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Novel Anti-microbials from Streptomyces albidoflavus SAC61 against Multidrug-Resistant Bacteria: Metabolomics and Molecular Modeling

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Abstract

Antibiotic-resistant pathogenic bacteria have attracted the attention of scientists for the last decade, especially in the search for bioactive compounds. Advanced techniques such as genomics and metabolomics to identify novel antibiotics extracted from actinomycetes bioactive metabolites have promising potential to defy the newly emerged resistant bacteria. We aimed to test metabolic organic extracts from 29 Actinomycete strains against four human pathogenic bacterial reference strains: Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterobacter aerogenes. Non-targeted metabolomics and molecular modeling technique (LC-QTOF-MS-MS) were used to identify the potential mechanisms of action and target sites of the produced antibiotic. The results highlighted the bioactivity of Streptomyces albidoflavus SAC61 with minimum inhibitory concentration (MIC) against P. aeruginosa (25 μg/ml) and E. coli (12.5 μg/ml) as a potential source of novel anti-microbial substances. Streptomyces albidoflavus SAC61 extracts were a good source of potent anti-microbial products. It emphasizes the need for further research to characterize and identify more active compounds and recognizes their mechanisms of action to combat antibiotic resistance.

Keywords: Actinomycetes, antibiotic resistance, bioactive compounds, Streptomyces albidoflavus

1. Introduction

During the past ten years, multidrug-resistant enteric bacteria and other pathogenic microorganisms have become a challenge in infection prevention and control, encouraging researchers to find new anti-microbials out of biologically active microorganisms, especially Actinomycetes. Actinomycetes are a rich source of bioactive compounds with diverse structures and potentials for pharmaceuticals and naturopathy uses **[1].** About 90% of commercially available antibiotics are derived from Actinomycetes **[2].** Approximately 75% of bioactive compounds are synthesized by Streptomyces spp. **[3].** The genus Streptomyces has proven to be a prolific source of most antibiotics, including streptomycin, macracidmycin, and kedarcidin, and produces about 75% of all antibiotics discovered to date **[4].**

Metabolomics is an analytical tool that provides qualitative and quantitative metabolites profiling, even at low-abundance molecular metabolites in cells and their environment at any cell-life stage. Time-course metabolite profiling provides a "static snapshot," which allows the integration

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of biological mechanisms and provides physiological insight **[5].** Accordingly, metabolomic analysis can potentially pursue more advanced and improved microbial metabolites associated with system biology and metabolic engineering. Advanced cultivation methods, genomics- and metagenomics-based approaches, and modern metabolomics-inspired methods help improve access to the novel natural product **[6].** The 16S ribosomal DNA sequencing is one of the most helpful techniques that provide evolutionary phylogenetic trees that allow precise investigation of the isolate's taxonomical position **[6].**

We aimed to screen for the antibacterial activities of selected Egyptian marine and terrestrial isolates of Actinomycetes and their secondary metabolites at the lowest minimum inhibitory concentration (MIC) against Multi-Drug Resistant (MDR) enteric bacterial pathogens. We also emphasized specific molecular modeling techniques and metabolomics used to identify the high-potential isolates and some of their activity mechanisms.

2. Materials and Methods

2.1. Actinomycetes strains and metabolites extraction

We have screened twenty-six isolates of well-known potential for bioactive products from Egyptian habitats: 10 marine Actinomycetes **[7] a**nd 16 terrestrial strains **[8].** Spore suspensions were inoculated into 30 ml of Starch Casein (SC) and incubated at 100 rpm, 28 ± 2°C/5–7 days. Equal volumes of the broth and ethyl acetate solvent were agitated vigorously in a separating funnel for 30 min. The less polar solvent layer with the dissolved organic molecules was separated and evaporated to dryness using a rotary evaporator (HS2005S-N - Hahn Shin Scientific Co, Korea) at $40^{\circ}C$. A 100 mg/ml stock concentration was prepared for antimicrobial activity screening by re-dissolving the extract in Dimethyl sulfoxide (DMSO).

