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Detection of Streptomycin Production Gene from Actinomycetes spp. Isolated From Agricultural Soils in Hillah City /Iraq

Ehsan F. Hussain Eman M. Jarallah*, Ali.H.Al-Saadi Babylon
University /College of Science /Dept. of Biology

Abstract: *One hundred of agricultures soil samples have been collected from different locations in Hillah city. 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 agents were extracted from these fermentations cultures and 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) on Muller Hinton Agar. tenStretopmycesspp from these isolates were found have antibacterial activity, these isolates were named as symbols such as S.K-5, S.M.A -17, S.N-22, S.M-34, S.S-46, S.H-52, S.H.A-65, S.K-72, S.K.A-83 and S.A-98. These isolates were identified by molecular method by used PCR for identified the 16S rDNA gene as well as the morphological methods, which included light microscope for study the aerial and substrate mycelia and electron microscope for study of spores surface morphology and the results revealed all these isolates were belonged toStreptomtces spp. In addition to used PCR for identification ofstrA streptomycin phosphortransferase gene which responsible for streptomycin production.*

***Keywords*:** Actinomycetes , Electron microscope, Genes

I. Introduction

Actinomycetes are gram positive bacteria that are distributed in water and some colonizing plants but widely found in soil, the types and species of these microorganisms in ecosystem are determined by numerous of physical, chemical and biological factors such as showing marked chemical and morphological diversity, also they produce branching mycelium, which are two kinds, substrate mycelium and aerial mycelium, in addition they are potential source of many bioactive compounds, which have large clinical effects and important applications in medicine (Varghese et al., 2012).

Consequently, the of isolating of novel Actinomycetes strains from the soils that used in searching for novel products has switched in way to obtain rarer genera of Actinomycetes or to well characterized ones that are found in unusual environments, however, novel genera can be isolated by taking into account several factors during the isolation procedures, such as, selection of ecological locations for sample collection, chemical and physical factors of the collection samples, like use of specific selective media, of culture conditions and genus or specific isolation methods (Khannaet al., 2011).

Actinomycetes, which are phylogenetically defined as a group within the high guanine (G) and cytosine (C) gram positive bacteria and they are one of the major populations of microbial communities in soils and more types of which have been isolated from this part of environment and also these microorganisms have been isolated from decaying and sound stone (Abdulla et al., 2008 ; Pan et al., 2013).

Marine environment form 70 % of the earth's surface which provides the largest inhabitable space for living organisms, particularly microbes, and as largely source for the isolation of new microorganisms with potentiality to produce active secondary metabolites, such Actinomycetes (Baskaranet al., 2011).

II. Materials and Methods

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).

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).

Amplification of 16S rDNA gene of Streptomyces spp

Tow 16S rDNA genes primes, forward and reverse were designed as show in the following table.

Table: 1. Primers sequence for identification Streptomyces App

Nucleotides Sequence	**Bases No.**	**Annealing Temp.**	**References**
F: 5´-AAGCCCTGGAAACGGGGT-3´	18 bp	55 ºC	Dehnadet al., 2010
R: 5´-CGTGTGCAGCCCAAGACA-3´	18 bp		

Gradient amplification of 16S rDNA gene at different annealing temperatures for Streptomyces spp

PCR amplification was performed using Master Mix (Bioneer, Korea), (2 µl) primers, (1 µl) for forward primer and(1 µl) reverse primer, (1.5 µl) chromosomal DNA, and the final volume was reached to (20 µl). Byadded (16.5 µl) of demonized water.

The PCR amplification was performed using the thermal cycler program as follows: 94ºC for 5 min as a primary denaturation step, 35 cycles of 94ºC for 1 min as denaturation step, (52.3 °C, 55 °C, 57.5 °C and 60 °C) for 1 min as annealing temperature and final extension was 72ºC for 10 min. The PCR products were visualized using gel electrophoresis on 1% agarose and compared with 1 kb DNA ladder.

Amplification of 16S rDNA gene at (55 ºC) annealing temperatures for Streptomyces spp

PCR amplification was performed using Master Mix (Bioneer, Korea), (2 µl) primers, (1 µl) for forward primer and(1 µl) reverse primer, (1.5 µl) chromosomal DNA, and the final volume was reached to (20 µl). By added (16.5 µl) of demonized water.

The PCR amplification was performed using the thermal cycler program as follows: 94ºC for 5 min as a primary denaturation step, 35 cycles of 94ºC for 1 min as denaturation step, (55 ºC) for 1 min as annealing temperature and final extension was 72ºC for 10 min. The PCR products were visualized using gel electrophoresis on 1% agarose and compared with 1 kb DNA ladder.

Amplification of strA gene that codes for biosynthetic Streptomycin phosphortransferase gene of Streptomyces spp

Tow strA streptomycin phosphortransferase genes primes, forward and reverse were designed as show in the following table:.

Table: 2. Primers sequence for identificationstrA gene

Nucleotides Sequence	**BasesNo.**	**Annealing Temp.**	**Refer.**
strA-F: 5´-ATGAGTTCGTCGGACCACAT-3´	20 bp	59.1ºC	Lim et al., 1989
strA-R: 5´-TCAGGGCTTCGCCAGCGCTT-3´	20 bp		

Gradient amplification of strA gene at different annealing temperatures for Streptomyces spp

PCR amplification was performed using Master Mix (Bioneer, Korea), (2 µl) primers, (1 µl) for forward primer and(1 µl) reverse primer, (1.5 µl) chromosomal DNA, and the final volume was reached to (20 µl). By added (16.5 µl) of demonized water.

The PCR amplification was performed using the thermal cycler program as follows: 94ºC for 5 min as a primary denaturation step, 35 cycles of 94ºC for 1 min as denaturation step, (52.8 °C, 57 °C, 59.1 °C and 61.3 °C) ºC for 1 min as annealing temperature and final extension was 72ºC for 10 min. The PCR products were visualized using gel electrophoresis on 1% agarose and compared with 1 kb DNA ladder.

Amplification of strA gene at (59.1 °C) annealing temperatures for Streptomyces spp

PCR amplification was performed using Master Mix (Bioneer, Korea), (2 µl) primers, (1 µl) for forward primer and(1 µl) reverse primer, (1.5 µl) chromosomal DNA, and the final volume was reached to (20 µl). By added (16.5 µl) of demonized water.

The PCR amplification was performed using the thermal cycler program as follows: 94ºC for 5 min as a primary denaturation step, 35 cycles of 94ºC for 1 min as denaturation step, (59.1 °C) ºC for 1 min as annealing temperature and final extension was 72ºC for 10 min. The PCR products were visualized using gel electrophoresis on 1% agarose and compared with 1 kb DNA ladder.

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 Actinomycetes isolates obtained from these samples. These isolates have been cultured and purified on international Streptomyces project type-2 (ISP-2) (Table: 3).

Table: 3. Number of Actinomycetes isolates that obtained from agricultures samples

No.	Site of Collection	No. of site soils samples	Sequence of site samples	ActinomycetesNo.	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 %**

Secondary screening test for Actinomycetes isolates against pathogenic bacteria

The size of inhibition zones of activity of Stretopmycessppcultures extracts againstpathogenicgram negative bacteria(Klepsilla Pneumonia, Escherichia coli, Pseudomonasaeruginosa ,Serratiamarcescens and Aeromonashydrophila)measured by milliliter and found the rage of these zones from zero mm to 22.5 mm ,this mean thecultures extracts of this bacteria have no or moderate or strong effect against these pathogenic bacteria (Table: 4).And in comparison to the size of inhibition zones of activity of Stretopmycessppcultures extracts against pathogenic gram positive bacteria (Staphylococcusaureus ,Streptococcuspyogenes and Staphylococcusalbus)measured by milliliter and found the rage of these zones from zero mm to 27.5 mm ,this mean the cultures extracts of this bacteria also have no or moderate or strong effect against these pathogenic bacteria (Table: 5).

