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Article

Antimicrobial activity of actinomycetes and characterization of actinomycin-producing strain KRG-1 isolated from Karoo, South Africa

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In the present study we reported the antimicrobial activity of actinomycetes isolated from aridic soil sample collected in Karoo, South Africa. Eighty-six actinomycete strains were isolated and purified, out of them thirty-four morphologically different strains were tested for antimicrobial activity. Among 35 isolates, 10 (28.57%) showed both antibacterial and antifungal activity. The ethyl acetate extract of strain KRG-1 showed the strongest antimicrobial activity and therefore was selected for further investigation. The almost complete nucleotide sequence of the 16S rRNA gene as well as distinctive matrix-assisted laser desorption/ionization-time-of-flight/mass spectrometry (MALDI-TOF/MS) profile of whole-cell proteins acquired for strain KRG-1 led to the identification of *Streptomyces antibioticus* KRG-1 (GenBank accession number: KX827270). The ethyl acetate extract of KRG-1 was fractionated by HPLC method against the most suppressed bacterium Staphylococcus aureus (Newman). LC//MS analysis led to the identification of the active peak that exhibited UV-VIS maxima at 442 nm and the ESI-HRMS spectrum showing the prominent ion clusters for $[M-H_2O+H]^+$ at *m/z* 635.3109 and for $[M+Na]^+$ at *m/z* 1269.6148. This information could be assigned to chromopeptide lactone antibiotic - actinomycin. Our results suggest that unexplored soils could be an interesting source for exploring antibacterial secondary metabolites.

Keywords: Actinomycin/antimicrobial activity. *Streptomyces*. Soil. 16S rRNA. MALDI-TOF MS.

INTRODUCTION

Actinomycetes are free living Gram - positive bacteria having high G+C content (>55%) in DNA (Kämpfer, 2012). The most important and dominant genus within Actinobacteria is *Streptomyces* (Ceylan, Okmen, Ugur, 2008). Members of this group referred to as the biological antagonistic types. They are of special interest since streptomycetes are the ones that are exploited and their metabolites are used in the manufacture of antibiotics (Kekuda, Shobha, Onkarappa, 2010). Streptomyces provides more than half (70%) of the naturally occurring antibiotics (Bérdy, 2005) with high commercial value and continue to be routinely screened for interesting bioactive substances (Takahashi, 2004; Meena *et al*., 2013).

The actinomycines belong to a family of chromopeptide lactone antibiotics that present antitumor and cytotoxic properties (Praveen *et al*., 2008). They represent an important class of natural products that, despite being discovered more than 70 years ago, continue to be a focus of many research areas, especially in the biological and medicinal sciences (Kurosawa *et al*., 2006). Among the actinomycines, actinomycin D has been studied most extensively and it is used for treatment of malignant tumors, such as Wilms' tumor (Green, 1977), and childhood rhabdomyosarcoma (Womer, 1997). The biological activity of actinomycin D is related to its ability to bind to the DNA duplex, these being associated with DNA functionality, leading to RNA and, consequently, protein synthesis inhibition (Martinez, Chacon-Garcia, 2005; Boer, Canals, Coll, 2009). The two main mechanisms are intercalation to DNA and the stabilization of cleavable complexes of topoisomerases I and II with DNA, or the drug penetrates to a place in the DNA structure where topoisomerase binds with DNA,

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respectively (Koba, Konopa, 2005). Actinomycin binds to the highest-energy beta-DNA form found within the boundaries connecting double-stranded B-DNA with single-stranded DNA in the transcription complex (Sobell, 2016) and physically obstructs the trascriptional complex (Huang *et al*., 2000).

Actinomycin D is produced by a range of *Streptomyces* species as part of a mixture of actinomycines (Kurosawa *et al*., 2006; Praveen *et al*., 2008).

Exploring new habitats is one of the most promising ways of isolating actinomycete producers of antibiotics endowed with antimicrobial activity (Zitouni *et al*., 2005; Khanna, Solanki, Lal, 2011; Wadetwar, Patil, 2013). Thus, we report here the antimicrobial activity of actinomycetes isolated from soil sample collected in Karoo, South Africa and characterization and identification of actinomycin-producing strain KRG-1.

