



A BRIEF STUDY ON STREPTOMYCES AS AN ANTIBACTERIAL SOURCE

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ABSTRACT:

In the realm of microorganisms, the genus *Streptomyces* is often considered to be among the most influential groupings. Numerous antibiotics are produced by *Streptomyces*. They inhabit a wide variety of ecosystems, from the deepest parts of the ocean to the highest peaks in the Andes. Facultatively anaerobic, filamentous, spore-forming bacteria that are members of the phylum *Bacillus* species. *Streptomyces* diverged from its closest relative, *Kitasatospora*, some 382 million years ago, at the start of the Devonian period. The scanning electron microscope (SEM) analysis of both isolates confirmed the existence of spherical spores with smooth surfaces. Both isolates had the type I cell envelope chemotype that is typical of *Streptomyces* bacteria. However, no distinctive sugar was found in the whole-cell crude extract, and the guard cells contained the L,L-DAP (diaminopimelic acid) isomer. Isolates thrived in temperatures ranging from 25 to 45 degrees Celsius (optimum temperature 28 degrees Celsius), pH ranges from 5 to 10 (optimum pH 7.0), and salt concentrations of up to 5%. They also used all of the tested carbon sources, including starch, glycerol, D-glucose, sucrose, lactose, D-fructose, raffinose, inositol, arabinose, and D-xylose. On the basis of these features alone, it was proposed that the isolates belonged to the genus *Streptomyces*.

KEYWORDS: *Streptomyces*, Antibacterial, *E. coli*, *K. pneumoniae*, Actinobacteria etc.

INTRODUCTION

In developing countries, existence bacterial infections are among the primary causes of significant health issues. Pathogenic bacteria are a key agent in the transmission of infectious illnesses. Due to the fact that antibacterial drugs medicine may cause some unpleasant side effects as well as the spread of diseases, there is an increased need for the creation of additional and more recent antimicrobial drugs that have action against this kind of bacteria.

bacteria in existence. *Streptomyces* is responsible for the production of a significant number of antibiotics. They may be found in practically every habitat, from the depths of the ocean to the In light of this fact, the species *Streptomyces* is regarded as one of the most significant groups of peaks of the highest mountains. They belong to the phylum Actinobacteria and have the characteristics of being Gram-positive, filamentous, and spore-forming bacteria".

Around 382 million years ago, during the early Devonian era, *Streptomyces* began to separate from their nearest cousin, *Kitasatospora*. This event occurred around the same time as terrestrial animals

first appeared. Streptomyces are non-motile microorganisms that grow by the production of hyphae that resemble threads and are capable of entering surface areas in search of nutrition. Streptomyces produces aerial hyphae that divide, creating spores that can tolerate unfavorable circumstances and are readily transferred to other locations or sources of nutrition. This process occurs when resources are restricted.

"Secondary metabolites" are substances that are not absolutely essential for growth or development but might provide the creature a comparative benefit. Streptomyces make bioactive molecules when they are in this growth phase. These metabolites assist the reproductive bacterial cells in a number of ways, including isolating metals such as iron (through siderophores), shielding them from ultraviolet radiation (by coloration), suppressing competition (via antibiotics), and promoting interaction with other organisms. This molecular variety is only conceivable in Streptomyces as a result of their very big genome, which may be up to four times the size of the chromosomes of certain other types of bacteria. In this article, we discuss the role that Streptomyces has played in the development of antibiotic treatment, recent advances in bioprospecting made possible by the connection between Streptomyces and conventional healers, and the ways in which this information has been applied to the search for new antibiotics.

Actinobacteria are the most useful prokaryotic cells from a biotechnology standpoint since they are accountable for the production of almost half of the known biologically active compounds. These compounds include antibiotics. Streptomycin was the first compound isolated from Streptomyces griseus represented an important milestone in natural product chemotherapy. The vast majority of clinically relevant antibiotics in use today are natural products derived from Streptomyces. (Table 1.1).