2.2. Anti-microbial screening of Actinomycete extracts

2.2.1. Modified disc diffusion method:

The disc diffusion protocol by the Institute of Clinical and Laboratory Standards **[9] w**ith modifications was used to evaluate the anti-microbial activities of the extracts against four reference bacterial cultures: E. coli NCMB 11943; P. aeruginosa NCMB 8295; K. pneumoniae ATCC 13883, and Enterobacter aerogenes NCTC 9735. Enteric bacteria strains were diagnosed, collected and characterized from patients in some Libyan hospitals (Al-Jalaa Hospital for Surgery and Accidents, the Center for Specialized Surgery, Benghazi, and the Kidney Services Center, Benghazi). Each sterile disc (Whatman, 6 mm) was impregnated with the reconstituted extract (3.2 μg). The discs were added onto Muller Hinton agar inoculated by (10^7 CFU/ml) of enteric bacteria. Extracts were assessed in duplicates. DMSO-saturated discs have represented the negative control. Positive controls were standard antibiotic discs, Cefepim for E. coli and E. aerogenes, Ciprofloxacin for P. aeruginosa, and Cefuroxime for K. pneumoniae. The plates were incubated at 37°C/18-24 h. The diameter of inhibition zones was recorded.

2.2.2. Determination of minimum inhibitory concentration (MIC)

The broth-dilution method was applied by inoculating a serial of twelve different dilutions of Actinomycetes metabolites (3.2 μg/ml to two mL) of 24 hrs culture of the enteric bacteria (10^7 CFU/mL) adjusted to 0.5 McFarland turbidity, then incubated at 37°C/ 24 hrs. MIC was considered the lowest dilution concentration with no detectable turbidity **[10].**

2.3. Phylogenetic characterization of bioactive actinomycetes strains

2.3.1. Isolation of genomic DNA:

The four potent strains with the highest antimicrobial activities were selected for their 16S rRNA gene sequencing. Actinomycete genomic DNA was extracted by salting out method with modifications **[11]**

The propagated mycelia on starch casein agar medium were harvested, homogenized, and resuspended in 0.5 ml SET buffer: 75mM NaCl, 25mM EDTA (pH8), 20mM Tris-HCl (pH7.5), lysozyme (15 μl of 1.5 mg/ml) was added and incubated for 60 min at 37°C. Seventeen μl Proteinase K (70 μl; 0.64 mg/ml) and Sodium dodecyl sulphate)SDS) (60 μl; 10%) were added and mixed (final concentration of SDS 1%), then incubated for two hours at 55°C with inversion mixing. Sodium chloride (200 μl; 5M) was added and mixed for 5 min, and then the mixture cooled at 37°C (final concentration of NaCl 1.3 M). Five hundred μl of Chloroform was added and mixed for 30 min at 20°C. Centrifugation (Scilogex SCI-12, USA) at 6000 rpm/15 min. The clear aqueous phase was transferred, adding 0.6 vol. isopropanol, and mixed. Cold ethanol (500 μl 70%) was added. The mixture was centrifuged at 6000 rpm/15 min, and the supernatant was discarded. The resulting DNA pellet was dissolved in 50 μl TE buffer at 55°C.

2.4. Sequencing of 16S rRNA gene:

16S rRNA gene sequencing was conducted at the Dept. of Microbiology and Genetics, University of Salamanca, Spain [12]**.** The resulting sequence aggregations were compared against the Ezbiocloud Database [13] **and** other public platforms (Genbank, EMBL, etc.) and aligned with ClustalX2 **[14]. M**EGA (v 7.0.14) was used for phylogenetic analyses **[15] an**d the Kimura 2-parameter for distances calculations; tree topologies were based on the Maximum Likelihood algorithm.

