**Identification and Antifungal Mechanism of a Novel Actinobacterium *Streptomyces huiliensis* sp. nov. against *Fusarium Oxysporum* f. sp. *Cubense* Tropical Race 4 of Banana**

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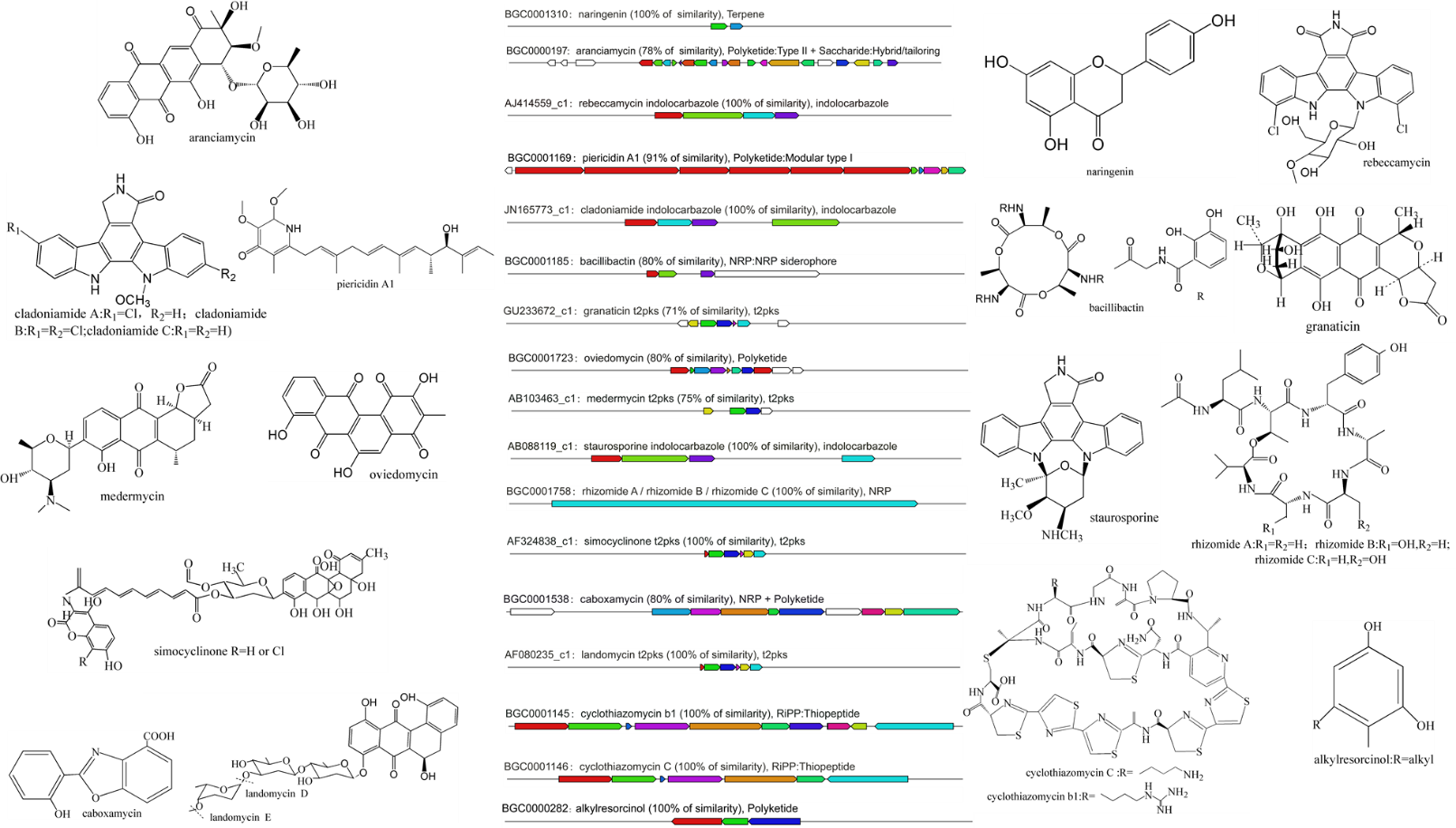


