دانشگاه علوم پزشکی و خدمات بهداشتی،درمانی کرمانشاه

Kermanshah University Of Medical Sciences

Investigation on taxonomy, secondary metabolites and antibacterial activity of *Streptomyces sediminicola* sp. nov., a novel marine sediment-derived *Actinobacteria*

Presentor: Maryam Mehrang
Supervisor: Dr. Yadollah Bahrami

مکان برگزاری : سالن امفی تاتر نوروز نژاد

The impact factor of microbial cell factories is 4.3

Zhang *et al. Microbial Cell Factories* (2024) 23:285
https://doi.org/10.1186/s12934-024-02558-z

Microbial Cell Factories

RESEARCH

Open Access

Investigation on taxonomy, secondary metabolites and antibacterial activity of *Streptomyces sediminicola* sp. nov., a novel marine sediment-derived *Actinobacteria*

Kun Zhang[1,2†], Wenping Ding[1†], Chenghui Han[1], Lijuan Long[1,3], Hao Yin[1*] and Jianping Yin[1,3*]

https://www.biologie.uni-halle.de/microbiology/sawers/streptomyces/

Why Marine Actinomycetes?

- Rich source of bioactive secondary metabolites (antibiotics, anticancer agents).
- Marine Streptomyces adapt to extreme conditions (high salinity, pressure), yielding unique compounds.
- Challenges: Rediscovery of known metabolites → Need for novel strains

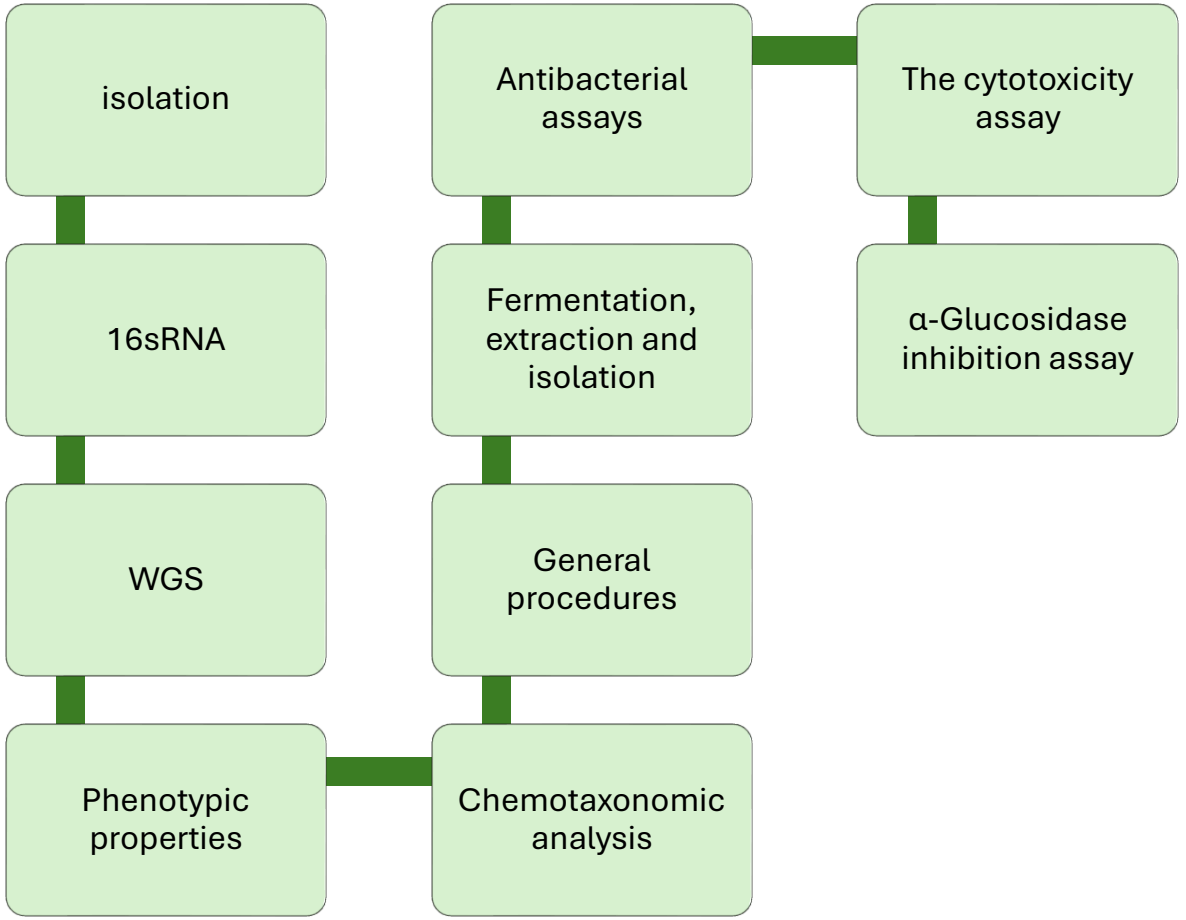
Objectives

1- Isolate and taxonomically characterize a novel Streptomyces strain.

