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ISOLATION AND IDENTIFICATION OF EGYPTIAN STRAINS OF *Serratia marcescens* PRODUCING ANTIBACTERIAL AND ANTIOXIDANT PRODIGIOSIN PIGMENT

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ABSTRACT: Prodigiosin is a natural red pigment, it is an alkaloid secondary metabolite with a unique tripyrrole structure produced by *Serratia marcescens,* it's motile, short-rod shaped, G^- bacteria, classified in the large family of *Enterobacteriaceae*. 13 isolates were isolated from 32 soil samples, which showed clear red pigmented colonies. The Egyptian *Serratia marcescens* isolates were identified based on morphological, biochemical and then only the two best isolates for pigment production (M1, S1) were identified by MALDI-TOF mass spectrometry technique. The two selected species gave a score value between 2.565 to 2.668 (100%) were correctly identified by MALDI-TOF- MS to the genus and species levels. The average daily rate of pigment production by M1 and S1 isolates estimated by 421.6 and 326.1 unit/cell, respectively. Antibacterial activity of prodigiosin was determined by disc diffusion assay against 7 types of G^- and 4 types of G^+ pathogenic bacteria. Minimum inhibitory concentration (MIC) and the concentration inducing 50% inhibition of the bacterial growth (IC_{50}) against the mentioned bacteria were determined and prodigiosin was proved to be effective in inhibiting bacterial growth. Prodigiosin possess antioxidant activity assayed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction method and this activity increased gradually with increasing concentrations.

Key words: *Serratia marcescens*, MALDI-TOF-MS, prodigiosin, antibacterial activity, antioxidant activity.

INTRODUCTION

Serratia marcescens bacteria is G^- bacilli belonging to the family *Enterobacteriaceae*, which are opportunistic to human, plant and insect. There are two types of *Serratia marcescens*; pigmented (red) and non-pigmented (white) strains **(Hardjito *et al.*, 2002)**.

Matrix-assisted laser desorption/ionization time of-flight mass spectrometry technique (MALDI-TOF MS) has been attracting attention as a tool for the automated identification of microorganisms. This device can identify microorganisms from prepared samples in about 10 min, and the obtained results show a high rate of concordance with 16S rRNA gene identification. The efficiency, rapidity, and cost-benefit of this technique for the identification of pathogenic bacteria are well documented (**Bizzini *et al.*, 2011; Tadros and Petrich, 2013; Yonetani *et al.*, 2016**).

The production of prodigiosin by *S. marcescens* is influenced by numerous factors including inorganic phosphate availability, medium composition, temperature, pH, and natural components in the media (**Iranshahi *et al.*, 2004 and Wei *et al.*, 2005**). Prodigiosin is a red pigment produced as a secondary metabolite by *Serratia marcescens*, *Pseudomonas magneslorubra* and *Vibrio psychroerythrous etc*. characterized with unique tripyrrole structure which is regarded as responsible for its reported pharmacological characteristics as anticancer, antimicrobial, antioxidant and immunosuppressant agents

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(Araújo *et al.*, 2010; Samrot *et al.*, 2011) and its unique characteristics as a natural based pigment for olefins and textiles **(Gulani *et al.*, 2012).** The effective clinical properties of prodigiosin imply it as antitumor (**Alihosseini *et al.*, 2008**), immunosuppressive (**Songia *et al.*, 1997**), phytopathogenic fungal inhibitor agents **(Someya *et al.*, 2003**).

The purified pigment extracted from the biomass of bacteria was analyzed by mass spectrophotometry and showed the expected molecular weight of 324 Da corresponding to prodigiosin **(Araújo *et al.*, 2010).** The large scale production of prodigiosin was achieved by using optimized growth conditions (time of incubation, temperature of incubation, pH of the medium, speed of agitation, carbon and nitrogen source used *etc.* **(Phatake and Dharmadhikari, 2016)**.

The major advantage of pigment production from microorganisms includes easy doubling time, specific growth rate, fast in the cheap culture medium, and optimal environmental parameters **(Su *et al.*, 2011)**. The standardization of culture medium and fermentation conditions plays a very crucial role in the maximum production of prodigiosin **(Su *et al.*, 2011)**.

The aim of the present study was to isolate indigenous strains of *Serratia marcescens* and identify them using MALDI-TOF MS, and extracts the red pigment from culturable bacteria to evaluate its antibacterial activity against a series of bacteria and antioxidant activities.

