

Exploring the Diversity and Antibacterial Potentiality of Cultivable Actinobacteria from the Soil of the Saxaul Forest in Southern Gobi Desert in Mongolia

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Table S1. Compositions of the ten different media used for the isolation of actinobacteria in the study.

NO.	Name	Composition (In 1.0 L distilled water)
M1	Modified Gauze's NO. 1 synthetic medium	Starch 2.0 g, KNO ₃ 0.5 g, KCl 1.7 g, MgSO ₄ •7H ₂ O 0.5 g, Na ₂ HPO ₄ 0.5 g, NaCl 0.5g, CaCO ₃ 0.02 g, FeSO ₄ •7H ₂ O 0.01 g, Vitamin mixture ^a 1.0 mL, Agar 20.0 g, pH 8.0
M2	ISP 2 medium	Yeast extract 4.0 g, Glucose 4.0 g, Malt extract 5.0 g, Vitamin mixture ^a 1.0 mL, Trace salt ^b 1.0 mL, Agar 20.0 g, pH 8.0
M3	R2A medium	R2A (BD) 18.6 g, Agar 12.0 g, pH 8.0
M4	Modified Cellulose-Casein medium	Cellulose 10.0 g, Casein 0.3 g, K ₂ HPO ₄ 0.2 g, FeSO ₄ •7H ₂ O 0.01 g, CaCO ₃ 0.02 g, KNO ₃ 2.0 g, MgSO ₄ •7H ₂ O 0.05 g, NaCl 10g, Agar 20.0 g, pH 8.0
M5	CMKA medium	Casein acids hydrolysate 0.5 g, Mannitol 1.5g, KNO ₃ 1.0 g, (NH ₄) ₂ SO ₄ 2.0 g, K ₂ HPO ₄ 0.5g, CaCO ₃ 0.5g, NaCl 10.0 g, KCl 5.0g, MgCl ₂ 1.0 g, Agar 20.0 g, pH 8.0
M6	Raffinose-Histidine medium	Raffinose 1.0 g, Histidine 0.1 g, Na ₂ HPO ₄ 0.5 g, KCl 1.7g, MgSO ₄ •7H ₂ O 0.05 g, FeSO ₄ •7H ₂ O 0.1 g, CaCO ₃ 0.02 g, Vitamin mixture ^a 1.0 mL, Agar 20.0 g, pH 8.0
M7	Trehalose-Proline medium	Trehalose 5.0 g, L-Proline 1.0 g, (NH ₄) ₂ SO ₄ 1.0 g, CaCl ₂ 2.0 g, NaCl 1.0 g, K ₂ HPO ₄ 1.0 g, MgSO ₄ •7H ₂ O 1.0 g, Vitamin mixture ^a 1.0 mL, Agar 20.0 g, pH 8.0
M8	Proline medium	L-Proline 5.0 g, Agar 20.0 g, Distilled water 1.0 L, pH 8.0
M9	Casein-Glucose medium	Casein 0.3 g, Glucose 10.0 g, KNO ₃ 2.0g, MgSO ₄ •7H ₂ O 0.05 g, K ₂ HPO ₄ 2.0 g, CaCl ₂ 1.0 g, FeSO ₄ •7H ₂ O 0.01 g, NaCl 50.0 g, KCl 20.0 g, MgCl ₂ •6H ₂ O 10.0 g, Agar 20.0 g, pH 8.0
M10	Casein-Glucose medium with 16% (w/v) multi-salts	Casein 0.3 g, Glucose 10.0 g, KNO ₃ 2.0g, MgSO ₄ •7H ₂ O 0.05 g, K ₂ HPO ₄ 2.0 g, CaCl ₂ 1.0 g, FeSO ₄ •7H ₂ O 0.01 g, NaCl 100.0 g, KCl 40.0 g, MgCl ₂ •6H ₂ O 20.0 g, Agar 20.0 g, pH 8.0

Note: ^a Vitamin mixture: thiamine 0.1 g, pyridoxine 0.1 g, riboflavin 0.1g, niacin 0.1 g, biotin 0.1 g, distilled water 100.0 mL.

^b Trace salt solution: FeSO₄•7H₂O 0.2 g, MnCl₂•4H₂O 0.01 g, ZnSO₄•7H₂O 0.01 g, distilled water 100.0 mL.

Table S2. Taxonomic statistics of the isolated 172 actinobacterial strains.