2.5. Non-targeted metabolomics and molecular modeling technique.

The secondary metabolites of the strong antagonizing strains of Actinomycetes were separated and identified by quadrupole-time-of-flight high-definition mass spectrometry (LC–Q-TOF-MS-MS) at the Metabolomic Unit in the Children's Cancer Hospital Egypt 57357. Each sample was injected in the Positive TOF-MS mode; 28 min were provided for the run. A total of 2584 cycles were performed with 0.6502 sec/cycle. The injection volume was 10 μL. The quadrupole-time-of-flight high-definition mass spectrometry was performed on a Triple TOF$^{\text{ő}}$ 5600+ system +, Sciex (ISO9001), Canada, fused two LC columns, performed In-Line filter disks Pre column (0.5 μm × 3.0 mm; Phenomenex Co., USA) and X Bridge C18 column (3.5 μm, 2.1 × 50 mm; Waters Co., USA) and kept at 40 °C. Some optimized parameters were used: curtain gas, 25 psi; nebulizer gas (gas 1), 45 psi; heater gas (gas 2), 45 psi; ion source heater 500 °*C*; ion spray voltage, 4.5 kV; de-clustering potential, 80 eV; collision energy, 35 eV; Collision energy spread, 20 eV. Molecular Operating Environment (MOE 2008-10 Chemical Computing Group, Canada) was the computational software for all molecular modeling studies. Receptor and ligand preparation procedures were carried out **[16].**

2.6. Data processing:

Master View was used for feature (peaks) extraction from Total Ion chromatogram **(TIC)**, using (Peak View 2.2 Software Sciex), based on the following criteria: features should have Signal-to-Noise greater than 5 (non-targeted analysis). Non-targeted peak findings and clustering were analyzed by (Marker View 1.3 software, Sciex). Features intensities of the sample-to-blank should be greater than 5. Marker View was used for feature annotation and removing isotopic peaks. Master View was used again to identify peaks based on their fragments using: The built-in database (Data Acquisition Analyst TF 1.7.1 software, Sciex) and online databases.

2.7. Molecular modeling:

All the molecular modeling studies were carried out on Intel® Core^{TM} i3 CPU, 2.40 GHz processor, and 3 GB memory with Windows 7 operating system using Molecular Operating Environment (MOE 2008-10 Chemical Computing Group, Canada) as the computational software. All procedures regarding receptor and ligand preparations were carried out according to **[16].**

For the docking studies, PDB codes of DNA Gyrase (1KZN) (https://www.rcsb.org/structure/1KZN **[17], T**opoisomerase VI (1S14) (https://www.rcsb.org/structure/1S14) **[18] we**re available at the freely accessible Protein data bank. The 2D compounds under study were constructed

by ChemBio-office 2015, converted to 3D by the builder interface of the MOE program, then subjected to energy minimization with MMFF94X force. MOE visualizing tool was employed to analyze the interaction in 2D and 3D images for each complex.

2.8. Statistical analyses

The data were collected, checked, revised, and organized in tables and figures using Microsoft Excel 2016. They were subjected to outliers' detections and statistical normality tests to detect whether the data were parametric or nonparametric. The data were analyzed for descriptive statistics, both graphical and numerical description. Inferential statistics for evaluating and comparing between different anti-microbial agents ($A_1, A_2, \ldots, A_{20}$) and different bacterial species time of investigations (B_1, B_2, B_3, ... B_8) were performed by repeated measures analysis of variance (ANOVA) or corresponding nonparametric analyses or corresponding statistical analyses for nonparametric data (e.g., Kruskal-Wallis and Chi-square test), at significance levels of 0.05. Chi-square test statistics performed differences between scores, positive and negative. Data analyses were conducted using the computer software Statistical Package for Social Science SPSS (IBM-SPSS ver. 28.0 for Mac OS).

3. Results

3.1. Anti-microbial screening of actinomycete extracts

All 26 Actinomycete extracts showed anti-microbial activity against at least one of the examined bacterial strains. Twenty-three strains had activity against K. pneumoniae, while 21 isolates were highly active against E. aerogenes (Table 1). The anti-microbial activities of Actinomycetes extract from terrestrial Streptomyces sp. SAC97, SAC61, and Streptomyces atrovirens SAC105 showed the highest inhibitory effect on all selected enteric pathogens.