Table 1.1: List of metabolites produced by Streptomyces

<i>Streptomyces</i> spp.	Metabolites produced	References
<i>Streptomyces antibioticus</i>	Actinomycins	Waksman and Woodruff, 1940
<i>Streptomyces venezuelae</i>	Chloramphenicol	Rebstock <i>et al.</i> , 1949
<i>Streptomyces nodosus</i> subsp. <i>Asukaensis</i>	Asukamycin	Hu and Floss, 2004
<i>Streptomyces hygroscopicus</i> strain 17997	Geldanamycin	He <i>et al.</i> , 2008
<i>Streptomyces virginiae</i>	Virginiamycin	Kitani <i>et al.</i> , 2010
<i>Streptomyces roseosporus</i>	Daptomycin	Baltz, 2010
<i>Streptomyces lydicus</i>	Selamectin	Selvakumar <i>et al.</i> , 2014
<i>Streptomyces aureofaciens</i>	Aureomycin	Francis, 2017
<i>Streptomyces rimosus</i>	Tetracyclines	Petkovic <i>et al.</i> , 2017
<i>Streptomyces venezuelae</i>	Chloramphenicol	Cytryn <i>et al.</i> , 2017
<i>Streptomyces</i> sp. HS-NF-1178	Lankamycin, Lankacidin C	Lu <i>et al.</i> , 2018
<i>Streptomyces rochei</i>	Grixazone B	Arakawa, 2018
<i>Streptomyces griseus</i>	Streptomycin	Lee <i>et al.</i> , 2018
<i>Streptomyces coelicolor</i>	Germicidin A, Albaflavenone	Zhao <i>et al.</i> , 2008; Čihák <i>et al.</i> , 2017
<i>Streptomyces clavuligelus</i>	Clavulanic acid	Flores-Gallegos and Nava-Reyna, 2019
<i>Streptomyces chrestomyceticus</i> BCC 24770	Chresdihydrochalcone	She <i>et al.</i> , 2020

AIM AND OBJECTIVES OF THE STUDY:-

- 1) Isolation of a species of *Streptomyces* from the soil samples that were gathered.
- 2) Evaluation of *Streptomyces* Cultural and morphological characteristics of isolates M7 and M4

MATERIALS AND METHODS

ISOLATION AND SCREENING OF *Streptomyces* ACTINOBACTERIA FOR ANTIMICROBIAL ACTIVITY

Sampling

The several areas of "Punjab, Jammu, and Himachal Pradesh" each contributed a sample of their soil to the overall collection. For the purpose of carrying out more research and experiments, soil samples ranging from a depth of 5 to 15 centimetres were taken into sterile glass screw - capped bottles and stored in a refrigerator with the temperature set between 4 and 8 degrees Celsius.

Actinobacteria isolation from soil sample

In order to provide ideal circumstances for isolating uncommon actinobacteria, soil samples were first allowed to air-dry before undergoing the pre-treatments of dry heating at 100 degrees Celsius for one hour and wet thermal treatment in a water bath at 70 degrees Celsius for thirty minutes. After performing dilution series of the treated soil up to a concentration of "10⁻⁶, aliquots of 0.1 ml from dilutions of 10⁻², 10⁻³, and 10⁻⁴ were spread on the surface of SCNA (starch casein nitrate agar) supplemented with antifungals (cycloheximide: 50 g/ml and nystatin: 25 g/ml) and antibacterial (nalidix Actinobacterial colonies that had been isolated were first subculture and then purified on SCNA plates." The strains were stored as stocks at a temperature of -20 degrees Celsius in glycerol containing 20%.

Screening of actinobacterial isolates for antimicrobial potential

Primary screening

The "Kirby Bauer antibiotic susceptibility test" was adapted for this purpose, and it was used for the first assessment of antimicrobial activities. Actinobacteria plugs of 6 millimeters in diameter that had been cultivated on "SCNA plates" were used in this experiment. The "Mueller Hinton Agar (MHA)" used in this experiment had previously been loaded with bacteria tested. After that, the plates were placed in an incubator set to 37 degrees Celsius, and after 24 hours incubation, the findings were reported as areas of inhibition according to Materials and Methods.

Secondary screening

Actinobacteria were grown on SCNA medium at a temperature of 28 degrees Celsius for a period of seven days. Following incubation, three discs of 7-day-old bacterial isolates growth, each measuring 6 millimetres in diameter, were placed aseptically into 50 millilitres of "starch casein nitrate broth (SCNB)", where they remained for another 48 hours in order to generate an inoculum. The seeds culture was introduced to the Erlenmeyer flasks at a density of 1%, and each flask had 50 cc of SCN broth. The flasks were then placed in an incubator at 28 degrees Celsius with 180 revolutions per minute for ten days. The flasks were collected after each period of 24 hours, and "the biomass was separated from the culture broth by centrifugation at 10,000 rpm for 20 minutes at a temperature of 4 degrees Celsius. For the purpose of determining the culture supernatant's antimicrobial activity, MHA and PDA plates were each seeded with 100 l of test bacterial [optical density (OD) equivalent to McFarland standard 0.5] and fungal suspensions (1 10⁶ spores), respectively. These plates were then punctured with a sterile cork borer to create wells of a size of 6 millimetres, and the supernatant was tested Following the addition of 100 l of culture supernatant to each well, the plates were placed in the refrigerator for one hour to allow for the diffusion of active metabolites. This was followed by incubation at temperatures of 37 °C and 28 °C, respectively, for bacteria and fungi. After 24 and 72 hours of incubation, respectively, the findings were measured in terms of inhibitory zones (mm) that surrounded the wells." These measurements were performed separately for bacterial and fungi.

