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Characteristics Salivaricins producing *Streptococcus salivarius* isolated from gingiva

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***Abstract*---**The bacteriocins study (Salivaricins) produced by *Streptococcus salivarius*. S. salivarius strains were isolated from healthy gingiva , The bacteriocin production under anaerobic conditions at 37°C for 24 hrs and inoculum (1.5×10^9 CFU/ml) and Salivaricins was purified by precipitation ammonium sulphate, (75)% chromatofocusing by using column Sephadex G-50 the antibacterial activity against streptococci oral and other bacterial oral species was detection positive and ngative strain showed a different activity. The molecular weight of Salivaricins to be 8 kDa. The cells viability was decreased with increasing the concentration of Bacteriocin and the IC50 values of the HdFn cells (2.5 µg/mL).In conclusion, *S. salivarius* bacteriocin could be a good antibacteral agent and acivity against other types of species isolation gingiva.

***Keywords*---**Salivaricins, *Streptococcus salivarius,* gingiva, HdFn cells

Introduction

Periodontal disease such Gingivitis is a resulting from microbial infection. Conventional periodontal therapy consists of oral hygiene instructions, scaling and root planning. favourably to conventional therapy and adjunctive therapeutic agents may be necessary.Antimicrobial and non-steroidal anti-inflammatory drugs have been studied as an adjunctive to mechanical plaque biofilm control. (1)

S.salivarius, a species predominant in the oral cavity and major producers of bacteriocin-which are active against other bacteria , reducing the number of colonization pathogens(2).

Bacteriocins, those derived from Gramnegative bacterial have a narrow inhibitory spectrum of activity against other species such mutans streptococci to produce like substances bacteriocin- a wide spectrum of antibacterial activity (3) LAB bacteriocins are inherently stress high thermal and their activity at wide pH range.(4) In the environment fragments bacteriocin not long in the human body , *Streptococcus salivarius* against medical pathogens including MDR isolated oral cavity . (5).

Materials and Methods
Samples of collection

100 samples collected from 100 patient gingiva . who had refrained from eating, drinking and oral hygiene for 2 hours before collection in AL Yarmouk hospital, Baghdad, in strile transport media then transport in cool box to the laboratory.
A/ Isolation procedures:
A stained with gram stain by doing smears and to bacteria isolated (6)

B/ used culture:
Spacemen was incubated into both (Blood and Mitis Salivarius); incubated under anaerobic conditions. differentiate between strain by Biochemical reactions (7)

Bacterial Isolates

Samples were streaked on selective Mitis Salivarius Agar to obtain isolated colonies of oral streptococci species.Samples were grown under anaerobic conditions (jar under co_2 at 37˚C for 24 hrs). Colonies demonstrating dissimilar colonial morphology with gram positive cocci arranged as chains were chosen in the present work and MRS medium Confirmation identification optochin sensitivity and by Vitech 2 system.(8)

Bacteriocin producer: The producer isolates *S.salivarius* were isolated from gingiva patients and identifien by biochemical test and confemation by vitic 2 system (9). and Protein estimation according to (10)

Dialysis

Dialysis membrane (SIGMA) of molecular weight cut off (MWCO) 1000 Da of suitable length was boiled in 2% (w/v) sodium bicarbonate (NaHCO3) and 0.05% EDTA (Ethyle diamine tetra acetic acid) solution for 5 min., cooled and again boiled for 10 minutes in sterile distilled water. Dialysis was carried at 4˚C over night (11) and activity assay against test microbial by AWD method. and Determination of the molecular weight of bacteriocin according to Schagger and Jagow (1987) at separating gel (15%)

Optimization of bacteriocin :

1- Determination of pH for the optimum

The different pH effect on production of bacteriocin rang of pH as (4, 5 , 6, 7,8) after change the culture of pH and the concentration protein measured.

2 -Determination of inoculum size

Producer by using different inoculum (3 × 10^8 CFU/ml), 2(6 × 10^8 CFU/ml), and 3(9 × 10^8 CFU/ml) standard for 24 hr. at 37° C.

3-Determination of the optimum incubation period the incubation period was testing at incubation time different between (24, 48 and 72) hr. at strain (1.5 × 10^9 CFU/ml).

