

**Isolation and Identification of Some Terrestrial Invertebrate's
Bacteria from Baghdad, Al-Jadriya.**

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Abstract

This study aimed to isolate and culture the bacteria from surfaces of some terrestrial healthy invertebrates from Al-Jadriya, Baghdad. Four major phyla of terrestrial invertebrates were obtained, Arthropoda genus *Porcellio sp.* and *Julus sp.*, Mollusca genus *Monacha cantiana*, Nematoda, genus *Seinura sp.* and Annelida genus *Pristina sp.*, the bacteria were characterized by using selective culture media and biochemical tests. The surface associated bacteria strains were identified as *Alteromonas putrefaciens* , *Bacillus pumillus* , *Citrobacter freundii* , *Actinomyces sp.* , *Bacillus azotoformans* , *Serratia marcescens* , *Yersinia sp.* , *Proteus vulgaris* , *Pasteurella pneumotropica* , *Edwardsiella tarda* , *Enterobacter aerogene* and *Micrococcus sp.* , The biological activities of the isolated microorganisms were checked against some of the important pathogenic bacteria like *Escherichia coli* and *Staphylococcus aureus* by Agar well diffusion assay. We found that *Porcellio sp.*, *Julus sp.* and *M. cantiana* which refer to (Arthropods and Mollusca) have higher biological activity isolated from their surface than other bacterial invertebrates compared with other isolates which were refer to, *Alteromonas putrefaciens* , *Bacillus azotoformans* and *Pasteurella pneumotropica* respectively.

Key words: Terrestrial invertebrates, bacterial isolation, biochemical test, antibacterial activity.

عزل وتشخيص البكتريا من بعض اللاقريات الارضية من منطقة الجادرية ابغداد.

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الخلاصة

Porcellio sp. و تهدف الدراسة الحالية الى عزل وتشخيص البكتريا المرافقة للاقريات الارضية التي تم جمعها من منطقة الجادرية ابغداد والتي تعود لشعب المفصليات مثل *Julus sp.* ، شخّصت البكتريا باستخدام الاوساط والخيطيات *Seinura sp.* و الحلقيات *Pristina sp.* والنواع *Alteromonas putrefaciens* ، الزرعية الاختيارية واجراء الاختبارات البايوكيميائية ، وكانت: *Bacillus pumillus* ، *Citrobacter freundii* ، *Actinomyces sp.* ، *Bacillus azotoformans* ، *Serratia marcescens* ، *Yersinia sp.* ، *Proteus vulgaris* ، *Pasteurella pneumotropica* ، *Edwardsiella tarda* ، *Enterobacter aerogene* و *Micrococcus sp.*

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اختبرت الفعالية الحياتية للعزلات المشخصة من اللاققریات تجاه بعض الانواع البكتيرية المرضية . اظهرت النتائج وجود فعالية مضادة لبعض العزلات البكتيرية المعزولة بطريقة الانتشار بالحفر تجاه بعض انواع البكتريا المرضية مثل *Staphylococcus aureus* و *Escherichia coli* وسجلت اجناس البكتريا *M. cantiana, Julus sp., Porcellio sp.* اعلى نتائج في التنبيت التي شخص على سطحها بكتريا

كلمات مفتاحية: العزلات البكتيرية , الاختبارات البايوكيميائية , الفعالية المضادة للبكتريا , اللاققریات البرية *Pasteurella pneumotropica , Bacillus azotoformans , Alteromonas putrefaciens*

Introduction

Soil refers to the outer loose material of the earth crust. It's composed of five major components, these include: mineral matter, water, organic matter, air and living organism. The various component of the soil environment constantly changed and the quantity of these constituents are not the same in the all soil but vary with locality, Living portion of the soil body includes small animals and microorganisms but It's generally considered the its microorganism that plays the most important role in release of nutrient and carbon dioxide for plant growth. (1)Also, invertebrates of soil are enormously diverse. According to recent estimation , soil animals my present as much as 23% of the total diversity of living organisms (7).The smallest nematodes and protozoa (protists) of micro fauna less than 20 mm on average live in the water filled porosity. Microarthropodes, Ecchytraeidae and many groups of the mesofauna (0.2-2) mm live in their filled soil porosity. The Largest Arthropods, Mollusca, Annelida and Crustacea include the macro fauna that live in the surface litter or in nests and burrows that they create in the soil.(11) This bio diversity is highly sensitive to any disturbance since the soil environment is their habitat and the source of all the resources they need.(12) However, the causal relationships between composition, diversity and abundance of soil organisms and sustained soil fertility are unclear. While the scientific community largely accepts that microbiological activities are important in hydric soil and wetlands development; there is little research on identifying the microbial community and determining their activities in the hydric soils. Microbial activities and biomass concentrations have been applied to serve as biological indicators of nutrient enrichment in constructed wetland.(5) In this paper we offer first dynamic interaction of soil between invertebrates and microorganisms present on the surface of invertebrates and the search for new drugs from nature (the surface of the living invertebrates wild), which may have a significant role in the production metabolize compounds minor a role pest-eating invertebrates ground, so we isolate bacteria cultural and indigenous cultural figures and study the biochemical and cultural identification of bacteria soil invertebrates. Because even today, reports on the bacterial communities of healthy invertebrates limited particularly on soil invertebrates.