SELECTION OF POTENT ACTINOBACTERIAL ISOLATES AND DETERMINATION OF THEIR TAXONOMIC POSITION

Based on their efficacy against a broad range of bacteria and fungus, including "MRSA and VRE, isolates M7 and M4" were chosen to keep the research going. These isolates were obtained from soil samples taken from the rhizosphere of *Stevia rebusiana* plants planted in the regions of "Palampur (32° 6' 37.9512" N, 76° 32' 10.4064" E), Himachal Pradesh, India, and Amritsar (31.63°"

Characterization of isolates on the basis classical and molecular approaches

Both traditional methods (such as morphology, microbiological, chemotaxonomical, physiologically, and pharmacological analysis), as well as molecular techniques, were used in order to ascertain the correct taxonomic placement of samples M7 and M4.

Cultural characteristics

On SCNA and various "International Streptomyces Project (ISP) media (Shirling and Gottlieb, 1966)", the national cultures of strains isolated have always been observed. These characteristics include the colour of aerobatic and adsorbent mycorrhizal fungi, improvement, morphogenesis, and manufacturing of soluble pigment.

RESULT

ISOLATION AND SCREENING OF ACTINOBACTERIA FOR ANTIMICROBIAL ACTIVITY

Actinobacteria isolation:

In the present research, a total of 66 isolates were acquired from the mycorrhizal soil samples taken from different areas of "Punjab, Jammu, and Himachal Pradesh, India". These isolates were characterized as actinobacteria on the basis of their morphometric and sociocultural factors. 36.4% of the isolates were found in the rhizospheric soil specimen of *Stevia rebusiana* (Palampur, Himachal Pradesh), followed by 24.2% of the strains isolated found in *Solanum tuberosum* (Gurdaspur, Punjab), 21.2% of the isolated strains found in *Citrus lemon* (Amritsar), and 18.2% of the isolates found in *Anacyclus pyrethrum* (Jammu). Twenty-two of these actinobacteria strains had antimicrobial activities when they were tested in the main screening. The antimicrobial properties of these active isolates was tested using the Kirby Bauer assay to determine whether or not it was present in the biosurfactant. In the secondary screening, 12 of these active isolates showed evidence of antibacterial activity, with different extents of suppression against the various test microorganisms.

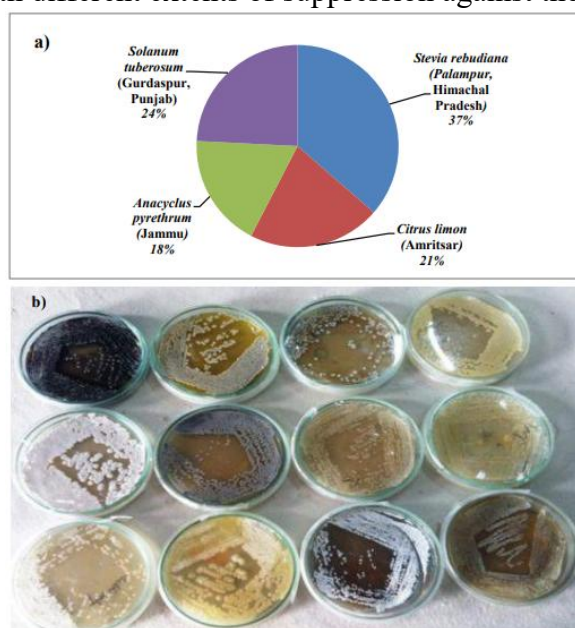


Fig. 1.1: a) Distribution of actinobacteria isolates in different rhizospheric soil samples; b) actinobacteria isolates grown on starch casein nitrate agar medium

SELECTION OF POTENT ACTINOBACTERIA ISOLATES

Two isolates i.e. M7 and M4, which showed strong antibacterial activity against all the tested bacteria, were selected for further experimental studies (Fig. 1.2).

