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Screening of Marcescin Producing Isolates from *Serratia marcescens*

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Abstract

The current study intends to investigate the production of bacteriocin from S. marcescens, a Gram-negative bacterium, it is a human pathogen and a main reason for contamination of medical equipment at hospitals. About 220 various samples were collected from patients which include blood, urine, abscess and sputum; Twenty -three isolates of S. marcescens were performed as per standard laboratory procedure and confirmed using VITEK-2 systems. The isolates of S. marcescens which produced marcescin detected by four methods : Spot –on-the – lawn method , Cup-assay method ,Well-diffusion method and Agar cross streaking assay , the production of bacteriocin was produced by adding mitomycin-c to the bacterial broth to increase the amount of bacteriocin. The most effective technique for this aim was the cup assay that gave the best and highest result of producing marcescin 86.95% and the second best method was well diffusion method that gave 82.60% producing marcescin.

Keyword: *Serratia marcescens* **,** marcescin , Cup-assay method , bacteriocin.

الكشف عن العزلات البكتيرية المنتجة للمارسيسين من بكتيريا Serratia marcescens

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الخلاصة

تهدف الدراسة الحالية إلى الكشف عن إنتاج البكتريوسين من بكتيريا S. marcescens ، وهي بكتيريا سالبة لصبغة جرام ، حيث تعتبر هذه البكتيريا أحد المسببات المرضية و ايضا احد اسباب تلوث المعدات الطبية في المستشفيات. تم جمع 220 عينة سرسرية مختلفة شملت الدم والبول والخراج والبلغم ، وتم إجراء ثلاثة وعشرين عزلة من S.marcescens وفقًا للإجراء ات المختبرية المعمول بها وتأكيد النتائج باستخدام نظام VITEK-2. تم الكشف عن عزلات S.marcescens التي أنتجت marcescin بأربع طرق : spot- on- the lawn ، cup assay ,well diffusion method , Agar cross streaking ، تم إنتاج بكتريوسين عن طريق إضافة ميتوميسين- c إلى المرق البكتيري لزيادة كمية البكتريوسين. وكانت افضل طريقة هي cup assay حيث كانت نسبة انتاج البكتريوسين 86.95%هي وثاني افضل طريقة هي well diffusion method حيث كانت نسبة انتاج البكتريوسين هي 82.60%.

الكلمات المفتاحية : بكتيريا Serratia marcescens، مارسيسين، طريقة Cup-assay، البكتريوسين.

Introduction

Serratia marcescens is a rod-shaped, mobile, psychrophilic, and facultatively anaerobic Gram-negative bacteria belonging to the Enterobacteriaceae family.[1] It can be inhibiting a wide range of environmental niches, including water, soil, plants, insects, and animals, some of which are linked to food degradation.[2]It can grow in a wide range of temperatures, from 5 to 40 °C. It differs from other Gram-negative bacteria in that it can hydrolyze casein, which enables it to create metalloproteinases that are thought to facilitate interactions between cells and the extracellular matrix. [3] *S. marcescens* is frequently the causative agent for "hospital acquired infections" in both patients and medical personnel. *S. marcescens* may grow quickly on medical devices and facilities, such as showers, toilets, and other items found in hospitals, and is thought to be the source of around 2% of all known instances of hospital acquired infection.[4] [5] A strain of *S. marcescens* was found to produce a marcescinthat inhibits the growth of certain *Escherichia coli* strains . Bacteriocin is a kind of antimicrobial peptide generated by bacteria that is ribosomally synthesized and has the ability to kill or inhibit bacterial strains that are related to or unrelated to the producing bacterium.A range of gram-positive and gram-negative bacterial strains generate bacteriocin, a different-molecular-weight protein that frequently remains linked to the cell surface.[6]Bacreiocins are proteins that have been divided into several groups based on their biological characteristics, including their

activity range, diffusibility, and susceptibility to proteolytic enzymes. [7] The expression of this bacteriocin was negligible unless induced with mitomycin C [8]

Aim : This study aimed to investigate the best method that used for screening marcescin-producing isolates.