Table: 4. Measurements of inhibition zones formed by Actinomycetes extract against pathogenic gram positive bacterial species

Mean of inhibition zones formed by Actinomycetes extract against pathogenic gram positive bacteria measured by millimeter (mm)±SE			
Antibacterial extracts of Actinomycetes	**S. aureus**	**S. pyogenes**	**S. albus**
S.K-5	-	-	7.5±0.3
S.M.A -17	15.5±0.2	17.5±0.2	-
S.N-22	14.5±0.1	24.5±0.3	-
S.M-34	19.5±0.3	19.5±0.1	6±0.2

S.S-46	6±0.2	-	-
S.H-52	25±0.3	-	8.5±0.5
S.H.A-65	-	5±0.4	8.5 ±0.2
S.K-72	27.5±0.2	20.5±0.1	24.5±0.2
S.K.A-83	-	5±0.2	10.5±0.4
S.A-98	24.5±0.2	21±0.1	11±0.3

***Each record represents mean of threereplicate**

This table explained the test of secondary screening activity of Actinomycetesspp extract against pathogenic gram positive bacteria and results showed the range was from zero mm to 27.5 mm.

Table: 5. Measurements of inhibition zones formed by Actinomycetes extract against pathogenic gram negative bacterial species

Inhibition zone formed by Actinomycetes extract against pathogenic gram negative bacteria measured by millimeter (mm)* ±SE					
Antibacterial extracts of Actinomycetes	**K. Pneumonia**	**E. coli**	**P. aeruginosa**	**S. marcescens**	**A. hydrophila**
S.K-5	-	20.5±0.1	2.5±0.2	6.5±0.5	-
S.M.A -17	-	-	-	13±0.3	-
S.N-22	9.5±0.2	9±0.1	-	3.5±0.1	1±0.1
S.M-34	2±0.1	-	2 ±0.1	6±0.2	2±0.1
S.S-46	6.5±0.2	-	6.5 ±0.2	11±0.2	8.2±0.2
S.H-52	1±0.2	13±0.5	15 ±0.2	-	2±0.2
S.H.A-65	-	8±0.5	1.5 ±0.3	-	-
S.K-72	9.5±0.1	10±0.2	16±0.2	19.5±	22.5±0.4
S.K.A-83	2.5±0.1	20±0.2	5.5 ±0.2	-	-
.A-98	6.5±0.3	11±0.3	22.5±0.1	21±0.1	9±0.6

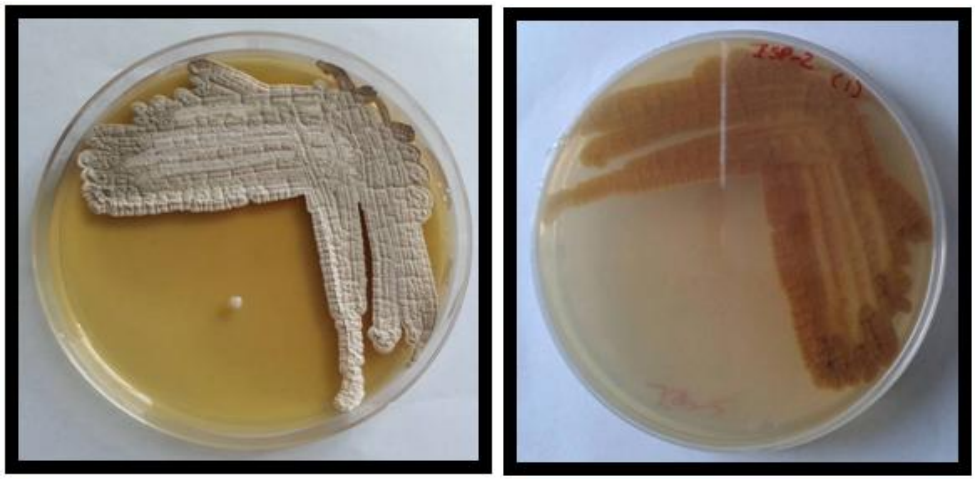
***Each record represents mean of three replicate**

This table explained the test of secondary screening activity of Actinomycetesspp extract against pathogenic gram negative and the results showed range was from zero mm to 22.5 mm.

When comparison of these results with the results of close study for Prabhahar and his group (2014) were isolated of Actinomycetes from different locations in Muthuppettai Mangrove areas in India and test antibacterial activity of these isolates against pathogenic bacteria, this found the size of inhibition zone range from zero mm to 18 mm against pathogenic negative bacteria and from zero mm to 23 mm against pathogenic gram positive bacteria (Prabhaharet al., 2014). And incomparison of the results with the results of different study for Singh and his workers that isolation of Actinomycetes from different soils samples in N Delhi in India and test antibacterial activity of these isolates against pathogenic bacteria, this found the size of inhibition zone range from zero mm to 14 mm against both of gram positive and negative pathogenic bacteria (Singh et al., 2012).

Morphological identification of Actinomycetes isolates

The morphological identification of Streptomycesspp involved the study of textures and colors of colonies on international Streptomyces project (ISP) systems as well as the study of aerial and substrate mycelia morphology under light microscope at 1000 X, in addition, the study of the morphological characteristics of spores with scanning electron microscope for identifications of spores surface properties.



A. Shape of aerial mycelium on ISP-2 agar

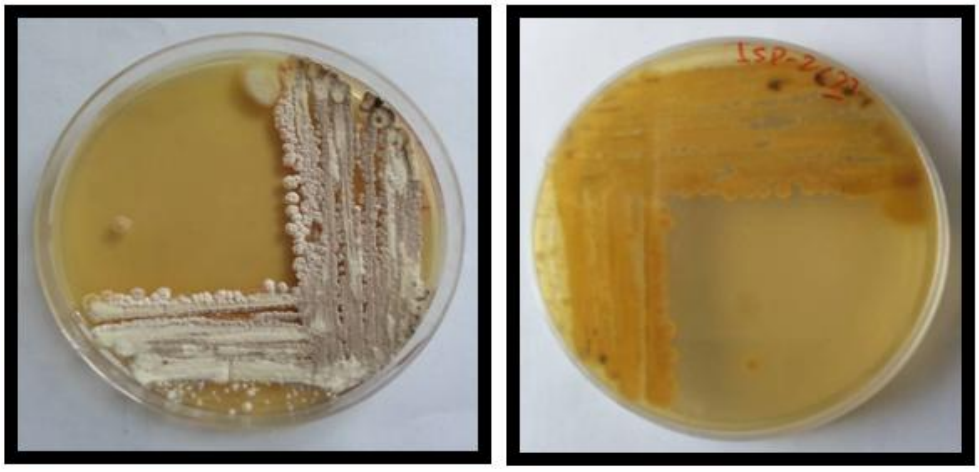
B. Shape of Substrate mycelium on ISP-2 agar

C. Shape of mycelium under light microscope at 1000 X with gram stain

D. Shape of spores under electron microscope at 21000 X

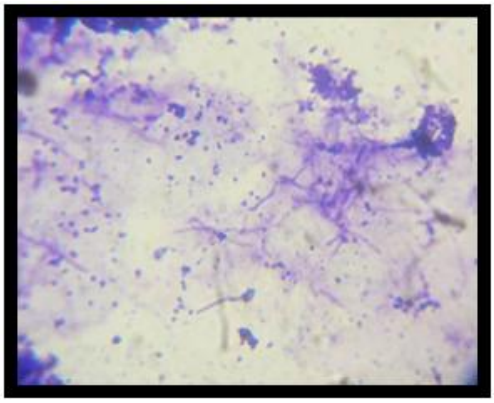
Figure: 1. Morphological properties ofS.K-5 isolate

Figure (1. A) showed the colors of aerial mycelium and figure (1. B) explained substrate mycelium in on ISP-2 agar, in addition figure (1. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (1. D) explained the shape and surface morphology of spores under electron microscope at 21000 X.