2- Identify secondary metabolites via genome mining and bioassays.

3-Evaluate antibacterial/biological activities of purified compounds.

Methods Overview



Methods

- Isolation and maintenance

  2216E agar

- 16S rRNA gene phylogeny

  Sequence similarity : (www.ezbiocloud.net/ident ify)

  Multiple sequence alignments and phylogenetic reconstructions : MEGA version 11

Whole genome sequencing

- Sequence assembly: Unicycler software
- quality of microbial genome: CheckM
- average nucleotide identity (ANI) : ANI Calculator
- Digital DNA-DNA hybridization (dDDH) : Genome-to-Genome Distance Calculator
- average amino acid identity (AAI) : AAI calculator tool
- The whole genome and orthologs genes : OrthoVenn 3
- Synteny analysis: progressive Mauve tool
- BGC: ANTI-ASMASH

Phenotypic properties

- Cell morphology
- Anaerobic growth: GasPak EZ anaerobic bag system (BD)
- Growth, temperature range, pH tolerance, NaCl tolerance
- Hydrolysis of starch, cellulose, gelatin, and Tweens (20, 40, 60, and 80) and H2S production, coagulation, and peptonization of milk, Catalase activity

- biochemical characteristics: enzymatic activities, carbon and energy source (API ZYM, API 20NE, Biolog GEN III

Chemotaxonomic analysis

- Menaquinones
- cell-wall diamino acid and whole-cell sugar
- Cellular fatty acid
- polar lipids

General procedures

- HRESIMS/NMR
- Column chromatography (CC) /HPLC

Fermentation, extraction and isolation

Media	Compositions
MA	Glucose (10 g/L), Yeast extract (5 g/L), Soluble starch (12 g/L), Bacterial peptone (5 g/L), NaCl (4 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $CaCO_3$ (2 g/L), Sea salt (30 g/L).
MK	Yeast extract (2 g/L), Bacterial peptone (2 g/L), Glucose (2 g/L), Mannitol (3 g/L), Malt extract (5 g/L), Peptone from soybean (5 g/L), Soluble starch (5 g/L), Sea salt (30 g/L).
2216E	2216E (37.4 g/L)
M2216E	2216E (37.4 g/L), Glucose (15 g/L), Sea salt (30 g/L).
AM3	Soluble starch (10 g/L), soybean meal (5 g/L), Bacterial peptone (15 g/L), Glycerin (10 ml/L), $CaCO_3$ (2 g/L), Sea salt (30 g/L).
MC3	Soluble starch (10 g/L), Yeast extract (4 g/L), Bacterial peptone (2 g/L), $CaCO_3$ (2 g/L), Sea salt (30 g/L).
MISP2	Yeast extract (4 g/L), Malt extract (10 g/L), Glucose (4 g/L), Sea salt (30 g/L).
MISP4	Soluble starch (10 g/L), KH_2PO_4 (2 g/L), $MgSO_4 \cdot 7H_2O$ (1 g/L), NaCl (1 g/L), $(NH_4)_2SO_4$ (2 g/L), $CaCO_3$ (3 g/L), TS (1 mL/L) [a], Yeast extract (0.5 g/L), Bacterial peptone (1 g/L), Sea salt (30 g/L).
MRA	Soluble starch (20 g/L), Glucose (10 g/L), Malt extract (10 g/L), Maltose (10 g/L), Corn steep liquor (5 g/L), $CaCO_3$ (2 g/L), Sea salt (30 g/L).
MJNP1A	Soluble starch (20 g/L), Peptone of fish powder (2 g/L), Trehalose Dihydrate (2 g/L), Beef extract (3 g/L), Sea salt (30 g/L).
MM18	Corn flour (5 g/L), Yeast extract (2 g/L), Soybean meal (5 g/L), Soluble starch (15 g/L), $(NH_4)_2SO_4$ (2 g/L), $CaCO_3$ (2 g/L), Sea salt (30 g/L).

