

Streptomyces smaragdinus* sp. nov., isolated from the gut of the fungus growing-termite *Macrotermes natalensis

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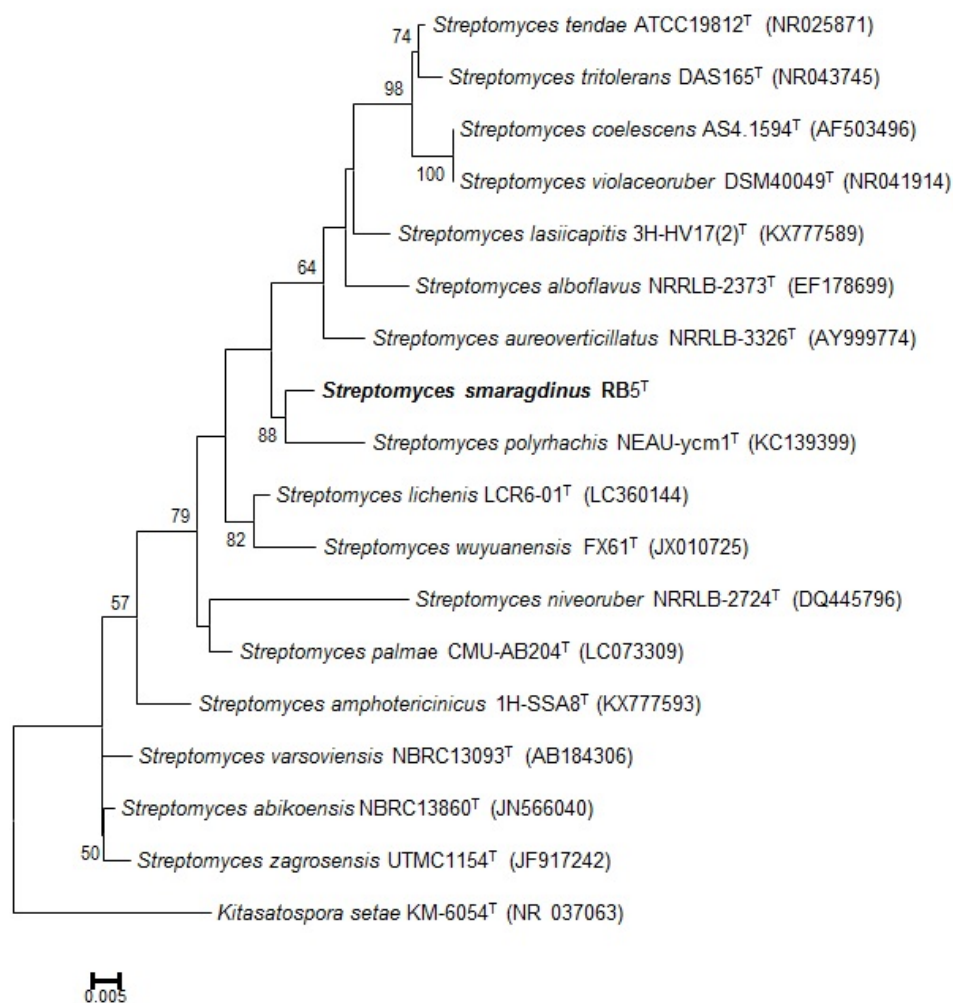


Figure S1. Maximum-likelihood phylogenetic tree showing the phylogenetic relationship of strain RB5^T and other closely related species based on 16S rRNA gene sequences. *Kitasatospora setae* KM-6054^T was used to root the tree. Only bootstrap values above 50% (1000 pseudoreplications) are shown. Bar length corresponds to 0.005 substitutions per nucleotide position.

