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MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *Streptomyces* spp. WHICH SUPPRESS PATHOGENIC FUNGI

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ABSTRACT

Streptomyces species are aerobes and chemoorganotrophic bacteria. These microorganisms produce a wide range of industrially significant compounds, specifically antibiotics and anti fungal substances. The objective of this study was to characterise soil-borne *Streptomyces* isolates using morphological and molecular traits in order to identify them to species level, and leverage from their potential to suppress the growth of *Aspergillus flavus, Fusarium oxysporum* and *Penicillium italicum*. Twenty-seven soil-borne putative *Streptomyces,* which elicited comprehensive antimicrobial activity against *Aspergillus flavus, Fusarium oxysporum* and *Penicillium italicum*, in a previous study, were evaluated. On the basis of morphology, the bacteria resembled the genus *Streptomyces*. Initially, colonies phenotypically appeared to have a relatively smooth surface but as growth progressed the bacteria developed a weft of aerial mycelium granular, powdery or velvety in appearance. Bacteria produced a wide variety of pigments which in turn were responsible for the colour of the vegetative and aerial mycelia, colour ranged from white to cream or buff shades and yellow to orange or brown. Microscopic analyses and morphological characteristics generated sub-groups of the isolates and clustered them according to their similarities. One bacterial strain was randomly selected from each cluster and investigated using molecular characteristics. Partial 16S rDNAs from the selected representative isolates from each subgroup, were sequenced and phylogenetic analysis performed. The 16S rDNA sequences of the isolates indicated that they were related to *Streptomyces* species: *S. bungoensis, S. thermocarboxydus, S. corchorusii* and *S. lasaliensis,* that are known secondary metabolite producers possessing antimicrobial activity against plant pathogens.

Key Words: Antimicrobial activity, phylogenetic analysis, secondary metabolites

RÉSUMÉ

Les espèces de *Streptomyces* sont des bactéries aérobies et chimio-organotrophes. Ces micro-organismes produisent une large gamme de composés d'importance industrielle, en particulier des antibiotiques et des substances antifongiques. L'objectif de cette étude était de caractériser les isolats de *Streptomyces* transmis par le sol à l'aide de traits morphologiques et moléculaires afin de les

identifier au niveau de l'espèce, et de tirer parti de leur potentiel à supprimer la croissance d'*Aspergillus flavus, Fusarium oxysporum* et *Penicillium italicum*. Vingt-sept *Streptomyces* putatifs transmis par le sol, qui ont suscité une activité antimicrobienne complète contre *Aspergillus flavus, Fusarium oxysporum* et *Penicillium italicum*, dans une étude précédente, ont été évalués. Sur la base de la morphologie, les bactéries ressemblaient au genre *Streptomyces*. Au départ, les colonies semblaient phénotypiquement avoir une surface relativement lisse, mais au fur et à mesure que la croissance progressait, les bactéries développaient une trame de mycélium aérien d'aspect granuleux, poudreux ou velouté. Les bactéries produisaient une grande variété de pigments qui à leur tour étaient responsables de la couleur des mycéliums végétatifs et aériens, la couleur variait du blanc au crème ou au chamois et du jaune à l'orange ou au brun. Des analyses microscopiques et des caractéristiques morphologiques ont généré des sous-groupes d'isolats et les ont regroupés en fonction de leurs similitudes. Une souche bactérienne a été sélectionnée au hasard dans chaque groupe et étudiée en utilisant des caractéristiques moléculaires. Des ADNr 16S partiels provenant des isolats représentatifs sélectionnés de chaque sous-groupe ont été séquencés et une analyse phylogénétique a été effectuée. Les séquences d'ADNr 16S des isolats ont indiqué qu'ils étaient apparentés aux espèces de *Streptomyces*: S. *bungoensis*, S. *thermocarboxydus*, S. *corchorusii* et S. *lasaliensis*, qui sont des producteurs de métabolites secondaires connus possédant une activité antimicrobienne contre les phytopathogènes.