- MC3 liquid: HPLC profiles and antibacterial zones
- The culture (30 L) : (2.5 g)

- EtOAc extract was fractionated using MPLC (C18 column) with a methanol/water gradient (10–100%), yielding 10 fractions (Fr.1–Fr.10).

Compound Purification (Semi-Preparative HPLC):

- Fr.5 (76mg)→ Compound 1 (Monaprenylindole A)
- Fr.7 (90mg)→ Compounds 2 (3-Cyanomethyl-6-prenylindole)&
- 3 (6-Prenyltryptophol)
- Fr.9 (74mg) → Compound 4 (Anthracimycin)
- Fr.10.4 (60mg) & Fr.10.5 (38 mg)→ Compounds 5 (2-Epi-anthracimycin)& 6 (β-Rubromycin)
- Fr.3.2 (40mg)→ Compound 7 (4-(Dimethylamino) benzoic acid)

Antibacterial assays

- Bacillus thuringiensis ATCC 10792, Staphylococcus aureus ATCC 29213, Bacillus subtilis BS0l, Acinetobacter baumannii ATCC 19606 and Escherichia coli ATCC 25922)
- (LB) agar plate at 37 °C
- The eleven EtOAc extracts were dissolved in MeOH at the concentration of 20 mg/ mL, the crude extract at concentration of 10 mg/mL, and the ten fractions (Fr.1–Fr.10) at concentration of 4 mg/ mL.

The cytotoxicity assay

- Target cell line: Human tumor cells (HL-60)
- compound 1

- Method: CCK-8 assay

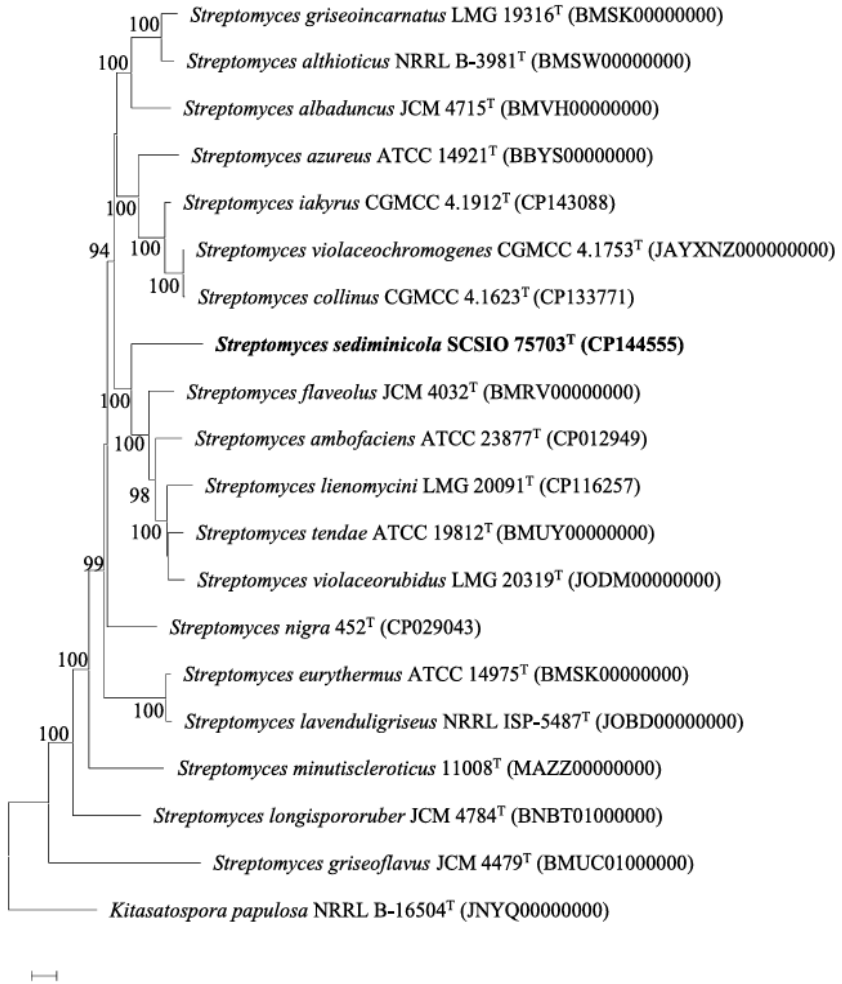
α-Glucosidase inhibition assay

- Test Compounds: Compounds 1–3 tested at 5 concentrations (starting at 100 µg/mL).
- Serial twofold dilutions prepared in DMSO.
- Positive Control: Acarbose (starting concentration: 14.3 µg/mL).
- triplicate.
- Analysis:IC50 GraphPad Prism 9.