Taxon			No. of isolates
Order (7)	Family (13)	Genus (22)	
<i>Streptomycetales</i>	<i>Streptomycetaceae</i>	<i>Streptomyces</i>	73
<i>Micrococcales</i>	<i>Microbacteriaceae</i>	<i>Agromyces</i>	23
		<i>Labeledella</i>	6
		<i>Microbacterium</i>	2
		<i>Agrococcus</i>	1
		<i>Kocuria</i>	17
	<i>Micrococcaceae</i>	<i>Nesterenkonia</i>	13
		<i>Arthrobacter</i>	4
		<i>Citricoccus</i>	4
		<i>Isoptricola</i>	7
		<i>Promicromonospora</i>	2
		<i>Krasilnikovella</i>	1
	<i>Dermabacteraceae</i>	<i>Brachybacterium</i>	2
	<i>Bogoriellaceae</i>	<i>Georgenia</i>	1
	<i>Gordoniaceae</i>	<i>Gordonia</i>	4
		<i>Williamsia</i>	1
	<i>Nocardiaceae</i>	<i>Rhodococcus</i>	4
	<i>Mycobacteriaceae</i>	<i>Mycobacterium</i>	2
<i>Streptosporangiales</i>	<i>Nocardiopsaceae</i>	<i>Nocardiopsis</i>	2
<i>Propionibacteriales</i>	<i>Nocardioidaceae</i>	<i>Aeromicrobium</i>	1
<i>Micromonosporales</i>	<i>Micromonosporaceae</i>	<i>Micromonospora</i>	1
<i>Glycomycetales</i>	<i>Glycomycetaceae</i>	<i>Glycomyces</i>	1
Total number of actinobacterial isolates			172

Table S3. Antibacterial activity of 23 strains isolated from soil of the saxaul forest in southern Gobi Desert in Mongolia.

[illegible]

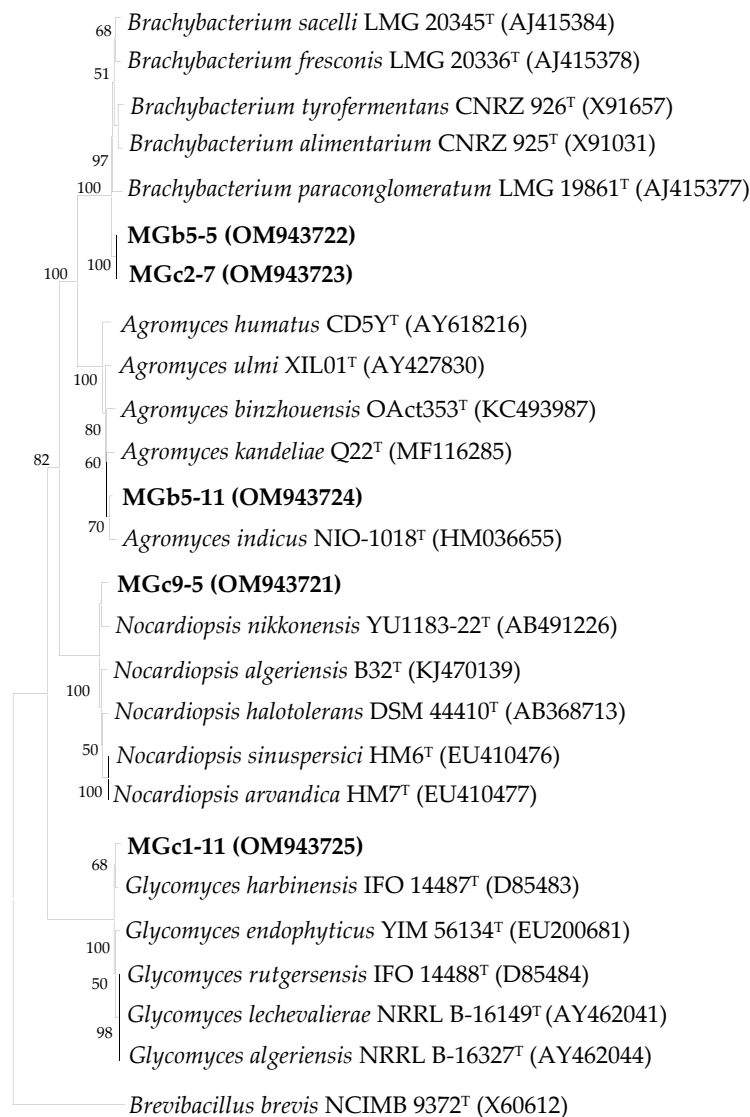
[illegible]