MATERIAL AND METHODS

Sample collection and processing

Soil sample was collected from Aquilla Safari in the Southern Karoo, South Africa (33° 21' 5.569"S; 19° 56' 8.23"E) from 10 - 15 cm depth and passed through a 2 mm sieve to remove debris and plants, in July 2015. Then the sample was dried for 45 min at 60 °C to eliminate the bacterial and fungal growth and stored in 4 °C until examination.

Isolation of actinomycetes and maintenance

Isolation and enumeration of actinomycetes were performed by the serial dilution method (10^{-2} - 10^{4}) and the spread plate technique on starch-casein medium (Poosarla, Ramana, Krishna, 2013) supplemented with cycloheximide (1 mg/mL). The plates were incubated at 30 °C for 7 days. Powdered colonies were picked up, transferred to ISP2 medium (Shirling, Gottlieb, 1966) and several times purified. Morphologically different isolates were named as KRG-1 - KRG-35 and stored in presence of 30% (v/v) glycerol at - 20 °C.

Preliminary screening of actinomycetes for antimicrobial activity by agar plug method

The actinomycete isolates were tested against eleven test microorganisms i.e. *Bacillus subtilis* (DSM 10), *Micrococcus luteus* (DSM1790), *Staphylococcus aureus* (Newman), *Mycobacterium smegmatis* (ATCC 700084), *Escherichia coli* (DSM 1116), *Escherichia coli* (TolC), *Pseudomonas aeruginosa* (PA14), *Chromobacterium violaceum* (DSM 30191), *Candida albicans* (DSM 1665), *Pichia anomala* (DSM 6766)] and *Mucor hiemalis* (DSM 2656) obtained from the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany and the American Type Culture Collection, Manassas, VA 20110, USA. Primary screening for antibiotic activity of the isolates was done by using the agar plug method (Eccleston, Brooks, Kurtböke, 2008). Determination of activity was carried out by preparing 4-6 h cultures of tested bacteria followed by dilution with Mueller- Hinton (MH) broth (Merck, Germany) to obtain 0.05 McFarland standard turbidity and 4-6 h culture of yeasts diluted with Mycosel broth (Cazin, Wiemer, Howard, 1989) to obtain 0.01 McFarland turbidity. Spores of *Mucor hiemalis* were collected in sterile distilled water and then adjusted to a spore density of approximately 10^{4} spores/mL. Plates were incubated at 30 °C and the zones of inhibition were determined after 24 h (bacteria and yeasts) and after 48 h (fungus) with the Haloes Caliper (IUL Instruments, USA).

Extraction of antimicrobial compounds and secondary screening

The selected antagonistic actinomycete isolates were inoculated into liquid 5254 medium (glucose: 15.0 g/L, soymeal: 15.0 g/L, corn steep: 5.0 g/L, $CaCO_3$: 2.0 g/L, NaCl: 5.0 g/L, deionized water: 1000 mL, pH 7) supporting metabolite production and incubated at 30 °C in a shaker at 250 rpm for five days. After incubation period, the broths were mixed with ethyl acetate (Sigma Aldrich, USA) in ratio 1:1 and centrifuged 10 min at 9000 rpm. Supernatants were transferred to bottom flasks and evaporated in rotary evaporator (Stuart, UK) at 40 °C. Finally, the extracts were dissolved in 1 mL of ethylacetate:acetone:methanol (1:1:1) solution, resulting in raw extracts of 1:100 concentration. The antimicrobial activities of those extracts were tested using the broth-microdillution method according to Wiegand, Hilpert, Hancock (2008) methodology against the same test microorganisms in 96-well microplates (BRAND, Germany). Dilution stages of raw extracts were determined by inhibited wells (A-H).

Taxonomy and characterization of the most potent strain KRG-1

The morphological, cultural, physiological and biochemical characterization as well as molecular and protein spectra identification of the isolate was carried out.

Morphological characteristics

Morphological signs were examined according to methods given by the International Streptomyces Project (ISP) (Shirling, Gottlieb, 1966). ISP2-ISP7 media were used to determine the speed growth, reverse colors, colors of aerial mycelium and colors of soluble pigments. Melanin pigment production was determined on ISP6 and ISP7 media. For the light microscopic classification of the strain sporophores, well -grown agar plate with GYM (Větrovský, Steffen, Baldrian, 2014) medium was used.