Results and discussion

Table 1 Results of dDDH, ANI, AAI values between strains SCSIO 75703^T and their most closely related species

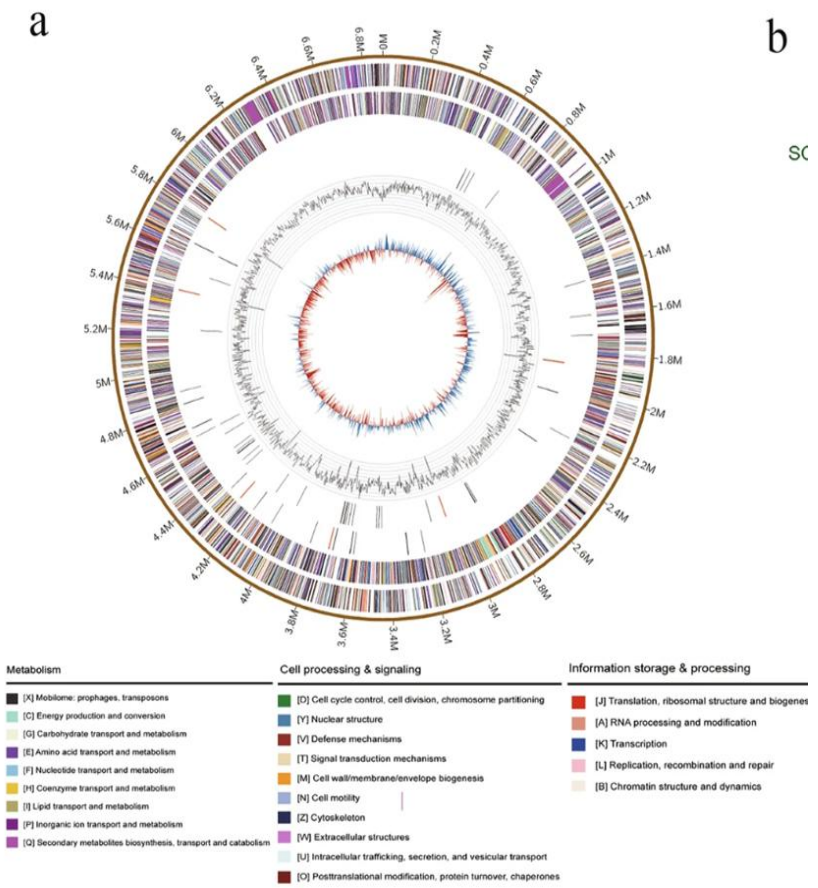
Strains	SCSIO 75703^T (CP144555)		
	dDDH (%)	ANI (%)	AAI (%)
S. flaveolus JCM 4032^T (BMRV00000000)	29.5	85.9	81.8
S. ambofaciens ATCC 23877^T (CP012949)	28.9	85.6	81.8
S. lienomycini LMG 20091^T (CP116257)	28.7	84.4	81.7



