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ORIGINAL ARTICLE

Investigation of diverse biosynthetic secondary metabolites gene clusters using genome mining of indigenous *Streptomyces* strains isolated from saline soils in Iran

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ABSTRACT

Background and Objectives: Bioactive secondary metabolites are the products of microbial communities adapting to environmental challenges, which have yet remained anonymous. As a result of demands in the pharmaceutical, agricultural, and food industries, microbial metabolites should be investigated. The most substantial sources of secondary metabolites are *Streptomyces* strains and are potential candidates for bioactive compound production. So, we used genome mining and bioinformatics to predict the isolates secondary metabolites, biosynthesis, and potential pharmaceuticals.

Materials and Methods: This is a bioinformatics part of our previous experimental research. Here, we aimed to inspect the underlying secondary metabolite properties of 20 phylogenetically diverse *Streptomyces* species of saline soil by a rationalized computational workflow by several software tools. We examined the Metabolites' cytotoxicity and antibacterial effects using the MTT assay and plate count technique, respectively.

Results: Among *Streptomyces* species, three were selected for genome mining and predicted novel secondary metabolites and potential drug abilities. All 11 metabolites were cytotoxic to A549, but ectoine ($p \leq 0.5$) and geosmin ($p \leq 0.001$) significantly operated as an anti-cancer drug. Metabolites of oxytetracycline and phosphinothricin ($p \leq 0.001$), 4Z-annimycin and geosmin ($p \leq 0.01$), and ectoine ($p \leq 0.5$) revealed significant antibacterial activity.

Conclusion: Of all the 11 compounds investigated, annimycin, geosmin, phosphinothricin, and ectoine had antimicrobial properties, but geosmin also showed very significant anti-cancer properties.

Keywords: *Streptomyces*; *Streptomyces* metabolite; Biological products; Bioinformatics

INTRODUCTION

Streptomyces (Actinobacteria) are often non-pathogenic and produce a variety of natural metabolites. These bioactive metabolites are the source of two-thirds of pharmaceutical compounds (1, 2). Nowadays, the researchers moved on to the new clinically beneficial bioactive compounds derived from the *Streptomyces* secondary metabolites, mostly spices from un-studied ecological niches (3). On the other

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hand, the WHO global health emergency predicted that the multidrug-resistant crisis would cause 10 million deaths in 2050 (4). Multidrug-resistant phenomena lead to growing public health trouble and the enduring need to discover novel treatments. So, the quest for biologically active compounds with distinct mechanisms of action or various cellular targets is further than ever.

Streptomyces, Gram-positive bacteria, with the competence of producing bioactive metabolites, are the crucial source of 80% of the antibiotics (5). The common bioactive secondary metabolites of microorganisms are structurally classified as Polyketides (PK), Non-ribosomal peptides (NRP), Terpenoids (TP), Ribosomally synthesized, post-translationally modified peptides (RiPPs), and PKs/NRPS hybrids (6). Once more, bacterial bioproducts typically can be categorized by pathway-specific regulatory networks and pleiotropic regulatory networks (7). According to bioinformatics analysis, of the further BGCs existing on the bacterial genome, only a few of them has been explored and explained by conventional methods (8).

Currently, genome mining has developed as a principal technology to explore and exploit bioproduct diversity, by analysis of numerous novel biosynthetic gene clusters (BGCs), encoded by some species (1, 8, 9). For sequencing new strain isolates, the best reference can be the most similar one. Additionally, a genomes collection of interdependent *Streptomyces* strains allows evaluation of the underlying mechanisms of genome plasticity and system compatibility (10). This study intended to characterize the properties of the bioactive metabolites of *Streptomyces* species isolated from the Howz-e Soltan saline soil in Iran, using bioinformatics approaches.

MATERIALS AND METHODS

Sample collection. *Streptomyces* species isolated from the Howz-e Soltan saline soil in Iran (Latitude: 35°00'17.8"N, Longitude: 50°56'21.6"E), (geo: 35.004935, 50.939344), located 85km south of Tehran (11). Isolation of strain from soil samples, and morphologic, phenotypic, and taxonomical analyses formerly scrutinized *in vitro*. The bioinformatics data were then analyzed.

