**A new albomycin-producing strain of *Streptomyces globisporus* may provides protection for ants *Messor structor***

***Supplementary Materials***

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**Table S1.** The isolation of Gram-positive bacteria strains from *Messor structor* individuals

|  |  |  |  |
| --- | --- | --- | --- |
| Caste | Isolated strains | Frequency of occurrence1,% | Identified as |
| Solders | M1, S1, S3, S4, S5, S51, Mdom1, Mdom2, Mdom3 (4-3) | 89 | *Streptomyces globisporus* subsp. *globisporus* strain 4-32 |
| Am1, Am2, Mr | 44 | *Staphylococcus gallinarum* L13 |
| Workers | M1, S1, S3, S4, S5, S51, Mdom1, Mdom2 | 50 | *Streptomyces globisporus* subsp. *globisporus* strain 4-3 |
| Am1, Am2, Mr | 88 | *Staphylococcus gallinarum* L1 |
| Larvae | MdomX1 | 7 | *Streptomyces globisporus* subsp. *globisporus* strain 4-3 |
| L1 | 85 | *Staphylococcus gallinarum* L1 |
| Pupae | MdomX1, MdomX2 | 21 | *Streptomyces globisporus* subsp. *globisporus* strain 4-3 |
| L1 | 85 | *Staphylococcus gallinarum* L1 |

1 -- the proportion of individuals of the studied caste in which this strain was isolated,

2 -- GenBank accession number for 16S rRNA gene sequence is ON326616,

3 -- GenBank accession number is ON326604

**Table S2.** General features of the genome of strain 4-3 and its closely related strains of *Streptomyces globisporus*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Genomic features  | 1 | 2 | 3 | 4 | 5 |
| Size (Mbp)  | 7,941,828 | 8,246,570 | 7,237,318 | 7,548,572 | 7,332,794 |
| Total gene  | 7,449 | 7,410 | 6,729 | 6,822 | 6,762 |
| Contigs | 139 | 59 | 594 | 54 | 51 |
| G +C content (%)  | 71.6 | 71.4 | 71.4 | 71.7 | 71.7 |
| No. of rRNA clusters | 3 | 1 | 5 | 3 | 5 |
| No. of tRNA clusters  | 65 | 68 | 63 | 65 | 63 |
| Contig N50 | 375,468 | 434,455 | 22,344 | 268,800 | 459,850 |
| Contig L50 | 9 | 6 | 100 | 7 | 5 |
| No. proteins | 7,142 | 7,495 | 6,258 | 6,622 | 6,593 |
| Completeness of genome\*, % | 99.1 | 99.1 | 95.3 | 99.1 | 97.2 |
| Quality of genome\*, % | 56.6 | 47.1 | 62.3 | 52.1 | 54.7 |

Strains, (GenBank assembly accessions are indicated in parentheses): 1, 4-3 (SAMEA13759927); 2, *Streptomyces globisporus subsp. globisporus* DSM 40199T (GCA\_014649555.1); 3, *Streptomyces globisporus subsp. globisporus* DSM 40136 (GCA\_002154385.1); 4, *Streptomyces rubiginosohelvolus* DSM 40176T (GCA\_014649875.1); 5, *Streptomyces pluricolorescens* DSM 40019T (GCA\_014650395.1).

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**Figure S1.** Neighbour-joining phylogenetic tree of strain 4-3, related *Streptomyces globisporus* subsp. *globisporus* and some actinobacteria species, firstly isolated from insects. The evolutionary distances were computed from 16S rRNA full-length sequences using the Tamura-Nei method. Bootstrap values for n=1,000 are given at the branching points in percentages for values higher than 60. Bar, 0,01 nucleotide substitutions per site. The analysis involved 17 nucleotide sequences with a total of 1472 positions in the final dataset. *Nocardia camponoti* DSM 100526T was used as the outgroup.

**Figure S2**. Maximum-parsimony phylogenetic tree based on almost complete 16S rRNA gene sequences (1445 positions in the final dataset) showing the position of 4-3 among the related strains of the genus *Streptomyces* and type actinobacterial species isolated from insects. Tree #1 out of 2 most parsimonious trees (length = 353) is shown. The consistency index is (0,661765), the retention index is (0,634921), and the composite index is 0,510814 (0,420168) for all sites and parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). The tree is drawn to scale , with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated.

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**Figure S3**. Maximum-likelihood phylogenetic tree of strain 4-3 and comparison with related strains of the genus *Streptomyces* and type actinobacterial species isolated from insects, based on almost complete 16S rRNA gene sequences. Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-3854,6731) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1445 positions in the final dataset.

