100 µl of enriched samples were spread on MRS agar. Then, they have anaerobically incubated in [Candle jar] incubator at 37 degree Celsius which is the optimum temperature for lactobacillus species for 24 hours. After the period of incubation 8, isolated colonies were grown. In that, 1 colony shows 100% resemblance with *lactobacillus acidophilus*. For long term storage Figure 1 shows cultural characteristics of Lactobacillus species. Figure 2 shows Gram staining under the compound microscope with a magnification 100x. Preliminary identification tests like catalase, oxidase, indole and motility were performed. Through antagonist activity of *Lactobacillus acidophilus* against common pathogens, bacteriocin producers were selected and used for further studies.⁷ Chloroform – Methanol (2:1 v/v) was used for crude Bacteriocin extraction

Purification of crude bacteriocin by HPLC method⁷

Figure 3 shows the Ammonium sulfate precipitation method. Bacteriocin cleansing Method of Precipitation with Ammo-

onium sulfate, Sep-pack C18 cartridge and converse stage HPLC on a C18 Nucleosil section. A 24-h-old culture (200 ml) of *L. acidophilus* ST31 was centrifuged for 15 min at 20,000 x g, 4°C. The dynamic supernatant was treated for 10 min at 80°C to forestall bacteriocin proteolysis. Ammonium sulfate (Kimax) was delicately added to the cell supernatant (kept up at 4°C) to acquire 60% immersion (1, 6), and blended for 4h. After centrifugation (1h at 20,000 x g, 4°C), the pellet was resuspended in 25 mM ammonium acetic acid derivation (pH 6.5) and stacked on a Sep-Pack C18 cartridge (Waters Millipore, MA, USA). The cartridge was washed with 20% I-propanol in 25 mM ammonium acetic acid derivation (pH 6.5) and the bacteriocin was eluted with 40% I-propanol in 25 mM ammonium acetic acid derivation (pH 6.5).

In the wake of drying under diminished tension (Speed-Vac; Savant, France), the divisions were halfway disintegrated in 0.1% trifluoroacetic corrosive (TFA) and tried for antimicrobial action. This dynamic portion was additionally cleaned by invert stage HPLC on a C18 Nucleosil segment (250 x 4.6 mm). Elution was performed by applying a straight slope from 0.1% TFA (dissolvable A) to 90% acetonitrile in 0.1% TFA (dissolvable B) in 65 min. Polypeptides, recognized by A220, were gathered physically. After drying under diminished tension and resuspension in 1 ml of de-ionized water, the watery polypeptide arrangements were put away at -20°C.

Isolation and Identification of Shigella species

The in-house strain of *Shigella dysenteriae* and *Shigella flexneri* were used for antimicrobial testing of Lactacin F with zinc oxide. To check the synergistic activity of Zinc oxide nanoparticles with lactacin F agar cup diffusion method was adapted.⁸

Agar cup Diffusion Method

Lactacin F and Zinc oxide nanoparticles of 20- 25nm size were added in 1:1 proportion of microgram per millilitre and tested against lawn culture of *Shigella* species by making wells in the medium.⁹

RESULTS AND DISCUSSION

In this study out of 25 isolates of Lactobacillus species, 18 were *Lactobacillus acidophilus*. All these strains had antagonistic activity against *Escherichia coli* ATCC 25922. American Type Culture Collection 10mL of Bacteriocin were extracted and purified from these isolates. Lactacin F was purified and the antimicrobial testing was done with and without zinc oxide nanoparticle, which shows that Lactacin F with zinc oxide nanoparticle had increased (>20mm) zone of inhibition to *Shigella* species when compared to

Lactacin F alone and the positive control with Gentamicin Disc.¹⁰ Figure 5 shows the agar cup diffusion testing of the synergy compounds.

Different approaches can be employed which includes herbal-based,¹¹ in silico based,¹² nanomaterial-based,¹³ and combination therapy.¹⁴ These approaches are tried recently against *A. baumannii* also and some of them shown promising results. ZnO NPs have also shown antimicrobial activity on skin-specific bacteria¹⁵ like *Streptococcus mutans*, *Streptococcus pyogenes*. This study showed effective antibacterial efficacy of zinc oxide even in presence of Lactacin F against *Shigella* species.

