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DETERMINE THE OPTIMUM CONDITIONS FOR PRODUCING PRODIGIOSIN PIGMENT FROM SERRATIA MARCESCENS CLINICAL AND ENVIRONMENTAL ISOLATES

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Abstract
Serratia marcescens isolates were isolated from various clinical and environmental sources, the 60 sample include 12 isolates were obtained from diverse clinical specimens and environment and all isolates produced prodigiosin. Following multiple screening stages, the local isolate *Serratia marcescens* SM5 was chosen due to its high prodigiosin dye production. Environmental and clinical samples were cultured on MacConkey agar medium. The media was incubated at 37 °C for 24 hours. The color and shape of the colonies in the culture media, as well as the cell shape and pigmentation in the microscopic examination, were used to determine the phenotypic traits. To confirm the type of isolates, biochemical tests were done. and the Api 20 E. method was used to identify the isolates. The best conditions for producing prodigiosin dye were discovered. The maximum dye output was discovered while employing a peptone-glycerol medium at pH 8 and 30 °C for 72 hours.

1. Introduction

Serratia marcescens bacteria are widely distributed in the environment, as evidenced by their isolation from soil and water, plant surfaces, and many types of seafood, milk, and other foods (Khanafari et al., 2006; Falkiner, 1997) as it causes respiratory and urinary tract infections, as well as eye infections, wounds, burns, arthritis, and meningitis (Su et al., 2003). *S. marcescens* bacteria are distinguished by the production of the prodigiosin dye during the stationary phase, which is a red dye that binds to the cell membrane of bacteria and is not disseminated in the medium, It does not dissolve in water, except that it dissolves in organic solvents. Among these are chloroform, alcohols, bromoform, benzene, and dimethyl sulfoxide, a three-ring pyrrole molecule with a tiny molecular weight of 323.4 daltons. It is synthesized by two metabolic pathways, the first ending with the formation of mono-pyrol (MAP) (2-methyl-3-amyl-pyrrol), and the other ending with the formation of dipyrrole (4-methyl-2,2-bipyrrole-5-carboxaldehyde, or MBC), which then combines at the cell wall with the help of the prodigiosin condensing enzyme (P.C.E.) to form the pigment prodigiosin (Williamson et al. 2005; Srijith, 2006). The dye is produced by Serratia bacteria thanks to a collection of genes found on either the cell chromosome or the plasmid, or both. Prodigiosin dye is very important since it has anticancer activity as well as activity against several types of bacteria., fungi and algae (Nakashima et al., 2005; Song et al., 2006). This study aimed to determine the optimal condition for prodigiosin by *Serratia marcescens*.

2. Materials and Methods

Environmental and clinical samples were cultured on MacConkey agar medium in a streaking manner using a sterile culture media , and the media was incubated at 37 °C for 24 hours. The color and shape of the colonies in the culture media, as well as the cell shape and pigmentation in the microscopic examination, were used to determine the phenotypic traits. To confirm the type of isolates, biochemical tests were done. Finally, the Api 20 E. method was used to identify the isolates.

2.1 Prodigiosin pigment production

According to Haddix and Werner (2000), the isolates were activated by growing them on Brain Heart Infusion (BHI) broth medium. A bacterial cell suspension was prepared and standardized to an optical density (OD) of 0.75 at a wavelength of 620 nm. And finally inoculating the Brain Heart Infusion broth with bacteria at a rate of 1% and two repeats for each isolate, while leaving an uninoculated control tube. After 72 hours of incubation at 37 °C, the absorbance of the bacterial culture was measured at wavelengths 499 and 620 nm, and the amount of dye was determined using the following equation:

Prodigiosin U/Cell = [OD_{499} – (1.3831 x OD_{620})] x 1000/ OD_{620}

OD_{499} represents the absorbance peak of the dye.

OD_{620} represents the uptake of bacterial cells.

1.3831 is a correction constant.

1000 is a scaling factor to avoid dealing with numbers smaller than one.

