IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 4 Ver. III (Jul - Aug. 2015), PP 46-52
www.iosrjournals.org

Antibacterial Activity of Actinomycetes Isolated From Agriculture Soils in Hillah /Iraq

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Abstract: *One hundred of agricultures soil samples have been collected from different locations in Hillah city during the period from September to December /2013. Twenty one Streptomyces isolates were obtained from these samples. These isolates have been cultured and purified on international Streptomyces project type-2 (ISP-2). Antibacterial activity of these isolates on Muller Hinton Agar were tested against gram positive bacterial species (Staphylococcus albus, Staphylococcus aureus and Streptococcus pyogenes) and gram negative species (Klepsilla Pneumonia, Escherichia coli, Pseudomonas aeruginosa, Serratiamarcescens and Aeromonashydrophila) . Thirteen Stretopmycesspp from these isolates were found to have antibacterial activity, these isolates were named as symbols such as S.K-3, S.K-5, S.M.A -17, S.N.22, S.M-34, S.M-35, S.S-43, S.S-46, S.H-52, S.H.A-65, S.K-72, S.K.A-83 and S.A-98. The results of biochemical tests revealed all these isolates were belonged toStreptomtces spp.*

Keywards: *Actinomycetes , Antibacterial activity , Soils.*

I. Introduction

Actinomycetes are microorganisms can be mediated in appearance and activity between bacteria and fungi and are group of gram positive bacteria with high guanine (G) and cytosine (C) ratio and more than 70% in their DNA (Ventura et al., 2007 ; Deepthiet al., 2012). In spit of, Actinomycetes are produce branching mycelium, are substrate and aerial mycelium (Johnson et al., 2012). Many strains of which are observed have several characters such as, presence or absence of aerial mycelium, fragmentation or not fragmentation of substrate and aerial mycelium, also the spore morphology such as shape and color (Kavitha&Vijayalakshmi., 2007). Also the aerial mycelia which is smaller than those of fungi and some species of these produce asexual spores called conidia (Mythili and Ayyappa Das., 2011).

However, Actinomycetes are found in all types of natural sources, but largely found in soil, the soils are the main natural habitat, and it is nutritionally, biologically, physically complex and variable and the result of this they are able to doing a large number of metabolic activities such as produce huge diversity of bioactive secondary metabolites like antibiotics (Sajidet al., 2011).

Actinomycetes, specially the Streptomyces spp contain a widely types of microorganisms that have ability to produce of secondary metabolites and enzymes that used in commercial importance for medical and agricultural applications (Narayana and Vijayalakshmi., 2009). The genus Streptomyces seems to provide big number of new and more active of antibiotics more than of any other genus, therefore, Streptomyces it is importance for both industrial application and human health care (Sajidet al., 2011).

Since Streptomyces are produce a large number of secondary metabolites specially antibiotics can be use in pharmaceutical companies nowadays, resulting in reaching of widespread investigation towards discovery of new antibiotics, note they are produce more than seventeen percent of all used and known antibiotics (Hongjuanet al, 2006).

II. Materials And Methods

Collection of samples

One hundred soils samples have been collected from different locations in Hilla city, about one gram of the soil samples were taken from soils top about 5 to 10 cm in depth. The soil samples when collected from these regions are taken with an auger and placed in dry and sterile polyethylene tubes and stored at 4°C until use. Soil samples were pretreated with calcium carbonate to reduce the number of vegetative bacterial cells and allowing Streptomyces spores to survive, this method was required for inhibiting unwanted bacteria and remain only test bacteria (Pordeliet al.,2013).

Preparation of (ISP-2) Medium:

Chemical compounds of (ISP-2) prepared as the following:.

Yeast Extract 4.0 g , Malt Extract 10.0 ,g. Dextrose.4.0 g, Agar Agar 20.0 g, Distilled Water1000 ml.

The pH of this medium adjust from 7.0 to 7.3, then the components liquefy by heating at 100° C. After this, sterilized by autoclaved at 121° C for 15 min (Priddhamet al.,1957).