Genomic analysis

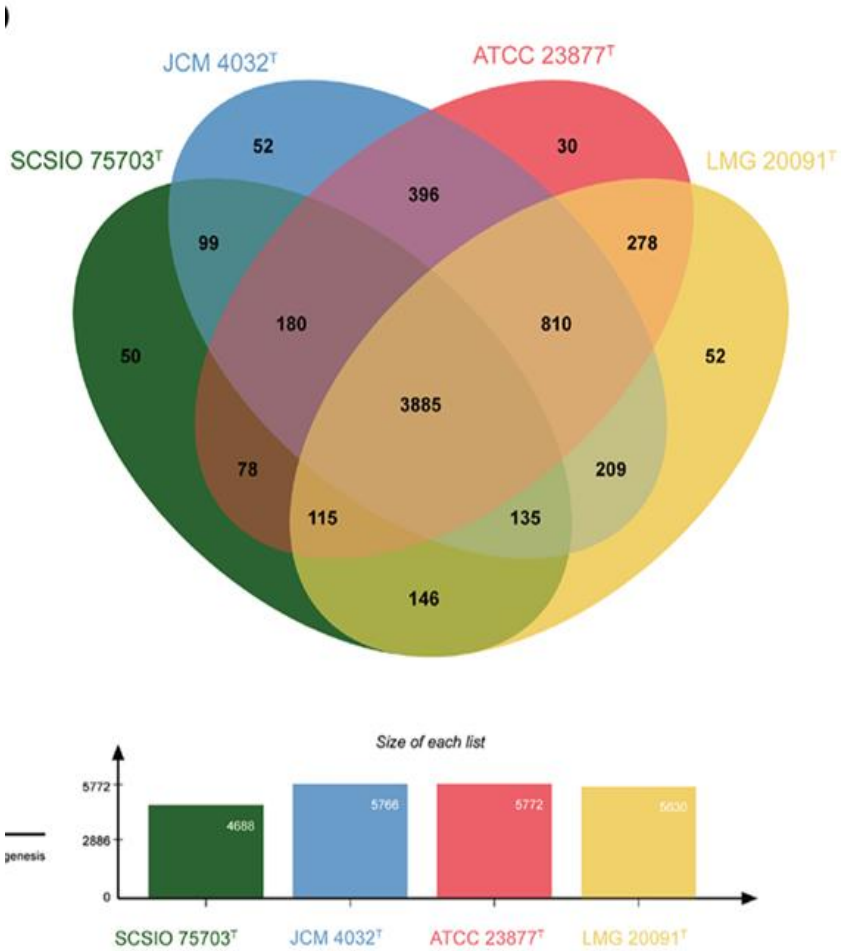
- 6,885,934 bp
- 73.5% G + C

5,937 genes were predicted from the genome of SCSIO 75703 T, which contained 72 tRNA, 6 5S rRNA, 6 16S rRNA (Similarity, 99.9–100%) and 6 23S rRNA genes.



genome completeness of strain SCSIO 75703 T is 99.89% (> 95%) with 0.94% contamination (< 5%)

3,885 gene clusters, with the three largest classes associated with hydrolase activity (n = 149), molecular function (n = 109) and oxidoreductase activity (n = 105)



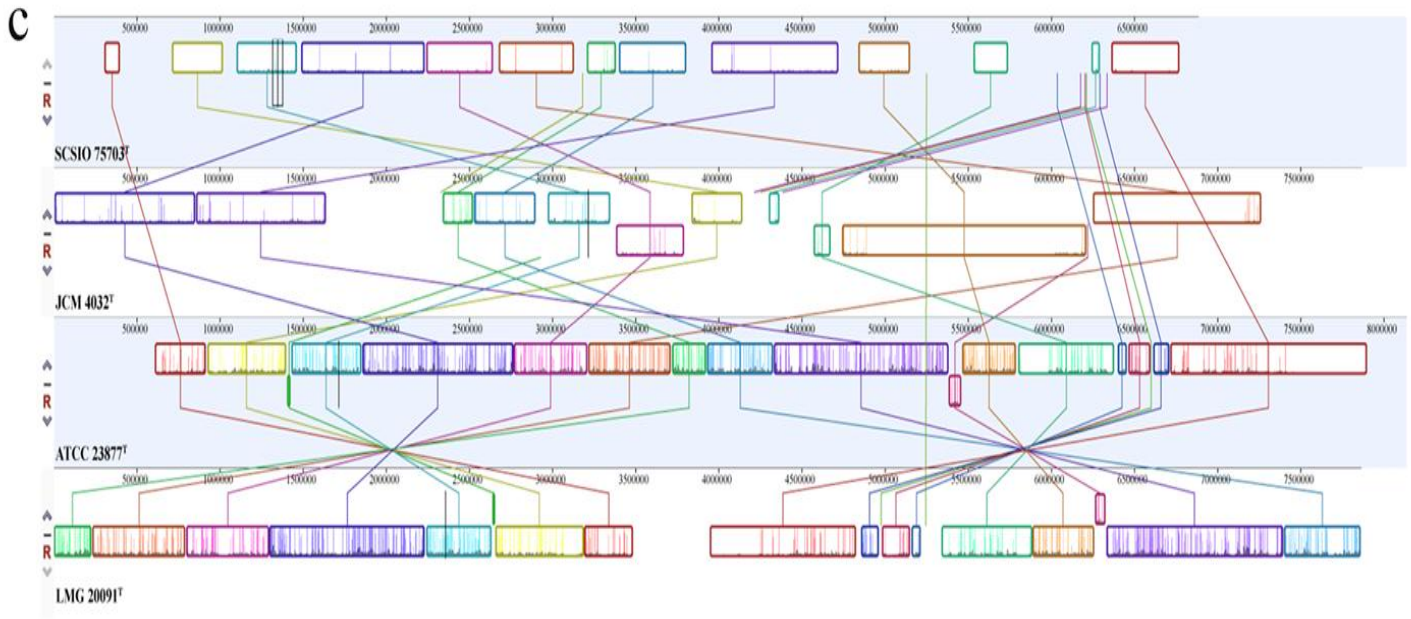


Fig. 2 Comparative genomics analysis. **a** Genome map of strain SCSIO 75703^T. **b** Venn diagram represents the core orthologs and unique genes for strain SCSIO 75703^T and the most closely related strains. **c** Genomic collinearity analysis between strains. Each contiguously colored locally collinear block (LCB) represents a region without rearrangement of the homologous backbone sequence

