Biopotency of Actinobacterial metabolites: A review

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ABSTRACT
The secondary metabolites isolated from microbes and exhibits either antimicrobial (antibacterial, antifungal and antiprotozoans), antitumor and/or antiviral activities, used to be called as antibiotics. Secondary metabolites are also known as bioactive metabolites which work against microbes. There are more than 70% of the antibiotics can be obtained from members of the *Actinomycetes* family and it has been suggested that a large number of *Actinomycetes* may still be unknown with a potential to produce antibiotics. The presence of Multidrug resistant bacteria (MDR) is also responsible for the research of novel antibiotics and novel microbes. Exhibiting various bioactivities, they provide valuable approved drugs in clinical use. Actinobacteria, which share the characteristics of both bacteria and fungi, are widely distributed in both terrestrial and aquatic ecosystems, mainly in soil. They are considered as the biotechnologically valuable bacteria that are exploited for its secondary metabolite production. Approximately, 10,000 bioactive metabolites are produced by Actinobacteria, which is 45% of all bioactive microbial metabolites discovered. Especially *Streptomyces* species produce industrially important microorganisms as they are a rich source of several useful bioactive natural products like biosurfactant, enzyme, bioherbicides, vitamins, pigments, bioremediation, phytohormone production, odor and flavor compounds production etc. with potential applications. In this survey, a large number of the actinomycetes inhabit natural substrates which have the capacity to inhibit the growth of bacteria and other microorganisms. The ability of these actinomycetes to exert an inhibiting effect upon microorganisms is highly specific. Antagonistic actinornycetes produce a variety of antibiotics that vary in chemical nature, in antimicrobial action, in toxicity to animals, and in their chemotherapeutic potentialities.

Keywords: Secondary metabolites; Multidrug resistant bacteria (MDR); Actinobacteria; natural products; antibiotics.

INTRODUCTION

Actinomycetes are high GC, gram-positive microorganism with plant life morphology. They're made supply of secondary metabolites with numerous biological activities. The gram-positive microorganism with high guanine+cytosine in their DNA [3] is 2 major phylogenetic divisions, "low-GC" and "high-GC". GC content is an abbreviation for the proportion of GC base pairs in an organism's DNA. People who have a low GC content, have additional AT base pairs in their DNA. GC content may be crude measure of the connection of microorganisms, however continues to be helpful for differentiating massive phylogenetic divisions. They exhibit a large vary of life cycles, that are distinctive amongst the prokaryotes. Gram-positive bacteria that are placed among the phylum Actinobacteria, class Actinobacteria, subclass Actinobacteridae, order Actinomycetales that presently consists of 10 suborders, quite 30 families and over 160 genera. Being an outsized cluster of microbial resources of wide sensible use and high industrial worth, actinomycetes contribute to around 70% of the supply of antibiotics and conjointly manufacture various non-antibiotic bioactive metabolites, like enzymes, enzyme inhibitors, immunological regulators, anti-oxidation reagents, and so on. Actinomycetes are cosmopolitan in natural habitats, particularly soil and ocean. [1] The marine environment harbors countless species of

microorganisms that play necessary role in mineralization of complex organic matter, degradation of dead plankton, plants, animals, degradation of pollutants and toxicants and primary and secondary productivity. Marine microorganisms have a diverse range of enzyme activity and capable of catalyzing various biochemical reaction with novel enzymes like amylase, lipase, deoxyribonuclease and protease. Among the marine microorganisms actinomycetes includes a very important cluster. The foremost cluster studied very well was the marine Streptomyces [2]. They're filamentous bacteria that manufacture 2 kinds of branching mycelium, particularly aerial and substrate mycelium. Factors influencing the number and kinds of actinomycetes present in explicit soil are a geographical location, like soil type, temperature, organic matter content, moisture content, cultivation and aeration. Actinomycetes act as a serious part of the microbial population in most of the soil. About 90% of the whole actinomycetes population consists of Streptomyces species [4]. Virtually 80% of the world's antibiotics are known to come back from actinomycetes, largely from the genera Streptomyces and Micromonospora [5]. The most multiple drug-resistant bacteria inflicting necessary community-acquired infections embody methicillin/oxacillin-resistant staphylococcus aureus (MRSA), vancomycin-resistant staphylococcus aureus (VRSA), vancomycin-resistant Enterococcus (VRE), extended-spectrum beta-lactamase (ESBL) manufacturing bacteria like E. coli and Klebsiella spp and penicillin-resistant Streptococcus pneumoniae (PRSP). Marine actinomycetes are established as an upscale supply of many secondary metabolites like novel bioactive molecules like antibiotics, antifungal, and anticancer compounds, plant growth hormones, industrially necessary enzymes, enzyme inhibitors, and pigments [6, 7]. α-Amylases (1, 4-α-D-glucan glucanohydrolases, E.C. 3.2.1.1) are one of the most necessary industrial enzymes. Alkaline α-Amylases have high catalytic efficiency and stability at the alkaline pH starting from 9.0 to 11.0 [8] and hydrolyze starch under high pH conditions within the starch and textile industries and conjointly as ingredients in detergents for automatic dishwashers and laundries [9, 10]. Most amylases according from Streptomyces sp. are active in the pH ranges of 5.0–7.5, with limitations for industrial applications [11–15].

