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CATALYSING THE PRODUCTION OF LIPOPROTEIN FROM *Pseudomonas aeruginosa* USING SOME HYDROCARBON MATERIALS

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ABSTRACT:

Lipoprotein is a bacterial cyclic surface active compound produced mainly by different microorganisms like bacteria, fungi and yeasts as secondary metabolites, Fourty eight bacterial isolates were obtained from 20 soil samples that oils contaminated from various locations in Al-Najaf city. About 30 samples have been identified as belonging to the Pseudomonas genus. All of these samples were succumbed to two tests: microscopy exam and cultural features. Approximately 16 samples were found to retain to the genus. *P. aeruginosa* .About 5 isolate were selected for the purpose of knowing their ability to produce lipoprotein and all these isolates were screened for their ability for lipoprotein production through blood haemolysis test on blood agar medium indicated that all isolates were β -haemolysis , then the isolates stimulated the production of lipoprotein by adding catalysts to the medium, which is the substances of kerosine and benezene .The results showed that the material is the most efficient in increasing production, reaching 0.38 g / l in the case of benzene and 0.31 g / l in the case of kerosene.

AIMS OF STUDY:

This study aimed to investigation the ability of *P. aeruginosa* to production of lipoprotein in hydrocarbon contaminated soil and stimulated by some petroleum catalysts.

INTRODUCTION:

Lipoprotein is a natural substance that arises from microbes. A number of yeast, bacteria, and filamentous fungus synthesize these lipophilic biological substances extracellular and found in cell membrane (1). One of the most important characteristics of these antibiotics is their ability to lower surface tension. Lipoproteins are important constituents in a variety of products, including antibacterial, polymeric, lubrication, and solvents.in detergents shampoo toothpaste, oil additives, and in many industrial products. The total lipoprotein production has exceeded 3 million tons in the year; the rate of expansion is linked to global consumption, which requires more than half of all lipoprotein production (2). Lipoproteins are complex lipophilic molecules that contain proteins, lipids, phospholipids, glycolipids, antimicrobials, and lipoproteins, among other chemical types. As a result, there is a growing interest in these microbial productions as chemical substitutes (3).

Several studies have been published on the synthesis of various types of lipoprotein by microbes using water-soluble substances as substrates, such as many saccharides, ethanol, or glycerol (4). The petroleum industry has been identified as one of the companies with a high potential for creating a microbe capable of making lipoprotein. Here, the emphasis has been on refining the method of lipoprotein manufacturing, which is critical in commercial applications.

METHODS:

Soil Samples Collection:

Twenty soil specimens were obtained

from various places of Al- Najaf city in October. Samples were taken from soil contain hydrocarbon compounds such as kerosene and benzene which taken below the soil surface, hydrocarbons soil beneath cars fuel stations were the prefer area for isolation bacteria .The specimens was placed in sterilized plastic and transported to the laboratory, where it was examined for bacteria isolation.

Isolation of Bacteria:

Five gm of hydrocarbon soil are placed in 50 ml of brain broth medium and incubated in 250 C° for 72 hrs. followed by incubation, the media was sequentially dissolved in distilled water and serially from 10^{-1} to 10^{-6}. l ml was placed to Petri dishes from the dilutions and 20 ml of brain agar was put over it. The platters then were inverted and incubated for 48 hrs. at 250 C°. The control and duplicate plates were kept the same. Following incubation, visually distinct colonies were chosen for further investigation.

Identification of *Pseudomonas sp*:

Diagnosed *Pseudomonas sp*. depending on the culture, microscopic, biochemical testing is as follows:

Morphological and Cultural Features:

After incubation for 24 hrs, the form, volume, coloring, and corner of bacterial cultures were examined on nutrient agar plates (5).

Microscopically Testing:

Gram's Stain:

Transferred a single pure culture from each bacteria that isolated to a sterile slide and fixed by flame, the smear was stained with Gram stain to study its Gram reaction and under the light compound microscope (6).

Biochemical Test:

Catalase Test:

Take the amount of bacterial culture 24 hrs. on a clean slide and then add one drops of H_2O_2 concentration (3%) on culture, formation of air bubbles is evidence of the positive result (7) .

Oxidase Test:

Filter paper was saturated with the substrate (Tetra-Methyl-P-phenylene diamine - dihydrochloride), and directly add bacterial colony to be tested age 24 hrs. by a sterile wooden stick on the filter paper ,the color change to dark purple during (2-10) sec. indicated a positive examination (8) .

Citrate Utilization:

This test was an indicator for utilization of citrate by bacteria a one carbon supply in which Simmon's citrate slant was infected with a young colonies and then incubated at 30ºC for 3 days , the formation of deep blue color indicated on positive result (9).

Sugar Fermented Test:

Inoculated a serial tube containing sugar fermented medium with bacterial culture by loop and incubated at 37 ºC for 24 hrs. , the result is positive when changing media color from red to yellow, a sign of the sugar fermentation and acid production. Also appearance of air bubble in durhum tube due to formation gas which indicated of positive result (6).

