

Effect of purified Pyocyanine produced by *Pseudomonas aeruginosa* on other microbes.

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Abstract

In this study *Pseudomonas aeruginosa* was isolated and purified from burns and wounded patients who attending and residents in some Baghdad hospitals. The high pyocyanine production isolates were selected and the identification of *Pseudomonas aeruginosa* according to biochemical & morphological tests were done.

The effect of physical conditions (pH and temperature) on pyocyanine production was studied.

The results showed that the optimal pH and temperature were (7.5; 37°C) Respectively. The different concentrations of (0, 25, 50, 100, 200, 300) µg/ml Showed the *Staphylococcus* and *streptococcus* were inhibited at 50 µg/ml, while the *clostridium* at 300 µg/ml, but the Genus *Pseudomonas* had not been affected at all The concentrations of the pigment.

		<i>Pseudomonas aeruginosa</i>	
		()	
		(37 7.5)	
<i>Staphylococcus</i>			
300	<i>clostridium</i>	/	50 <i>streptococcus</i>
		<i>Pseudomonas</i>	/

1-Introduction

1-1- *Pseudomonas aeruginosa*.

This bacterium is a cylindrical an aerobic organism, gram negative, non-forming spores, motile by mono-polar flagellum, living on different media with optimal temperature more than 37°C, resistant to many antiseptics and antibiotics (Gupte, 1998).

This bacterium is an opportunistic pathogen in human, causing many adverse effects, like cystic fibrosis(Williams *et al.*,2007) or direct tissue damage with a greater cytotoxic potential like Urinary Tract Infection (Buonanno and Damweber, 2006), pneumonia(Sadikot *et al.*, 2005).

Many virulent factors produced by this pathogen effects the human body during infection causing both acute and chronic diseases, these factors are either enzymes like elastase (Prithiviraj *et al.* ,2005) or maybe toxins like LPS that suppresses host immune response causing persistent infection(Cryz *et al.*,1984),and sometimes poison pigments which is considered a by- products of this pathogens like a redox cycling phenazine pyocyanine (Price-Whelan *et al.*,2007) and its derivative 1-hydroxyphenazine(Muller and Sorrell,1995), and they have anti-microbial growth behavior specially Gram negative bacteria.

Medical treatment may reduce colonization of many pathogens including *Pseudomonas aeruginosa* and Immunoprophylaxis against *Pseudomonas* is being investigated (Döring and Pier, 2008).

1-2-Pyocyanine.

It is green-blue pigment, water soluble, secondary metabolite, and considered as one of the phenazine derivatives and contain methyl group, and when this pigment loses this methyl group becomes another pigment called 1-Hydroxyphenazine with golden color and without charge (Budaver *et al.*, 1989).

The pyocyanine is one of the toxic virulent factors of *Pseudomonas aeruginosa* (O'malley *et al.*, 2003) associated with direct damage to the host tissue and impacting the host immune response (Buret and Cripps, 1993), causing patho-physiological adverse effects in many organs and tissues (Look *et al.*, 2005).

This pigment influenced the diversity of the microbial community and suggests the importance of understanding how interspecies interactions influence the capability of a microbial community (Norman *et al.*, 2004). Degradation.

So the aims of this study firstly is to find out the effect of this pigment on different species of microbes like (*Staphylococcus*, *Streptococcus*, *Escherichia coli*, *Clostridium* and *Penicillium*, *Pseudomonas aeruginosa*) and secondly to know the optimal circumstances like (pH, Temperature) for this pigment production.

2-Materials and Methods

2-1-Materials and Instruments

Glycerol
Peptone
Magnesium sulfate
Citric acid
Glucose
Potassium phosphate
Chloroform
Incubators
Spectrophotometer

2-2-Preparation of King-A medium.

This medium was prepared according to (King *et al.*, 1954).

2-3-Isolation and Diagnosis of *Pseudomonas aeruginosa*.

Fifty isolates were collected of this bacterium from Burns and wounds of patients who attending and residents in some Baghdad hospitals according to (Holt *et al.*, 1994) method, and just one isolate which highly pyocyanine productivity was chosen.

