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**Research Article**


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# Molecular Characterization of Mesenterocin as a Novel Drug against Bacterial Uropathogens of Male Infertility

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## ABSTRACT

Microbial infections have been associated with male infertility for many years. These infections may affect fertility in several ways by damaging sperm, hampering thier motility, altering the chemical composition of the seminal fluid or by producing an inflammatory structure in the tract. Bacteriocin from *leuconostoc mesenteroides* was extracted by APS and partially purified by Dialysis and HPLC. The anti bacterial activity of partially purified mesenterocin was analysed against the semenal isolate using agar well diffusion assay. The peptide sequencing of the bacteriocin was done using MALDI-TOF which revealed the exact nature of the bacteriocin which can pave way for the mesenterocin to be used as a novel drug molecule..

**Key words:** Bacteriocin, Mesenterocin, *Leuconostoc mesenteroides*, MALDI-TOF, HPLC, Antimicrobial activity, Uropathogen, Male Infertility

## INTRODUCTION

Presence of pathogenic micro organisms in semen, which may be related to a breach in the integrity of the blood-tests barrier, may provide early warning signals of impairment of male fertility (Friday *et al*., 2005). Uropathogens are Multi Drug Resistants (Meena *et al*., 2005). It was reported that detection of bacteria in semen does not necessarily suggest infection since bacterial isolates in seminal fluid may represent contamination, colonization of the urethral orifice or infection.

The presence of uropathogenic micro oraganisms in sperm might result in Bactreiospermia and also it may act as factor to lead out several 'spermia' such as Normozoospermia, oligozoospermia, asthenzoospermia, teratospermia, oligosthenozoospermia,azoospermia,aspermia,nechrospermia,and golobosperia.To over come this a novel drug of choice, the bacteriocin from *Leuconostoc mesenteroides* was obtained. ( Mogra *et al*., 1981)

Bacteriocins are proteinaceous antibacterial compounds that exibit bactericidal activity against species closley related to the producer strain (De vuyst and Vandamme, 1994). Depending on the producer organism and classification criteria, bacteriocins can be classified into several groups (Mc Auliffe *et al*., 2001) in which classes I and II are the most thoroughly studied. Class I, termed lantibiotics, constitute a group of small peptides that are characterized by their content of several unusual amino acids. (Ennahar *et al*., 2006) The class II bacteriocins are small, non modified, heat stable peptides (Nes and Holo., 2000).

The class III bacteriocins are large heat liable peptides and the fourth class of bacteriocins are composed of an undefined mixture of protiens, lipids and carbohydrates. The existance of the fourth class was supported mainly by the observation that some bacteriocin activities obtained in cell free supernatant.(Jorge and Klaenhammer, 2003). These bacteriocin substances are produced by many species and among them are the lactic acid bacteria (LAB). Bacteriocin named mesenterocin is produced by *Leuconostoc mesenteroides*. These bacteriocins also have antibacterial activity, antiviral activity and antitumor activity to that of some bacteriocins that have been discovered.

## MATERIALS AND METHODS

### Isolation and Identification of Bacterial Pathogens

The semen samples were allowed to liquefy for 2 hours in room temperature. The samples were directly swab streaked to the nutrient agar plates and were incubated for 24 hours. After incubation the cultures were identified by performing biochemical tests and confirmed by performing 16S rRNA sequencing.

### Antimicrobial Suscepility Test

To analyze the antibiogram of the isolates, antimicrobial susceptibility test was done by Kirbey Bauer disc diffusion method using 26 antibiotics.

#### Isolation and Identification of Leuconostoc Mesenteroides

The surface of chicken sausage were swabbed and plated on MRS agar plates. The plates were incubated for 16 hours at 30°C. After incubation the cultures were identified by performing biochemical tests and confirmed by performing 16S rRNA sequencing.

### Detection of Bacteriocin

The confirmed culture of *Leuconostoc mesenteroides* are subcultured and used to determine its capacity to produce Bacteriocin with help of stab overlay assay.

### Bacteriocin Extraction and Partial Purification

Bacteriocin from *leuconostoc mesenteroides* was extracted by Ammonium Sulphate Precipitaion and partially purified by Dialysis and HPLC.