	(OM943737)	90018 ^T , 99.8%	W	—	—	—	—	—	—	—	—	—	0.8	0.7	—	—
			M	—	—	—	—	—	—	—	—	—	—	—	—	—
17	MGb9-13	<i>Streptomyces sparsus</i> YIM	E	—	—	—	—	—	—	—	—	—	0.7	—	—	2.8
	(OM943738)	90018 ^T , 99.8%	W	—	—	—	—	—	—	—	—	—	—	—	1.5	1.8
			M	—	—	—	—	—	—	—	—	—	—	1.0	—	1.0
18	MGc5-5	<i>Streptomyces tuirus</i> NBRC	E	—	—	—	—	—	—	—	—	—	0.9	0.8	1.1	1.5
	(OM943739)	15617 ^T , 99.5%	W	—	—	—	—	—	—	—	—	—	—	—	—	—
			M	—	—	—	—	—	—	—	—	—	—	—	—	—
19	MGa5-5	<i>Streptomyces lateritius</i> LMG	E	—	—	—	—	—	—	0.7	0.7	1.2	1.4	2.3	0.9	2.0
	(OM943740)	19372 ^T , 99.7%	W	—	—	—	—	—	—	—	—	0.7	1.2	1.3	—	—
			M	—	—	—	—	—	—	—	—	—	—	—	—	—
20	MGc2-5	<i>Streptomyces huasconensis</i>	E	—	—	—	—	—	—	—	—	—	1.8	—	—	—
	(OM943741)	HST28 ^T , 99.5%	W	—	—	—	—	—	—	—	—	—	1.2	1.5	—	—
			M	—	—	—	—	—	—	—	—	—	1.5	1.8	—	—
21	MGa1-3	<i>Streptomyces venezuelae</i> ATCC	E	1.8	1.6	1.7	—	2.0	1.5	1.2	1.3	2.0	0.9	1.3	2.1	
	(OM943745)	10712 ^T , 99.7%	W	—	—	—	—	—	—	—	—	—	—	—	—	—
			M	—	—	—	—	0.9	—	—	—	—	—	—	—	—
22	MGc5-6	<i>Streptomyces coeruleoprunus</i>	E	—	—	—	—	1.1	—	—	—	—	0.9	—	—	—
	(OM943742)	NBRC 15400 ^T , 99.6%	W	—	—	—	—	—	—	—	—	—	—	—	—	—
			M	—	—	—	—	—	—	—	—	—	—	—	—	—
23	MGa2-5	<i>Streptomyces althioticus</i> NRRL	E	—	—	—	—	2.0	—	—	—	—	0.8	1.3	0.7	1.4
	(OM943743)	B-3981 ^T , 98.8%	W	—	—	—	—	—	—	—	—	—	—	—	—	—
			M	—	—	—	—	—	—	—	—	—	—	—	—	—

^a E: crude sample extracted with ethyl acetate; M: crude sample from mycelium; W: crude sample from water layer.

^b The diameters of the inhibition zones: cm; —, no inhibition.

Figure S1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of five potential novel strains. Numbers at nodes indicate the level of bootstrap support (> 50%) based on 1000 replications. Bar, 2 nt substitutions per 100 nt.



0.02

Figure S2. UPLC-MS/MS spectra of the putative granaticin A (compound **1**) from strain MGa5-5 and the authentic standard of granaticin A. **(A)** TIC plots of the TLC band (R_f 0.71-0.79) from the ethyl acetate of cultural broth of strain MGa5-5 and authentic granaticin A. **(B)** MS/MS spectra of the putative granaticin A (**1**) and authentic granaticin A.

