**Isolation and characterisation of *Streptomyces sp.* strain GLD25 with antimicrobial and antioxidant effects from** **Gueldaman cave (GLD1), Akbou-Algeria**

Fatima Zohra Djebbah 1,\* Naif Abdullah Al-Dhabi2,\* Mariadhas Valan Arasu2, Larbi Belyagoubi 3, Farid Kherbouche 4, Djamel Eddine Abdelouahid1, Balasubramani Ravindran5

1Laboratoire de Microbiologie Appliquée à l’Agro-alimentaire, au biomédical et à l’Environnement (LAMAABE), Département de Biologie, Université Abou Bekr Belkaid, BP 119, Imama, 13000 Tlemcen, Algeria.

2Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box

2455, Riyadh 11451, Saudi Arabia

3Laboratoire des Produits Naturels (LAPRONA), Département de Biologie, Université Abou Bekr Belkaid, BP 119, Imama, 13000 Tlemcen, Algeria

4Centre National de Recherches Préhistoriques, Anthropologiqes et Historiques (CNRPAH), 3 rue Franklin Roosevelt, 16000 Alger, Algeria

5Department of Environmental Energy and Engineering, Kyonggi University Youngtong-Gu, Suwon, Gyeonggi-Do, 16227, Republic of Korea

Correspondions Autours :

fatimazohra.djebbah@univ-tlemcen.dz; naldhabi@ksu.edu.sa

**The information of the media composition is given below.**

1. Media ingredients/ 1000 mL distilled water, pH=7±0,2

**ISP1:** tryptone (5 g), yeast extract (3 g), agar (15 g).

**ISP2:** yeast extract (4 g), glucose (4 g), malt extract (10 g), agar (15 g).

**ISP3:** Oatmeal (20 g), agar (15 g).

**ISP4:** K2HP04 (1g), MgS04.7HzO (1g), NaCl (1g), (NH4)2S04 (2.0g), CaC03(2g), salts solution1 (1mL), agar (15 g).

**ISP5:** L-asparagine (1g), glycerol (10g), salts solution1 (1mL), agar (15 g).

**ISP6:** peptone (15g), proteose peptone (5g),ferric ammonium citrate (0,5g),sodium thiosulfate (0,08g),yeast extract (1g), K2HPO4 (1g), agar (15 g).

**ISP7:** Glycerol (10g), L-tyrosine (0,5g), L- asparagine (1g), K2HP04 (0,5g), MgS04.7HzO (0,5g), NaCl (0,5g), FeSO4.7H2O (0,01g), agar (15 g).

**ISP9:** KH2PO4 (2,38g), K2HPO4 (5,65g), MgSO4.7H2O (1g), (NH4)2 SO4 (2,64g), salts solution2 (1ml), agar (15 g).

**Salts solution 1:** FeSO4.7H2O (1g),ZnSO4.7H2O (1g),MnCl2.4H2O (1g).

**Salts solution 2:** FeSO4.7H2O (1,1g), CuSO4.5H2O (6,4g),ZnSO4.7H2O (1,5g),MnCl2.4H2O (7,9g).

**GYP (GLP):** Extrait de levure (5g), peptone (5g), glucose (3g), CaCO3 (7,5g), Agar (15g).

**GLM:** Yeast extract (3 g), malt extract (3 g), peptone (10 g), glucose (20 g), agar (15 g).

**M2 (Wiliams medium):** starch(10 g), casein(0.3 g), potassium nitrate (2 g), sodium chloride (2 g), dipotassium phosphate (2 g), magnesium sulphate (0.05 g), calcium carbonate (0.02g), FeSO4.7H2O (0.01 g), agar (15 g).

**AF:** yeast extract (4g), malt extract (10 g), glucose (2 g), sodium chloride (2.5 g), calcium carbonate (1 g), agar (15 g).

**Bennett:** yeast extract (1g), beef extract (1g), casein peptone (2 g), glucose (10 g), agar (15 g).

**Table S1.** Physiological and biochemical characters of GLD25

|  |
| --- |
| **Biochemical tests** |
| Degradation of Starch | **+** |
| Degradation of Casein | **++** |
| Degradation of Gelatin  | **+** |
| Degradation of Glucose | **-** |
| Degradation Lactose | **-** |
| Degradation Saccharose | **+** |
| Degradation Citrate | **+++** |
| H2S production | **-** |
| Production of Urease | **+** |
| Mannitol | **-** |
| Catalase | **++** |
| Action on Skimmed milk  | **-** |
| **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% | **+** |

-: Absent; +: Moderate; ++: Good; +++: Signifiant.

**Table S2.** Results of media optimization

|  |  |  |
| --- | --- | --- |
| **Tested microbes** | **Inhibition zone (mm)±SD of the strain** | **Inhibition zone (mm)±SD** of the standard compounds |
| **Bennett medium**  | **M2 medium** | **GLM medium** | **AF medium** | **Streptomycin****(10µg/disc)** | **Nystatin****(100µg/disc)** |
| *Staphylococcus aureus* (ATCC 6538) | 12±0 | 00±0 | 00±0 | 00±0 | 20±0 | ND |
| *Bacillus cereus* (ATCC 25921) | 20±1 | 00±0 | 00±0 | 00±0 | 21±0 | ND |
| *Bacillus subtilis* (ATCC 6633) | 14±0 | 00±0 | 00±0 | 00±0 | 21±0 | ND |
| *Escherichia coli* (CIP 53.126/ ATCC 8739) | 00±0 | 00±0 | 00±0 | 00±0 | 18±0 | ND |
| *Pseudomonas aeruginosa* (ATCC 27853) | 00±0 | 13±1 | 00±0 | 00±0 | 15±0 | ND |
| *Klebsiella pneumoniae* (IBMC Strasbourg) | 00±0 | 00±0 | 00±0 | 00±0 | 14±0 | ND |
| *Candida albicans* (CIP 444) | 00±0 | 00±0 | 00±0 | 00±0 | ND | 25±0 |
| *Candida albicans* (ATCC 10231) | 00±0 | 00±0 | 00±0 | 00±0 | ND | 20±0 |
| *Aspergillus fumigatus* (MNHN 566) | 00±0 | 00±0 | 00±0 | 00±0 | ND | 30±0 |
| *Fusarium oxysporum* (MNHN 963917) | 00±0 | 00±0 | 00±0 | 00±0 | ND | 11±0 |

