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CHARACTERIZATION OF ANTIBIOTIC PRODUCING *ACTINOMYCES* FROM RIVER TANA AND LAKE ELEMENTAITA.

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ABSTRACT

The aquatic environment has many Actinomyces such as Streptomyces species, that produces anti-tumors, enzymes, antibiotics, antivirals, and antifungals. Actinomyces species have produced antibiotics such as chloramphenicol, streptomycin, gentamycin, among others. The increased prevalence of bacterial infections has been a major challenge to the human population with devastating high morbidity and mortality rates. This situation has been worsened by increasing antibiotic resistant strains of pathogenic bacteria, reduced effectiveness of antibiotics in the market, and the emergence of new bacterial infections. This study aimed at isolation and molecular identification of antibacterial Actinomyces species. The experiments for this study was laid out in Complete Randomized Design and replicated three times to determine the difference between the inhibition zones (mm) of isolates against the tests organisms. A total of six antibiotic producing Actinomyces species were isolated from river Tana and lake Elementaita and identified through morphological and molecular characterizations. There was significant (p<0.05) difference on antibacterial activity of Actinomyces isolates against Escherichia coli, Salmonella typhi and Staphylococcus aureus. The findings of this study can help in developing new or alternative antibiotics that can be used for treatment of pathogenic and resistant bacteria. **Keywords:** Six *Actinomyces*, antibiotic resistance, antibacterial activity.

INTRODUCTION

Natural products have been extracted from plants, animals and bacteria and used in various industries such as pharmacy, medicine, agriculture, and food. Natural products from microbes have acted as a major source of antibiotics used to treat many bacterial infections worldwide. Starting from penicillin discovered in 1929 by Alexander Fleming, studies have indicated that microbes are a great source bioactive compounds (Mohr *et al.,* 2016). The increased prevalence of bacterial infections has been a major challenge to the human population with devastating high morbidity and mortality rates. This situation has been worsened by increasing antibiotic resistant strains of pathogenic bacteria, reduced effectiveness of antibiotics in the market, and the emergence of new bacterial infections (Nordenfjäll *et al.,* 2014).

The yearly report of Global Antimicrobial Resistance and Use Surveillance System (GLASS) indicate rising cases of antibiotic resistance for treatment of sepsis, sexually transmitted diseases, urinary tract infections, and diarrhea (WHO, 2022). A good example is percentage resistance against ciprofloxacin meant to treatment urinary tract infection is between 4.1% - 79.4% in *Klebsiella pneumoniae* and 8.4% - 92.9% in *Escherichia coli* (GLASS, 2021). Studies show 64% mortality rate amongst people infected with Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Escherichia coli* and MRSA have developed resistance to third generation cephalosporins. *Neisseria gonorrhea* has shown resistance to sulphonamides, fluoroquinolones, tetracycline, macrolides and penicillins. It is only susceptible to extended spectrum cephalosporins ceftriaxone is effective. In 2018 WHO reported about 500,000 incidences of rifampicin resistance and multidrug resistant *Mycobacterium tuberculosis* (WHO,2022).

The *Actinomyces* species such as *Streptomyces*, *Actinoallumurus and Micromonospora* isolated from various parts of the world has revealed various secondary metabolites of polyketide, cyclo dipeptides, alkaloids and terpenes that have antibiotic activity against pathogenic bacteria (Rao *et al.,*2017). The *Streptomyces* sp. SA32 isolated from India was found to produce biomolecules that were active against multi drug resistance strains of *E. coli*, *K. pneumonia, S. aureus*, E. *cloacae* and *Enterococcus* species *(Sanghvi et al., 2014).* Eleven species of *Actinomyces* obtained from sediments and water of Lake Tana in Ethiopia had antimicrobial metabolites with activity against *P. aeruginosa*, *S. aureus E. coli,* and *S. typhi* (Gebreyohannes *et al.,* 2013). *The species of Streptomyces isolated from soil samples in* Bangladesh had zones of inhibition against *Bacillus subtilis* (Ripa *et al.,*2009). *The bacteria isolated from River Wiwi and* Lake Bosomtwe showed activity againsst *Proteus vulgaris*, *B. thuringiensis*. *P. aeruginosa, S. aureus,* and *Bacillus Subtilis* (Tawiah *et al.,*2012). This study aimed at isolation and identification and characterization of antibacterial *Actinomyces* from river Tana and lake Elementaita.

