دانشگاه علوم پزشکی کرمانشاه

Isolation and characterization of novel Streptomyces strain from Algeria and its in-vitro antimicrobial properties against microbial pathogens

Journal club

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- Scope:
- **infection prevention and control**
- **Microbiology**
- **infectious diseases**
- **public health**
- **application of healthcare epidemiology to the evaluation of health outcomes**

introduction

- A constant need for the development of antibiotic from natural products is must.
- 22/000 active natural product from microorgamism

- Microorganisms like:

1. Actinomycetes → %45 (specially from Streptomyces)
2. Pseudomonas
3. Myxobacteria
4. Cyanobacteria
5. Filamentous fungi

introduction

- Gram +, high C+G genome, Filamentous, aerobic.
- Genera Streptomyces and Micromonospora produce %70 of anitibiotics.

Material and method

V

Isolation of actinomycetes

All culture media from sigma company

- Different samples such as sediments and water were collected aseptically from different locations from Gueldaman cave
- Water ⟶ Sterile tube (4 C)
- sediments ⟶ sterile bags (air dried 7 days in petri dishes)
- Culture media:

1. Bennett agar
2. TSA (Tryptic soy agar)
3. GLM (Yeast extract-malt extract agar)
4. Chitin-vitamins agar
5. ISP2 (international streptomyces project)
6. GLP (Glucose-Yeast Extract-Peptone)

cycloheximide (50 g ml−1)

Nystatin (30 g ml−1)

nalidixic acid (30 g ml−1)

- 10 gr /10ml added to 90ml NS ⟶ 100µl added to culture media
- Incubate 2-4 weeks at 28˚ C
- suspected actinomycetes colonies were identified based on morphological characters and purified on Bennett medium for routine laboratory studies

