

## Isolation and identification of antibiotic producing bacteria from local soil in Babylon province

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### Abstract :

In this research study the capacity of *Bacillus subtilus* and *Bacillus cereus* isolated from soil to inhibit the growth of pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus* , *Klebsiella pneumonia* , *Pseudomonas aeruginosa* ) isolated from urinary tract infection by producing antibiotic and study the effect of some environmental factors (temperature ,salt ,PH) on growth of bacteria . the diameter of inhibition zone of *B.subtilius* was ( 25,30,22,19 ) while *B.cereus* was(18,15,20,11) against *E. coli*, *S. aureus*, *K. pneumonia*, *P.aeruginosa* respectively. The result showed the ability of *B. cereus* to growth between 20-45° C while *B. subtilus* growth between 15-40° C and showed the optimum pH to growth of *B. subtilus* was 7 while the optimum pH to growth of *B. cereus* was 5. The result showed the *B. subtilus* was grown in 5% and 10% of NaCl concentration while the *B. cereus* was grown in 5% NaCl concentration. Concluded the ability of bacilli bacteria to produced antibiotic inhibit the growth urinary tract infection bacteria .

Key word : antibiotic ,Inhibition zone , urinary tract infection.

### الخلاصة :

تم دراسة قدرة بكتريا *Bacillus subtilus* و *Bacillus cereus* المعزولة من التربة على تثبيط نمو البكتيريا الممرضة (*Escherichia coli*, *Staphylococcus aureus* , *Klebsiella pneumonia* , *Pseudomonas aeruginosa* ) المعزولة من التهاب المسالك البولية من خلال إنتاج المضادات الحيوية ودراسة تأثير بعض العوامل البيئية (درجة الحرارة ، التركيز الملحي، الرقم الهيدروجيني) على نمو البكتيريا. كان قطر منطقة التثبيط لبكتريا *B.subtilius* (25,30,22,19) في حين كان قطر التثبيط لبكتريا *B.cereus* (18,15,20,11) ضد *E. coli*, *S. aureus*, *K. pneumonia*, *P.aeruginosa* على التوالي. وأظهرت النتائج قدرة بكتريا *B.cereus* على النمو بين 20-45 درجة مئوية في حين أن نمو *B.subtilius* بين 15-40 درجة مئوية ، وأظهرت النتائج ان درجة الحموضة المثلى لنمو *B.subtilius* هي 7 في حين أن الرقم الهيدروجيني الأمثل لنمو *B.cereus* هو 5 . وتستطيع بكتريا *B.subtilius* النمو في تركيز ملحي 5% و 10% بينما بكتريا *B.cereus* تنمو في تركيز 5% . واستنتج من الدراسة قدرة بكتريا العسوية المعزولة من التربة على إنتاج المضادات الحياتية المثبطة لنمو البكتريا المسببة لالتهاب المسالك البولية.

الكلمة الرئيسية: المضادات الحيوية، منطقة تثبيط، التهاب المسالك البولية

## Introduction

Soil microbial community structure and activity depend to a large extent on the status of their soil habitat (Sarker *et al.*,2010). Within this habitat, soil organisms are eating, respiring, competing, cooperating, and responding to changes in their immediate environment(Sandhya *et al.* ,2014). Indeed, the majority of the microbial community may be dormant at any given time in most soils, ready to respond as conditions for a particular group become favorable (Sarker *et al.*,2010). The soil habitat is perhaps best envisioned as a complex matrix with pores and soil aggregates of differing sizes(Anita *et al.*,2012) .

Microorganisms in a particular location are exposed to many overlapping gradients of nutrients and various other environmental factors(Sarker *et al.*,2010). Microorganisms will grow in 'microenvironments' until an environmental or nutritional factor limits growth(Anita *et al.*,2012) . The growth of a microorganism depends on both the nutrient supply and its tolerance of the environmental conditions(Sandhya *et al.* ,2014).

As the antibiotic resistance is spreading, the need for new antibiotics is increasing. One of the possible ways to increase the chance of finding novel antibiotics is to find new approaches for isolating interesting bacteria and fungi or at least make the existing methods more efficient (Anita *et al.*,2012). One of the more promising techniques is to use the resistance and enhance the self-protection mechanism antibiotic producers need to have, in order to avoid suicide (Mohamed *et al.*,2015).