Figure S1

Table S1 Allele sequence accession numbers of the Streptomyces used for the present study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Strains** | **atpD** | **gyrB** | **recA** | **rpoB** | **trpB** |
| *Streptomyces huiliensis* | SCA2-4 T | JAIQLH000000000 | JAIQLH000000000 | JAIQLH000000000 | JAIQLH000000000 | JAIQLH000000000 |
| *Streptomyces mobaraensis* | NBRC 13819 T | NZ\_AORZ01000143.1 | NZ\_AORZ01000007.1 | AORZ01000041.1 | NZ\_AORZ01000093.1 | NZ\_AORZ01000083.1 |
| *Streptomyces abikoensis* | NBRC 13860 T | NZ\_BMRT01000003.1 | NZ\_BMRT01000009.1 | BMRT01000001.1 | NZ\_BMRT01000012.1 | NZ\_BMRT01000008.1 |
| *Streptomyces orinoci* | NRRL\_B-3379 T | NZ\_PHNC01000031.1 | NZ\_PHNC01000031.1 | NZ\_PHNC01000035.1 | NZ\_PHNC01000029.1 | NZ\_PHNC01000029.1 |
| *Streptomyces angustmyceticus* | NRRL B-2347 T | NZ\_MUAY01000056.1 | NZ\_MUAY01000018.1 | NZ\_MUAY01000653.1 | NZ\_MUAY01000135.1 | NZ\_MUAY01000591.1 |
| *Streptomyces griseocarneus (Streptomyces alboverticillatus)* | NRRL B-24281 T | NZ\_MUFU01000328.1 | NZ\_MUFU01000060.1 | NZ\_MUFU01000022.1 | NZ\_MUFU01000051.1 | NZ\_MUFU01000181.1 |
| *Streptomyces catenulae* | NRRL B-2342 T | NZ\_JODY01000004.1 | NZ\_JODY01000023.1 | NZ\_JODY01000039.1 | NZ\_JODY01000013.1 | NZ\_JODY01000007.1 |
| *Streptomyces ochraceiscleroticus* | NRRL ISP-5594 T | NZ\_JOAX01000003.1 | NZ\_JOAX01000001.1 | NZ\_JOAX01000012.1 | NZ\_JOAX01000002.1 | NZ\_JOAX01000011.1 |
| *Streptomyces varsoviensis* | NRRL ISP-5346 T | NZ\_JOBF01000022.1 | NZ\_JOBF01000004.1 | NZ\_JOBF01000041.1 | NZ\_JOBF01000008.1 | NZ\_JOBF01000015.1 |
| *Streptomyces caniferus* | NBRC 15389 T | NZ\_BLIN01000002.1 | NZ\_BLIN01000005.1 | NZ\_BLIN01000002.1 | NZ\_BLIN01000005.1 | NZ\_BLIN01000005.1 |
| *Streptomyces sioyaensis* | NRRL B-5408 T | NZ\_JABZEL010000008.1 | NZ\_JABZEL010000009.1 | NZ\_JABZEL010000008.1 | NZ\_JABZEL010000009.1 | NZ\_JABZEL010000016.1 |
| *Streptomyces rimosus* subsp*. rimosus* | ATCC 10970 T | NZ\_ANSJ01000029.1 | NZ\_ANSJ01000024.1 | NZ\_ANSJ01000088.1 | NZ\_ANSJ01000031.1 | NZ\_ANSJ01000105.1 |
| *Streptomyces libani* subsp.*libani* | NBRC 13452 T | NZ\_BLIP01000001.1 | NZ\_BLIP01000001.1 | NZ\_BLIP01000001.1 | NZ\_BLIP01000001.1 | NZ\_BLIP01000001.1 |
| *Streptomyces xinghaiensis* | S187 T | NZ\_CP023202.1 | NZ\_CP023202.1 | NZ\_CP023202.1 | NZ\_CP023202.1 | NZ\_CP023202.1 |
| *Streptomyces nigrescens* | AS 4.1410（NBRC 12894）T | FJ406135.1 | FJ406191.1 | FJ406247.1 | FJ406303.1 | FJ406358.1 |
| *Streptomyces pseudoechinosporeus* | NBRC 12518 (NRRL B-16931) T | KT384693.1 | KT385043.1 | KT385394.1 | KT389014.1 | KT389362.1 |
| *Streptomyces hiroshimensis* | NRRL B-1823（NBRC 3839）T | KT384596.1 | KT384945.1 | KT385294.1 | KT388916.1 | KT389265.1 |