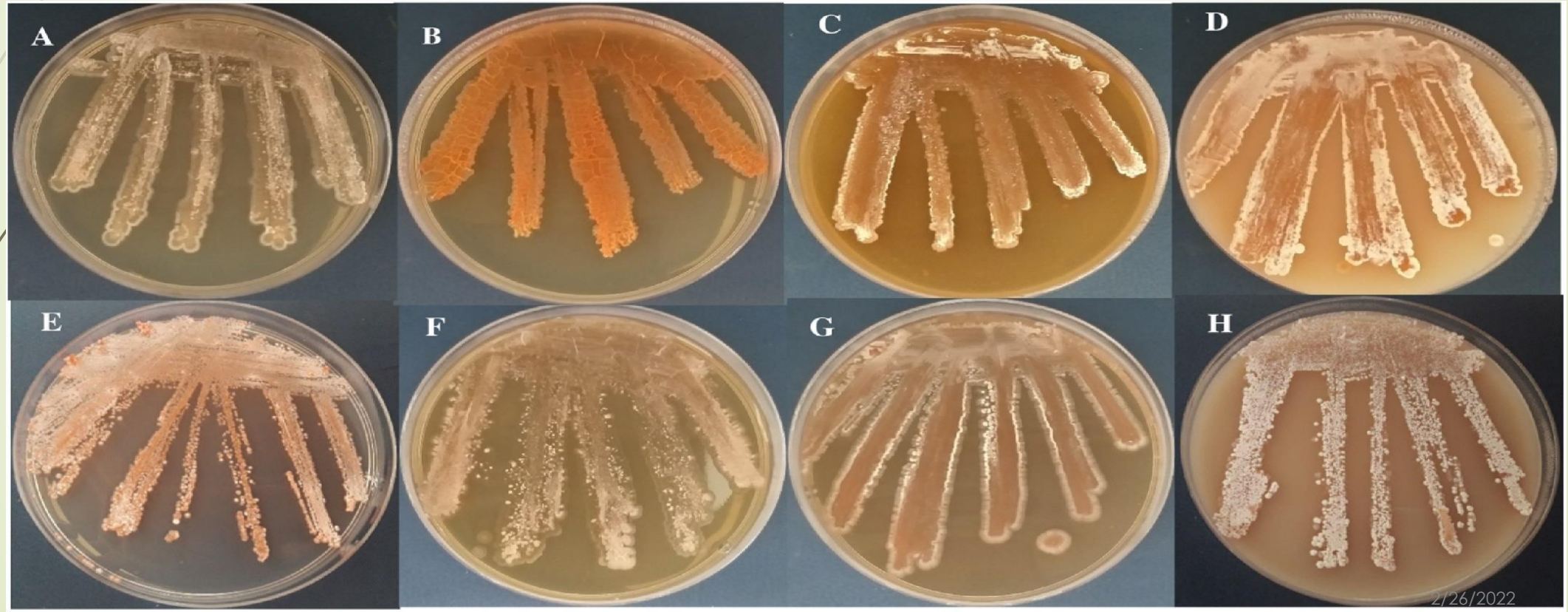
❑ Morphological features

1. Gram staining
2. Light microscopy

- For morphological characteristic (Mycelium and pigment):

Character	Different cultivation medium								
	ISP-1	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6	ISP-7	ISP-9	GLP
Growth	++	+++	+++	+++	+++	+++	+++	+	+++
Colour of substrate mycelium	Beige	Orange	Orange	Orange	Orange	Orange	Orange	White	Orange
Colour of aerial mycelium	White	Orange	White	White	White	Beige	Beige	White	White
Soluble pigments produced	–	–	Orange	Orange	Orange	–	–	–	Orange

+; moderate growth, ++; good growth, +++; good growth, -; no growth.



DNA Extraction

- GenJET Genomic DNA purification kit (Thermofisher company)
- PCR :

Primers:

1. 785 F 5´ GGATTAGATACCCTG GTA 3´
2. 907R 5´ CCGTCAATTCMTTTRAGTTT 3´

- Evolutionary tree

Using Mega X

Isolation of GLD22

- ISP2 medium

Physiological tests

Physiological tests		
Gram-staining		+
Melanoides pigments production on ISP6		–
Melanoides pigments production on ISP7		–
Growth temperature	25 ° C	++++
	35 °C	–
	45 ° C	–
	55 °C	–
Optimum pH	5	+
	7	+++
	9	++++
	10	++
NaCl tolerance (W/V)	3%	+
	5%	+++
	7%	+
	10%	+