2.2 Optimal conditions for production

The effect of some factors was studied to determine the optimal conditions for the production of prodigiosin pigment, including:

1. Determining the optimum incubation
   The peptone-glycerol broth media was prepared and incubated after inoculation with 1% of the activated bacterial culture at a temperature of 30 °C for periods of time: (24, 48, 72) hours, after which the amount of dye produced was estimated.

2. Determining the optimum temperature
   Peptone-glycerol broth media were created and inoculated with 1% of the bacterial culture of the selected and activated isolate, and the media were incubated at different temperatures of 26, 28, 30, 32, and 36 for 72 hours, and the amount of color produced was estimated.

3. Determination of the optimum pH
   Peptone-glycerol broth media with different pH numbers (6, 7, 8, 9 were prepared using sodium hydroxide and hydrochloric acid, and the media were inoculated with 1% of the bacterial culture of the selected and activated isolate and incubated at 30 °C for 72 hours, the amount of dye produced was estimated.

3. Result and Discussion

The phenotypic characteristics of bacterial isolates grown on the MacConkey agar medium at 37°C were studied in terms of the shape and size of colonies. The shape of the bacteria colonies is circular in shape, sticky in texture, and pink to red in color from fermented sucrose lactose. A microscopic inspection of a smear collected from a single colony of bacteria after Gram staining revealed that they are short, rod-shaped cells, red in color, and Gram stain negative. (Bayona et al., 2009 ; Holt et al., 1994). Twelve Serratia bacterium isolates were identified. Two urine samples, two sputum samples, and two eye puss samples and six from wounds were among the positive clinical samples. According to studies, this bacteria can be identified in instances of urinary tract infection (Shih et al., 2005; Ania, 2008). Other research suggests that Serratia bacteria can contaminate wounds caused by surgery or accidents. (Kumar and Worobee, 2005).

3.1 The best isolate test for prodigiosin pigment production

The isolates were cultivated in the heart and brain infusion broth for 72 hours at 73 degrees Celsius, and the best isolate was determined to yield prodigiosin dye. This disparity in prodigiosin dye production between isolates is attributed to genetic variances or the diversity of their isolation sources, which comprise clinical sources (urine, sputum, wounds), environmental sources (soil, milk).

3.2 Factors affecting the production of prodigiosin pigment

3.2.1 Determining the optimal incubation

The bacteria were cultured for 24 hours, 48 hours, and 72 hours to determine the appropriate incubation period for the generation of prodigiosin dye (Figure 1). That is, the cells require time for the dye to begin production. After 24 hours of incubation, a small amount of dye is produced, so the middle is a light pink, and the color of the medium darkens as the incubation period increases until the dye production reaches its peak in the stage of stability at 72 hours because the dye is attached to the cell wall, It will be released following the death of some cells during the stability process, and the dye will be released to the medium, resulting in the dye appearing clearly, as mentioned by Williams, (1973) and Cang et al., (2000).

