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The Effect of Prodigiosin Extracted from *Serratia marcescens* on DNA Fragmentation of Human Peripheral Blood Lymphocytes Cells

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Abstract

Prodigiosin, is a natural red pigment produced by various bacteria that firstly characterized from *Serratia marcescens*. It is an alkaloid secondary metabolite with a unique tripyrrol structure.This pigment is a promising drug owing to its reported characteristics of having antifungal, immunosuppressive and anti-cancer activity. In this study prodigiosin was produced by *Serratia marcescens*., which was isolated from soil identified and characterized by morphology, Gram's staining, biochemical and carbohydrate fermentation tested and confirmed by the API 20E test.

From these samples, six isolates of *Serratia marcescens*(24) % were obtained out of 25 soil samples. Ability of these isolates in prodigiosin production was examined to select the efficient isolate in prodigiosin production.Results showed that all of these isolates are prodigiosin producers, and among them one isolate was the most efficient in prodigiosin production.

The apoptotic effect of the pigment was assessed in blood lymphocytes cells by DNA fragmentation analysis assay collected from anticoagulated human blood , prodigiosin at all concentrations were significantly increased the percentage of fragmented DNA of peripheral blood Lymphocyte cells compared to control. This study reveals that Prodigiosin has possessed apoptotic property.

Keywords: *Serratia marcescens*, Prodigiosin, apoptosis, DNA Fragmentation, Human Peripheral Blood and Lymphocytes Cells.

تاثير البرودجيوسين المستخلص من *Serratia marcescens* على تجزؤ الدنا للخلايا اللمفيه للدم المحيطي للانسان

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الخلاصة

البرودجيوسين، صبغه طبيعيه حمراء اللون تنتج من انواع مختلفه من البكتيريا خاصه بكتريا *Serratia marcescens*. الصبغه ناتج قلوي للايض الثانوي ذات تركيب احادي التربين .هذه الصبغه اعتمدت كعلاج نسبة لصفاتها الضد فطريه ومثبطة للمناعه والضد سرطانيه .في هذه الدراسه صبغة البرودجيوسين المنتجه من بكتريا*Serratia marcescens* والمعزوله من التربه والمشخصة مظهريا، بصبغة كرام ،الفحوصات البايوكيميائيه وتخمر الكربوهيدرات واخيرا اكدت بواسطة اختبار نظام API 20 .ستة عزلات من بكتريا *Serratia marcescens* (24%) تم الحصول عليها من مجمل العينات التي تمثل 25 عينه تربه .فحصت

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قابلية هده العزلات على انتاج البرودجيوسين لاختيار العزله الاكثر كفاءة في الانتاجيه. اظهرت النتائج ان جميع العزلات منتجه للبرودجيوسين لكن عزله واحد كانت الاكثر كفاءه في انتاج البرودجيوسين.

درس تاثير الصبغه في الموت المبرمج لخلايا الدم اللمفاويه من خلال اختبار تجزؤ الدنا التي جمعت من عينات دم الانسان.

وكان البرودجيوسين بجميع التراكيز المستخدمه كان له تاثير معنوي في زيادة نسبة تجزؤ الدنا للخلايا اللمفيه للدم المحيطي للانسان مقارنة بنموذج السيطره . اثبتت هذه النتائج ان للبرودجيوسين قابليه على احداث الموت المبرمج للخلايا اللمفيه للدم المحيطي للانسان.

Introduction

Serratia spp. are Gram negative bacteria, classified in the large family of *Enterobacteriaceae*. *Serratia* spp can be distinguished from other genera by its production of three special enzymes DNAase, lipase and gelatinase [1]. *Serratia* spp occur in water, soil, on plant, in insects, in man and animal [2]. *Serratia* spp. is responsible for1.4% of nosocomial septicemia. It is also responsible for 2% of lower respiratory, urinary tract and surgical wound infections. *Serratia* spp. can be a cause of meningitis, especially after surgical intervention [3].

Another characteristic feature of the *Serratia* is the production of cell associated red color pigment called prodigiosin. Isolated from infected adults generally does not synthesize prodigiosin [4,5].