On ISP6-ISP7

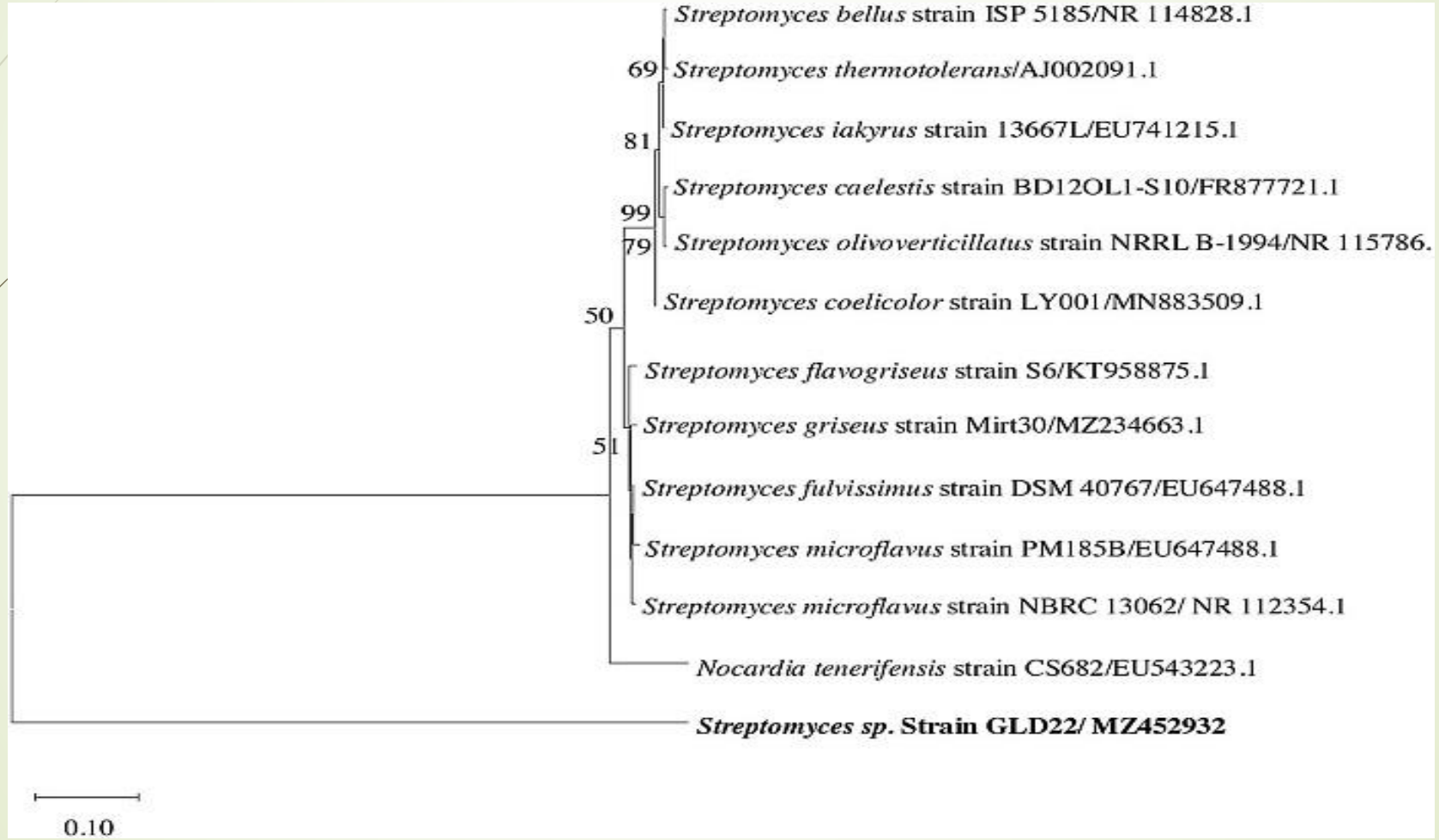
+; Positive, –; negative.

Biochemical tests

Table 2
Physiological and biochemical characters of *Streptomyces* strain GLD22.

Biochemical tests	Results
Degradation of Starch	+
Degradation of Casein	++
Degradation of Gelatin	+
Degradation of Glucose	–
Degradation Lactose	–
Degradation Saccharose	+
Degradation Citrate	+++
H_2S production	–
Production of Urease	+
Mannitol	–
Catalase	++
Action on Skimmed milk	–