Materials and Methods

Collection and Identification of bacteria

The (220) clinical samples were gathered from several Baghdad´s hospitals; which including Al-Kindy Hospital, Imam Ali Hospital, and Private Nursing Hospital. The collection was performed during the period of Feb-2022 to June-2022. It is worth mentioning that the specimens include: blood, urine, and sputum and Abscess. The samples cultured on blood agar as part of the hospitals` routine clinical laboratory protocol identification. Using methods including microscopic examination, culture characteristics, and biochemical tests, the bacterial samples are identified according to the Bergey´ s Manual of Determinative Bacteriology's approved features. [9]

Bacterial isolates and media

The selected isolates of *S. marcescens* and *E. coli* have already been grown in 5 ml of Brain Heart Infusion Broth(BHIB) for activating the bacteria .After picked 4-5 colonies from original culture and measurement of turbidity which set to McFarland 0.5 standard suspension (1.5×10^8), then added 2 µg/ml mitomycin C for *Serrstia* broth with 10 % glycerol after 14 h incubation in shaking incubator at temperature 37 ºC.

Screening for bacteriocin-producing isolates

- **A Spot –on- the- lawn method:**

In a sterile 5 ml culture tube with ventilation cover containing BHIB broth, prepare of the desired strain of *S. marcescens*, and incubate it statically at 37 °C for 18 hours.

Diluted the indicator (sensitive) strain, and obtained a concentration of approximately MaCfarland (0.5) when the top layer of soft-agar has solidified, pour onto the surface of a plate and leave at room temperature. Prepare several inoculums of delicate bacteria and spread out on the agar, letting dry at room temperature. Drop 20µl aliquots of two-fold serially over the

target strain-containing agar plate overlay. Before putting the plates in the incubator, wait until all of the drips have been absorbed.Observe the inhibitory zone surrounding the site after 18 hours of incubation at 37 °C [10] [11]

- **Cup assay**

The isolates under examination were incubated at 37 °C for 18 hours after being cultivated in brain heart infusion broth with 5% glycerol added. • On medium with 5% glycerol added to brain heart infusion agar, the bacterial isolates were severely streaked (mat streaking) and then incubated at 37 °C for 18 hours. The preparation of 5 ml of the indicator isolate suspension and the comparison with the McFarland (0.5). For each isolate, duplicate plates were utilized, and the indicator isolate suspension was placed on the surface of the nutritional agar and dried at 37°C for 10 minutes.• Making discs with producer isolate on Brain Heart Infusion Agar using cork holes with a diameter of 5 mm. Discs were taken out of the agar medium and carefully put onto the nutrient agar surface that had been distributed by the indicator isolate. They were then incubated at 37° C for an entire night. The best marcescin-producing isolate was chosen because it had a larger inhibition zone around the agar disk against the indicator isolate. [12]

- **Wells diffusion Agar method**

Using the Mueller-Hinton agar (MHA) and well diffusion technique under aseptic conditions, a bacterial strain (was seeded into MHA agar plates as a suspension with a turbidity of McFarland (0.5).100µl of the test samples were then added to wells with a 6 mm diameter, and they were incubated at 37 °C for 24 hours.The diameter of the growth inhibition zones was measured following the incubation period. [13] [14]

- **Agar cross-streaking method**

A marcescin-producing isolate was used to streak a plate of thick Tryptic soy agar (8–10 mm deep). After 48 hours of incubation at 37°C, the plate was exposed to chloroform vapor for one hour in order to destroy the bacteria. The bacterial growth was then removed with a glass slide and the plate was left exposed for an hour at room temperature to completely remove all remnants by chloroform vapor. The chloroform-treated medium was streaked with the marcescin-sensitive isolate that had previously been grown in TSB for three to four hours

at37ºC, and the plate was then left at4 ºCat least for four hours or overnight. The plate was tested for signs of growth suppression of marcescin - sensitive isolates following incubation [15]

Results

Collection and Identification of bacteria

There were (183) clinical isolates which include 23clinical isolates of *S. marcescens*(12.56%) from (220) clinical samples collected from different source ; clinical isolates included blood, urine, sputum and abscess as show in(Table -1). All isolates of *S. marcescens* were identified according to morphological characteristics of growing colony on blood, nutrient agar and MacConky agar, microscopically characteristics, in addition to biochemical test and Vitek 2 system. [9] [16]

Table 1: Incidence of *S marcescens* isolates according to the collection source

Clinical source	Isolates No.	%
urine	11	47.82
Blood	8	34.78
Sputum	2	8.69
Abscess	2	8.69
Total	23	100

Screening of marcescinproducing isolates

A Spot –on- the- lawn method

Using the "Spot on the Lawn"method, the production of marcescin was assessed in all *S. marcescens* isolates. The findings showed that following the incubation period, inhibition zone appeared surrounding expanding colonies with various diametersand revealed that 16 (69.56%) of 23 isolates of *S. marcescens* had the ability for producing marcescinand7(30.43%)couldn't produce marcescin as in table (2).

