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Antimicrobial extract produced by *Streptomyces rimosus*-OG95 and its inhibitory activity against indicator strains

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Abstract: The aim of this study was to profile the antimicrobial metabolites synthesized by *Streptomycesrimosus*-OG95 (KU934251) and to assess the inhibitory activity of the strain against some indicator strains. The production of the antimicrobial compounds was carried out by submerged fermentation. Partial purification of the antimicrobial metabolites was carried out by column chromatography using silica gel while chemical characterization was determined out using FTIR and GC-MS. The Minimum Inhibitory Concentration (MIC) of the partially purified antimicrobial extract was determined by macro broth dilution method. Eleven antimicrobial compounds were identified in the partially purified antimicrobial extract of *Streptomyces rimosus*-OG95. Bis (2-ethylhexyl) phthalate had the highest abundance at 46.55 %. The MIC ranged between 3.12 to 12.5 mg/L. The antimicrobial compounds produced by *Streptomyces*rimosus-OG95 possessed antimicrobial activity against Gram positive and Gram negative bacteria.
Keywords: Actinomycetes; antimicrobial metabolites; Broad spectrum activity; *Streptomyces rimosus*-OG95; Minimum Inhibitory Concentration

INTRODUCTION

Streptomyces are distinct soil bacteria, filamentous and known to excrete many bioactive compounds which are between 65 – 80 percent of the identified bioactive compounds (Berdy, 2005). *Streptomyces* have been reported to produce antimicrobial metabolites such as unsaturated alkyl compounds, Nonadecene and Tetradecene (Kalaivani *et al*., 2013. Furthermore, previous reports by Maheswari *et al*. (2016) and Mangamuri *et al*. (2016) had shown that Phenol 2, 4-bis (1,1-dimethylethyl and Dibutyl phthalate have been identified in the solvent extract of different strains of *Streptomyces*. However, due to resistance to commercially available antibiotics, there is need to search for new strains of Actinomycetes, screening and developing novel antimicrobial agents that are potent against a wide range of resistant microorganisms. Therefore, this study was carried out to profile the antimicrobial metabolites produced by *Streptomyces rimosus*-OG95 and to assess its antimicrobial activity against some selected test organisms.

MATERIALS AND METHODS

Source of the strain

The strain used for this study *Streptomyces rimosus*-OG95 (KU934251) was collected from the stock culture at Microbial Physiology and Biochemistry Laboratory, Department of Microbiology, University of Ibadan, Ibadan.

Indicator organisms

The following bacterial strains *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 8309, *Pseudomonas aeruginosa* ATCC 9077, *Staphylococcus aureus* ATCC 700699 and *Salmonella typhimurium* ATCC 13311 were used as indicator organisms for Minimum Inhibitory Concentration.

Production of antimicrobial compounds

Streptomycesrimosus-OG95 culture on starch casein slant was harvested and cultured in 200 mL starch casein broth for 48 h. This broth was used as seed inoculum to inoculate 8.0 litre of starch casein broth (g/l) (soluble starch 15.0, potassium phosphate dibasic 2.0, Potassium nitrate 2.0, Sodium chloride 2.0, Casein 0.30, Magnesium sulphate heptahydrate 0.05, Calcium carbonate 0.02, iron II sulphate heptahydrate 0.01 at pH 7.2 (Sharon *et al*., 2014).

The inoculated broth was incubated on orbital shaker (Platform Shaker MSZ-100A) at 150 rpm at room temperature (28-30^{o}C) for ten days and then filtered using Whatman No 1 filter paper. The filtrate was treated with 50 % (w/v) ammonium sulphate so as to remove proteinous materials and improve antimicrobial activity. The treated filtrate was centrifuged at 4000 rpm for 20 min. The supernatant was then pooled together and equal volume (1:1) of supernatant and ethyl acetate were mixed and shaken vigorously in a separating funnel for 30 min. The separating funnel was allowed to stand for another 30 min. The supernatant containing the antimicrobial extract was concentrated at 60^{o}C using a Rotary evaporator (Rotary evaporator, RE-52A) (El-Naggar *et al*., 2001). The crude concentrate was then transferred to a water bath at 50^{o}C to dry off the solvent.