TYPES OF ACTINOBACTERIA

Thermophilic Actinobacteria

Number of studies has been applied by the researchers to substantiate the existence of extremophilic and extreme tolerant soil Actinobacteria (acid tolerant and alkali tolerant, psychrotolerant and thermotolerant, and halotolerant and haloalkalitolerant or xerophilic). Mesophilic Actinobacteria will grow at associate in Nursing best temperature from 20^0C to 42^0C, among that thermotolerant species exist, which might survive at 50°C. Moderately thermophilic Actinobacteria have associate in nursing optimum growth at 45°C–55°C [19], whereas strictly thermophilic Actinobacteria grow at 37°C–65°C with the optimum temperature at 55°C–60°C [16]. Incubation temperatures of 28°C, 37°C, and 45°C are thought of best for isolation of soil mesophilic, thermotolerant, and moderately thermophilic Actinobacteria.

Acidophilic Actinobacteria

Acidophilic Actinobacteria, that are common in terrestrial habitats like acidic forest and mine drain soil, grow within the pH vary from regarding three.5 to 6.5, with optimum rates at pH 4.5 to 5.5 [17, 18].

Halophilic Actinobacteria

Extreme halophiles grow best in media containing 0.5–5.2 M salt, whereas borderline extreme halophiles grow best in media containing 1.5–4.0 M salt, moderate halophiles grow best in media containing 0.5–2.5 M salt, and finally halotolerants that don't show an absolute demand to salt for growth however grow originate to usually terribly high salt concentrations and tolerate 100 g/l salt (equivalent to 1.7 M NaCl) a minimum of. Seawater, saline soils, salt lakes, brines, and alkaline saline habitats are thought of because the best habitats for isolating halophilic Actinobacteria like a few of genera, together with Micromonospora, Rhodococcus, and Streptomyces [20].

Endophytic Actinobacteria

Endophytic Actinobacteria are defined as those who inhabit the interior part of plants, inflicting apparently no visible changes to their hosts. These Actinobacteria play specific roles, for instance, protective the host plants against insects and diseases.[21] Actinobacteria include Streptomyces, however the genera Streptoverticillium, Nocardia, Micromonospora, Kitasatospora, Pseudonocardia, Microbispora,

Kibdelosporangium, Actinopolyspora, Nocardioides, Brevibacterium, Actinomadura, Glycomyces Plantactinospora, Polymorphospora, Promicromonospora, and Streptosporangium are found within the plants, like Palicourea longifolia, Calycophyllum acreanum, Monstera spruceana, Croton lechleri, Cantua buxifolia, Siparuna crassifolia, and Eucharis cyaneosperma.

Symbiotic Actinobacteria

About 15% 1945 of the world's nitrogen is fixed naturally by the symbiotic relationships between numerous species of the Frankia belonging to the family of Actinobacteria. The plants that form symbiotic relationships with Frankia are referred to as actinorhizal plants.[22]

Endosymbiontic Actinobacteria

An endosymbiont is any organism that lives inside the body or cells of another organism. Endosymbiosis method is typically obligate, that is, either the endosymbiont or the host cannot survive without the other. Members of the phylum Actinobacteria are known as abundant members of sponge-associated microbial communities. Mycobacterium along with Micrococcus, Micromonospora, Microbacterium, Brevibacterium, Kocuria, Corynebacterium, Rhodococcus, Brachybacterium, Rubrobacter, Streptomyces, Dietzia, Salinispora, Actinokineospora, Gordonia, Arthrobacter, Nocardiopsis, and Rothia species were found to measure as endosymbionts in marine sponges Callyspongia aff. Implexa, Aplysina aerophoba, Spheciospongia vagabunda, Hemimycale culumella, Hyrtios erecta, Dysidea tupha, Callyspongia sp., Dysidea avara, Amphimedon sp., and Negombata magnifica.

Gut Actinobacteria

Tan et al [23] isolated Streptomyces, Nocardiopsis, and Oerskovia from healthy goat feces. The ability of the probiont Streptomyces sp. JD9 from gut of chicken possesses all the characteristics required to satisfy the indigenous Actinobacterial probiont for increased broiler production [24].

