Optimum conditions for Prodigiosin production by *Serratia marcescens* S11

تعيين الظروف المثلى لانتاج البرودجيوسين بوسطة بكتريا *Serratia marcescens* S11

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Abstract

Different nutritional and cultural factors were studied to determine the optimum conditions for prodigiosin production by *Serratia marcescens* S11 in a batch culture of brain-heart infusion broth medium. These factors include carbon source and its concentration, nitrogen source and its concentration, phosphate source, temperature and pH. Results showed that the optimum conditions for prodigiosin production were achieved when the production medium was supplemented with olive oil and casein hydrolysate as a carbon and nitrogen sources respectively in a concentration of 1.5% for broth, KH_2PO_4 as a phosphate source at initial medium pH8, and incubation at 28°C for 24 hours. Under these optimal conditions, prodigiosin activity produced by *Serratia marcescens* S11 in culture medium was increased from 200 U/cell before optimization to 3000 U/cell.

المستخلص

درست بعض العوامل التغذوية والمزرعية المؤثرة لتعيين الظروف المثلى لانتاج البروديجيوسين بوساطة بكتريا *Serratia marcescens* S11 في وسط مرق نقيع الدماغ القلب باستخدام مزرعة الدفعة . وقد شملت هذه العوامل كل من نوع وتركيز المصدر الكاربوني ، نوع وتركيز مصدر النايتروجين ، نوع مصدر الفوسفات، درجة الحرارة ، والرقم الهيدروجيني لوسط الانتاج . أظهرت النتائج ان الظروف المثلى لانتاج البروديجيوسين تضمنت تدعيم وسط الانتاج بزيت الزيتون ومتحلل الكازئين كمصدر كاربوني ونايتروجيني على التوالي بتركيز 1.5 % لكل منهما ، واستخدام فوسفات البوتاسيوم ثنائية الهيدروجين مصدرا فوسفاتيا ، وكان الرقم الهيدروجين الامثل لوسط الانتاج pH8 ، ثم الحضن بدرجة 28 مئوي لمدة 24 ساعة . وقد ازدادت فعالية البروديجيوسين المنتج من بكتريا *Serratia marcescens* S11 تحت هذه الظروف من 200 وحدة/خلية الى 3000 وحدة/خلية .

Introduction

Prodigiosin is a secondary metabolite (red pigment) produced by *Serratia marcescens* and many other grams negative and positive bacteria [1]. It was of great interest in medicine due to its antifungal, antibacterial, antiprotozoal, antimalarial, immunosuppressive, and anticancer activities [2, 3].

There are many conditions that affect the productivity of prodigiosin pigment, which include pH, temperature, carbon source, nitrogen source, and phosphate source. Optimum growth of all strains of *Serratia* has been observed at pH 7 and optimum pH for prodigiosin production is between (8.0-8.5) [4]. The temperature is considered as one of the most important factors affecting pigment productivity and the growth of the microorganisms. Optimum growth of all strains of *Serratia* has been observed at a temperature from (20-37) ˚C, while the optimum temperature of prodigiosin production was at 30˚C [5]. Microorganisms differ in their needs to carbon sources

according to their nutrient nature; the use of pure carbon sources e.g. (glucose, sucrose and fructose) is expensive from the economical case, so the industrial fermentation try to use cheap carbon sources especially industrial and a variety of plant seed oils have also been used as carbon substances for prodigiosin production and displayed stimulatory effects on the production by *S. marcescens*. Mineral salts have an effect on the production of prodigiosin and there are several studies which demonstrated that synthesis of prodigiosin by non-proliferating cells of *S. marcescens* is depends on the presence of inorganic phosphate (Pi) concentrations [6,7]. According to the importance of prodigiosin in different applications, this study was aimed to determine the optimum conditions for prodigiosin production by local isolate of *S. marcescens*

Materials and Methods

S. marcescens S11 was obtained from previous study [8], and was maintained on slants of Nutrient agar medium kept at 4ºC**.** Fresh cultures were obtained by inoculating Nutrient broth medium with a loopful of stock culture, and incubated at 28 ºC for 16 hours in a shaker incubator (150 rpm)**.**

Optimum conditions for prodigiosin production

Optimization of prodigiosin production by *S. marcescens* S11 was carried out aerobically in the production medium (brain-heart infusion broth) under batch culture conditions. Optimum conditions include type and concentration of carbon source, type and concentration of nitrogen source, type of phosphate source, temperature, and pH.