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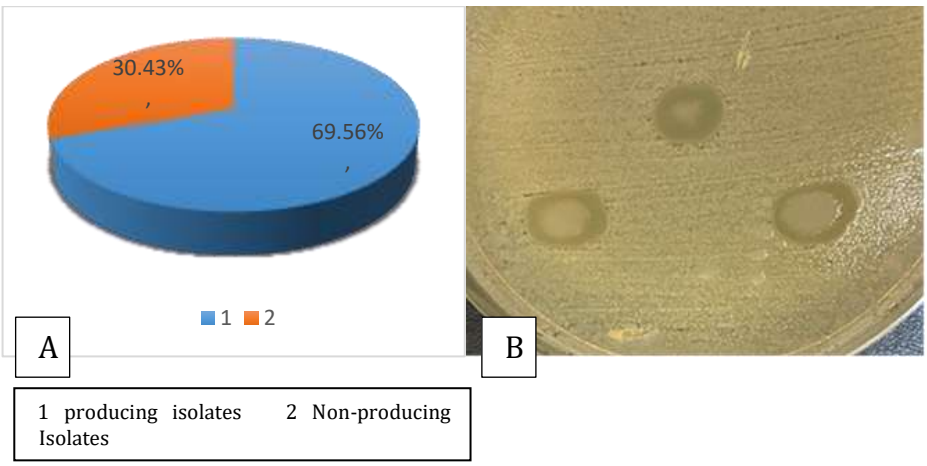

Figure 1 A: Percentages of marcescin –producing isolate by spot method

B: Spot on the lawn method

Cup assay method

All isolates of *S. marcescens* were subjected to screening process in order to choose the higher marcescin- producer isolates. The ability of these bacterial isolates for marcescin production were detected by cup assay. The results showed that 20 isolates (86.95%) of the 23 clinical isolates were marcesin producers as in table (2), and 3 isolates only were none marcescin-producer (13.04%).

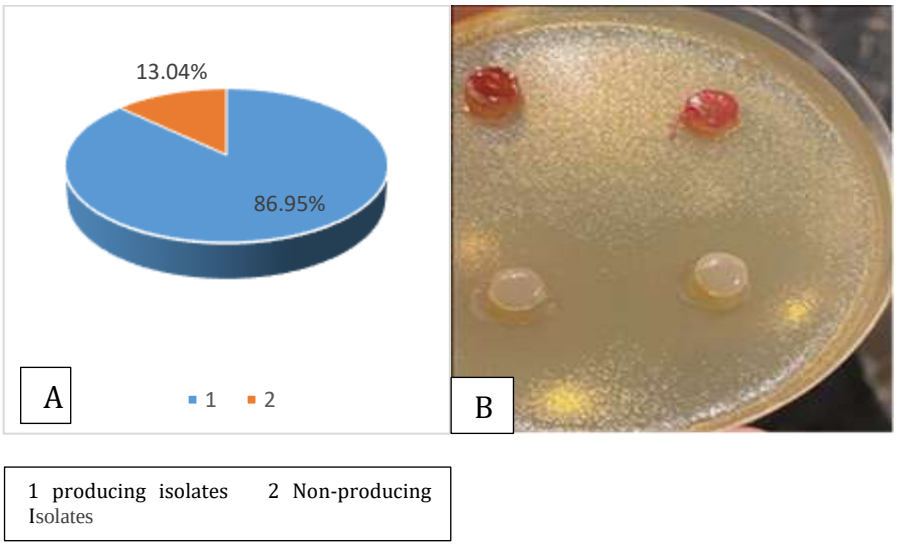

Figure 2 A: Percentages of marcescin –producing isolates by cup assay

B: Cup assay

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Agar well diffusion method

To validate the synthesis of marcesin, 23 isolates of *S. marcesens* were grown on BHIB and free-cell supernatant liquid will take to detect the marcescin ,current study reveled that 19 (82.60%) of the 23 isolates were marcesin- producers as in table (2) , and (17.39%) 4 isolates were not producing marcescin

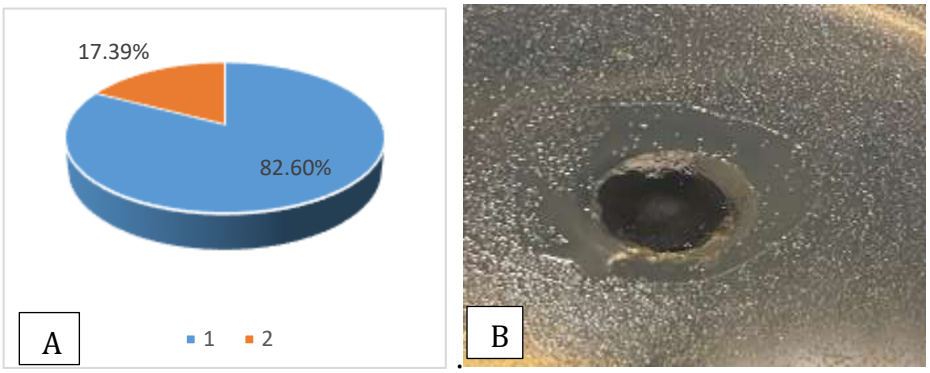

Figure 3 A: Percentages of marcescin –producing isolates by agar well diffusion method