4- Determination of the optimum temperature Study the effect of incubation temperature (25, 30, and 37) °C

Results and Discussion

Isolation of Bacterial Isolates

In gram positive the type of bacteria isolated belong to, Table (1) the isolated were the aerobic bacteria eight strains according *Streptococcus* spp ,

Table 1: Number and type of bacteria isolated from teeth caries

Type of microorganism (Grams positive)	Number of microorganism (100%)
Streptococcus mutans	37
Streptococcus salivarius	33
Streptococcus. Sanguis	28
Streptococcus mitis	25

All samples collected on Mitis Salivarius Agar were streaked a selective medium used to isolate oral streptococci and differential because each species of streptococcus has a distinguished colony morphology. The medium contains selective that bactericidal Gve+ bacilli except streptococci and enterococci. *S. salivarius* metabolizes and colonies develops with a “gum-drop” appearance. *S. mitis* and enterococci. Colony morphology of Streptococcus species obtained on Mitis salivarius agar, as seen under a microscope (Figure 1) and blood agar .

colonies Single on the surface of MS-agar by subculturing and purification on blood agar .Gve + included cocci which are (spherical or ovoid) chains under light microscope

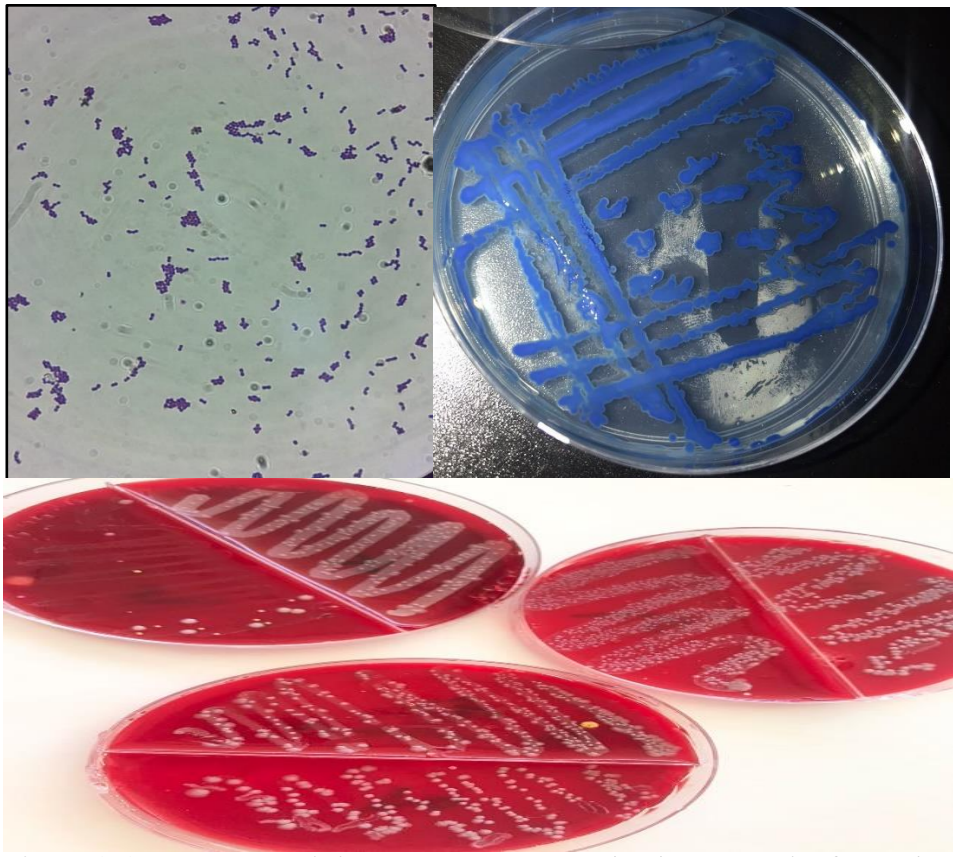


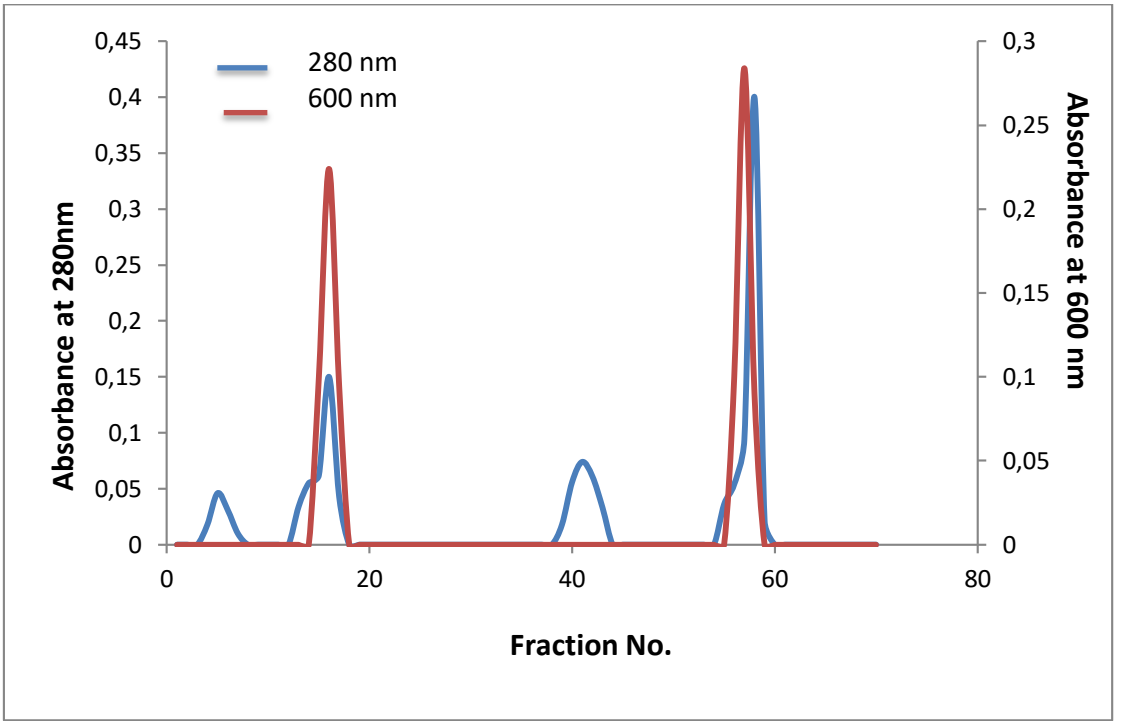
Figure (1) A: Gram staining – *S. mutans*, B: Blood agar at 37c for 24 hrs

Streptococcus mutans was the highest counts was obtained from carries samples followed by complete denture group .

Ammonium sulfate precipitation and desalting by dialysis

Salivaricins was by ammonium sulphate at 75% the activity compare crude dimeter inhibition zone about (5)mm.they result agree (12)(13) .

Purification of bacteriocins the final to next step chromatography gel filtration the (Sephadex G-50) column.stage for applection Salivaricins after precipitation and dialysis,. The curve between was plotted the absorbance fraction numbers which gave one protein peaks. was (14, 15, and 17) ,and Figure (2).

