

Exploring the Antimicrobial and Antioxidant Activities of *Streptomyces* sp. EIZ2 Isolated from Moroccan Agricultural Soil

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1. General methods

1.1 Recommended media for *Actinobacteria* isolation

M2: 10 g of starch, 0.3 g of casein, 2 g of KNO₃, 2 g of NaCl, 0.05 g of MgSO₄·7H₂O, 2 g of K₂HPO₄, 0.02 g of CaCO₃, 0.01 g of FeSO₄·7H₂O, 1 g of glucose, 15 g of agar, 1L distilled water, and pH adjusted at 7.2 [1].

GA: 10 g of Glucose, 0.5 g of asparagine, 0.5 g of K₂HPO₄, 15 g of agar, 1L distilled water, and pH adjusted at 6 [1].

GLM: 10 g of glucose, 3 g of yeast extract, 3 g of malate extract, 5 g of tryptone, 15 g of agar, 1L distilled water, and pH adjusted at 7.2 [2,3].

Bennett : 10 g of glucose, 2 g of yeast extract, 1 g of meat extract, 2 g of tryptone, 15 g of agar, 1L distilled water, and pH adjusted at 7.2 [4].

1.2 Recommended media for testing antimicrobial activity [5]

ISP1: 3 g of yeast extract, 5 g of tryptone, 16 g of agar, 1L of distilled water, and pH adjusted at 7.15.

ISP2: 4 g of yeast extract, 4 g of glucose, 10 g of malate extract, 16 g of agar, 1L of distilled water, and pH adjusted at 6.51.

GYES: 10 g of yeast extract, 10 g of glucose, 16 g of agar, 1L of distilled water, and pH adjusted at 6.96.

Bennett: 10 g of glucose, 2 g of yeast extract, 1 g of meat extract, 2 g of tryptone, 15 g of agar, 1L distilled water, and pH adjusted at 7.2.

1.3 Recommended medium for testing NaCl tolerance

YEA medium: 3 g of yeast extract, 5 g of peptone, 15 g of agar, 1L of distilled water, and pH adjusted at 7.2.

1.4 Recommended medium for the production of melanoid pigment

ISP9: 2.64 g of (NH₂) SO₄, 2.38 g of KH₂PO₄, 5.65 g of K₂HPO₄, 1 g of MgSO₄·7H₂O, 20 g of agar, and 1L of distilled water.

100 mL salt solution: 4 g of CuSO₄·5H₂O, 1.1 g of FeSO₄·7H₂O, 7.9 g of MnCl₂·4H₂O, 1.5 g of ZnSO₄·7H₂O.

GYES: 10 g of yeast extract, 10 g of glucose, 16 g of agar, 1L of distilled water, and pH adjusted at 6.96.

