microorganisms

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Kermanshah University of Medical Sciences

Article

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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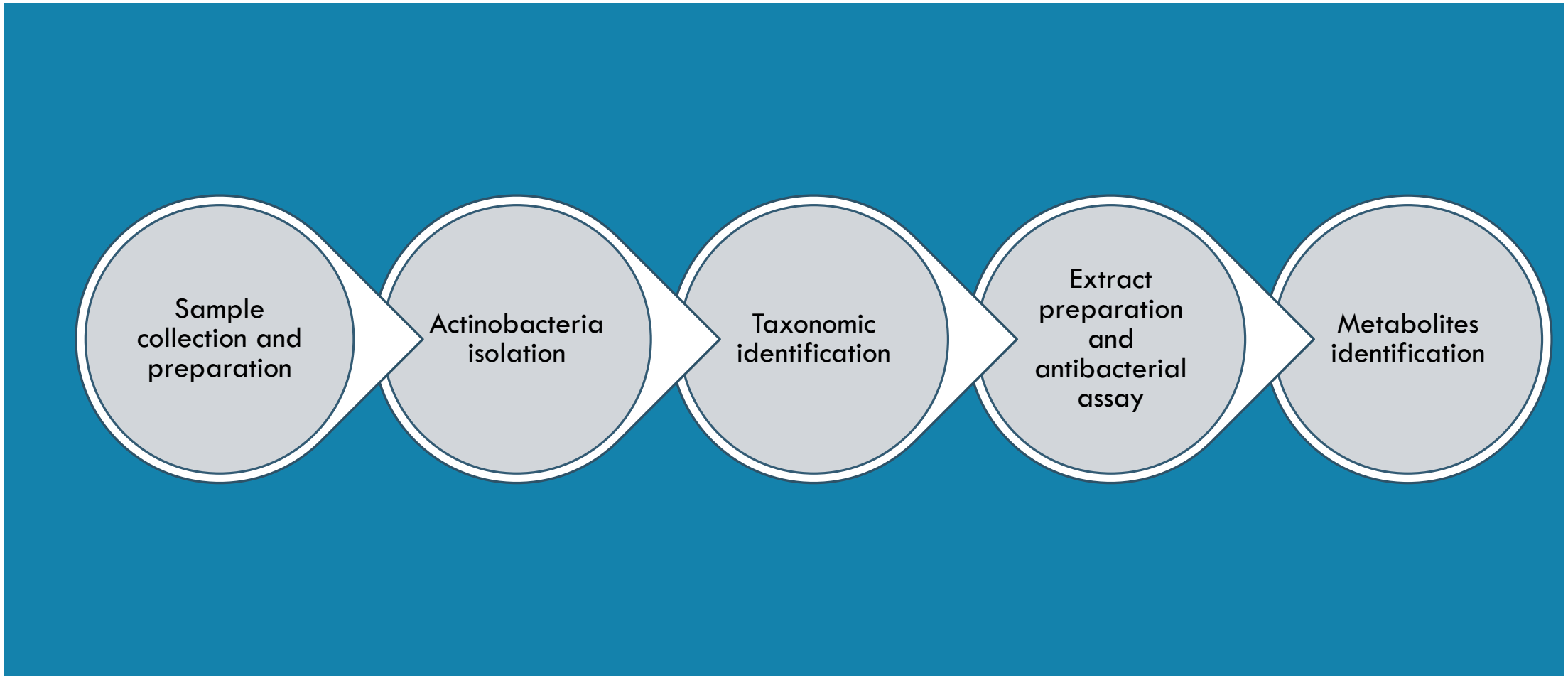
INTRODUCTION

- Emergence of Antimicrobial resistance
- Necessity for antibiotic discovery
- Actinobacteria
- Mongolia and Saxual forest