The activity of lactocidin against *Salmonella enteritidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* led to speculate that *L. acidophilus* producer strains were essential in controlling undesirable flora in the intestinal tract. However, antibacterial activity at pH 7.0 was lost with further purification as described by Vincent in his study.¹⁵ Reports also says that Bacteriocin produced by *L. acidophilus* has a broad-spectrum antagonistic property.¹⁶

CONCLUSION

Thus, along with probiotics, nanoparticles can be incorporated and it can play a key role in easy absorption and highly efficient elimination of pathogens in the intestine. Instead of antibiotics, Lactacin F combined with zinc oxide nanoparticles can be effective against enteric pathogens. In future, it helps to reduce the intake of antibiotics and creating community-acquired drug resistance in microbes. This combination may even help in the skin and a soft tissue infection which needs future research work.

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Conflict of Interest: None declared

Ethical Approval: The study was approved by the Institutional Ethics Committee, No. SU216/2018

AUTHORS CONTRIBUTION

Kavyasree and Malini Evangeline rose made substantial contributions to conception, acquisition of data, took part in drafting the article or revising it critically for important intellectual content, Monica, Nithya, Shelina namperipakkam

and Muthumariyammal made HPLC analysis [Graphical data] and final approval of the version to be published and agreed to be accountable for all aspects of the work

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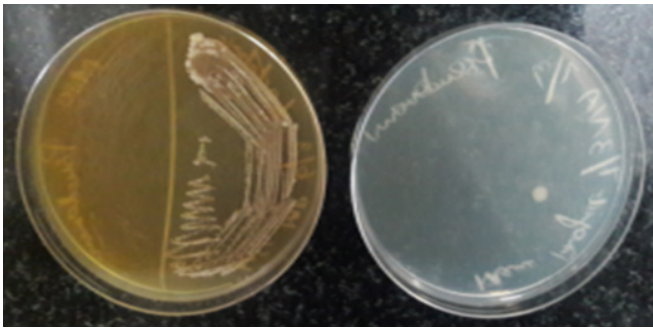


Figure 1: Cultural characteristics of *Lactobacillus acidophilus* in MS agar.

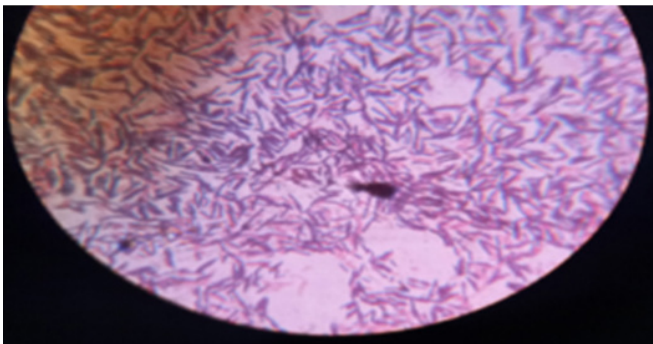


Figure 2: Gram staining appearance of *Lactobacillus acidophilus*- Gram positive bacilli.

