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Unique secondary metabolites of a Streptomyces strain isolated from extreme salty wetland show antioxidant and antibacterial activities

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ORIGINAL ARTICLE

Unique secondary metabolites of a *Streptomyces* strain isolated from extreme salty wetland show antioxidant and antibacterial activities

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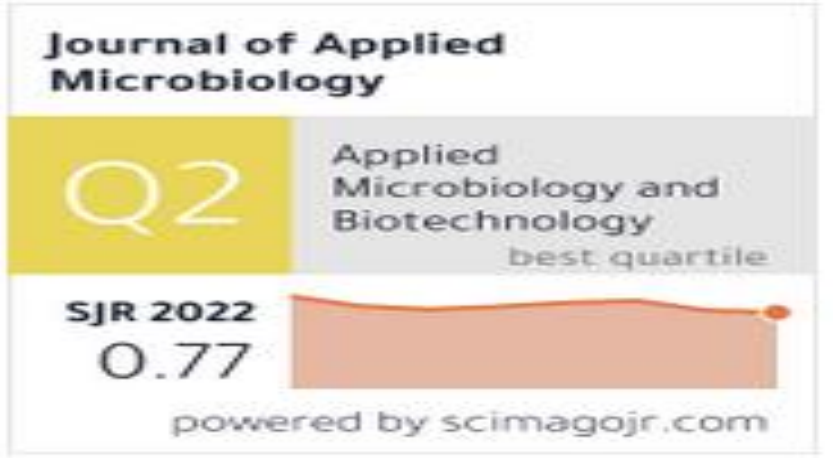

Impact Factor

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Keywords

antimicrobials, environmental, metabolites, sediment, soil, *Streptomycetes*.