METHODS AND MATERIALS



SAMPLE COLLECTION

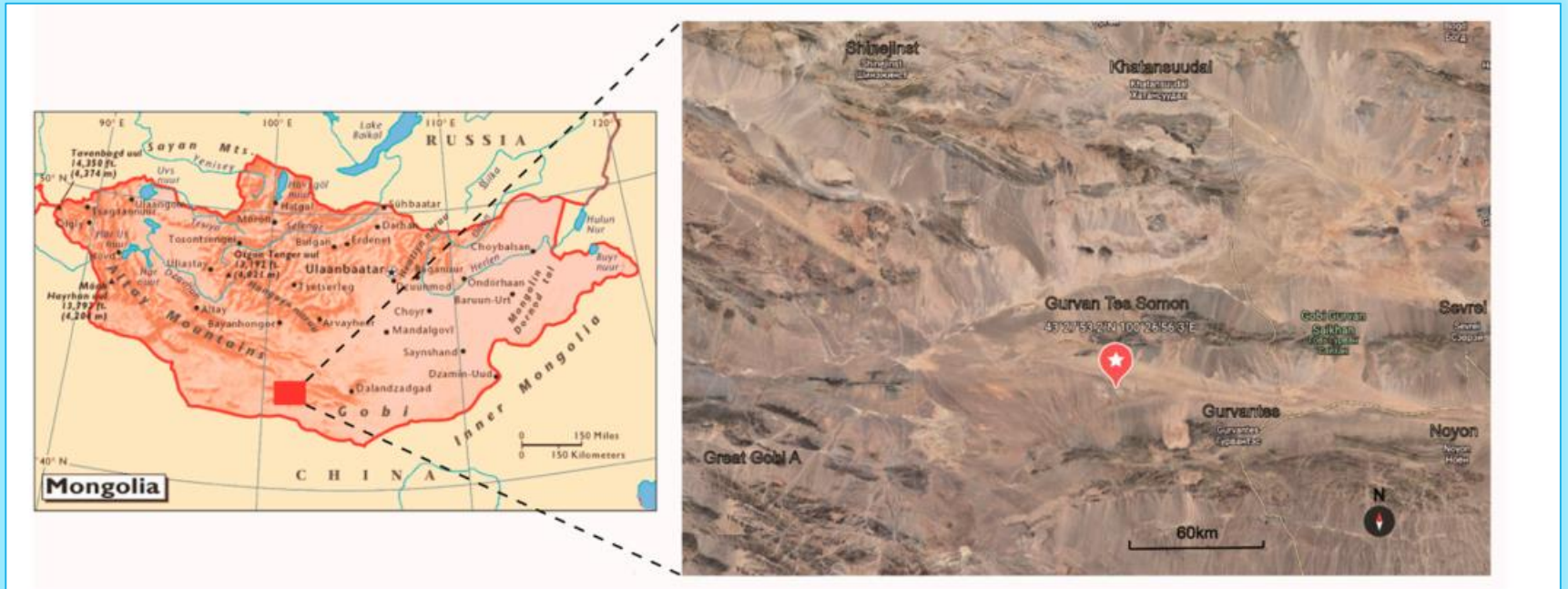
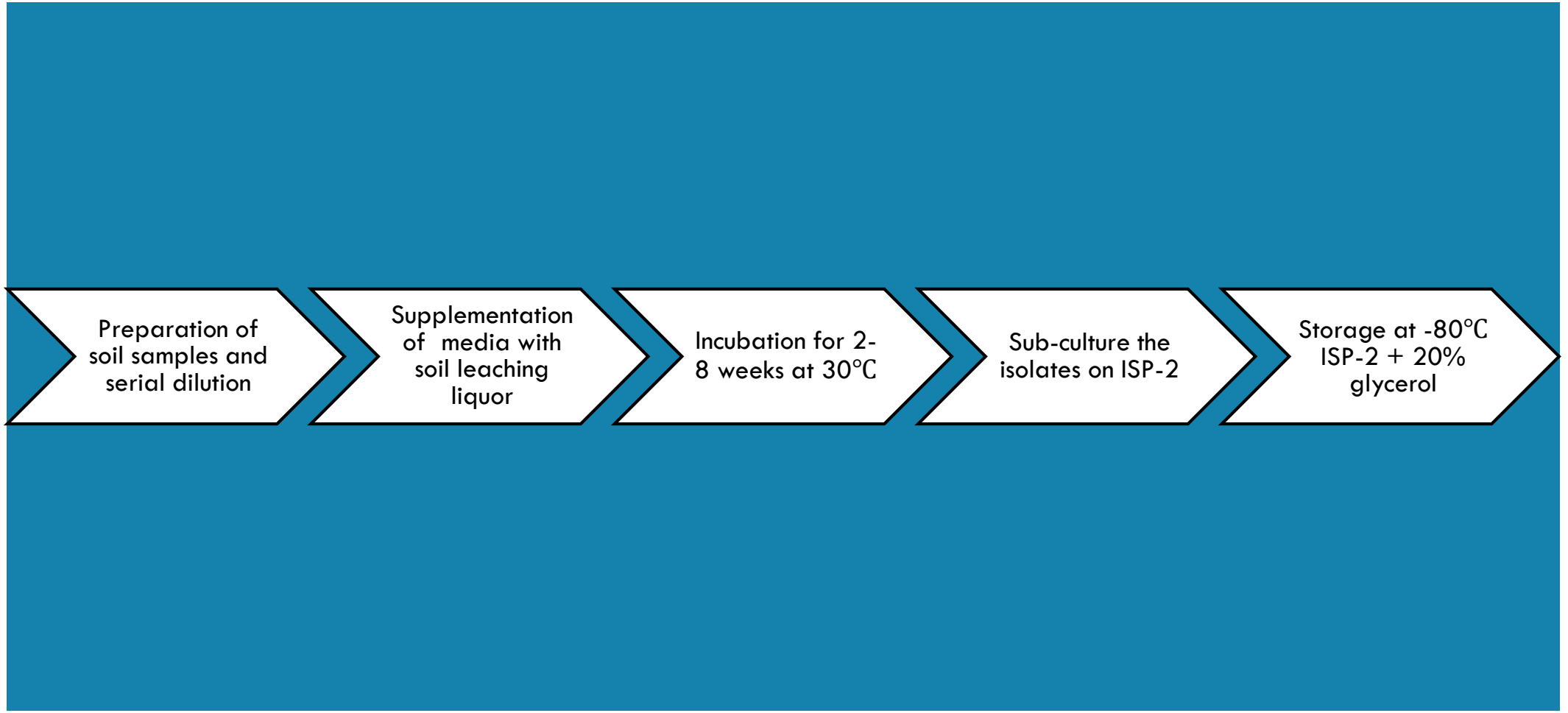


Figure 1. Sampling site. The map shows the sample collection site in Gurvan Tes Somon (Gurvan Tes County) in the Southern Gobi Aimak (Southern Gobi Province) in Mongolia.