Isolation and purification of Streptomyces spp from soil samples:

The air dried samples are mixed and passed through the 2mm sieve filter to remove gravel, large stone and debris. After this the samples were incubated at 55°C in an Incubator for 5 min. 1g of soil was dissolved in 9ml of distilled water and successive dilutions was made up to 10^5. Serial dilutions were spread plated on ISP-2 media (International Streptomyces Project type-2 media) using Dilution plate technique. Antibiotics like nalidixic acid and nystatin were added to minimize microbial contamination. All the plates were incubated at 30°C in an Incubator for 5-7 days. After incubation, the Actinomycete growing colonies were selected and purified by subculturing on ISP-2 agar medium plates according to type and forms of these colonies.Then the purified colonies examined under light microscope at (10x). After this, the typical growing colonies of Streptomyces cultured on International Streptomyces Project type-2 agar slants and stored at 4°C for further uses (Deepthiet al.,2012).

III. Results And Discussion

Isolation of Streptomyces from agricultures soils

One hundred agricultures soil samples have been collected from different locations in Babylon city, twenty one Streptomyces isolates obtained from these samples. These isolates have been cultured and purified on international Streptomyces project type-2 (ISP-2) (Table: 1).

Table.1. Number of Actinomycetes isolates that obtained from agricultures samples

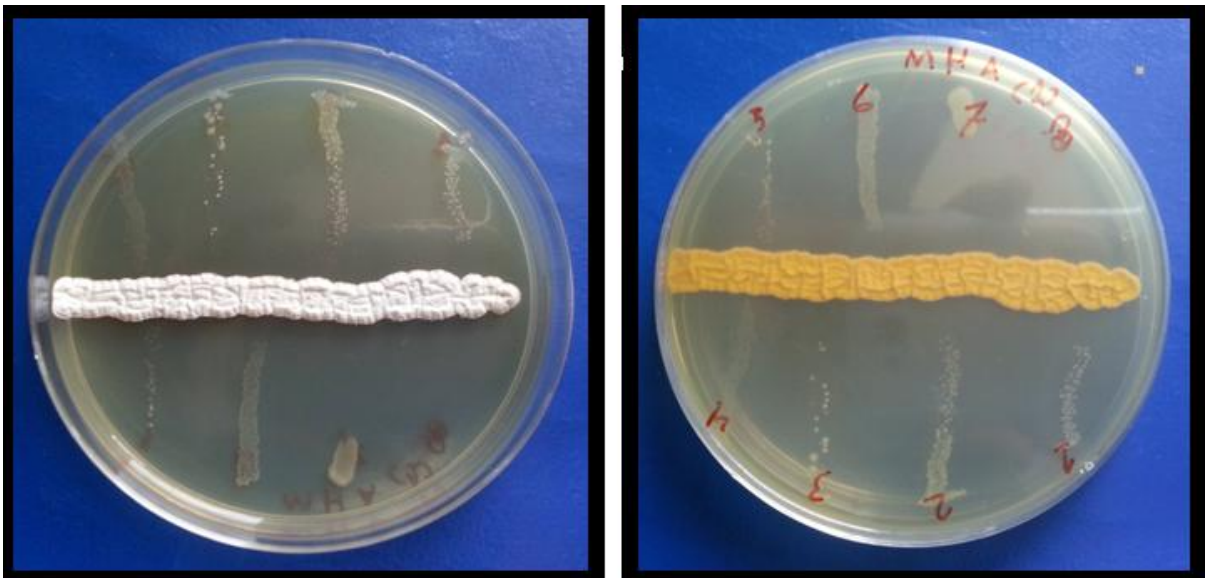
No.	Site of Collection	No. of site soils samples	Sequence of site samples	StreptomycesNo.	Samples %
1	Al-Kothar	10	1 - 10	3	14.286%
2	Al-Mahaweel	10	11 - 20	2	9.524%
3	Al-Nile	10	21 - 30	2	9.524%
4	Al-Mussaiab	10	31 - 40	4	19.046%
5	Al –Sadda	10	41 - 50	3	14.286%
6	Al-Hashmia	10	51 - 60	1	4.762%
7	Al-Hamza	10	61 - 70	1	4.762%
8	Al-Kasim	10	71 – 80	2	9.524%
9	Al-Karama	10	81 – 90	1	4.762%
10	Al-Annana	10	91 – 100	2	9.524%
Total		**100**	**1 - 100**	**21 isolates**	**21 %**

Primary screening test for Streptomyces isolates against pathogenic bacteria

Thirteen Streptomyces isolates showed antimicrobial activity against bacteria of gram positive (Staphylococcus albus, Staphylococcus aureus and Streptococcus pyogenes) and gram negative (Klepsilla Pneumonia, Escherichia coli, Pseudomonas aeruginosa, Serratiamarcescens and Aeromonashydrophila) when tested on Muller Hinton Agar (Table -2)

Table: -2. Number of Streptomyces isolates that show positive primary screening test