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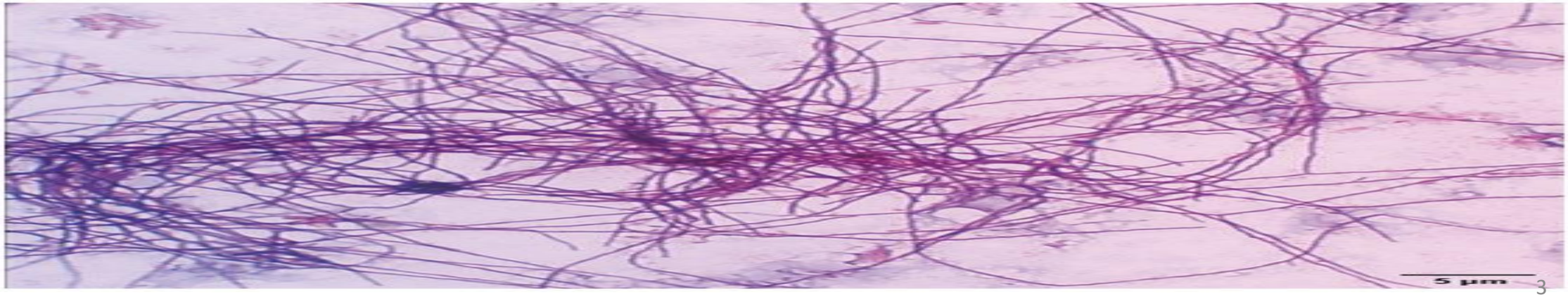
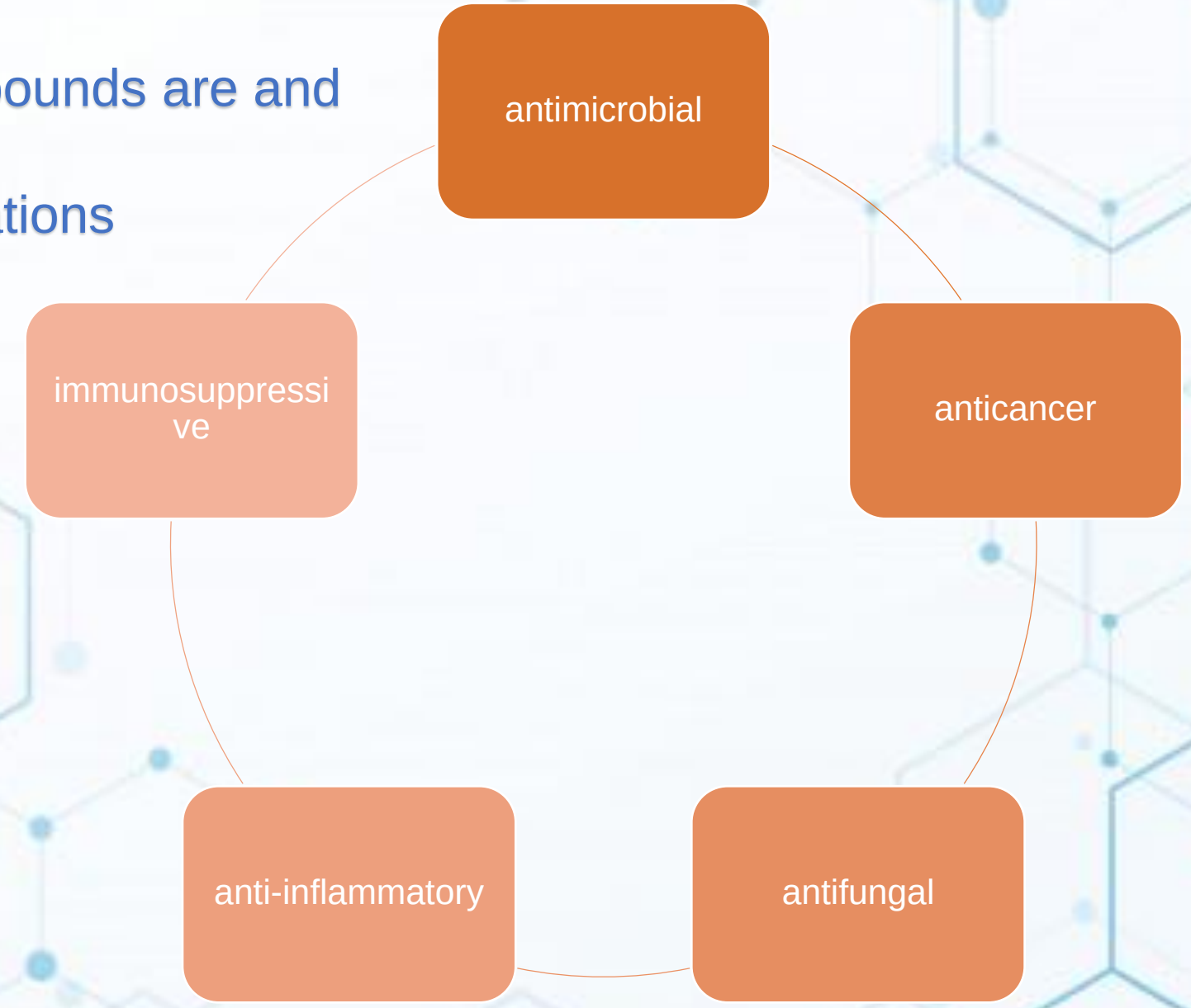
Introduction

- ✓ The phylum Actinobacteria, which encompasses bacteria that have guanine plus cytosine-rich DNA , contains major producers of antibiotics and represents the most economically and biotechnologically valuable prokaryotes.

- ✓ Among them, members of the genus Streptomyces have received particular attention to their ability to produce a plethora of secondary metabolites with well-renowned biological activities.



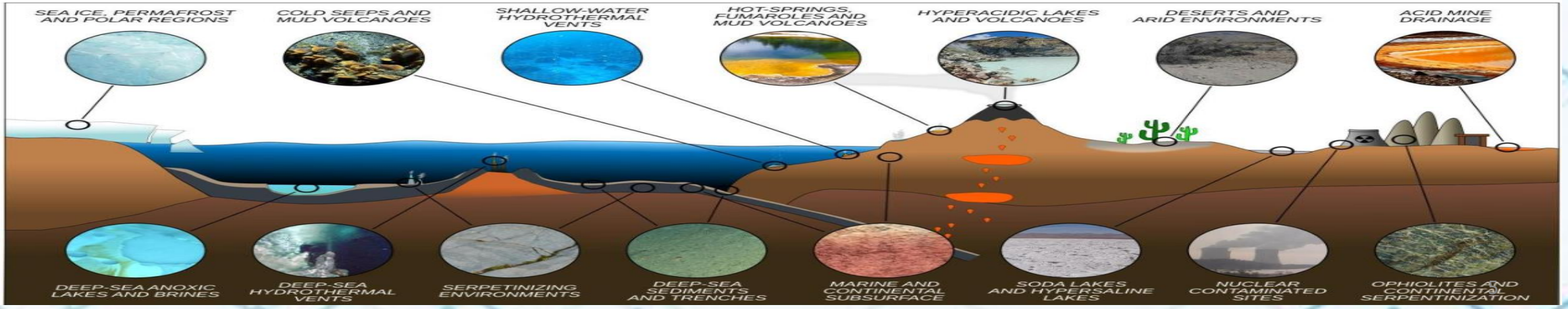
Streptomyces-derived compounds are and their pharmaceutical applications