Physiological tests

The physiological characteristics included pH tolerance (pH 2-10) and determination of optimal temperature (4, 25, 28, 30, 37 and 42 °C) in liquid ISP2 medium. Sodium chloride tolerance (0; 2.5; 5; 7.5 and 10%) was tested using six-well microtiter plates (Kutzner, 1981). Carbon utilization test (Shirling, Gottlieb, 1966) was done with 10 different carbon sources (glucose, arabinose, inositol, cellulose, mannose, fructose, galactose, rhamnose, sucrose, xylose) using twelve well microplate technique. The morphological and physiological characteristics of the strain were compared with the four phylogenetically most related species.

Biochemical characteristics

Biochemical features were examined using commercially available test kits ApiZym® and ApiCoryne® (bioMérieux, France). Api stripes were inoculated following by manufacturer's manual. Stripes were incubated for 1 day at 30 °C. After incubation period, reagents were added to cupules. After 5-10 min the stripes were evaluated according to the manual criteria.

Molecular identification

Molecular identification included extraction of the genomic DNA, PCR reaction and sequencing of 16S rRNA gene. Genomic DNA was extracted according to Deininger (1990) methodology**.** The isolated DNA was amplified by PCR reaction using primers according to Cook, Meyers (2003). Reaction mixture was made in total volume of 50 µL. Each reaction contained 5 µL of 10 × DreamTaq Green PCR buffer, 5 µL of 2 mmol.dm^{-3}dNTP, 2 µL of each 10 µmol.dm^{-3} primer, 0,3 µl Taq DNA polymerase and 0,5 µL of template DNA (approximately 20 ng). The PCR reaction ran in the thermo cycler Biometra T Personal under the following conditions: 95 °C for 3 min, 40 cycles of 95 °C for 30 sec, 56 °C for 30 sec, 72 °C for 90 sec and final extension at 72 °C for 10 min. The purified PCR product was sequenced with the help of the MacroGen Genomics, Korea. Gaps and unidentified base positions were edited using BioEdit (Hall, 1999).

Phylogenetic analysis

Phylogenetic analysis was performed using the Maximum-Likelihood method (Felsenstein, 1981) using PhyML (Guindon, Gascuel, 2003) with bootstrap values based on the 1000 replications.

MALDI-TOF MS analysis

For the MALDI-TOF MS identification, cellular proteins were extracted according to Loucif *et al*. (2014). Four extraction procedures were performed, and 1 µL of each suspension was deposited on the MALDI-TOF steel target plate (Bruker Daltonics, Germany) in three replicates. The plate was allowed to dry at room temperature and then overlaid with 1 µL of matrix solution containing α-cyano-hydroxycinnamic acid (SigmaAldrich, USA). Raw spectra obtained by the MALDI-TOF MS LT Microflex (Bruker Daltonics, Germany) were measured in linear positive mode and controlled by flexAnalysis 3.4 (Bruker Daltonics, Germany) for maximal purity. Every controlled mass spectrum was transferred to the MALDI Biotyper OC 3.1. For the clustering of streptomyces species, a mean spectra projection (MSP) dendrogram was created from mass ranging from 2-20 kDa. Obtained spectra were observed by the flexAnalysis 3.4 software (Bruker Daltonics, Germany). Evaluation of peaks was done by centroid detection algorithm with a signal-to-noise threshold of 1, a relative intensity threshold of 0%, a minimum intensity threshold of 0, a peak width of 0.2 m/z, a height of 80%, a Tophat baseline substraction, smoothing with the Savitzky-Golay algorithm, a width of 0.2 m/z, and cycle of 1.

HPLC fractionation and LC/MS analysis of secondary metabolite produced by the KRG-1

The most potent extract was fractionated by the HPLC technique (Agilent 1100 with an analytical column X-Bridge 3.5 µm, 2.1x100 mm; Waters, Milford, USA) and eluted at 0.3 mL/min flow rate. For the HPLC measurement and separations we used following buffers: A2: 950 mL H_2O, 50 mL acetonitrile + 0.05 mM (385 mg/L) ammonium acetate + 40µL acetic acid; B2: 50 mL H_2O, 950 mL acetonitrile, 0.05 mM (385 mg/L) ammonium acetate + 40 µL acetic acid and a DAD detector (200-400 nm). Each fraction with volume 0.15 mL from the HPLC column was collected in a 96-well plate every 0.5 min. The fractions in the 96-well plate were dried for 60 min at 40 ºC in MiniVap (Porvair Sciences, UK).