MATERIALS AND METHODS

Study Area

The samples for this study were collected from Lake Elementaita and River Tana. River Tana is the longest river in Kenya which is about 1000 km long. Its GPRS coordinates are -2°35'56.42" S 40°20'19.04" E. Lake Elementaita is a soda lake located in the Great Rift Valley about 120 kilometers from Nairobi. Its GPRS coordinates are 0°26'59.99" N 36°14'60.00" E.

Isolation and Identification of Antibacterial *Actinomyces* Species from River

Tana and Lake Elementaita Sample Collection and Inoculation

Using a simple random sampling method, sampling and specific sampling sites identification at R. Tana and L. Elementaita were carried out. During sampling, a total of 64 water and sediment samples were collected into sterile five hundred millilitre screw cap bottles and spaces left to allow mixing and proper aeration. During collection of water samples, the 500 ml bottle were submerged to a depth of approximately 10 cm of water and opened to allow water to fill, closed and removed from water. The water and sediment material were collected at the shallow parts of the lake and river. The water samples were packed in cooler box at 4°C. The packed samples were transported to the Animal Science Laboratory in Chuka University and stored at same temperature awaiting processing (Tawiah *et al.*, 2012). All the samples were tested within 6-24 hours after collection. *Actinomyces* were isolated by serial dilution from sediments and water samples. Stock solution was prepared by diluting 1g of sediment in 9 ml of sterile normal saline (8.5g/l) and shaken well using vortex mixer. From stock solution 1 ml was used to prepare the final dilutions of 10^{-1},10^{-2} and 10^{-3} by serial dilution. The 0.1 ml of suspension from 10^{-3} and 10^{-2} were spread on starch casein agar while observing aseptic conditions (Gebreyohannes *et al.,* 2013). For water samples, 1 ml of the stock solution was used to make ultimate volume of 10^{-1},10^{-2} and 10^{-3} by serial dilution. Only the 0.1 ml of 10^{-3} and 10^{-2} suspension were spread on starch casein agar using L-shaped glass rod. To reduce the growth of other bacteria and fungi, starch casein was supplemented with doxycline 100 mg/L and 100 ml of nystatin during preparation. The plates were incubated at 28˚C and monitored after 24hrs, 36hrs and 48 hrs. The resultant colonies were repeatedly streaked on nutrient agar to get pure colonies. The pure colonies were maintained in nutrient agar at 4˚C (Gebreyohannes *et al.,* 2013).

Testing for Antimicrobial Activity of *Actinomyces* Isolates

The isolates were screened for antibacterial activity by agar well diffusion method. To prepare the inoculum, test organisms were grown separately on nutrient agar plates and resultant colonies transferred into 3 ml of normal saline in test tubes (Tawiah *et al.*, 2012). The surface of the Muller-Hinton agar plate was evenly inoculated with *Escherichia coli* with a sterile swab. Then by means of sterile wet swab Muller-Hinton agar plates were inoculated by even streaking of the plate surface (Tawiah *et al.*, 2012). This was repeated for *Salmonella typhi* and *Staphylococcus aureus.* The agar wells (eight milmetres) were made in the inoculated agar using a sterile cork borer and applied with 0.2ml of pure culture of the isolates. The experiment was replicated three times for each of the tests organisms. The plate was incubated at 37˚Cfor 24 hours. The diameter of the zone of growth-inhibition produced were measured using a ruler and the mean value calculated (Tawiah *et al.*, 2012).

Molecular characterization of *Actinomyces* isolates. Extraction of genomic DNA

The genomic DNA was extracted using a protocol described by Azadi *et al* (2020) with modifications. The *Actinomyces* were allowed to grow in nutrient broth at 37˚C for three days after which the cells pelleted through centrifugation. The *Actinomyces* cells were lysised by pretreating with lipase (200mg/ml) and sonication. The cell wall was disrupted with concentration of lysozyme (200mg/ml) and proteinase K (300mg/ml) in the presence of sodium dodecyl sulfate (SDS) and eventually treated using guanidium isothiocyanate (Azadi *et al.,* 2020). The extracted DNA was purified by phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol) and chloroform-isoamyl alcohol (:24:1, vol/vol) then precitated with ethanol and sodium acetate at 20˚C. The precipitated DNA was washed with 70% ethanol and resuspended in 200 ml of mill-Q water (Azadi *et al.,* 2020). The purified DNA was quantified by agarose gel electrophoresis after staining with ethidium bromide (Rajivgandhi *et al.,*2016).