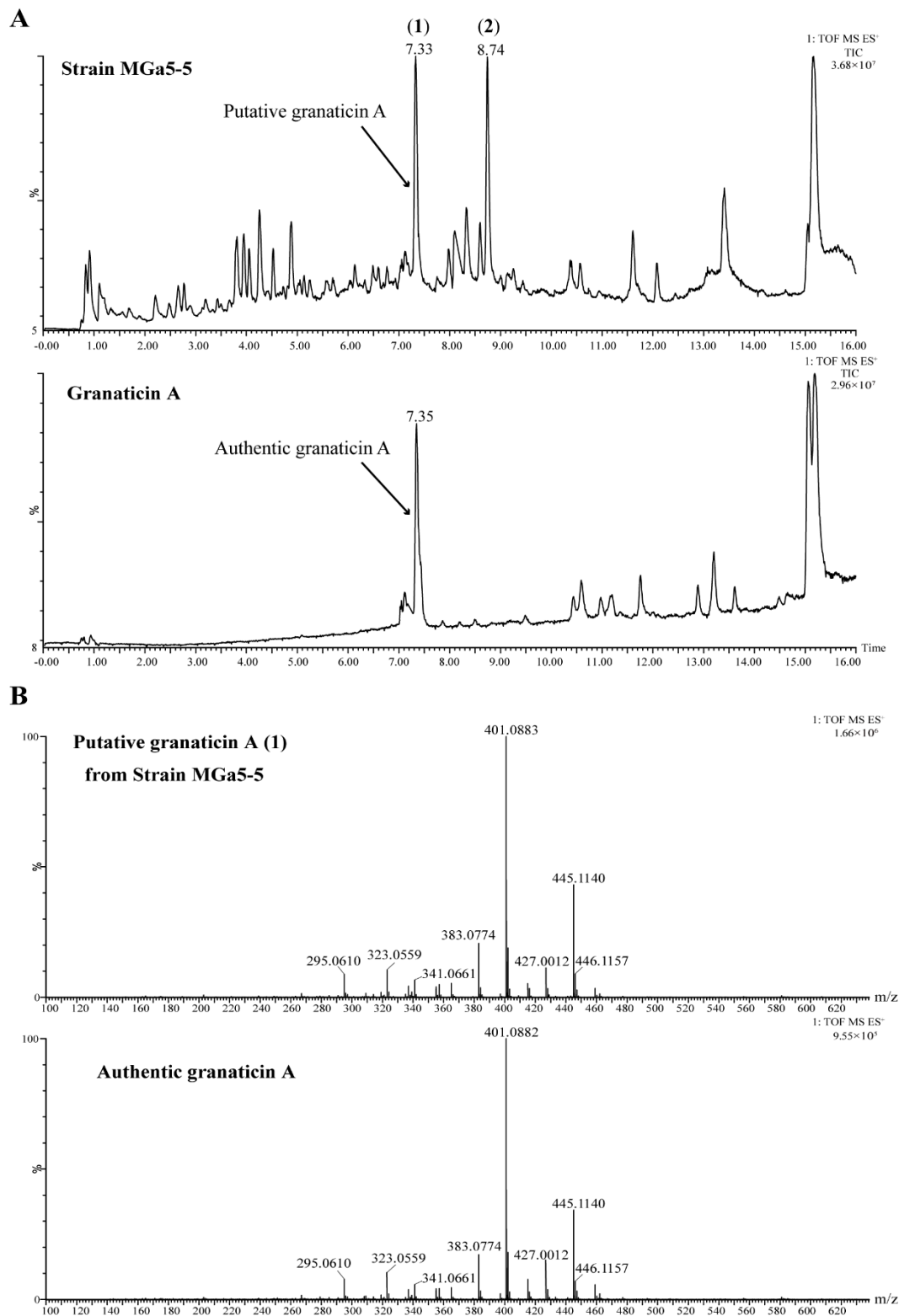


Figure S3. UPLC-UV-HRMS/MS spectra and identification of bioactive compounds from EA extract of strain MGa2-5. (A) UPLC-UV chromatogram at 254 nm of the bioactive TLC band (R_f 0.62) extracted from strain MGa2-5, and one major compound (peak 3) was labelled in the UPLC spectrum. (B) TIC plot of the bioactive TLC band (R_f 0.62) extracted from strain MGa2-5. (C) MS/MS spectrum and the annotated fragmentation pattern of peak 3 (althiomycin).