Assay of prodigiosin activity

Prodigiosin activity (U/cell) was measured spectrophotometrically according to Haddix [9], by inoculating brain-heart infusion broth with 1% of mid-exponential phase culture of *S. marcescens* S11 and incubated with shaking at 150 rpm in a shaker incubator at 28 ºC until the optical density of the growth medium was reaches 0.75 at 620nm. At the same time, optical density of the growth medium was also measured at 499nm (λ-max of prodigiosin absorbance), then prodigiosin activity was calculated according to the following equation against blank of growth medium:

$$\text{Prodigiosin activity (U/Cell)} = \frac{[O.D_{499} - (1.3831 \times O.D_{620})] \times 1000}{O.D_{620}}$$

Results and Discussion

Optimum conditions for prodigiosin production

Effect of Carbon Source

Six carbon sources (fructose, glucose, sucrose, lactose, olive oil, and sunflower oil) were used as a sole source of carbon and energy to determine the optimum in prodigiosin production by *S. marcescens* S11, these carbon sources were added to the production medium in a concentration of 2%.

The results in Figure (1) showed that the maximum production of prodigiosin was obtained when the culture medium supplemented with olive oil as a sole source for carbon and energy. The prodigiosin activity in culture medium was 440.3 U/cell. This may due to olive oil which contains many nutrients essential for growth requirements for the microorganism such as fatty acids, growth factors, vitamins and minerals [10]

In addition, *S. marcescens* has lipase activity and thereby was capable for hydrolyzing oil substrates to liberate fatty acids as a sole source for carbon and energy [11], while glucose may inhibit prodigiosin production due to catabolic repression or by lowering medium pH during growth and fermentation [12].

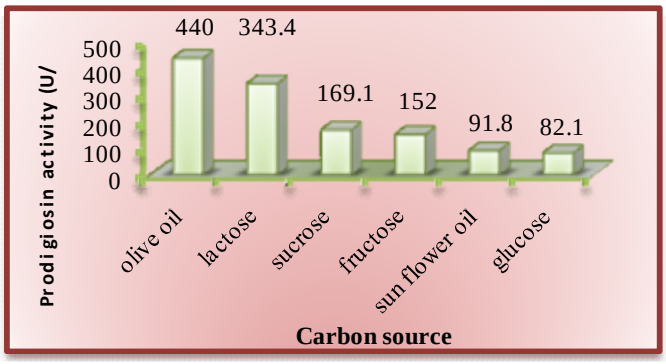

Fig (1): Effect of carbon source on prodigiosin production by *S. marcescens* S11 after incubation in shaker incubator (150 rpm) for 24hrs at 28°C.

Effect of carbon source concentration

The optimum carbon source (olive oil) was used to supplement the production medium in a concentration of (1, 1.5, 2, 2.5, 3, 3.5) % (v/v) to determine the optimum concentration for production of prodigiosin by *S. marcescens* S11.

Figure (2) showed that the maximum production of prodigiosin was obtained when the concentration of olive oil was 1.5%, at this concentration the prodigiosin activity in culture medium was 833.3 U/cell. This may indicate that this carbon source concentration was the best for providing the microorganism with the needed energy for growth and maximum production of the pigment. Other studies indicated that the optimum carbon and energy source for the prodigiosin production varies between different concentrations of olive oil, for example [13] referred that 4% (w/v) was the optimum carbon source for prodigiosin production by *S. marcescens* SMΔR.

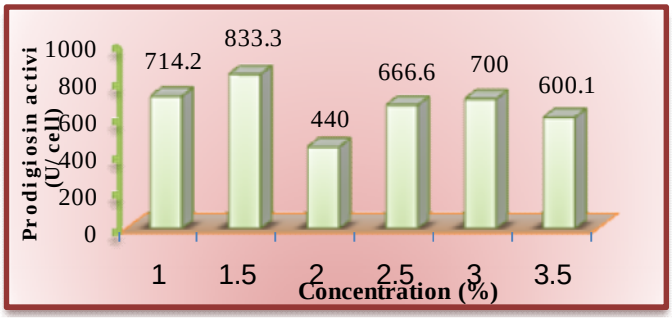

Fig (2): Effect of olive oil concentration on prodigiosin production by *S. marcescens* S11 after incubation in shaker incubator (150 rpm) for 24 hrs at 28°C.

Effect of nitrogen source

Five nitrogen sources were used to supplement the production medium for enhancing prodigiosin production by *S. marcescens* S11, three of these nitrogen sources are organic (peptone, tryptone, and casein hydrolysate), and two inorganic (ammonium nitrates and ammonium sulphate). These sources were added to the production medium instead of tryptone and yeast extract in a concentration of 1.5%.