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Materials and Methods

Samples collection

Samples have been collected from Al-Jadriya District in Baghdad during November, 2013 from different areas of the soil till (10-15) centimeters' deep ,the samples were diagnosis in the laboratory of advanced invertebrates and bacteriology lab at the College of Science for women – Baghdad university as follows:

Nematoda & Annelida

Samples were taken from the area and put in plastic containers and moved to the lab where they were cleaned form remain of plants that can be seen clearly. Then the samples were examined in the lab by using Dissecting Microscope, type (wild M3B) under the power (16,604) after they were moved into petri dishes and after water was added to them to be washed from glasses. Then water was added to them to be washed from dust and they were moved into test tubes that contain the Brain Heart Infusion media.

Arthropoda & Mollusca

They were picked up from soil by a special tweezers and put in closed containers. After being cleaned, they were moved into test tubes.

Isolation of bacteria:

Samples were taken out and incubated at 37C for 24 hours and considered as stock culture. A series of dilutions were made from the broth of stock culture. volume of 100 microliter of diluted culture was spread on Brain Heart Infusion agar incubated at 37c overnight. Single bacterial colonies with different morphological characteristics such as colony color, shape, margin and surface texture were isolated and transferred onto fresh Brain Heart Infusion agar plates. The purified isolates were then sub cultured onto 1mf of (BHI) Broth and incubated at 37C overnight. (10)

Morphological and phenotype characterization.

Bacterial cultures grown on (BHI) agar were examined based on their Gram stain by conventional staining technique series of selective media which are MacConkey Agar, Eosin Methylene Blue (EMB), S.S. Agar and blood agar were used to characterize these isolates. Motility test was performed using Modified SIM medium ,Isolated strains were characterized by conventional microbiological methods(4,16)involving following characteristics assays: catalase , oxidase , Methyl Red test ,Voges Proskauer test , Indole production test ,Degrading of starch , Urea , Casein, Gelatin , utilization of citrate , Blood hemolysis , Triple Sugar Iron test and growth in present of NaCl in Mannitol salt agar medium . in this assay , the bacteria were grown on the specific medium according to the standard preparation protocol then we were identified bacteria according to Berge's Manual of Determinative Bacteriology (2&3).

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Preparation of Concentrated Bacterial Pellet (CBP):

Preparation of concentrated bacterial pellet (CBP) was prepared by cells from a 48 hours old culture grown in (BHI) medium were pelleted by centrifugation at 6000 ground per 20 minutes and re suspended into (BHI) medium the suspension was incubated for 24 hours at 37C, the cells were harvested by centrifugation at 6000 ground per 20 minutes, the supernatant was filter sterilized and used for testing antimicrobial activity. (14)

Antimicrobial Activity:

The antibacterial activity against one gram positive pathogenic strain *Staphylococcus aureus* and one gram negative pathogenic strain *Escherichia coli* using well agar diffusion method of 24 hours old test culture, we inoculated the test pathogenic bacteria by sterilized cotton swabs and made wells by sterilized cork borer 5mm and added 500 microliter from concentrated bacterial pellet (CBP) to each well and compared with control well which had distilled water. The relative activity was calculated by measuring the diameter of inhibition zone after incubation for 24 hours at 37 C. (15)

Results and Discussion

Table (1) Types of isolated terrestrial invertebrates explain their classification, morphology and extence.

Classification	<i>Seinura sp.</i>	<i>Pristina sp.</i>
Phylum Class Order Family	Nematoda Secreneta Aphelenchida Aphelenchidae	Annelida Clitellata Haplotaxida Naidiaa
Morphology	<ul style="list-style-type: none"> - Caved flossier unsegment body. - Transparence. - The anterior part of the body had a flossier spine and the posterior end little deflected. 	<ul style="list-style-type: none"> - Each segment has four bundles of chaeta with on the underside and the other on the sides, dorsal seta bundles with 1-3 needles like seta simple, bifurca.
Existence	<ul style="list-style-type: none"> - In the clay sediments for the fresh water and humid soil. 	Able to swim found in fresh water and humid terrestrial habitats.