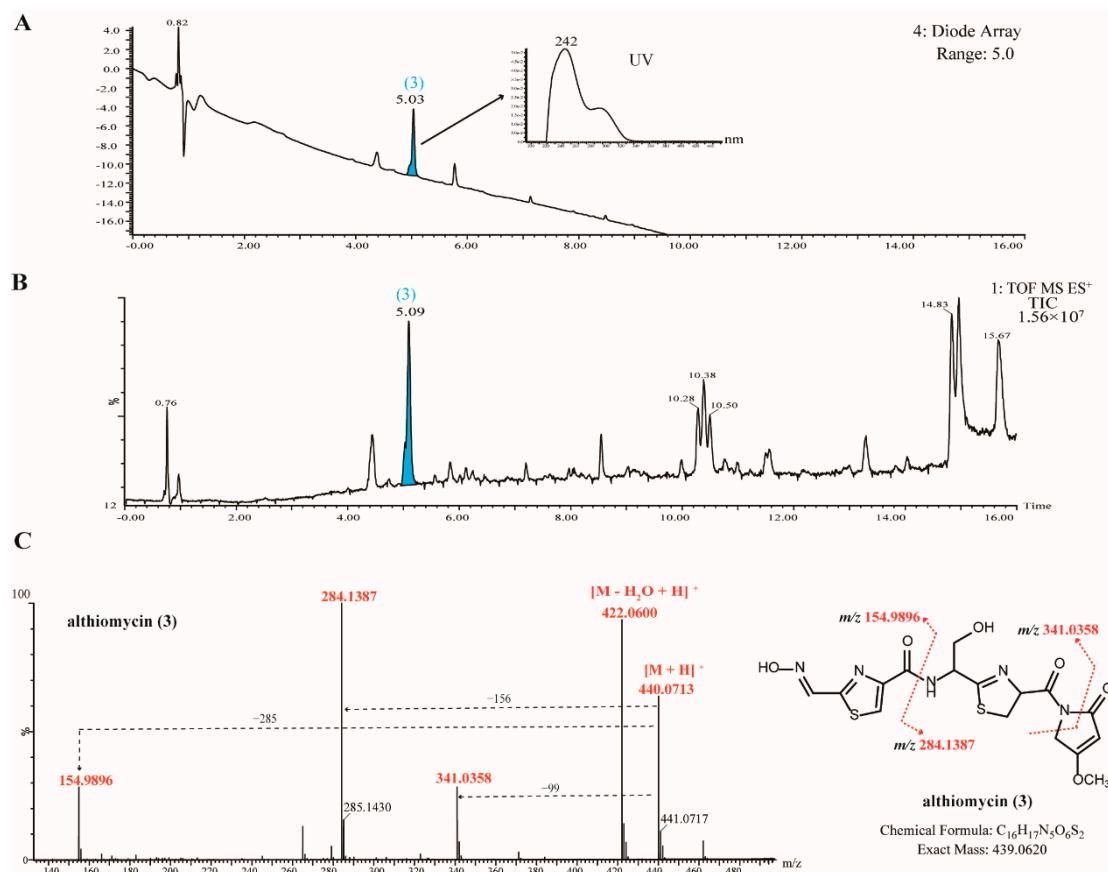


Figure S4. UPLC-UV-HRMS/MS spectra and identification of bioactive compounds from EA extract of strain MGa1-3. (A) UPLC-UV chromatogram at 280 nm of the bioactive TLC band (R_f 0.63) extracted from strain MGa1-3, and two major compounds (peak 4 and 5) were labelled in the UPLC spectrum. (B) TIC plot of the bioactive TLC band (R_f 0.63) extracted from strain MGa1-3. (C) MS/MS spectra and the annotated fragmentation patterns of peak 4 (corynecin III) and 5 (chloramphenicol).