APPLICATIONS AND IMPORTANCE OF ACTINOMYCETES

The attention given to the actinomycetes in biotechnological applications may be a natural result of the great metabolic diversity of those organisms and their long association with the surroundings. Actinomycetes are a novel cluster of organisms within the prokaryotes having completely different morphological, cultural, biochemical and physiological characters [26]. They have the ability to degrade a good range of hydrocarbons, pesticides, and aliphatic and aromatic compounds. They perform microbial transformations of organic compounds, a field of great commercial value.

Antibiotics

Actinomycetes are referred to as the best supply of antibiotics. 2/3 of today"s antibiotics are obtained from actinomycetes. The necessary antibiotics from actinomycetes include anthracyclines, aminoglycosides, beta-lactams, chloramphenicol, macrolides, tetracyclines, nucleosides, peptides and polyethers. Untill 1974 antibiotics of actinomycetes origin were virtually completely confined to Streptomyces. Recently efforts are created to explore rare actinomycetes like Actinomadura, Actinoplanes, Ampullariella, Actinosynnema and Dactylosporangium for the search of latest antibiotics [27].

Antimicrobials

Particularly, Streptomyces species produce around 7600 compounds, several of that are secondary metabolites that are potent antibiotics, which has created streptomycetes the first antibiotic-producing organisms exploited by the pharmaceutical industry [28, 29]. The antibiotics from Actinobacteria are differentiated into several major structural categories, like aminoglycosides (e.g., streptomycin and kanamycin), ansamycins (e.g., rifampin), anthracyclines (e.g., doxorubicin), β-lactam (cephalosporins), macrolides (e.g., erythromycin), and tetracycline. One of the first antibiotics used is streptomycin produced by Streptomyces griseus [30]. Some Actinobacteria produce more than one antibiotic substance (e.g., S. griseus), as well as the same antibiotic may be produced by different species of Actinobacteria (e.g, actinomycin, streptothricin) (Table 2).

Table.2: List of antibiotics produced from Actinobacteria

Antibiotic compound	Application	Actinobacteria
1,8-Dihydroxy-2-ethyl-3	Antitumor	*Streptomyces* sp.

methylanthraquinone		
1-Hydroxy-1-norresistomycin	Antibacterial; anticancer	*Schisandra chinensis*
2-Allyloxyphenol	Antimicrobial; food preservative; oral disinfectant	*Streptomyces* sp.
Anthracyclines	Antitumor	*S. galileus*
Arenicolides A–C	Mild cytotoxicity	*Salinispora arenicola*
Arenimycin	Antibacterial; anticancer	S. arenicola
Avermectin	Antiparasitic	Streptomyces avermitilis
Bafilomycin	ATPase inhibitor of microorganisms, plant and animal cells	S. griseus, Streptomyces halstedii
Bisanthraquinone	Antibacterial	Streptomyces sp.
Butenolides	Antitumor	Streptoverticillium luteoverticillatum
Carboxamycin	Antibacterial; anticancer	Streptomyces sp.
Chinikomycins	Anticancer	Streptomyces sp.
Chloramphenicol	Antibacterial, inhibitor of protein biosynthesis	Streptomyces venezuelae
Cyanospraside A	Unknown	Solieria pacifica
Daryamides	Antifungal; anticancer	Streptomyces sp.
Frigocyclinone	Antibacterial	S. griseus
Glaciapyrroles	Antibacterial	Streptomyces sp.
Hygromycin	Antimicrobial, immunosuppressive	Streptomyces hygroscopicus
Lajollamycin	Antibacterial	Streptomyces Nodosus
Lincomycin	Antibacterial, inhibitor of protein biosynthesis	Streptomyces lincolnensis
Marinomycins A–D	Antimicrobial; anticancer	Marinispora
Mechercharmycins	Anticancer	Thermoactinomyces sp.
Mitomycin C	Antitumor, binds to doublestranded DNA	Streptomyces lavendulae
Pacificanones A & B	Antibacterial	S. pacifica
Piericidins	Antitumor	Streptomyces sp.
Proximicins	Antibacterial; anticancer	Verrucosispora sp.
Rapamycin	Immunosuppressive, antifungal	S. hygroscopicus
Resistoflavin methyl ether	Antibacterial; antioxidative	Streptomyces sp.
Saliniketal	Cancer chemoprevention	S. arenicola
Salinispyrone	Unknown	S. pacifica
Salinispyrone A & B	Mild cytotoxicity	S. pacifica
Salinosporamide A	Anticancer; antimalarial	Salinispora tropica
Salinosporamide B & C	Cytotoxicity	S. tropica
Sesquiterpene	Unknown	Streptomyces sp.
Staurosporinone	Antitumor; phycotoxicity	Streptomyces sp.
Streptokordin	Antitumor	Streptomyces sp.
Streptomycin	Antimicrobial	S. griseus
Streptozotocin	Diabetogenic	S. achromogenes
Tetracyclines	Antimicrobial	Streptomyces achromogenes Streptomyces rimosus
Tirandamycins	Antibacterial	Streptomyces sp.