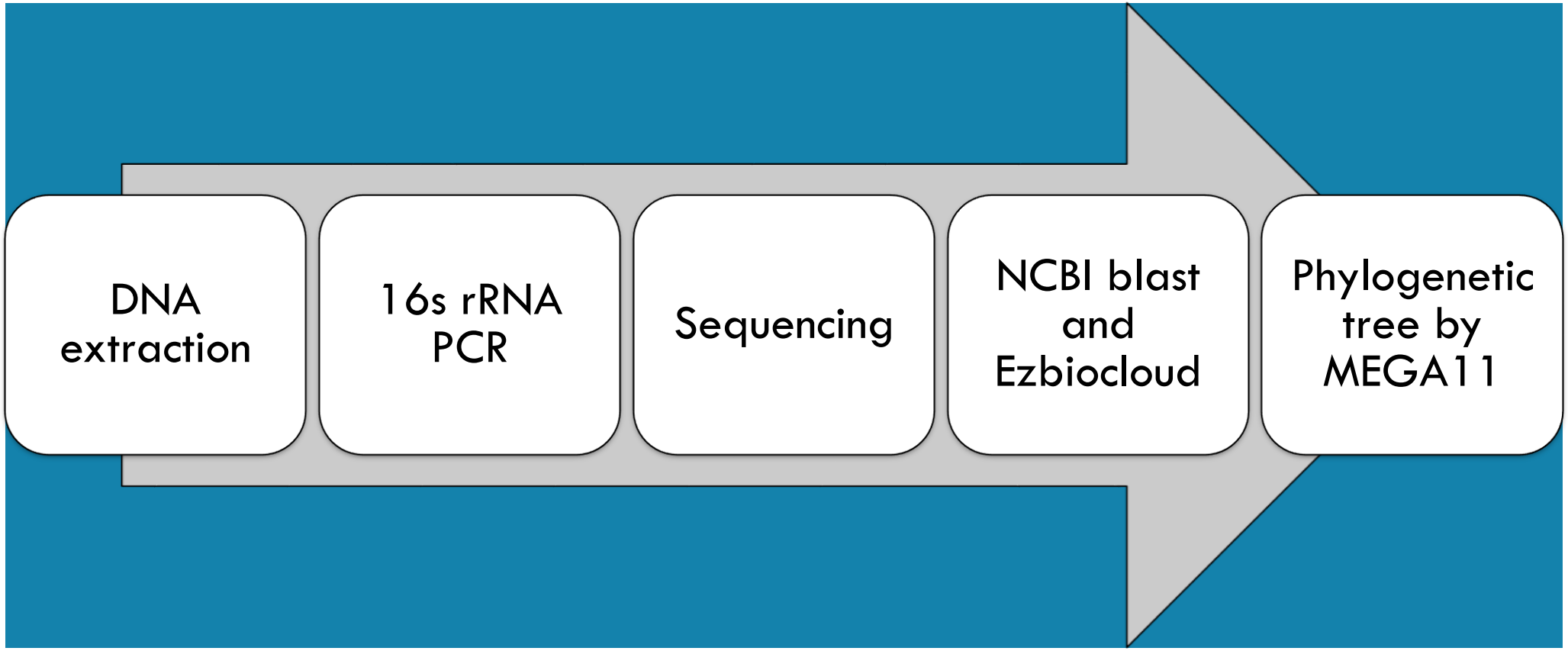
ACTINOBACTERIA ISOLATION



SELECTIVE MEDIA

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, $MgSO_4 \cdot 7H_2O$ 0.5 g, Na_2HPO_4 0.5 g, NaCl 0.5g, $CaCO_3$ 0.02 g, $FeSO_4 \cdot 7H_2O$ 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_2HPO_4 0.2 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, $CaCO_3$ 0.02 g, KNO_3 2.0 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, NaCl 10g, Agar 20.0 g, pH 8.0
M5	CMKA medium	Casein acids hydrolysate 0.5 g, Mannitol 1.5g, KNO3 1.0 g, $(NH_4)_2SO4$ 2.0 g, K_2HPO_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_2HPO_4 0.5 g, KCl 1.7g, $MgSO_4 \cdot 7H_2O$ 0.05 g, $FeSO_4 \cdot 7H_2O$ 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, $(NH4)_2SO_4$ 1.0 g, $CaCl_2$ 2.0 g, NaCl 1.0 g, K_2HPO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 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, $MgSO_4 \cdot 7H_2O$ 0.05 g, K_2HPO_4 2.0 g, $CaCl_2$ 1.0 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, NaCl 50.0 g, KCl 20.0 g, $MgCl_2 \cdot 6H_2O$ 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, $MgSO_4 \cdot 7H_2O$ 0.05 g, K_2HPO_4 2.0 g, $CaCl_2$ 1.0 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, NaCl 100.0 g, KCl 40.0 g, $MgCl_2 \cdot 6H_2O$ 20.0 g, Agar 20.0 g, pH 8.0

TAXONOMIC IDENTIFICATION



ANTIMICROBIAL ASSAY

Culture of 49 isolates out 172 on TSB or ISP-2 medium (30°C, 180 RPM, 3-14days)

Ethyl acetate extraction with separating funnel

Drying the extracts and resolving in methanol/water solution

Antimicrobial assay (paper disk diffusion) against ESKAPE group

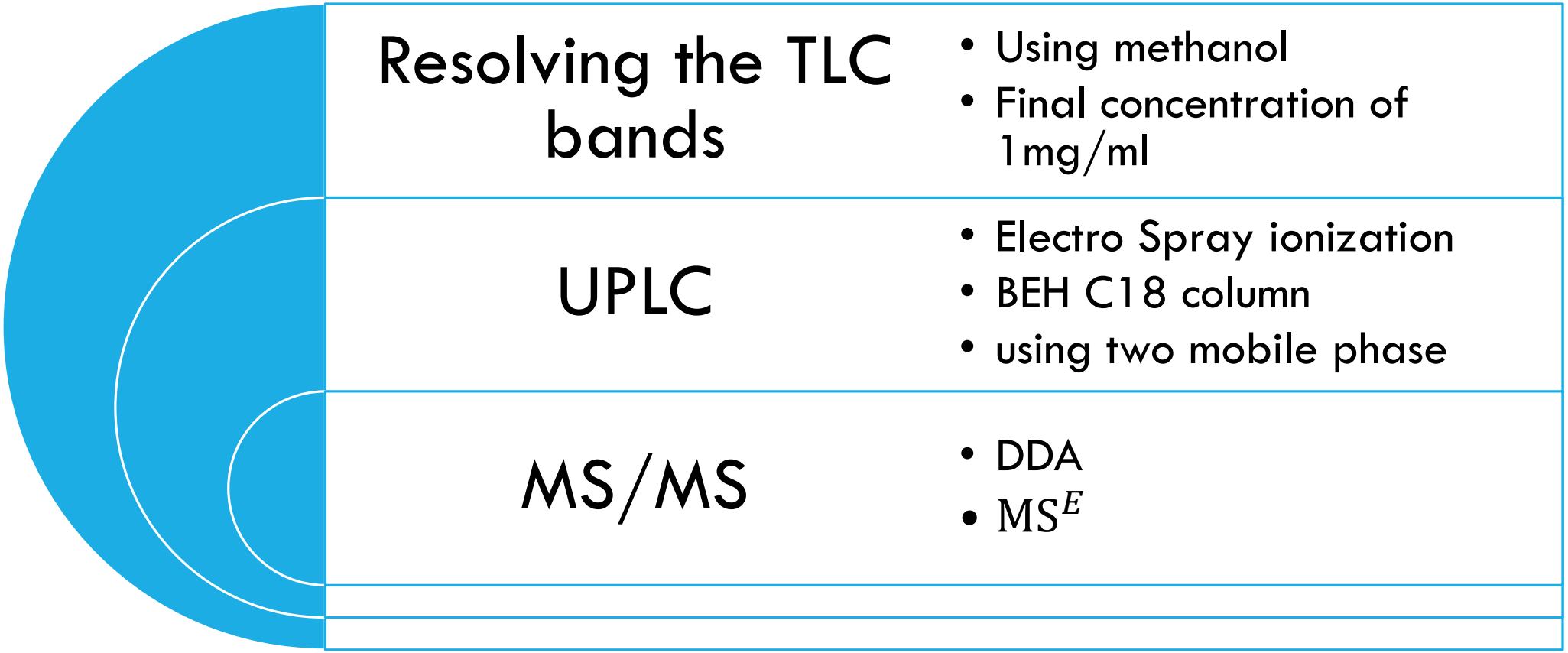
DETERMINATION OF ANTIMICROBIAL MECHANISM