Amplification of *Actinomyces* DNA

The 16SrRNA gene of *Actinomyces* were amplified with universal primesrs 27F (5'-AGAGTT TGATCMTGGCTCAG-3') to 1492R (5'-CGGTTACCTTGTTACGACTT-3') (Rajivgandhi *et al.*, 2016). The

thermocycler Biometra T Personal (Germany) PCR amplifications were done in 20µl volume of reaction mixture containing 10µl of master mix (10 × DreamTaq Green PCR buffer, 3dNTP and Taq DNA polymerase), 1µl of each

primer (20 pmol/µl), 1µl (approximately 200 ng) of template DNA and 7µl of sterile Millipore water. There were 30 cycles of amplification (94˚C for 5min, 94˚C for 40 sec, 52˚Cfor 1 min and 72˚C for 90 sec and final extension at 72˚C for 10 min) (Rajivgandhi *et al.*,2016).

Sequencing similarities and Phylogenetic Analysis

The PCR products was subjected to Sanger sequencing using ABI3730 genetic analyzer (USA). Then BLAST program (www.ncbi.nlm.nih.gov/blst) was used to determine the degree of DNA similarity (Rajivgandhi *et al.*,2016). Multiple sequence alignment was done using clustalW and molecular phylogenetic tree drawn MEGA X version 11.0. (Kumar *et al.,* 2018).

Statistical Analysis

The measurement of the zones of inhibition (mm) for determination of antibacterial activity of *Actinomyces* was analysed using Kruskal Wallis test in SAS version 9.4 because the the data was not normaly distributed even after it was log transformed Significance means were compared using Wilcoxon with Bonferroni correction at alpha = 0.05.

RESULTS

Isolation of Antibacterial *Actinomyces*

Growth Morphology of *Actinomyces* Isolates.

A total of 11 *Actinomyces* colonies were isolated from 64 samples collected at River Tana and Lake Elementaita. Isolate A, B, C, D, E, F and G were isolated from River Tana while isolates H, I, J and K were isolated from Lake Elementaita. The morphological characteristics on starch casein agar and nutrient agar and images of each colony are indicated in the Table 1 and plate 1 below. The isolates had smooth and crenated edges, raised, mucoid, cream -yellow and produced earth like odour.

Table 22*:* Growth Characteristics of *Actinomyces* Isolates on Starch Casein Agar Plates.

Isolates	Outline	Elevation	Consistency	Colour	Smell	Media alteration
A	Entire	Raised	Mucoid	Cream	Earth like odour	Clear
B	Entire	Raised	Mucoid	Creamish yellow	Earth like odour	Clear
C	Crenated	Raised	Mucoid	Creamish yellow	Earth like odour	Clear
D	Crenated	Raised	Mucoid	Creamish yellow	Earth like odour	Clear
E	Entire	Raised	mucoid	Cream	Earth like odour	Clear
F	Crenated	Raised	Mucoid	Cream	Earth like odour	Clear
G	Entire	Raised	Mucoid	Cream	Earth like odour	Clear

H	Entire	Raised	Mucoid	Cream	Earth like odour	Clear
I	Entire	Raised	Mucoid	Cream	Earth like odour	Clear
J	Entire	Raised	Mucoid	Cream	Earth like odour	Clear
k	Entire	Raised	Mucoid	Cream	Earth like odour	Clear
Control	None	None	None	None	None	None