The anti-microbial activities of Actinomycetes extract from S. albidoflavus SAC61 were tested against eight strains of multidrug-resistant clinical enteric pathogens. Those strains were E. coli, K. pneumoniae, P. aeruginosa, and E. aerogenes, as presented in Table (2).

3.2. Phylogenetic characterization of bioactive Actinomycetes stains

The 16S rRNA gene sequences of the most potent Streptomyces isolates were compared to the most related Streptomyces species. In all cases, 99.50–99.72% similarity values **(Table 3)**.

A phylogenetic tree was constructed for the three Streptomyces strains (**Figure 1**). The maximum Likelihood method based on the Tamura 3-parameter model **[19] wa**s used to infer the evolutionary history of the isolates.

3.3. Determination of minimum inhibitory concentration (MIC)

The Actinomycete strain S. albidoflavus SAC61 (50 to 6.5 μg/ml) was selected for the determination of MIC against E. coli sp., Pseudomonas sp., the extract concentrations ranged from 50 to 6.5 μg/ml., K. pneumoniae, P. aeruginosa, and E. aerogenes. The extract from S. albidoflavus SAC61 showed the lowest MIC against the test bacteria Pseudomonas sp. (25 μg/ml) μg/ml, then E. coli (12.5 μg/ml).

3.4. Non-targeted metabolomics and Molecular modeling of Streptomyces. albidoflavus SAC61 metabolites

We have focused on exploring the unexplored yet, efficient ParE enzyme in which E. coli fluoroquinolones preferentially bind to it more than GyrB. As summarized in Figure (2), Cyclopentyl-acetic acid and Queuine were docked inside both proteins with binding energies and ligand-receptor interactions. They were tested for binding affinity towards DNA Gyrase and Topoisomerase VI. Cyclopentyl-acetic acid was docked inside 1KZN protein. Queuine was docked inside 1KZN protein.

4. Discussion

This experiment evaluated the antibacterial activity spectra of 29 selected strains of actinomycete extracts isolated from Egyptian habitats on some enteric bacteria with multiple-antibiotics

Table 1: **1: Screening of Actinomycete metabolites for its anti- microbial activity**