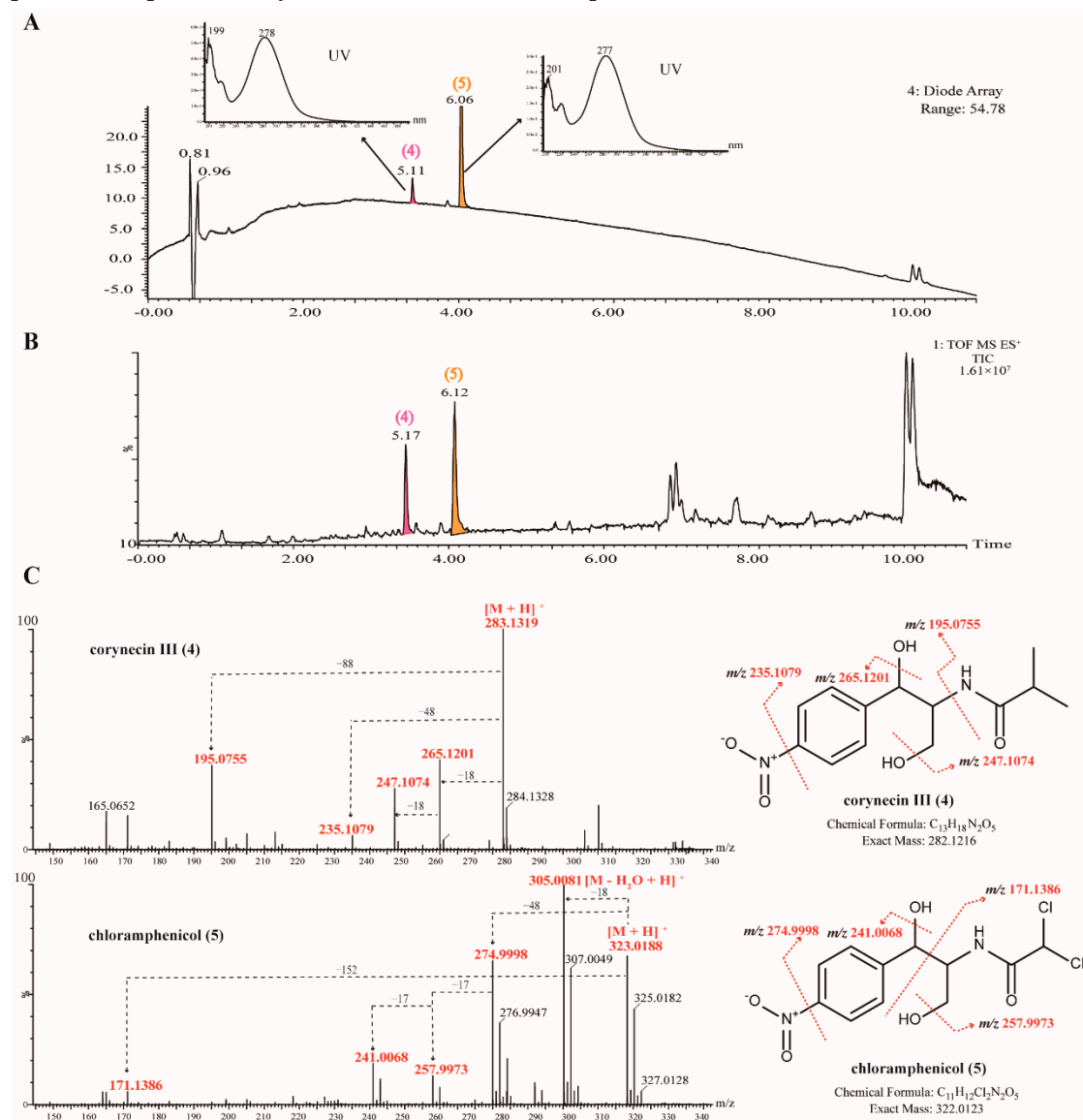


Figure S5. UPLC-MS/MS spectra of the putative chloramphenicol (compound 5) from strain MGa1-3 and the authentic standard of chloramphenicol. **(A)** TIC plots of the TLC band (R_f 0.63) from the ethyl acetate of cultural broth of strain MGa1-3 and authentic chloramphenicol. **(B)** MS/MS spectra of the putative chloramphenicol (5) and authentic chloramphenicol.