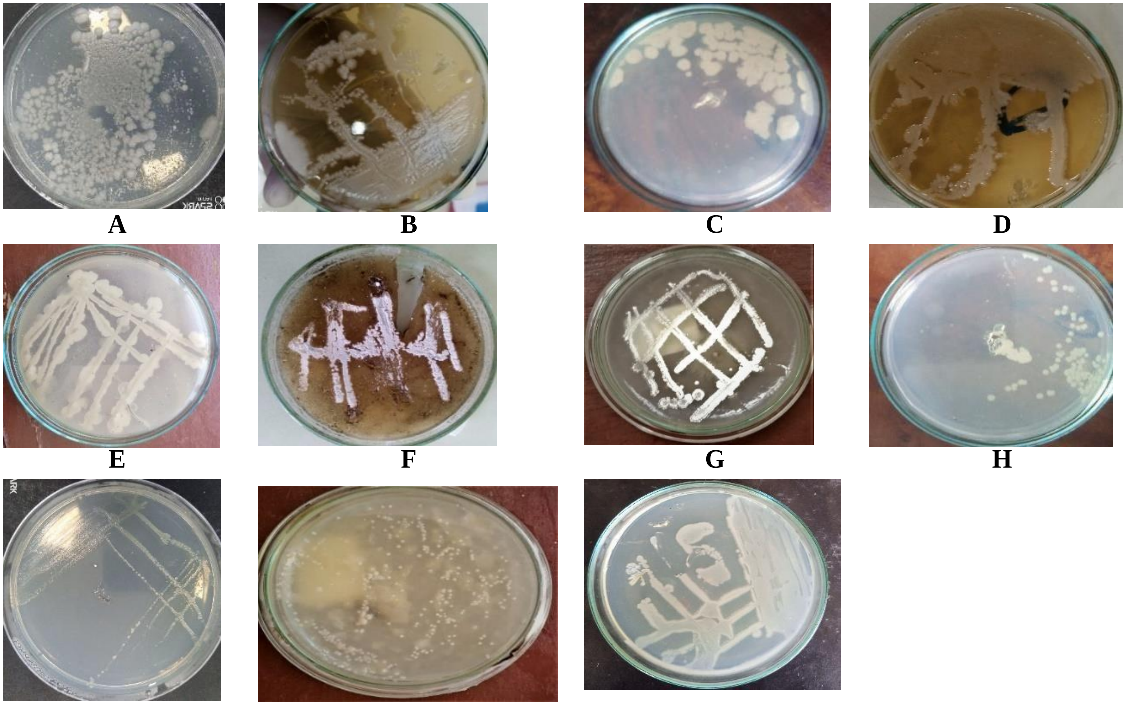
A **B** **C** **D**
E **F** **G** **H**
I J K

Plate 3: Images of *Actinomyces* isolates on starch casein agar plates. . Isolate A, B, E, G, H, I, J and K had smooth margins, mucoid, raised and white while isolate C, D, F had crenated margins, mucoid, raised and white.

Bioactivity of Selected *Actinomyces* Isolates from River Tana and Lake Elementaita against Selected Test Organisms

Bioactivity of *Actinomyces* Against *E. coli*

There was significant ($p<0.05$) difference in zones of inhibition of *Actinomyces* isolates and standard drugss against *E. coli*. Isolate B (8.8 mm) had the largest zone of inhibition compared to all other *Actinomyces* isolates followed by isolate G (4.2 mm) (Table 5). Isolate B performed lower than standard gentamycin (10 mm), cotrimazole (9.9 mm) and chloramphenicol (9 mm) but performed better than standard streptomycin (5 mm), kanamycin (5 mm), tetracycline (2 mm), sulphamethoxazole (0.0 mm), and ampicillin (0.0 mm). Isolate A, D and H had the lowest zones of inhibition (2 mm) against *E. coli* however they performed better than ampicillin and equal to tetracycline (2 mm). In total 54.5 % of isolates had activity against *E. coli* (Table 2).

Table 23:The Bioactivity of *Actinomyces* Isolates Against *E. coli*

	Analysis Variable : INHIBITION				
Isolates and controls	**N**	**Mean**	**Median**	**Minimum**	**Maximum**

A	3	2.333	2.0^{c}	2.000	3.000
Ampicillin	3	0.000	0.0^{d}	0.000	0.000
B	3	8.767	8.8^{a}	8.600	8.900
C	3	0.000	0.0^{d}	0.000	0.000
D	3	2.633	2.0^{c}	2.000	3.900
E	3	3.333	3.0^{c}	3.000	4.000
F	3	0.000	0.0^{d}	0.000	0.000
G	3	4.300	4.2^{c}	3.800	4.900
Gentamycin	3	9.933	10.0^{a}	9.800	10.000
H	3	2.000	2.0^{c}	1.500	2.500
I	3	0.000	0.0^{d}	0.000	0.000
J	3	0.000	0.0^{d}	0.000	0.000
K	3	0.000	0.0^{d}	0.000	0.000
	Analysis Variable : INHIBITION				
Isolates and controls	**N**	**Mean**	**Median**	**Minimum**	**Maximum**
Tetracycline	3	1.967	2.0^{c}	1.800	2.100
chloramphenicol	3	8.967	9.0^{a}	8.800	9.100
Cotrimazole	3	9.800	9.9^{a}	9.500	10.000
Kanamycin	3	4.900	5.0^{b}	4.700	5.000
Saline	3	0.000	0.0^{d}	0.000	0.000
Streptomycin	3	4.967	5.0^{b}	4.700	5.200
sulphamethoxazole	3	0.00	0.00^{d}	0.00	0.00