MATERIALS AND METHODS

This study was carried out at the laboratories of Agric. Microbiology Dept., Faculty of Agriculture, Zagazig University, Egypt, during the period of 2017 to 2019, in order to select efficient indigenous isolates of *Serratia marcescens* to produce a red pigment (prodigiosin). Thirty two moistured soil samples were collected from 8 locations from different districts at Sharkia Governorate. These districts namely Zagazig (Z), Belbais (B), Mashtool El-Sook (MS), Fakous (F), El-Hessenia (H), Menia El-Kamih (M), Abo Kabeer (AK) and Salhya (S). The aforementioned moisturized soil samples were used for isolation of different isolates of *S. marcescens*.

Isolation and Purification of *S. marcescens*

Pure cultures of *S. marcescens* isolated from soil samples, collected from aforementioned districts in Sharkia Governorate, were obtained according to **Gulani *et al.* (2012)**. Each soil sample (10 g of soil) was transferred to sterile 250 ml conical flasks containing 90 ml 0.1% peptone water. Samples were serially diluted with peptone water up to 10^{-7} and plated on nutrient agar then incubated at 30˚C for 2-3 days. After incubation the plates were examined for red pigmented colonies and streaked on the same medium for purification. Pure colonies were subjected to slant agar and kept at 4˚C.

Morphological and Biochemical Characteristics of *S. marcescens*

The direct microscopic examination of stained smears of pure bacterial isolates was carried out for studying size, shape, Gram staining and hanging drop method which was used to study the motility of the bacterial cells. Biochemical and physiological testes of cultures were also examined according to **(Grimont and Grimont, 2005)** in the Bergey's Manual of Systematic Bacteriology 2nd Ed., volume two, part B (the proteobacteria), the gammaproteobacteria.

Identification of *S. marcescens* using MALDI – TOF – MS

Identification of *S. marcescens* had been confirmed by using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) in peptide and protein analyses. One large colony from each of selected bacterial isolate (enough to fill about one half of a 10-µl inoculating loop) was suspended in 70% ethanol in a 1.5 ml micro-centrifuge tube and loaded three times onto ground steel MALDI target according to the manufacturer′s instruction (Bruker Daltonics, Breman, Germany). Matching between experimental MALDI-TOF-MS profiles obtained from bacterial isolates and the reference MALDI-TOF-MS profiles is expressed by a BioTyper according to a Log (score) and an associated-color code (green, yellow and red) was recorded. Micro Flex mass spectrometeries were performed at Academic Park, Faculty of Medicine, Alexandria University, Egypt, according to **Biswass and Rolain (2013) and Nacef *et al.* (2016).**

Presumptive Test for Prodigiosin Confirmation from *S. marcescens*

The culture broth of selected *S. marcescens* (M1). was subjected to centrifugation process at 5000 xg for 15 min and 10 ml of 95% methanol was added to the cell pellet and centrifuged under the same conditions. Debris was removed and the 2 ml of the supernatant was taken in two test tubes. The content of the test tube was acidified with a drop of concentrated HCl (30 - 34%) and the other alkalinized with a drop of concentrated ammonia solution (30 - 32%). A red or pink color in the acidified solution and a yellow or tan color in the alkaline solution indicated a positive, presumptive test for prodigiosin presence **(Gerber and Lechevalier, 1976).**

Screening and Determination of Prodigiosin Produced by *S. marcescens* Isolates

Individual isolates of *S. marcescens* were transferred into 250 ml conical flasks with 100 ml of nutrient broth medium (seed cultures are 10% of the total volume of the fermentation medium) and incubation at 30°C. The prodigiosin was extracted, determined and the growth was followed by measuring the optical density (OD) at 620 nm after 48, 72 and 96 hr., of incubation.