Afterwards, each well of plate was filled with 150 µL of the *Staphylococcus aureus* diluted with MH broth. After incubation time, the inhibited wells were visible, and therefore the extract was applied to LC-MS system [(Agilent 1200 series with DAD detector (200-600 nm) in connection with a maXis UHR-TOF mass spectrometer (Bruker Daltonics, USA)] for peak-activity correlation. Sample was analyzed using the Waters ACQUITY UPLC BEH C18 Column, 2.1 x 50 mm, 1.7 µm. Chromatographic conditions for LC/MS analysis were set: column oven temperature 40 ºC, flow rate 0.6 mL/min, solvent A (H_2O with 0.1% formic acid), solvent B (CH_3CN with 0.1% formic acid), gradient profile 0.5 min, 5% B; in 19.5 min to 95% B, 10 min, 95% B (maXis gradient). Equilibration time between samples was 5 min. Active compound was identified with the "Data Analysis" software included in the Compass-software from Bruker (USA).

RESULTS AND DISCUSSION

Antimicrobial activity of isolated actinomycetes

Eighty-six actinomycete cultures were isolated from soil sample collected in Karoo, South Africa. Out of them thirty-four morphologically different isolates were subjected to primary screening using the agar plug method. The identical isolates were scored out. It was determined that out of 34 tested isolates, 21 produced inhibitory substances against at least one tested microorganism. According to the results, the inhibition zones against Gram-positive bacteria were larger and clearer compared to the Gram-negative bacteria, yeasts and fungus, indicating that these tested microorganisms were less susceptible to active substances produced by our isolates. Among the active isolates, 21 inhibited Gram-positive bacteria with inhibition zones ranging from 16 - 40 mm in the following order: *Bacillus subtilis* (21 isolates), *Staphylococcus aureus* (18 isolates), *Micrococcus luteus* (14 isolates) and *Mycobacterium smegmatis* (11 isolates). Eleven isolates inhibited Gram-negative bacteria, mostly *Chromobacterium violaceum* (11 isolates) and *Escherichia coli* (TolC) (7 isolates) with inhibition zones 12-20 mm. Growth of *Pseudomonas aeruginosa* and *Escherichia coli* (DSM1116) were suppressed by 2 strains with 12 mm inhibition zones. Ten active isolates were active against yeasts with 12-19 mm inhibition zones and only five strains suppressed the growth (12-14 mm) of *Mucor hiemalis*.

Our results support previous studies with previous studies, which showed that the most isolated *Streptomyces* spp. had activity against Gram-positive bacteria (Thakur *et al*., 2007; Euanorasetr *et al*., 2010). The study of Baskaran, Vijayakumar, Mohan (2011) also reported the highest antagonistic activities against *Staphylococcus aureus* and *Bacillus subtilis*. Another investigation revealed the antimicrobial activity of actinomycetes active against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas vulgaris*, with no effect against *Pseudomonas aeruginosa* and *Candida albicans* (Dalin *et al*., 2010).

The potential actinobacteria were selected based on the results of preliminary screening. The inhibition potential discovered during the primary screening was different from secondary screening. During the tests, it was found out that 40 % of the strains had lost their inhibition potential. According to Robinson, Singh, Nigam (2001) there are two explanations for this. Firstly, the cultivation on solid and liquid media may lead to the production of different active secondary metabolites. Secondly, some compounds may be lost during the organic solvent extraction method, because active components may become inactivated during the extraction step.

One strain, namely KRG-1, exhibited a broad inhibitory effect against bacterial and fungal pathogens, and therefore was selected as the potent strain and used for identification and fermentation process.

Taxonomic identification of the most active strain KRG-1

The isolate was taxonomically characterized based on the morphological and physiological signs, genotypic data, phylogenetic analysis and analysis of protein spectra. Strain KRG-1 was a Gram-positive soil bacterium. Morphological examination of the 7 day-old culture revealed that both aerial and substrate mycelium were abundant. The aerial mycelium was well-developed and mature sporulating aerial mycelium was grey on majority of the tested media. The substrate mycelium was yellow. Diffusible yellow pigment was determined only on ISP2 medium and melanin was not produced. The detailed cultural and physiological characteristics and comparative analysis with the four most related strains are summarized in Table I.