Bioactivity of *Actinomyces* Against *S. aureus*

There was significant (*p<0.05*) difference in antibacterial activity of *Actinomyces* isolates against *S. aureus* (Table 6). Isolate H (25 mm) had the largest zone of inhibition against *S. aureus* followed by isolate A (8.5 mm) (Table 6). Isolate H performed better than standard gentamycin (7 mm), chloramphenicol (6 mm), cotrimazole (6 mm), tetracycline (4 mm), sulphamethoxazole (4 mm), kanamycin (3 mm), streptomycin (3 mm) and ampicillin (3 mm). Isolate G (1.5 mm) had the lowest zone of inhibition against *S. aureus* performing lower than all the standard antibiotics in the study except ampicillin. In total 45.5% of isolates had bioactivity against *S. aureus* (Table 3).

Table 24: Bioactivity of *Actinomyces* Isolates Against *S. aureus*

	Analysis Variable : INHIBITION				
Isolates	**N**	**Mean**	**Median**	**Minimum**	**Maximum**
A	3	9.133	8.5^{b}	8.000	10.900
Ampicillin	3	3.900	4.0^{c}	3.700	4.000
B	3	3.933	3.9^{c}	3.800	4.100
C	3	0.000	0.0^{e}	0.000	0.000
D	3	0.000	0.0^{e}	0.000	0.000
E	3	6.667	7.0^{b}	6.000	7.000
F	3	0.000	0.0^{e}	0.000	0.000
G	3	1.667	1.5^{de}	1.500	2.000
Gentamycin	3	6.967	7.0^{b}	6.700	7.200
H	3	25.333	25.0^{a}	21.000	30.000
I	3	0.000	0.0^{e}	0.000	0.000
J	3	0.000	0.0^{e}	0.000	0.000
K	3	0.000	0.0^{e}	0.000	0.000
Tetracycline	3	3.900	4.0^{c}	3.700	4.000
chloramaphenical	3	5.867	6.0^{b}	5.600	6.000
Cotrimazole	3	5.967	6.0^{b}	5.800	6.100
Kanamycin	3	2.867	3.0^{c}	2.600	3.000
Saline	3	0.000	0.0^{e}	0.000	0.000
Streptomycin	3	3.033	3.0^{c}	2.700	3.400

sulphamethoxazole	3	4.000	4.0^{c}	3.800	4.200

Bioactivity of *Actinomyces* against *S. typhi*

There was significant (*$p<0.05$*) difference in antibacterial activity of *Actinomyces* isolates against *S. typhi* (Table 4). Isolate A (8.6 mm) had the largest zone of inhibition against *S. typhi* followed by isolate B (7 mm). Isolate A performed better than standard cotrimazole (8mm), sulphamethoxazole (7 mm), chloramphenicol (7 mm), kanamycin (6 mm), gentamycin (5mm), streptomycin (4mm) and ampicillin (5.0 mm). Isolate H and G (4 mm) had the lowest zones of inhibition which are equal to standard streptomycin and better than ampicillin. In total 45.5% of isolates had bioactivity against *S. typhi* (Table 4).

Table 25: Zones of Inhibition of Actinomyces Isolates Against *S. typhi*

	Analysis Variable : INHIBITION				
Isolates	**N**	**Mean**	**Median**	**Minimum**	**Maximum**
A	3	9.167	8.6^{a}	8.400	10.500
Ampicillin	3	5.033	5.0^{b}	4.800	5.300
B	3	7.000	7.0^{a}	5.000	9.000
C	3	0.000	0.0^{c}	0.000	0.000
D	3	5.367	5.1^{b}	5.000	6.000
E	3	0.000	0.0^{c}	0.000	0.000
F	3	0.000	0.0^{c}	0.000	0.000
G	3	3.500	4.0^{b}	2.000	4.500
Gentamycin	3	5.033	5.0^{b}	4.800	5.300
H	3	3.500	4.0^{b}	1.500	5.000
I	3	0.000	0.0^{c}	0.000	0.000
J	3	0.000	0.0^{c}	0.000	0.000
K	3	0.000	0.0^{c}	0.000	0.000
Tetracycline	3	5.033	5.0^{b}	4.800	5.300
chloramphenicol	3	6.933	7.0^{a}	6.700	7.100
Cotrimazole	3	7.900	8.0^{a}	7.500	8.200
Kanamycin	3	5.933	6.0^{ab}	5.800	6.000
Saline	3	0.000	0.0^{c}	0.000	0.000
Streptomycin	3	3.933	4.0^{b}	3.800	4.000
sulphamethoxazole	3	7.100	7.0^{a}	6.900	7.400

Molecular Characterization of Selected *Actinomyces* Isolates DNA Extraction and Polymerase Chain Reaction.