Data. The 16S rRNA gene sequence were documented in GenBank with accession numbers (https://www.ncbi.nlm.nih.gov/genbank/tbl2asn2/) using the tbl2asn online program (11, 12). The nucleotides, proteins, and annotations data were taken on Aug 15, 2021.

Prediction of secondary metabolite BGCs of isolates. The identified metabolites structures were designed using ChemDraw Ultra 8.0 software (13). The biosynthetic gene clusters or BGCs distribution and diversity in the genome of three isolated *Streptomyces* genomes were specified using antiSMASH the public web version 6.0.1, (available at http://antismash.secondarymetabolites.org) (14). MIBiG, an online tool associated with the antiSMASH, provides minimum in- formation about a biosynthetic gene cluster (https://mibig.secondarymetabolites.org) (14).

Scrutinizing the secondary metabolites of *Streptomyces* species is associated with the exploration of their chemical information and then identification of structures and functions. Consequently, compounds names were searched in PubChem (https://pubchem.ncbi.nlm.nih.gov/) (15) and UniProtKB (16). Then to find the potential ligands, biomolecules were searched in the Protein Data Bank (PDB) (https://www.rcsb.org/).

Drug ability of secondary metabolites. To measure the amount of drug, compounds having a molecular weight of more than 500 Daltons were disqualified by Lipinski's rule of five. Then enter the Canonical SMILES code of each compound in the pkCSM online software at http://biosig.unimelb.edu.au/pkcsm/ prediction to determine the pharmacokinetic properties of the possible ligands. Looking for ligands with the same features (ligand-based) is a pharmacophore.

Drug able metabolites *in vitro* assays. Secondary metabolites were isolated using preparative HPLC and GC-MS analysis (Data not shown). Then anticancer and antibacterial effects were determined by MTT and MIC assays, respectively.

Determination of metabolites' cytotoxicity by MTT assay. Human lung carcinoma cells of line A549 were cultured in a 96-well plate with an approximate seeding density of 10^4 cells with supplemented RPMI 1640. Following two days of incubation

at 37°C and 5% CO_2, cells were treated with equal amounts (50 µM) of oxytetracycline, annimycin, phosphinothricin, ectoine, and geosmin. The media were discarded from the A549 cell culture plate. Then 80 µL of the RPMI 1640 fresh serum-free media was aspirated in, and 20 µl MTT solution (5mg/mL) was added to each well. The plate was incubated for 3 hours at 37°C. Then the medium was removed and add 100 µL DMSO (solubilization solution) was added to each well and placed in darkness to fully dissolve the MTT formazan. The colorimetric evaluation was performed using a microtiter ELISA reader (Awareness Technology Inc, Stat Fax 2100, USA) at a wavelength of 570 nm (17).

Determination of metabolites' antibacterial effects by plate count technique. The direct metabolites' antibacterial effects were investigated using the bacterial growth inhibition assay and colony-forming units (CFUs) counting (18). Briefly, the suspension of 100 µl= 10^6 CFUs *S. aureus* (ATCC 29213) per well was exposed to 10 µM of each metabolite and incubated overnight at 37°C. After overnight incubation, 10 µl of the bacterial suspensions were diluted (1:1000) and spread on blood-agar to count the CFUs (logarithmic) the next day (19).

Statistical analysis. The collected data were analyzed in Excel (2019) using ANOVA at a significance level $p \leq 0.05$.

RESULTS

The search for this research was conducted through Google Scholar, Science direct, NCBI PubMed, BMC, Expasy, and some other helpful databases, with more than 70 articles reviewed seeking the most recent techniques. The amplified 16S rRNA genes were commercially sequenced through the Sangar technique. Comparing the concerned sequences to other microbial sequences performed sequence similarity searches using the BLAST in the NCBI.

Nucleotide sequence accession numbers allocation. The 16S rRNA gene sequences were introduced to GenBank to designate the specific accession numbers. Hence, they were *Streptomyces griseoflavus* (MN846650) (20), *Streptomyces calvus* (MN853595) (20), and *Kitasatospora phosalacineus* (MN853650) (20). Genomic data comprising accession numbers, genome size, %GC content, number of genes, and the number of protein-coding genes, were predicted by the most similar one, and are presented in Table 1.