Quantitative Estimation of Prodigiosin

The absorption pattern over various wavelengths was initially checked and it was found that the absorption maxima were at 499 nm where prodigiosin also absorbs maximally**.** At this wave length the absorptions were recorded on methanolic extract and 95% methanol was used as a blank. Extracted prodigiosin was estimated using the following equation **(Mekhael and Yousif, 2009).**

$$\text{Prodigiosin unit/cell} = ([\text{OD } 499 - (1.381 \times \text{OD } 620)]) \times 1000 / \text{OD}620$$

Where:

OD 499–pigment absorbance, OD 620–bacterial cell absorbance, 1.381 – constant

Prodigiosin Bioassay

Antibacterial activity of prodigiosin

Disc-diffusion assay on Mueller Hinton Agar (MHA) was used to determine the prodigiosin antibacterial activity against 4 types of G^+ bacteria (*Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecium and Bacillus cerues*) and 7 types of G^- bacteria (*Salmonella enterides, Proteus vulgaris, Pseudomonas aeruginosa, klebsiella pneumoniae, Aeromonas hydrophila, E. coli and E.coli* O157:H7) as described by **Ivanova *et al.* (2000)**. These bacteria were obtained from the Agric. Microbiology Dept. Fac. Agric., Zagazig Univ. The pure cultures of the tested indicstors were sub-cultured in Mueller Hinton broth (MHB) and incubated at 37˚C on a rotary shaker at 200 rpm for 24 hr. Each culture was spread uniformly onto individual Mueller Hinton Agar (MHA) plates using spread plate method. 6 mm diameter sterile filter paper discs were impregnated individually with 50 µl of methanolic extract of prodigiosin concentrations (25, 50, 100, 200 and 400 µg ml^{-1}). All the discs were dried and placed on the surface of the tested pathogenic bacteria plates. After incubation at 37˚C for 24 hr., the different resulting inhibition zones diameter were measured (in mm). Treatments using 95% methanol were considered as a control.

Minimum inhibition concentration of prodigiosn (MIC)

Minimum inhibition concentration (MIC) which identified as the lowest concentration of the test compound which inhibits the visible growth and IC_{50} (the concentration inducing 50% inhibition of the bacterial growth) of prodigiosin were determined against the mentioned bacteria using the conventional broth dilution assay **(Murray *et al.*, 1995)**. Serial dilutions of the tested prodigiosin were adjusted to final concentrations of 25, 50, 100, 200 and 400 µg ml^{-1} of Mueller-Hinton broth (MHB) and 5 ml of each dilution was transferred into 10 ml test tube. To each tube, 100 µl of the bacterial inoculum was added and incubated for 24 hr., at 37˚C. The turbidity (visible growth) was determined at 600 nm using JENWAY – England 6405 UV/VIS Spectrophotometer after 24 hr. At the end of the incubation time, MIC was visually identified and the IC_{50} was mathematically concluded from the obtained data. All tests were performed in triplicate. Tests using sterilized distilled water were used as negative control.

Antioxidant activity of prodigiosn

The extracted pigment was dissolved with 95% methanol to prepare the stock solution (2000 µg/ml). The tested samples were prepared from stock solution by diluting with methanol to a desired concentration of 1000, 400, 200, 100, 50 and 25 µg/ml, respectively. Diluted tested samples (1ml) were added to 1ml of a 0.004% methanol solution of DPPH and mixed. After 30 min incubation in the dark, the decrease in absorbance was measured at 517 nm using spectrophotometer. Ascorbic acid was used as a standard **(Renukadevi and Vineeth, 2017)**. The optical density was recorded and inhibition (%) was calculated according to the formula:

Inhibition of DPPH activity (%) =

$(ABS_{control} - ABS_{sample}) / ABS_{control} \times 100$

RESULTS AND DISCUSSION

Identification of the Selected *S. marcescens*

Thirty two soil samples were collected from Sharkia Governorate, Egypt and screened for red pigment (Prodigiosin) producing organisms. Among the 32 soil samples, Only 13 bacterial isolates which showed clear red pigmented colonies were recovered, purified and slanted. The isolated colonies were identified based on their Phenotypic characteristics and using MALDI-TOF-MS. (matrix-assisted laser desorption ionization-time of flight mass spectrometry). The isolates were confirmed as *Serratia marcescens*.

Phenotypic Characterization

Morphological and biochemical characteristics were used to identify the 13 selected bacterial isolates as described by **Grimont and Grimont (2005)**. Results in Table 1 show that all thirteen bacterial isolates were Gram negative, motile, catalase positive, non-produced of Indole, Voges – proskauer test positive, non-production H_2S, grow in 3% of NaCl and non-spore forming bacteria. Also, most the tested isolates can use the citrate as one source for carbon except two isolates (Z3 and S1), most of isolates can grow in 7% of NaCl except B1, B2, F2 and AK isolates. Identifying *Serratia marcescens* isolates was made mainly on the results of 11 carbohydrate fermentation tests.