Mots Clés: activité antimicrobienne, analyse phylogénétique, métabolites secondaires

INTRODUCTION

Streptomyces, previously termed *Actinomyces,* was discovered by Waksman and Henrici (1943). These were later extensively studied and found to be spore-forming, Gram-positive bacteria with a filamentous form (Compant *et al.,* 2005). *Streptomyces* is a genus of over 500 species of Gram-positive bacteria in the phylum *Actinobacteria*, order *Streptomycetales* and family *Streptomycetaceae*. Phylum *Actinobacteria* to which *Streptomyces* belong is one of the largest taxonomic units among all the 18 major lineages of studied bacteria (Ludwig, 2012). Over time, the taxonomy of *Actinobacteria* has evolved significantly as more bacteria are being studied. The order in which *Streptomyces* belong, *Actinomycetales*, was established in 1917 by Buchanan.

Streptomyces species are aerobes and chemoorganotrophic bacteria. These microorganisms produce a wide range of industrially significant compounds, specifically antibiotics and anti-fungal substances (Berdy, 2012; Compant *et al.,* 2016). Their genomes have a high GC content of about 70-78% (Kavitha *et al.,* 2010). *Streptomyces* have very small filaments and spores, which usually have a diameter of 1 μm or less (Willemse *et al.,* 2011). *Streptomyces* colonies are slow-growing and in most cases, they release a soil-like odour attributed to the production of geosmin a volatile metabolite (Jüttner and Watson, 2007). Initially, *Streptomyces* colonies phenotypically appear to have a relatively smooth surface, but as growth progresses, they develop a weft of aerial mycelium visually different, and may be granular, powdery or velvety in appearance (Ambarwati *et al.,* 2012).

Streptomyces are ubiquitous in soil habitats and aquatic sediments (Gontang *et al.,* 2007). They are widely distributed and abundant in soil, predominantly in composts and decaying vegetation.

Streptomycetes are also capable of degrading cellulose, lignocellulose, chitin and many other organic compounds in biogeochemical cycles (Lewin *et al.,* 2012). *Streptomyces* degrade adenine, casein, esculin, gelatin, hypoxanthine, starch, and ltyrosine (Smaoui *et al.,* 2011). They also are catalase positive and have the ability to reduce nitrates to nitrites (Smaoui, *et al.,* 2011). Within the temperature range of 25 – 35 °C and a pH in

the neutral range 6.5 - 8 *Streptomyces* grow best. They occur in the same habitats as fungi (Ikeda *et al.,* 2003).

Streptomyces produce a variety of pigments, which in turn are responsible for the colour of the vegetative and aerial mycelial appearance (Flärdh *et al.,* 2009). Their colour ranges from white to cream or buff shades; yellow to orange or brown; pink to cinnamon, red or pinkish tan to lavender; and green to grey or blue.

A wide range of organic compounds are available as carbon sources for *Streptomyces* energy and growth.

Developing zero-chemical master-plans for the control of plant pathogens is one of the major issues that have caught global attention in recent years.

Unfortunately, only a few attempts have been done so far on morphological and molecular characterisation of *Streptomyces*. The objective of this study was to characterise soil-borne *Streptomyces* spp. isolates, morphological and molecular, to identify them and to leverage from their potential to suppress the growth of *Aspergillus flavus, Fusarium oxysporum and Penicillium italicum.*

METHODOLOGY

***Streptomyces* strains.** Twenty-seven *Streptomyces* spp. isolates, collected from Chinhoyi University of Technology Farm soils (Goredema *et al.,* 2020), were examined for their growth characteristics and morphology in agar media.

Morphological characterisation. For the characterisation, *Streptomyces* isolates were grown in OA amended with cycloheximide (50 µl/ ml), then incubated at 28 °C for 3 days. The variables that were assessed at day 14 in culture were growth rate, mycelium colour, mycelia surface appearance, mycelia texture, pigmentation, opacity, spore shape and spore colour (Davelos *et al.,* 2004).