Table 1.2: In vitro antibacterial activity of cell free culture supernatant of active isolates

Isolates	Test microorganisms				
	<i>K. pneumoniae</i> sub sp. <i>pneumoniae</i>	MRSA	<i>E. coli</i> S1-LF	<i>B. subtilis</i>	VRE
Zone of inhibition in mm					
M-1	-	13.16 ± 0.29	-	13.5 ± 0.40	10.8 ± 0.19
M-2	-	14.23 ± 0.31	11 ± 0.33	12.2 ± 0.34	-
M-3	-	13 ± 0.33	11.96 ± 0.44	11.23 ± 0.35	-
M-4	15.3 ± 0.41	15.5 ± 0.31	14.13 ± 0.39	17.5 ± 0.23	16.1 ± 0.29
M-5	-	12.33 ± 0.52	-	12.66 ± 0.41	-
M-6	13.6 ± 0.41	-	14.2 ± 0.28	13 ± 0.33	-
M-7	17 ± 0.33	20.16 ± 0.34	16.83 ± 0.44	16.73 ± 0.26	18.5 ± 0.38
M-8	11.93 ± 0.44	-	13.16 ± 0.34	13.76 ± 0.41	-
M-9	11.67 ± 0.41	13.60 ± 0.32	-	12.20 ± 0.19	10.67 ± 0.25
M-10	-	13.56 ± 0.32	-	12.06 ± 0.18	-
M-11	-	13.16 ± 0.34	-	11.23 ± 0.34	-
M-12	-	11 ± 0.33	-	13.70 ± 0.28	-

Values are mean ± SD (n=3); - indicates no zones of inhibition

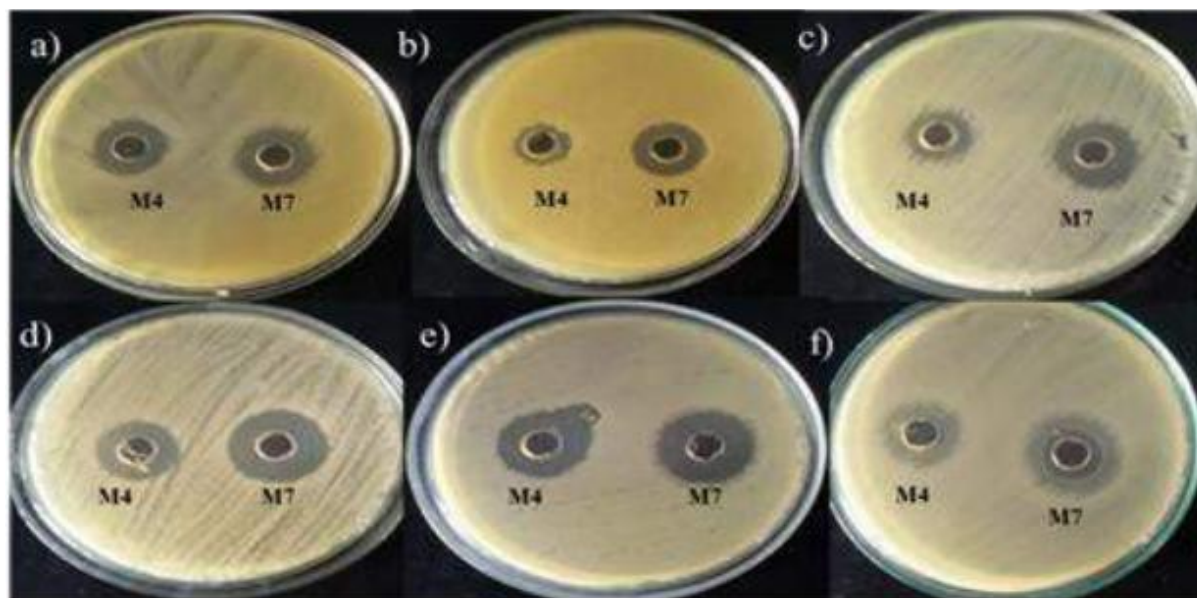


Fig. 1.2 Antibacterial activity of isolates M7 and M4 against bacterial pathogens; a) *E. coli* S1-LF; b) *S. typhi*; c) *K. pneumoniae* sub sp. *pneumoniae*; d) *B. subtilis*; e) MRSA, f) VRE