Strain KRG-1 utilized glucose, inositol, fructose and rhamnose, moderate growth was determined in presence of xylose and mannose. This utilization was different from the patterns of all strains used for comparison, except for *Streptomyces antibioticus*. Growth of strain KRG-1 was observed at a wide range of temperature (25-37 °C), although the optimal temperature was at 28 °C. The initial pH range, for which growth was observed, was between pH 5-8, while the optimal pH value was determined to

TABLE I - Morphological and physiological characteristic of strain KRG-1 and phylogenetically related *Streptomyces* species

Sign	*Streptomyces* strain				
	KRG-1	**1**	**2**	**3**	**4**
Spore chain	RF	RF	RF	SP	ND
Aerial mass color					
ISP2	Umbra grey	Grey	Grey	Grey	Silk grey
ISP3	Umbra grey	Grey	Grey	Grey	Traffic grey
ISP4	Grey beige	Grey	Grey	Grey	Light ivory
ISP5	Yellow grey	Grey	Grey	Grey	Light ivory
ISP6	None	None	White	None	None
ISP7	Platinum grey	Grey	White	Grey	None
Reverse side color					
ISP2	Maize yellow	Yellow	Red	Brown	Orange brown
ISP3	Honey yellow	Yellow	Red	Brown	Honey yellow
ISP4	Honey yellow	Yellow	Red	Brown	Ochre brown
ISP5	Honey yellow	Yellow	Black	Brown	Golden yellow
ISP6	Honey yellow	Yellow	Red	Brown	Green brown
ISP7	Sand yellow	Yellow	Red	Brown	Sand yellow
Pigment	ISP2 honey yellow	None	ISP7 brown	ISP7 brown	ISP2,3,4,6, yellow
Melanin	None	None	+	+	-
Carbon source utilization					
Glucose	+	+	+	+	+
Arabinose	-	-	+	(+)	+
Sucrose	-	-	-	-	+
xylose	(+)	-	-	(+)	+
Inositol	+	+	-	-	+
Mannose	(+)	(+)	-	(+)	+
Fructose	+	(+)	(+)	-	+
Rhamnose	+	(+)	(+)	-	-
Raffinose	-	-	-	-	+
Cellulose	-	ND	-	-	+
NaCl tolerance	2.5	ND	5	5	ND
Temperature	28	28	28	28	28
pH	6-8	ND	ND	ND	ND

1 – *Streptomyces antibioticus*, 2 – *Streptomyces griseoruber,* 3 – *Streptomyces longwooedensis*, 4 – *Streptomyces galbus*, positive (+) growth, negative (-) growth, intermediate ((+)) growth, ND - no data available

be 7. Strain KRG-1 was also capable of growth in the presence of 2.5% NaCl.

According to the Api Zym activity of the extracellular enzymes, we found out that isolate KRG-1 showed high (> 40 nmol) alkaline phosphatase, leucinearylamidase and acid phosphatase activity. Moderate activity was determined in case of esterase-lipase, naphtol-AS-BI-phosphohydrolase and glucosidase. Contrary, the least occurring enzymes were lipase, trypsin, chymotrypsin, glucuronidase and fucosidase. These results are in line with findings of Vítezová (2013) who reported, that the most common enzymes detected in ApiZym stripes are alkaline phosphatase and valinearylamidase and glucuronidase was the least occurring. Other enzymes and fermentation tests were determined by the ApiCoryne® stripes. Isolate KRG-1 showed positive activity for nitrate reduction,

alkaline phosphatase, β-galactosidase, α-glucosidase and gelatine hydrolysis. Majority of streptomycete species showed positive activity for the mentioned activities (Khan *et al*., 2010; Sakiyama *et al*., 2014).

The nucleotide sequence of the 16S rRNA gene was determined on the both strands (1239 bp) and has been assigned to the GenBank under the accession number KX827270. Obtained sequence was subjected to similarity searches using the BLAST tool to deduce phylogenetic relationships of this strain. The phylogenetic tree (Figure 1) from representative and the most similar strains indicated that strain KRG-1 belongs to the genus *Streptomyces*. In the comparison of 16S rRNA gene, KRG-1 was mostly related with *Streptomyces antibioticus* (99% similarity) and *Streptomyces griseoruber* (99% similarity). The MSP dendrogram (Figure 2) constructed using our local *Streptomyces* database spectra clustered the strain KRG-1with *Streptomyces antibioticus.* Scientific information from the literature highlights the fact that *Streptomyces antibioticus* is one of the most productive strains of antibiotics. Examples of secondary metabolites produced by this strain are given in Table II.