Phylogenicity



Antibiotic assay

- Culture media for metabolite production

1. Modified Nutrient Glucose broth (MNG)
2. Bennett broth
3. AF broth

PH= 7±/2

120 rpm shaker

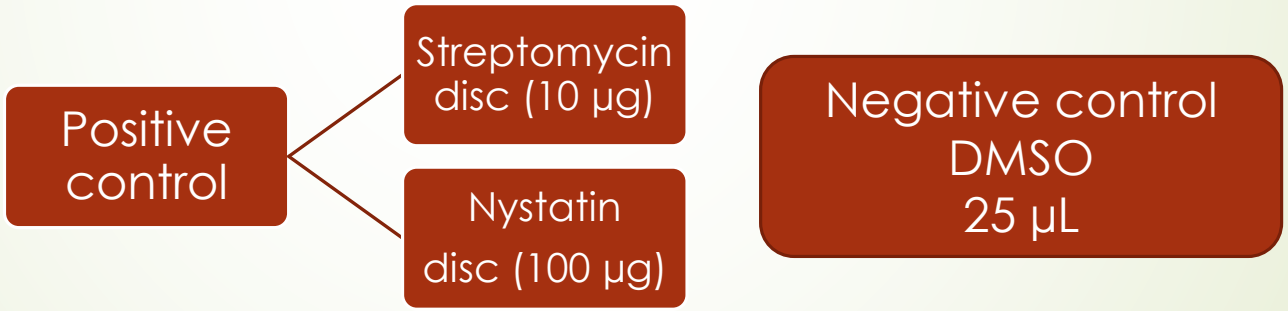
2 weeks

28° C

- Use Ethyl acetate as solvent – concentrate using rotary Evaporator at 45°C

Antibiotic assay

- Microorganism were cultured on Mueller-Hinton broth, M2, GLM, AF consistency of 10^8 CFU/ml for bacteria and 10^6 CFU/ml for fungi.
- The Antibiogram was assay using well diffusion method.
- Mueller-Hinton swabbed with suspension of pathogenic bacteria, the crude extract (30mg) was dissolved in 250µL DMSO- 25µL in wells.