- Dual-fluorescent receptor system “JW5503-pDualrep2”
- Reporter strain “E. coli JW5503 ΔtolC”
- ChemiDoc Imaging System with two channel
- First channel :“Cy3-blot” (553/574 nm, green pseudocolor) for red fluorescent protein (RFP) fluorescence
- Second channel : “Cy5-blot” (588/633 nm, red pseudocolor) for Katushka2S fluorescence
- Induction of expression of Katushka2S is triggered by translation inhibitors, while RFP is upregulated by DNA damage-induced SOS response
- Erythromycin and Levofloxacin were used as positive controls for inhibitors of protein and DNA biosynthesis, respectively

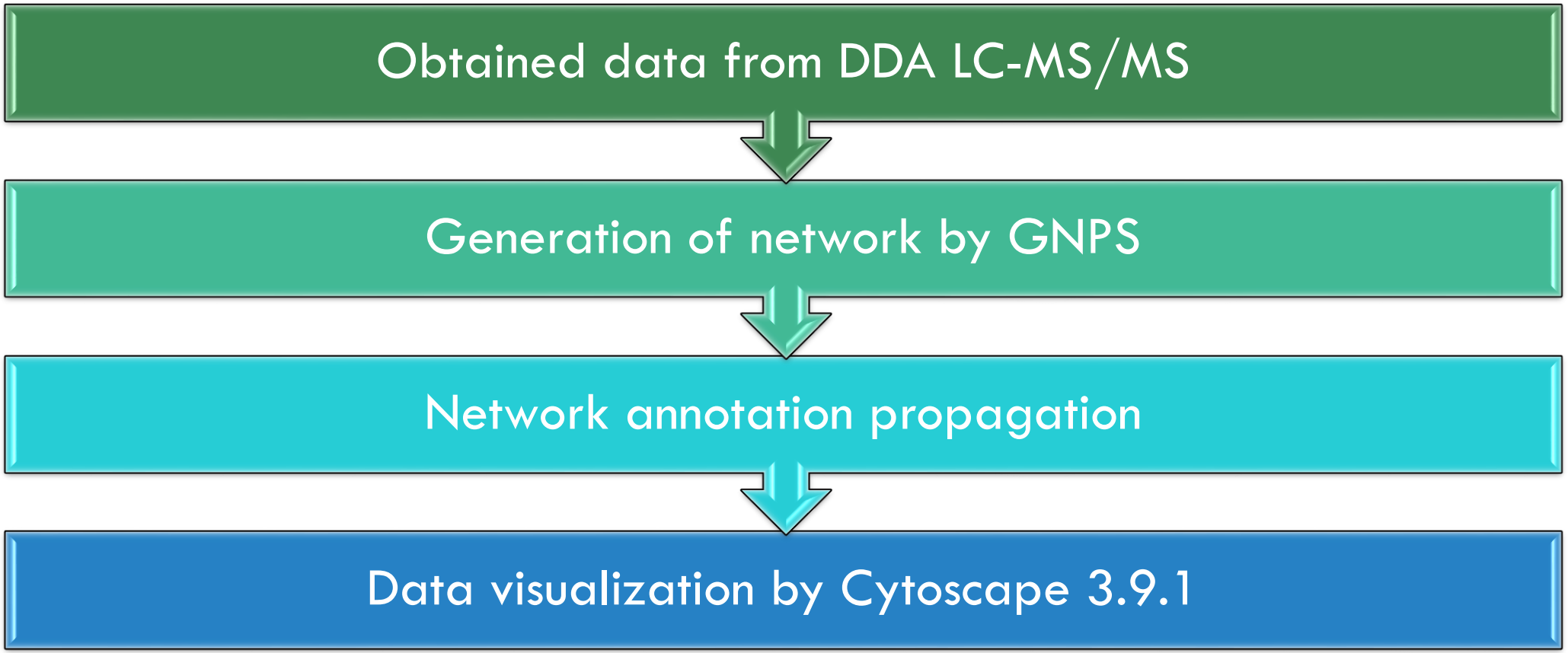
THIN LAYER CHROMATOGRAPHY (TLC)

TLC	Ethyl acetate extract of cultural broth
	Silica gel 60 F_{254}
	Methanol/ dichloromethane(v/v, 1/9)