Direct Identification of some Tested Bacteroial Isolates using MALDI-TOF-MS

The selected isolates were identified at Academic Park Fac. Medicine Alex. Univ., Egypt, using MALDI-TOF-MS. Using this advanced method, this identification process was considered as accurate approach to bacterial isolates identification and the prospective strains were identical with their numbers as conserved in the International Cultural Center for Microorganisms (ICCM). The score values for the bacterial isolates are shown in Table 2. All of the isolates showed a score value between 2.565 to 2.668 (100%) and were correctly identified to genus and species levels. Two tested bacterial isolates were type strains that are included in the Bruker Database, and all spectrum scores were greater than 2.0. Thus, all of the tested isolates were correctly identified to genus and species levels with biotype software score values **(Bizzini *et al.*, 2010; Wang *et al.*, 2013)** greater than 2.0, and all of them had high degree of precision.

Screening and Determination of Prodigiosin Produced by *S. marcescens* Isolates

Colorimetric estimations of the prodigiosin pigment in bacterial cultures of *Serratia marcescens* isolates are shown in Table 3. The highest value of prodigiosin recorded in culture was obtained by M1 isolate (830.2 unit/cell) followed by S1 isolate (635.5 unit/cell) after 72 hr., of incubation, and the calculated average daily and hourly rates production in the case of M1 isolate and S1 isolate were found to be 421.6, 17.5, 326.1 and 13.5 unit/cell, respectively. The lowest value of prodigiosin recorded in culture was obtained by B2 isolate (211.8 U/cell) followed by D1 isolate (215.8.5 unit/ cell) after 48 hr., of incubation. Generally, highly amount of prodigiosin obtained after 72 hr., of incubation. These results are in agreement with previous results reported by **Kamble and Hiwarale (2012)**. They found that the prodigiosin production is increasing gradually after 48 hr., and were maximum towards 72 hr., thereafter the production was decreasing towards 92 hr., in nutrient broth and peptone glycerol broth.

Table 1. Biochemical characteristics of *S. marcescens* bacteria isolated from soil samples from Sharkia Governorate

Characteristic	Bacteria isolate												
	Z1	Z2	Z3	B1	B2	MS	F1	F2	S1	H1	M1	M2	AK
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-
Voges – Proskauer	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	-	+	+	+	+	+	-	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-
Lipase production	-	+	+	+	+	-	+	+	+	+	+	+	+
Growth in NaCl 3%	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in NaCl 7%	+	+	+	-	-	+	+	-	+	+	+	+	-
Lactose	-	-	-	-	-	-	-	-	-	+	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	-	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2. Rate of classification results as determined by Bruker Daltonik MALDI Biotyper

Isolate code	Analyte name	Organism(best match)	Score value
M1	B17 **(+++) (A)**	*Serratia marcescens* ssp *marcescens* DSM 30121T DSM	2.565
S1	B15 **(+++) (A)**	*Serratia marcescens* DSM 30122 DSM	2.668

- Category A= species consistency (2.300-3.000). DSM: Deutsche Sammlung von Mikroorganismen.

Table 3. Changes in amounts of prodigiosin (unit/cell) produced by different *S. marcescens* isolates as affected by incubation time

Serratia isolate	Prodigiosin (unit/cell)				
	48 hr.	72 hr.	96 hr.	Average daily rate production	Average hourly rate production
MS	272.6	457.4	348.7	269.6	11.2
AK	357.9	506.4	434.1	324.6	13.5
B1	257.2	341.9	234.9	208.5	8.6
B2	211.8	467.2	392.9	267.9	11.1
M1	552.8	830.2	303.4	421.6	17.5
M2	251.6	430.3	280.5	240.6	10.0
H1	215.8	448.9	426.0	272.6	11.3
S1	336.9	635.5	332.0	326.1	13.5
Z1	289.4	487.4	309.7	271.6	11.3
Z2	301.4	450.7	353.3	276.3	11.5
Z3	404.2	586.0	361.7	337.9	14.0
F1	387.0	453.7	395.0	308.9	12.8
F2	416.7	504.2	297.4	304.5	12.6

Antibacterial Activity of Prodigiosin

Prodigiosin extracted from Egyptian *Serratia marcescens* strains were used to study their action as antibacterial agent against 4 G^+ bacteria (*S. aureus, L. monocytogenes, E. faecium* and *B. cerues*) and 7 G^- bacteria (*S. enterides*, *P. vulgaris*, *P. aeruginosa, K. pneumonia, A. hydrophila, E. coli* and *E. coli* O157 : H7).