- 37° C – 24hr for bacteria / 28° C 24-48hr for Fungi

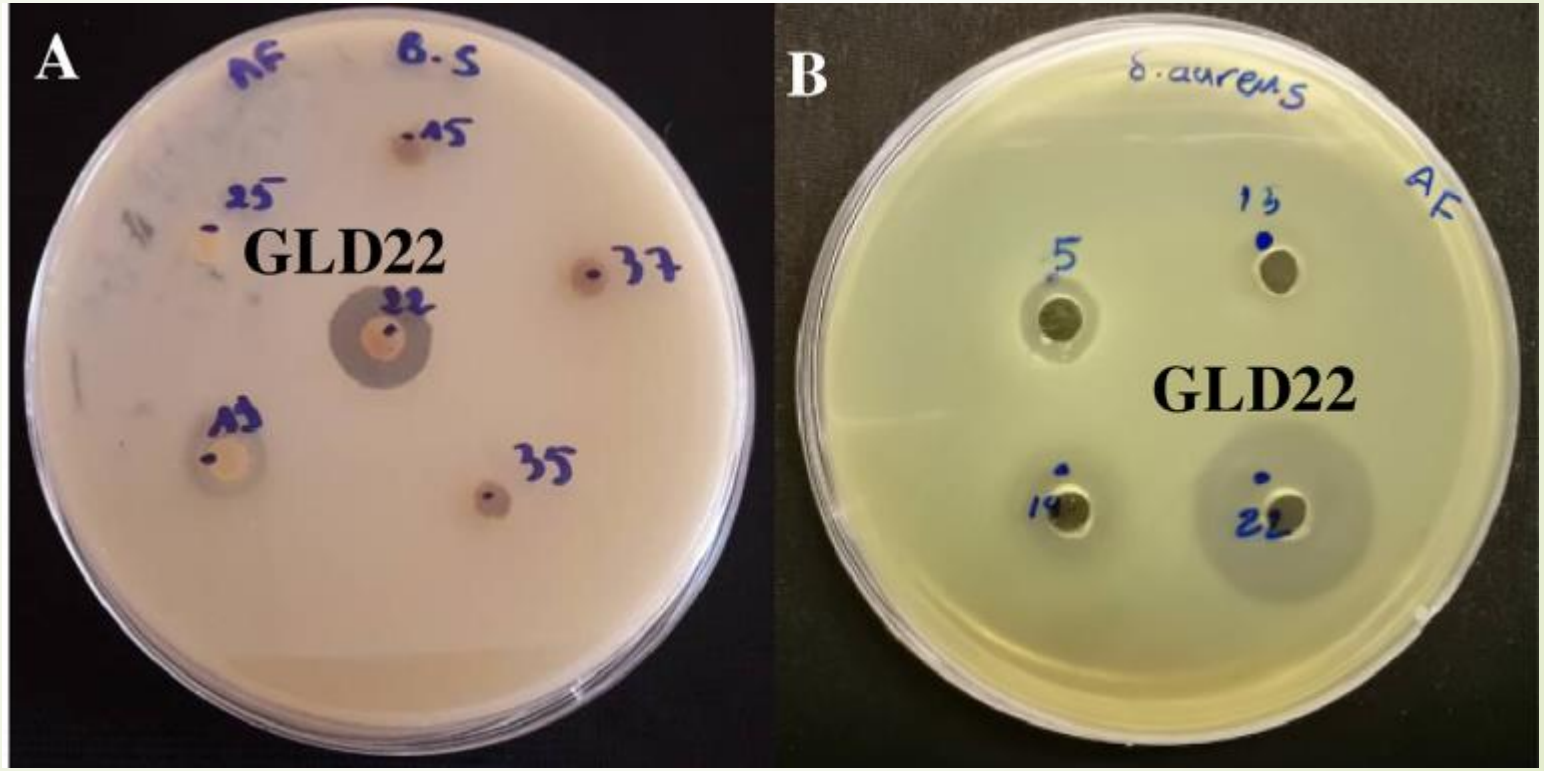
Antibiotic assay

Staphylococcus aureus	ATCC 6538
Bacillus cereus	ATCC 25921
Bacillus subtilis	ATCC 6633)
Pseudomonas aeruginosa	ATCC 27853
Escherichia coli	CIP 53.126 / ATCC 8739
Klebsiella pneumoniae	IBMC Stras-bourg
Candida albicans	CIP 444 / ATCC 10231
Aspergillus fumigatus	MNHN 566
Fusarium oxysporum	MNHN 963917

National Museum of Natural His-tory (Paris, France) and Laboratories of Natural Products (Tlemcen, Algeria)

Antibiotic assay

Pathogens	Diameter of inhibition zone (in mm)					
	Bennett	M2	GLM	AF	Chlormophenicol (10 µg/disc)	Nystatin (100 µg/disc)
Gram-positive bacteria						
Staphylococcus aureus (ATCC 6538)	22	18	0	14	30	ND
Bacillus cereus (ATCC 25921)	20	11	0	12	25	ND
Bacillus subtilis (ATCC 6633)	21	7	0	14	25	ND
Gram-negative bacteria						
Escherichia coli (CIP 53.126/ ATCC 8739)	10	0	0	0	25	ND
Pseudomonas aeruginosa (ATCC 27853)	8	0	0	0	24	ND
Klebsiella pneumoniae (IBMC Strasbourg)	0	0	0	0	19	ND
Fungi						
Candida albicans (CIP 444)	0	0	0	0	ND	25
Candida albicans (ATCC 10231)	0	0	0	0	ND	20
Aspergillus fumigatus (MNHN 566)	0	0	0	0	ND	30
Fusarium oxysporum (MNHN 963917)	0	0	0	0	ND	11


A
GLD22
B
GLD22

Chemical composition of metabolites

- GC MS-QP2010 (gas chromatograph mass spectrophotometer)