**Table S3.** Morphological, physiological and biochemical characteristics of strain 4-3 and closest *Streptomyces globisporus* subsp. *globisporus* strains

|  |  |  |  |
| --- | --- | --- | --- |
|  **Properties** | **1** | **21** | **31** |
| Morphological and physiological characteristics |
| Spore surface | Smooth | Smooth | Smooth |
| Spore chains morphology | RF | RF | RF |
| Temperature-range and optimum (°C) | 8-37 (28) | (28) | (28) |
| NaCl tolerance (%) | 5 | n/d2 | n/d |
| Acid from carbohydrates  |
| Arabinose | + | + | + |
| Dulcite | ﹣ | n/d | n/d |
| Fructose | + | + | + |
| Galactose | + | n/d | n/d |
| Glucose | + | ﹣ | + |
| Inositol | ﹣ | ﹣ | ﹣ |
| Lactose | ﹣ | n/d | n/d |
| Maltose | + | n/d | n/d |
| Mannitol | + | + | + |
|  Raffinose | ﹣ | ﹣ | ﹣ |
| Rhamnose | + | + | + |
| Sorbitol | ﹣ | n/d | n/d |
| Sucrose | ﹣ | ﹣ | ﹣ |
| Xylose | + | ﹣ | ﹣ |
| Degradation of and enzymes |
| Starch | + | + | + |
| Gelatin | + | + | + |
| Cellulose | ﹣ | + | + |
| Urea | + | + | + |
| Citrate | + | + | + |
| β-glucosidase | + | + | + |
| L-ornithine decarboxylase | ﹣ | + | + |
| L-arginine decarboxylase | ﹣ | + | + |
| L-lysine decarboxylase | + | + | + |

1 Data for *Streptomyces globisporus* subsp. *globisporus* DSM 40199T (2) and *Streptomyces globisporus* subsp. *globisporus* DSM 40136T (3) are from<https://bacdive.dsmz.de/>

2 n/d, not determined

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**Figure S4**. Cultural properties of 4-3 on ISP media after 14 days at 28℃.



**Figure S5.** Utilization of different sugars (1.0%, w/v) as sole carbon source by strain 4-3.

Top row: arabinose, base agar without sugar, cellulose, fructose, galactose;

middle row: glucose, inositol, lactose, mannitol, mannose;

bottom row: raffinose, rhamnose, sorbitol, sucrose, xylose.



**Figure S6.** RPHPLC of an active fraction of culture liquid eluted from the LPS500H sorbent and UV spectrum of an active metabolite peak at 11.5 min. Column: Phenomenex Luna 5 μm C18 (2) 100 Å 4.6\*250 mm; eluent: 4% of MeCN, 10 mM AcONH4, Flow rate 1ml/min, Temperature 25C, UV260 nm





**Figure S7.** LC-MS data for albomycin δ2 (top down): UV profile at 287 nm; TIC collected at positive ion mode, MS1 and MS2 for the peak at 7.2 min.

**Table S4.** Some of the secondary metabolite gene clusters in *Streptomyces globisporus* subsp. *globisporus* 4-3

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Region | Type | From (bp) | To (bp) | Most similar known cluster | Similarity, % |
| 1.1 | NRP | 287,765 | 324,504 | Streptophenazines B/C/E/H/G biosynthesis gene cluster | 100 |
| Polyketide | 324,738 | 347,987 | Mayamycin biosynthesis gene cluster | 100 |
| 2.1 | RiPP:Lanthipeptide | 58,226 | 81,129 | Labyrinthopeptins A2/A1/A3 biosynthetic gene cluster | 40 |
| 4.1 | Polyketide | 277,202 | 399,632 | Viguiepinol biosynthetic gene cluster | 73 |
| 7.2 | Siderophore | 84,661 | 96,439 | Desferrioxamine B biosynthetic gene cluster | 100 |
| 8.1 | Terpene | 301,258 | 327,831 | Hopene biosynthetic gene cluster  | 69 |
| 9.1 | RiPP: Lassopeptide | 350,361 | 373,055 | Keywimysin biosynthetic gene cluster | 100 |
| 10.1 | Other: Melanin | 81,003 | 91,476 | Melanin biosynthetic gene cluster | 100 |
| 10.2 | Polyketide | 129,829 | 170,881 | Alkylresorcinol biosynthetic gene cluster | 100 |
| 14.1 | Terpene | 23,415 | 48,421 | Isorenieratene biosynthetic gene cluster | 100 |
| 14.3 | Terpene | 128,324 | 174,416 | Geosmin biosynthetic gene cluster | 100 |
| 14.4 | NRPS: Siderophore | 211,154 | 258,557 | Streptobactin biosynthetic gene cluster | 100 |
| 21.1 | RiPP:Lanthipeptide | 148,549 | 166,688 | Lanthipeptide(s) Putative Class III | 100 |
| 24.1 | NRPS: Siderophore | 1 | 36,289 | Coelichelin biosynthetic gene cluster | 81 |

**Table S5**. Antimicrobial activity of *Streptomyces globisporus* subsp. *globisporus* 4-3, isolated from *Messor structor* ants

|  |  |
| --- | --- |
| Test organisms | Zones of growth inhibition\*, mm |
| *Bacillus subtilis* ATCC 6633 | 5±0,6 |
| *Staphylococcus aureus* ATCC 25923 | 10±2,0 |
| *Aspergillus niger* INA 00760 | 5±0,8 |
| *Candida albicans* CBS 8836 | 0 |
| *Paenibacillus alvei*  VKM B-502 | 20±1,6 |
| *Bacillus thuringiensis* VKM B-6650 | 15±2,4 |
| *Beauveria bassiana* VKM F-1357 | 12±1,4 |
| *Entomophthora coronata* VKM F-1359 | 15±3,6 |

\*   Data are means ± standard error over five replicates.