Based on the taxonomic and morphological attributes, they are related to the genus *Streptomyces*. From the molecular point of view, these results offered distinct *Streptomyces* species from the saline soils. Blasting three strains altogether with *Streptomyces* (taxid:1883) exhibited high levels (97-99%) of sequence similarity with microorganisms of the given family as strain 1 to *Streptomyces griseoflavus*, Strain 2 to *Streptomyces calvus*, and strain 3 to *Kitasatospora phosalacineus.* So, we can explain it as *Streptomyces griseoflavus* strain SAEM 16S ribosomal RNA gene, partial sequence GenBank: MN846650, 237 bp DNA linear (BCT 23-DEC-2019) with 99% similarity. *Streptomyces calvus* strain Qom1 16S ribosomal RNA gene, partial sequence GenBank: MN853595.1, 540 bp DNA linear (BCT 25-DEC-2019) with 98% similarity. *Kitasatospora phosalacinea* strain Qom 2 16S ribosomal RNA gene, partial sequence GenBank: MN853650.1, 1007 bp DNA linear (BCT 25-DEC-2019) with 97% similarity (20).

Prediction of secondary metabolite BGCs of isolates. The basis for the selection of these references was the antiSMASH and MIBiG databases. The public web version 6.0.1 of antiSMASH was refined by secondary metabolism-related functional domains from further analysis. The antiSMASH, comparing regions to the reference database, showed that there are 57 regions in the *S. griseofluvus* genome expressing secondary metabolites. While there was 1 region in each of *S. calvus* and *K. phosalacineus* for this purpose. We decided to merge clusters that were more than 80% similarity, except for *K. phosalacinea* (75% similarity). Predicted clusters and backbone biosynthetic enzymes were identified. After one-by-one cluster analysis, the most similarities were related to *Streptomyces* sp. (Table 2).

The function of secondary metabolites. As mentioned earlier, we used several databases such as PubChem, UniProtKB, and PDB to achieve the structure and function of secondary metabolites. The results have stated as follows in the order mentioned in Table 2.

Ectoine, as a cytoprotectant during osmotic stress and extreme temperature, is a compatible solution and chaperone commonly synthesized by bacteria and

Table 1. Accession numbers and genomic information of isolates

Strains	*S. griseoflavus*	*S. calvus*	*K. phosalacineus*
Accession ID	MN846650.1	MN853595.1	MN853650
Genome size (Mbp)	7.58	7.77	7.62
%GC in genome	72.3	72.5	74.1
# Genes # ORFs	6841	6614	6895
# CDC	6554	6389	6596
Most similar to ID	MT760553.1	NZ_CP022310.1/CP022310.1	NR_040808.2

Table 2. MIBiG accession of predicting the most similar bioactive secondary metabolites of *Streptomyces* species, their sources, and activities

Compound (S)	MIBiG accession	Similarity score	Type	Resource Organism	Activity
Ectoine	BGC0000853.1	0.88	Other	*Streptomyces* sp. *Streptomyces anulatus*	Antibacterial/anti-inflammatory
Rimosamide	BGC0001760.1	0.92	NRP	*Streptomyces rimosus* subsp. *rimosus* ATCC 10970	Anti-antibiotic
Desferrioxamine E	BGC0001478.1	0.85	Siderophore	*Streptomyces* sp. ID38640	Antibacterial
Isorenieratene	BGC0000664.1	0.71	Terpene	*Streptomyces griseus* subsp. *griseus* NBRC 13350	Antioxidant/Anticancer
Lagmysin	BGC0001645.1	0.81	RiPP	*Streptomyces* sp. NRRL S-118	Antibacterial/ Anticancer/Anti-HIV
Geosmin	BGC0001181.1	0.75	Terpene	*Streptomyces coelicolor* A3 (2)	Antibacterial/ Anticancer
Oxytetracycline	BGC0000254.1	0.89	Polyketide	*Streptomyces rimosus*	Antibacterial
Tyrobetaine	BGC0001813.1	0.87	NRP	*Streptomyces* sp. NRRL WC-3703	Antibacterial/ Anticancer
Isocomplestatin	BGC0000326.1	0.62	NRP	*Streptomyces lavendulae*	Antibacterial/ Anticancer
4-Z-annimycin	BGC0001298.1	0.94	Polyketide	*Streptomyces calvus*	Antibacterial
Phosphinothricin tripeptide	BGC0000406.1	0.76	NRP	*Streptomyces viridochromogenes*	Antibacterial

some Archaea. Ectoine is a carboxamidine heterocycle, a highly water-soluble monocarboxylic acid, and a tautomer of an Ectoine zwitterion (Fig. 1) (21).