Table S2 MLSA distance values for selected strains in this study

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Strains | MLSA distance (Kimura two-parameter) | | | | | | | | | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | 0.024 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 | 0.066 | 0.065 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 4 | 0.619 | 0.625 | 0.607 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 5 | 0.079 | 0.082 | 0.085 | 0.631 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 6 | 0.068 | 0.073 | 0.041 | 0.597 | 0.079 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7 | 0.081 | 0.086 | 0.076 | 0.630 | 0.054 | 0.078 |  |  |  |  |  |  |  |  |  |  |  |
| 8 | 0.085 | 0.087 | 0.084 | 0.609 | 0.069 | 0.085 | 0.068 |  |  |  |  |  |  |  |  |  |  |
| 9 | 0.076 | 0.081 | 0.079 | 0.601 | 0.082 | 0.071 | 0.080 | 0.081 |  |  |  |  |  |  |  |  |  |
| 10 | 1.020 | 1.019 | 1.011 | 1.092 | 1.042 | 1.003 | 1.024 | 1.015 | 0.991 |  |  |  |  |  |  |  |  |
| 11 | 0.081 | 0.081 | 0.088 | 0.623 | 0.039 | 0.086 | 0.063 | 0.066 | 0.081 | 1.030 |  |  |  |  |  |  |  |
| 12 | 0.082 | 0.088 | 0.082 | 0.606 | 0.073 | 0.083 | 0.071 | 0.061 | 0.077 | 1.026 | 0.070 |  |  |  |  |  |  |
| 13 | 0.089 | 0.089 | 0.089 | 0.635 | 0.040 | 0.087 | 0.064 | 0.073 | 0.088 | 1.055 | 0.035 | 0.070 |  |  |  |  |  |
| 14 | 0.623 | 0.623 | 0.622 | 0.790 | 0.645 | 0.612 | 0.630 | 0.627 | 0.621 | 0.291 | 0.636 | 0.622 | 0.651 |  |  |  |  |
| 15 | 0.608 | 0.613 | 0.626 | 0.140 | 0.626 | 0.620 | 0.645 | 0.626 | 0.604 | 1.137 | 0.620 | 0.631 | 0.625 | 0.809 |  |  |  |
| 16 | 0.617 | 0.620 | 0.632 | 0.131 | 0.609 | 0.625 | 0.642 | 0.643 | 0.625 | 1.132 | 0.611 | 0.630 | 0.587 | 0.798 | 0.117 |  |  |
| 17 | 0.608 | 0.617 | 0.602 | 0.093 | 0.618 | 0.594 | 0.611 | 0.603 | 0.591 | 1.090 | 0.617 | 0.603 | 0.623 | 0.774 | 0.123 | 0.122 |  |