- A total of 25 secondary metabolite BGC
- 13 clusters : antibiotic Streptothritin, Collinomycin,
- Anthracomycin, Tirandamycin, Lomofungin, and others.
- terpenes, peptides, polyketides

Phenotypic characteristics

Category	Characteristics
Morphology	Gram-stain-positive, aerobic actinomycete with spiral spore chains; elliptic spores (0.7–1.2 × 0.5–0.7 μm).
Growth Conditions	- **Temperature:** 10–37°C (optimum: 28–35°C) - **pH:** 6.0–10.0 (optimum: 7.0–8.0) - **NaCl Tolerance:** 0–10% (optimum: 1.0–3.0%).
Growth Media	**Good growth:** ISP2, ISP3, ISP4, ISP7, 2216E, NA, TSA **Weak growth:** ISP5, R2A.
Biochemical Tests	- **Catalase:** Positive - **Oxidase:** Negative - **Hydrolysis:** Positive for Tween 80, gelatin, starch - Negative for Tween 20, 40, 60, cellulose, milk coagulation/peptonization.
Distinguishing Features	Differs from related strains in optimal growth conditions, Tween hydrolysis, carbohydrate utilization, and enzymatic activities (see Table S4).

Chemotaxonomic characterization

Category	Details
Major Fatty Acids	- iso-C16:0 (predominant) - iso-C15:0 - anteiso-C15:0
Menaquinones	- MK-10(H10): 74.7% - MK-10(H8): 25.3%
Cell Wall Component	- LL-2,6-Diaminopimelic acid
Whole-Cell Sugars	- Galactose - Glucose - Ribose
Polar Lipids	- Diphosphatidylglycerol (DPG) - Phosphatidylethanolamine (PE) - Phosphatidylmethylethanolamine (PME) - 1 unidentified amino phospholipid (APL) - 3 unidentified phospholipids (PL) - 1 unidentified lipid (L)