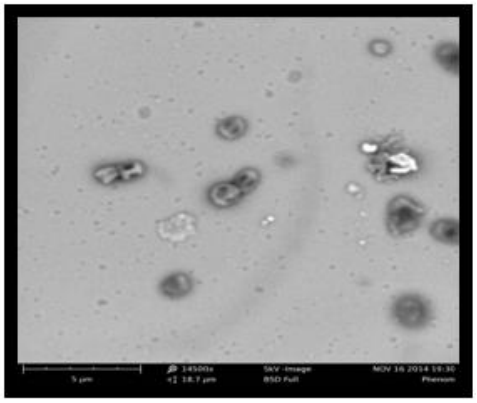


A. Shape of aerial mycelium on ISP-2 agar

B. Shape of Substrate mycelium on ISP-2 agar



C. Shape of mycelium under light microscope at 1000 X with gram stain

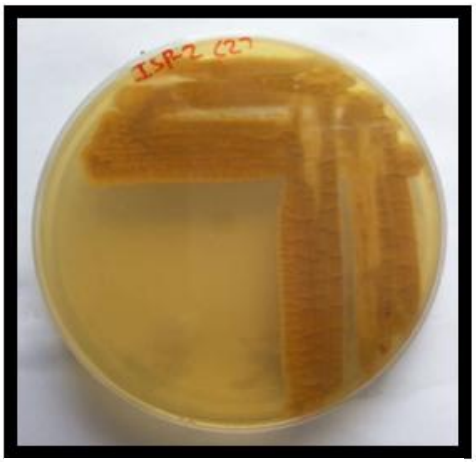


D. Shape of spores under electron microscope at 14500 X

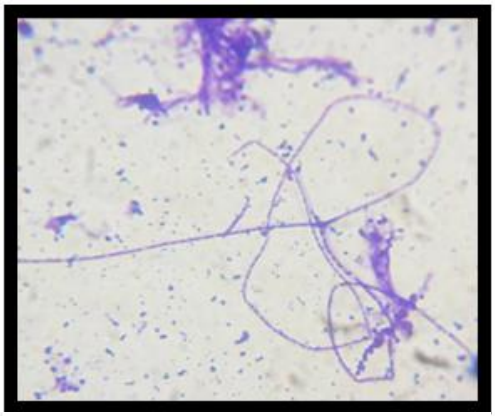
Figure: 2. Morphological properties ofS.M.A-17isolat**e**

Figure (2. A) showed the colors of aerial mycelium and figure (2. B) explained substrate mycelium in on ISP-2 agar, in addition figure (2. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (2. D) explained the shape and surface morphology of spores under electron microscope at 14500 X.

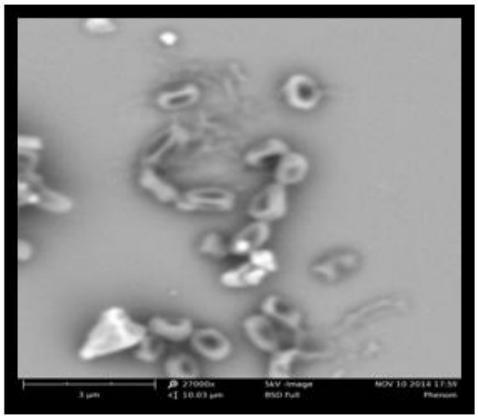
A. Shape of aerial mycelium on ISP-2 agar



B. Shape of Substrate mycelium on



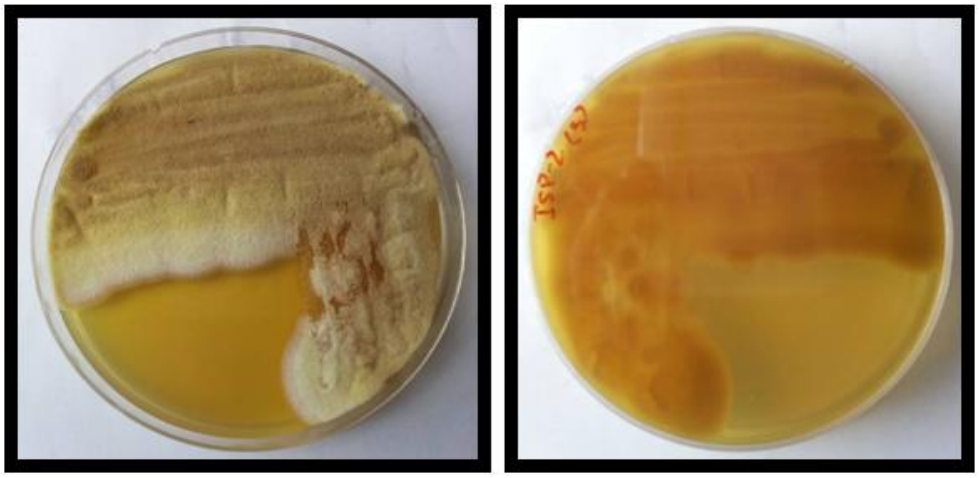
C. Shape of mycelium under light microscope at 1000 X with gram stain



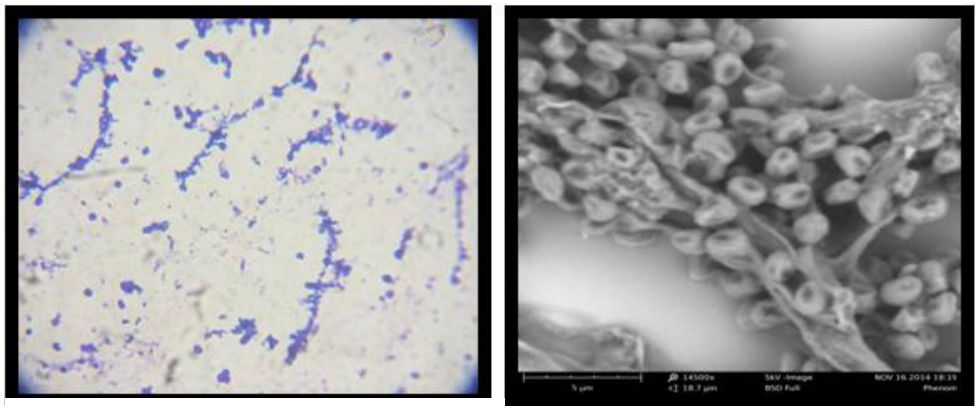
D. ISP-2 agar Shape of spores under electron microscope at 27000 X

Figure: 3. Morphological properties ofS.N-22 isolate

Figure (3. A) showed the colors of aerial mycelium and figure (3. B) explained substrate mycelium in on ISP-2 agar, in addition figure (3. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (3. D) explained the shape and surface morphology of spores under electron microscope at 27000 X.