The prodigiosin group of natural products Prodigiosin,is a natural red pigment produced by produced by *Serratia sp, Pseudomonas spp, Streptomyces* spp. and *Vibrio* spp. The prodigiosin group is a family of tripyrrole red pigments that contains a common 4-methoxy, 2-2 bipyrrole ring system. The biosynthesis of the pigment is a bifurcated process in which mono and bipyrrole precursors are synthesized separately and then assembled to form prodigiosin].Prodigiosin have been shown to be associated inextracellular vesicles,cell associated or present in intracellular granules[6,7].

This pigment is a promising drug owing to its reported characteristics of having antifungal, immunosuppressive and anti-proliferative activity [8],prodigiosin is a promising drug owing to its reported characteristics of having antibacterial, antimycotic and immunomodulatory activities, prodigiosin also has a therapeutic use as potential anticancer drug.

S. marcescens have wide range of applications but the major being prodigiosin as anticancer agent. It is already stated that the pigmented strains are less virulent. prodigiosin production can be carried out safely from *S. marcescens* [9].

Effect of prodigiosin on human carcinoma cells was investigated by Kavitha and significant results were found [10] .

Prodigiosins are found to selectively act on cancerous cells with a very less or no effect on normal cells. Drug resistant cells can particularly be targeted by prodigiosin [10].

Prodigiosin is a promising drug owing to its reported characteristics of having antibacterial, antimycotic and immunomodulatory activities [11].

Prodigiosin also has a therapeutic use as potential anticancer drug. Biological mechanism and pharmacology of prodigiosin therefore have attained considerable interest in recent years. Some members of this family are potent apoptosis inducers. Thus prodigiosin induces apoptosis in various human hematopietic cancer cell lines. Interestingly prodigiosin has no marked toxicity in nonmalignant cell lines [12].

The elucidation of the mechanisms involved in the apoptotic action of prodigiosin and its evaluation as a possible anticancer drug warrants further investigation [13].

Materials and Methods

Samples collection: twenty five soil samples were collected from different locations in a farms in Baghdad city. Upper layer of soil l was collected by spatula in to clean sterile plastic bags and stored at 4°C prior to use. The soil samples were air-dried (20°C) and passed through a sieve (mesh number 2mm). Ten gram of sieved soil was suspended in 20 ml of basic salt medium (BSM) [14].

Isolation & characterization of *Serratia marcescens*: One loopfull of suspension soil samples was plated on blood agar and MacConkeys agar, then incubated at 28°C for 18-24 h. Bacterial isolates were identified according to using morphological, cultural and biochemical tests. API 20E test system (Bio- Merieux) are used to confirm identifications [14].

Production and estimation of Prodigiosin

Prepared prodigiosin was extracted according to [15,16]

A saline suspension of organism was made .Culture was Spread on five sterile nutrient agar plate, and Incubation at room temperature 28°C for two days. Observation the confluent growth of organism with brick red pigment. The growth was scraped off in sterile saline and was collected it in centrifuge tubes from all plates. The cells were Sedimented at 3000 rpm for 20 min by centrifuge Celsl were digested mass in glass test tube by adding 1N NaOH,two times, the volume of cell mass put in water bath along 1hr at 100°C. The pigment was extracted from the digest in equal volume of absolute ethanol. The tubes were centrifuged at 3000 rpm for 20 min. to get turbidity free color solution them was mixed well with equal volume of petroleum ether ,latervi gorously were shake mixed with vortex mixer. The tubes were let to settle for 10 min for separating layers.

The upper layer were collected in an evaporating dish. An obtained solvent was evaporated by heating with water bath at 100°C till dry residue remains.

Recover the residue in 5 ml acidified ethanol. With spectrophotometer absorbance fixed at 535 nm the concentration of prodigiocin calculated by using $51\chi10^3$ liters per g per cm as specific absorbance of the pigment. Concentration expressed as micrograms of prodigiocin produced per milliliter of culture supernatant were determined from the equation.