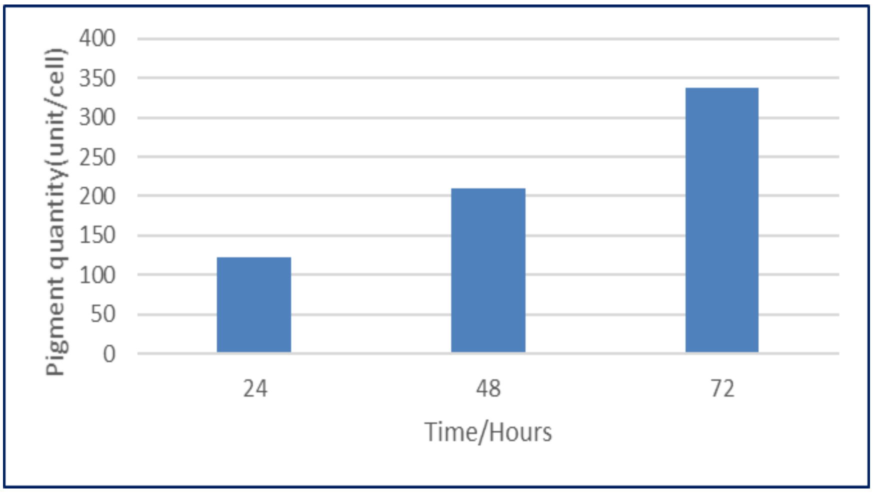

Figure 1. Production of prodigiosin pigment from isolate SA5 and its growth in the medium of peptone-glycerol broth at 30°C and with different periods of time.

3.2.2 Determining the optimal incubation

The temperature of the environment or surroundings in which the bacteria live has a significant impact on their metabolic activities, as the effect of different temperatures (26, 28, 30, 32, and 36°C) was studied in the production of prodigiosin dye, and the results (Figure 2) showed that the dye production was good within the range 28–32 ° C, but the amount of dye produced decreased when the temperature was reduced to 25 ° C or increased to 36 °C. It was also discovered that a temperature of 30 ° C was the best for dye production, which is supported by the findings of certain researchers. (Robert et al., 1971; Holt et al., 1994; Greenwood et al., 2002), while other studies indicated that the best temperature for dye production is 28 °C (Cang et al., 2000) ; Giri et al., 2004; Al-Murjani, 2005). This temperature difference may be due to the nature of the culture medium's components, as the optimum temperature for dye production when growing bacteria in peptone-glycerol broth is 30 ° C, while the optimum temperature for dye production when growing bacteria in nutritious broth is 28 °C. When cultured in media containing oilseed extracts such as pistachio seeds, sesame seeds, and sunflower seeds, this bacteria may produce the color at temperatures greater than 42° C (Giri et al. , 2004).

The lack of prodigiosin pigment production at high temperatures can be attributed to several factors, including: the culture medium and high temperature, which may lead to suppression of the genes responsible for pigment production expression without affecting bacterial growth (Haddix and Werner, 2000), or high temperature may lead to suppression of pigment production genes. The action of the enzyme responsible for the intensification of dye-generators (P. C. E.), as it is sensitive to high temperatures (Furstner, 2003), and the growth of bacteria at high temperatures may lead to a lack of dye production as it is a secondary metabolite that is not required for the survival of bacteria, and this explains why the isolates were found to be lacking in dye production. The dye does not produce the dye because its growth at the human body temperature of 37°C is not at the optimum temperature for its production, which is a stressful factor for the bacteria and causes them to save the energy required for the dye's formation in the production of other substances that aid in the colonization of the bacteria in the host (Vining, 1990; Carbonell et al., 2000; Nystrom, 2004).