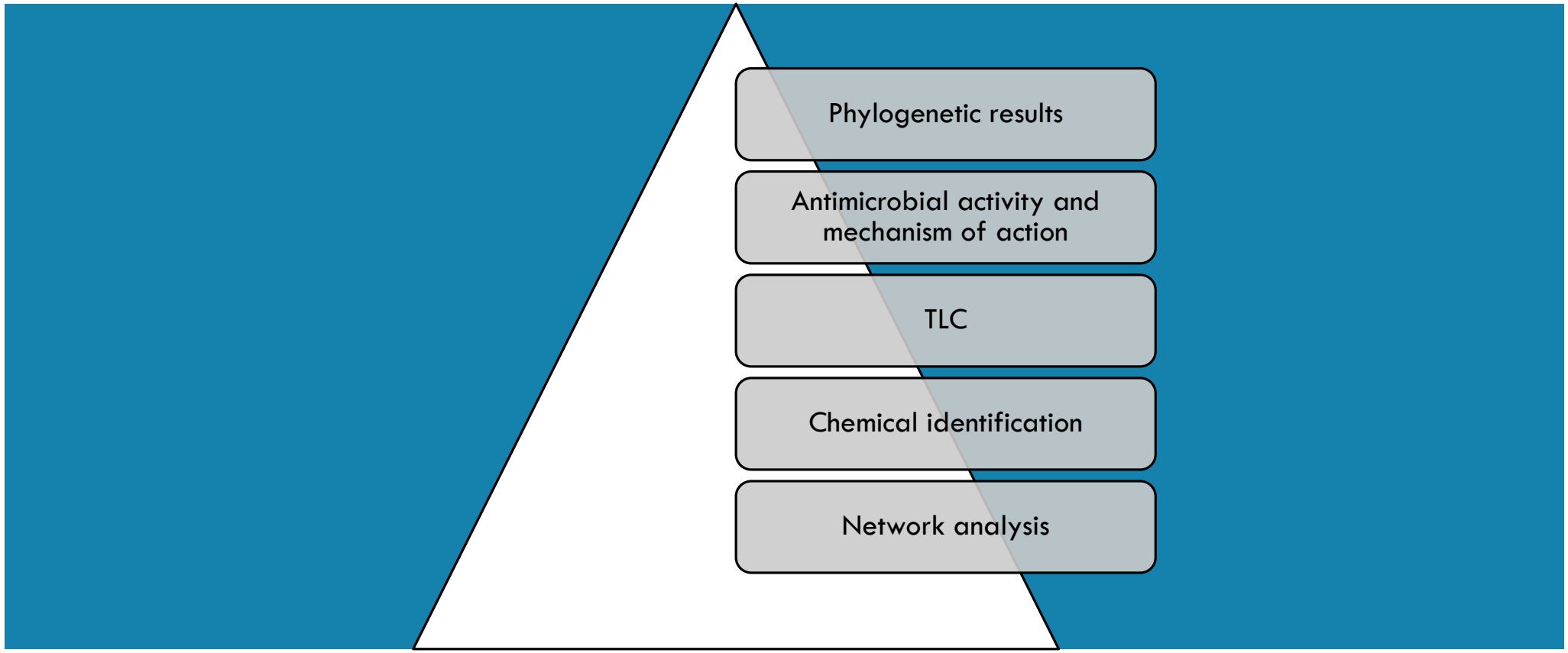
UPLC/QTOF-MS/MS ANALYSIS



MOLECULAR NETWORKING



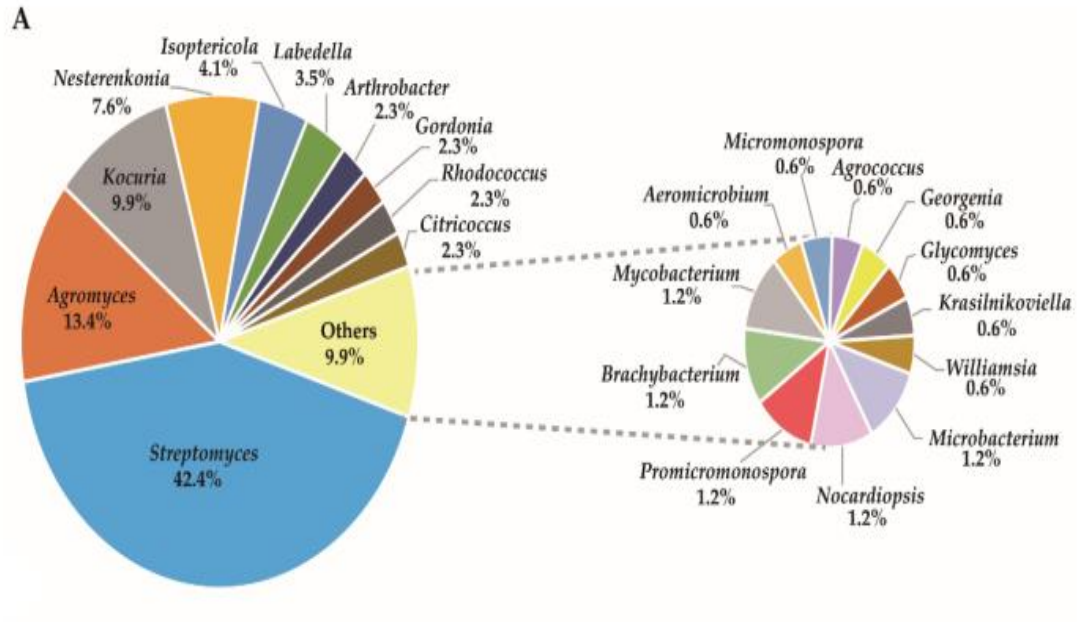
RESULTS

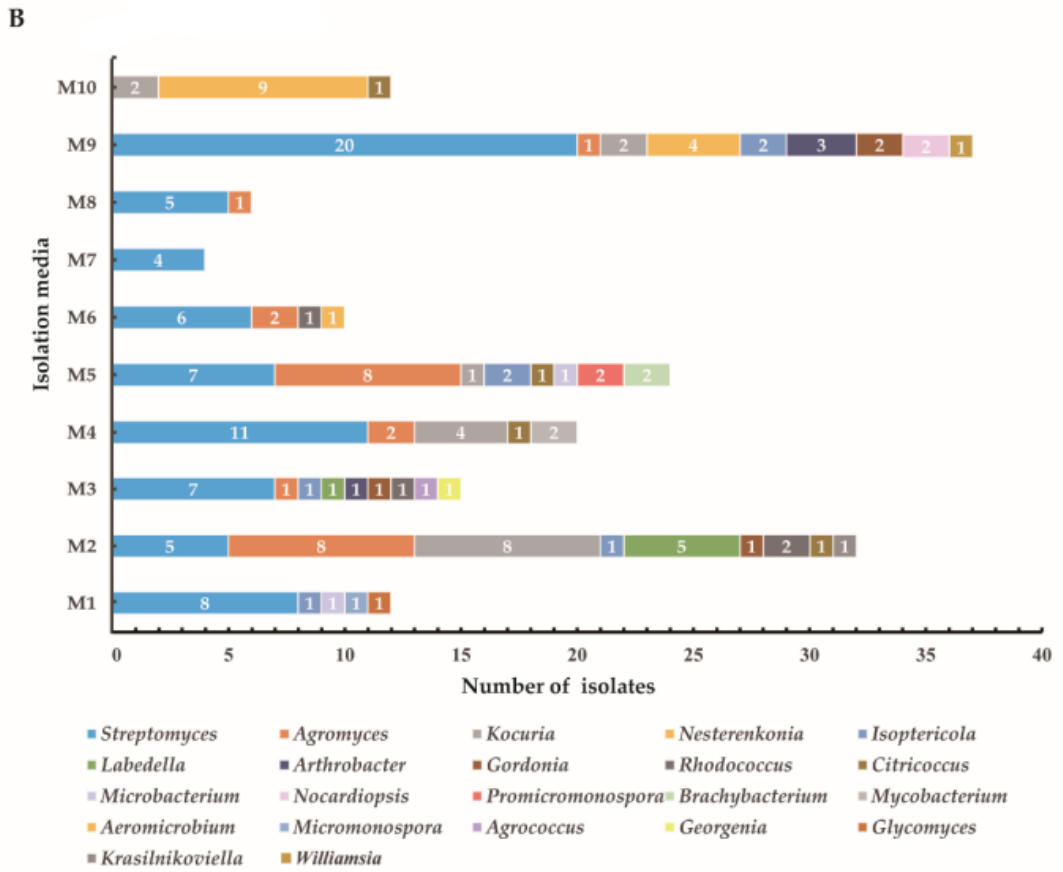

Phylogenetic results
Antimicrobial activity and mechanism of action
TLC
Chemical identification
Network analysis

STATISTICS OF ISOLATES

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