The morphological traits that were assessed were grouped into categories and each category was given a number from 0-5 depending on the number of sub categories within a specific group. The growth rates of *Streptomyces* isolates were determined by measuring their radii on the agar plate, relative to each other. There were three *Streptomyces* isolates with growth rate categories with the largest diameters which were ranked as fast growers; while those with the least growth were ranked as slow growers. Growth rate categories were assigned number from 0-2 from the slowest growers to the fastest growers.

Mycelia colour of *Streptomyces* isolates growing on Oatmeal Agar (OA), was assessed visually and the four colour categories were yellow to orange, brown, greenish to grey, and white to cream, with each colour category assigned a number from 0-3, respectively.

Mycelia texture was also visually assessed and classified into three categories, which were mucoid, viscid or buttery. *Streptomyces* spp. pigmentation was grouped into four categories which were colourless, white, brown and yellow. Opacity (visible light impenetrability) was categorised as opaque or translucent. (Davelos *et al.,* 2004).

Surface appearance of the *Streptomyces* isolates was classified into three categories, namely smooth, rough or glistering. Spore shapes as observed under a light microscope (Leica dm500) at a magnification of 100x and 400x, were classified as either globose or rod shaped. Colours of spores were classified in four groups, namely white, grey, brown and yellow as described by Davelos *et al.* (2004).

Statistical data analysis. Data were subjected to cluster analysis using Minitab software for windows version 16.1. Variable comparison was performed using Correlation Coefficient Distance and Average Linkage amalgamation steps.

Molecular characterisation. For the molecular characterisation, selected *Streptomyces* isolates were grown on oatmeal agar (OA) amended with cycloheximide (50

µl ml^{-1}); then incubated at 28 °C for 3 days. A ZR Genomic DNA II Kit was used for DNA isolation. Cells were then trypsinised off the surface of growth plates. The cell suspension was centrifuged at approximately 500 x g for 5 minutes. The supernatant was removed and 500 µl of Genomic Lysis Buffer added directly to the pellet. The pellet was re-suspended by vortexing. The mixture was then transferred to a Zymo-Spin-column in a collection tube and centrifuged at top speed (10 000 rpm) for one minute.

The collection tube with the flow-through was discarded. Five hundred microliters of g-DNA wash buffer were added to the spin-column in a new collection tube and spinned at 10 000 rpm for one minute. The collection tube with the flow-through was discarded again. The spin-column was transferred to a clean micro-centrifuge tube and 35 µl water were added to the spin-column. After one minute, to elute the DNA, the spin-column was centrifuged briefly at top speed. The DNA pellet was air-dried and immediately collected for storage at < -20 ºC (Dodd *et al.,* 2013)**.**

***Streptomyces* DNA identification by 16S rDNA sequencing.** The 16S target region was amplified using OneTaq Quickload 2 x Master Mix (NEB, Catalog No. M0486) with the primers (Table 1). The PCR products were run on a gel and extracted with a Zymoclean Gel DNA Recovery Kit (Zymo Research, Catalog No. D4001). The extracted fragments were sequenced in the forward and reverse directions (Nimagen, Brilliant Dye Terminator Cycle Sequencing Kit V3.3, BRD3 - 100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean up Kit, Catalog No. D4050). The purified fragments were analysed on the ABI 3500XL Genetic Analyser (Applied Biosystems, Thermo-Fisher Scientific) for each reaction and sample. CLC Bio Man Workbench v7.6 was used to analyse the .ab1 files generated by the ABI 3500XL Genetic Analyser; and results were obtained by a Basic Local Alignment Search Tool (BLAST) search (NCBI) (Altschul *et al.,* 1997).

Sequence processing and DNA-analysis. Sequences were aligned by hand in Bioedit (Hall, 1999). The final dataset consisted of 15 sequences for 15 species of the sequences were newly sequenced and 10 were obtained from GenBank, which had been part of earlier studies (Goredema *et al.,* 2020). Evolutionary analyses were conducted in MEGA X (Kumar *et al.,* 2018).