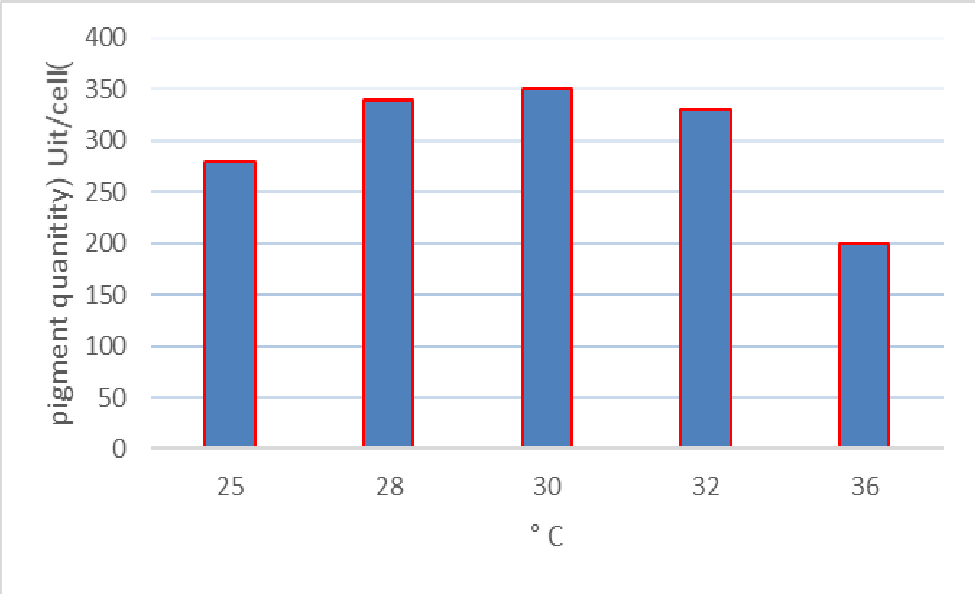

Figure 2. Production of prodigiosin pigment from isolate SA5 when grown in peptone-glycerol broth medium at different temperatures for 72 hours.

3.2.3 Determining the optimum pH

Because the pH of the media in which the bacteria grow affects dye production, five different pH levels (5–9) were used to discover the best pH for dye production. And Wei et al. (2005b) reported the reason for this is that pH 8 inhibits the activity of the enzyme Proline Oxidase, which causes the catabolism of proline, which is an essential amino acid in the synthesis of the pigment pyrrole binary MBC, as inhibiting this enzyme leads to an increase in proline acid concentration. Proline concentrations in the media and their application in the formation of the MBC's pyrrole ring. In addition, a high or low pH of 8 leads to disruption and disruption in the vital pathways leading to dye synthesis by affecting the activity of enzymes responsible for dye synthesis (Sole et al., 1997).

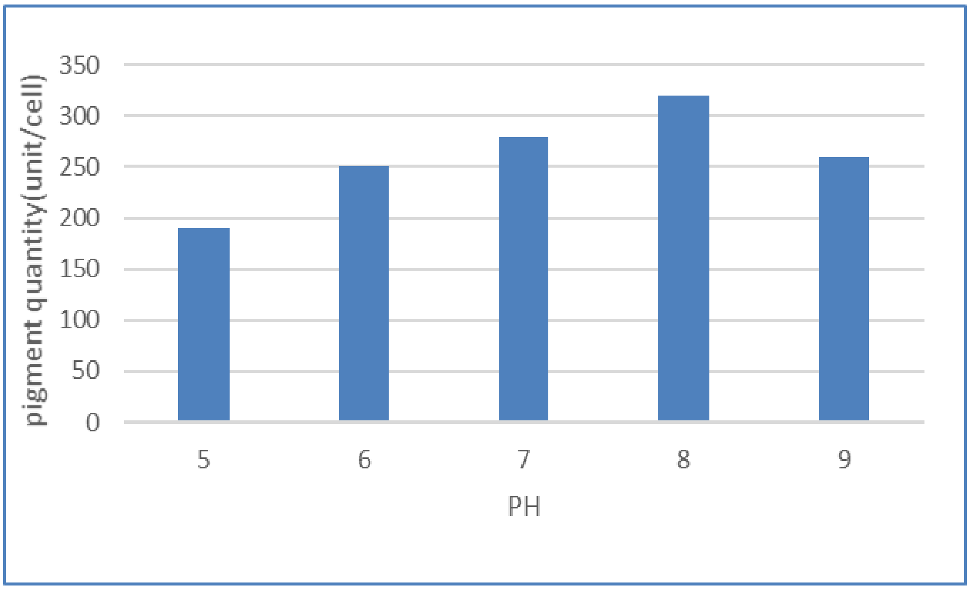

Figure 3. Production of prodigiosin dye from isolate SA5 when grown at different pH numbers at 30°C for 72 hours.

4. Conclusion

Serratia marcescens bacteria are distinguished by the production of the prodigiosin dye during the stationary phase, clinical and environmental isolates can produce the prodigiosin pigment at optimal condition for 72 hours in peptone-glycerol broth medium at 30°C and pH 8.

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