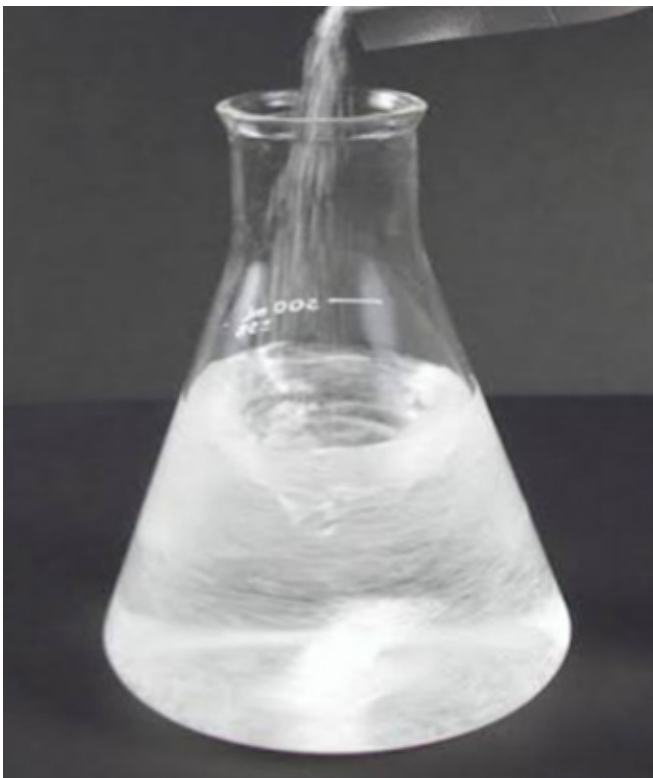


Figure 3: Ammonium sulfate precipitation for purification of Bacteriocin.

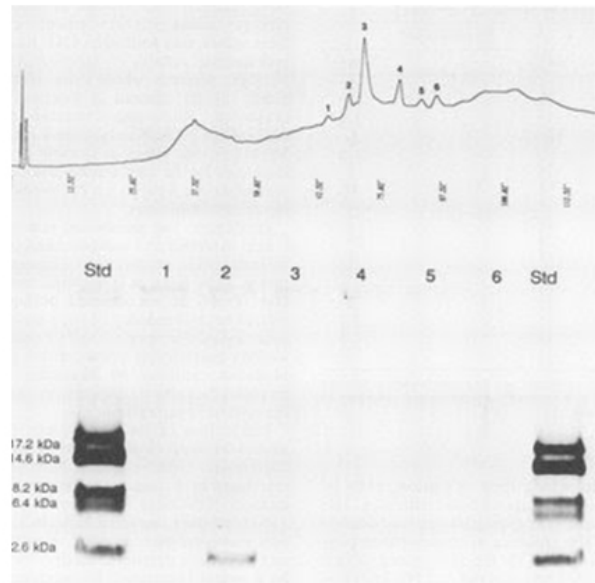


Figure 4: HPLC results showing the retention factor of Lactacin F.

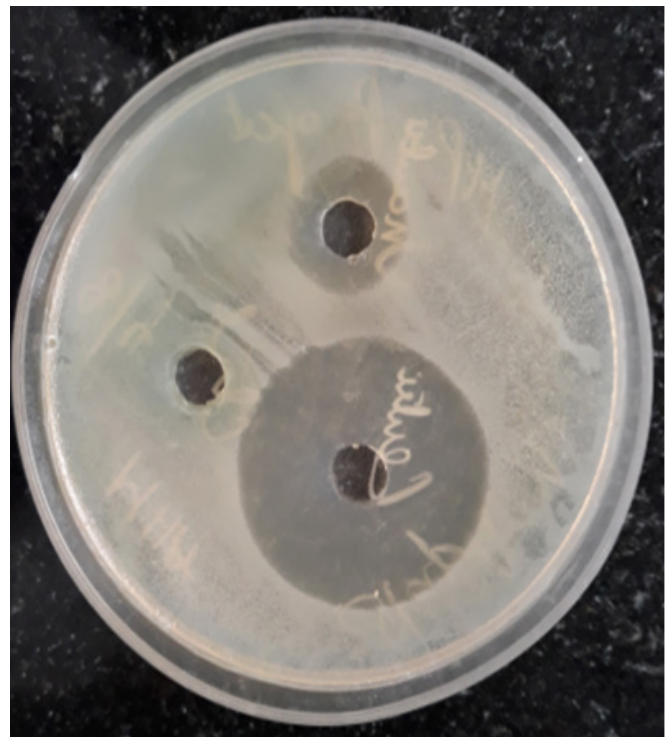


Figure 5: Antibacterial effect of Lactacin F in synergy with zinc oxide nanoparticles against *Shigella dysenteriae*.