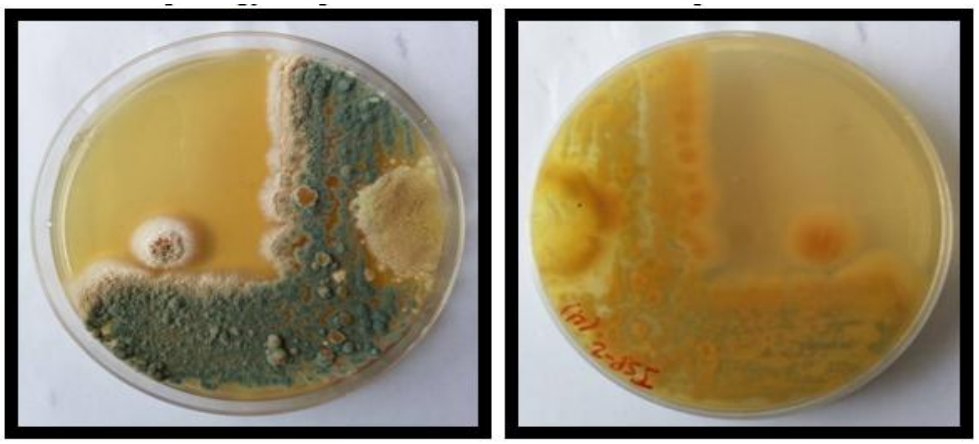
A. Shape of aerial mycelium on ISP-2 agar **B.** Shape of Substrate mycelium on ISP-2 agar



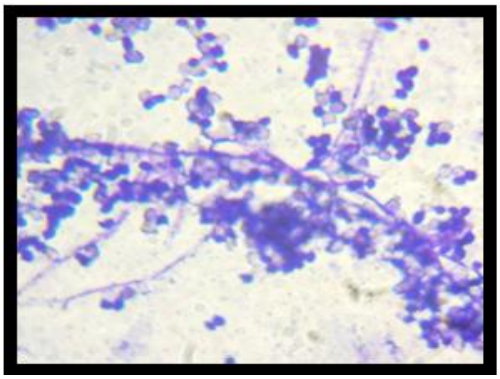
C. Shape of mycelium under light microscope at 1000 X with gram stain **D.** Shape of spores under electron microscope at 14500 X

Figure: 4. Morphological properties ofS.M-34 isolate

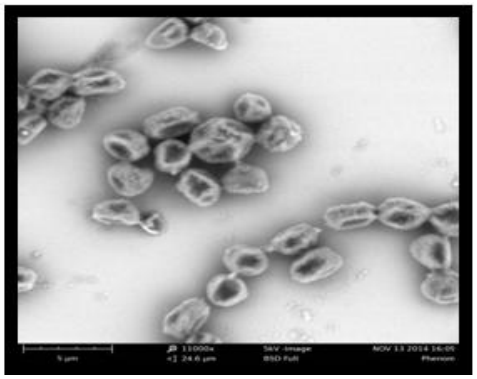
Figure (4. A) showed the colors of aerial mycelium and figure (4. B) explained substrate mycelium in on ISP-2 agar, in addition figure (4. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (4. D) explained the shape and surface morphology of spores under electron microscope at 14500 X.



A. Shape of aerial mycelium on ISP-2 agar **B.** Shape of Substrate mycelium on ISP-2 agar



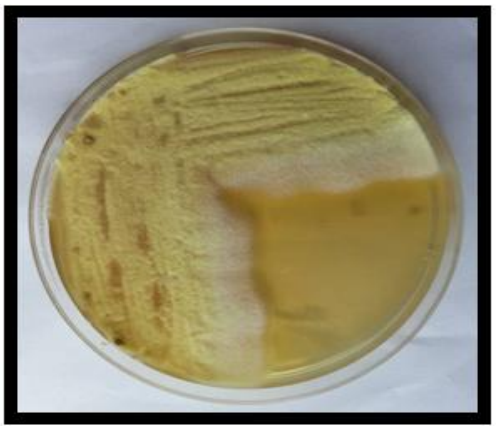
C. Shape of mycelium under light microscope at 1000 X with gram stain



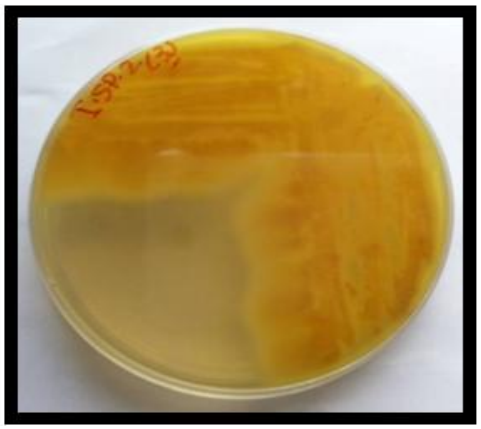
D. Shape of spores under electron microscope at 11000 X

Figure: 5. Morphological properties ofS.S-46 isolate

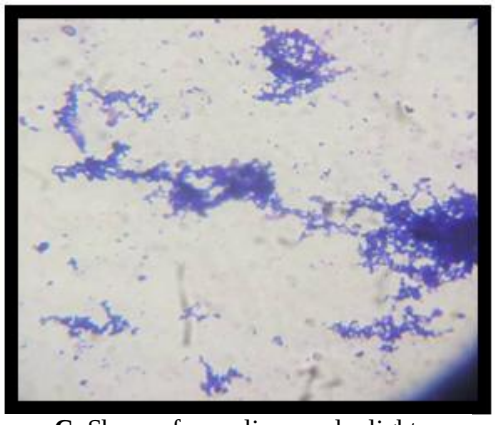
Figure (5. A) showed the colors of aerial mycelium and figure (5. B) explained substrate mycelium in on ISP-2 agar, in addition figure (5. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (5. D) explained the shape and surface morphology of spores under electron microscope at 11000 X.



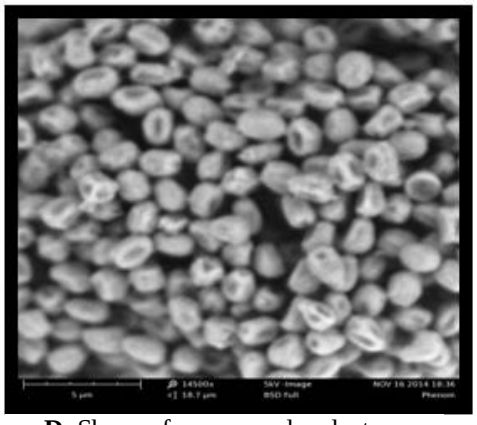
A. Shape of aerial mycelium on ISP-2 agar