RESULTS

Isolation and morphological. Even though all *Streptomyces* isolates evidently formed an extensive network of primary vegetative mycelium branching, which differentiated into aerial mycelium that in turn produced spores, species specific variances in growth rates, mycelium colour, mycelia surface appearance, mycelia texture, pigmentation, opacity, spore shape and spore colour were observed. *Streptomyces* isolate characteristics were defined and further categorised in clusters containing strains with similar traits.

Cluster analysis strains from soil and compost. Twenty-seven *Streptomyces* isolates obtained from CUT Farm soils that suppressed growth of fungal pathogens in *in-vitro* tests, were clustered on the basis of their morphological characteristics. The isolates

TABLE 1. The 16S rDNA primer sequences used in the study

Name of primer	Target	Sequence (5' to 3')
16S - 27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S - 1492R	16S rDNA sequence	CGGTTACCTTGTTACGACTT

were grouped into five distinct clusters (Fig. 1).

Cluster 1 comprised of *Streptomyces* isolates 1, 8, 9, 13, 14 and 20 (Fig. 1), characterised by whitish or greyish colonies, with smooth viscid surfaces. These *Streptomyces* produced yellow-opaque pigmentation, spores with globose and yellow colour (Table 1).

In vitro pathogen inhibition assay studies using the same *Streptomyces* spp. isolates showed that *CUT-Streptomyces* 20 in this cluster exhibited antimicrobial activity against *F. oxysporum* and *P. italicum* (Goredema *et al.,* 2020).

Cluster 2 contained *CUT-Streptomyces* 2, 3, 4, 23, 25 and 26 (Fig. 1). *Streptomyces* isolates under cluster 2 were mostly fast-growing grey and white colonies without pigmentation. They had viscid glistering surfaces, which were opaque (Table 2). *Streptomyces* spores in this class were mostly grey and globose. Four *Streptomyces* spp. in this cluster were classified as the best antibiotic producers; and possessing the best antagonistic activity against fungal pathogens in *in vitro* pathogen inhibition assays carried out in studies earlier on (Goredema *et al.,* 2020). *CUT-Streptomyces* 23 and *CUT-Streptomyces* 26 had the best *F. oxysporum* and *P. italicum* fungal pathogen antagonistic activity (Goredema *et al.,* 2020). *CUT-Streptomyces* 2, 4 and 25 also showed antimicrobial activity to selected fungal pathogens. Antimicrobial activity possessed by *Streptomyces* isolates in Cluster 2 show that their morphological characteristics can be used as criteria for selecting *Streptomyces* spp. with antimicrobial activity against fungal pathogens.

CUT-Streptomyces 5 and 21 were classified under Cluster 3. *Streptomyces* in this cluster were characterised by slow-growing grey and white glistering colonies, with whitish pigmentation. The *Streptomyces* appeared to be translucent and their surfaces viscid in texture. Spores of *Streptomyces* in Cluster 3