No.	Site of Collection	Actinomycetes Symbol No.	Antimicrobial activity
1	Al-Kothar	S.K-3	+
2	Al-Kothar	S.K-5	+
3	Al-Mahaweel	S.M.A -17	+
4	Al-Nile	S.N.22	+
5	Al-Mussaiab	S.M-34	+
6	Al-Mussaiab	S.M-35	+
7	Al-Sadda	S.S-43	+
8	Al-Sadda	S.S-46	+
9	Al-Hashmia	S.H-52	+
10	Al-Hamza	S.H.A-65	+
11	Al-Kasim	S.K-72	+
12	Al-Karama	S.K.A-83	+
13	Al-Annana	S.A-98	+
Total		**13**	



A-Front shape **B.** Rear shape

Figure: 1. Antibacterial activity ofS.K-5againstpathogenic gram positive and negative bacteria

A-Front shape **B.** Rear shape

Figure: 2. Antibacterial activity ofS.M.A-17againstpathogenic gram positive and negative bacteria

1. **Klepsilla Pneumonia.**
2. **Escherichia coli.**
3. **Pseudomonas aeruginosa.**
4. **Serratiamarcescens.**
5. **Aeromonashydrophila.**
6. **Staphylococcus aureus.**
7. **Streptococcus pyogenes.**
8. **Staphylococcus albus.**

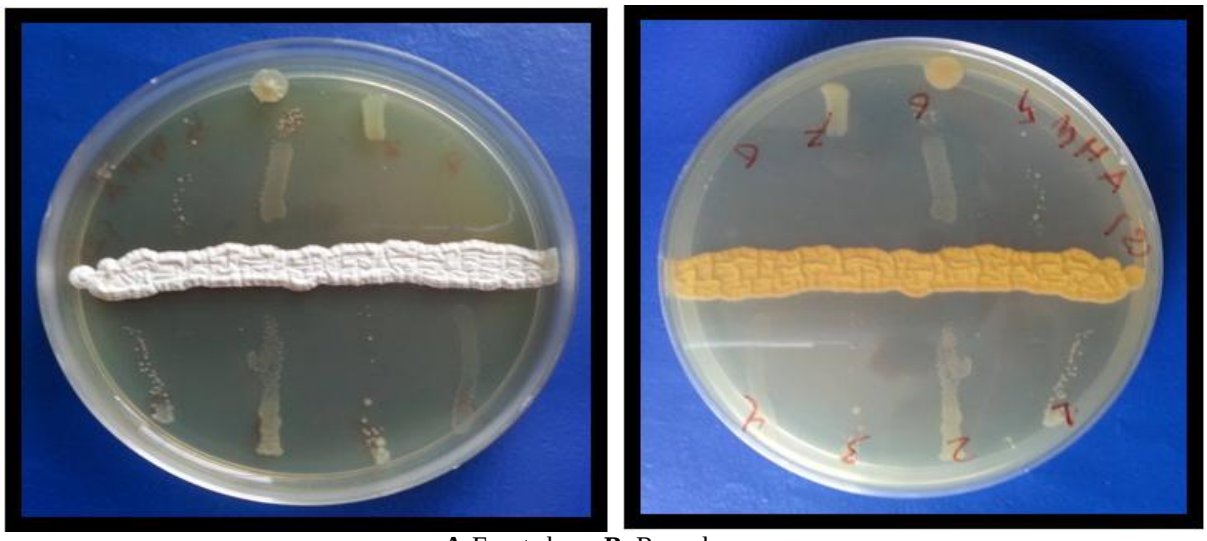


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Figure: 3. Antibacterial activity ofS.N.22againstpathogenic gram positive and negative bacteria