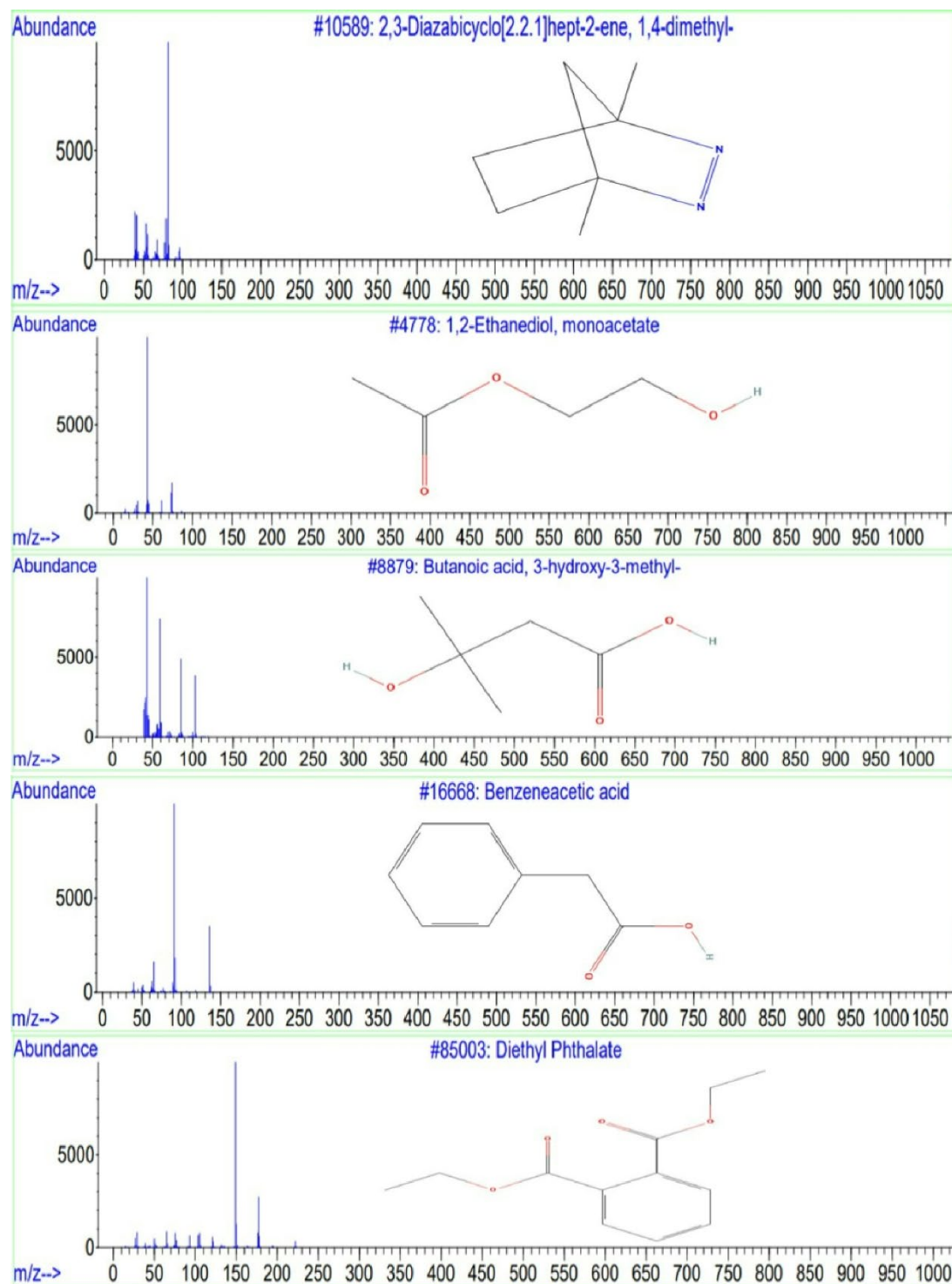
1.5 Other media used in this study

PDA medium: 4 g of potato extract 20 g of glucose, 15 g of agar, 1L distilled water, and pH adjusted at 7.5.