The maximum production of prodigiosin was noticed in medium was supplemented with casein hydrolysate as an organic nitrogen source. The prodigiosin activity in culture medium using this nitrogen source was 922 U/cell Figure (3). This result may be attributed to the type of nitrogen source and its growth factors contents that supplements bacterial requirements for growth, production and secretion of prodigiosin to culture medium as mentioned by [13]. Furthermore, the production of prodigiosin in culture medium by *S. marcescens*S11 using organic nitrogen sources (peptone, tryptone, casein hydrolysate) was better than the prodigiosin production using inorganic nitrogen sources (ammonium sulfate, ammonium nitrate) under the same condition. The increase in the production of prodigiosin using the casein hydrolysate may be attributed to its natural component that provide the medium with nitrogen source which contributed in the supporting of bacterial biomass, also it contains trace elements such as Ca, Mg, and carbohydrates that provide the optimum condition for pigment activities especially those enzymes responsible for biosynthesis of prodigiosin, and as it was mentioned by [14].

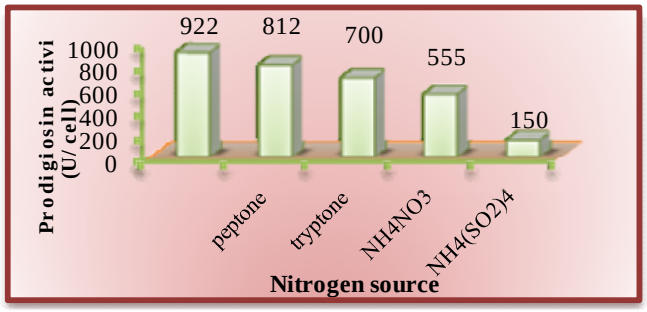

Fig (3): Effect of nitrogen source on prodigiosin production by *S. marcescens* S11 after incubation in shaker incubator (150 rpm) for 24hr at 28°C.

Effect of nitrogen source concentration

Different concentrations (0.5, 1, 1.5, 2, 2.5) % w/v of the optimum nitrogen source, casein hydrolysate were used to supplement the production medium.

Figure (4) showed that the maximum production of prodigiosin was recorded in concentration of 1.5% of casein hydrolysate, the prodigiosin activity was 1000 U/cell. On the other hand, the increase or decrease of casein hydrolysate concentrations above or below the optimum concentration value causing a decrease in prodigiosin production, this may due to the change in the C/N ratio in production medium that affects different secondary metabolites pathways especially those responsible for prodigiosin production and as it was mentioned by [13].

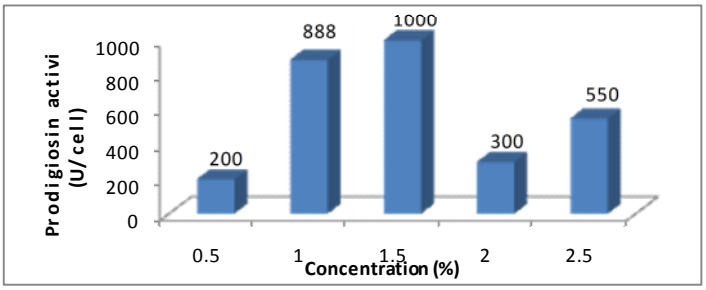

Fig (4): Effect of casein hydrolysate concentration on prodigiosin production by *S. marcescens* S11 after incubation in shaker incubator (150 rpm) for 24

Effect of phosphate source

Different phosphate sources were also studied to determine the optimum one for prodigiosin production by *S. marcescens* S11. Two types of phosphate sources (KH_2PO_4 and K_2HPO_4) were added to the production medium at a concentration of 0.1% , and a mixture of them (0.07% of KH_2PO_4 and 0.03% of K_2HPO_4) were also used. The results indicated that addition of KH_2PO_4 to production medium was showed an increase in prodigiosin activity (1500 U/cell) in comparison with K_2HPO_4 or when used both of them Figure (5). The presence of phosphate in culture medium works as a buffering capacity when medium become alkaline due to the biosynthesis of prodigiosin [15].