PHYLOGENETIC RESULTS





ANTIMICROBIAL ACTIVITY AND MECHANISM OF ACTION

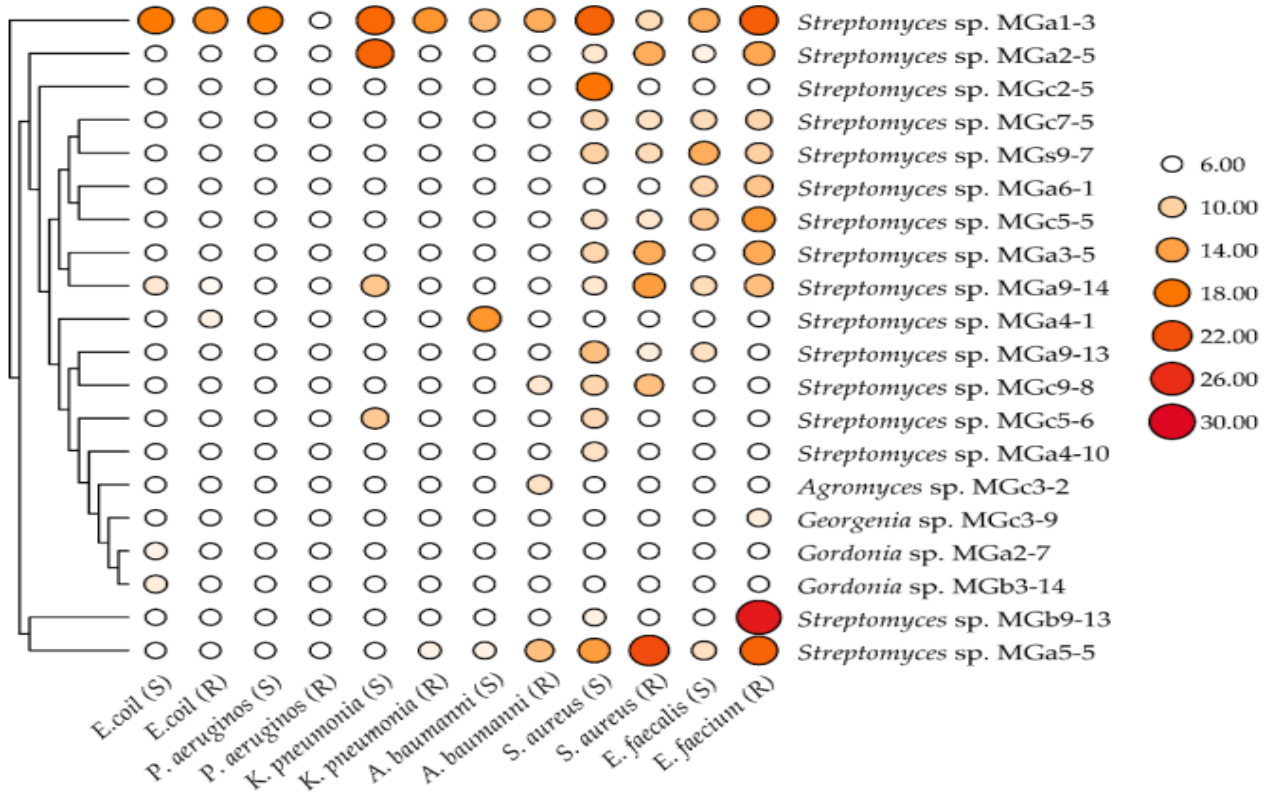


Figure 3. Antibacterial activity of bioactive ethyl acetate extracts from cultural broth of 20 strains. Numbers alongside the circles represent the diameter (mm) of the inhibition halos. 6.00 mm, no inhibitory activity; S, drug-sensitive; R, drug-resistant.