Valinomycin	Ionophor, toxic for prokaryotes and eukaryotes	S. griseus
ZHD-0501	Anticancer	Actinomadura sp.
Elaiomycins B and C	Antitumor	Streptomyces sp.
BK 190 N-[2-hydroxyphenyl)-2-phenazinamine (NHP),	Anticancer; antifungus	Nocardia dassonvillei
Chromomycin B, A2, A3	Antitumor	Streptomyces coelicolor
1,4-dihydroxy-2-(3-hydroxybutyl)-9, 10-anthraquinone 9, 10-anthrac	Antibacterial	Streptomyces sp. RAUACT-1

Enzymes for industrial use

Wide styles of biologically active enzymes are created by both marine and terrestrial Actinobacteria (Table 3). They secrete amylases to the skin of the cells that helps them to hold out extracellular digestion. This accelerator is of nice significance in biotechnological applications like food business, fermentation, and textile to paper industries owing to their ability to degrade starch [31]. Another necessary aspect of Actinobacteria is the production of cellulases that are a set of hydrolytic enzymes that hydrolyze the glucosidic bonds of polyose and connected cello-digosaccharide derivatives. Lipase is created from varied Actinobacteria, bacteria, and fungi and is employed in detergent industries, foodstuff, oleochemical, diagnostic settings, and conjointly in industries of pharmaceutical fields [32]. Many Actinobacteria are isolated from varied natural sources, still as in plant tissues and rhizospheric soil. Similarly, Actinobacteria are unconcealed to be a superb resource for L-asparaginase, which is produced by a variety of Actinobacteria, mainly those isolated from soils, such as S. griseus, Streptomyces karnatakensis, Streptomyces albidoflavus, and Nocardia sp. [33, 34]. The roots and rhizomes of many Thai healthful plants like lemon grass (Cymbopogon citratus) and ginger (Zingiber officinale) have long been utilized in Thai ancient medication for abdomen ache and respiratory disorder treatment [35].

Table.3: Enzymes and their industrial applications

Enzyme	Actinobacteria	Use	Industry of application
Protease	Thermoactinomyces sp.	Detergents	Detergent
	Nocardiopsis sp.	Cheese making	Food
	Streptomyces pactum	Clarification- low calorie beer	Brewing
	Streptomyces thermoviolaceus	Dehiding	Leather
	Streptomyces sp.	Treatment of blood clot	Medicine
Cellulase	Streptomyces sp.	Removal of stains	Detergent
	Thermobifida halotolerans	Denim finishing, softening of cotton	Textile
	Streptomyces sp.	Deinking, modification of fibers	Paper and pulp
	Thermomonospora sp.		
	Streptomyces ruber		
Lipase	Streptomyces griseus	Removal of stains	Detergent

		Stability of dough and conditioning	Baking
		Cheese flavoring	Dairy
		Deinking, cleaning	Textile
Xylanase	Actinomadura sp.	Conditioning of dough	Baking
	Streptomyces spp.	Digestibility	Animal feed
		Bleach boosting	Paper and pulp
Pectinase	Streptomyces lydicus	Clarification, mashing	Beverage
		Scouring	Textile
Amylase	Streptomyces sp.	Removal of stains	Detergent
	Streptomyces erumpens	Softness of bread softness and volume	Baking
	Nocardiopsis sp.	Deinking,drainage improvement	Paper and pulp
	Thermobifida fusca	Production of glucose and fructose syrups	Starch industry
	Nocardiopsis sp.	Removal of starch from woven fabrics	Textile
Glucose oxidase	Streptomyces coelicolor	Strengthening of dough	Baking
Keratinase	Nocradiopsis sp. SD5	Feather degradation	Animal feed
Phytase	Streptomyces luteogriseus R10	Phytate digestibility	Animal feed

MATERIALS AND METHODS

Collection of soil samples

The soil samples were collected from various locations in habitats like ponds, dyke areas, river shores, swampy soils etc. Then they are dried separately at 45-60 °C for 1 h in a hot air oven and then cooled to room temperature. The soil sample (1 g) was added to a conical flask containing 100 ml of sterile water. The flasks were shaken for 30 min and their contents designated stock cultures.