A=KCL ( ) g/ml then equivaluts into µg/ml [15,16]

A: absorbance

K: constant ($51\chi10^3$)

C: concentration

L: length of light=1

Lymphocyte transformation assay

Apoptotic property of Prodigiosin was confirmed by the Lymphocyte transformation assay by using Peripheral human blood Lymphocyte cells, different concentrations of prodigiosin were prepared as following (5,15,25,50 µg/ml) . This assay was done according to [17] with few modifications: A volume of 500 µl of heparinized blood was cultured in complete RPMI-1640 medium (Sigma), mitogen Phytohemagglutinin (PHA) (obtained from the Iraqi center for Cancer and Molecular Genetics Research), four concentrations of prodigiosins in sterile silicon coated tubes.

The ability of prodigiosin to induce apoptosis of Peripheral human blood Lymphocyte cells treated with or without prodigiosin at various concentrations and two periods of exposure was studied. Two cultures were prepared, and incubated for 24 hr and other was incubated for 48 hr .

DNA frgmentation analysis

This assay was done according to [18]

After the end of incubation periods(24,48) the tubes spined at 1000 rpm for 10 min (this tubes were labeled with litter A).The supernatant was removed to another tube and were labeled with letter (B) One ml of (TTE) solution was added to pellet in the tubes (A) and mixed well. The tubes (A) were spined at 14000 rpm for 10 min (in 4°C)

The supernatant of tubes (A) were removed to another tubes and were labeled with letter (c).

One ml of TritonX-100 Ttris EDTA (TTE) solution were added to the pellet in the tubes (A).

One ml of TCA (25%) were added to the tubes (A,B,C) and were mixed well. Incubation the tubes were incubated overnight at (4°C). At the end of incubation period the tubes were spined at 14000 rpm for 10 min in order to precipitate DNA. The supernatant was discard.

Avolum of 320 µL of Trichloro acetic acid TCA (5%) were added to the tubes and were put in water bath at 90°C along to 15 min.

After the end of bathing , 640 µl of(DPA Diphenylamine) reagent were added and mixed tubes gently then incubated over night at 37°C the color will change to blue.Reading of O.D at 600 nm with spectrophotometer.

The DNA fragmentation percentage measured as formula:

$$\% F = \frac{B + C}{A + B + C} \times 100$$

f% : DNA fragmentation percentage

A: O.D of A tubes

B: O.D of B tubes

C: O.D of C tubes

Results and Discussion

Isolation and characterization of *S. marcescens*

Six isolates of 24 % were obtained out of 25 soil samples. The isolated colonies were identified based on their morphology and biochemical characteristics. The isolates were confirmed by the API 20E test system.

Sundaramoorthy, [19] showed that 20 samples of *S. marcescen*s isolated from soil and only 5 of them were produced red pigment and lipase as virulence factor. Other researches refered that *S. marcescen*s isolated from soils and it produced many virulence factors like lipase, chondroitinase, prodigiosin and proteases [1,20-22].

This recovery rate in current study was similar to Zaki *et.al*., [23] .

Production of prodigiosin pigment

These isolates were screened for prodigiosin producing organism. One isolate was selected according to high prodigiosin production.

The prodigiosin pigment extracted from the culture broth of *S. marcescen*s, and Optical densities of densities of the extracted prodigiosin was estimated by UV spectrophotometer at 535 nm, different concentrations of prodigiosin were prepared(5,15,25,50 µg/ml) [1,16]

The present study focused on isolation of prodigiosin producing organisms from soil, Williams & Hussain Quadri [24] reported that no prodigiosin was produced when cultures were incubated at 38°C; however pigment production was observed when the temperature was shifted to 27°C. A complete block in prodigiosin was observed in most of the basically used media tested at 37°C was similar to the result observed by Pryce & Terry (2000) [25].

DNA fragmentation analysis

Prodigiosin pigment induces apoptosis on Peripheral blood Lymphocyte.Peripheral blood Lymphocyte cells treated with or without tested pigment at various concentrations and incubated at two periods (24 and, 48).