Strains 1.*Streptomyces huiliensis* SCA2-4 T, 2. *Streptomyces mobaraensis* NBRC 13819 T, 3. *Streptomyces abikoensis* NBRC 13860 T, 4. *Streptomyces orinoci* NBRC 13466 T, 5. *Streptomyces angustmyceticus* NRRL B-2347 T, 6. *Streptomyces griseocarneus* (*Streptomyces alboverticillatus*) NRRL B-24281 T, 7. *Streptomyces catenulae* NRRL B-2342 T, 8. *Streptomyces ochraceiscleroticus* NRRL ISP-5594 T, 9. *Streptomyces varsoviensis* NRRL ISP-5346 T, 10. *Streptomyces caniferus* NBRC 15389 T, 11. *Streptomyces sioyaensis* NRRL B-5408 T, 12. *Streptomyces rimosus* subsp.*rimosus* ATCC 10970 T, 13. *Streptomyces libani* subsp.*libani* NBRC 13452 T, 14. *Streptomyces xinghaiensis* S187 T, 15. *Streptomyces pseudoechinosporeus* NBRC 12518 (NRRL B-16931) T, 16. *Streptomyces nigrescens* NBRC AS 4.1410 (12894) T, 17. *Streptomyces hiroshimensis* NRRL B-1823 (NBRC 3839) T

Table S3 Nitrogen-source utilization and antibiotic sensitivity of strain SCA2-4T

|  |  |
| --- | --- |
| **Characteristics** | **Results** |
| **Growth on sole nitrogen sources (1.0%, w/v)** |  |
| L-phenylalanine | + |
| Ammonium sulfate | + |
| L-hydroxyproline | + |
| L (+)-cysteine | + |
| Histidine | + |
| Glycine | + |
| Valine | + |
| Ammonium oxalate | + |
| Ammonium acetate | - |
| Ammonium nitrate | - |
| Ammonium molybdate tetrahydrate | - |
| L-arginine | - |
| Glutamate | - |
| **Antibiotic sensitivity (µg/ml)** |  |
| Ampicillin (0.5) | R |
| Chloramphenicol (1.5) | R |
| Streptomycin (0.5) | R |
| Penicillin-G (0.5) | R |
| Gentamicin (1.0) | R |
| Nystatin (5) | R |
| Tetracycline (1.5) | R |
| Neomycin sulfate (0.5) | R |
| Kanamycin sulfate (0.5) | R |
| Rifampicin (0.5) | S |

+: positive reaction; –: negative reaction; S: sensitivity; R: resistance.

Table S4 Functional cluster of orthologous genes (COG) classification of predicted genes in strain SCA2-4T