Disc diffusion assay was performed in this respect. The antibacterial activity of different concentrations of prodigiosin (25, 50, 100, 200 and 400 µg/ml) was assayed against all the aforementioned pathogenic tested indicators by measuring the area of the inhibition zones after incubation at 37˚C for 24 hr. Results in Table 4 show that the growth of all aforementioned bacteria was inhibited by all concentrations of prodigiosin used in this experiment. Furthermore, the diameter of the inhibition zones increased proportionally with the increase of prodigiosin concentrations. The clear zone diameter in all tested bacteria ranged between 7 to 29 mm under different prodigiosin concentrations. The highest inhibition zone was obtained in the case of *K. pnuemonia* (29 mm) at a concentration of 400 µg/ml of prodigiosin. The inhibitory action of prodigiosin against the G^- bacteria (especially *S. enterides* and *K. pnuemonia*) was always higher than that of all tested bacteria. The prodigiosin has an antibacterial, antiprotozoal, antitumor and antiinflammatory activity (**Perez-Tomas and Vinas 2010**). It has been demonstrated that prodigiosin inhibits growth of a wide spectrum of Gram positive bacteria (*B. subtilis* and *S. Aureus*) as well as Gram negative (*E. coli, S. enterica* and *Erwinia carotovora*) (**Danevčič *et al.*, 2016)**. The antimicrobial study of prodigiosin revealed that it is a potent inhibitor of G^+ bacteria as well as G^- bacteria (**Chauhan *et al.*, 2017)**.

Table 4. Disc diffusion assay of the antibacterial action of prodigiosin (25 -400µg/ml) against 4 G^+ and 7 G^- bacteria

Concentrations of prodigiosin	Inhibition of bacterial growth (mm)										
	Gram positive bacteria				Gram negative bacteria						
	S. aureus	*L. monocytogens*	*E. faecium*	*B. cerues*	*S. enterides*	*P. vulgaris*	*P. aeruginosa*	*K. pnuemonia*	*A. hydrophila*	*E. coli*	*E. coli O157:H7*
25 µg/ml	8	8	9	10	13	9	7	15	9	9	9
50 µg/ml	8	10	10	10	15	12	10	18	10	10	11
100 µg/ml	9	11	10	11	17	14	12	22	13	10	11
200 µg/ml	10	11	10	12	20	14	18	26	13	11	12
400 µg/ml	10	12	11	14	24	17	20	29	15	12	12

Minimum Inhibition Concentration of Prodigiosin (MIC)

Antibacterial inhibiting action of different concentrations of *Serratia* prodigiosin was assayed as described by **Murray *et al.* (1995)**. Results in Table 5 show that the antibacterial action was observed when adding prodigiosin at different concentrations (25 – 400 µg/ml) to the aforementioned bacteria (4 G^+ bacteria and 7 G^- bacteria) inoculated in MHB tubes and incubated at 37^0C for 24 hr. Prodigiosin concentration corresponding to 50 % inhibition of G^+ bacteria (IC_{50}) was in the range of 26.18 to 51.54 µg/ml of prodigiosin and 20.31 to 69.71 µg/ml of prodigiosin for G^- bacteria. Red pigment prodigiosin whish produced by *Serratia marcescens* showed intracellular antibacterial action higher susceptibility against Gram positive than Gram negative bacteria. The same characteristics were observed in other antimicrobial studies on plant and cyanobaterial extracts against pathogenic bacteria (**Tong *et al.*, 2014; Sitohy *et al.*, 2015)**.

Antioxidant Activity of Serratia prodigiosin

Changes in DPPH radical scavenging activity (RSA) of prodigiosin at different concentrations (25, 50, 100, 200, 400, 1000 µg/ml) are presented in Table 6. It can be noted that, the antioxidant activity of prodigiosin increased gradually with increasing concentration. At different concentrations, the extracted *Serratia* prodigiosin was tested for the radical scavenging effect against DPPH. The DPPH free radicals scavenging activity of prodigiosin were found to be 51.79, 59.82, 60.49, 62.05, 63.17 and 92.63% at 25, 50, 100, 200, 400 and 1000 µg/ml of *Serratia* prodigiosin, respectively. Total antioxidant capacity was reported as ascorbic acid equivalents **(Renukadevi and Vineeth, 2017)**. The result indicates that the antioxidant activity of prodigiosin is lower than that of standard ascorbic acid.