Isolation of actinomycetes

A series of culture was spread on sterile starch-casein agar and Nutrient agar (NA) medium plates aseptically. The plates were incubated at 36-37°C for 72 h. The plates were observed intermittently during incubation. The distinctive colony of actinomycetes were selected and purified by multiple streaking methods. The stock cultures of each selected strain was prepared and maintained in transport swab media (Nutrient agar media) at 36-37 °C for the further studies.

Screening of strains for antagonistic activity

1. Primary screening

Preliminary screening for antibacterial activity was done by the cross-streak method, on NA media. Actinobacteria from swab media were streaked across one-third of the plates and the plates incubated at 36^0c for 3-4 days. After incubation the test bacteria were streaked perpendicular to the actinomycete, and the plates were further incubated at 37 ^{0}c for 24 h. The inhibitory effect was determined by the failure of test bacteria to grow near producing actinomycete [36].

Crawford et al. (1993) method was followed for primary screening of antifungal activity. On the PDA, the actinomycetes from transport swab media were streaked onto one side of plates and the plates were incubated at 30 ^{0}C for 72 h, at which time the colonies had just become visible. Then, agar plug from 10 day old culture of test fungi was transferred onto the other side of each plate. Fungal plugs were also

placed on un inoculated PDA plates separately as uninhibited controls. Cultures were incubated at 30^0C, and the plates were examined for inhibition of growth after 5 days [37].

2. Secondary screening

Based on the zone of inhibition, secondary antimicrobial screening was done under submerged fermentation conditions by agar well diffusion assay .The antimicrobial producing actinomycetes were grown on the PDB with pH 8 at 30 0c for 10 days. To obtain the cell-free filtered supernatant after incubation, the culture broth was centrifuged at 8000 xg for 10 min and filtered by Millipore filter (0.22). On the muller hinton agar the test microorganisms were swabbed by sterile transport swab, the wells (6.0 mm diameter, 2.0 cm apart) was cut from MHA using a sterile cork borer and 100 µl of filtered supernatant was loaded into each well for the assay of antagonistic activity. The dishes were pre incubated at 4°C for 2 h to allow uniform diffusion into the agar. After pre incubation, the plates were incubated at 37°C for 24h for bacteria and at 30°C for 48 h incubation for fungi. The antimicrobial activity was evaluated by the measuring of inhibition zones diameter.

Characterization of antibiotic-producing isolate at the genus level

Morphological identification

The Actinomycetes isolate was stained with gram stain and examined by light microscope at 10x, 40x and 100x magnification. The colony on the NA was examined under light microscope at 40x, to observe the aerial mycelium at the margins of the colony.

Cultural and physiological identification

Cultural features of Actinobacterial strain isolates were characterized following the directions given by the International Streptomyces Project (ISP) [38]. The Tryptoneyeast extract agar (ISP 1), yeast extract-malt extract agar (ISP-2), oatmeal agar (ISP-3), inorganic salt-starch agar (ISP-4), glycerol-asparagine agar (ISP-5), peptone-yeastextract- iron agar (ISP-6), tyrosine agar (ISP-7) were inoculated with Actinobacterial strain isolates and incubated at 36^0c for 3-4 days. The color of aerial mycelium, substrate mycelium color (reverse side of plate) and density of growth were observed after incubation period.

Physiologically, to determine whether the isolate resistance to lysozyme or not, lysozyme basal media with different concentrations of lysozyme (10, 25, 50, 75 and 100 µg/ml) was cultured with Actinomycetes isolate and incubated at 30 0c for 10 days. The resistance was determined by the presence of growth. [39]

Genomic DNA extraction

The pure cultures of the actinobacterial isolates were grown in Nutrient broth at 36°C with shaking at 200 rpm for 2–3 days. The mycelia were then separated from the broth culture by centrifugation at 10000 rpm for 15 min at 4°C. One g of mycelium was aseptically crushed in mortar-pestle using liquid nitrogen. The crushed mycelium (~500 mg) was used for genomic DNA extraction using QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The DNA content was quantified spectrophotometrically using a nanodrop spectrophotometer. The extracted DNA was stored at room temperature until required.

Molecular identification of actinobacterial isolates

The selected actinobacterial isolates were identified by 16S rRNA gene sequencing followed by a sequence similarity search. 16S rRNA gene was amplified using the universal eubacterial primers 27F (5′-AGAGTTTGA TCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTT GTTACGACTT-3′) [40]. The PCR reactions were performed in Proflex PCR System (Applied

Biosystems, USA) using the following reaction conditions: initial denaturation at 94°C for 5 min, followed by 35-45 cycles at 94°C for 1 min 30 s, 54°C for 30 s and 72°C for 1 min, and final extension at 72°C for 10 min. The amplified fragments were analyzed by 1.5% (w/v) agarose gel electrophoresis and further purified using GenElute PCR Clean-Up Kit (Sigma Aldrich, USA) following manufacturer's instructions. To designate the taxonomic status of the isolates, the quality-checked, assembled sequences were queried against NCBI's non-redundant, reference RNA sequence database (refseq_rna) in the nucleotide BLAST tool using the mega blast algorithm. The isolates were assigned species-level taxonomy using the prescribed 98.7% similarity threshold [41, 42].