Rimosamide. BGCs encode rimosamides consisting of rimosamide A, B, C, and D, which are of nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) components. These families with exclusive privileged bioactivity, are similar to dtoxins, demonstrating anti-antibiotic activity. They have an antagonistic effect on the antimicrobial activity of blasticidin S, by inhibiting its active uptake (Fig. 1).

Desferrioxamine E (Nocardamine) is a cyclic hydroxamic acid siderophore with pyridoxal-dependent decarboxylase activity. It is produced by several microorganisms and shows antitumor activity, anti-mycobacterial, antioxidant, and siderophore (as iron (Fe) chelating compound) (24). It was far ahead and extended to these compounds derivatives and today commonly embraces totally synthetic compounds (Fig. 1) (23).

Isorenieratene. Some carotogenic species of *Streptomyces* yield a typical product in the carotenoid biosynthesis pathway as isorenieratene. The biosynthesis of isorenieratene has been verified in the lineage of actinobacteria. Carotenoids, as colored terpenoids, have antioxidant properties and are helpful to cope with oxidative stress and antioxidant protection systems (Fig. 1) (24).

Lagmysin is almost a new lasso peptide (class II) of the RiPP cluster with a macrocycle made by a macrolactam band and contains a unique N-terminal Leu (Fig. 1) (6).

Geosmin is an uncompleted sesquiterpene responsible for the unpleasant odor of moist soil (Fig. 1) (25).

Oxytetracycline (or Terramycin, Oxacycline, Oxyterracine, Abbocin, Adamycin), is a tetracycline with

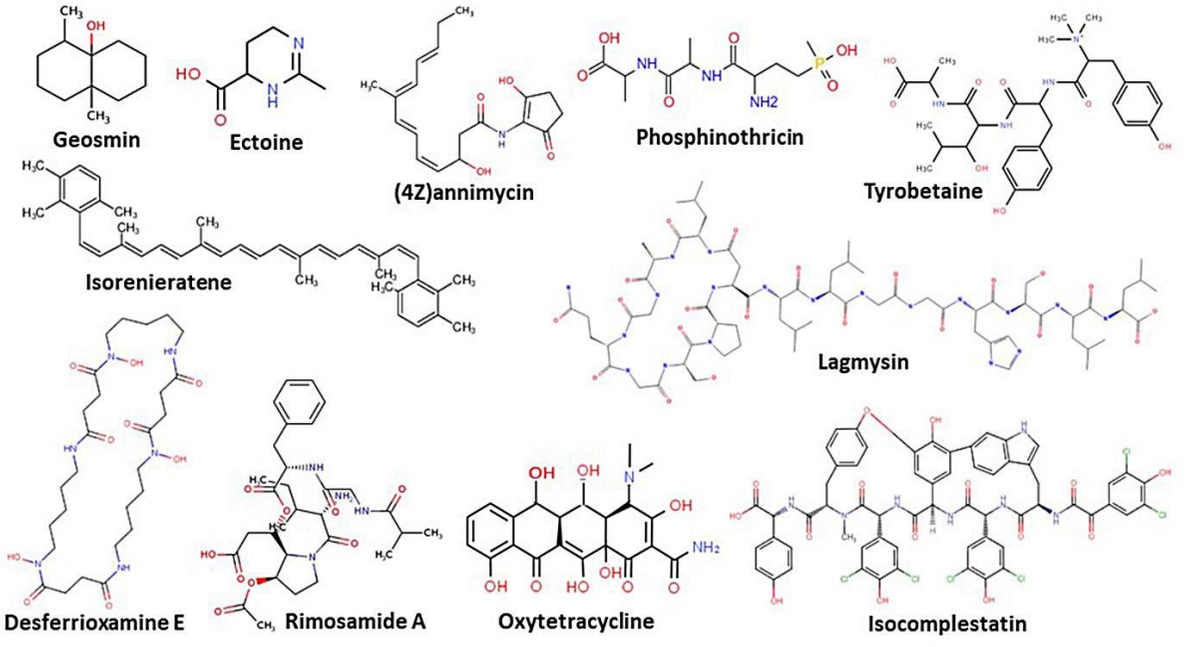

Fig. 1. The structures of predicted secondary metabolites encoded in *Streptomyces* sp. genome (13)

a bacterial aromatic polyketide structure. The BGC of oxytetracycline encodes PKS enzymes such as OxyS, OxyE, and OxyP (Fig. 1) (26, 27).