Partial Purification of the antimicrobial compounds produced by *Streptomyces rimosus*-OG95

One gram (1.0 g) of the crude antimicrobial compound was dissolved in 3.0 mL of ethyl acetate. The dissolved crude antimicrobial extract was introduced into a silica gel column chromatography (100-200 mesh, column dimension 2.0 cm inner diameter x 25 cm length. The column was be eluted with n-hexane and ethyl acetate (1:4 v/v) and fractions of 3.0 ml each were collected. All fractions were screened for antimicrobial activity and fractions that exhibited inhibitory activity were pooled together and subjected to the second round of purification using column chromatography. Fractions were collected and spotted on silica gel coated plates. Spots complex were observed when plate were exposed to iodine crystals. Fractions that possessed activity and similar retention factor were pooled together as well as concentrated at 60^{o}C using Rotary evaporator (Rotary evaporator, RE-52A). Partially purified antimicrobial extract concentrate was chemically characterized and assessed for antimicrobial activity against test organisms

Chemical Characterization of the antimicrobial compounds

The chemical characterization of the partially purified antimicrobial compounds was determined using Fourier Transformed Infrared (FTIR). The sample was homogenized with potassium bromide (KBr). The spectrum of the partially purified antimicrobial compound was recorded on Shimadzu AUX220 spectrophotometer that was in the range of 4000 cm^{-1} to 400 cm^{-1} (Sanghvi *et al*., 2014). The chemical structures, formulae and names of the antimicrobial compounds were determined using gas chromatography-mass spectrophotometer (GC-MS) Shimadzu QP 2010 (Anupriya *et al*. 2016).

Determination of MICs) and MBC of the partially purified antimicrobial compounds

The Minimum Inhibitory Concentrations (MICs) of the partially purified antimicrobial extract was determined by a 2-fold dilution method using Mueller Hinton broth in mg/L after 18-24 h incubation at 37^{o}C(Andrews, 2001). The tubes observed for the least concentrations without turbidity were sub-cultured on Nutrient Agar and incubated plates without growth were recorded as Minimum Bactericidal Concentrations (MBCs).

RESULTS AND DISCUSSION

The retention factor (Rf) of solvent antimicrobial extract of *S. rimosus*-OG95 was 0.94 (Table 1), which differs from previous reports of Khattab *et al*. (2016) and Maheshwari *et al*. (2016). This difference could be attributed to the molecular weights of the compounds present in the extracts.

The FTIR spectrum of antimicrobial metabolites produced by *Streptomyces rimosus*-OG95 revealed the presence of aromatic rings at wave numbers 484.15 cm^{-1} and 651.96 cm^{-1},(Coates, 2000) and unsaturated aliphatic group,hydroxyl group was at wave numbers 1288.49 cm^{-1} and 1381.08 cm^{-1}respectively (Figure 1).

The peaks at wave numbers 3720.81 cm^{-1} and 1728.28 cm^{-1} revealed that both amine and carbonyl functional groups representing amide compounds were observed. The observed similarity with previous reports of Ayari *et al* (2016 and Barakat and Beltagy (2015) could be attributed to compounds present in the extract.

Table 1: Retention factor of partially purified compounds produced by *Streptomyces rimosus*-OG95

Isolate	Retention factor
Streptomyces rimosus-OG95	0.94

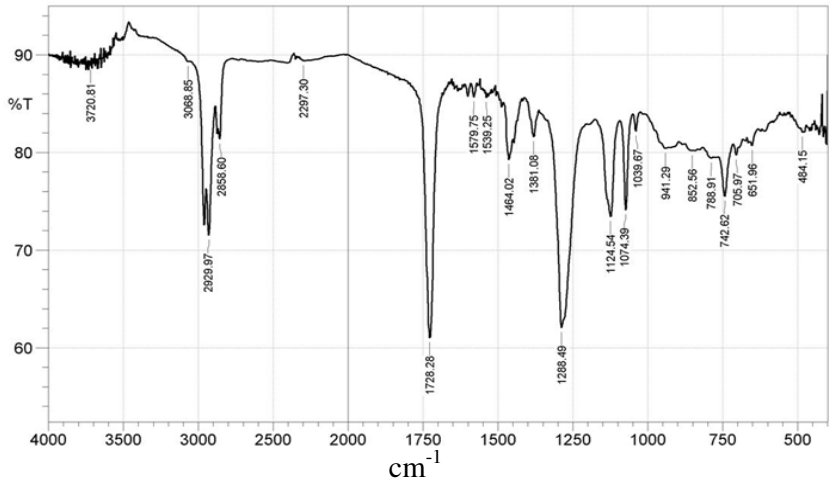


Figure 1:FTIR spectral analysis of antimicrobial compounds produced by *Streptomyces rimosus*-OG95