Phylogenetic evaluation

The identified 16S rRNA gene sequences and the CDS of the antimicrobial biosynthetic genes were aligned using Clustal X algorithm implemented in MEGA6 software along with the sequences of the nearest known taxa [46]. Neighbour joining tree was constructed based on the evolutionary distance calculated using Kimura-2-parameter substitution model. The consistency of the tree was verified by bootstrap scrutiny with 1000 resamplings using p-distance model.

Minimum inhibitory concentration of actinomyctes against test microbial strains

Minimum inhibitory concentration (MIC) assay was performed [47, 48] with little modifications. A stock solution ethyl acetate extract of 7 days old culture of the isolate was prepared in 10% dimethyl sulfoxide (DMSO). Different concentrations of the extract (10 µg - 80 µg) were transferred to a 96-well plate and freshly grown test microbial strains were added to each well. Different antibiotics like Ampicillin and fluconazole (5 µg each)etc. were used as the positive controls, and 10% DMSO served as the negative control. The plates were aseptically incubated for 24 h at 37 °C for bacteria and 28 °C for fungus, and absorbance was measured at 620 nm using a UV-Vis spectrophotometer (Varioskan Flash, Thermo Scientific, San Jose, CA, USA). The plates were then incubated for 2 h at room temperature after adding 30 µl of 0.015% resazurin. The concentration at which no colour change was observed (blue) was considered as MIC value of the extract. Cells (10 µl) from the wells where blue colour was observed were spread on MHA and SDA plates and incubated for 24 h. The concentrations at which no visible bacterial or fungal growth was observed were noted as minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC), respectively.

RESULTS AND DISCUTIONS

Actinomycetes isolation

Actinomycetes were isolated and the morphological appearance of isolates is shown in Figure 1.

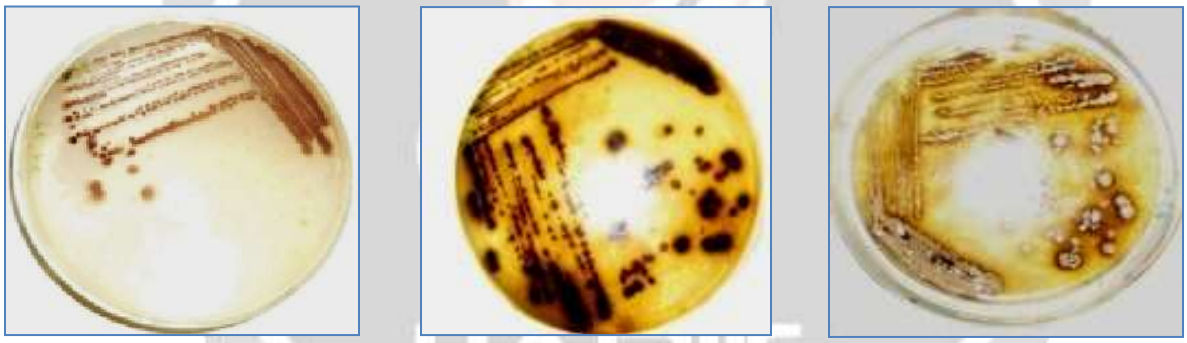


Figure.1: Isolation of Actinomycetes strains from soil samples [54]

Primary screening

Among the actinomycetes isolated from soil samples, 3-4 isolates showed antibacterial activities against at least one of the tested bacteria. In pour plating method, results revealed that the isolates of actinomycetes exhibited broad spectrum activities against tested bacteria. The isolates showed potential activity against E. coli, S. typhi, Kleb and S. aureus. The results were shown in Table.1.[53]

Table.1: Zone of inhibition (mm) of the Isolates against tested bacteria using pour plating method.