B. Shape of Substrate mycelium on ISP-2 agar



C. Shape of mycelium under light microscope at 1000 X with gram stain

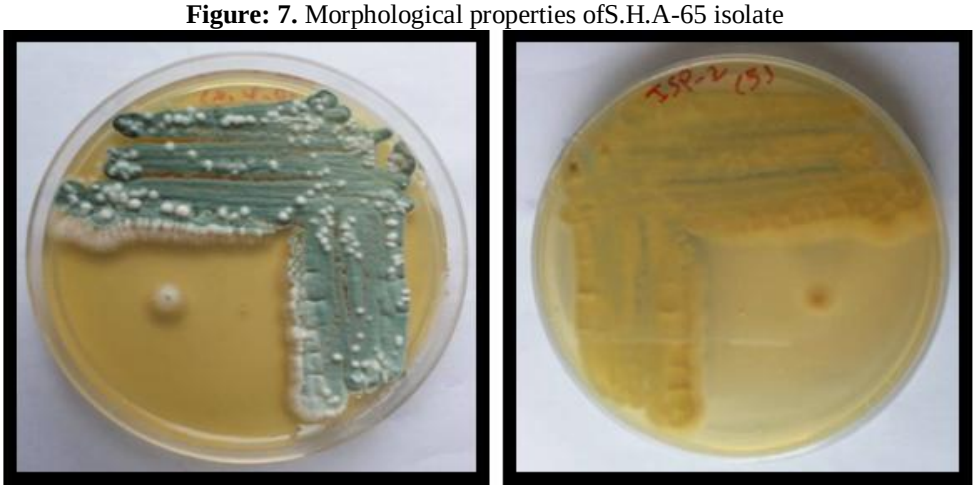


D. Shape of spores under electron microscope at 14500 X

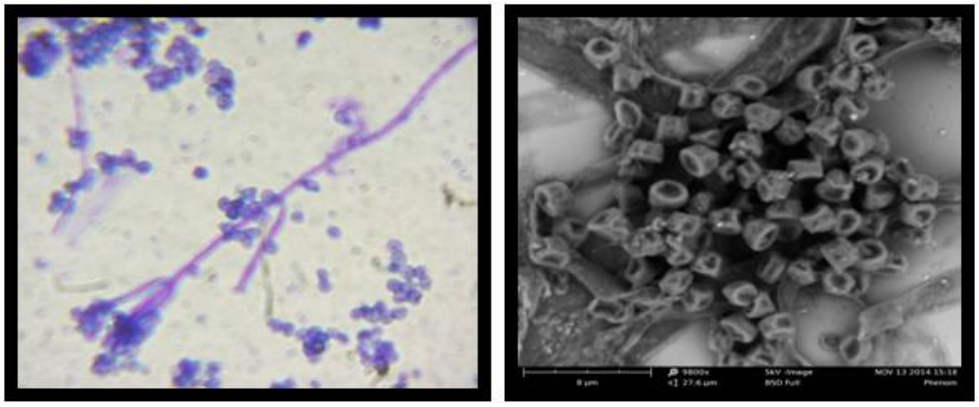
Figure: 6. Morphological properties ofS.H-52 isolate

Figure (6. A) showed the colors of aerial mycelium and figure (6. B) explained substrate mycelium in on ISP-2 agar, in addition figure (6. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (6. D) explained the .shape and surface morphology of spores under electron microscope at 14500 X.

Figure: 7. Morphological properties ofS.H.A-65 isolate



A. Shape of aerial mycelium on ISP-2 agar **B.** Shape of Substrate mycelium on ISP-2 agar

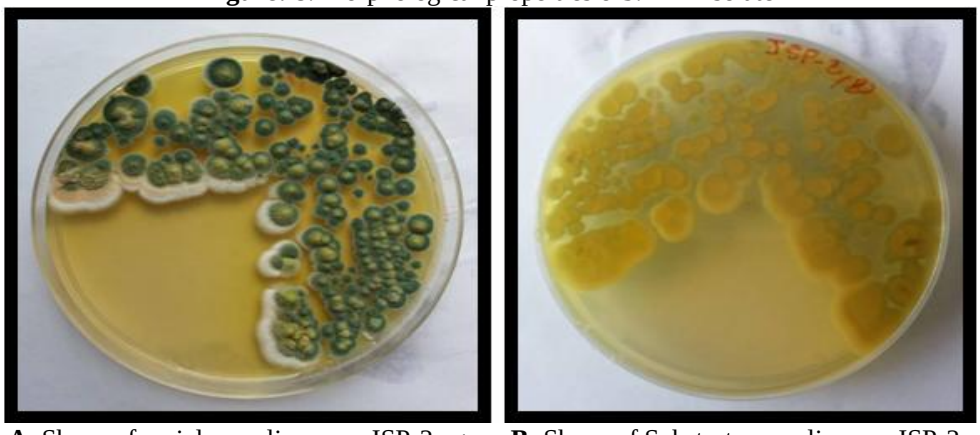


C. Shape of mycelium under light microscope at 1000 X with gram stain

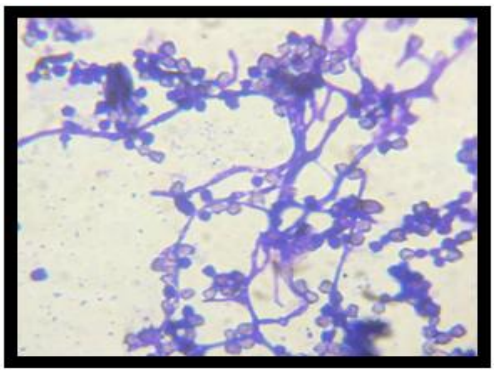
D. Shape of spores under electron microscope at 9800 X

Figure (7. A) showed the colors of aerial mycelium and figure (7. B) explained substrate mycelium in on ISP-2 agar, in addition figure (7. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (7. D) explained the shape and surface morphology of spores under electron microscope at 9800 X.

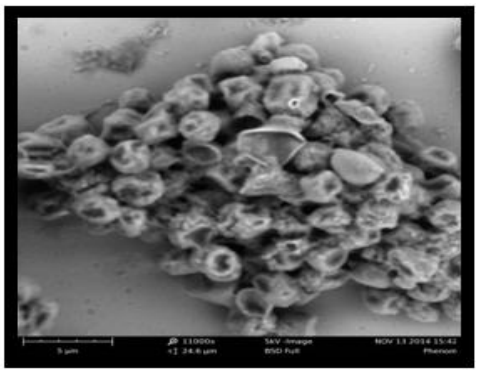
Figure: 8. Morphological properties ofS.K-72 isolate



A. Shape of aerial mycelium on ISP-2 agar **B.** Shape of Substrate mycelium on ISP-2 agar



C. Shape of mycelium under light microscope at 1000 X with gram stain

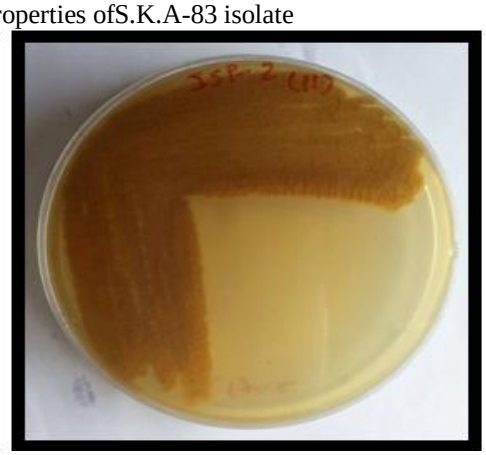


D. Shape of spores under electron microscope at 18500 X

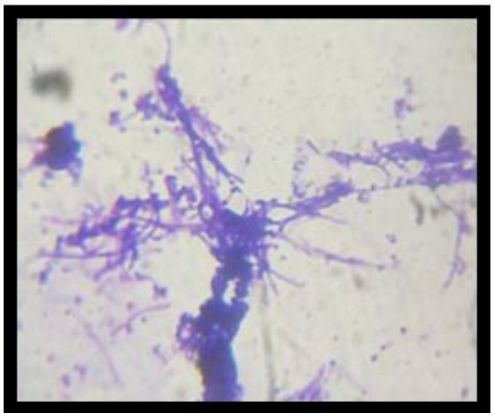
Figure (8. A) showed the colors of aerial mycelium and figure (8. B) explained substrate mycelium in on ISP-2 agar, in addition figure (8. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (8. D) explained the shape and surface morphology of spores under electron microscope at 18500 X.