Antibacterial activity-oriented separation

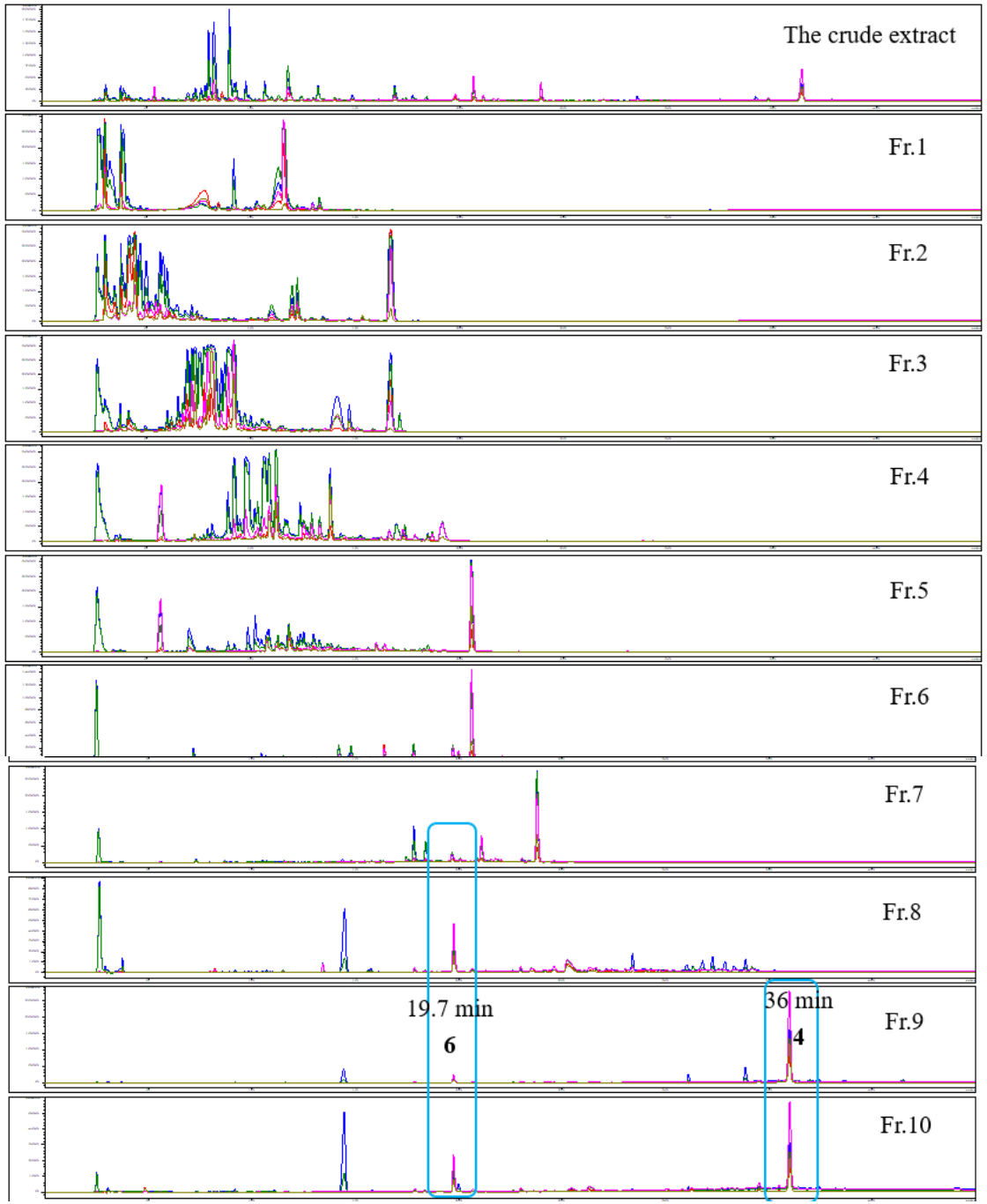
- (Fr.1–Fr.6) did not display antibacterial effect, but the next four fractions (Fr.7 -Fr.10) exhibited obvious inhibitory activity against the
- inhibition zones of 0.8 ~ 1.5 cm / 4 mg/mL

Table 2 Antibacterial activities of eleven extracts

Sample	*B. thuringiensis* ATCC 10792	*S. aureus* ATCC 29213	*B. subtilis* BS0l	*E. coli* ATCC 25922
2216E	+ + +	+ + +	+ +	-
MC3	+ + +	+ + + +	+	-
MRA	+ +	+ + +	+ +	-
MA	+ +	+ + +	+ +	-
M2216E	+ +	+ + +	+ +	-
MISP2	+ +	+ + +	+ +	-
MM18	+ +	+ + +	+	-
AM3	+ +	+ + +	+ +	-
MJNP1A	+ +	+ +	+ +	-
MK	+ +	+ + +	+	-
MISP4	+ +	+ +	+ +	-
Gentamicin [a]	+ +	+ + +	+ + + +	-

[a] Gentamicin is used as positive control at the concentration of 2 mg/mL (5 µL), and methanol is used as negative control

s β-rubromycin
and anthracimycin



Structural elucidation

Compound	Identification	Biosynthetic Gene Cluster (BGC)	Key Notes
1–3	Monaprenylindole A (1), 3-cyanomethyl-6-prenylindole (2), 6-prenyltryptophol (3)	**Indole BGC (Cluster 24)**	Identified via spectral comparison with literature.
4	Anthracimycin (4)	**TransAT-PKS BGC (Cluster 21)**	First confirmed by **single-crystal X-ray diffraction** (Cu Kα radiation).
5	2-epi-anthracimycin (5)	**TransAT-PKS BGC (Cluster 21)**	Spectral match with prior reports.
6	β-Rubromycin (6)	**T2PKS BGC (Cluster 16)**	Cluster 16 has **96% similarity** to collinomycin (α-Rubromycin) BGC.
7	4-(dimethylamino)benzoic acid (7)	**Unknown BGC**	Not linked to characterized clusters.