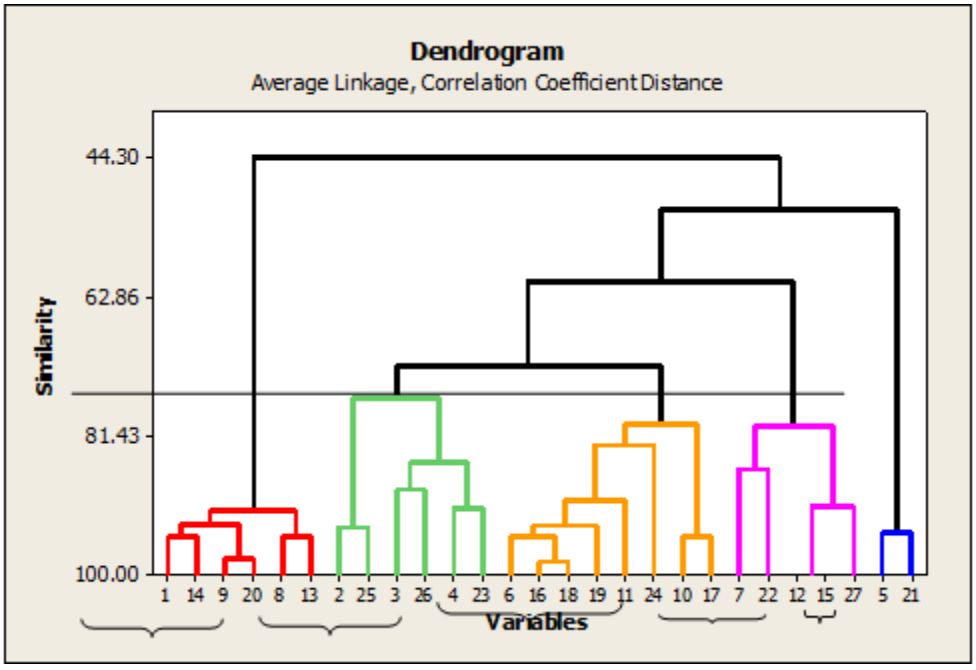

Figure 1. Relationship among 27 *Streptomyces* isolates that suppress growth of fungal pathogens classified based on morphological characteristics. The cut off point for the similarity coefficient was 80%.

TABLE 2. Morphological characterisation of *Streptomyces* isolates used in this study

Isolate	Growth rate	Grey colour	White colour	Brown colour	Pigmentation	Surface	Opacity	Texture	Spore shape	Spore
1	Moderate	Present	Present	Abstent	Yellow	Rough	Opaque	Viscid	Rod	Yellow
2	Fast	Present	Present	Abstent	Colourless	Glistering	Translucent	Viscid	Globose	Grey
3	Moderate	Present	Abstent	Abstent	Colourless	Glistering	Opaque	Viscid	Globose	Grey
4	Fast	Abstent	Abstent	Present	Colourless	Glistering	Opaque	Viscid	Globose	Brown
5	Slow	Present	Present	Abstent	whitish	Glistering	Translucent	Viscid	Globose	Grey
6	Fast	Present	Present	Abstent	whitish	Smooth	Translucent	Viscid	Rod	White
7	Moderate	Abstent	Present	Present	Colourless	Smooth	Translucent	Viscid	Rod	Grey
8	Moderate	Present	Present	Present	Yellow	Rough	Opaque	Viscid	Globose	Yellow
9	Moderate	Present	Present	Abstent	Yellow	Smooth	Opaque	Viscid	Globose	Yellow
10	Fast	Present	Abstent	Present	Brown	Rough	Translucent	Viscid	Rod	Brown
11	Fast	Present	Abstent	Abstent	Colourless	Rough	Translucent	Buttery	Globose	Brown
12	Moderate	Abstent	Present	Abstent	Colourless	Smooth	Opaque	Buttery	Globose	Grey
13	Moderate	Present	Present	Present	Yellow	Smooth	Opaque	Mucoid	Globose	Yellow
14	Moderate	Present	Abstent	Abstent	Yellow	Rough	Opaque	Viscid	Globose	Yellow
15	Moderate	Abstent	Present	Abstent	Colourless	Smooth	Opaque	Buttery	Globose	Grey
16	Fast	Present	Present	Abstent	Colourless	Rough	Translucent	Viscid	Globose	White
17	Fast	Abstent	Abstent	Present	Brown	Rough	Opaque	Viscid	Globose	Brown
18	Fast	Present	Present	Abstent	Colourless	Smooth	Translucent	Viscid	Globose	White
19	Fast	Present	Present	Abstent	Whitish	Smooth	Opaque	Buttery	Globose	White
20	Moderate	Present	Present	Abstent	Yellow	Smooth	Opaque	Buttery	Globose	Yellow
21	Slow	Present	Present	Abstent	Colourless	Glistering	Translucent	Viscid	Globose	Grey
22	Slow	Abstent	Present	Present	Colourless	Smooth	Translucent	Buttery	Rod	Grey
23	Moderate	Abstent	Abstent	Present	Colourless	Glistering	Opaque	Viscid	Globose	Brown
24	Moderate	Abstent	Abstent	Present	Colourless	Smooth	Opaque	Viscid	Globose	White
25	Fast	Present	Present	Abstent	Colourless	Rough	Opaque	Viscid	Globose	Yellow
26	Moderate	Abstent	Present	Abstent	Colourless	Glistering	Opaque	Viscid	Globose	Grey
27	Moderate	Abstent	Present	Present	Colourless	Smooth	Translucent	Buttery	Globose	Grey

were rod shaped and grey in colour (Table 2). *Streptomyces* spp. in this cluster showed no evidence of antagonistic or antimicrobial activity against *A. flavus*, *F. oxysporum* and *P. italicum* (Goredema *et al.,* 2020).