2-4-Effect of Temperature and pH on pigment production.

Three flasks were containing King-A media with this isolate, and each flask with different pH (5.5, 6.5, 7.5) and each flask was put in shaker incubator for (11) days, then, the effect of temperature on pyocyanine production was studied by inoculation of these three flasks with this bacterium and incubated at different temperatures (25°C, 37°C, 42°C).

2-5-The effect of different pyocyanine concentrations on other microbes.

Different concentrations of pyocyanine were prepared (0, 25, 50, 100, 200, 300) µg/ml to see their effects on different microbes (*Staphylococcus*, *Streptococcus*, *Escherichia coli*, *Clostridium* and *Penicillium*, *Pseudomonas aeruginosa*).

3-Results.

The results showed that the best and optimal effect of pH on pyocyanine productivity was 7.5 and the pigment concentration was measured by µg/ml

According to the following equation.

$$\text{Pyocyanine concentration} = \frac{\text{Optical density with wave length 690nm}}{4.3 \times 10^6}$$

The results indicated that the optimal temperature for pyocyanine production was 37°C after (11) days incubation (Table-2), meanwhile (Table-3) showed the effect of different concentrations on different microbes (Bacteria and fungi) and the results showed differences in the inhibition activity of pyocyanine on these microbes growth, and the *Pseudomonas aeruginosa* was able to grow in the all concentrations of pyocyanine which means that it was not affected by the pigments which it produces.

Table-1: Effect of pH on pyocyanine production.

Pyocyanine concentration pH	Absorption pyocyanine Reading(nm)	Pyocyanine concentration µgm/ml
5.5	0.42	0.021
6.5	0.61	0.030
7.5	0.73	0.034

Table-2: Effect of temperatures on pyocyanine production.

Temperature/ °C	Pyocyanine concentration µgm/ml
25°C	0.022
37°C	0.035
42°C	0.025

Table-3: Effect of different Pyocyanine concentrations on microbes' inhibition.

Pyocyanine concentration µgm/ml	Inhibited microbes types
0.0	No inhibited microbe
25	No inhibited microbe
50	<i>Staphylococcus, Streptococcus</i>
100	<i>Escherichia coli</i>
200	<i>Penicillium</i>
300	<i>Clostridium</i>

4-Discussion

Pseudomonas aeruginosa is considered on of mesophilic for neutral pH (Weak basic and weak acidic), so the results was found that the pyocyanine production was higher in pH (7.5) and this results agree with (Palumbo, 1972) who indicated that the pyocyanine production influenced by the pH and the best pH for its production is (7-8.2), and the presence of sulfur in the media increases its production, while the presence of iron decreases its production.

Some strains of this bacterium have severe pathogenesis, so that it prefer the 37°C temperature, and 50% of the *Pseudomonas aeruginosa* pathogenic strains produce the pigment at 37°C and numbers of amino acids exist singly or with glycerol leads for Pyocyanine production (Knight *et al.*, 1979)

The pyocyanine showed high inhibition activity for *Penicillium* at 200µgm/ml because the pyocyanine possess broad inhibition spectrum against micro-Organisms (Muller, 1995).

The pyocyanine pigment has inhibitory effects for Gram positive bacteria like *Staphylococcus* and *Streptococcus*, in addition to that it also has inhibitory effect for Gram negative bacteria like *Escherichia coli* except Genus *Pseudomonas* which resist The effect of this pigment and this result agree with (Sorensen and Klinger, 1987) who they said that this pigment has inhibitory effect for Gram negative bacteria.

The *Pseudomonas aeruginosa* pyocyanine is toxic redox cyclic agent for many types of cells like T lymphocytes (Epelman *et al.*,2002),B lymphocytes(Ulmer *et al.*,1990), macrophages (Thomassen *et al.*,1997) or generally Causing direct tissue damage(Abusriwil and Stockley,2007), and also has another effect on many other organisms like nematode killing (Gallagher *et al.*,2002).

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