Figure: 9. Morphological properties ofS.K.A-83 isolate

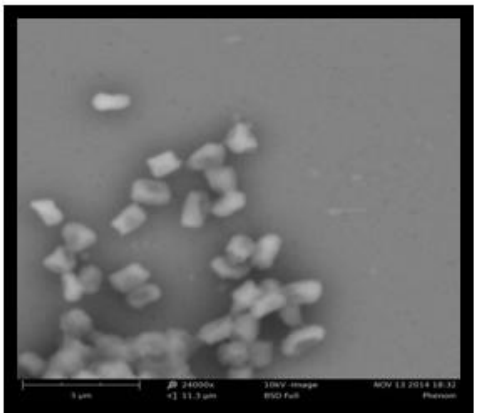
A. Shape of aerial mycelium on ISP-2 agar



B. Shape of Substrate mycelium on ISP-2 agar



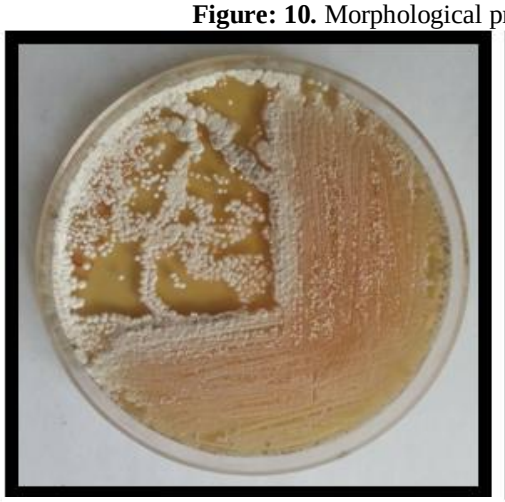
C. Shape of mycelium under light microscope at 1000 X with gram stain



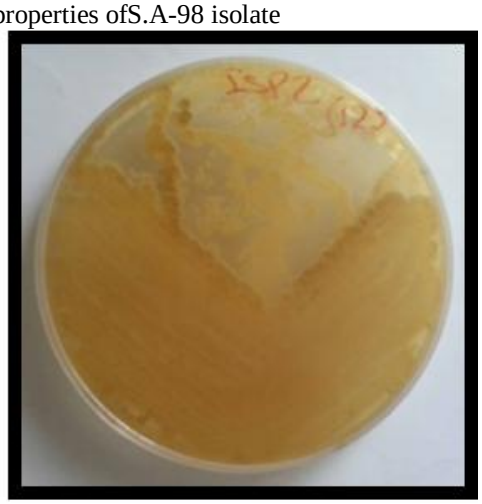
D. Shape of spores under electron microscope at 24000 X

Figure (9. A) showed the colors of aerial mycelium and figure (9. B) explained substrate mycelium in on ISP-2 agar, in addition figure (9. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (9. D) explained the shape and surface morphology of spores under electron microscope at 24000 X.

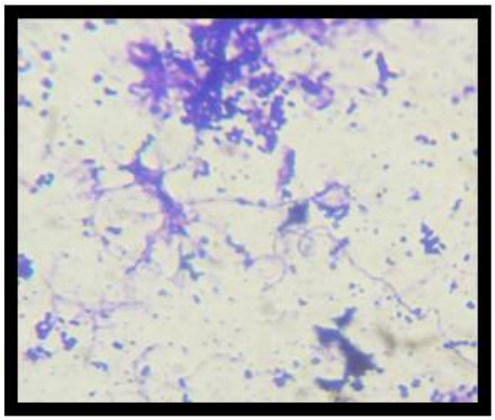
Figure: 10. Morphological properties ofS.A-98 isolate



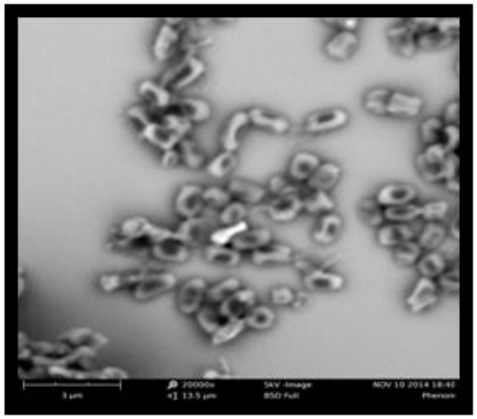
A. Shape of aerial mycelium on ISP-2 agar



B. Shape of Substrate mycelium on ISP-2 agar



C. Shape of mycelium under light microscope at 1000 X with gram stain



D. Shape of spores under electron microscope at 20000 X

Figure (10. A) showed the colors of aerial mycelium and figure (10. B) explained substrate mycelium in on ISP-2 agar, in addition figure (10. C) show the color and morphology of mycelium under light microscope at 1000 X with gram stain, while figure (10. D) explained the shape and surface morphology of spores under electron microscope at 20000 X.

The present results of colonies characteristics approximately agreed with the results of Zin and his group (2011) were isolated the Streptomycesspp from the soil of peripheral area in University Putra in Malaysia and study the shapes of colonies and aerial with substrate mycelia colors for these microorganisms on ISP-2 media (Zinet al., 2011).Also the presented results approximately agreed with the results of Balasubramaniam and his students (2011) were isolate the Actinomycetes from soils in India and study the morphological characteristics for these bacteria (Balasubramaniamet al., 2011).

The current results of mycelia morphology agreed with the results of Rakshanya and his students (2011) were isolated the Actinomycetes from soils and study the antibacterial against different human pathogen bacteria and, also study the mycelia morphological characteristics with oil immersion under light microscope for these bacteria (Rakshanyaet al., 2011). Also the current results of mycelia morphology agreed with the results of Janardhan and his group (2014) were collected the Actinomycetes from soil in Nellore region of Andhra Pradesh in India, and screened for these ability to produce bioactive compounds and, also study the morphological characteristics of mycelia for these bacteria (Janardhanet al., 2014).

The results of scanning electron microscope approximately agreed with the Atta and his group (2012) were isolated Streptomyces form soils in Egypt and examined of spores morphology with scanning electron microscope at magnification bower 15000 X, and El-Batal and his student (2014) were isolated Streptomyces form sediments in Egypt and examined of spores with scanning electron microscope at magnification bowers 8500 X and 14000 X , both of them found these spores have smooth surface (Atta et al.,2012 ; El-Batalet al.,2014).

Molecular identification of Streptomyces spp isolates by amplification of 16S rDNA gene and optimization of amplification conditions for this gene : After identification of Streptomyces by morphological characteristics, these isolates then subjected to molecular identification depended on amplification of 16S rDNA gene by polymerase chain reaction. PCR performed at different annealing temperatures for amplification of genomic DNA by using the (St-F) and (St-R) primers in order to determine the appropriate annealing temperature for identification of Streptomycesisolates. The results revealed the 55 °C was the suitable annealing temperature. After this, all isolates then identified by amplification of thegenomic DNA at 55 °C. The gradient amplification of 16S rDNA genefor three Streptomycesisolates were S.K-5, S.H-52 and S.A-98 at four different annealing temperatures were done in order to determine the optimal amplification temperature. Four temperatures were tested **(52.3 °C, 55 °C, 57.5 °C** and **60 °C)**. The results exhibited the **(55 °C)** it specific temperature for amplification 16S rDNA gene, (Figurer: 11). The amplification of 16S rDNAgenefor Streptomyces spp isolates at**(55 °C)**annealing temperatures by the polymerase chain reaction and the product was approximately **(1500 bp)**.However, this used for identification of Streptomyces species isolates,(Figurer: 12).