- ✓ Because of their potential therapeutic, industrial and biotechnological importance, several studies were carried out in order to isolate new Streptomyces species with desirable characteristics.

- ✓ By exploring new habitats (e.g. marine, halophilic, acidophilic), these studies have successfully isolated new species, and concomitantly new useful secondary metabolites (e.g. polyketides, pigments, flavonoids) have been characterized

- ✓ polyketides are one of the most abundant classes of compounds with remarkable structural diversity and biological activities



- ✓ Most of the newly isolated active metabolites from Streptomyces originate from marine habitats and extreme environments, namely with high salinity, and were characterized by their unique efficiency deeply involved in the mitigation of the deleterious effects of high salinity.

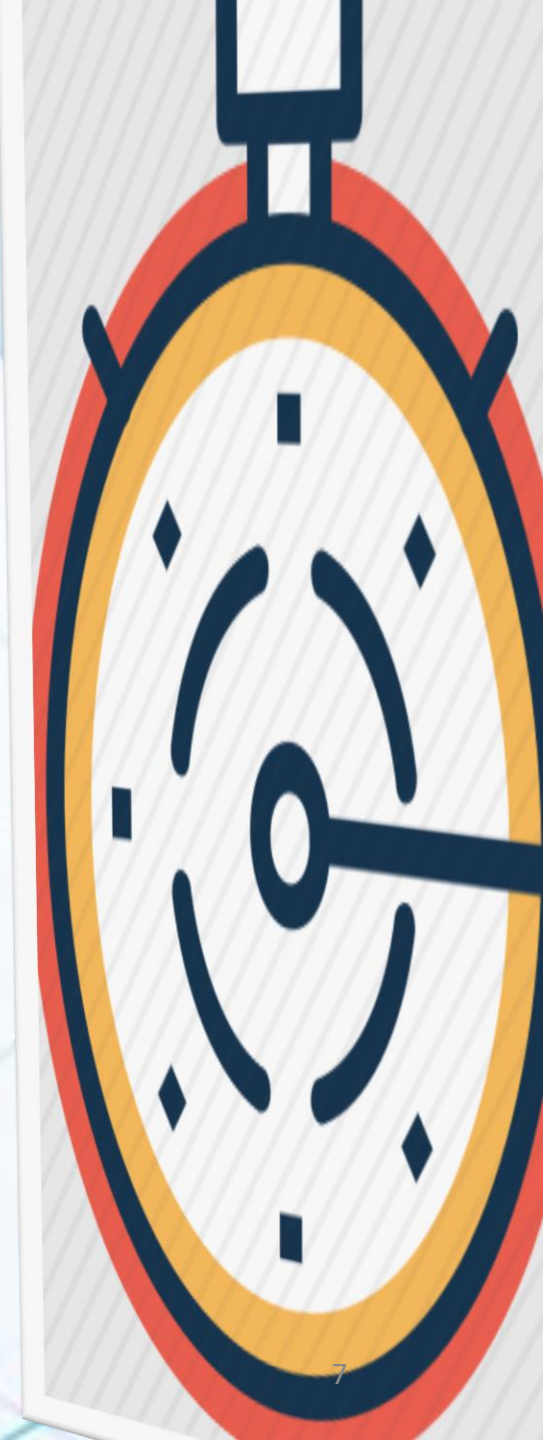
- ✓ These promising biomolecules are also undoubtedly revolutionary in terms of biotechnological uses. Among them, we can mention antibiotics, enzymes and compatible solutes, resulting from several mechanisms of adaptation to extreme conditions and unique metabolic biochemistry.

research goals

Describe the isolation and investigation of antibacterial activity of an extract from a *S.lanatus* strain (AR2) from a salty wetland.