DETERMINATION OF TAXONOMIC POSITION OF THE SELECTED POTENTIAL ISOLATES

Cultural and morphological characteristics of isolates M7 and M4

The cultural features of the isolated M7 and M4 grown on SCNA and ISP medium are shown in Tables 1.3. On the SCNA, isolate M7 had yellow-colored substratum mycelium and white-colored aerial mycelium. It also contained grey sporulation. On the SCNA medium, the isolate M7 had a yellow pigmentation, while on the ISP6 and ISP7 media, brown pigmentation could be seen. The

isolate M7 was found to have formed branching "substratum mycelium and rectiflexibilis-type spore chains, and it bore 25-38 smooth cylindrical spores on aerial mycelium". These findings were uncovered by microscopic examination. On the other hand, isolate M4 displayed grey spore germination with gray apical and surface mycelia when grown on SCNA medium, and it grew well on a variety of ISP media. On none of the medium, we could find any trace of a pigment that might diffuse. Isolate M4 developed branching substratum mycelium as well as aerial hyphae that contained retinaculiaperti helical spore chains that bore between 26 and 35 smooth surface spherical spores.

Table 1.2: Cultural characteristics of isolate M7 on SCNA and ISP media

Medium	Growth	Aerial mycelium	Substrate mycelium	Sporulation	Diffusible Pigmentation
SCNA	Good	White	Yellow	Grey	Yellow
ISP1	Good	Grey	Brown	White	Light brown
ISP2	Good	White	Creamish	Grey	-
ISP3	Good	White	Yellow	Grey	-
ISP4	Good	White	Pale yellow	Light grey	-
ISP5	Good	White	White	Grey	-
ISP6	Good	White	Light brown	Dark grey	Brown
ISP7	Good	White	White	Grey	Brown

SCNA: Starch Casein Nitrate Agar, ISP: International *Streptomyces* Project, (-) Absent

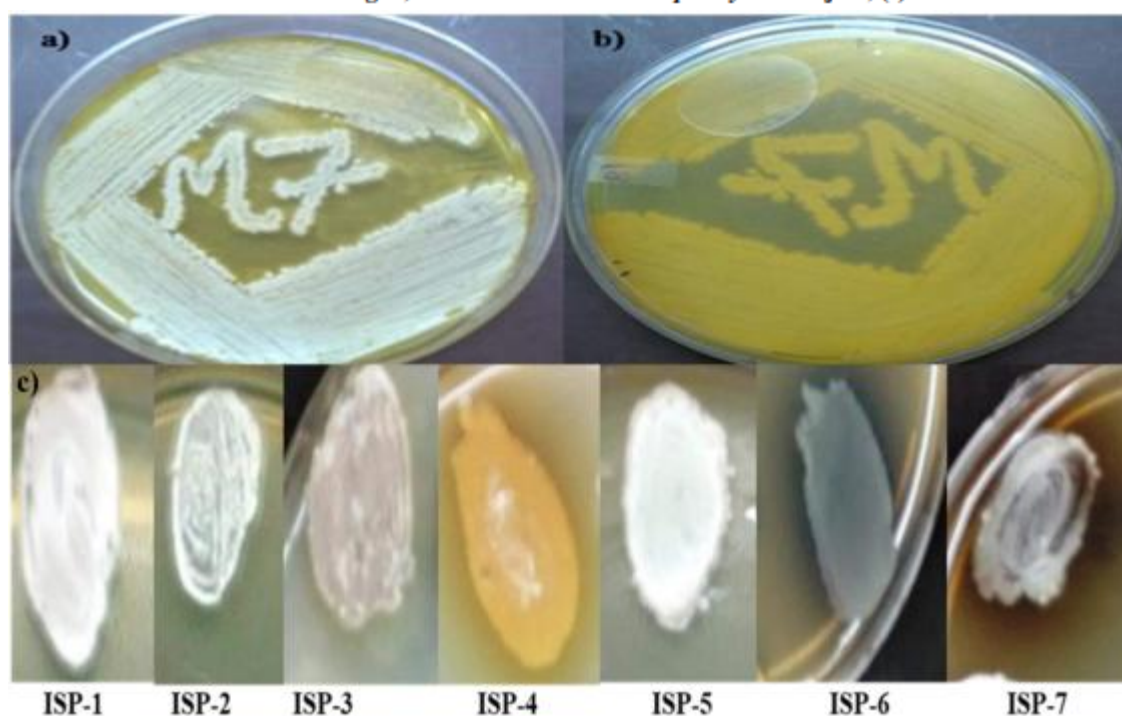


Fig. 1.3 Cultural characteristics of isolate M7 (a) aerial mycelium, (b) substrate mycelium on SCNA medium, (c) on different ISP media