The extract Contain about 14 compound

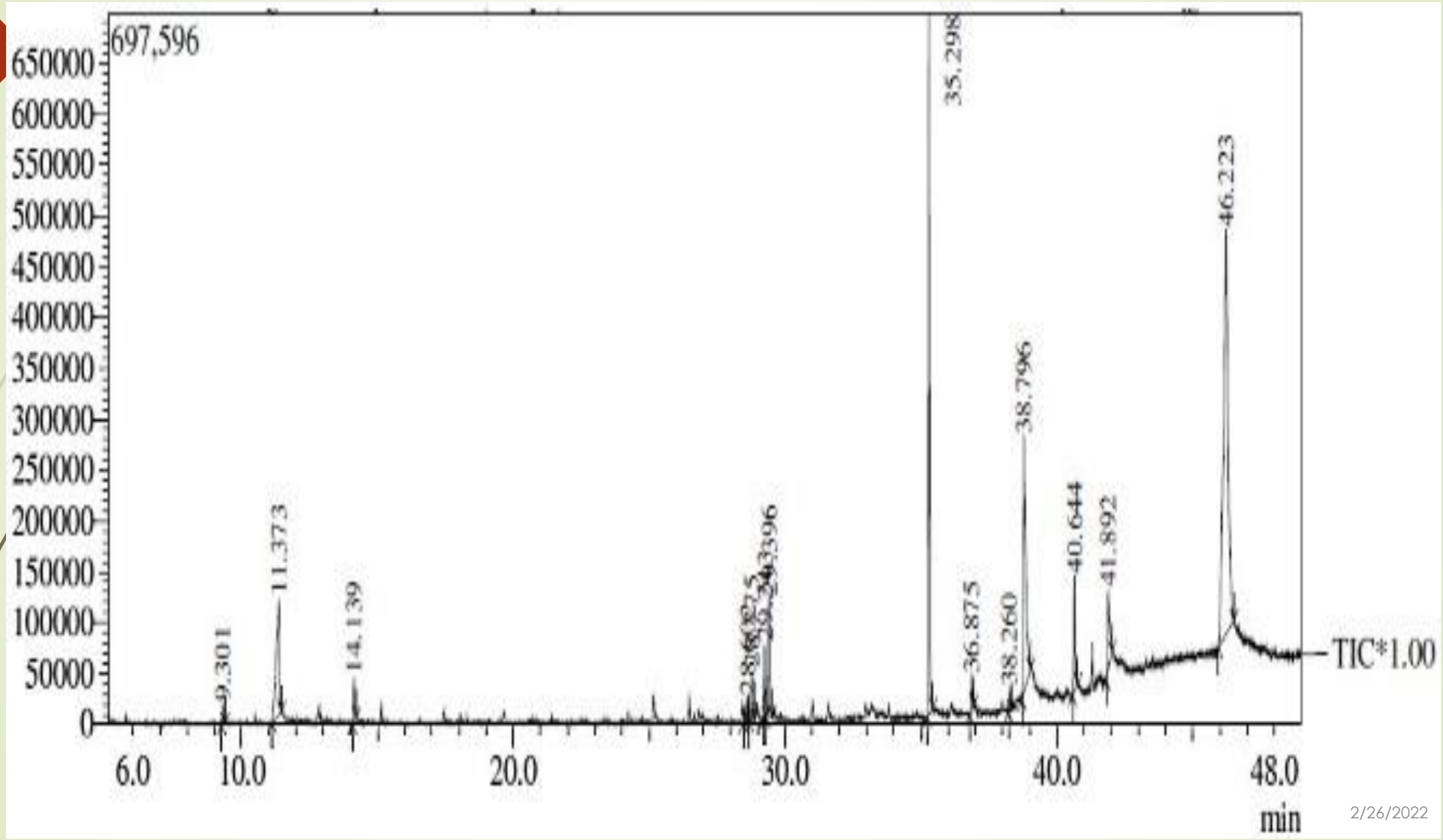
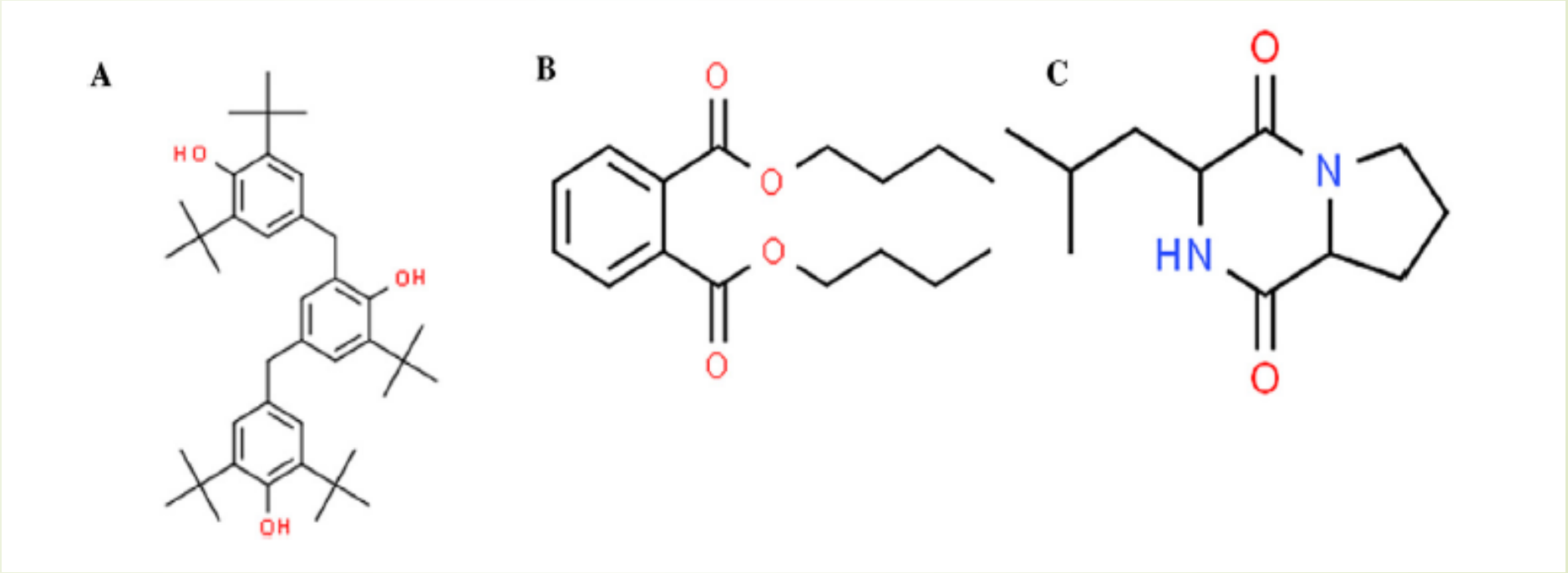