Table 2 MIC and MBC of partially purified antimicrobial extract and Gentamicin (Control)

Indicator strains	Antimicrobial extract		Gentamicin (Control)	
	MIC (mg/L	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
Bacillus cereus ATCC 10876	6.25	12.5	0.78	1.56
Staphylococcus aureus ATCC 700699	3.12	12.5	0.78	1.56
E. coli ATCC 35218	6.25	12.5	3.12	6.25
Salmonella typhimurium ATCC 13311	12.5	25.0	0.78	1.56
Klebsiella pneumoniae ATCC 8309	12.5	25.0	0.78	1.56
Pseudomonas aeruginosa ATCC 9077	12.5	25.0	0.78	1.56

The GC-MS spectral analysis for solvent antimicrobial compound extract produced by *Streptomyces rimosus*-OG95 revealed eleven antimicrobial compounds were characterized (Table 3 and Figure 2). This was a shift from previous report by Mangamuri *et al*. (2016) who identified two compounds from the antimicrobial extract produced by *Pseudonocardia endophytica*-VUK-10,Abd-Elnaby *et al*. (2016) in their work identified eight compounds while Maheshwari *et al*. (2016) and Awla *et al*. (2016) that identified twenty and twenty two bioactive compounds from different solvent extract of Actinomycetes broth. The observed difference could be as a result of the genetic and physiological state of different strains used and the production medium.

Furthermore, one of the antimicrobial metabolites observed in this study, Dibutyl phthalate has been reported by Sulistyani *et al*. (2016) and Roy and Sen (2013) to exhibit broad spectrum antimicrobial activity.

Similarly, Bis (2-ethylhexyl) phthalate synthesized by *Streptomyces rimosus*-OG95 has been reported by Silber *et al*. (2016) to be inhibitory against *Micrococcus luteus*, *Pseudoalteromonas piscida* and *Vibrio harveyi*.

In addition, unsaturated aliphatic alkenes identified in the antimicrobial extract such as 9-Octadecenamide, (Z) (3g) and 1-Nonadecene (Figure 3a) have been reported by Selvin *et al*., (2009) and Naragani *et al*. (2016) as antimicrobial compounds respectively.

The partially purified antimicrobial compounds synthesized by *S. rimosus*-OG95 had MIC against test bacterial strains that ranged between 3.12 and 12.5 mg/L and MBC that was between 12.5 and 25.0 mg/L. The observed values were higher than those reported by Al-Bari *et al*. (2006) and Arasu *et al*. (2014). The variations could be due to the level of purity as well as the test organisms used.

Table 3: Profile of antimicrobial compounds and other secondary metabolites produced by *Streptomyces rimosus*.-OG95

Peak	Retention time	Area	Area%	Height%	Molecular weight	Chemical formula	Name
1	8.246	966233	0.95	1.04	200	$C_{13}H_{28}O$	n-Tridecan-1-ol
2	10.631	1449660	1.42	1.61	310	$C_{16}H_{29}F_3O$	Tetradecyltrifluoroacetate
3	12.802	1514913	1.49	1.66	266	$C_{19}H_{38}$	1-Nonadecene
4	15.837	8465915	8.30	5.12	376	$C_{23}H_{36}O_4$	Phthalic acid, butyl undecyl ester
5	16.359	3659034	12.03	11.74	278	$C_{17}H_{22}O_4$	Dibutyl phthalate
6	16.518	3659034	3.59	3.28	292	$C_{17}H_{24}O_4$	Phthalic acid, isobutyl 2-pentyl ester
7	16.839	10350206	10.15	10.31	278	$C_{16}H_{22}O_4$	Dibutyl phthalate
8	16.993	6685523	6.56	5.93	334	$C_{20}H_{30}O_4$	1, 2-Benzenedicarboxylic acid, butyl octyl ester
9	17.270	2603772	2.55	2.08	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid methyl ester, (E)-
10	17.684	297491	2.92	2.31	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid, (E)-
11	20.059	3547709	3.48	3.18	281	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)-
12	20.955	47461371	46.55	51.74	390	$C_{24}H_{33}O_4$	Bis (2-ethylhexyl phthalate