Cluster 4 included *CUT-Streptomyces* 6, 10, 11, 16, 17, 18, 19 and 24 (Fig. 1). *Streptomyces* isolates in Cluster 4 were identified by their grey and or whitish fast-growing colonies with rough viscid surfaces. *Streptomyces* in this Cluster produced whitish or colourless translucent pigmentation, with spores of globose and brownish colour.

CUT-Streptomyces 7, 12, 15, 22 and 27 were grouped into Cluster 5 (Fig. 1). *Streptomyces* isolates in this cluster were unique by their moderate growth rates, white-brownish smooth colonies, with colourless pigmentation. The *Streptomyces* colonies appeared to be translucent and their surfaces buttery in texture. Spores in this group were mostly rod shaped and grey in colour.

Table 3 summaries five *Streptomyces* spp. morphological clusters established using growth rates, colour, pigmentation, opacity, texture, spore shape and spore colour

TABLE 3. Morphological characteristics of the isolated *Streptomyces* spp. defined as major clusters according to Correlation Coefficient Distance and Average Linkage systems using Minitab

Growth rate		Clusters				
		1	2	3	4	5
	1	-	-	+	-	-
	2	+	-	-	-	+
	3	-	+	-	+	-
Grey colour	4	+	+	+	+	-
White colour	5	+	+	+	+	+
Brown colour	6	-	-	-	-	+
Pigmentation	7	-	+	-	-	+
	8	-	-	+	+	-
	9	-	-	-	-	-
	10	+	-	-	-	-
Surface	11	+	-	-	-	+
	12	-	-	-	+	-
	13	-	+	+	-	-
Opacity	14	+	+	-	-	-
	15	-	-	+	+	+
Texture	16	-	-	-	-	-
	17	+	+	+	+	-
	18	-	-	-	-	+
Spore shape	19	+	+	-	+	-
	20	-	-	+	-	+
Spore colour	21	+	-	-	-	-
	22	-	-	-	+	-
	23	-	+	+	+	+
	24	-	-	-	-	-

+ = Positive, - = Negative, 1 = Slow, 2 = Moderate, 3 = Fast, 4 = Grey colour, 5 = White colour, 6 = Brown colour, 7 = Colourless, 8 = Whitish, 9 = Brown, 10 = Yellow, 11 = Smooth, 12 = Rough, 13 = Glistering, 14 = Opaque, 15 = Translucent, 16 = Mucoid, 17 = Viscid, 18 = Buttery, 19 = Globose, 20 = Rod, 21= Yellow, 22 = Brown, 23 = White, 24 = Orange

similarity. This was done using the Correlation Coefficient Distance and Average Linkage systems by Minitab data analysis software. Sequences obtained from *CUT-Streptomyces* DNA identification by 16S rDNA sequencing were subjected to BLAST. BLAST results showed that the selected *Streptomyces* isolates were similar to already discovered secondary metabolite producing *Streptomyces* strains (Table 4).

BLAST findings showed that, of the five sequenced isolates, four *CUT-Streptomyces* isolates were from the genus *Streptomyces*; while one strain was identified as *Stenotrophomonas maltophilia* (Table 4).

Phylogenic analysis. In the course of characterising twenty-seven novel antibiotic-producing *Streptomyces* isolated from Chinhoyi University of Technology Farm soils and composts, five *Streptomyces* clusters were developed (Fig. 1). One *Streptomyces* isolate was randomly selected from each of the five morphological clusters and the 16S rDNA sequenced were queried against Genbank sequences. Similar sequences which appeared to be highly similar to *CUT- Streptomyces* 16S rDNA sequences were retrieved. The basis of their selection was identity similarity (Table 4). BLASTN results showed that the selected *Streptomyces* isolates were similar to already discovered secondary metabolite producing *Streptomyces* strains (Table 4).