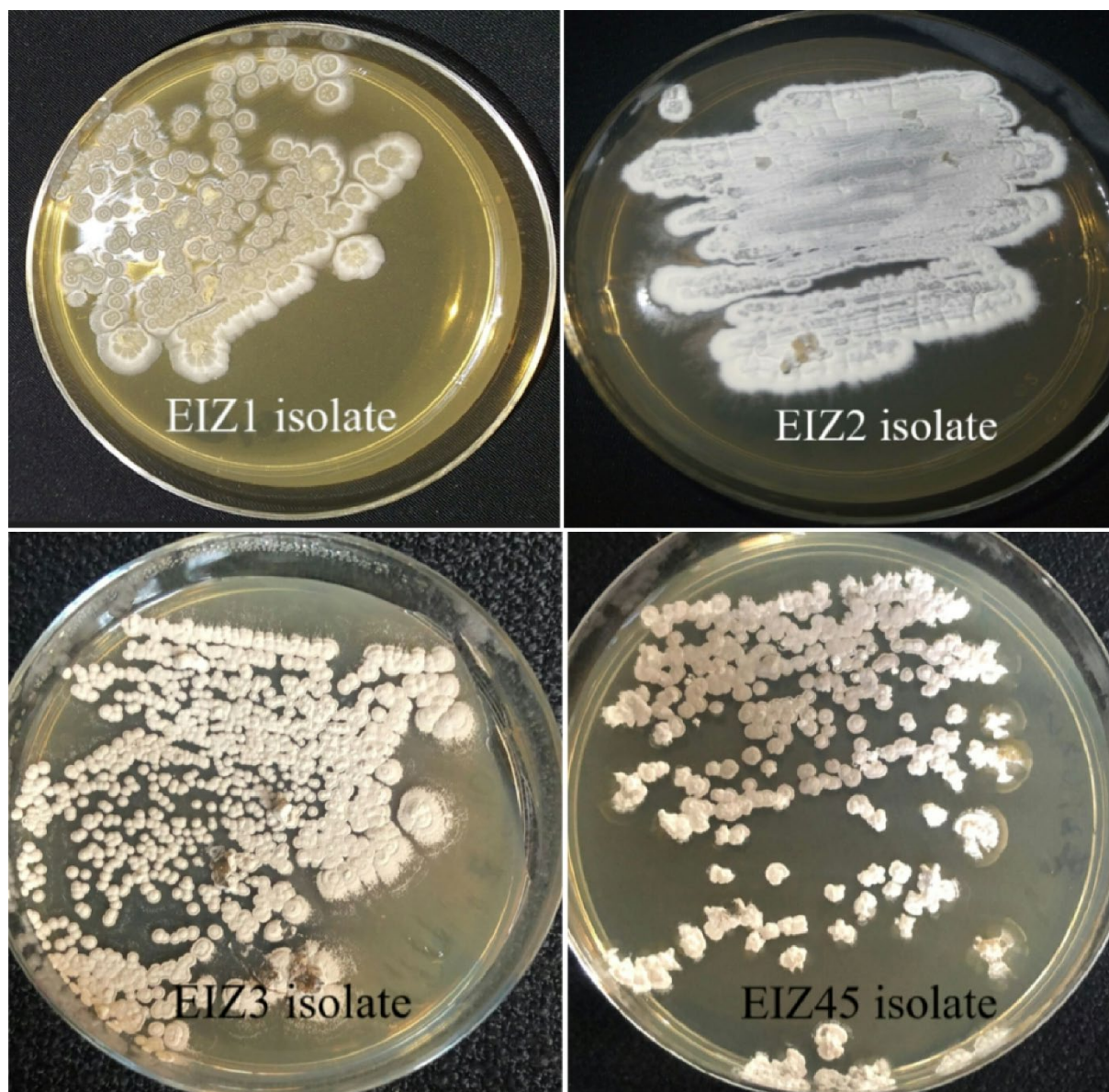
Muller Hinton Agar medium: Ready to use

CLED medium: Ready to use.

2. Supplementary figures



Supplementary Figure S1. GC-MS spectra of 5 important secondary metabolites present in ethyl acetate extract of EIZ2 strain.



Supplementary Figure S2. Macroscopic Appearance of *Actinobacteria* isolates (EIZ1, EIZ2, EIZ3, and EIZ45).

3. Supplementary tables

Supplementary Table S1. Resistance profile of multi-drug resistant (MDR) clinical bacteria.