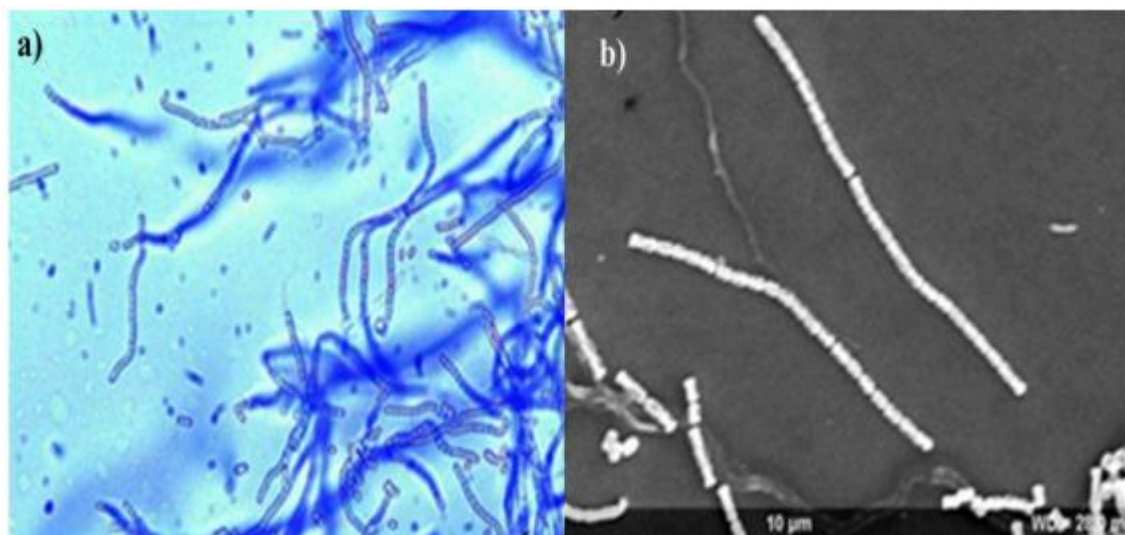


Fig. 1.4 Morphological characteristics of isolate M7 (a) light micrograph at 100X bearing rectiflexibilis-type spore chains on aerial hyphae, (b) scanning electron micrograph (3500X) showing smooth spore surface

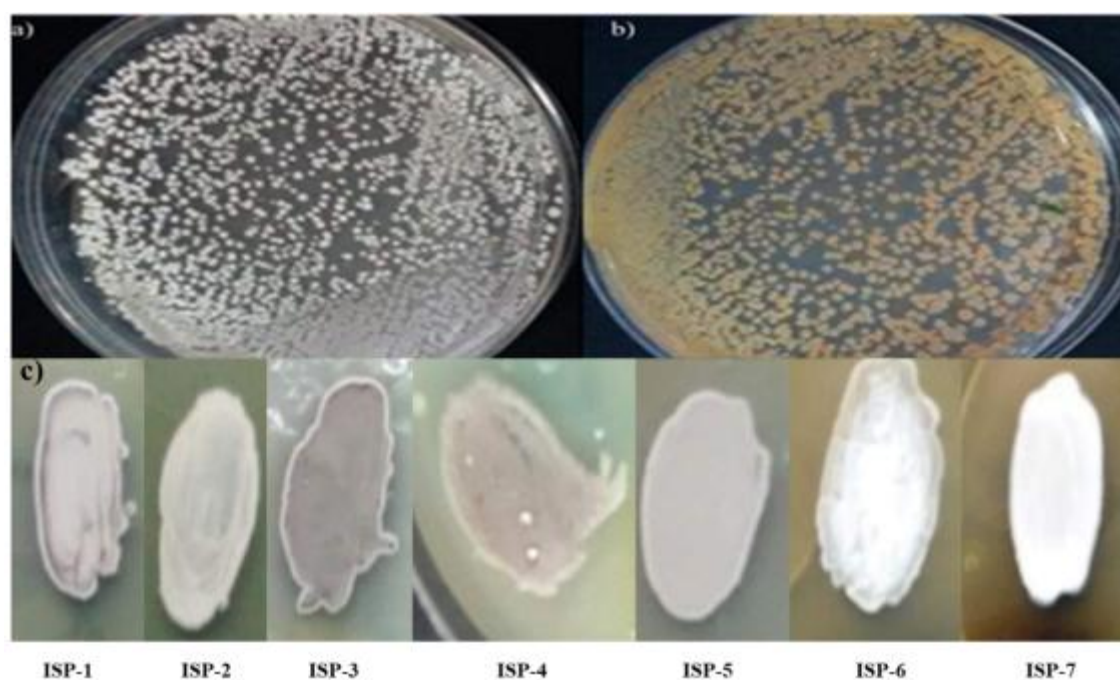


Fig. 1.5: Cultural characteristics of isolate M4 (a) aerial mycelium, (b) substrate mycelium on SCNA medium, (c) on different ISP media