ANTIMICROBIAL ACTIVITY AND MECHANISM OF ACTION

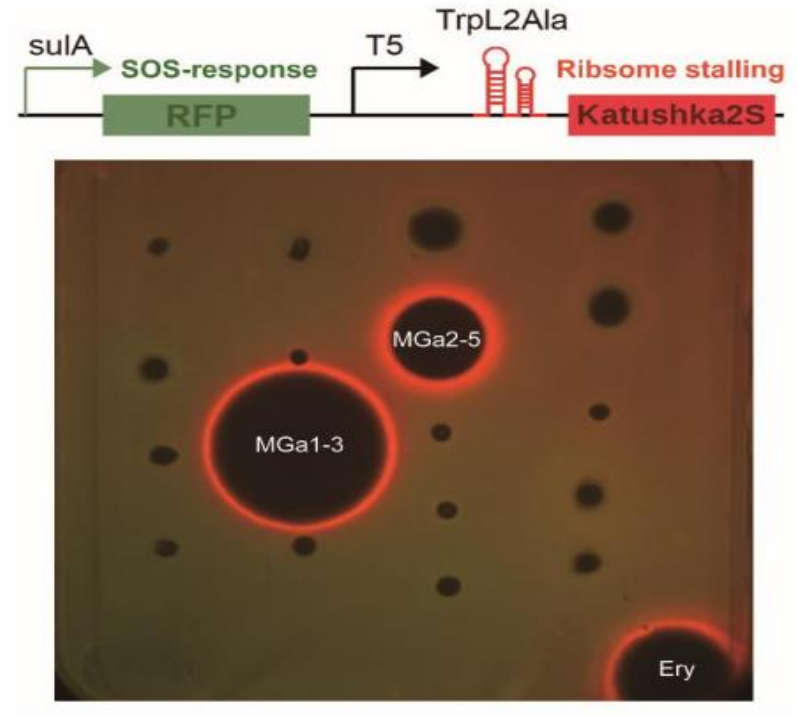


Figure 4. The dual-fluorescent reporter "pDualrep2" system sensitive to inhibitors of ribosome progression or DNA replication. Spots of erythromycin (Ery) and test samples were placed on an agar plate coated with a layer of *E. coli* JW5503 Δ*tolC* strain that was transformed with the pDualrep2 plasmid. Induction of the expression of Katushka2S is triggered by translation inhibitors, whereas RFP expression occurs on DNA-damage induced SOS-response. The plate was scanned under the ChemiDoc Imaging System at 553/574 nm and 588/633 nm channels to detect RFP (green pseudocolor) and Katushka2S (red pseudocolor) fluorescence, respectively.

THIN LAYER CHROMATOGRAPHY

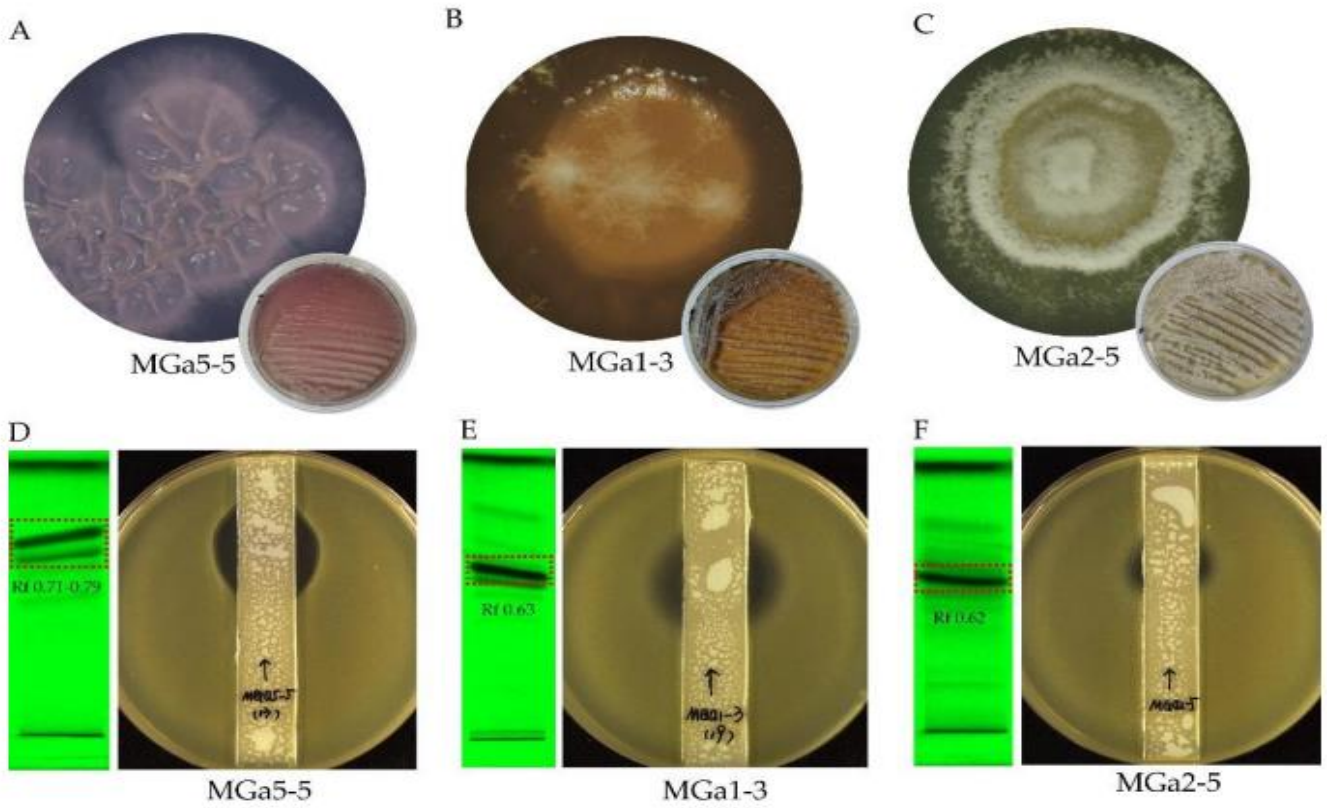
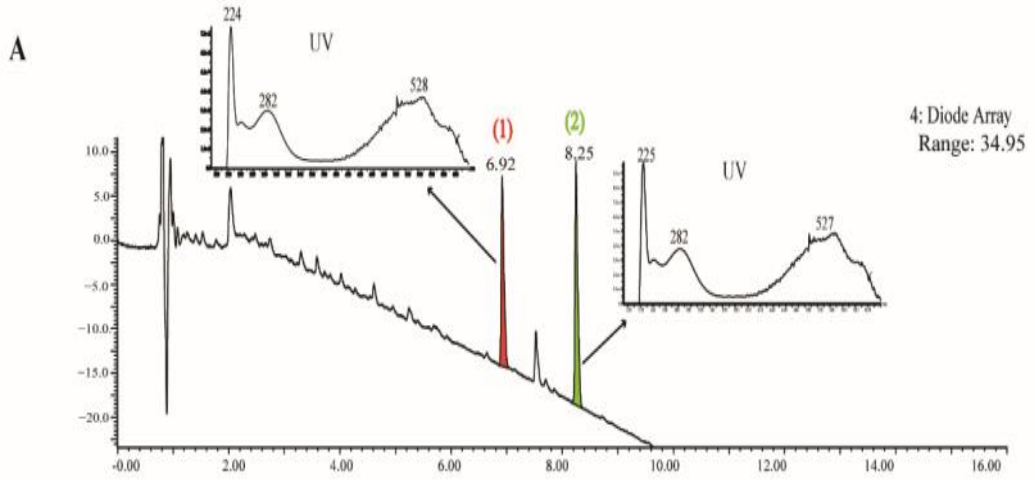
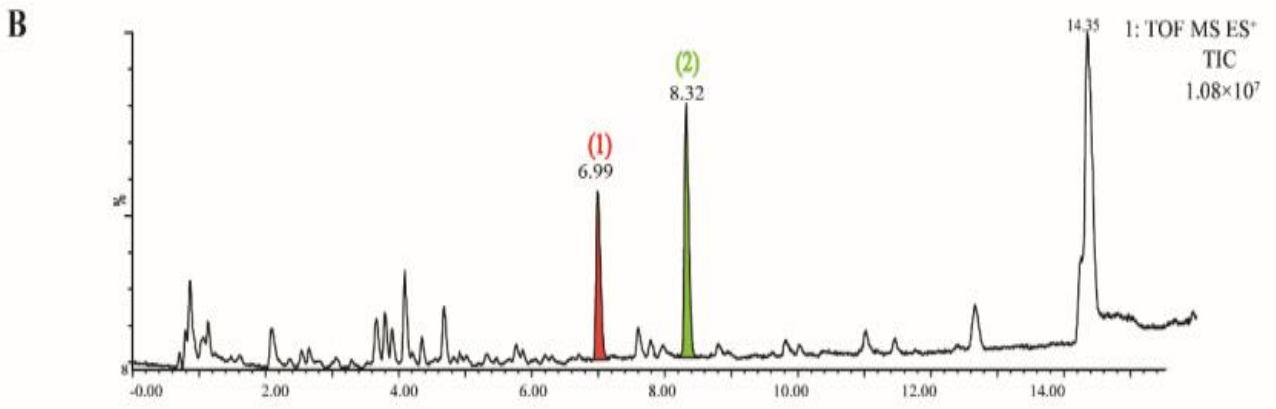