Tyrobetaine is an NRP with an uncommon N-terminal trimethylammonium tyrosine residue (Fig. 1). It might have a synergistic effect with oxytetracycline co-expression in the same strain and acts as a protease inhibitor of its structural similarity to some protein inhibitors. Tyrobetaine potentially possesses protease inhibitory, and antibiotic/anticancer activity (28).

Isocomplestatin (an axial-chiral isomer of Complestatin) is a potent natural product with rigid bicyclic hexapeptide, owning anti-HIV-1 activity. Isocomplestatin represses HIV-1 integrase, a unique enzyme required for viral replication. It also has anti-Complement activity (Fig. 1) (29).

4-Z-annimycin from *S. calvus* is a product of the *bldA* gene that regulates sporulation and induces the polyene 4-Z-annimycin expression. A single BGCs of 35 kb encodes the annimycin, the 2-Amino-3-hydroxycyclopentenone or C5N group (*ann1* to *ann5*), of five open reading frames. Its antimicrobial activity is associated with the inhibition of several actinobacteria sporulation (Fig. 1). The biosynthesis of annimycin is an unusual formation of PKS modules and N-acetylcysteamine thioester substrates, catalyzed with Ann3, Ann2, and Ann1 enzymes. The 5ALA or 5-aminolevulinic acid is an important intermediate (30).

Phosphinothricin tripeptide (Bialaphos, Bilanafos, Antibiotic SF 1293, Glufosinate); *Kitasatospora* sp. produces a secondary metabolite, phosphinothricin tripeptide (PTT). PTT is an antimicrobial agent comprising one L-phosphinothricyl and two L-alanyl units with transferase activity (31) (Fig. 1). PTT biosynthesis pathway is a continuous process catalyzed by the BGC products *phsA*, *phsB*, and *phsC*. In summary, bioactive secondary metabolites, their sources, and their activities are sorted in Table 2.

Drug ability of secondary metabolites. To determine the drug ability of secondary metabolites, we should inspect their pharmacokinetic properties. So, the Canonical SMILES codes were entered in pkCSM online software. These compounds are considered potential ligands or leads. The pharmacokinetic properties of the possible ligands were evaluated in an excel worksheet (data not shown).

In this project, oxytetracycline was considered as a control and threshold. Oxytetracycline is a real drug of the Tetracycline family. Compounds that had a molecular weight of more than 500 Daltons were ineligible following Lipinski's law of five, including rimosamide, nocardamine, tyrobetaine, and isocomplestatin. The same compounds are excluded from the drug ability hypothesis due to their hepatoxicity activity. Then, the AMES test was negative for all compounds,

revealing no mutagenicity. Ligands below the threshold of blood-brain-barrier permeability are removed. Intestinal absorption was sorted from more to less. Ligands are better that have higher adsorption and therefore more distribution and bioavailability. Phosphinothricin tripeptide excepted for the low intestinal absorption (almost 18%). Then, the compounds' water solubility properties of ranging from the smallest to the largest. Hydrophilic or hydrophilic compounds having polar structures tend to dissolve in water and other aqueous solvents. In other words, for a drug compound to be well absorbed, it must be dissolved in water (slightly polar) to be able to pass through the intestine. In measuring the solubility of water, hydrophilic compounds, which have polar structures, tend to dissolve in water and should have a lower solubility value in water (log mol/L). Here all compounds are qualified from this point of view. The Caco2 permeability column was then sorted from largest to smallest, which indicates the intestinal absorption of edible drugs (Table 3).

Biosynthesis gene clusters encode for druggable secondary metabolites in Fig. 2, in order (4Z) annimycin, phosphinothricin, ectoine, and geosmin. They were derived from the BiG-FAM database available at https://bigfam.bioinformatics.nl/dataset/0 (33).