Test strains MDR	Resistance to	Sensitivity to	Intermediate to
Clinical <i>Klebsiella pneumoniae</i> 19K 929	P, AMP, AML, KF, CXM, CRO, CIP, FMQ, OFX, E, TE, SXT, F	AN, CN, IPM	AMC, NA
<i>Proteus vulgaris</i> 16C1737	P, AMP, AML, AMC, CXM, CTX, SXT, FD, F	CN, CRO, OFX,	CIP, AK
Clinical <i>Klebsiella pneumoniae</i> 20B1572	P, AMP, AML, AMC, KF, CXM, CRO, CN, CIP, NA, FMQ, OFX, E, TE, SXT, F	AN, IPM	-
Clinical <i>Proteus</i> sp. 19K1313	P, AMP, AML, KF, CXM, CIP, NA, FMQ, OFX, E, TE, SXT, F	AMC, CRO, AN, IPM	CN
Clinical <i>Escherichia coli</i> 19L2418	P, AMP, AML, AMC, KF, CXM, CRO, CN, CIP, NA, FMQ, OFX, E, TE, SXT, F	AN, IPM	-
			AMC :

Amoxicillin + Ac. clavulanic; P: penicillin G; AMP: Ampicillin; KF: Cefalotin; CRO: Ceftriaxone; AN: Amikacin; CN: Gentamicin; CIP: Ciprofloxacin; NA: Nalidixic acid; FMQ: Flumequine; OFX: Ofloxacin; E: Erythromycin; TE: Tetracycline SXT: Trimethoprim+Sulfamide; FD: Fusidic acid; F: Nitrofurantne; CXM: Cefuroxime; VA: Vaomycin; CTX: Cefotaxime. IPM: Imipenem; CAZ: Ceftazidim; TM: Tobramycin; FOX: Cefoxitin; TIC: Ticarcillin.

Supplementary Table S2. Molecular identification of the selected EIZ2 *Actinobacteria* isolate based on 16S rRNA gene sequencing.

Isolate Name	Query Length (bp)	Nearest known species^a	Percent identity (%)	Identification^b (Scientific Name)	NCBI GenBank accession number
EIZ2	1332	<i>Streptomyces bacillaris</i> (T) strain NBRC 13487 (NR 041146.1)	99.06	<i>Streptomyces bacillaris</i>	OR 731196

^aNCBI's nucleotide BLAST program was used to find the closest match against the non-redundant reference RNA sequence database (refseq_rna). The strain number, strain type (T), and GenBank accession number are all listed after the species name.

^bIsolates with a greater percentage of identity than 98.78% and only one closest match for the same identity characteristics are classified up to the species level.

References

1. Bouaziz, S.; Messis, A.; Bettache, A.; El Hadj, M.D.O.; Benallaoua, E.S. Antifungal Activity of *Streptomyces* sp. 14 Strain Isolated from Ouargla (Southeast of Algeria): Identification, Production and Characterization of the Active Substance. *Int. J. Biosci* **2016**, *9*, 45–56.
2. Das, R.; Romi, W.; Das, R.; Sharma, H.K.; Thakur, D. Antimicrobial Potentiality of *Actinobacteria* Isolated from Two Microbiologically Unexplored Forest Ecosystems of Northeast India. *BMC Microbiol.* **2018**, *18*, 1–16.
3. Thakur, D.; Yadav, A.; Gogoi, B.K.; Bora, T.C. Isolation and Screening of *Streptomyces* in Soil of Protected Forest Areas from the States of Assam and Tripura, India, for Antimicrobial Metabolites. *J. Mycol. Med.* **2007**, *17*, 242–249.
4. Lee, E.J.; Hwang, K.Y.; Lee, H.-S.; Chung, N. Characterization of a New *Streptomyces* sp. A1022 as a Potential Biocontrol Agent. *J. Korean Soc. Appl. Biol. Chem.* **2011**, *54*, 488–493.
5. Badji, B.; Riba, A.; Mathieu, F.; Lebrihi, A.; Sabaou, N. Activité Antifongique d'une Souche d'*Actinomadura* d'origine Saharienne Sur Divers Champignons Pathogènes et Toxinogènes. *J. Mycol. Med.* **2005**, *15*, 211–219.