### Molecular Weight Determination

The dialyzed bacteriocin and medium molecular mass standard were subjected to sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

## Peptide Mass Finger Printing of Bacteriocin
## Amino Acid Sequence Determination

For the amino acid sequence determination, the protien band was excised from SDS-PAGE was sent to NxGenBio, New Delhi, for MALDI- TOF (Matrix Assisted Laser Desorption Ionization- Time of Flight) assay.

## Sequence Analysis by Mascot Search Tool

Homology searches were performed against the swiss port, NR and databases with the basic local alignment search tool (BLAST) provided by the NCBI server (http:www.ncbi.nim.nih.gov/BLAST). Peptide mass, amino acid composition, isoelectric point and hydropathicity were predicted by the Expert Protien Analysis System (ExPASy) proteomics server of the swiss institute of Bioinformatics (http; //www.expasy.chl) (Jorge *et al*.,2003)

**Application of partially purified Bacteriocin against the Bacterial pathogen of male Infertility agar well Diffusion Method**

To pre poured sterile Mueller Hinton agar plated the isolated predominant bacterial uropathogen was swabbed, and wells were made using sterile well punture, 50µl of partially purified bacteriocin preparation was added, and the plates were incubated at 37°C for 24hrs**.** The zone of inhibition formed by the isolates was measured.

# RESULT AND DISCUSSION

## Isolation and Identification of Bacterial Pathogens

The results showed the pathogen to be *Escherichia coli* in the agar and the results were identified by biochemicals were confirmed to be the same through 16s rRNA direct sequencing.

## Antimicrobial Susceptibility Test

The zone of inhibition was measured and upon comparison with standard CLSI charts, reported as sensitive, moderately sensitive and resistant to the antibiotics

## Isolation and Identification of Leuconostoc Mesenteroides

The results was identified to be *Leuconostoc mesenteroides* and confirmed the same through 16s rRNA direct sequencing.

## Detection of Bacteriocin

On inspecting the stab-overlay assay plate after incubation, it showed clear zone of inhibition of the indicator strain.

## Bacteriocin Extraction and Partial Purification

Bacteriocin extraction was done at 80% saturation of ammonium sulphate. The bacteriocin extracted in the supernatant after centrifugation was partially purified by dialysis. The crude bacteriocin preparation obtained after ammonium sulphate precipitation and dialysis were subjected to HPLC analysis. The results revealed main peak with retention time of 2.932.

## Molecular Weight Determination by Sds-Page

The molecular weight of dialyzed bacteriocin was analyzed by sodium dodesylsulphate polyacrylamide gel electreophoresis (SDS-PAGE) with 12% polyacrylamide gel. As shown in the stained bands of gel band had an apparent molecular mass of about 3.8 k Da, as estimated by calculating the relative migration values of standard proteins.

## Peptide Mass Finger Printing of Bacteriocin [MALDI_TOF]

On MALDI-TOF analysis the bacteriocin, was found to have 19 peak fractions and about 10 peaks were taken into consideration. On Mascot analysis, the total amino acid sequence obtained was 152.

**Sequence Analysis by Mascot Search Tool:** Sequence analysis was done by BLAST tool and ExPASy.

Application of Partially Purified Bacteriocin as A Drug Molecule Against Uropathogens of Male Infertility

The inhibitory spectrum of bacteriocin was determined by agar well diffusion method. Inhibitory activity was observed against the isolate, where high level of activity was observed against the isolate**.**