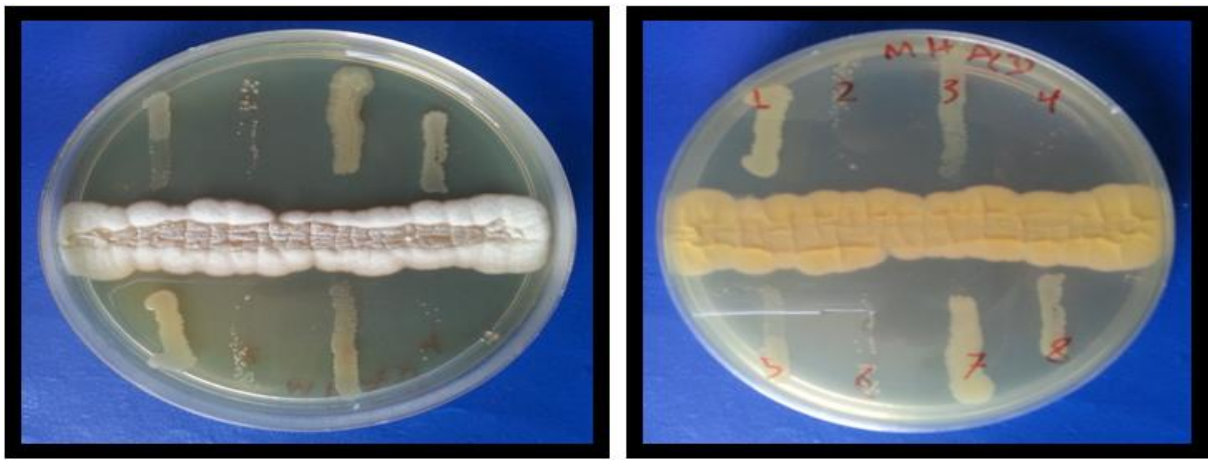

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Figure: 4. Antibacterial activity ofS.M-34 againstpathogenic gram positive and negative bacteria

A-Front shape **B.** Rear shape

Figure: 5. Antibacterial activity ofS.S-46againstpathogenic gram positive and negative bacteria

A-Front shape **B.** Rear shape

Figure: 6. Antibacterial activity ofS.H-52againstpathogenic gram positive and negative bacteria

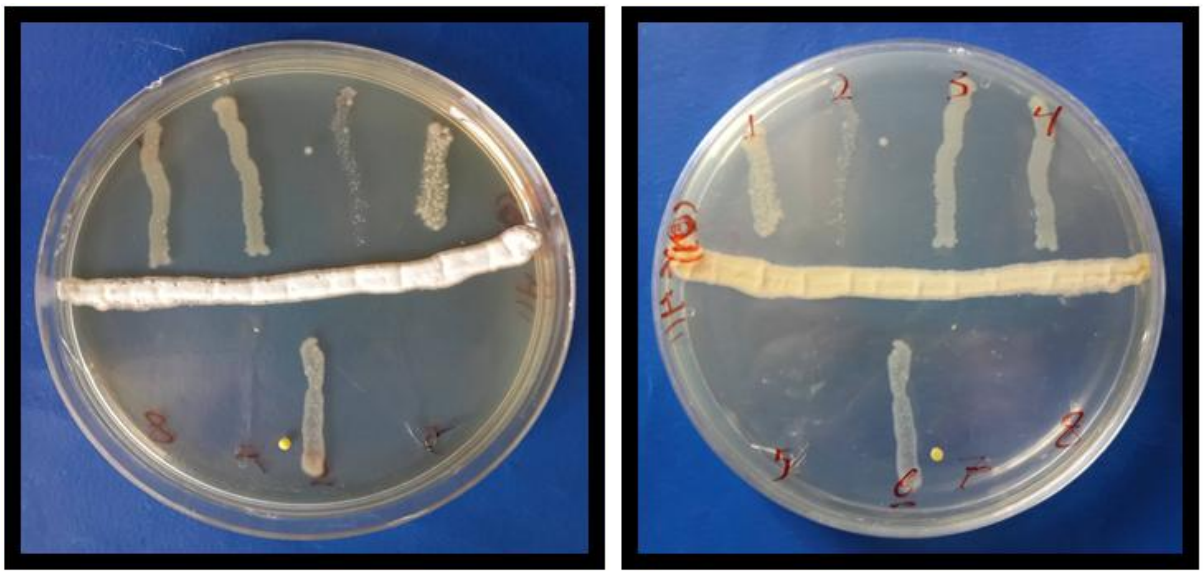

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Figure: 7. Antibacterial activity of S.H.A-65againstpathogenic gram positive and negative bacteria