identification of novel secondary metabolites with high potential applications

investigated the antioxidant activity of the extract using Saccharomyces cerevisiae as eukaryotic cell model



Materials and methods

4

Bacterial strains, media and growth conditions

- ✓ Luria–Bertani medium (LB)
- ✓ Tryptic Soy Broth medium (TSB)

3

Molecular identification of strains

- ✓ 16S rRNA seuencing

2

Isolation of Streptomyces, media and culture growth conditions

- ✓ Sterilization
- ✓ Isolation culture
- ✓ Different amounts of NaCl

1

Site description, sampling and pretreatment

- ✓ Samples with 10-cm depth were taken in Sabkhat Seijoumi, a salty wetland in Northern Tunisia.

8

Electron/Hydrogen Donation

- ✓ DPPH assay

7

Determination of total phenolic (TPC) and total flavonoid Contents (TFC)

- ✓ TPC: Folin–Ciocalteu method
- ✓ TFC: aluminium chloride colorimetric method

6

Preparation of the extract

- ✓ ISP2 medium supplemented with 3% (w/v) NaCl, at pH 9 and was incubated at 37°C, 150 rev min 1, for 48 h.

5

Antibacterial activity screening

- ✓ agar well diffusion Method

12

Statistical analysis

- ✓ All experiments were performed in triplicate, and the results are expressed as mean ±SD.

11

Profiling of phenolic compounds by HPLC-PDA-ESIMS/ MS

- ✓ Mobile phase:
- ✓ A: 01% acetic acid
- ✓ B: acetonitrile
- ✓ flow rate: 0.25 ml/min

10

Reducing Power

- ✓ FRAP assay

9

Iron-chelating activity

- ✓ method of Decker and Welch (1990)

RESULTS

Actinomycetes isolation based on specific morphological characteristics

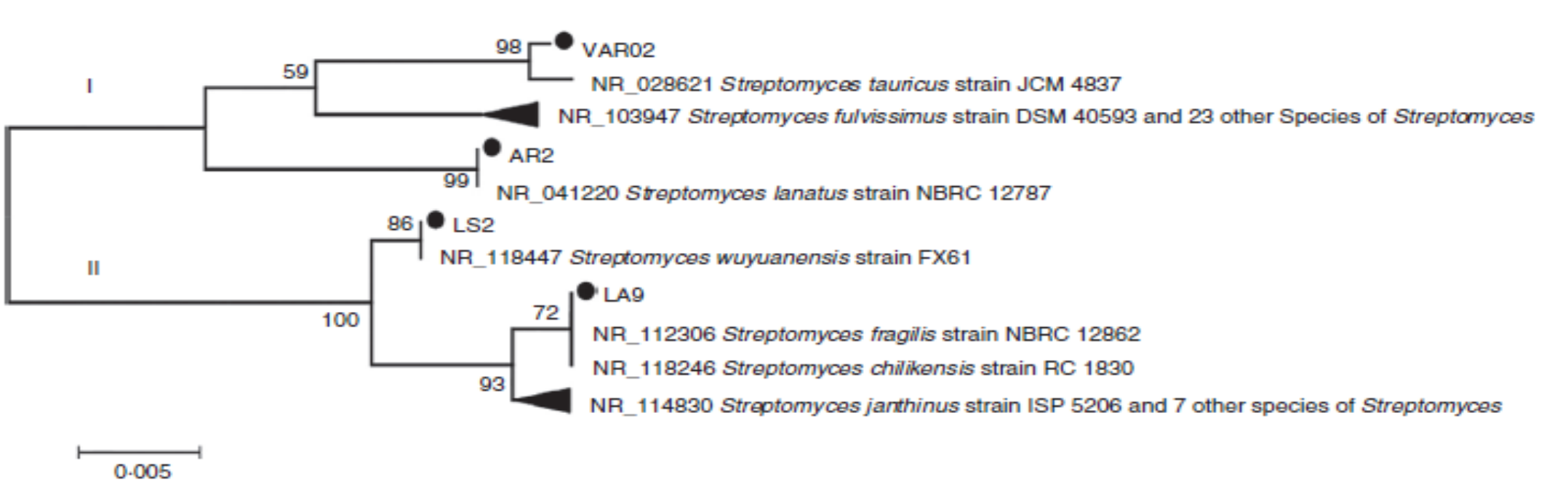
strain AR2, was markedly distinguished from the remaining isolates;

1. heavy growth on ISP2 medium
2. specific colours of aerial (olive green)
3. and vegetative mycelia (yellowish brown).