**Phylogenitic tree of *E .coli***

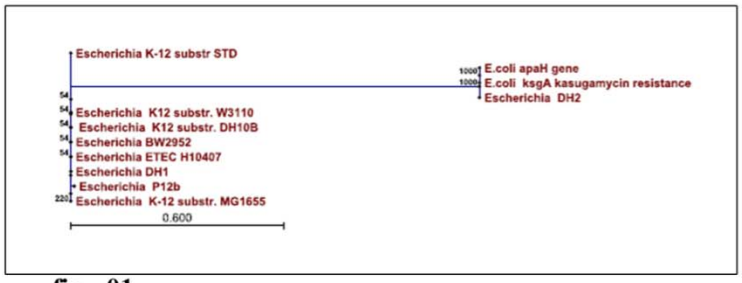


**fig - 01**

**Antibiogram of the seminal isolate ( *E.coli*)**

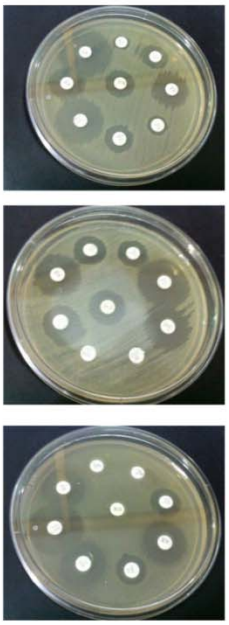

**fig - 02**

**Phylogenitic tree of *Leuconostoc mesenteroides***

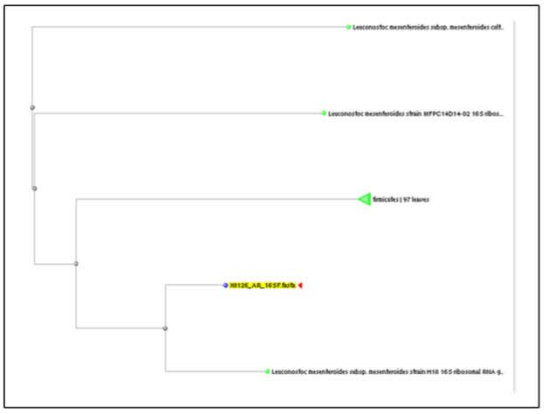

**fig - 03**

**Stab overlay assay**

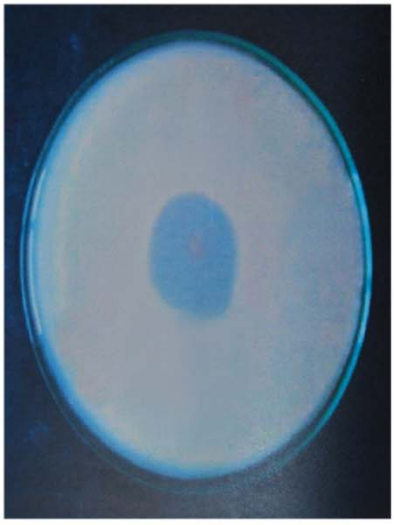

**fig - 04**

**HPLC**

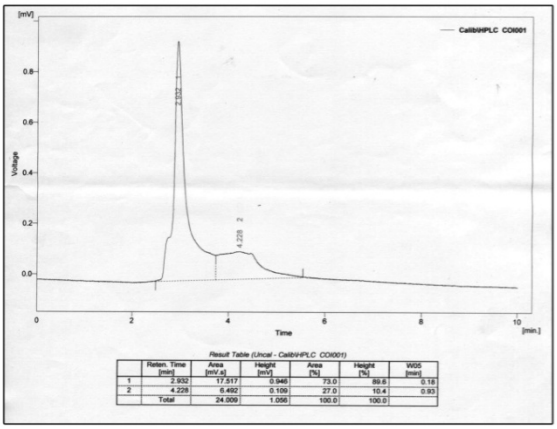


**fig - 05**

**MALDI-TOF**

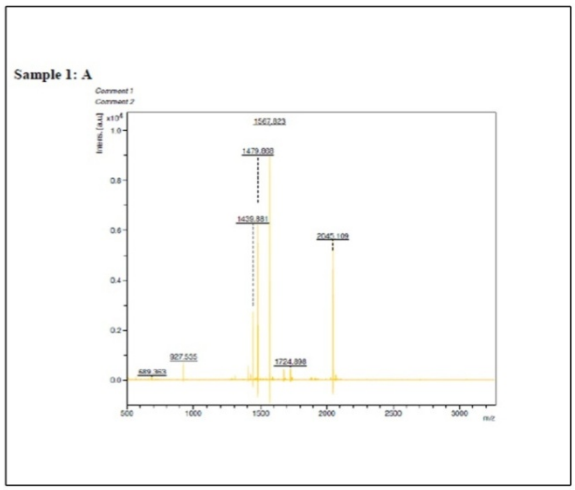


**fig - 06**

**INSILICO ANALYSIS OF THE BACTERIOCIN AMINO ACID SEQUENCE**
**PHYSIOlOGICAL DATA OF AMINO ACID SEQUENCE**

| S.NO | Charecteristics | Results |
|---|---|---|
| 1 | Mass | 16561.4 |
| 2 | PI | 4.93 |
| 3 | Net charge | -5 |
| 4 | Basic residues | 23 |
| 5 | Acidic residues | 28 |
| 6 | Hydrophobic residues | 53 |
| 7 | Polar residues | 36 |
| 8 | Aliphatic residues | 28 |
| 9 | Tiny residues | 43 |
| 10 | Boman Index | -393.58 |
| 11 | Hydropathy Index | -0.565 |
| 12 | Aliphatic Index | 76.45 |

**fig - 07**

**Antimicrobial activity of partially purified Bcateriocin**

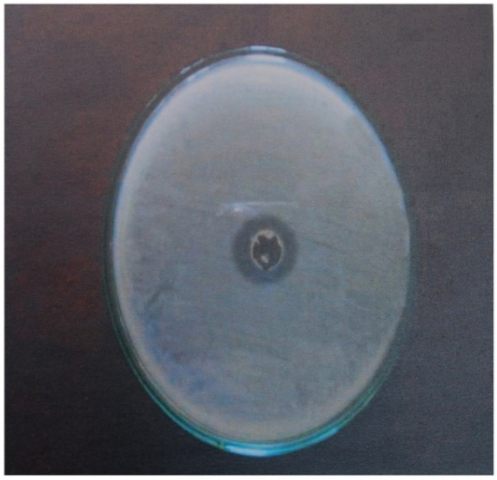

**fig - 08**

## CONCLUSION

Male urogenital tract infection plays an important role in male infertility. Most of the microbial infections in uropathogen includes several bacteria and fungi like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherchia coli, Klebsiella pnuemonia, Mycoplasma hominis, Candida albicans, Candida tropicalis, Cryptococcus neoformans, Saccaromyces cereviceae etc.*,In this study out of four semen samples one sample had the bacterial isolate. It was found to be *Escherchia coli.* It is found that micro organisms in uropathogen were highly resistant to antibiotics, which is also reflected in the study. High level of resistance was seen in the isolate against the 12 antibiotics used . All these factors insist the need of new drug molecule which will have a wide activity spectrum and that are capable of inhibiting MDRO.