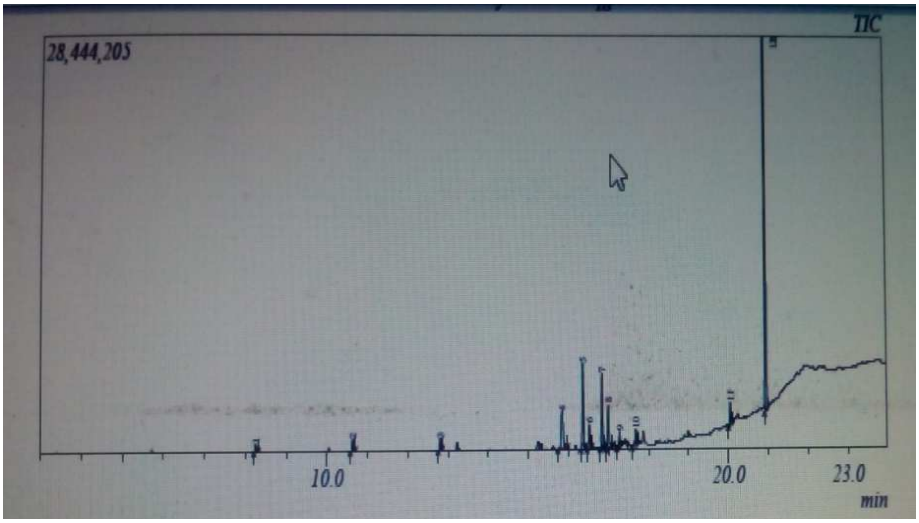
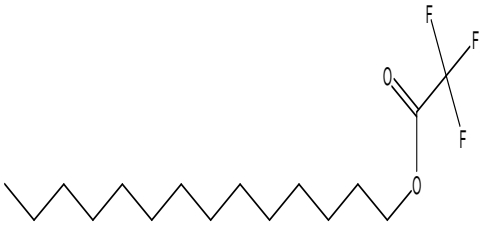


Figure 2 GC-MS spectrum of antimicrobial metabolites produced by *Streptomycesrimosus*-OG95



n-Tridecan-1-ol

3a



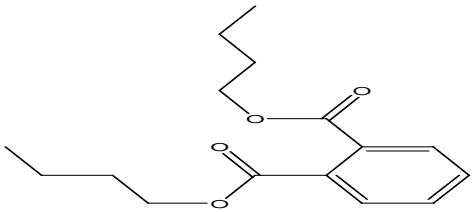
Tetradecyl trifluoroacetate

3b



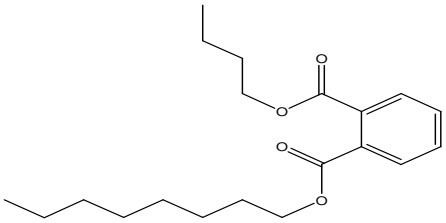
1-nonadecene

3c



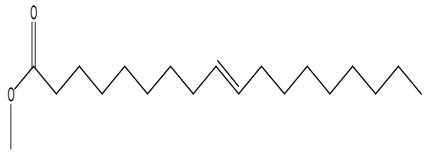
Dibutyl phthalate

3d



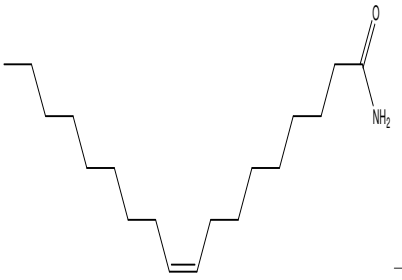
1,2- Benzenedicarboxylic acid, butyl octyl ester

3e



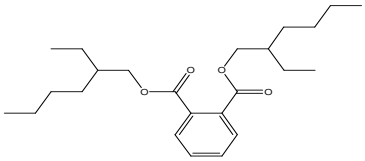
9-Octadecenoic acid, methyl ester

3f



9-Octadecenamide,(Z)-

3g



3h Bis (2-ethylhexyl) phthalate

Figure 3a-3h Chemical structures of some antimicrobial metabolites produced by *Streptomyces rimosus*-OG95 identified from solvent extract

CONCLUSION

In conclusion, eleven antimicrobial metabolites were synthesized by *Streptomyces rimosus*-OG95. Among the metabolites produced are unsaturated aliphatic alkenes, esters, amides and phtahlic compounds. These antimicrobial metabolites are known to possess inhibitory activity against pathogens. The strain *S. rimosus*-OG95 exhibited broad spectrum antimicrobial activity bacterial strains.

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