Phylogenetic analysis

- ✓ The partial 16S rRNA gene sequence of AR2 covered a stretch of 1240 bp.
- ✓ The nucleotide sequence was submitted to GenBank (accession number MG008497)
- ✓ The homology of the 16S rDNA sequence provided 100% identity with *S. lanatus*.

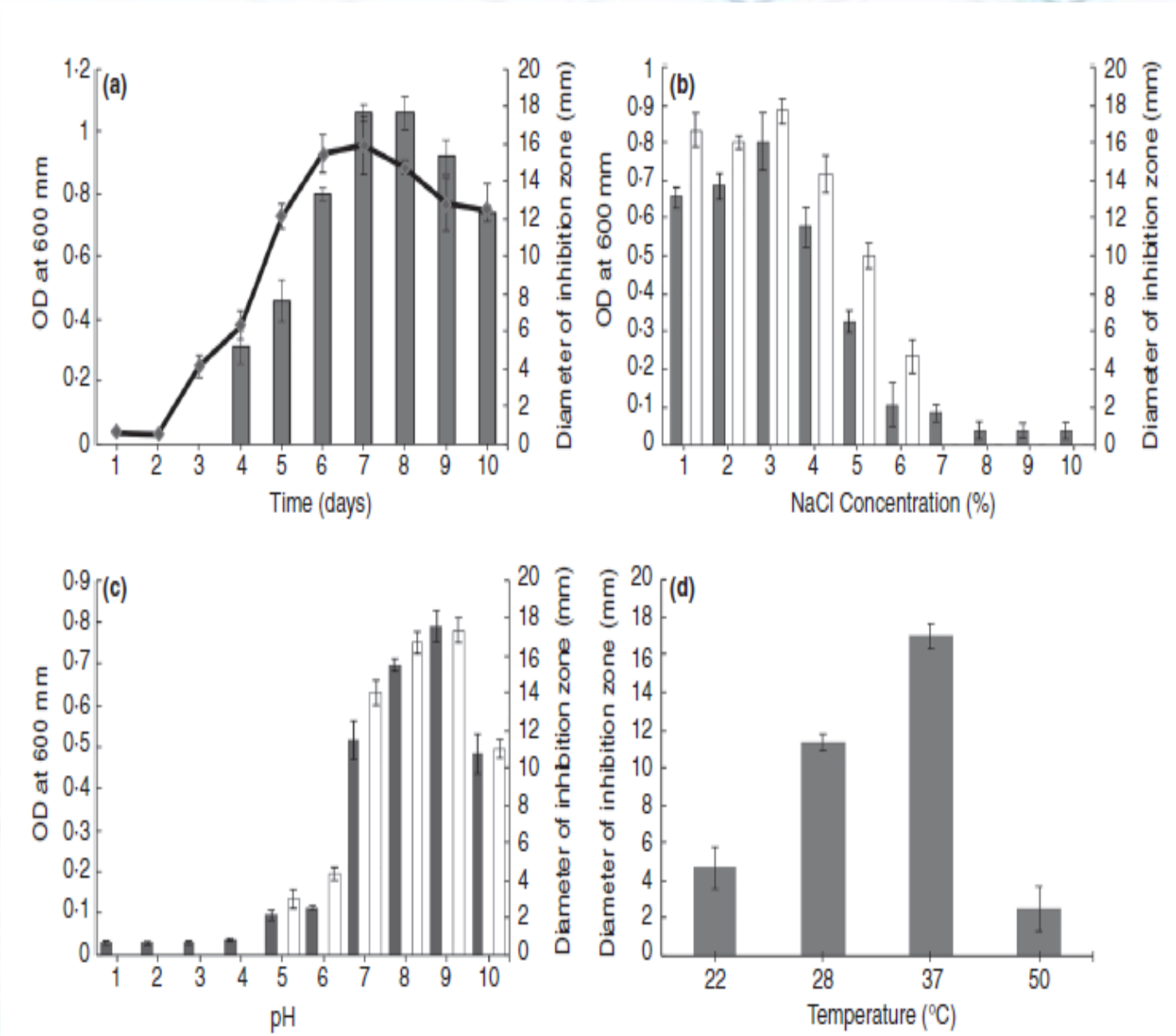
Neighbour-joining tree based on 16S rDNA sequences showed that strain AR2 was positioned in a separate subgroup within the group I harbouring another isolated strain (VAR02) *of Streptomyces tauricus* and *Streptomyces fulvissimus strains*.