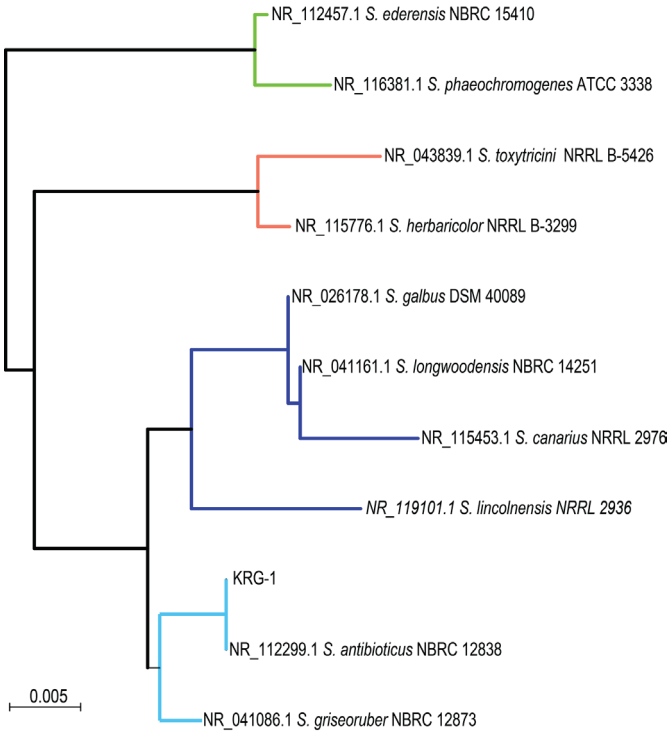

FIGURE 1 - Phylogenetic position of isolate KRG-1 and related Streptomyces species based on the 16S rRNA gene sequences.

Antimicrobial activity and compound identification of the most potent strain KRG-1

During the secondary screening, the range of inhibited wells of the selected strain revealed, that the extract exhibited the strongest antimicrobial activity against Gram-positive bacterium *Staphylococcus aureus* (inhibited wells until H) (Table III) and therefore it was subjected to the HPLC fractionation against this bacterium.

Inhibited wells were visible at retention times 20.0-23.5 min, where active fractions were located and therefore the extract was applied to LC/MS system for identification of active substances.

Chromatographic analysis revealed that extract contained at least one active compound active against suppressed bacterium. The peak-activity-correlation of the LC/MS analysis revealed that the active fractions were located between retention times 13.67-13.94 min. The peak appearing at this retention times exhibited UV-VIS maxima at 442 nm and the ESI-HRMS spectrum showing the prominent ion clusters for $[M\text{-}H_2O+H]^+$ at *m/z* 635.3109 and for $[M+Na]^+$ at *m/z* 1269.6148 (figure 3). According to the available databases, this result could be assigned to actinomycin D. The actinomycines are chromopeptide lactones produced by various *Streptomyces* strains (Praveen *et al*., 2008). They consist of a phenoxazinone chromophore (actinocin) with two pentapeptide lactone rings attached in amide linkage (Sakiyama *et al*.,