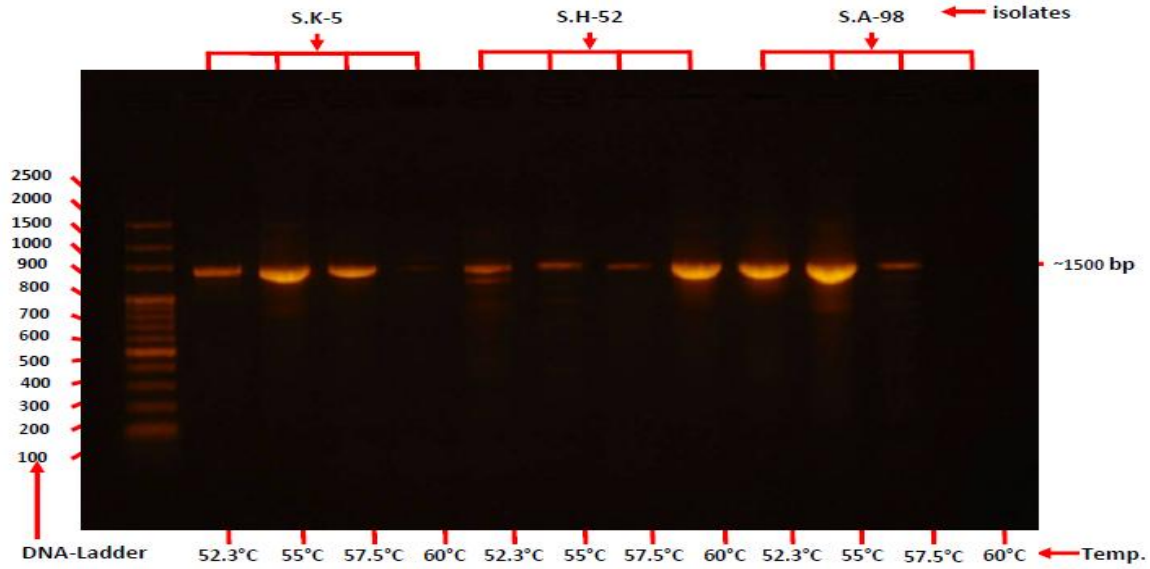


Figure: 11. Gradient amplification of 16S rDNA genes for three Streptomyces isolates at different annealing temperatures

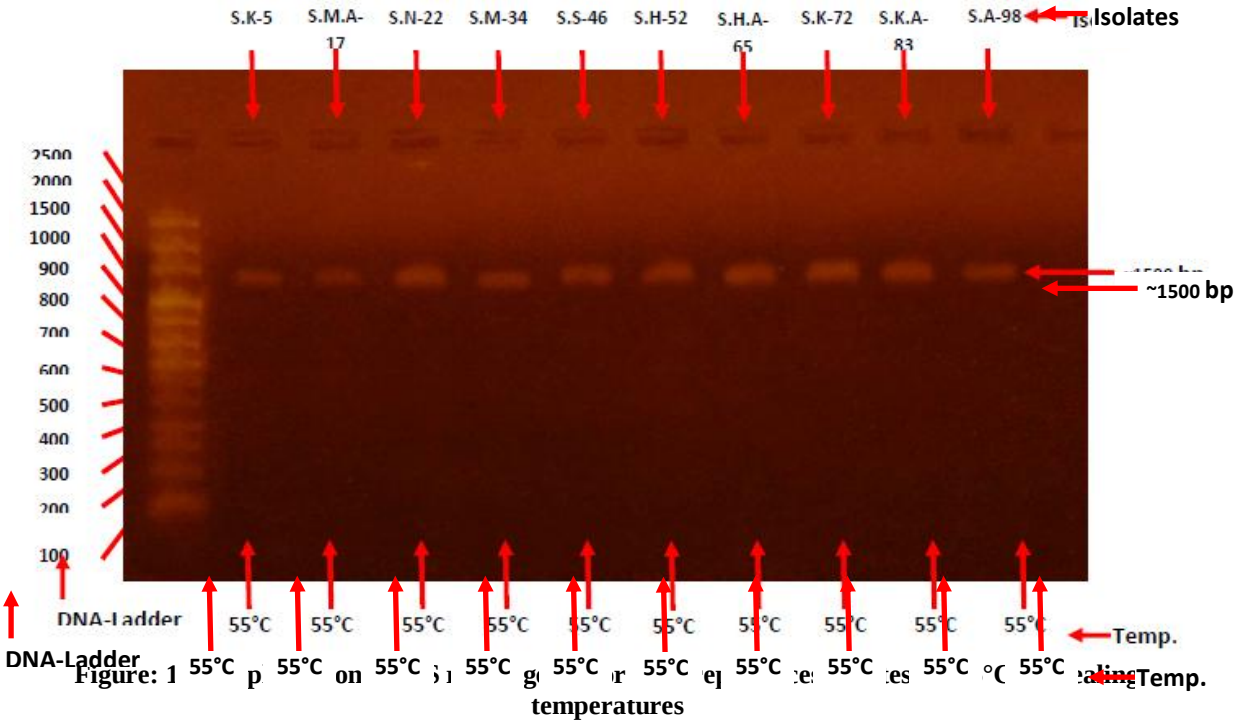


Figure: 1 p on g r ep es es °C aling temperatures

These results similar to Maleki (2013) who used same the 16S rDNA gene (St-F) and (St-R) primers for identification of high antimicrobials Stretopmycesspp isolates, were isolated from soils in northwest of Iran and these gave DNA bands ~1500 bp in length (Malekiet al., 2013). However, in the genetic application the 16S rDNA gene is more simple, specific and yet efficient in identification of new Streptomyces strains (Anderson andWellington.,2001).

In addition, the 16S rDNA gene has less changes and transformation through evolution, and for these it is deemed for taxonomic studies as well as the 16S rDNA gene containing of five variable regions which are α, β, δ, ε, and particularly variable γ part which shows relatively high polymorphism at the 5′ end of its structure (Stachet al., 2003). Therefore, the identification of new strains of Streptomyces by using amplification of hyper variable regions have been frequently described (Pandeyand Mishra., 1995).The 16S rDNA gene is mainly used for molecular identification of bacteria, some regions of this gene are highly conserved and common to all types of bacteria while others are specific to species of these microorganisms (Jihaniet al.,2012). And for this the 16S rDNA analysis has great advantage for the molecular identification of the Actinomycetes(Provost et al., 1997). The 16S rDNA used for molecular identification of new strain of Streptomyces with high capacity for antibiotic production (Oh et al., 2005).

Molecular identification ofstrA gene for Streptomyces spp isolates and optimization of amplification conditions for this gene

The Streptomyces isolates then subjected to molecular identification of the strA streptomycin phosphortransferase genes by polymerase chain reaction. PCR performed at different annealing temperatures for amplification of genomic DNA by using the (strA-F) and (strA-R) primers for known appropriate annealing temperature. The results found the 59.1 °C was the suitable annealing temperature. After this, all isolates then identified by amplification of thegenomic DNA at 59.1 °C.