The search ended on bacteriocin, which are protien complex antibiotics produced by a wide range of bacterial species. By conventional definitions, bacteriocins differ from most other antibiotics in that, the producer strain is immune to the action of its own bacteriocin. Bacteriocins demonstrate enormous possibilities in treating and containing target bacteria and may be a part of novel approaches for replacing classiscal antibiotics at a time when many pathogens are no longer susceptible to the existing antibiotics.The ability to develop novel bacteriocins based drugs aimed at potential target cells, prokaryotic as well as eukaryotic, may open new possibilities for the design of improved antibiotics possesing refined characteristics.

In this study the organism of choice for bacteriocin production was *Leuconostoc mesenteroides,* which is lactic acid bacteria and is highly useful in food industry and it produce various bacteriocins, lantibiotics and mesenterocines. There are several indicator strains that remain sensitive to bacteriocins. The ability of microorganisms to produce bacteriocin can be screened by stab overlay assay. On stab overlay assay it was clearly found that *Leuconostoc mesenteroides* has the potential to produce bacteriocin. Partial purification of the bacteriocin was done by salt precipitaion method and solvent extraction method.

Mass spectroscopy analysis of the bacteriocin was done by MALDI-TOF; the results obtained were analyzed with BACTIBASE database where, several properties like similarity serach, Physiological properties, molecular mass etc were characterized. It also showed that the bacteriocin extracted belongs to ClassII group bacteriocin.

Thus the characterized and partially purified bacteriocin was checked for its antibacterial activity against the Uropathogenic isolate *Escherchia coli.* It remains hopeful as a drug molecule as it is active against the organism. These factors suggest the bacteriocin from *Leuconostoc mesenteroides* can be used as a new drug of choice against male infertility caused by uropathogens.

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