#	Actinomycete strain	Habitat	Mean zone of inhibition (mm) (± SD)[a]			
			Enterobacter aerogenes NCTC 9735	Klebsiella pneumoniae ATCC 13883	Echerichia coli NCMB 11943	Pseudomonas aeruginosa NCMB 8295
Streptomyces strains						
1.	**Streptomyces sp. F9**	**terrestrial**	6.5 ± 0.7	7 ± 0.0	–	–
2.	**Streptomyces sp. SAC97**	**terrestrial**	9.5 ± 3.5	7.5 ± 1.4	7.5 ± 0.7	9.5 ± 1.4
3.	**Streptomyces sp. 20-7A**	**Marine**	7 ± 0.0	6.5 ± 0.7	–	–
4.	**Streptomyces sp. 23-2B**	**Marine**	9 ± 1.4	–	–	6.5 ± 0.7
5.	**Streptomyces atrovirens SAC105**	**terrestrial**	9.5 ± 0.7	10.5 ± 2.1	9.5 ± 0.7	10 ± 0.0
6.	**Streptomyces sp. S35**	**terrestrial**	10.1 ± 1.4	9.5 ± 0.7	10.5 ± 2.1	8.5 ± 0.7
7.	**Streptomyces sp. 26-1B**	**Marine**	7 ± 0.0	6.5 ± 0.7	-	-
8.	**Streptomyces sp. 28-2B**	**Marine**	-	9 ± 1.4	-	10 ± 0.0
9.	**Streptomyces sp. SAC61**	**terrestrial**	**9.5 ± 0.7**	**9 ± 2.8**	**12 ± 0.0**	**12 ± 0.7**
10.	**Streptomyces sp. 30-4B**	**Marine**	8 ± 0.0	8 ± 0.0	–	9 ± 0.0
11.	**Streptomyces sp. S26**	**terrestrial**	8 ± 1.4	10 ± 0.0	10.5 ±0.7	10 ± 1.4
12.	**Streptomyces sp. 15-3A**	**Marine**	-	6.5 ± 0.7	6.5 ± 0.7	–
13.	**Streptomyces sp. S11**	**terrestrial**	9.5 ± 0.7	6.5 ± 0.7	9 ±1.4	10 ± 1.4
14.	**Streptomyces sp. F78**	**terrestrial**	-	-	1.13 ± 0.1	7 ± 1.4
15.	**Streptomyces sp. F61**	**terrestrial**	0.7 ± 0.0	6.5 ±0.7	1.3 ± 0.1	-
16.	**Streptomyces sp. S6**	**terrestrial**	10 ± 0.0	10.5 ± 0.7	11 ± 1.4	9 ± 0.0
17.	**Streptomyces sp. DCF4**	**terrestrial**	8 ± 0.0	8 ± 0.0	7 ± 0.0	9 ± 0.0
Nocardioides strains						
18.	**Nocardioides 13-1A**	**Marine**	7 ± 1.4	6.5 ± 0.7	–	9 ± 0.0
19.	**Nocardioides 14-2A**	**Marine**	7.5 ±2.1	–	–	–
Kitasatospora strains						
20.	**Kitasatospora 18-5A**	**Marine**	8 ± 0.0	7.5 ± 0.0	8 ± 1.4	–
21.	**Kitasatospora sp. Q1**	**terrestrial**	7 ± 1.4	9 ± 1.4	10.5 ± 0.7	8 ± 0.0
22.	**Kitasatospora sp. F67.**	**terrestrial**	8 ± 0.0	6.5 ± 0.7	1.9 ± 0.1	6.5 ± 0.7
Other actinomycete strains						
23.	**Nocardia sp. 19-6A**	**Marine**	7.5 ± 2.1	7 ± 0.0	9 ± 1.4	-
24.	**Nocardiopsis sp. F1**	**terrestrial**	–	9 ± 0.0	1.2 ± 0.0	8.5 ± 3.5
25.	**Microbispora sp. F55**	**terrestrial**	8.5 ± 3.5	8.5 ± 3.5	1.6 ±0.1	–
26.	**Microbispora sp. F50**	**terrestrial**	-	9 ± 1.4	1.6 ± 0.1	7 ± 0.0
Total active strains			**21**	**23**	**18**	**17**

[a]Average (avg) of inhibition zone diameter in mm ± standard deviation (SD).