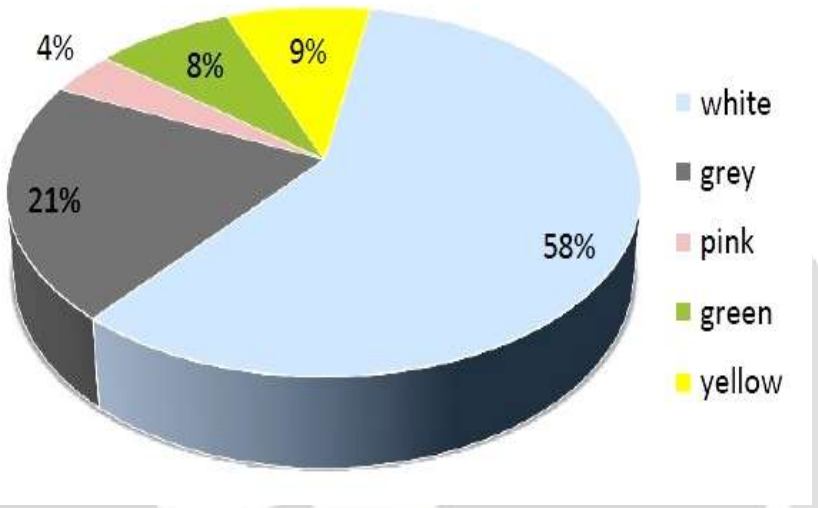
Test Organism	Control	Actinomycetes metabolite
Staphylococcus aureus	26mm	10.6mm
Escherichia coli	27mm	7.5mm
Klebsiella pneumonia	25mm	7.2mm
Salmonella typhi	26mm	7.2mm

Morphological identification

Results of morphological characteristics of the selected isolates revealed that the growth of the isolates was in starch casein agar and glycerol yeast extract. In Nutrient agar and starch casein agar growth was

excellent for actinimycetes isolates. The aerial and substrate mycelium colour varied among the isolates such as some were observed with blackish pigment and some were observed with yellowish pigment shown in a graphical picture in Figure.2.[52]

Figure.2:



Morphological characteristics of Actinomycetes by mycelium colour varied among the isolates

Gram staining of Actinomycetes isolates:

For characterizing and identification of actinomyctes strains gram straining of the colonies through the glass slides were done where the structural and morphological characterization was taken place. Figure.3 showing the some isolates structure. [52]

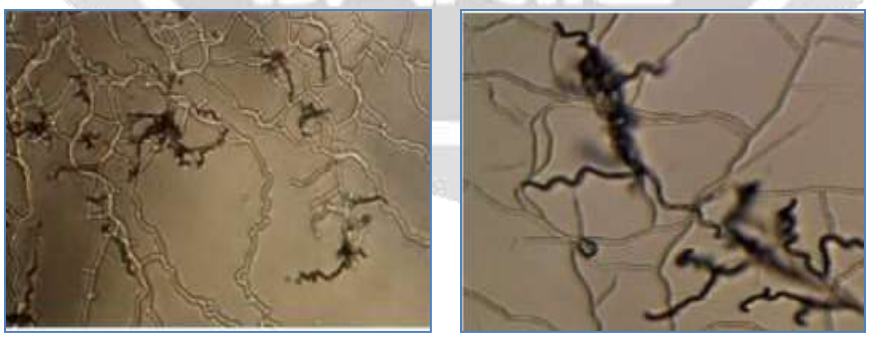


Figure.3: The figures showing the structure of the Actinomycetes strain

Physiological and biochemical characteristics of isolates

Physiological and biochemical characteristics result indicates that all isolates showed the ability of starch and urea hydrolysis. The isolates were able to hydrolysis celatin and casein some cases. The positive

utilization of citrate was also recorded for the isolates. The optimum temperature for the growth of most isolates was between 25-30 °C and exceed up to 40 °C (Table.2).[53]

Table.2: Physiological and biochemical identification of isolates

S.L No	TESTS	MORPHOLOGICAL AND BIOCHEMICAL TESTS
1	Grams staining	+
2	Shape	+ **(Branched Rod)**
3	Spore formation	**+(White, Yellow,Green & Black)**
4	Motility	+
5	Gelatin	-
6	Indole production	+
7	Citrate utilization	+
8	Mannitol	+
9	Methyl red (MR)	-
10	Voges-Proskauer (VP)	-
11	Oxidase	-
12	Catalase	-
13	Starch hydrolysis	+
14	Nitrate reduction	-
15	Casein hydrolysis	-
16	Gas production from glucose	-

Phylogenetic evaluation

The identified 16S rRNA gene sequences and the CDS of the antimicrobial biosynthetic genes were aligned using Clustal X algorithm implemented in MEGA6 software along with the sequences of the nearest known species. (Figure.4)[55]