A simplified neighbour-joining tree based on partial 16S rDNA sequences showing the evolutionary relationships between the different local isolates (VAR02, AR2, LS2 and LA9; type strains of different species of Streptomyces were used as references. The evolutionary distances were computed using the Kimura two-parameter method. All positions containing gaps and missing data were eliminated. The significance of each branch is indicated by the bootstrap value calculated for 500 replicates. The bar indicates the number of substitutions per site).

Optimization of growth conditions and antibacterial activity

Optimization of growth conditions and antibacterial activity of strain AR2 against Staphylococcus aureus ATCC 6538. Growth kinetics (line) of *Streptomyces lanatus* culture in ISP2 at 37°C, 150 rev min 1 and antibacterial activity (bars; a). Effect of NaCl on growth of *S.lanatus* strain cultures after 7 days of incubation in ISP2 medium at pH 9, 37°C, 150 rev min 1 (black bars) and antibacterial activity (white bars; b). Effect of pH on S. lanatus strain after 7 days of incubation in ISP2 medium with 3% (w/v), at 37°C, 150 rev min 1 (black bars) and antibacterial activity (white bars; c). Effect of temperature on *S.lanatus* strain AR2 antibacterial activity after 7 days of incubation in ISP2 medium at 37°C with 3% (w/v), pH 9 and 150 rev min 1 (d). Growth was monitored by OD600 and antibacterial activity against *S.aureus* ATCC 6538 was measured by the agar diffusion method and was recorded as diameter of zone of inhibition in millimetres. Average values and standard deviations were calculated from three independent experiments.



Total phenolic and flavonoid contents and antioxidant activity of S. lanatus AR2 crude extract

Phenolic compounds and antioxidant capacity IC50 (mg ml 1) of AR2 crude extract

Phenolic compounds		Antioxidant capacity IC_{50} (mg ml^{-1})		
Total polyphenol mg GAE/g extract	Total flavonoids mg QE/g extract	DPPH	Reducing power	Iron chelating
0·397 ± 0·02	0·071 ± 0·01	0·74 ± 0·02	1·12 ± 0·01	1·84 ± 0·030

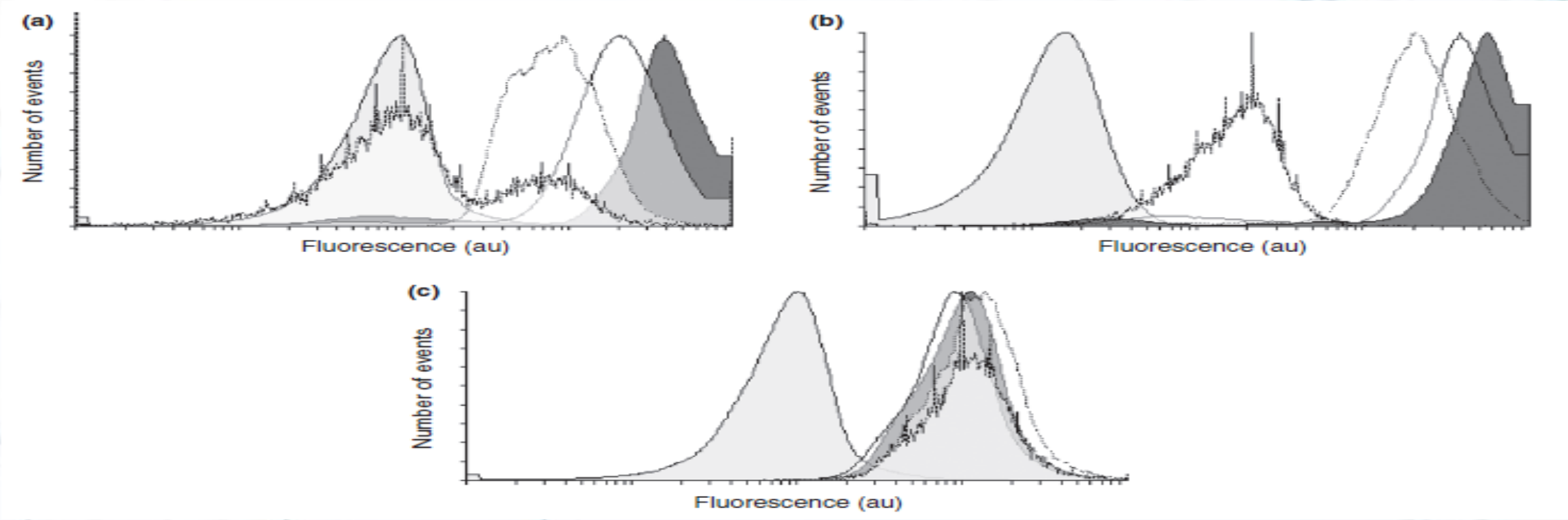
GAE, gallic acid equivalents; QE, quercetin equivalents.