So, the obvious drug ability values of these compounds vary from oxytetracycline as a real drug to 1. annimycin, 2. geosmin, 3. phosphinothricin, and 4. ectoine. In between, annimycin and phosphinothricin had antimicrobial activity. Ectoine had antimicrobial and anti-inflammatory activities. Geosmin had antibacterial and anticancer functions.

Metabolites drug ability *in vitro* assays. The isolated metabolites with drug ability were extracted by preparative HPLC and GC-MS analysis (Data not shown). Then, anticancer and antibacterial effects were determined by MTT and colony count assay, respectively.

Cytotoxic metabolites (MTT assay). Positive control was included A549 cell line with no treatment. Negative control only was the culture medium. While oxytetracycline is considered a well-known drug with anticancer activity. However, all the tests' samples were cytotoxic to A549, ectoine ($p \leq 0.5$), and geosmin ($p \leq 0.001$) significantly acted as anticancer drugs (Fig. 3a).

Table 3. Pharmacokinetic properties and Drug ability of secondary metabolites from new *Streptomyces* predicted by pkCSM online software

Property/ Model Name	Ectoine	Rimosamide	Nocardamine	Isorenieratene	Lagmysin	Geosmin	Oxytetracycline	Tyrobetaine	Isocomplestatin	(4Z)-Annimycin	Phosphinothricin
MW	142.1	604.7	600.7	528.8	1516	182.3	460.4	587.7	1328	331.4	323.3
logp	-0.14	0.778	0.853	11.71	-7.27	3.117	-1.4	0.533	9.197	3.01	-1.30
Water solubility	-0.16	-3.13	-4.37	-5.91	-2.89	-2.8	-2.56	-2.97	-2.89	-3.93	-1.79
Caco2 permeability	1.115	-0.36	0.087	1.119	-0.14	1.487	0.23	-0.60	-1.46	0.87	-0.52
Intestinal absorption	86.56	34.14	33.96	91.67	0	93.79	41.32	15.91	54.46	93.46	17.97
Skin Permeability	-2.73	-2.7	-2.76	-2.73	-2.73	-2.45	-2.73	-2.73	-2.73	-3.24	-2.73
BBB permeability	-0.34	-1.5	-1.69	1.084	-2.20	0.395	-0.81	-1.13	-2.9	-0.60	-1.17
CNS permeability	-3.42	-3.9	-3.40	-0.50	-6.79	-2.96	-4.17	-4.11	-3.74	-2.75	-4.14
AMES toxicity	No	No	No	No	No	No	No	No	No	No	No
Max. tolerated dose	1.497	0.947	0.734	0.16	0.44	0.687	0.546	0.232	0.438	0.297	1.002
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No	No	No	No	Yes	No	No
Oral Rat Acute Toxicity (LD50)	1.547	2.488	2.798	2.304	2.482	1.947	2.291	2.627	2.482	2.085	1.84
Oral Rat Chronic Toxicity (LOAEL)	2.409	2.379	0.844	0.325	7.137	1.309	3.492	4.109	9.739	2.468	2.723
Hepatotoxicity	No	Yes	Yes	No	Yes	No	No	Yes	Yes	No	No
Skin Sensitisation	No	No	No	No	No	Yes	No	No	No	No	No

Antibacterial metabolites (colony count technique). Following exposure to the metabolites, the diluted bacterial suspensions were illustrated in the logarithmic trend line, and colony-forming units were calculated in Log 10^3 (Fig. 3b).

DISCUSSION

Natural products, the trigger of drug discovery, have been the leading sources of many antimicrobials and chemotherapeutics drugs. *Streptomyces* genome mining analysis revealed the encoding of various interesting secondary metabolites by diverse biosynthetic gene clusters (34). Kai Blin et al. in 2021, reported and evaluated the available web-based bioinformatics tools for genome mining to determine the new natural bioactive products (14). Therefore, we did the same as defined using the antiSMASH, available at http://antismash.secondarymetabolites.org/. Incon-