A urinary tract infection is a bacterial infection that affects any part of urinary tract. In most cases bacteria travel to the urethra and multiply causing kidney infection if not treated (Mansour *et al.*, 2009). Despite the advances in various field of medicine, urinary tract infections are still considered as serious public health problems and inflict a major burden to health care services around the world and especially in developing countries (Anita *et al.*,2012).

This study was aims to test the ability of *B. subtilus* and *B. cereus* isolated from soil to producing antibiotic and study the effect of some environmental factors (temperature ,salt ,PH) on the growth of bacteria.

## Material and Method

- **Soil sample :**

25 local soil sample were collected from 5 different site in Babylon province included (citrus , tomatoes, Okra, Jet and rice farms) by take a 1 cm<sup>3</sup> of the soil surface and a depth ranging from 1-5 cm and the amount of sample taken was 100 g from each sites included in the study and placed in a sterile bag and scored garret information (sample number, type of soil, the type of crop grown, the site) and brought to the lab (Mansour *et al.*,2009). Then weighed 1 g of soil and dissolved in 10 ml of sterile distilled water and then attended a series of dilution to obtain the concentrations 1:10 , 1: 100, 1: 1000, then used the spreading method to isolate the bacteria by taking 0.1 from each dilution and spreading by spreader in nutrient agar after incubation period of 24 hours at 37 ° C . The diagnoses of the bacteria based on a gram's stain and biochemical tests (Sarker *et al.*,2010).

- **Urine sample :**

25 urine samples were collected from patients with urinary tract infection reviewers Teaching Hilla hospital by using clean and sterile containers and brought to the microbiology laboratory to isolate the bacteria that causes infection of the urinary tract by using appropriate media ( blood agar ,EMB agar, MacConky agar, Nutrient agar) and incubated at 37 ° C for 24 h in aerobic condition for each type of bacteria and making gram stain in addition to biochemical tests to diagnosis of bacteria isolated (Mohamed *et al.*,2015).

## Testing the ability of bacteria to

### 1- producing antibiotic

Testing was conducted by using the agar well diffusion method . After the growth of bacteria ( *B. subtilis* , *B. cereus* ) in the nutrient broth for 48 hours then placed in the centrifuge at fast 6,000 rpm for 10 minutes afterwards the sediment was collect (Muthna,2008). It was taken 0.1 from each diagnosed pathogenic bacteria (*E.coli*, *Sta. aureus* , *K.pneumonia* , *P.aeruginosa* ) and spreading in Muller Hinton Agar by using cotton swap and make hols in the media by using cork holes then added 100 ML of sediment of bacteria ( *B. subtilis* , *B. cereus* ) in the hole and then read results after 24 hours of incubation at 37°C by measuring the diameters of inhibition zones (Ronald,2011).

## 2- growth in different temperature

Used 12 test tubes containing nutrient broth, then divided into two groups , first group (6 tubes ) inoculated with *B. subtilus* and second group inoculated with *B. cereus* then also divided in to two groups , first group incubated at 40,45,50 °C for two days and second group incubated at 5,10,20 °C for 2-7 days . Present turbidity in broth media indicator of positive result (Mansour *et al.*,2009) .

## 3- growth in different concentration of salt

Add 3% 0.7 % 0.10 % 0.15 % concentrations of sodium chloride to the 6 tubes contain nutrient broth, then divided in to two groups , first group (3 tubes ) inoculated with *B. subtilus* and second group (3 tubes ) inoculated with *B. cereus* , then incubated for 2-4 days . Present turbidity in broth media indicator of positive result(Mansour *et al.*,2009) .

## 4- growth in different value of PH

Attended eight test tube containing nutrient broth Adjust the PH (3,7,9,11) and inoculation with bacteria ( *B. subtilus* , *B. cereus* ) then incubated for 24 hs . Present turbidity in broth media is considered positive result(Muthna,2008) .