Antibacterial activity and MIC

Minimum inhibitory concentration (MIC) of the Streptomyces AR2 crude extract (10 mg ml 1; CE). As an indication of the strength of antibacterial activity, inhibition zones of the extract and gentamicin (25 lg ml 1) are also displayed. Data are expressed as mean SD (n = 3)

Gram reaction	Test micro-organisms	Inhibition zone diameter (mm)		MIC (μg/ml)
		CE AR2	Gentamicin	CE AR2
Gram-positive	*Bacillus subtilis*	12·7 ± 0·4	22	25
	Bacillus megaterium	12·0 ± 0·0	21	25
	Bacillus cereus	12·0 ± 0·9	21	25
	Staphylococcus aureus	18·7 ± 0·5	20	5
	Micrococcus luteus	18·0 ± 0·0	16	5
	Listeria monocytogenes	12·0 ± 0·6	20	50
Gram-negative	*Escherichia coli*	-	ND	ND
	Salmonella typhimurium	-	ND	ND
	Proteus vulgaris	-	ND	ND

-, no inhibition zone; ND, not determined.



The AR2 crude extract attenuates intracellular oxidation induced by H2O2 in wild-type (a) Saccharomyces cerevisiae BY4741 and yap1 mutant (b) but not in the ctt1 mutant strain (c). Cells were loaded with the fluorochrome dichlorofluorescein diacetate for 1 h, at 30°C, in thedark, washed with PBS, incubated for 20 min at 30°C with 5 mmol l 1 H2O2 or 5 mmol l1 H2O2 with AR2 extract at 100 µg ml 1 (continuous line, empty), 200 µg ml 1 (dotted line, empty) or 500 µg ml 1 (dashed line, empty). Controls were made with PBS (negative; light grey) or with 5 mmol l 1 H2O2 (positive; dark grey). The results of a representative experiment from three independent replicas are presented; au means arbitrary units.

Retention time, maximum UV absorption (kmax), mass spectral data and tentative identification of the compounds in the ethyl acetate extract of Streptomyces lanatus strain AR2 performed by HPLC-PDA-ESI-MS/MS

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1}$	MS2	Tentative identification	References
1	2·591	255; 347	445	269	Genistein-7-*O*-glucuronide	Yang *et al.* (2010)
2	2·766	258; 347	579	271	Naringenin-7-*O*-rutinoside (narirutin)	Gattuso *et al.* (2007)
3	3·128	484; 568	682	369	γ-Actinorhodin	Čihák *et al.* (2017)
4	3·302	273; 311	195	151	Germicidin	Petersen *et al.* (1993)
5	3·523	260; 308	559	515	Geldanamycin	Shin *et al.* (2008)
6	5·546	261; 484	542	392	Doxorubicin	Desai *et al.* (2004)
7	5·930	267	135	91	Phenyl acetic acid	Martínez-Huélamo *et al.* (2015)
8	7·351	217; 290	271	135	7,3′,4′-Trihydroxyflavanone	Ye *et al.* (2012)
9	10·281	288	227	185	Resveratrol	Presta *et al.* (2009)
10	11·197	288; 484	317	287	Mycophenolic acid	Upadhyay *et al.* (2014)
11	11·641	290	561	518	4,5-Dihydrogeldanamycin	Lin *et al.* (2017)
12	13·55	278	197	153	Germicidin analogous	Petersen *et al.* (1993)
13	15·380	463; 568	217	97	Albaflavenone	Lin and Cane (2009)
14	24·490	205	589	235	Lasalocid	Olejnik *et al.* (2010)
15	28·885	206	529	-	Antimycin B2	Han *et al.* (2012)
16	47·406	217; 234	806	-	Linearolide A	Ueki *et al.* (2013)
17	49·335	216	807	-	Ascomycin	Karapirli *et al.* (2012)