Screening of Lipoprotein Production Blood Haemolysis Test:

Blood agar base was inoculated by bacterial culture using streaking methods, then dishes were incubated at 37 ºC for 24 hours ,morphology of a definite area (β-haemolysis) in all sides of the colonies indicated of concerned *Pseudomonas* colonies

were selected(10).

Production of Lipoprotein:

The inoculum of bacteria used for inoculated of 50 mL brain broth with 1 mL Benzene and 1 mL gasoline in another brain broth followed by incubated the culture at 250°C for week with stirring using shaker incubator , after the end of the incubation period ,centrifugation was used to extract the cells of bacteria (sedimentation) and leave it . The stench is taken and The pH of it was lowered to 2 with 1MH2SO4, and now an equal amount of chloroform: methanol was added (2:1). This solvent with stench was well mixed before being allowed overnight to evaporate. As a result of the process, a gray-color participate was produced it was considered "Lipoprotein".

Estimation of Lipoprotein Dry Weight:

The weight of lipoprotein was estimated using a sterile slender. Then the sedimentation now was spreading on the surface of the plates. Then left to dry in oven for 30 min at 60^0C, followed by weighted the slender once they had dried. The drying weight of production can measured by equation below:

Weight of lipoprotein = weight the slender after drying - weight the clear slender.

RESULTS ANDDISCUSSION:

Identification of Lipoprotein producing *Pseudomonas* sp.:

Microscopic and Culture features of *Pseudomonas* sp.:

In microscopic examination bacteria appear to be single or pairs, slender , motile by polar flagella .During laboratory tests for the growth of bacterial isolates on nutrient agar colonies appeared to be very in size, circular, opaque, with irregular edge, creamy colored colony and dry strength of colonies .green color in plates indicate for pyocyanin production by bacteria Table (1) .

Table (1) Results of biochemical tests of *Pseudomonas aeruginosa*

NO.	Biochemical Tests	Results
1	Hemolysis	Beta
2	Catalase	+
3	Oxidase	+
4	Citrate Utilization	+
5	Glucose	+
6	Lactose	–
7	Sucrose	—
8	Maltose	+
9	Motile	+

Isolation of Lipoprotein - producing Pseudomonas species:

Microscopic and conventional biochemical test were carried out for identification of bacterial isolate, the results showed 30 isolates were belonged to *P. aeruginosa* which appear as gram negative bacilli , high percentage of this genus were expected because the *Pseudomonas* have activity to production lipoprotein is backed up by evidence (11),hydrocarbon contaminated soil were chosen for isolation of lipoprotein producing *Pseudomonas* since they create a selective niche to such microorganisms that have stimulated ability to utilize hydrocarbons as nutrition source (12).

Screening of Lipoprotein Producing Isolates:

Haemolysis has been used as an initial selection criterion for the primary isolation of lipoprotein producing bacteria. Haemolytic activity was regarded as indicator for production and used as a rapid method for bacterial screening; the results indicated that all 30 bacterial isolates were capable to haemolyse blood. Spreading the solid medium

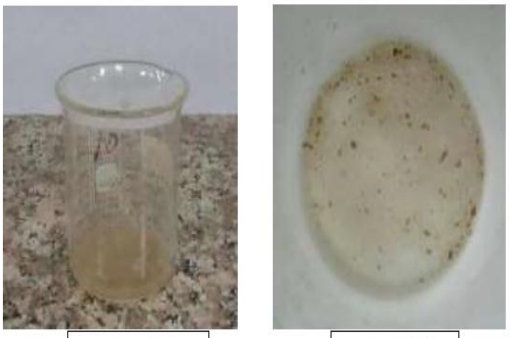
with bacteria producing β-haemolytic result. Culture with β-haemolytic can manufacture lipoprotein. Which associated with result of scientist Rashedi (13) who utilize this experiment in order to improving lipoprotein producing organisms ,other plates showed α-haemolytic activity ,when grew on blood agar plates . These conclusions of results may be linked to the observed that blood haemolysis capable to cause by other microbial products such as virulence factors. Such results were recorded by Carrillo (14) .

Extraction and Production of Lipoprotein and Estimation of Dry Weight:

All selected isolates of *P. aeruginosa* were grown separately in R2B medium the results showed, all isolates gave the lowest production after 24 hrs. of incubation, followed by an increase, but at a low rate in production with increasing time resulting in proportional increase in the number of bacteria producing lipoprotein in the broth medium and thus increase the bacterial consumption of the carbon-source in the medium in both addition with gasoline or with benzene .The maximum yields (g/l) of lipoprotein produced by *P. aeruginosa* were achieved after 72 hrs of incubation. The solid powder of the lipoprotein was guessed and evaluated the highest percentage of the production of the agents after 72 hrs. of incubation it has been obtained in the broth medium containing benzene it was reached to(0.38)g/ yield, while productively was lower than incased of kerosene as it reached (0.31)g/l, figure(3.1)and (3.2). This means that benzene is the best catalyst for the production of lipoprotein from kerosene.

Figure (3.1) Lipoprotein production with benzene before and after drying

Before drying After drying



Before drying After drying

Figure (3.2) Lipoprotein production with kerosene before and after drying

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