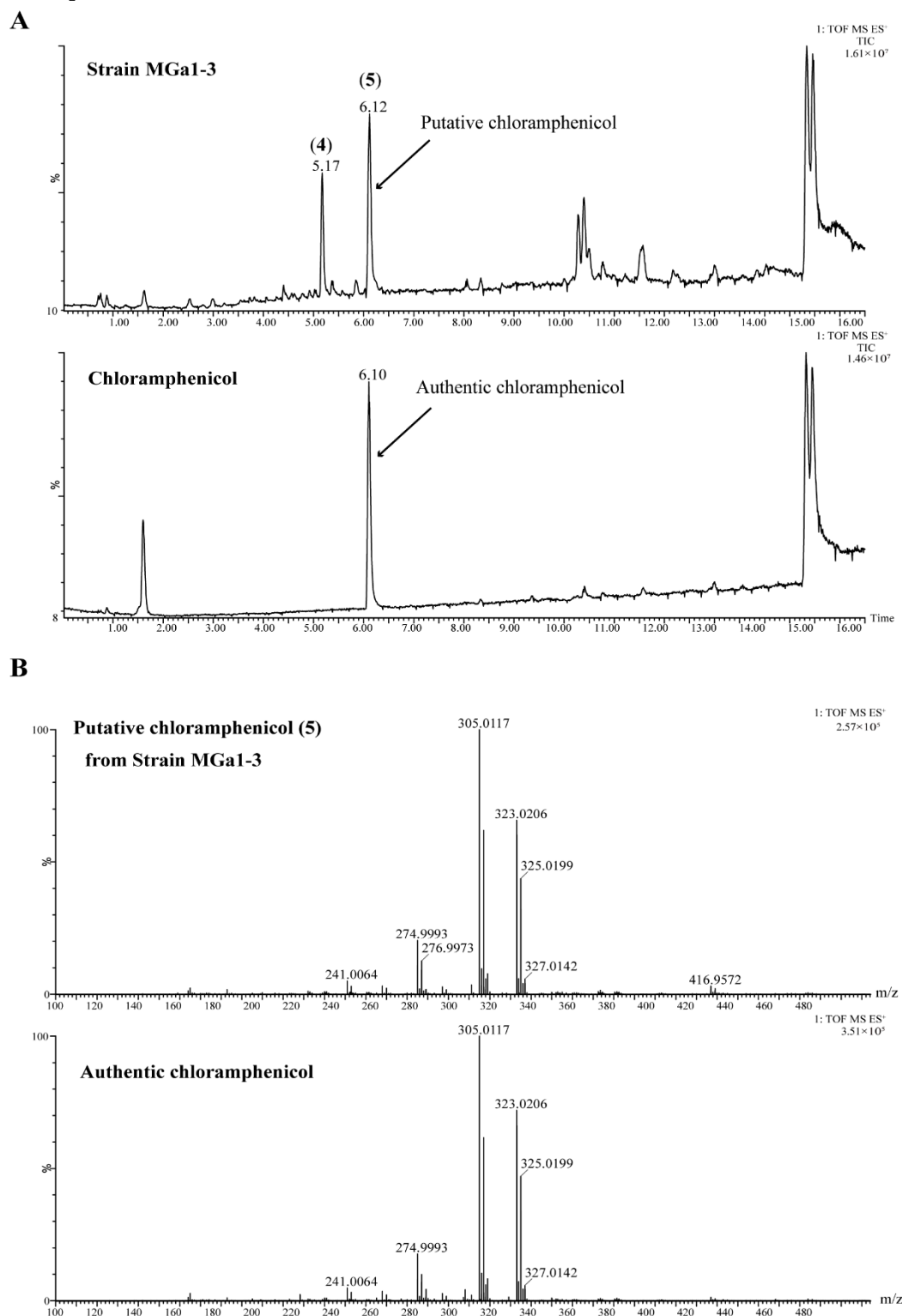


Figure S6. UPLC-MS/MS spectra acquired by MSE method and possible fragmentation patterns of putative granaticin compounds (6-10) from strain MGa5-5. (A) TIC plot of the ethyl acetate extract from the cultural broth of strain MGa5-5. (B) MS/MS spectra of five putative granaticin compounds 6-10 and possible fragmentation patterns of compounds 6-8.

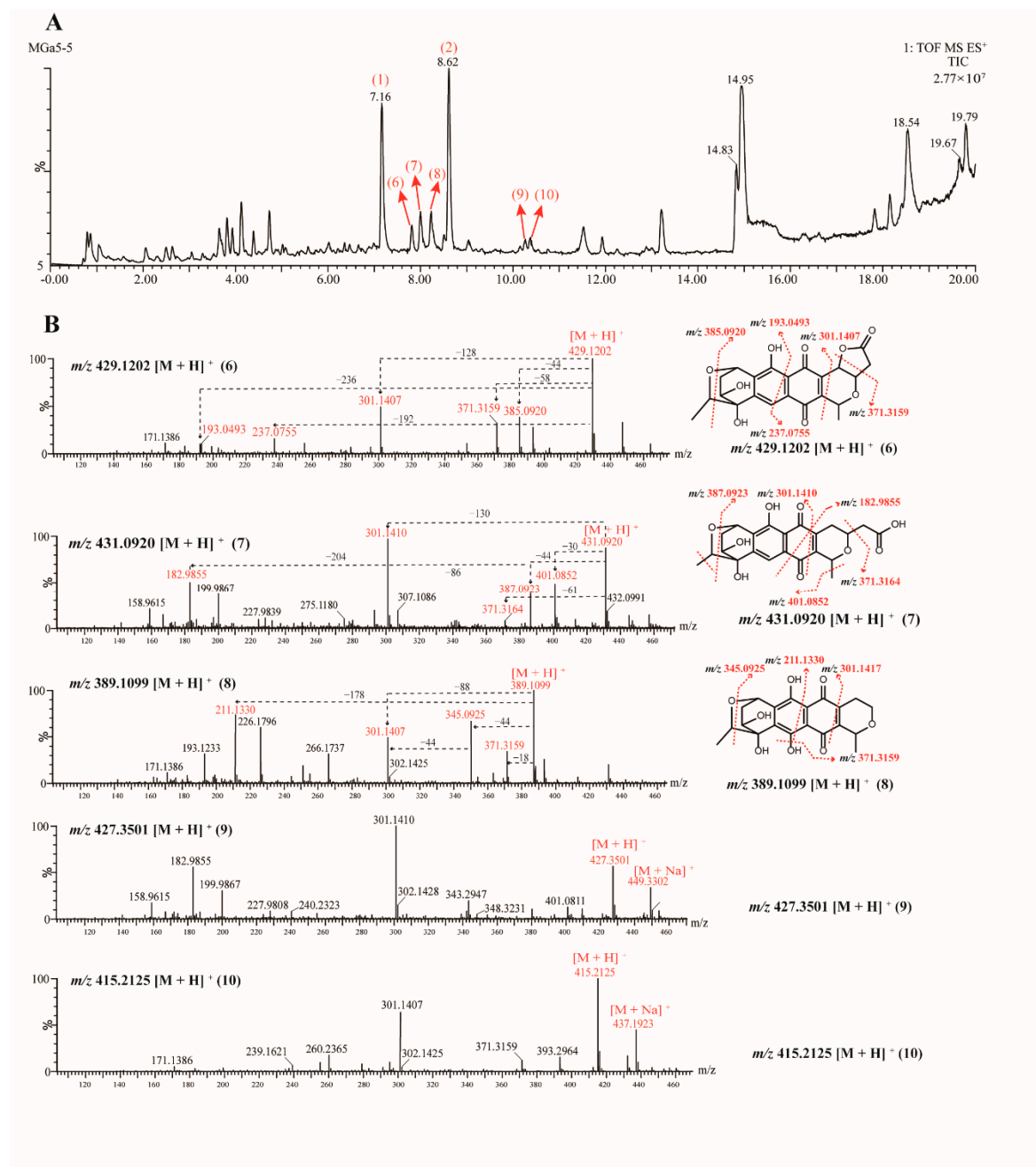


Figure S7. UPLC-MS/MS spectra acquired by MS^E method and possible fragmentation patterns of putative chloramphenicol derivatives (**11-13**) from strain MGa1-3. (A) TIC plot of the ethyl acetate extract from the cultural broth of strain MGa1-3. (B) MS/MS spectra and possible fragmentation patterns of three putative chloramphenicol derivatives **11-13**.