The genomic DNA of the six isolates was extracted and underwent polymerase chain reaction of 16S rDNA and electrophoresis on 1% agarose gel stained with ethidium bromide and observed under UV transilluminator. A positive amplification was seen as a bright band at 1500 bp which is the size of the 16S rDNA gene of *Actinomyces* species (1250-1500bp) as shown in Figure 1 below.

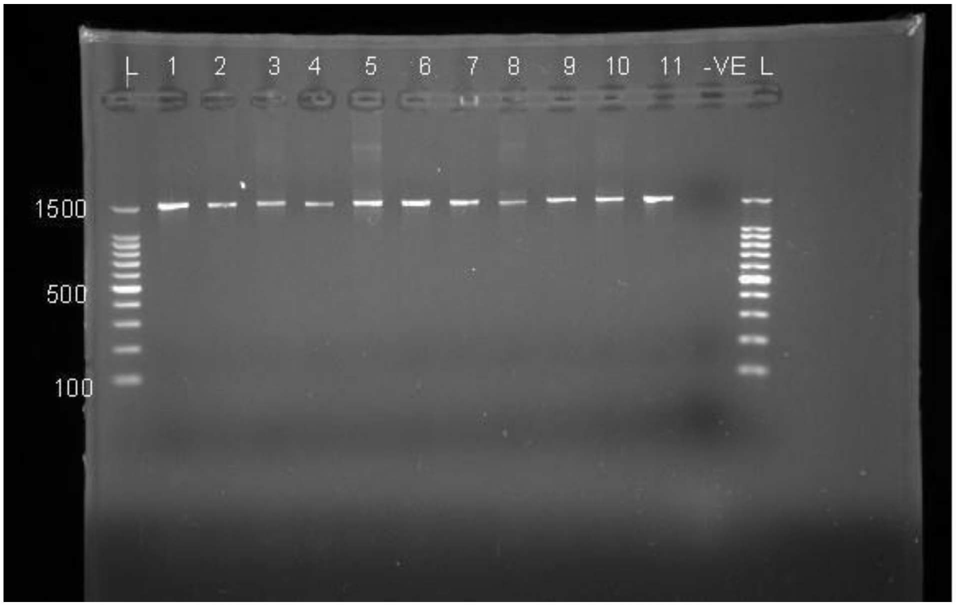

Figure 6: Ethidium bromide stained agarose gel (1%) containing PCR products, primer and template DNA from selected antibiotic producing *Actinomyces* species. L=molecular ladder, 1=isolate A, 2=Isolate B, 3=isolate C, 4=Isolate D, 5=Isolate E, 6=Isolate F, 7=Isolate G, 8=Isolate H, 9=Isolate I, 10=Isolate J, 11=Isolate J, and - VE=Negative control. All the isolates had a bright band at 1500 kb.

Molecular Sequencing of the 16S rRNA Gene

The results of the molecular sequencing of 16S rRNA gene indicated that all the six isolates belong to order *Actinobacterium* as shown above.

Table 26: The results of molecular sequencing showing percentage similarity of related known organisms to isolates.

Isolate	GeneBank Accession No.	% similarity	Scientific name	Country
A	OK483100.1	99.83	*Streptomyces sp.*	India
B	MT039501.1	74.88	*Streptomyces flavomacrosporus*	China
D	LC425654.1	84.59	*Streptomyces sp.*	Nepal
E	JQ437562.1	84.22	*Actinomycetales bacterium*	China
G	KP53721.1	100	*Actinobacteria bacterium*	China
H	KM588147.1	91.66	*Streptomyces intermedius*	Iran

The phylogenetic tree divided the six isolates into four dendrograms. The isolate G and E formed the first dendrograms, isolate H formed the second dendrograms, isolate D and isolate B formed the third dendrograms and isolate A formed the last dendrograms as shown in the Figure 2 below.