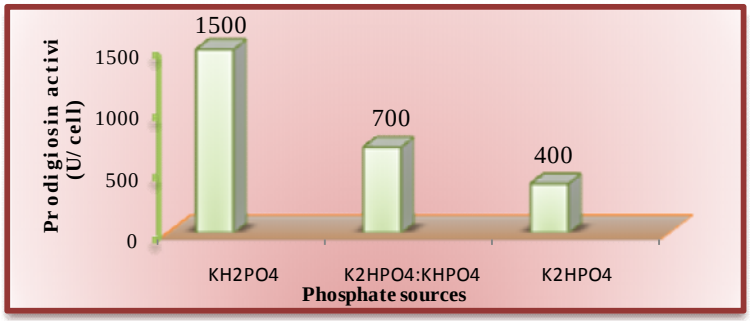

Fig (5): Effect of phosphate sources on prodigiosin production by *S. marcescens* S11 after incubation in shaker incubator (150 rpm) for 24hrs at 28°C.

Effect of Temperature

In order to determine the optimum incubation temperature for prodigiosin production by *S. marcescens* S11, different incubation temperatures (24, 28, 32, 36, 40) ºC were used for this purpose.

From Figure (6) it was found that the maximum production of prodigiosin was obtained when the temperature of fermentation medium was 28˚C. At this temperature, prodigiosin activity in culture medium was 2714 U/cell. This temperature was the optimum for bacterial metabolism and prodigiosin production as a secondary metabolite. Other studies showed that the optimum temperature for prodigiosin production was 30˚C [16,17].

A block in prodigiosin production occurred above 30°C in culture medium, while the presence of fatty acids in culture medium supported prodigiosin production up to 42°C [8].The increase in temperature to 40°C led to decrease in the bacterial growth rate and made the conditions unsuitable for prodigiosin production and finally led to repress the expression of genes responsible for prodigiosin with less effect on bacterial growth (10), or may repress the genes responsible for Prodigiosin Condensing Enzyme (PCE) which are sensitive to high temperature [18].

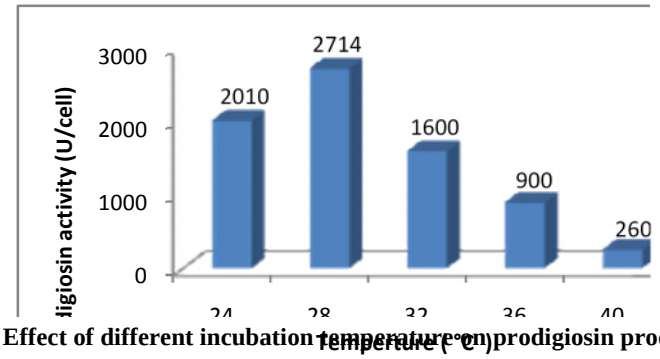

Fig (6): Effect of different incubation temperature on prodigiosin production by *S. marcescens* S11 after incubation in shaker incubator (150 rpm) for 24hrs at 28°C.

Effect of pH

Different pH values (7.0, 7.5, 8.0, 8.5, 9.0) were used to determine the optimum for prodigiosin production by *S. marcescens* S11. As it was shown in Figure (7), the maximum production of prodigiosin was obtained when the pH value was 8.0, at this pH the prodigiosin activity in culture medium was 3000 U/cell. This result was in agreement with [18] who noticed that the same result when the production medium was adjusted to alkaline pH. In addition, results showed that the increase or decrease in the pH value of the production medium above or under the optimum pH decrease prodigiosin production, this may due to the alteration of the activities of all genes responsible for prodigiosin biosynthesis [12].

The effect of pH value on pigment productivity is due to two reasons, the first is the effect on the properties of the culture medium including the solubility of the nutrients, transport and ionization, and the second is the effect of pH on the stability of the pigment.

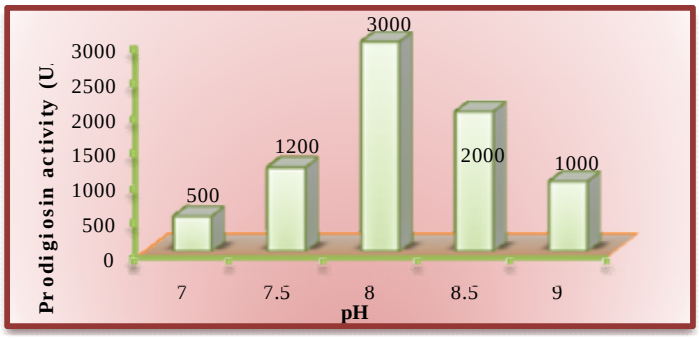

Fig (7): Effect of medium pH on prodigiosin production by *S. marcescens* S11 after incubation in shaker incubator (150 rpm) for 24hrs at 28°C.

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