The evolutionary history was inferred by using the Maximum Likelihood method and Jukes-Cantor model. The tree with the highest log likelihood (-6058.89) is shown in Figure 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances, estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Codon positions included were

TABLE 4. BLASTN results showing sequence similarities between the queried sequences and biological sequences within the NCBI database

Query sequence	Genbank highly similar sequence	Percentage identity
CUT-Streptomyces 4	*Stenotrophomonas maltophilia* strain M16*Stenotrophomonas maltophilia* strain A32	98.43, 98.17
CUT-Streptomyces 5	*Streptomyces thermocarboxydus* strain HD13*Streptomyces thermocarboxydus* strain HD08	97.95, 97.95
CUT-Streptomyces 10	*Streptomyces bungoensis* strain HBUM174567*Streptomyces bungoensis* strain DPS-98	98.76, 98.56
CUT-Streptomyces 20	*Streptomyces corchorusii* strain 088-68-1*Streptomyces corchorusii* strain AUH-1	99.06, 99.52
CUT-Streptomyces 22	*Streptomyces lasaliensis* strain*Streptomyces galbus strain* DSM 40089	99.52, 99.40

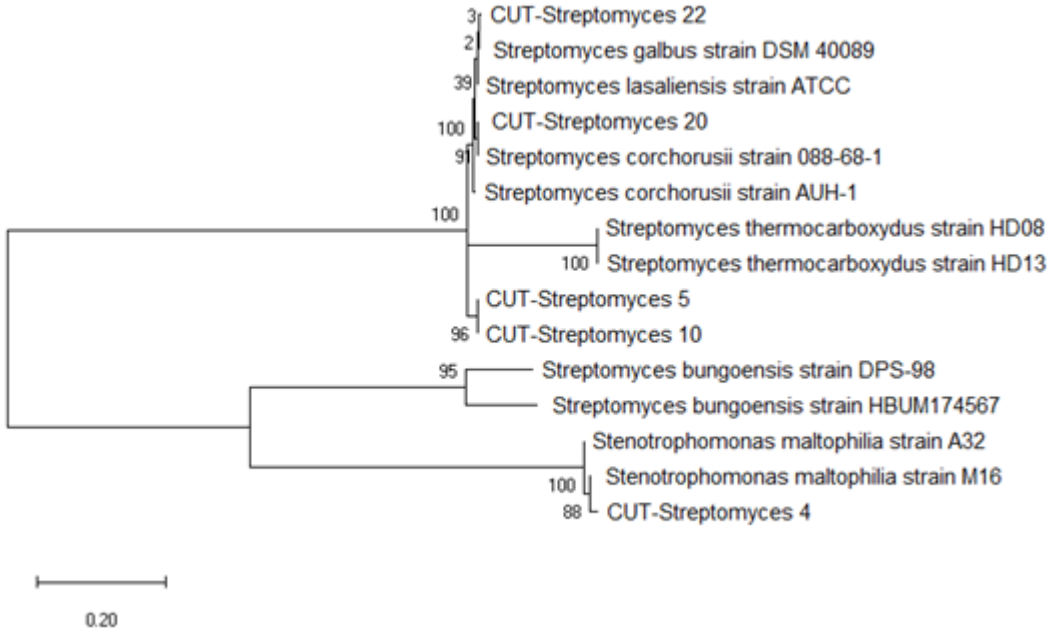

Figure 2. Dendogram showing evolutionary relationships between isolated *CUT-Streptomyces* and bacterium from the Genbank. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

1st+2nd+3rd+Noncoding. There were a total of 1478 positions in the final dataset.