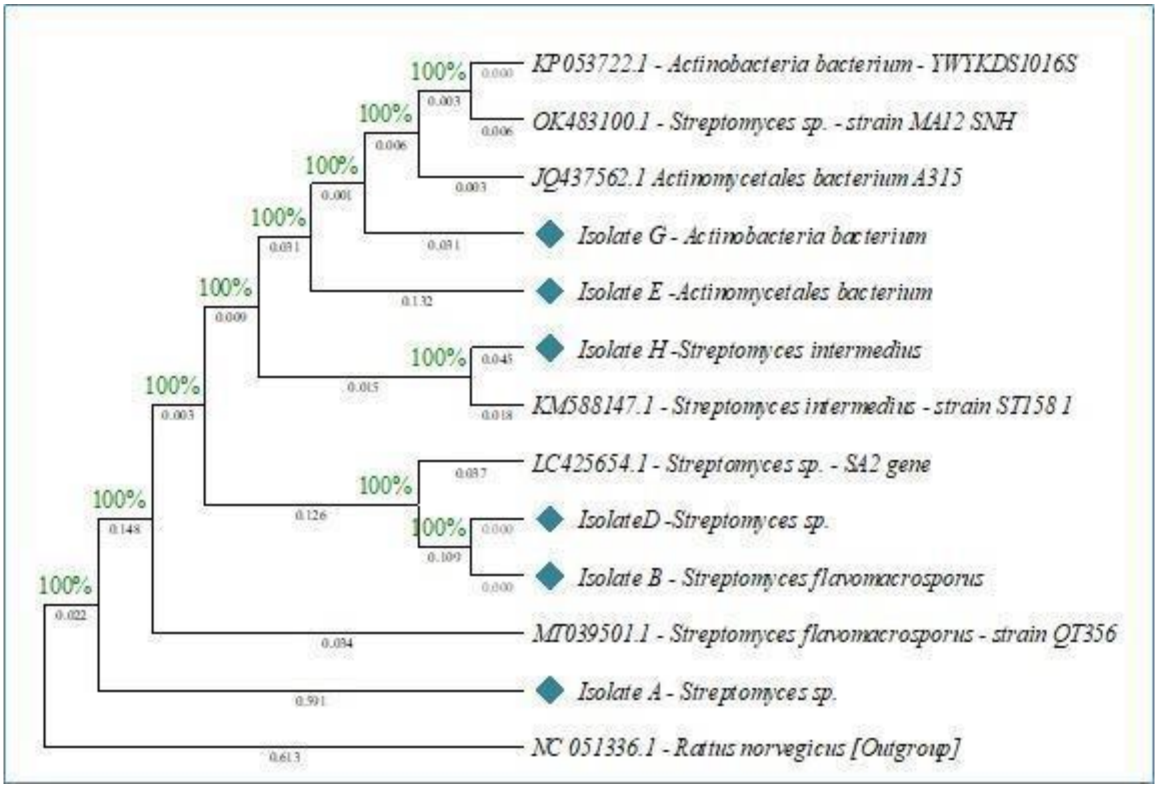

Figure 7: The phylogenetic tree showing the six isolates divided into four dendrograms. The evolutionary distance matrix of the *Actinomyces* isolates and various *Actinomyces* in the gene bank in the above phylogenetic tree is shown in Table 6 below.

Table 27: A matrix showing relationship amongst six isolates.

Actinomyces	1	2	3	4	5	6	7	8	9	10	11	12
LC425654.1-*Streptomyces_sp.*_SA2	0											
IsolateD -*Streptomyces sp.*	0.21											
JQ437562.1-*Actinomycetales bacterium*-A315	0.22	0.29										
IsolateE-*Actinomycetales bacterium*	0.33	0.42	0.16									
IsolateG - *Actinobacteria bacterium*	0.24	0.36	0.06	0.28								
KP053722.1– *Actinobacteria-bacterium*-YWYKDS1016S	0.23	0.35	0.01	0.28	0.10							
Isolate H -*Streptomyces intermedius*	0.27	0.38	0.11	0.34	0.19	0.20						
KM588147.1-*Streptomyces-intermedius*-strain-ST158	0.25	0.27	0.05	0.25	0.07	0.04	0.11					
OK483100.1-*Streptomycessp*.strain-MA12-SNH	0.23	0.35	0.01	0.28	0.10	0.00	0.20	0.05				
Isolate A -*Streptomyces sp.*	0.55	0.55	0.51	0.54	0.51	0.49	0.52	0.50	0.50			
MT039501.1-*Streptomyces_flavomacrosporus*_strain_QT356	0.24	0.36	0.07	0.31	0.16	0.05	0.20	0.03	0.05	0.50		
Isolate B -*Streptomyces*	0.1	0.0	0.2	0.3	0.3	0.2	0.3	0.2	0.2	0.5	0.2	