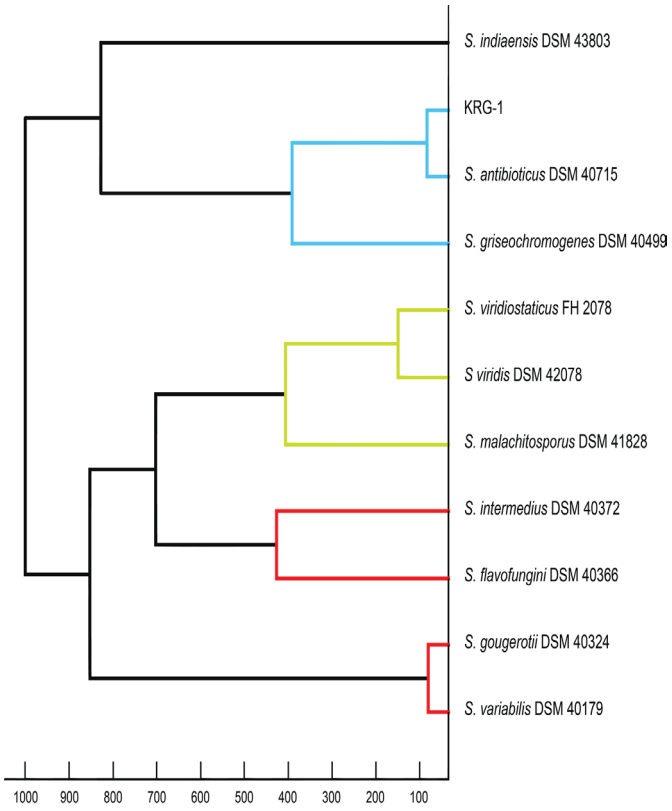

FIGURE 2 - MSP dendrogram of isolate KRG-1 based on the protein spectra with local *Streptomyces* database of type cultures.

TABLE II - Chemical structure and mode of action of selected antibiotics produced by *Streptomyces antibioticus*

Name of antibiotic	Chemical structure and mode of action	References
Actinomycin	chromopeptid, antitumor activity	Jones, 2000
Boromycin	polyether macrolide, antibacterial, coccidiosstat activity	Shibata *et al.*, 1985
Chlorothricin	macrolide, antibiotic activity	Waksman, Tishler, 1942
Cinerubin	anthracycline, antibacterial, antiviral, antitumor activity	Hütter *et al*., 1967
Esmeraldine A	diphenazine, antibacterial, antitumor activity	Keller-Schierlein *et al*., 1988
Oleandomycin	macrolide, broad spectrum antibacterial agent	Semenitz, 1977
Simocyclinones	angucyclinone, antitumor activity	Schimana *et al*., 2000
Desferroferrithiocin	alkaloid	Naegeli, Zahner, 1980

TABLE III – Antimicrobial activity of isolate KRG-1 in primary and secondary screening

Indicator test microorganism	Primary screening (mm)	Secondary screening (A-H)
Bacillus subtilis (DSM10)	30	G
Chromobacterium violaceum (DSM30191)	16	C
Escherichia coli (DSM116)	12	A
Escherichia coli (TolC)	16	B
Micrococcus luteus (DSM1790)	20	F
Pseudomonas aeruginosa (PA14)	12	-
Mycobacterium smegmatis (ATC700084)	16	D
Staphylococcus aureus (Newman)	35	H
Mucor hiemalis (DSM2656)	14	A
Pichia anomalia (DSM6766)	12	B
Candida albicans (DSM1665)	12	-

Diameter of the inhibition zone using the agar plug method excluding the diameter of *Streptomyces* agar block (8 mm)

2014). Since the discovery of actinomycin D, it has become an important tool in molecular and cell biology (Kurosawa *et al*., 2006) because of its unique structure and biological properties by intercalating into duplex DNA, which results in inhibition of DNA-dependent RNA polymerase and thus protein synthesis (Mukhtar, Ijaz, Ul-Haq, 2012). To our knowledge, *Streptomyces* and *Micromonospora* species were reported capable of producing actinomycins (Kurosawa *et al*., 2006; Praveen, Tripathi, 2009). Antimycines were isolated for example from *Streptomyces parvulus* RSPSN2 (Keller *et al*., 2010), *Streptomyces sindenensis* (Praveen *et al*., 2008), *Streptomyces avermitilis* MS449 (Watkins *et al*., 1998), *Streptomyces griseoruber* (Shetty *et al*., 2014), *Streptomyces nasri* (Chen *et al.*, 2012), *Streptomyces chrysomallus* (Sakiyama *et al*., 2014) and *Streptomyces triostinicus* (Praveen *et al*., 2009). There was a previous reports stating that also *Streptomyces antibioticus* produced actinomycin (El-Naggar, 1988; Singh *et al*., 2009) - however, not from South Africa and different species reported by different research groups may be the same species due to a lack of standard strain and not enough information regarding the characteristics of each strain (Shirling, Gottlieb, 1966).