|  |  |  |  |
| --- | --- | --- | --- |
| **COG functional categories** | **Type** | **Gene No.** | % of Gene No. |
| **Information storage and processing** |  | **921** | **17.58%** |
| RNA processing and modification | A | 1 |  |
| Chromatin structure and dynamics | B | 1 |  |
| Translation, ribosomal structure and biogenesis | J | 180 |  |
| Transcription | K | 539 |  |
| Replication, recombination and repair | L | 200 |  |
| **Metabolism** |  | **1795** | **34.26%** |
| Energy production and conversion | C | 282 |  |
| Amino acid transport and metabolism | E | 385 |  |
| Nucleotide transport and metabolism | F | 88 |  |
| Carbohydrate transport and metabolism | G | 282 |  |
| Coenzyme transport and metabolism | H | 153 |  |
| Lipid transport and metabolism | I | 181 |  |
| Inorganic ion transport and metabolism | P | 216 |  |
| Secondary metabolites biosynthesis, transport and catabolism | Q | 208 |  |
| **Cellular processes and signaling** |  | **748** | **14.28%** |
| Cell cycle control, cell division, chromosome partitioning | D | 31 |  |
| Cell wall/membrane/envelope biogenesis | M | 179 |  |
| Cell motility | N | 1 |  |
| Posttranslational modification, protein turnover, chaperones | O | 141 |  |
| Signal transduction mechanisms | T | 250 |  |
| Intracellular trafficking, secretion, and vesicular transport | U | 35 |  |
| Defense mechanisms | V | 111 |  |
| **Poorly characterized** |  | **1775** | **33.88%** |
| Function unknown | S | 1775 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cluster ID** | **Type** | **genomic location** | | **Gene No.** | **Most similar known cluster** | **similarity** |
| **Start** | **End** |
| Cluster1 | PKS III-siderophore | 63608 | 104726 | 45 | naringenin biosynthetic gene cluster | 100% |
| Cluster2 | fused | 33740 | 56237 | 22 | ketomemicin B3 / ketomemicin B4 biosynthetic gene cluster | 100% |
| Cluster3 | arylpolyene | 108244 | 130094 | 25 | hygromycin A biosynthetic gene cluster | 31% |
| Cluster4 | terpene | 32261 | 53172 | 22 | 2-methylisoborneol biosynthetic gene cluster | 100% |
| Cluster5 | lantipeptide-lassopeptide | 55186 | 94236 | 39 | moomysin biosynthetic gene cluster | 50% |
| Cluster6 | PKS III | 39841 | 80933 | 44 | - | - |
| Cluster7 | lassopeptide | 1589 | 24191 | 30 | anantin C biosynthetic gene cluster | 75% |
| Cluster8 | terpene | 94506 | 106083 | 10 | - | - |
| Cluster9 | PKS I | 29003 | 73058 | 37 | 5-isoprenylindole-3-carboxylate β-D-glycosyl ester biosynthetic gene cluster | 19% |
| Cluster10 | lantipeptide-NRPS | 1 | 58889 | 47 | SapB biosynthetic gene cluster | 75% |
| Cluster11 | PKS II | 1 | 29654 | 28 | aranciamycin biosynthetic gene cluster | 78% |
| Cluster12 | other | 65173 | 88774 | 27 | salinamide A biosynthetic gene cluster | 21% |
| Cluster13 | ladderane | 1194 | 43602 | 36 | 5-isoprenylindole-3-carboxylate β-D-glycosyl ester biosynthetic gene cluster | 14% |
| Cluster14 | PKS I | 29840 | 73751 | 42 | meilingmycin biosynthetic gene cluster | 3% |
| Cluster15 | PKS I | 45204 | 83115 | 35 | - | - |
| Cluster16 | other | 1 | 31807 | 23 | - | - |
| Cluster17 | indole | 48576 | 72322 | 19 | AT2433/Rebeccamycin/Staurosporine/K-252a/Cladoniamide biosynthetic gene cluster | >75% |
| Cluster18 | siderophore | 3215 | 14909 | 11 | kinamycin biosynthetic gene cluster | 20% |
| Cluster19 | PKS I | 1 | 32504 | 32 | hygromycin A biosynthetic gene cluster | 6% |
| Cluster20 | PKS I | 1 | 60356 | 48 | A-503083 A / A-503083 B / A-503083 E / A-503083 F biosynthetic gene cluster | 7% |
| Cluster21 | PKS III | 1 | 35286 | 29 | violapyrone B biosynthetic gene cluster | 28% |
| Cluster22 | PKS III-terpene | 8574 | 64323 | 48 | flaviolin biosynthetic gene cluster | 50% |
| Cluster23 | terpene | 8554 | 29790 | 19 | - | - |
| Cluster24 | NRPS | 1 | 31692 | 27 | bacillibactin biosynthetic gene cluster | 80% |
| Cluster25 | lassopeptide | 1 | 46295 | 54 | lagmysin biosynthetic gene cluster | 80% |
| Cluster26 | terpene-NRPS | 1 | 56046 | 40 | geosmin biosynthetic gene cluster | 100% |
| Cluster27 | terpene | 2849 | 23769 | 18 | - | - |
| Cluster28 | terpene | 1 | 38268 | 42 | curacomycin biosynthetic gene cluster | 15% |
| Cluster29 | PKS III | 10816 | 45677 | 29 | flaviolin biosynthetic gene cluster | 50% |
| Cluster30 | NRPS | 1560 | 44843 | 40 | arylomycin biosynthetic gene cluster | 22% |
| Cluster31 | bacteriocin | 14255 | 25119 | 14 | - | - |
| Cluster32 | PKS I | 1 | 29812 | 19 | piericidin A1 biosynthetic gene cluster | 91% |
| Cluster33 | otherks | 11880 | 39620 | 11 | - | - |
| Cluster34 | PKS I -NRPS | 1 | 38417 | 17 | microsclerodermin biosynthetic gene cluster | 21% |
| Cluster35 | PKS II | 1 | 32445 | 33 | Oviedomycin/Landomycin/Simocyclinone/Granaticin/Medermycin biosynthetic gene cluster | >71% |
| Cluster36 | siderophore | 17638 | 29297 | 12 | - | - |
| Cluster37 | terpene | 14701 | 33076 | 25 | - | - |
| Cluster38 | NRPS | 1 | 29103 | 23 | rhizomide A / rhizomide B / rhizomide C biosynthetic gene cluster | 100% |
| Cluster39 | lantipeptide | 1 | 14141 | 18 | - | - |
| Cluster40 | PKS I | 1 | 24417 | 20 | A54145 biosynthetic gene cluster | 6% |
| Cluster41 | lantipeptide | 1 | 21354 | 16 | chejuenolide A / chejuenolide B biosynthetic gene cluster | 7% |
| Cluster42 | PKS I | 1 | 19986 | 7 | piericidin A1 biosynthetic gene cluster | 50% |
| Cluster43 | terpene | 945 | 19474 | 19 | hopene biosynthetic gene cluster | 53% |
| Cluster44 | PKS I | 1 | 19130 | 10 | - | - |
| Cluster45 | NRPS | 1 | 16852 | 15 | matlystatin A biosynthetic gene cluster | 38% |
| Cluster46 | thiopeptide | 1 | 16821 | 12 | cyclothiazomycin b1/ cyclothiazomycin c biosynthetic gene cluster | 100% |
| Cluster47 | otherks | 1 | 16490 | 12 | caboxamycin biosynthetic gene cluster | 80% |
| Cluster48 | NRPS | 1 | 14825 | 2 | cysteoamide biosynthetic gene cluster | 18% |
| Cluster49 | PKS III | 1 | 9899 | 8 | alkylresorcinol biosynthetic gene cluster | 100% |
| Cluster50 | other | 1 | 9715 | 9 | cadaside A / cadaside B biosynthetic gene cluster | 19% |
| Cluster51 | terpene | 1 | 7754 | 6 | - | - |