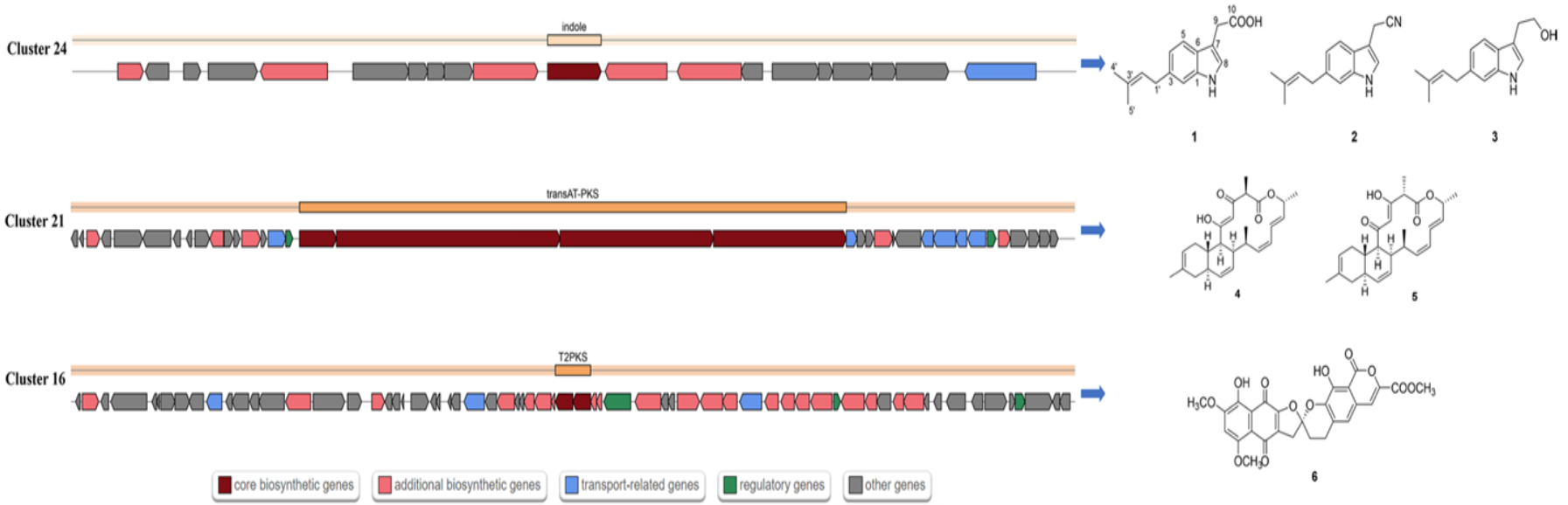


Fig. 5 Chemical structures and corresponding biosynthetic gene clusters in strain SCSIO 75703^{T}

Biological activities

Table 4 The antibacterial activities of compounds **4–6**

Bacteria	MICs (μg/mL)	Anthracimycin (4)	2-*epi*-anthracimycin (5)	*β*-rubromycin (6)
B. subtilis BS0l	2	4	1	1
S. aureus ATCC 29213	0.125	8	0.125	0.5
B. thuringiensis ATCC 10792	0.125	8	0.125	1
Eb. Profundum DH012	0.125	4	0.5	1
Ec. faecalis ATCC 29212	0.25	16	0.5	1
A. baumannii ATCC 19606	>32	>32	>32	1
V. alginolyticus XSBZ14	>32	>32	>32	1

[a] Ciprofloxacin is used as positive control

cytotoxicity

Table 5 Inhibitory activity of the compounds **1**–**3** and acarbose against α-glucosidase (n = 3)

Compounds	IC_{50} (μg/mL)[a]
1	83.27
2	86.21
3	45.4% inhibition at 100 μg/mL
Acarbose[b]	2.32

[a] IC_{50} is the concentration producing 50% inhibition of the enzyme activity

[b] Acarbose is used as a positive control

Conclusion

Category	Key Points
Novel Species Identification	- Strain **SCSIO 75703 T** represents a novel species: ***Streptomyces sediminicola* sp. nov.** (supported by 16S rRNA, ANI, AAI, dDDH, phylogeny, phenotype, and chemotaxonomy).
Discovery Strategy	- **OSMAC method** effectively explored metabolic profiles via HPLC. - **Bioassay-guided fractionation** isolated 7 compounds (**1–7**).
Bioactive Compounds	- **Anthracimycin (4)** and **β-rubromycin (6)**: Potent vs. Gram-positive bacteria (**MICs = 0.125–16 μg/mL**). - **Compounds 1 & 2**: Inhibit α-glucosidase (**IC50 = 83.27 & 86.21 μg/mL**).
Significance of Anthracimycin	- A **promising drug lead** with outstanding antimicrobial activity. - Strain is a **potential high-yield producer** of anthracimycin.
Other Metabolites	- **Prenylated indole alkaloids** (variable bioactivity based on side chains). - Minor production of **2-epi-anthracimycin** detected.
Future Work	- Further study needed to optimize anthracimycin production and explore analogues.

THANK YOU