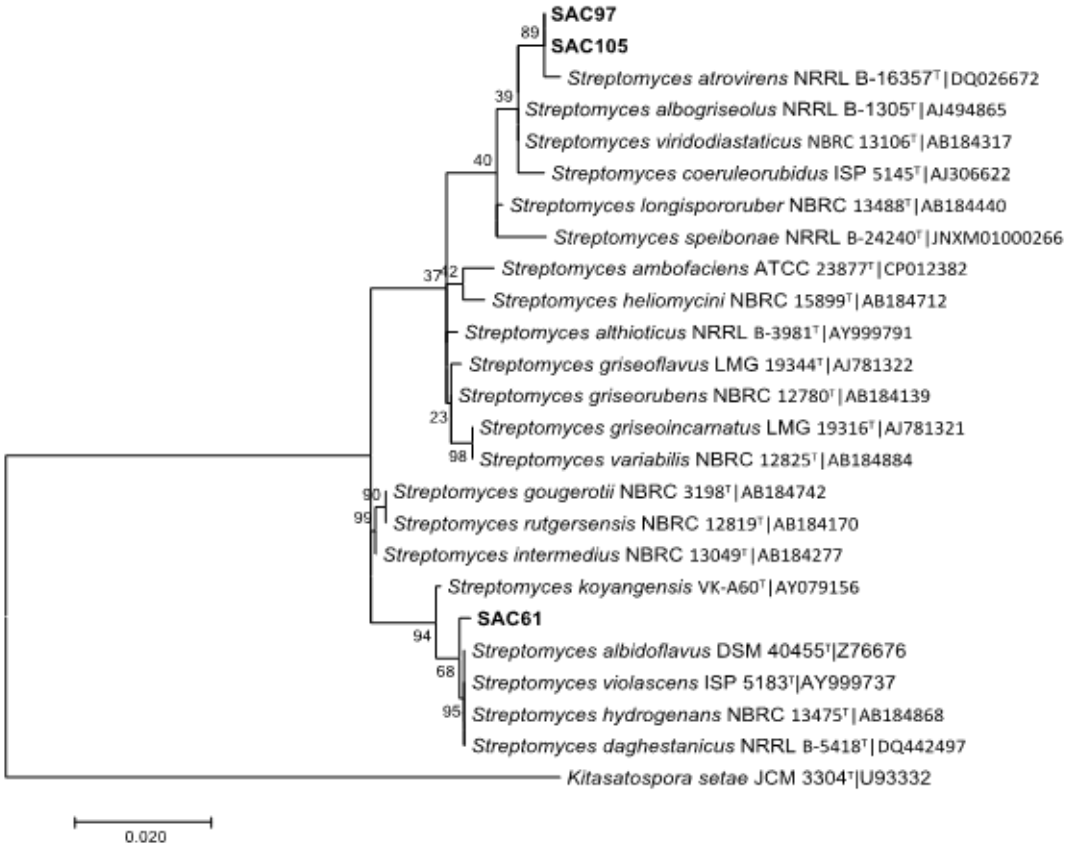


Figure 1: **1: Molecular Phylogenetic analysis of Streptomyces spp. strains by the Maximum Likelihood method**

Table 2: **Anti-microbial activities of selected metabolic extracts against clinical MDR bacteria**

Bacteria (code)	**Streptomyces albidoflavus SAC61**
	mean
Enterobacter aerogenes (1252)	± 13.5
Klebsiella pneumoniae (1759)	± 7.5
K. pneumoniae (1373)	± 7
K. pneumoniae (1652)	± 6.5
E. coli (38)	± 12
E. coli (23)	± 14.5
E. coli (51)	- ve
Pseudomonas aeruginosa (4)	± 13

resistance. The results revealed S. albidoflavus SAC61 as a potential source of novel anti-microbial substances. The promising inhibitory activities of Streptomyces sp. were consistent with previous studies identifying it as a significant source of antibiotics and other bioactive compounds **[3, 20, 21]. Streptom**yces spp. has been acknowledged as a valuable source of bioactive compounds, including anti-microbial compounds **[22], a**nti-cancer and antifungal activity **[23, 24], and im**munosuppressive agents **[22, 25, 26]. Therefore**, the genetic identification of the selected isolates was a critical step toward discovering new bioactive compounds. The 16S rRNA gene analysis of the selected actinobacterial strains confirmed their identity as S. albidoflavus (SAC61) and S. atrovirens (SAC97 and 105). The identified strains had shown high similarity to previously reported strains in the GenBank database.

In our study, MIC of Streptomyces sp. SAC61, ranging from 50 to 6.5 μg/ml, was evaluated for

Table 3: **Top-hit taxa of the sequenced actinomycete strains**

Strain code	GenBank accession number	Top-hit taxon	Similarity (%)	Completeness (%)	length (bp)
SAC61	MG930072	Streptomyces albidoflavus	99.72	99.50	1448
SAC97	MG930073	Streptomyces atrovirens	99.65	99.30	1447
SAC105	MG930074	Streptomyces atrovirens	99.65	99.00	1443