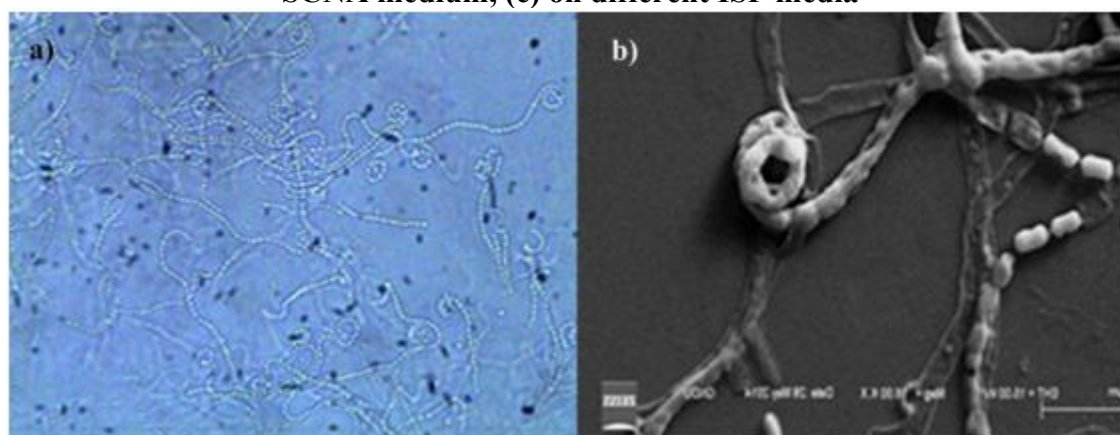


Fig. 1.6: Morphological characteristics of isolate M4 (a) light micrograph at 100X bearing retinaculiaperti coiled spore chains on aerial hyphae (b) scanning electron micrograph (16000X) showing smooth spore surface

Table 1.3: Cultural characteristics of isolate M4 on SCNA and ISP media

Medium	Growth	Aerial mycelium	Substrate mycelium	Sporulation	Pigmentation
SCNA	Good	White	Grey	Grey	-
ISP1	Good	White	Grey	Grey	-
ISP2	Good	White	Grey	Grey	-
ISP3	Good	White	Grey	Grey	-
ISP4	Good	White	Grey	Grey	-
ISP5	Good	White	Grey	Grey	-
ISP6	Good	White	Creamish	Grey	-
ISP7	Good	White	Grey	Grey	-

SCNA: Starch Casein Nitrate Agar, ISP: International *Streptomyces* Project, (-) Absent

DISCUSSION

Streptomyces is the preeminent genus within the actinobacteria phylum, which is responsible for the discovery of 65 percent of the new bioactive compounds found in the area of medication research. These molecules participate in a wide variety of biological functions (Azman et al., 2015; Kemung et al., 2018; Sharma and Thakur, 2020). The group of Gram-positive bacteria known as *Streptomyces* was first described by Waksman and Henrici in 1943. According to bacterio.net, there are a total of 843 species and 38 subspecies belonging to this genus (Parte, 2018). The first strain of *Streptomyces* to be isolated from soil, *Streptomyces griseus* was responsible for the spontaneous production of the antibiotic streptomycin, which was later shown to be effective in the treatment of TB (Woodruff, 2014). The contributions that *Streptomyces* have made to conventional medicine are irreplaceable. Seventy-five percent of the antibiotics that are clinically utilised in humans are derived from *Streptomyces*, but only sixty percent of these antibiotics are used in animals (Janardhan et al., 2014; Lee et al., 2014b; Ser et al., 2015a; Ser et al., 2015b; Law et al., 2017a; Law et al., 2017b). It has been discovered that *Streptomyces* spp. can survive in harsh soil conditions, such as those seen in biodiverse tropical regions all the way up to the range of the Arctic Circle. Microorganisms, such as bacteria, fungus, and yeasts, may be found in soil. *Streptomyces* spp. are the most common microorganisms in soil and are known to create bioactive substances that restrict other types of microflora by preventing the development of microbes or eliminating their rivals (Hibbing et al., 2010; Ilic-Tomic et al., 2015). Physical treatments (wet heat, heat dry, and UV) and chemical treatments (phenol, sodium dodecyl, calcium chloride, and calcium carbonate) are administered to generate vegetative cells and hinder the development of other microflora in order to improve the selective actinobacteria isolation (Khanna et al., 2011; Janaki et al., 2014). A wide range of *Streptomyces* isolates have been retrieved by a number of researchers from a variety of locations, and these isolates are being tested for their antibacterial ability against drug-resistant bacteria (Nguyen and Kim, 2015; Bhakyashree and Kannabiran, 2018; Bhakyashree and Kannabiran, 2020). 15 of the 117 actinobacterial strains that were obtained from soil samples collected in Ghaziabad were shown to have antibacterial activity against at least two of the different bacteria that were examined by Kumar et al. (2010). In 2011, Sharma et al. gathered soil samples from the states of Punjab and Himachal Pradesh. From those samples, they recovered 134 distinct actinobacterial strains. 51 of these isolates showed antimicrobial activity against one or more test pathogens, including MRSA (Sharma et al., 2011). Antibacterial activity of an actinobacterium identified as MITS1005, isolated from Gwalior soil sample, was reported by Singh et al. in 2012. The actinobacterium was effective against MRSA, VRE, *E. coli*, *S. aureus*, and MRSA and VRE, with zones of inhibition of 13-14 mm (Singh et al., 2012). 158 actinobacterial strains were identified