DISCUSSION

The five representative isolates that were characterised were *CUT-Streptomyces 4, 5*, 10, 20 and 22 found in this study belongs to the genus *Streptomyces* and was confirmed by the identification method of Atalan *et al.* (2000) (method for identification of *Streptomyces* species and subspecies). *CUT–Streptomyces* 5 isolate portrayed a sequence similarity to that of *S. thermocarboxydus* species from Jacob *et al.* (2008). *Streptomyces thermocarboxydus* has strong antagonistic activity against pathogenic microbes, enabled by its ability to degrade living cells of pathogenic microorganisms such as fungi and bacteria; thus biologically protecting plants (Kim *et al.,* 1998). Furthermore, this strain has the ability to secrete enzymes that take part in the breakdown and mineralisation of plant, animal and other naturally-occurring organic substances; and in so doing, release important nutrients for plant growth and development (Jacob *et al.,* 2008).

CUT-Streptomyces 20, an isolate that showed high antagonistic activity (Goredema *et al.,* 2020) to *F. oxysporum*, was also found to belong to genus *Streptomyces*. The isolate showed sequence resemblance to that of *S. corchorusii*. El-Shanshoury *et al.* (1996) showed that supplementation of chemical control agents with *S. corchorusii* and *S. mutabilis* increased their inhibitory effects against *P. solanacearum* and *F. oxysporum*. Adinarayana *et al.* (2006) isolated a bioactive *Streptomyces* from marine sediment samples collected from the Bay of Bengal, India. Taxonomically, the isolate was related to *S. corchorusii*. It also demonstrated *in vitro* potent cytotoxic activity and antibacterial activity against Gram-positive and Gram-negative bacteria. Furthermore, Tamreihao *et al.* (2016) found *S. corchorusii* to be affirmative for the production of fungal cell wall degrading enzymes such as chitinase, β-1,3-glucanase,

β-1,4-glucanase, lipases and proteases. This evidence confirms the antagonistic activity of *CUT– Streptomyces* 20 against pathogenic microbes.

Isolate 10, showing antimicrobial activity against *F. oxysporum* growth by depriving the fungus of nutrients and space, was shown to belong to the genus *Streptomyces.* Molecular identification of the isolate showed its likeness to *S. bungoensis*. Work by Atta in 2010 in screening *actinomycetes* for the production of bioactive substances, led to the discovery of *Streptomyces* spp. AZ-Z710 strain with 88% similarity with *S. bungoensis*.

Similar to *CUT-Streptomyces* 10, AZ-Z710 elicited the ability to produce broad-spectrum antibiotics. Such antibiotic compounds inhibited the growth of pathogenic microbes. Cheng (2013) isolated and selected isolate MJM2077 for its strong anti-*Staphylococcus aureus* activity. Based on the analysis of its 16S rDNA, isolate MJM2077 too labelled as *S. bungoensis*. This evidence supports findings of the ability of *CUT-Streptomyces* 10 in possessing antimicrobial activity against fungal pathogens because of its similarity to *S. bungoensis.*

Through morphological and molecular characteristics, *CUT-Streptomyces* 22, an isolate that elicited potential antimicrobial activity against *P. italicum* and *F. oxysporum* was also found belonging to the genus *Streptomyces*. *CUT-Streptomyces* 22 exhibited a sequence close to *S. lasaliensis*. Kinashi (1987), identified giant linear plasmids in *S. lasaliensis* which code for antibiotic biosynthesis genes. These were confirmed by Smaoui *et al.* (2012) to be genes for methylenomycin biosynthesis. This is evidence that *CUT-Streptomyces* 22 had the ability to elicit antimicrobial activity possibly be attributed to methylenomycin as shown in *S. lasaliensis.*

It is clear from the present study that *CUT–Streptomyces* 5, 10, 20 and 22 showed antagonistic activity *against F. oxysporum, P. italicum* and *A. flavus*. These isolates were also shown to belong to genus *Streptomyces* and were taxonomically related to already known; *Streptomyces* strains possessing antimicrobial activity against plant pathogens. Results of this study provide strong backing to earlier studies which have already proved Actinomyces to be one of the principal bacteria present in soil and also that *Streptomyces* spp. are a novel source of secondary metabolites that can be used for the control of plant diseases.

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