## Result & Discussions

The culture ,microscopic and biochemical result showed the bacteria isolated from soil belong to the genus *B. cereus* and *B.subtilius* and the bacteria isolated from the urine belong to the genus *S. aureus*, *K. pneumonia*, *P. aeruginosa* , *E. coli* . the same bacterial type also isolated by Mansour *et al* ., from urinary tract infection (Mohamed *et al.*,2015) .

When study the ability of *B. cereus* , *B.subtilius* to produced antibiotic inhibited the growth of urinary tract infection bacteria present the diameter of inhibition zone of *B.subtilius* was 25,30,22,19 against *E. coli*, *S. aureus*, *K. pneumonia*, *P.aeruginosa* respectively Table (1) larger than diameter of inhibition zone of *B. cereus* against the same bacteria these result similar the result obtained from (Ronald,2011, Musliu & Salawudeen, 2012) . When present the ability of *Bacillus* to inhibition the growth of *S. aureus* and *Pseudomonas* species.

**Table (1)the Diameter of inhibition zone (mm) against pathogenic bacteria**

Isolated bacteria	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>
	Diameter of inhibition zone mm	
<i>Escherichia coli</i>	18	25
<i>Staphylococcus aureus</i>	15	30
<i>Klebsiella pneumonia</i>	20	22
<i>Pseudomonas aeruginosa</i>	11	19

The growth of microorganisms is greatly affected by the chemical and physical nature of their surroundings. The major physical factors which affect microbial growth are solutes and water activity, pH, temperature, oxygen level, pressure and radiation (Mohamed *et al.*,2015).

The result showed the ability of *B. cereus* to growth between 20-45° C while *B. subtilis* growth between 15-40° C Table (2). Temperature profoundly affects microorganisms as the most important factor influencing the effect is temperature sensitivity of enzyme-catalyzed reactions. Beyond a certain point of higher temperature, slow growth takes place and damages the microorganisms by denaturing enzymes, transport carriers and other proteinsv(Aparanji *et al.*,2013 ).

The plasma membrane also is disrupted as lipid bilayer simply melts and the damage is such an extent that it cannot be repaired. At very low temperature, membranes solidify and enzymes don't work rapidly (Fakhar *et al.* ,2016).

**Table (2) effect of the temperature on growth of isolated bacteria**

Isolated bacteria	Low temperature			High temperature		
	10°C	15°C	20°C	40°C	45°C	50°C
<i>Bacillus cereus</i>	-	-	+	+	+	-
<i>Bacillus subtilis</i>	-	+	+	+	-	-

The pH values effect on the growth of bacteria though the effect on enzyme involved in growth processes . The result showed the optimum pH to growth *B. subtilis* was7 while the optimum pH to growth *B. cereus* was 5 Table (3). The pH

scale ranges from 1.0 to 14.0 and most microorganisms grow to vary widely from pH 0 to 2.0 at the acid end to alkaline lakes and soil that may have pH value between 9.0 and 10 (Aparanji *et al.*,2013). The pH can affect the growth of microorganisms and each species has a definite pH growth range and pH growth optimum (Fakhar *et al.* ,2016). Acidophiles have their growth optimum between pH 0 and 5.5; neutrophiles between 5.5 and 8.0 and alkalophiles prefer pH range of 8.5 to 11.5. (Bala *et al.*,2012).

**Table (3) Effect of the pH value on growth of isolated bacteria**

Isolated bacteria	pH value			
	3	5	7	9
<i>Bacillus cereus</i>	-	-	++	+
<i>Bacillus subtilus</i>	-	++	+	-

The result showed the *B. subtilus* was growth in 5% and 10% of NaCl concentration while the *B. cereus* was growth in 5% NaCl concentration Table (4). Changes in osmotic concentration of the surroundings can affect microbial growth as a selectively permeable plasma membrane separates the microorganisms from their surroundings(Fakhar *et al.* ,2016). Microorganisms need to keep the osmotic concentration of their cytoplasm somewhat above that of the habitat by the use of compatible solutes, so that the plasma membrane is always pressed firmly against their cell wall(Bala *et al.*,2012).

**Table (4) Effect of the NaCl concentration on growth of isolated bacteria**

Isolated bacteria	NaCl concentration			
	5%	10%	15%	20%
<i>Bacillus cereus</i>	++	+	-	-
<i>Bacillus subtilus</i>	++	-	-	-

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