697,596
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
9.301
11.373
14.139
35.298
36.875
38.260
38.796
40.644
41.892
46.223
TIC*1.00
6.0
10.0
20.0
30.0
40.0
48.0
min

Table 6
Metabolite profiling of *Streptomyces* strain GLD22 using GC–MS analysis.

Peak[a]	Time[b]	Area	Area%	Height	Height%	Name	Base *m/z*
1	9.301	57,200	0.53	19,916	0.98	Maltol	126.10
2	11.373	926,272	8.61	114,162	5.63	1H-Pyrrole-2-carboxylic acid	111.05
3	14.139	138,931	1.29	43,670	2.16	Propanoic acid	74.00
4	28.602	101,194	0.94	24,911	1.23	7,9-Di-*tert*-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	57.05
5	28.775	135,670	1.26	49,042	2.42	Cyclo(leucyloprolyl)	154.00
6	29.243	203,348	1.89	72,449	3.58	Hexadecanoic acid	73.00
7	29.396	362,352	3.37	117,568	5.80	Dibutyl phthalate	149.00
8	35.298	1,735,966	16.14	687,870	33.95	2-Propenoic acid, pentadecyl ester	55.00
9	36.875	99,214	0.92	33,182	1.64	Dihydroergotamine	125.05
10	38.260	63,341	0.59	22,694	1.12	4-(2-*tert*-Butyl-5-oxo-1,3-dioxolan-4-yl)butylformamide	130.10
11	38.796	1,474,607	13.71	250,401	12.36	Glycerol. beta.-palmitate	57.00
12	40.644	376,639	3.50	114,790	5.67	Lauryl. beta.-mercaptopropionate	57.00
13	41.892	411,239	3.82	77,354	3.82	Glycerol. beta.-palmitate	57.05
14	46.223	4,670,248	43.42	398,034	19.65	2-*tert*-Butyl-4,6-bis(3,5-di-*tert*-butyl-4-hydroxybenzyl)phenol	57.05

[a] Chromatogram peak number.
[b] Retention time.


A
HO
OH
OH
B
O
O
O
O
C
O
N
HN
O

Discussion

- Lower activity against gram negative may due to their cell wall composition
- ISP2, TSA 28°C in other studies. In caves for metabolite production

1. Maciejewska et al : %71 opposite Gram negative/ %94 against Gram positive (conflict)
2. Belyagoubi et al : %62 of all isolates active against yeast, filamentous fungi, Gram positive and Gram negative bacteria (conflict)

- Nevertheless, this study needs furtherapproaches to identify the bioactive molecules present in theextract

Conclusion

- The actinomycetes GLD22 isolated from cave can bepotentially used for the production of commercially importantnew biomolecules