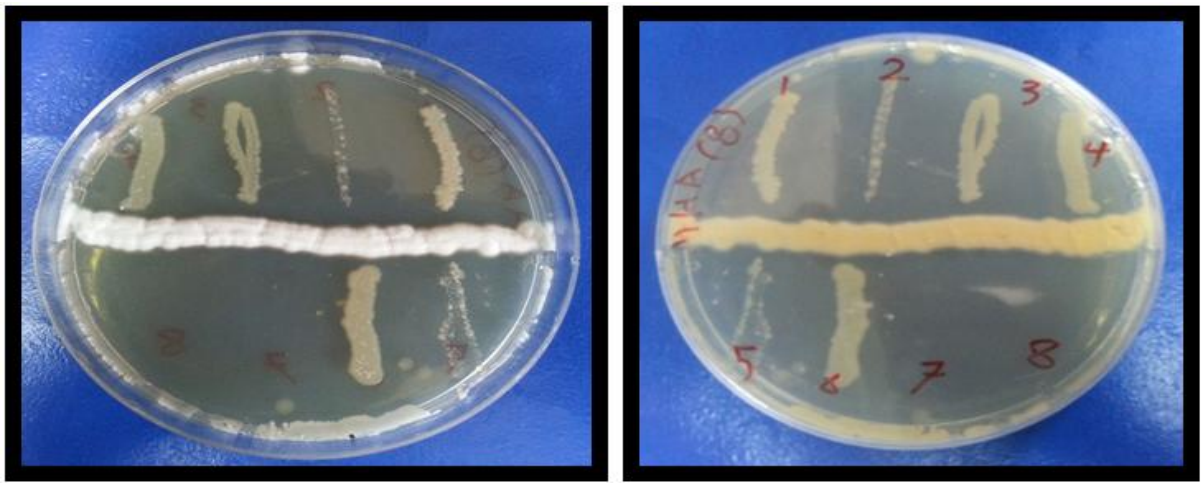

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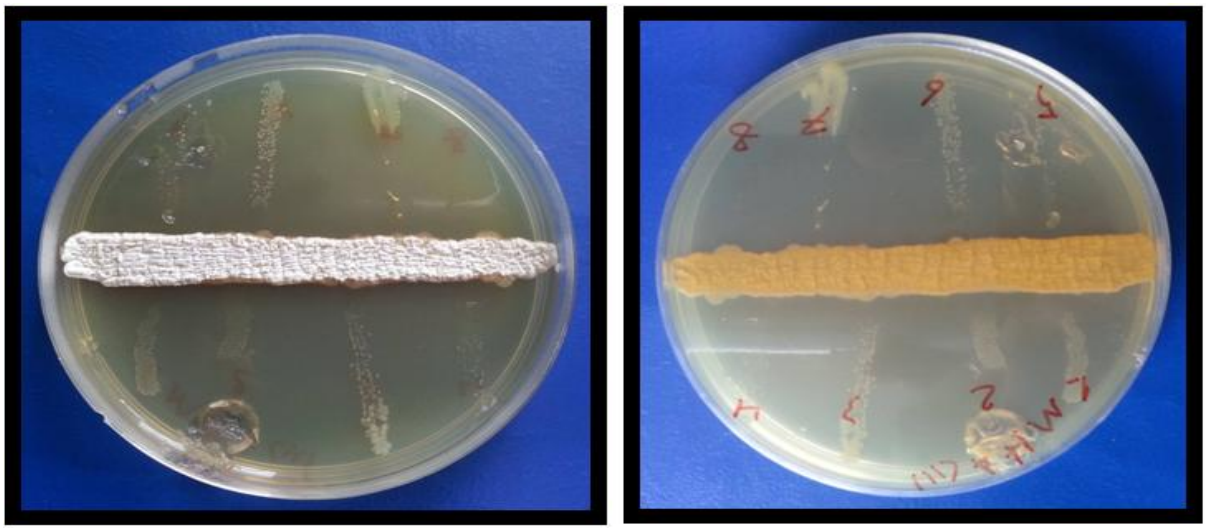
Figure: 8. Antibacterial activity ofS.K-72againstpathogenic gram positive and negative bacteria



A-Front shape **B.** Rear shape

Figure: 9. Antibacterial activity ofS.K.A-83against pathogenic gram positive and negative bacteria