Discussion

1

- ✓ This study demonstrates that Seijoumi wetland harbours Actinomycetes with relevant biological activities. The morphological characteristics of strain AR2 were similar to that described in Bergey's manual of systematic bacteriology (Lechevalier et al. 1989), suggesting that AR2 strain belongs to the genus Streptomyces.

- ✓ Sequencing of 16S rDNA correlated with the preliminary genus identification based on morphological characteristics.

2

- ✓ The extract of the AR2 isolate identified as S. lanatus exhibited pronounced antibacterial activity against Gram-positive bacteria and showed an antioxidant potential, as it was able to scavenge the free radical DPPH, to have reducing power capacity and to chelate iron.

- ✓ the profiling of metabolites by HPLC-PDA-ESIMS/ MS led to identify several antibiotics and phenolic compounds with potential antioxidant activity.

3

- ✓ In terms of growth conditions for antibacterial activity, the AR2 strain displayed similar behaviour as other Streptomyces strains as reported in the literature, such as Streptomyces sp 201 for the time of incubation (Thakur et al. 2009); *Streptomyces sannanensis* strain RJT-1 for the requirement of 3% NaCl (Vasavada et al. 2006); and the halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8 and the salt-tolerant and alkaliphilic S*. sannanensis* strain RJT-1 for the requirement of pH 9 (Vasavada et al. 2006; Thumar et al. 2010).

- ✓ Secondary metabolism of Streptomyces has been compared with that of plants in terms of metabolic pathways (Bode 2003). So, the presence of polyphenols and flavonoids is not surprising in Streptomyces AR2 strain , such as in *Streptomyces lavendulae* strain SCA5 (Kumar et al. 2014) and in *Streptomyces cellulosae* strain TES17 (Rani et al. 2018).

4

- ✓ Not surprisingly, antioxidant activity, measured with the DPPH assay, was remarkable in AR2 strain (Table 1), as is the case of S. lavendulae strain SCA5 (Kumar et al. 2014), although examples exist with lower (Streptomyces strain TES17; Rani et al. 2018) and higher antioxidant activity (Streptomyces sp. VITMSS05; Revathy et al. 2013).

- ✓ iron chelation seems to be another antioxidant mechanism involved in the antioxidant activity of the AR2 extract as this extract has the ability to chelate iron. As in many plant extracts, these two mechanisms are present, presumably due to scavenging and metal-chelating properties of polyphenols.

5

- ✓ The presence of antibacterial activity of AR2 extract against only the tested Gram-positive bacteria is in agreement with the report of Gebreyohannes et al. (2013) regarding the crude extracts of Actinomycetes isolated from water and sediments of Lake Tana, Ethiopia.

- ✓ According to the chemical analysis, the crude extract of Streptomyces AR2 contains germicidins and c-actinorhodin that were reported in the literature as selective bactericidal against Gram-positive pathogens, including B. subtilis (the case of germicidin; Cihak et al. 2017) and S. aureus (the case of c-actinorhodin; Nass et al. 2017). Therefore, it is reasonable to conclude that the antibacterial activity of AR2 extract is mainly directed against Gram-positive bacteria.

Conclusion

- ✓ The diversity of secondary metabolites and their reported bioactivities, together with the antioxidant and antibacterial properties we report here, makes *S. lanatus* strain AR2 a candidate to use as a source of compounds with great potential for applications in human health.
- ✓ The unique compounds detected in the extract, namely resveratrol, highlight the potentialities of this strain and constitute an indication of the enormous variety of secondary metabolites in Streptomyces with potential for human applications, similarly as those from plants.
- ✓ As stressful habitats are likely to impose selective pressure to organisms that have developed adaptive responses, such as increased production of secondary metabolites, salty wetlands and other extreme environments are promising sources of strains like AR2.

Thank you!