from soil samples by Lim et al. (2018), and six of the isolates demonstrated antibacterial capability against one of the seven MDR bacteria (multidrug resistant bacteria). Baniya et al. (2018) obtained 40 actinobacteria isolates from the rhizosphere of *Alnus nepalensis* from Godawari. Of these, 14 isolates demonstrated antibacterial activity against multidrug-resistant bacteria, including MRSA (methicillin-resistant *Staphylococcus aureus*), VRE (vancomycin-resistant *Enterococcus faecalis*), vancomycin-resistant *Klebsiella pneumoniae*. In 2019, Kumar et al. discovered 31 actinobacterial isolates from rhizospheric soils collected from Western Ghats, Doddabetta, and Tamil Nadu, India. Out of these isolates, 18 isolates displayed poor to excellent action against tested bacteria, which included MRSA (Kumar et al., 2019). Recently, Ahmed et al. (2020) reported that four different isolates exhibited antagonistic activity against ten different multidrug-resistant bacteria. These bacteria included *Acinetobacter boumanii*, *E. coli* ATCC 25922, ESBL-producing *E. coli*, *K. pneumoniae*, *S. aureus* ATCC 25923, *Proteus mirabilis*, *P. aeruginosa*, clinical MRSA, MRSA ATCC 43300, and methicillin-sensitive *A. total*. A total of 66 isolates were recovered from the soil samples that were gathered from three distinct states in India (Punjab, Jammu, and Himachal Pradesh) for the present research project, which was conducted in an effort to identify powerful actinobacterial strains. During the initial screening, there was evidence that these isolates had antibacterial properties against 33 percent of the total bacteria used in the test. Nevertheless, only 12 of the isolates (or 18 percent) that were subjected to a secondary screening showed any antibacterial activity. Of those 12 isolates, 10 were active against MRSA and 4 were active against VRE.

CONCLUSION:-

The emergence of superbugs, or germs that are resistant to many antibiotics, has become a worldwide crisis. The development of resistance to antimicrobial drugs happens when they are no longer effective in killing or inhibiting microbial growth. The CDC (Center for Disease Control and Prevention) classifies drug-resistant bacteria into four categories for 2019: urgent, severe, worrying, and watch list. Because of the prevalence of antibiotic-resistant bacteria in healthcare settings, scientists are constantly on the lookout for new antibacterial compounds to combat superbugs like methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and Extended Spectrum β -lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* spp. However, phytopathogens (including fungus, insects, and nematodes) are a major concern for both the quantity and quality of food produced worldwide. A number of nations have outlawed the use of certain agrochemicals in agriculture because of their harmful impact on human health and the environment. Since many drug-resistant bacteria and plant pathogens may be combated using natural chemicals, notably secondary metabolites from microorganisms, they are being investigated as promising compounds. When it comes to natural chemicals having biological functions, actinobacteria, and notably the *Streptomyces* species, are a rich source, accounting for 75% of all known compounds. Consequently, screening new streptomycetes strains is crucial to obtaining novel chemicals with a wide range of biological properties. With *Streptomyces*' importance in both the pharmaceutical and agricultural industries in mind, this study was conducted with *S. antibioticus* strain M7 and a phylogenetically novel *Streptomyces* sp. M4 that produces metabolites active against drug-resistant bacteria and exhibits biocontrol potential against phytopathogens.

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