CONCLUSION

This manuscript deals with the isolation of streptomycetes from poorly studied aridic soil sample collected from South Africa – it is a bioassay activity against human pathogens, exact identification of actinomycin-producing streptomycete strain KRG-1 and it gives a clear idea that unexplored soils could be an interesting source for exploring antibacterial secondary metabolites. The search for the very effective actinomycines, and mainly actinomycin D, requires the screening and the exact identification of many Streptomyces strains. The use of the MALDI-TOF MS

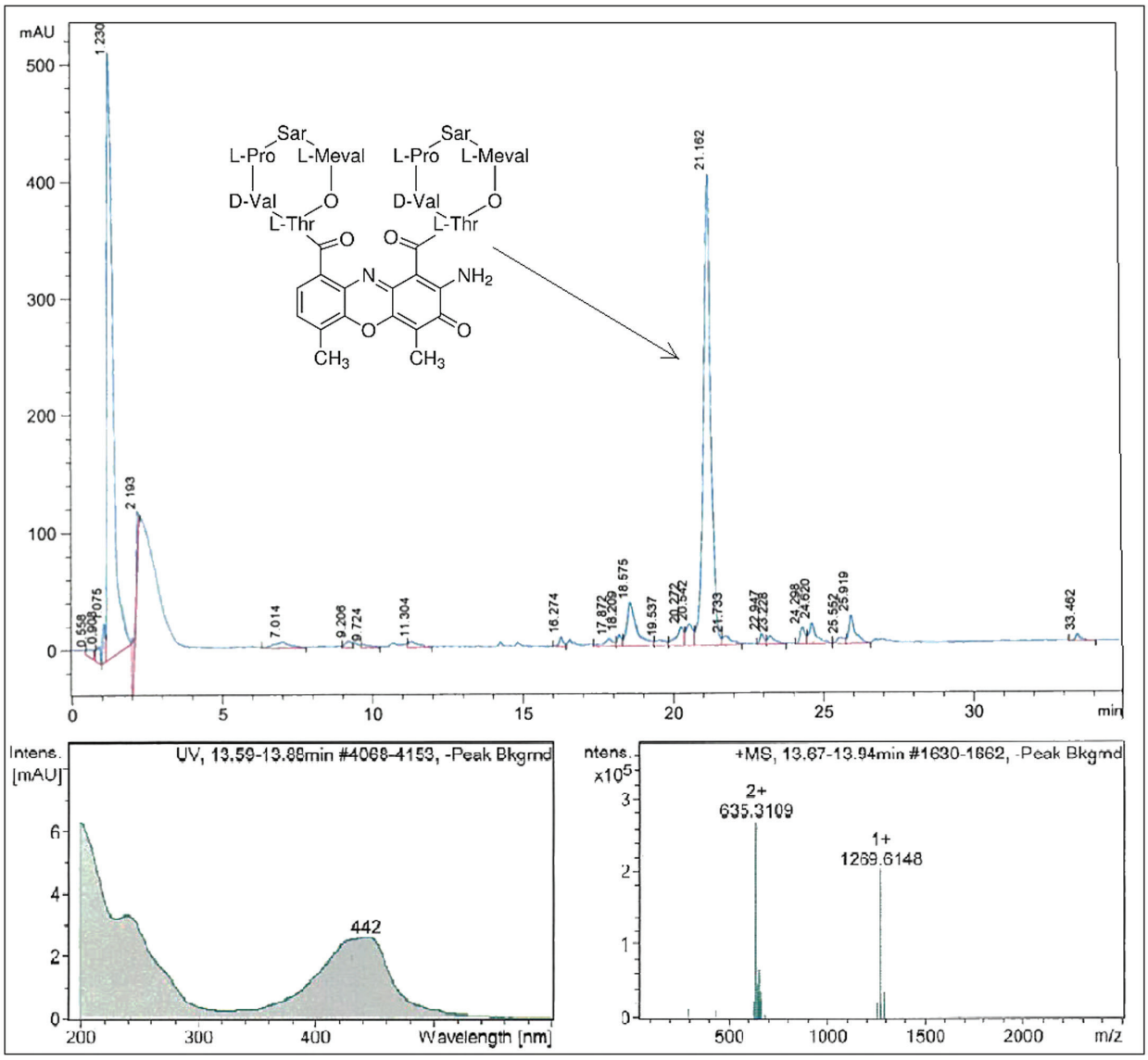

FIGURE 3 - Fractionation RP-HPLC chromatogram of detected antibiotic actinomycin D with UV spectrum and prominent ion clusters

has led to the rapid and powerful technique with a high accuracy for the more exact identification of the KRG-1 strain. This analysis together with the 16S rRNA sequence as well as morphological, physiological and biochemical features of the two most similar strains revealed that the active isolate was indeed to S. antibioticus. The strain KRG-1 is a promising strain and can be used for the industrial applications.

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