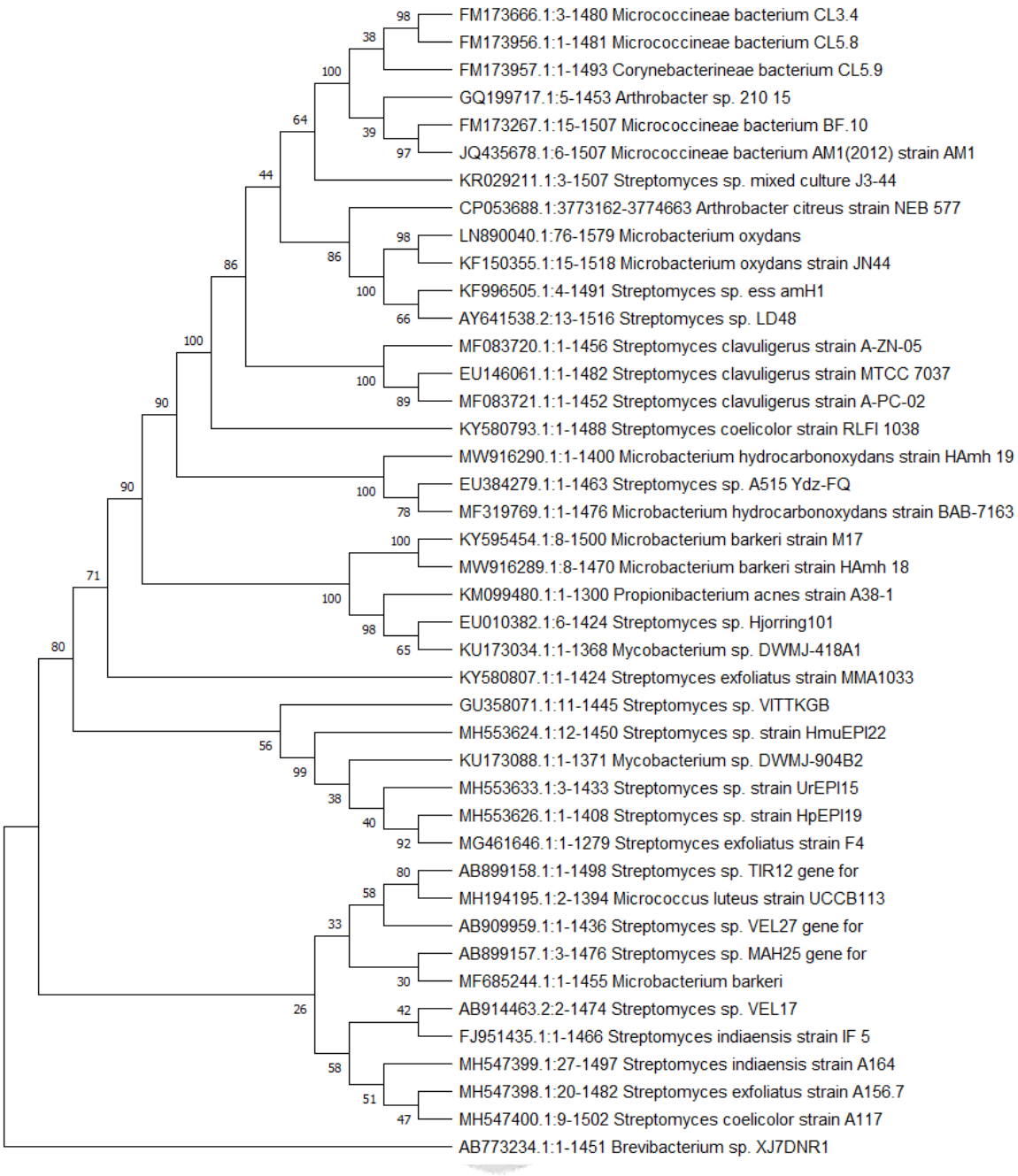


Figure.4: Phylogenetic tree for actinobacterial species whose genomes have been sequenced

Minimum inhibitory concentration (MIC) assay:

Minimum inhibitory concentration (MIC) assay was performed with little modifications. A stock solution ethyl acetate extract of 7 days old culture of the isolate was prepared in 10% dimethyl sulfoxide (DMSO).(Figure.5)[55]

Figure.5: Preparation for MIC of actionomycetes against *Staphylococcus aureus*

Factors affecting

CONCLUSION

Actinobacteria is one amongst the dominant groups of microorganisms that produced industrially necessary secondary metabolites. A wide range of antibiotics within the market is obtained from Actinobacteria. Merchandise like enzymes, herbicides, vitamins, pigments, larvicides, phytohormones, and surfactants area unit produced by these many genera of Actinobacteria, which are of great commercial value. They're capable of degrading a good range of hydrocarbons, pesticides, and feather waste, and their metabolic potential offers a strong area for analysis and research work. However, several of the rare genera of Actinobacteria are neither discovered from unknown locations nor used for their biotechnological and industrial potential. Thus, studies on distinctive ecological environments may yield molecules that might become future harbingers of green technology.

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