Table 5. Minimum inhibition concentration (MIC) and IC_{50} of prodigiosin (25 - 400µg/ml) against 4 G^+ and 7 G^- bacteria

Concentrations of prodigiosin	Inhibition of bacterial growth (OD)										
	Gram positive bacteria				Gram negative bacteria						
	S. aureus	*L. monocytogens*	*E. faecium*	*B. cerues*	*S. enterides*	*P. vulgaris*	*P. aeruginosa*	*K. pnuemonia*	*A. hydrophila*	*E. coli*	*E. coli O157:H7*
00 µg/ml (control)	1.15	1.34	0.94	0.76	1.39	1.28	1.61	1.34	1.19	1.25	1.15
25 µg/ml	0.95	0.92	0.61	0.33	0.94	0.67	1.45	0.74	0.95	0.61	0.53
50 µg/ml	0.51	0.65	0.27	0.26	0.80	0.63	1.33	0.69	0.90	0.56	0.10
100 µg/ml	0.16	0.18	0.16	0.20	0.12	0.11	1.29	0.14	0.24	0.14	0.09
200 µg/ml	0.08	0.09	0.05	0.07	0.03	0.03	0.38	0.10	0.16	0.10	0.01
400 µg/ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
IC_{50} (µg/ml of prodigiosin)	**51.17**	**51.54**	**26.18**	**33.61**	**56.56**	**50.81**	**69.71**	**48.63**	**66.98**	**44.20**	**20.31**

Table 6. Antioxidant activity of prodigiosin

Concentrations	Prodigiosin SA	Ascorbic methanol SA
25 µg/ml	51.79	98.66
50 µg/ml	59.82	99.33
100 µg/ml	60.49	99.55
200 µg/ml	62.05	99.55
400 µg/ml	63.17	101.56
1000 µg/ml	92.63	108.93

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عزل وتعريف سلالات مصرية من الـ سيراتيا مارسيسنس المنتجة لصبغة البروديجيوزين المضادة للبكتريا ومضادة للأكسدة

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تم عزل وتنقية ١٣ عزلة من بكتريا الـ *Serratia marcescens* التابعة لعائلة الـ *Enterobacteriaceae* والمنتجة لصبغة الـ prodigiosin من ٣٢ عينة من أراضي محافظة الشرقية والتي أظهرت مستعمرات حمراء واضحة وتم تعريفها علي أساس الصفات المورفولوجية والبيوكيميائية المختلفة وتم اختيار ٢ عزلة الأكثر كفأة في إنتاج الصبغة للتحقق منها عن طريق تطبيق تقنية MALDI-TOF MS للتعريف والذى تم بواسطته تعريف العزلات إلى مستوى الجنس والنوع، تم فصل صبغة الـ prodigiosin من الخلايا وكان معدل إنتاج الصبغة اليومي لكلا من M1 و S1 هو ٤٢١,٦ و ٣٢٦,١ وحدة/ الخلية علي التوالي وتم دراسة تأثير الصبغة كمضاد للميكروبات المرضية علي ٧ أنواع من البكتريا السالبة لصبغ جرام و٤ أنواع من البكتريا الموجبة لصبغ جرام وكذلك تم دراسة أقل تركيز مثبط والتركيز المثبط لـ ٥٠% من نمو البكتريا والذي يتراوح بين ٢٦,١٨ – ٥١,٥٤ ميكروجرام/مل في البكتريا الموجبة لصبغ جرام و٢٠,٣١ – ٦٦,٩٨ ميكروجرام/مل في البكتريا السالبة لصبغ جرام وقد أظهرت الصبغة أن لها تأثير فعال في خفض أو تثبيط نمو البكتريا في التركيزات المختلفة، تم دراسة التأثير المضاد للأكسدة بإستخدام دليل الـ DPPH وقد تبين أن الصبغة تعمل على زيادة النشاط المضاد للأكسدة بزيادة التركيز.

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