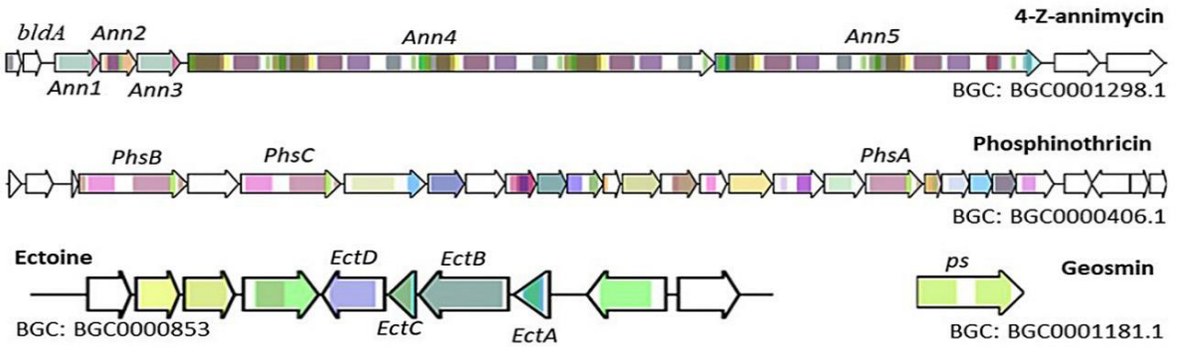

Fig. 2. Biosynthetic gene clusters of 4-Z-annimycin, phosphinothricin, ectoine, and geosmin derived from the BiG-FAM database (32)

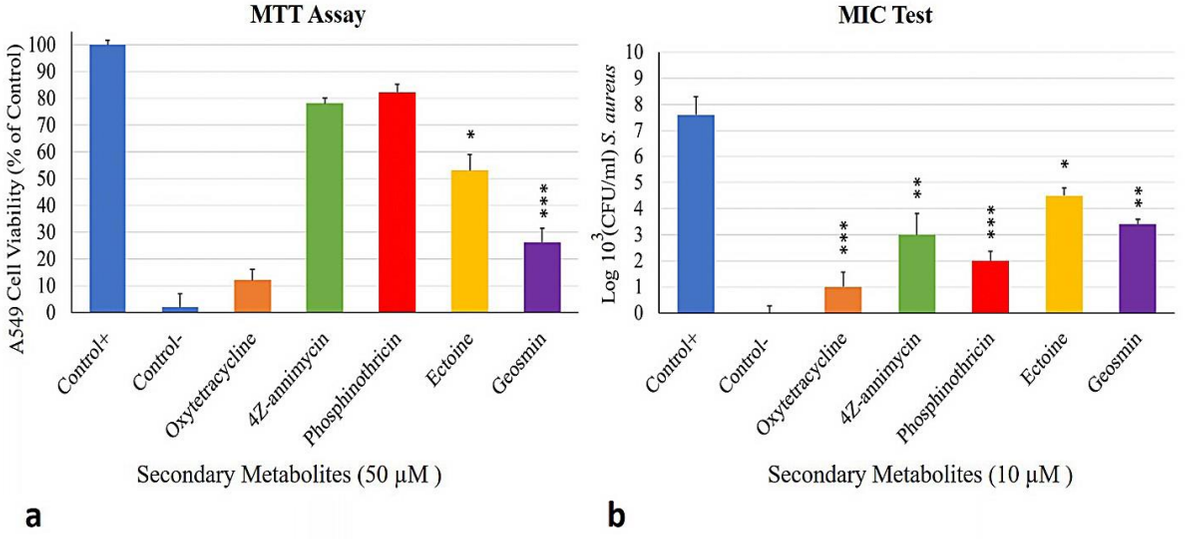

Fig. 3. *In vitro* drug ability of *Streptomyces* secondary metabolites. a. Metabolites cytotoxicity on A549 cell-line. Positive control as A549 cell-line with no treatment, negative control as the culture medium, and oxytetracycline with anticancer drug ability were all controls. From the tests samples as annimycin, phosphinothricin, ectoine, and geosmin, just ectoine ($p \leq 0.5$), and geosmin ($p \leq 0.001$) showed significant anticancer effects. b. *Streptomyces* secondary metabolites antibacterial activity on *S. aureus.* All samples revealed significant antibacterial activity oxytetracycline and phosphinothricin ($p \leq 0.001$), 4Z-annimycin and geosmin ($p \leq 0.01$), and Ectoine ($p \leq 0.5$). All tests were performed in triplicates and the data represents as mean ± SD. * P <0.05; ** P <0.01; *** P <0.001 are considered as significance.

sistent with that research, we investigated the potency of lead compounds to be a drug.