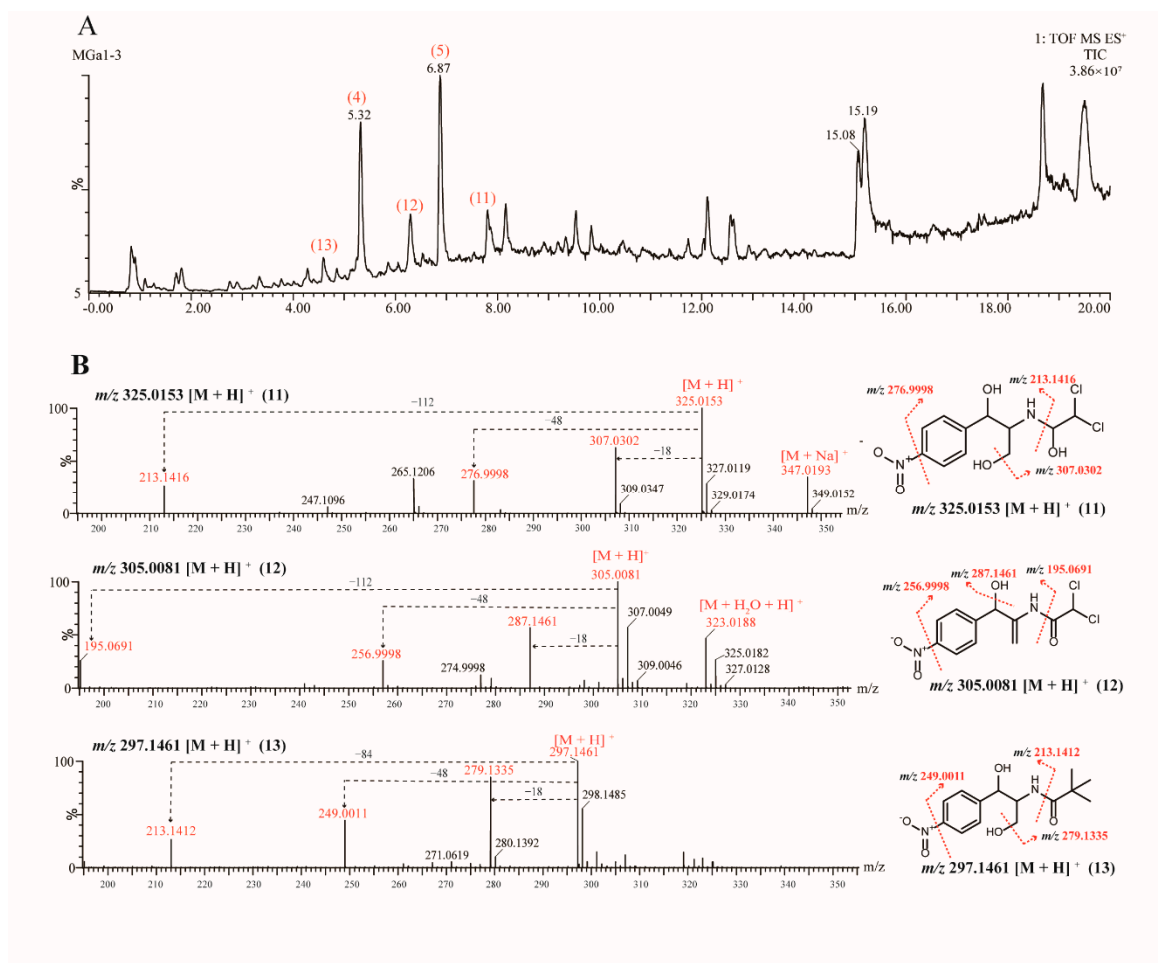


Figure S8. UPLC-MS/MS spectra acquired by MSE method and possible fragmentation patterns of putative althiomycin compounds (**14-16**) from strain MGa2-5. **(A)** TIC plot of the ethyl acetate extract from the cultural broth of strain MGa2-5. **(B)** MS/MS spectra of three putative althiomycin compounds **14-16** and possible fragmentation patterns of compounds **14-15**.