B: Agar well diffusion method

Agar cross-streaking method

The synthesis of marcesin by a locally isolated *S. marcescens* was evaluated by Agar cross-streaking assay which revealed that 15 isolates from 23 isolates (65.21%) were marcesin –producer as in table (2) , and 8 isolates (34.78%) were not producing marcescin .

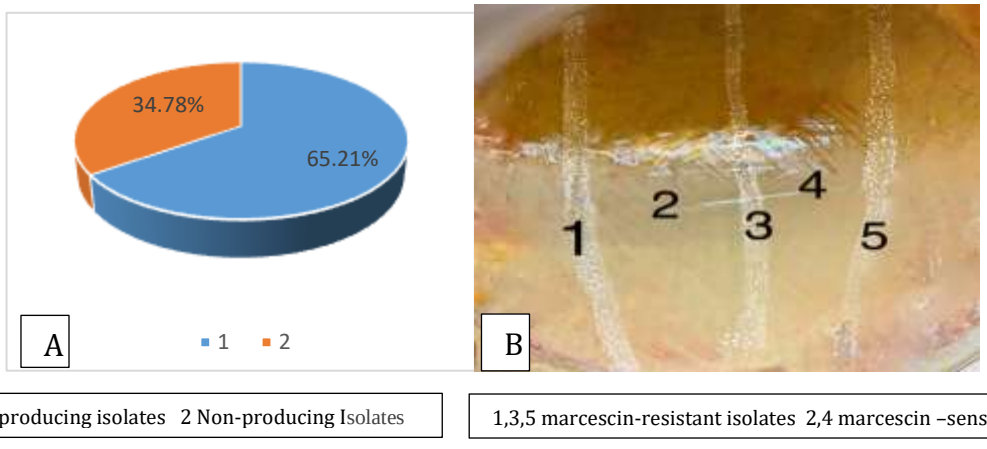

Figure 4 A: percentages of marcescin –producing isolates by agar cross -streaking method

B: agar cross -streaking method

Table 2: percentages and number of marcescin –producer isolates

Screening methods	Isolates No.	%
Spot on the lawn	16	69.56
Cup assay	20	86.95
Well diffusion	19	82.60
Agar cross streaking	15	65.21

Discussion

In order to choose the isolate with the highest marcescin production and the most effective detection technique, all *S. marcescens* isolates underwent a screening process. Four techniques were used to assess the isolation of *S. marcescens'*ability to synthesize marcesin :Spot –on- the-lawn method, cup assay ,agar well diffusion method and Agar cross-streaking assay, the best method for detecting marcescin –producer isolates was cup assay that gave the best and higher result :(86.95%) 20 isolates were producing marcescin. Several studies were used this method to detect bacteriocin producing isolates asrevealed by *Del Campo et al* ,who mentioned that The percentage of bacteriocin–producer isolates was higher pathogenic clinical isolates (63.2%)[17]. It was higher than what reported by each of Laftah and Aggarwal who found that the percentage of bacteriocin producing isolates by clinical specimens were (34.7, 30.9) % respectively [13]. [18]. Another local study done by Al Charrakh*et al* ., which used many liquid and solid culture media during four detection methods for kleobcin –producing isolates showed that the best culture media is brain heart infusion medium with 5 % glycerol andThe most effective technique for this aim was the cup test method.[19] [20] .The second best method was well diffusion method that gave the high result of marcescin- producer isolates (82.60%) ,this method reveled significant inhibition zone around the wells with diameter about (7-22mm) . Qayyum*et al*., used 25 lactic acid bacterial (LAB) strains and screened it ability to produce bacteriocin, among them five strains (20%) could producing bacteriocin by agar well diffusion method test against pathogenic organisms [12]. Rebuffat , showed that bacteriocin can diffuse in solid media and it have receptor –mediated mechanism of antibacterial activity ,that depending mainly on the sensitive bacterial isolates[21].

Conclusions

The most effective technique for this aim was the cup assay that gave the best and higher result and the second best method was well diffusion method.

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