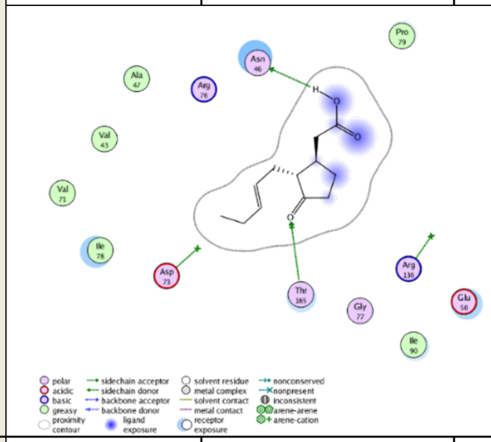
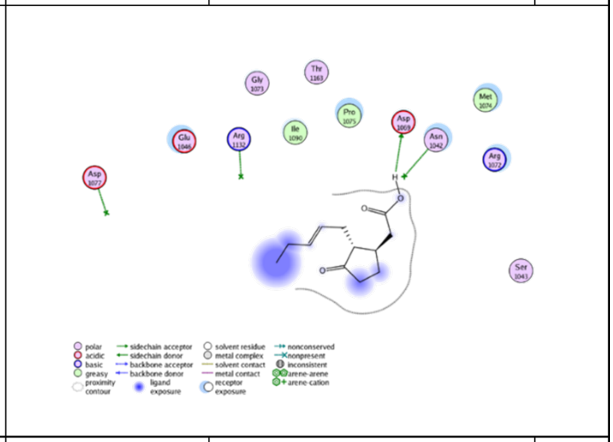
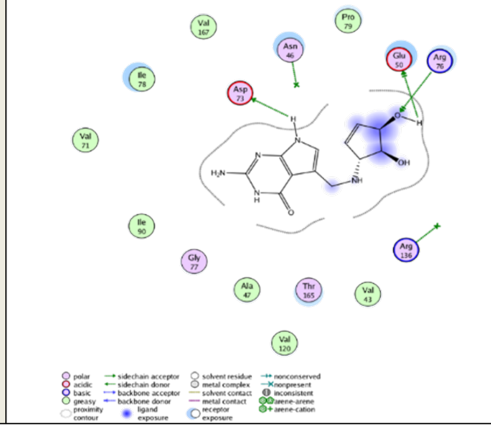
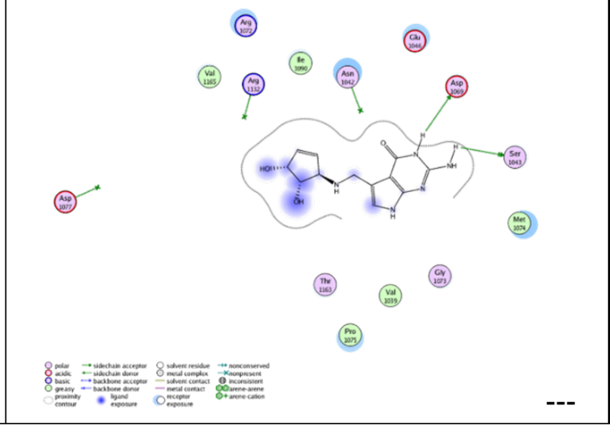
Comp.	DNA Gyrase (1KZN)			Topoisomerase VI (1S14)		
Co-crystallized ligand	**2** H-bonds with **Arg 136** and **Asn 46**			**3** H-bonds with **Asn 1042, Arg 1132,** and **Asp 1068**		
Cyclo-pentyl-acetic acid	-11.13 Kcal/mol	**1**HB with **Asn 46**	--	-11.7 Kcal/mol	1 HB with **Asp 1069**	--
						
Queuine	-18.46 Kcal/mol	**1** HB with **Asp 73**	--	-16.6 Kcal/mol	1 HB with **Asp 1069**	--
						---

Figure 2: Ligand-receptorinteractions of the tested compounds towards the two proteins of anti-microbialactivity; DNA Gyrase and Topoisomerase VI

its anti-microbial activity against E. aerogenes, K. pneumoniae, E. coli, and P. aeruginosa.The extract from Streptomyces sp. SAC61 had the lowest MIC against P. aeruginosa (25 μg/ml) and E. coli (12.5 μg/ml). These findings indicated that strain SAC61 could be a source of anti-microbial compounds against these pathogenic bacteria. Diverse studies have reported the anti-microbial activity of Actinomycetes against various pathogenic bacteria. For example, a study by **[27] st**ated that Streptomyces sp. isolated from soil samples showed significant anti-microbial activity against multidrug-resistant enterobacteria, including P. aeruginosa, E. coli, and K. pneumoniae.