Prodigiosin at all concentrations was significantly increased the percentage of fragmented DNA of Peripheral blood Lymphocyte cells compared to control figure-1 and(-2), results showed that this pigment was induced apoptosis in a concentration-dependent fashion but not in time fashion because the activity of pigment did not depend on the time of exposure (24hr ,48 hr) .

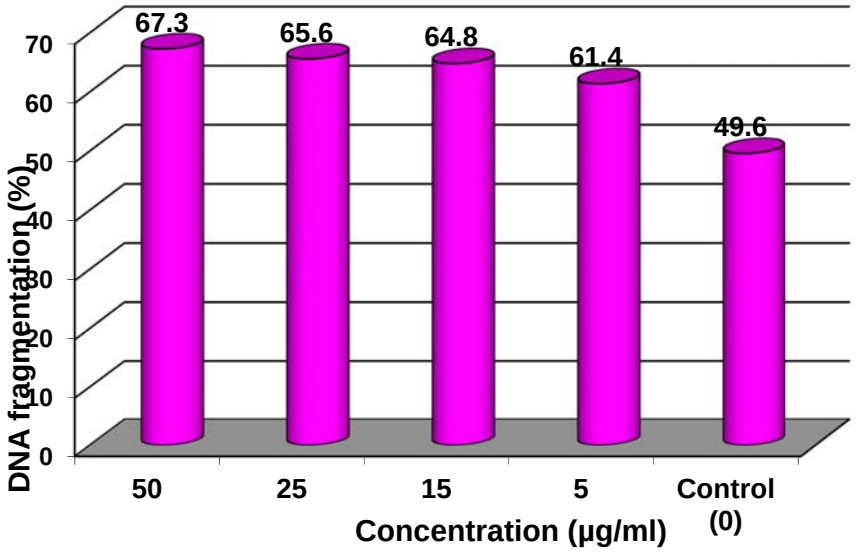

Figure 1- DNA fragmentation of Peripheral blood Lymphocyte cells after exposure to different concentrations of prodigiosin at incubation period 24 hr .

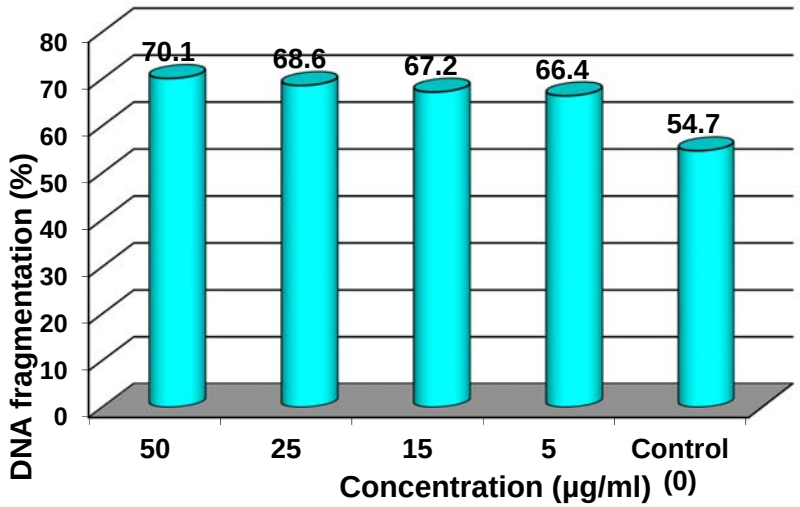

Figure 2- DNA fragmentation of Peripheral blood Lymphocyte cells after exposure to different concentrations of prodigiosin at incubation period 48 hr .

Indeed, the mechanism of prodigiosin pigment on DNA of cancer cells is not completely known. However, It may induce DNA breaks by cleavage poly(ADP-ribose) polymerase (PARP) which in turn activate caspases such as –9,–8, and –3 and this action in directly induces apoptosis[26].

Biopigments produced by bacteria possess enormous efficiency as medicinally important products. Prodigiosin, a red pigment, belongs to the family of tripyrrole was found to exhibit antibacterial, antimycotic, immunomodulating, anti-tumor and anti-malarial properties. These pigments were found associated with the cell wall vesicle of the bacteria.[19].

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