Table S5 Biosynthetic gene clusters of secondary metabolites of strain SCA2-4*T* on the online antiSMASH v 6.0.1

Table S6 Cluster number and gene number shown in different cluster types of strain SCA2-4T

|  |  |  |
| --- | --- | --- |
| **Cluster Type** | **Cluster Number** | **Gene Number** |
| PKS I | 9 | 250 |
| terpene | 8 | 161 |
| NRPS | 5 | 107 |
| PKS III | 4 | 110 |
| other | 3 | 59 |
| lassopeptide | 2 | 84 |
| PKS II | 2 | 61 |
| siderophore | 2 | 23 |
| otherks | 2 | 23 |
| lantipeptide | 2 | 34 |
| PKS III-siderophore | 1 | 45 |
| fused | 1 | 22 |
| arylpolyene | 1 | 25 |
| lantipeptide-lassopeptide | 1 | 39 |
| lantipeptide-NRPS | 1 | 47 |
| ladderane | 1 | 36 |
| indole | 1 | 19 |
| PKS III-terpene | 1 | 48 |
| terpene-NRPS | 1 | 40 |
| bacteriocin | 1 | 14 |
| PKS I-NRPS | 1 | 17 |
| thiopeptide | 1 | 12 |
| **total** | **51** | **1276** |

Table S7 Inhibitory activity and MIC of extracts of strain SCA2-4 T against Foc TR4

|  |  |
| --- | --- |
| **Index** | **results** |
| Inhibition zone (mm) | 31.83±2.36 |
| Mycelial inhibition (%) | 42.47 |
| MIC of extracts (µg/ml) | >6.25 |
| MIC of cycloheximide (µg/ml) | >1.563 |
| MIC of nystatin (µg/ml) | >6.25 |