The non-targeted metabolomics and molecular modeling of Streptomyces sp. SAC61 showed that various cyclopentanone-derived compounds were produced using a 1,3-dipolar cycloaddition methodology. The identified compounds are found to be with good anti-microbial properties, and some of them even showed properties more significant than the reference commercially known antibiotics. The attachment of cyclopentanone anion to spiro-pyrrolidines enhanced the anti-microbial properties against several pathogens, as referred by **[28].**

Since E. coli was a pioneer organism in the identification of the complete set of tRNA modifications and corresponding modifying enzymes **[29, 30], prote**in translation compatibility errors were recorded at a rate of 1 to 1,000 - 10,000 due to missense errors during protein polymerization event **[31, 32]. Antib**iotic treatment is one of the triggering factors for missense error reflection **[33–35].**

E. coli queuosine is among the most elaborate of the known RNA modifications. It was first identified in hydroxylate extracts of tyrosyl tRNA from E. coli and was given the single letter abbreviation of Q, from which the now common name of queuosine or Q-nucleoside derives. Q-nucleoside is specific to tRNA acceptors for the amino acid tyrosine, asparagine, aspartic acid, and histidine. In addition, the Q modification has also been detected in aspartyl tRNA from the rat's mitochondria using the 32P-post labeling technique, represents a non-essential, hypermodified guanosine nucleoside found in the anticodons of four E. coli tRNAs as an anticodon modification that could potentially be exploited to improve sense codon reassignment **[36, 37]. Queuo**sine base is a guanine analog found in the first tRNA anticodon. Q- modification occurred post-transcription modification by an irreversible insertion during maturation of specific tRNAs (tyrosine, histidine, aspartate, and asparagine tRNAs). Queuine is abundant among a wide range of living systems, from prokaryotes to eukaryotes. Q-modification has also been detected in rat's mitochondrial aspartyl tRNA using the 32P-post labeling technique **[38].**

DNA topoisomerases are essential bacterial targets, posing all prerequisites for efficient bacterial cell growth **[39, 40]. ATP-d**ependent topoisomerases in bacteria include DNA gyrase (Gyr A and Gyr B) and topoisomerase IV (ParC and ParE). DNA gyrase and topoisomerase IV are vital to replication and cellular proliferation **[41]. D**NA Gyrase is a member of class II topoisomerase, which introduces negative supercoils into restricted DNA strands. Topoisomerase IV disentangles newly replicated DNA and enables the segregation of daughter chromosomes, which are concurrent processes In bacteria **[42]. F**luoroquinolone class is one of the first-line therapeutic drugs, with the most toxic side effects and resistance-developing drugs, that inhibits the catalytic subunits of DNA gyrase and topoisomerase IV **[43, 44].**

Our study highlights the potential of Actinomycetes extracts as a source of novel anti-microbial agents. Further studies are needed to isolate and identify the active compounds in these extracts and determine their mechanisms of action. Using Actinomycetes-derived compounds to develop new antibiotics could be a promising solution to the growing problem of antibiotic resistance.

5. Conclusion

• Our study was conducted to find new natural sources of antibiotics to control multidrug-resistant enteric bacteria. Twenty-nine Actinomycetes were screened for their ability to produce secondary metabolites that could control MDR pathogenic enterobacteria. Streptomyces albid-

oflavus. SAC61 was identified as having the highest inhibitory effect on MDR enteric bacteria. Our findings suggest conducting further studies to isolate and identify more potential anti-microbial compounds from Actinomycetes, which could be developed into new antibiotics to combat antibiotic resistance. Using non-targeted metabolomics approaches can lead to the discovery of new compounds with anti-microbial properties and molecular modeling techniques to design new antibiotics.

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