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

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**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