ATCC : American Type Culture Collection ; [MNHN : Muséum National d’Histoire Naturelle ; CIP : [Collection de l’Institut Pasteur](https://www.pasteur.fr/fr/sante-publique/biobanques-collections/collection-institut-pasteur-cip);IBMC :Institut de Biologie Moléculaire et Cellulaire;](https://www.mnhn.fr/) ND: not determined.

**Table S3.** determination of antimicrobial activity by well diffusion method

|  |  |  |  |
| --- | --- | --- | --- |
| **Tested strains** | **Inhibition zone (mm) ±SD of the****crude extract****3mg/well** | **Inhibition zone (mm) ±SD of the Streptomycine****10µg/well** | **Inhibition zone (mm) ±SD of the Nystatin****100µg/well** |
| *Staphylococcus aureus* (ATCC 6538P) | 30±0 | 17±0 | ND |
| *Bacillus cereus* (ATCC 25921) | 23±1 | 19±0 | ND |
| *Bacillus subtilis* (ATCC 6633) | 30±1 | 23±0 | ND |
| *Escherichia coli* (CIP 53.126/ ATCC 8739) | 18±1 | 20±0 | ND |
| *Klebsiella pneumoniae* (IBMC Strasbourg) | 12±1 | 16±0 | ND |
| *Pseudomonas aeruginosa* (ATCC 27853) | 08±0 | 15±0 | ND |
| *Candida albicans* (ATCC 10231)  | 12±1 | ND | 20±0 |
| *Candida albicans* (CIP 444) | 13±1 | ND | 23±0 |
| *Aspergillus fumigatus* (MNHN 566) | ND | ND | ND |
| *Fusarium oxysporum* (MNHN 963917) | 9±0 | ND | 11±0 |

ND: not determined.

**Table S4.** Metabolites composition of GLD25 using GC-MS analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.no | Name | RT | Area% | Area |
| 1 | 9-oxononanoic acid | 9.54 | 1.120 | 448714 |
| 2 | Butanoic acid | 12.25 | 1.020 | 407765 |
| 3 | Lactic acid | 12.73 | 2.130 | 852173 |
| 4 | Benzeneacetic acid | 19.66 | 5.250 | 2101398 |
| 5 | Propanoic acid | 21.27 | 1.290 | 517767 |
| 6 | Decanoic acid | 23.74 | 0.520 | 207130 |
| 7 | 3-(3,5-di-tert-butyl-4- hydroxyphenyl)propionic acid | 25.91 | 4.420 | 1220993 |
| 8 | Undecanoic acid | 28.16 | 1.070 | 426898 |
| 9 | 2-bromosebacic acid | 28.57 | 0.570 | 226880 |
| 10 | 6-hydroxy-heptanoic acid | 29.83 | 23.490 | 9410264 |
| 11 | 9-eicosene | 32.82 | 0.640 | 258225 |
| 12 | Tetradecanoic acid | 33.21 | 2.570 | 1030586 |
| 13 | D-glucose | 34.82 | 1.420 | 569530 |
| 14 | 1h-indole-2-carboxylic acid | 35.36 | 1.340 | 535208 |
| 15 | 1-octadecanol | 37.10 | 0.570 | 227581 |
| 16 | Hexadecanoic acid | 37.38 | 5.610 | 2246713 |
| 17 | Propanoic acid | 38.17 | 0.540 | 215975 |
| 18 | 11-eicosaenoic acid | 38.95 | 0.550 | 222014 |
| 19 | Heptadecanoic acid | 39.39 | 1.580 | 631423 |
| 20 | Octadecanoic acid | 41.65 | 1.950 | 781317 |
| 21 | Acetyl tri-n-butyl citrate | 41.87 | 1.990 | 796008 |
| 22 | Androst-5-en-16-one | 42.19 | 0.910 | 364219 |
| 23 | Myristin | 42.35 | 0.910 | 365149 |
| 24 | Oleamide | 44.53 | 0.190 | 74297 |
| 25 | Diisooctyl-phthalate | 46.49 | 30.660 | 12282666 |
| 26 | Palmitinic acid | 47.08 | 2.650 | 1060958 |
| 27 | 2-monostearin | 49.34 | 0.250 | 98519 |
| 28 | Stearinic acid | 49.84 | 4.720 | 1890565 |
| 29 | Erucylamide | 50.17 | 0.730 | 292671 |

**A**

7.55

12.55

17.55

22.55

27.55

32.55

37.55

42.55

47.55

52.55

57.55

Time

0

100

%

31.78

18.89

25.90

46.49

36.60

**Fig.S1.** Gas chromatography spectrometry (GC-MS) spectrum of the metabolite from GLD25