The results showed the gradient amplification of strA genefor tow Streptomyces spp isolates were S.K.A-83and S.A-98 at four different annealing temperatures in order to determine the optimal amplification temperature. These temperatures were **(52.8 °C, 57 °C, 59.1 °C** and **61.3 °C)**. The amplification results found the **(59.1 °C)** was specific temperature for amplification of strAgene, (Figurer: 13).

The results revealed the amplification of strA genefor Streptomyces isolates. The best annealing temperatures was **(59.1 °C)**. The polymerase chain reaction product approximately **(920 bp)**.However, this used for identification ofstrA streptomycin phosphortransferase gene for Streptomyces species isolates and found the S.A-98 isolate gave DNA bands **(~920 bp)**in length, (Figurer: 14).

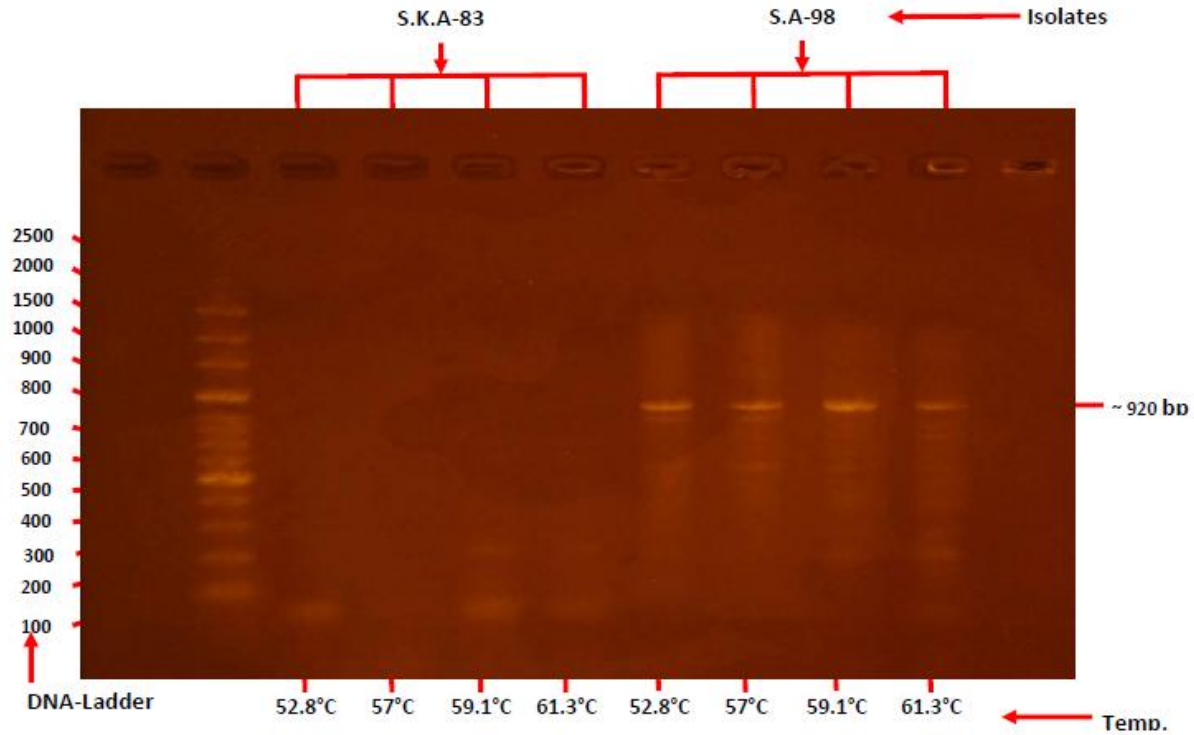


Figure: 13. Gradient amplification of strA genes for two Streptomyces isolates at different annealing temperatures

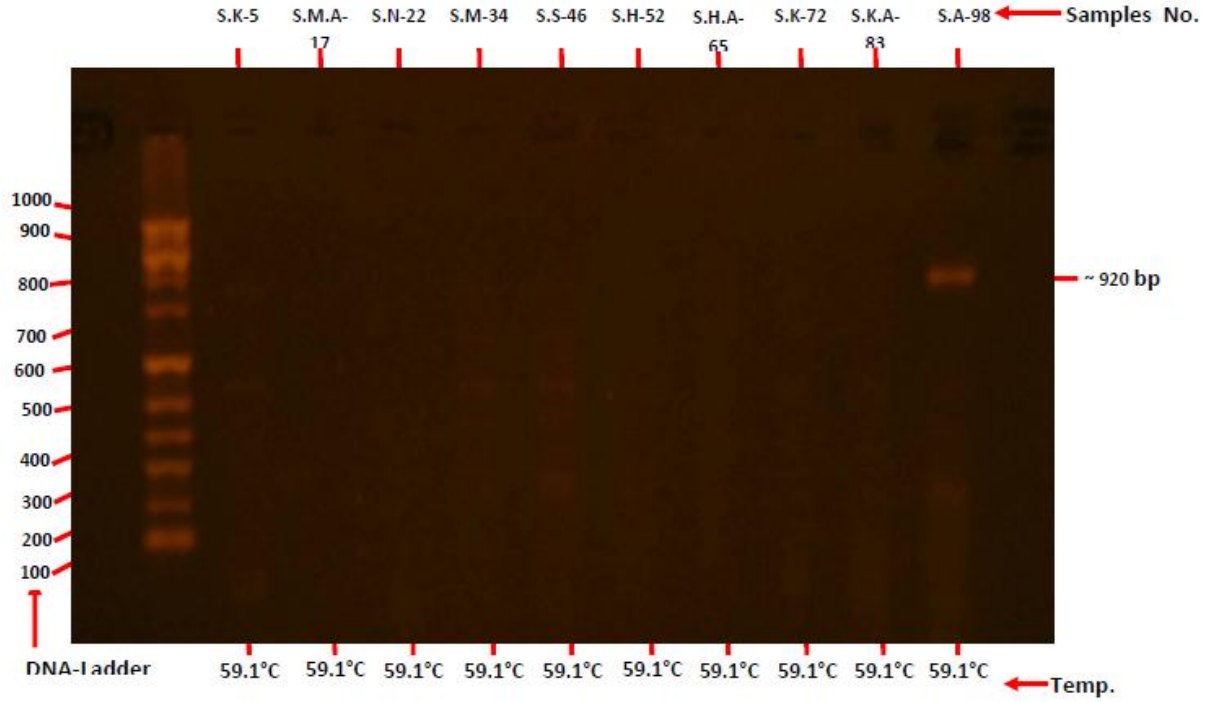


Figure: 14. Amplification of strA genes for all Streptomyces isolates at (59.1°C) annealing temperatures.

These results were agreed with Huddleston and his group (1997) were collected fifty-three isolates from rhizosphere soil in Brazilian and found approximately half of these strains were streptomycin resistant, these Streptomyces griseus strains then screened for the presence of strA by using same our (strA-F) and (strA-R)primers for identification of Streptomycin producing and found only twelve of thesestrains produced streptomycin and gave DNA fragments at ~ 920 bp in length (Huddleston et al., 1997).

The strA genes were identified by number of PCR methods (Piccard et al., 1992). These genes were isolated from Streptomyces (Tolbaet al., 2002). Anumber of S. griseus strains possess the strA streptomycin resistance gene within the streptomycin biosynthetic gene cluster to avoid suicide (Laskariset al., 2010). The production of streptomycin associated with determine of streptomycin phosphotransferasestrA gene that the presence in Streptomycesgriseus, there were also significant differences in the strA of homologue groups between different soil sites, demonstrated that the composition of the populations of these genes varies across different regions (Laskariset al., 2012).

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