flavomacrosporus	6	0	9	4	0	8	1	9	8	5	9	
MH028054.1-	0.5	0.5	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.6	0.4	0.4
*Nigrospora_sphaerica*_strain_E6_{outgroup}	0	1	2	1	6	4	9	5	5	0	5	8

DISCUSSION

Morphological Characteristics and Antibacterial properties of Actinomyces Isolates

The *Actinomyces* isolates (A-K) had excellent growth in both starch casein and nutrient agar this can be due to sufficient nutrients and minerals in the two media (Gebreyohannes *et al.*, 2013). On starch casein, *Actinomyces* isolates appeared smooth edged, raised, mucoid, soil like odour, and white to cream. These morphological characteristics are similar to Gebreyohannes *et al*. *Actinomyces* isolates from Lake Tana in Ethiopia. *Actinomyces* isolates in Gebreyohannes *et al*. had excellent growth in starch casein agar appearing as white or cream colonies that were Gram positive (Gebreyohannes *et al.*, 2013). Observed morphological characteristics of Gouse *et al.* (2017) indicated *Actinomyces* that were white yellow or cream in colour, wrinkled or smooth edged and Gram positive, this is comparable with the findings of this study. *Actinomyces* produce volatile essential oils which makes them to have characteristic soil-like odour (Hasani *et al.*, 2014).

There was significantly ($p<0.05$) different in antibacterial activity of *Actinomyces* isolates against *E. coli* (54.5%), *S. aureus* (45.5%) and *S. typhi* (45.5%). This results are comparable to other studies worldwide which have revealed that *Actinomyces* have potential to produce metabolites with antibacterial activities against pathogenic bacteria. The study carried out by Gouse *et al.* (2017) indicated that 59% of *Actinomyces* isolates had antibacterial activity against test organisms. A study by Charousová *et al*. (2019) results indicates that 39.5% of *Actinomyces* isolates had bioactivity against *Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa.* Eleven species (35.5%) of *Actinomyces* isolated in Lake Tana Ethiopia had bioactivity against *E. coli, S. typhi, k pneumonia, Pseudomonas aeruginosa* and *S. aureus*.

There was significant ($p<0.05$) difference in the antibacterial activities between ethyl acetate and chloroform extracts. Ethyl acetate extracts had a higher inhibition against test organisms than chloroform extracts. This is contrast to Sanghvi *et al.*, (2014) who reported that *Actinomyces* isolates from India had chloroform extracts with broad spectrum but ethyl acetate extract had limited activity.The secondary metabolites are more soluble in ethyl acetate than chloroform.The antibacterial difference between these extracts can be attributed to large number and high concentration of antibacterial metabolites of *Actinomyces* isolates in ethyl acetate extracts than in chloroform (Bernier *et al.,* 2013).

Molecular Characterization of the *Actinomyces* Isolates

The sequencing of 16S rRNA gene of isolate A (99.83%) revealed that it is closely related to *Streptomyces sp.* OK483100.1. The isolate B (74.88%) is closely related to *Streptomyces flavomacrosporus* MT039501. The *Streptomyces flavomacrosporus* has been reported to have antibacterial activity against *B. subtilis, E. coli, S. typhi,*

P. aeruginosa and antifungal activity against *Apergillus species* (Khalid *et al.,* 213). The isolate D (84.59 %) was identified to be closely related to *Streptomyces sp.* LC425654.1. The *Streptomyces sp.* LC425654 isolated from the soil samples in Nepal had bioactivity against extended spectrum beta- lactamase producing *E. coli, S. typhimurium,* *K. pneumonia* and *S. aureus* (Khadayat *et al.,* 2020).

The *Actinomyces* isolate E (84.22%) and G (100%) was found to be closely related to *Actinobacteria bacterium* JQ437562 and KP53721 respectively. The isolate H (91%) is closely related to *Streptomyces intermedius* KM588147.1 which is closely related to haloalkaliphilic *Streptomyces intermedius* isolated in India that activity against *E. coli* and *B. subtilis* (Dayma *et al.,* 2019).

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