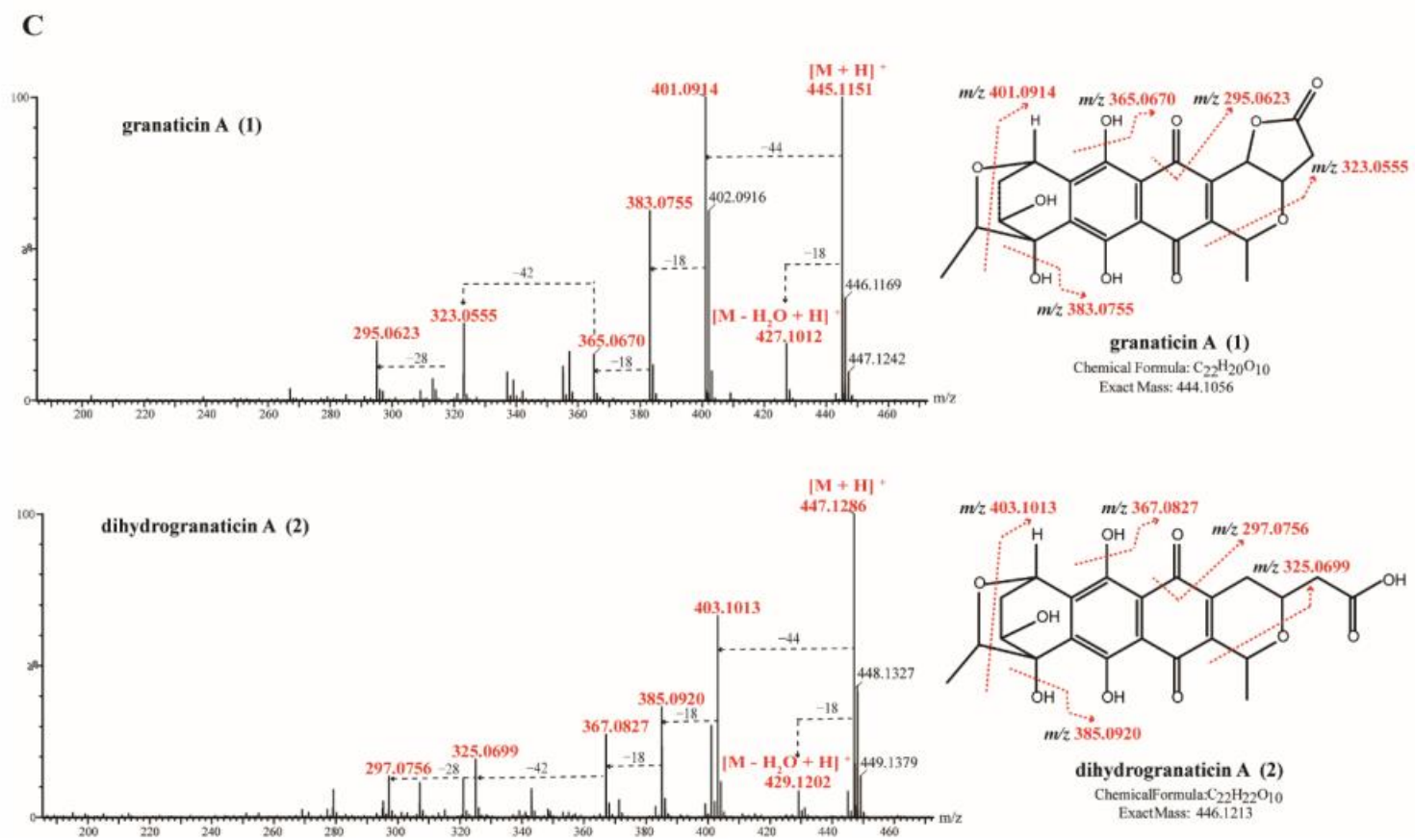
Figure 5. Colony morphology of *Streptomyces* isolates MGa5-5 (**A**), MGa1-3 (**B**) and MGa2-5 (**C**), and TLC analysis of the EA extracts from fermentation broth (**D–F**). Colony morphology was photographed after growing on ISP2 media for about 7 days. TLC analysis was coupled with anti-MRSA assay, and TLC bands was visualized at 254 nm.

CHEMICAL IDENTIFICATION





CHEMICAL IDENTIFICATION



MOLECULAR NETWORKING