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Classification	<i>Seinura sp.</i>	<i>Pristina sp.</i>
Phylum	Nematoda	Annelida
Class	Secreneta	Clitellata
Order	Aphelenchida	Haplotaxida
Family	Aphelenchidae	Naidiaa
Morphology	<ul style="list-style-type: none"> - Caved flossier unsegment body. - Transparence. - The anterior part of the body had a flossier spine and the posterior end little deflected. 	<ul style="list-style-type: none"> - Each segment has four bundles of chaeta with on the underside and the other on the sides, dorsal seta bundles with 1-3 needles like seta simple, bifurca.
Existence	<ul style="list-style-type: none"> - In the clay sediments for the fresh water and humid soil. 	Able to swim found in fresh water and humid terrestrial habitats.

Table (2) Morphological and biochemical characterization of bacteria from terrestrial invertebrates.

Characteristics	Sample (1) <i>Porcellio sp.</i>	Sample (2) <i>Porcellio sp.</i>	Sample (3) <i>Porcellio sp.</i>	Sample (4) <i>Julus sp.</i>	Sample (5) <i>Julus sp.</i>	Sample (6) <i>Julus sp.</i>
Morphology of organism	Cocco bacilli	Bacilli forming spore	Short Rod	Bacilli filamentous	Bacilli forming spore	Short Rods
Colony color	Mucoid whitish & utilize the ager	Mucoid light Brown	Mucoid light Brown	Mucoid whitish and yellowish	Dry whitish	Dry whitish
Gram stain	G ^{-ve}	G ^{+ve}	G ^{-ve}	G ^{+ve}	G ^{+ve}	G ^{-ve}
Growth on Mac Conkey Agar	+ non fermented	+ non fermented	+non fermented	+ non fermented	+ non fermented	+ fermented
Growth on EMB Agar	+ pink with Black center	+ pink with Black center	+ pink with Black center	+ pink	+ metallic greenish	+ pink
Growth on S.S. Agar	+ black center colony	+black center colony	+ black center colony	+ colorless	+ black center colony	+ colorless
Simmons citrate utilization	-	-	-	+	+	+
Urease activity	-	-	-	-	-	-

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Kleglir Sugar Iron						
Gas						
H ₂ S production						
slope /bottom	+	+	+	+	+	+
	+	-	+	-	+	-
	Red / yellow	Red / yellow	Red / yellow	Red / yellow	Yellow / yellow	Yellow / yellow
Casein hydrolysis	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-
Gelatin hydrolysis	-	-	-	-	-	-
VP test	-	+	-	-	-	-
MR test	-	-	+	-	+	-
Indole production	-	-	-	-	+	-
Blood hemolysis	+α	+α	+α	+β	+α	+α
Mannitol Salt Agar	-	+	-	-	-	-
Tentatively identified Genus	<i>Alteromonas putrefacims</i>	<i>Bacillus pumillus</i>	<i>Citrobacter freundii</i>	<i>Actinomyces sp.</i>	<i>Bacillus azotoformans</i>	<i>Serratia marcescens</i>

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Characteristics	Sample (7) <i>M. contiana</i>	Sample (8) <i>M. contiana</i>	Sample (9) <i>M. contiana</i>	Sample (10) <i>Seinura sp.</i>	Sample (11) <i>Seinura sp.</i>	Sample (12) <i>Seinura sp.</i>	Sample (13) <i>Pristina sp.</i>	Sample (14) <i>Pristina sp.</i>	Sample (15) <i>Pristina sp.</i>
Morphology of organism	Rods	Rods	Cocco bacilli Rods	Short Rods	Cocco Rods	Cocco Rods	Rods	Cocci	Cocci
Colony color	Mucoid whitish & utilize ager	Mucoid whitish & utilize ager	Mucoid whitish & utilize ager	Mucoid whitish & utilize Agar	Mucoid whitish	Mucoid whitish	Dry whitish	Dry whitish	Dry whitish
Gram stain	G-ve	G-ve	G-ve	G-ve	G-ve	G -ve	G-ve	G +ve	G +ve
Growth on Mac conkey Agar	+ non fermente d	+ non fermented	+ fermented	+ non fermented	+ non fermente d	+ non fermente d	+ fermen ted	+ non fermente d	+ non ferment ed
Growth on EMB Agar	+ pink	+ pink	+ metallic greenish	+ pink	+ metallic greenish	+ metallic greenish	+ metalli c greenis h	+ pink with Black center	+ pink with black center
Growth on S.S. Agar	-	+black center colony	+black center colony	+black center colony	+black center colony	+black center colony	+colorl ess	+black center colony	+black center colony
Simmons citrate utilization	-	-	-	+	+	+	+	-	+
Urease activity	+	+	+	-	-	-	-	-	-