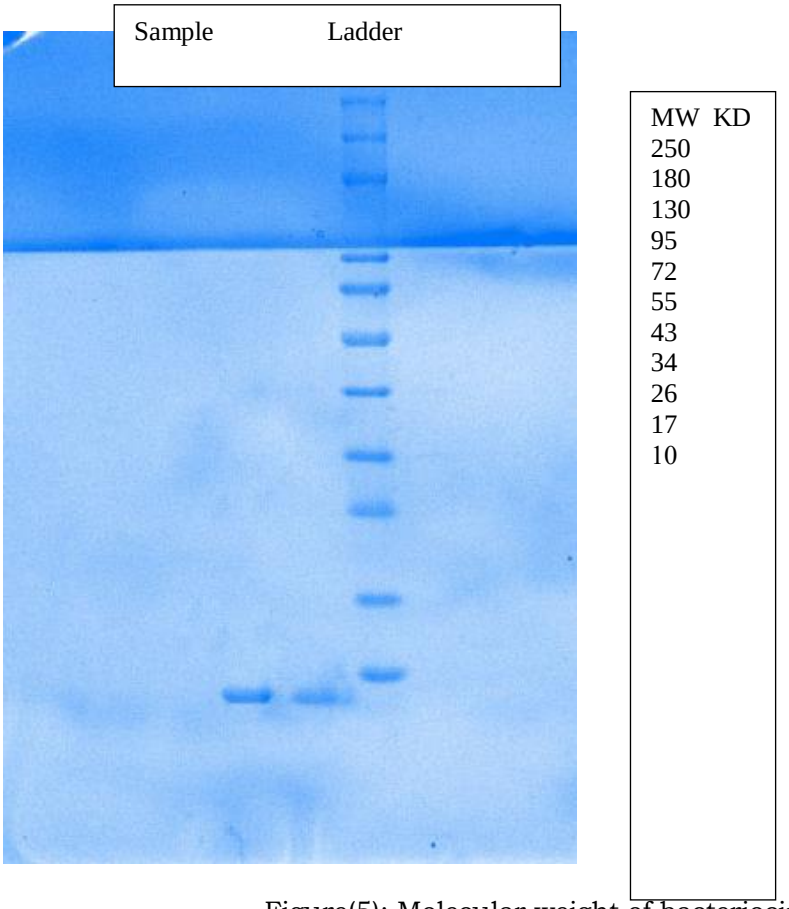


Figure(2): Purification of bacterocin by Sephadex G-50 column (red)

The result agree with (14) and(15) the isolated bacteria tonsillitis were streptococcus species which included *Streptococcus pyogenes* and *Enterococcus faecium*, while *mutancin* produces bacteriocins that show antibacterial activity against widely range bacteria (16) The result disagree with showen **(17)** bacteriocin (Pyocin) crud from was heated to denaturant any proteases and heat-sensitive proteins was not wide spectrum activity against gram negative bacteria **.**

Molecular weight:

The purified sample collected from different steps of purification were analyzed by Tricine-SDSPAGE. A single target band appeared (Figure, indicating that the resultant bacteriocin is a single active peptide in nature. After calculating the gel length and the migration distance of the sample compared with Protein standard (Broad range 10- 250 kDa), the molecular weight of was about 8 kDa .



Figure(5): Molecular weight of bacteriocins

The peptide *S.gallolyticus*. was range (6kDa), an oligonucleotide the peptide purified , thas characteristics antibacterial peptide that class II bacteriocins of gram-positive bacteria (18) It is another result that the molecular weight of enterocin is 7.9 kDa. produced by *E. faecalis* RJ-11 (5 kDa) (19) (20) while bacteriocin produced by *E. faecium* with highly bacteriocin ability was isolated from different source . (21)

- **Cytotoxicity Test of bacteriocin Using Cancer Cell Lines**:

The MTT assay of cytotoxicity analysis of cells after treating to be 45% at MIC (2.5 µg/mL) of bacteriocin. preperation dilution is shown in Figure 3. The viability of cells was dependent on the bacteriocin at concentration.the inhibitory percentage against the cancer (HdFn) compare normal (MG63) cells.

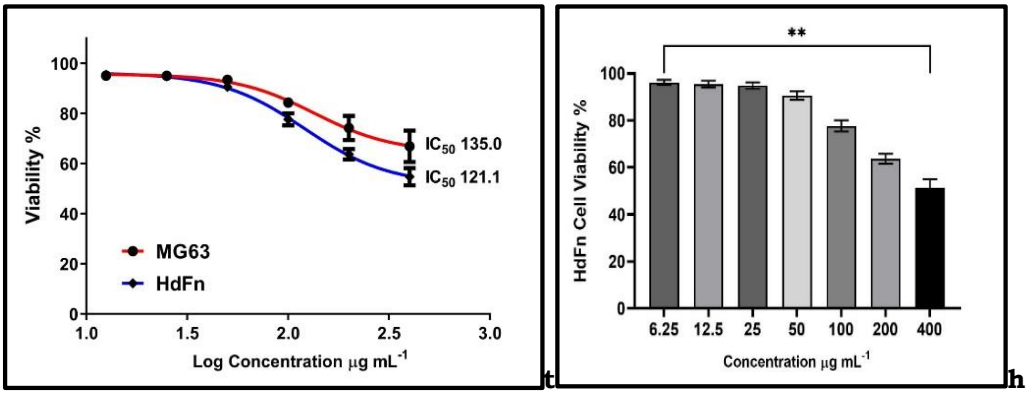


Fig. 6. Effect of the viability by bacteriocin on HdFn Cells evaluated by MTT assay.

The other result cytotoxic study of CFS bacteriocin conducted showed decrease viability at the MIC with untreated cells. The agree with bacterocin of *B. subtilis* reach 6% cytotoxicity (22). Bacteriocins have been cytotocity mammalian cell line and can affect the binding of the peptide(23).

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