Today, the novel secondary metabolites from cryptic gene clusters can be predicted from available genomic data, accompanied by computational approaches. However, automated bioinformatics provides new fields in possible drug leads discovery from the bacterial metabolites, the identified compounds do not necessarily have therapeutic activity (33).

The novelty of the study is the drug ability checkup of more than 10 *Streptomyces* metabolites through ligand-based drug discovery. In the current study, 20 diverse saline-soil-derived *Streptomyces* were detected. In between, 3 *Streptomyces* isolates were selected for the secondary metabolism analysis *in vitro*. The research aimed to genome mining of diverse metabolites biosynthetic gene clusters in novel *Streptomyces* species. For this purpose, we used bioinformatics databases, as mentioned earlier in the text. The lead structures, predicted in this step, went for more analyses and exploring the new pharmaceutical agents as hits. So, finding the converting of leads to hits, we analyzed the pharmacokinetic properties of the given compounds.

Wittmar J. et al. pointed out that ectoine is a natural metabolite with anticancer activity (34). The mechanism might be for the ectoine strong molecular interaction with DNA molecule, leading to dehydration and destabilizes the dsDNA (34). Where the direct interaction takes between the ectoine nitrogen atoms and DNA phosphate groups and causes a breakdown of hydrogen bonds between bases in the double helix (35). On the other hand, the antitumor activity evaluated by the MTT assay confirmed the ectoine ($p \leq 0.5$) significant antitumor activity. The *in vitro* experiment of antibacterial assay approved the ectoine significant ($p \leq 0.5$) antibacterial activity.

Our results on geosmin were consistent with what was introduced as a volatile compound to be an antitumor agent. The *in vitro* experiment of the MTT assay approved the geosmin significant ($p \leq 0.001$) antitumor activity. Our *in vitro* experiment of antibacterial assay confirmed the geosmin significant ($p \leq 0.01$) antibiotics activity.

Oxytetracycline has antibacterial activity, we also predicted the antibiotic property of oxytetracycline. Our *in vitro* experiment of antibacterial assay approved the oxytetracycline and phosphinothricin significant ($p \leq 0.001$) antibiotics activity.

In parallel with Lindsay Kalan et al. (30), 4Z-annimycin showed antibiotic effects. The *in vitro* experiment of antibacterial assay approved the 4Z-annimycin significant ($p \leq 0.01$) antibiotics activity. The observations reported before support the results of this research.

Genome mining proposed a comprehensive BGCs analysis of given isolates. *Streptomyces griseoflavus* produces ectoine, rimosamide, desferrioxamine E, isorenieratene, lagmysin, geosmin, oxytetracycline, tyrobetaine, and isocomplestatin. *Streptomyces calvus* produces 4-Z-annimycin, and *Kitasatospora phosalacineus* can produce phosphinothricin tripeptide.

Considering pharmacokinetics properties, drug ability was refined by excluding compounds with high molecular weight (Lipinski's rules), hepatotoxicity, mutagenicity, intestinal absorption, blood-brain-barrier, water solubility, and Caco2 permeability. The results indicated that 4 compounds had obvious drug ability in this order; 1. Annimycin, 2. geosmin, 3. phosphinothricin, and 4. ectoine. As all 4 had antimicrobial functions, and geosmin had also anticancer activity. Of course, excluding the rest from these properties doesn't mean that they cannot be hits (drugs), as mentioned before, they all are leads (potential drugs). There are strategies to improve them to the real drugs.

Investigating un-explored environments (niches) worldwide leads to discovering potential distinctive antimicrobials, anticancer, and many other drugs.

CONCLUSION

This study emphasizes the crucial importance of discovering halotolerant microorganisms as a bioresource. They supported the excellent promise of antibacterial and chemoprotective drug development. The novelty of the work is the drug ability examination of more than ten streptomyces metabolites through ligand-based drug discovery. Of all 11 compounds studied, annimycin, geosmin, phosphinothricin, and ectoine had antimicrobial functions. In addition to antimicrobial, geosmin has anticancer activity, too.

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