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Kleglir Sugar Iron									
Gas									
H ₂ S production	+	+	+	+	+	+	+	+	+
slope /bottom	- / yellow	+ / yellow	+ / yellow	+ / yellow	+ / yellow	+ / yellow	+ / yellow	- / yellow	- / yellow
Casein hydrolysis	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	-	-	-	-	-	-	-	-	-
VP test	-	-	-	-	-	-	-	-	-
MR test	+	+	-	+	+	+	+	+	+
Indole production	+	+	+	+	-	-	+	+	+
Blood hemolysis	+α	+α	+α	+β	+α	+α	+β	+α	+α
Mannitol Salt Agar	+	-	+	+	-	-	-	-	+
Tentatively identified Genus	<i>Yarsinia sp.</i>	<i>Proteus vulgaris</i>	<i>Pasteurella pneumotropic a</i>	<i>Edwardsiella tarda</i>	<i>Enterobacter aerogene</i>	<i>E. aerogene</i>	<i>Citrobacter diversus</i>	<i>Micrococcus sp.</i>	<i>Micrococcus sp.</i>

Table (3) biological activity of bacteria from terrestrial invertebrates measured by millimeters.

samples	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
<i>E. coli</i>	10	-	8	4	18	-	-	-	12	-	-	-	-	-	-
<i>S. aureus</i>	18	6	-	-	-	4	-	-	-	-	-	-	-	-	-

The results showed in table (1) Four major phyla of terrestrial invertebrates were obtained, Arthropoda genus *Porcellio sp.* according to what has been mentioned (8) and *Julus sp.* classified on the basis of Smith (2001), Mollusca genus *Monacha cantiana* classified on the basis of Dallinger (1994), Nematoda, genus *Seinura sp.*

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classified on the basis of (18) and Annelida genus *Pristina sp.* classified on the basis of (9). Then we began an analysis interaction between the bacteria associated with the surfaces of some healthy terrestrial invertebrates in soil in Baghdad, Al-Jadriyah gardens. Bacteria can be isolated using standard culture methods, By Inoculating Whole body of invertebrate in to culture media; it has shown that surfaces of invertebrates harbor diverse and abundant bacterial communities. A total of 15 isolates which demonstrated some conspicuous attributes in their conventional test results and which were presumptively identified on the basis of a biochemical profile most closely resembling that of a particular species and genus were recovered in Table (2). Cultural and biochemical characteristics of the entire isolates and gram reaction are variable. The majority of bacteria lie in the previous finding by gram negative, which 67% of total bacteria in some terrestrial invertebrates were gram negative and 33% of total bacteria gram positive this was similar with (17,20) ; we know that all species are not everywhere. There are biogeographic patterns for animals and microbes in soil (19). Bacteria may play crucial role between mucus and cuticle surface are an extremely good medium for bacterial growth , such as some genus of Millipedes' were identified aerobic bacteria had ability to lysis of cellulose, Where art Watkins & Simkiss (1990) reached that alimentary tract is a major site of interaction between an animal's ecosystem and It's physiology. It may be intermittently or permanently populated by microorganisms from Its environment that could be benefic or pathogenic in their influence. These bacteria produce of new pharmacologically active constituents as an evolving source of new bioactive natural products.(14), In searching for new antibiotics, relatively simple and rapid methods have been developed for screening microorganisms for antibiotic producing ability. Antibiotic isolated may be bactericidal or bacteriostatic in nature. Production of antibiotic by microorganisms from healthy invertebrates' surface shows antibiotic activity under normal growth condition. It is possible that these commensal bacterial species are on the bodies of these invertebrates produce efficient compounds protected from rot and diseases caused by microbes in the soil surrounding that. and we found these bacteria have ability for inhibiting some pathogenic bacteria such as *Bacillus pumillus* , *B. azotoformans* and *Actinomyces sp.* and some gram negative organism both *Alteromonas putrefacims* and *Pasteurella pneumotropica* shows antibacterial activity against *E. coli* and *S. aureus* these bacteria were isolate from Arthropods and Mollusca which have cuticle that accepts with Macrovet *et al.*(2012) who showed that Millipedes produce toxic fluids from Its pores in surface cuticle contain some active compounds : alkaloids , phenols, benzoic acids and terpenoids ,while the other's isolates proved to be resistance to all the isolates that is there is no clear zone of inhibition observed against the bacteria.

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It has been hypothesized that bacteria associated with terrestrial invertebrates and vertebrates secrete a number of antibacterial agents that may provide a level of immunity to themselves this is commonly known among us as 'probiotic'. So we recommend Extraction and purification method can be employed for the pure antibiotic production.

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