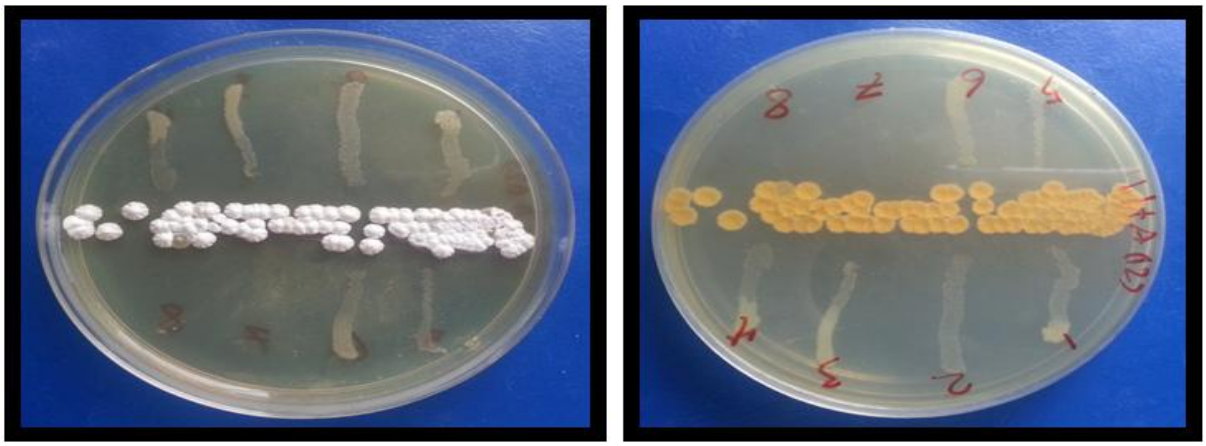


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Figure: 10. Antibacterial activity ofS.A-98 against pathogenic gram positive and negative bacteria

Table: 3. primary screening of Streptomyces isolates against pathogenic bacteria

Inhibition space between Streptomyces spp and pathogenic bacteria measured by Millimeter (mm)								
Actinomycetes Isolate	Pathogenic bacterial numbers							
	1	2	3	4	5	6	7	8
S.K-5	6	5	5	4	1	5	19	18
S.M.A -17	4	5	3	14	11	10	4	4
S.N.22	5	6	21	4	3	6	19	17
S.M-34	5	13	4	19	5	6	7	8
S.S-46	4	13	6	3	2	5	10	25
S.H-52	6	4	3	7	4	5	3	1
S.H.A-65	4	30	3	2	23	6	25	20
S.K-72	3	4	3	1	2	1	30	25
S.K.A-83	6	4	7	21	14	7	20	25
S.A-98	17	4	18	20	3	8	18	20

1. **Klepsilla Pneumonia.**
2. **Escherichia coli.**
3. **Pseudomonas aeruginosa.**
4. **Serratiamarcescens.**
5. **Aeromonashydrophila.**
6. **Staphylococcus aureus.**
7. **Streptococcus pyogenes.**
8. **Staphylococcus albus.**

This table explained the summary of tests of primary screening

This table explained the summary of tests of primary screening activity of Stretopmycesspp against pathogenic gram positive and negative bacteria growing together on Muller Hinton Agar that showed in figures numbered from (1 to 20) and measured by millimeter.

Twenty one Actinomycetes isolates have been isolated from one hundred agriculture soil samples collected from ten different sites in Babylon city, this involved collected ten samples for each site, table (1). Thirteen Stretopmycesspp from these isolates found have antimicrobial activity against pathogenic gram positive and gram negative bacteria, these named as symbols such as S.K-3, S.K-5, S.M.A -17, S.N.22, S.M-34, S.M-35, S.S-43, S.S-46, S.H-52, S.H.A-65, S.K-72, S.K.A-83 and S.A-98 by take the first letters from the wards from bothStretopmyces and collectedsitesthen added the number of serial sample, table (2). Ten samples from these used for further investigation by test the antimicrobial activity against pathogenic bacteria of the gram negative (Klepsilla Pneumonia, Escherichia coli, Pseudomonas aeruginosa, Serratiamarcescens and Aeromonashydrophila) and pathogenic gram positive bacteria (Staphylococcus aureus, Streptococcus pyogenes and Staphylococcus albus) when cultured togetheron Muller Hinton Agar, figures (1) to (10) and the size of inhibition growth of these bacteria measured by millimeter and summarized, (Table: 3).

When comparison these results with the results of close study in Malaysia, that involved isolation of Actinomycetes from Signy Island soils and the number of isolates was ninety five found that forty six samples from these isolates showed antimicrobial activity against gram positive and negative pathogenic test bacteria (Pan et al,. 2013). And in other study in Jordan that involved isolation and investigation of antimicrobial activity of twenty eight samples of Stretopmycesspp that isolates from soils in north part of Jordan found that eight isolates have antimicrobial activity (Abussaudet al., 2013). But in deferent study in Iran that involve isolation one hundred fortyStretopmycesspp isolates from soils in northwest of Iran found that only twelve isolates have antimicrobial activity against gram positive and gram negative pathogenic bacteria (Malekiet al., 2013).

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