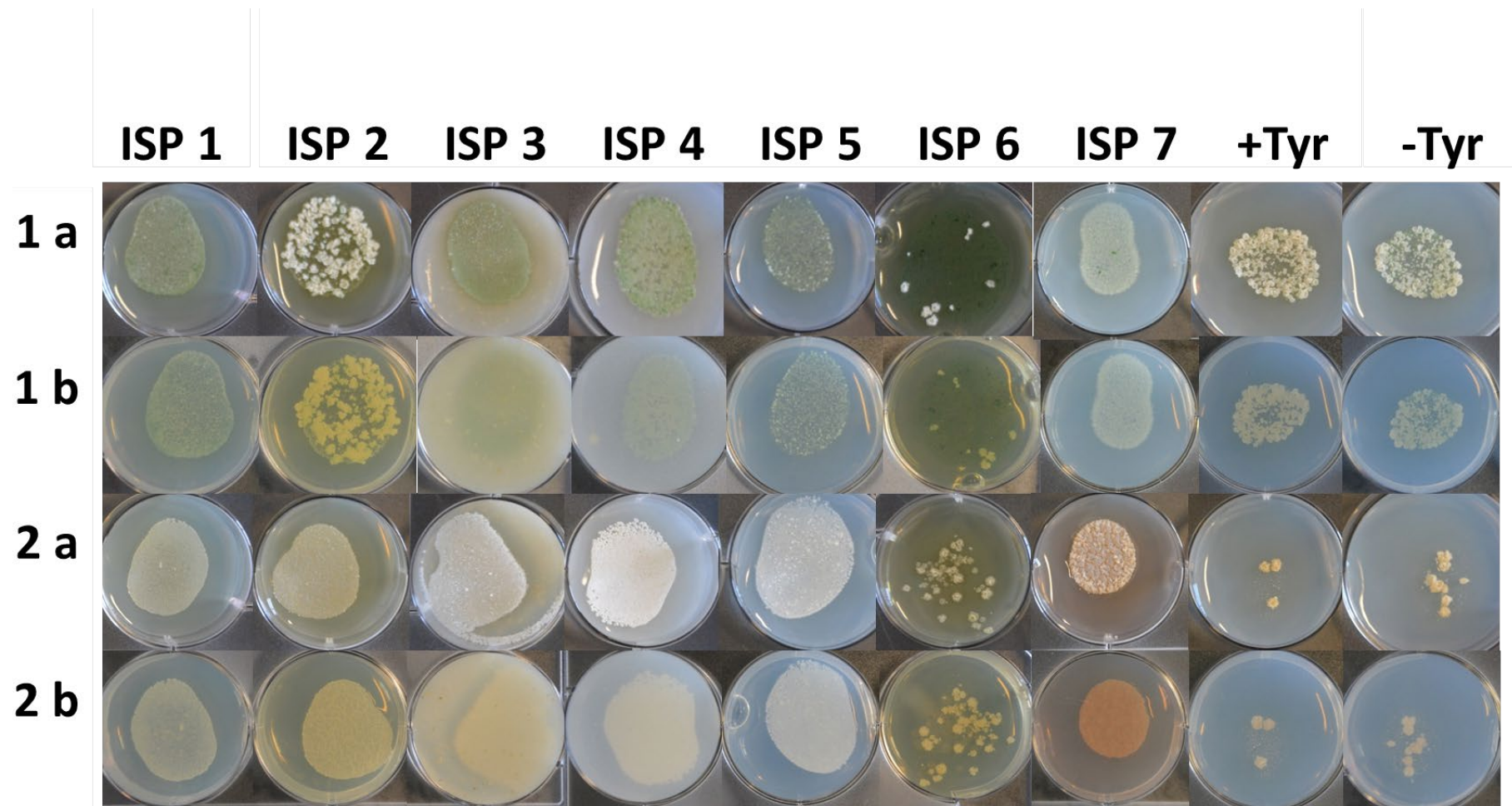


Figure S1. Morphology of strains grown on different ISP-media and Suter-Medium (with: +Tyr [1 g/L] and without tyrosine: -Tyr) for 12 days at 28 °C. Strains: RB5^T (1a: above, 1b: reverse); *Streptomyces polyrhachis* DSM42102^T (2a: above, 2b: reverse).

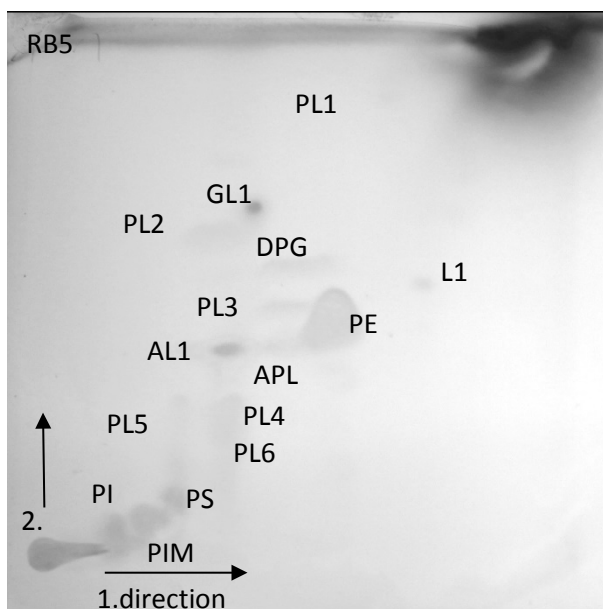


Fig S3. Polar lipid profile of strain RB5^T after two-dimensional TLC and detection with molybdotophosphoric acid. The solvent system consisted of chloroform-methanol-water (65:25:4, by vol.) for the first development and of chloroform-methanol-glacial acetic acid-water (80:12:15:4, by vol.) for second development. DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PS, phosphatidylserine; AL1, unknown aminolipid; APL, aminophospholipid; PL1-PL6, unidentified phospholipids; GL1 unidentified glycolipid; L1, unidentified lipid

Table S1. Calculated sequence similarities of strain RB5^T and *Streptomyces* strains

| Strain (Accession number) | Similarity [%] |
|--|----------------|
| <i>Streptomyces polyrhachis</i> NEAU-ycm1 ^T (KC139399) | 98.87 |
| <i>Streptomyces aureoverticillatus</i> NRRL B-3326 ^T (AY999774) | 98.52 |
| <i>Streptomyces alfalfae</i> XY25 ^T (KR080524.1) | 98.37 |
| <i>Streptomyces variegatus</i> LMG 20315 ^T (AJ781371) | 98.37 |
| <i>Streptomyces tendae</i> ATCC 19812 ^T (D63873) | 98.27 |
| <i>Streptomyces pluripotens</i> MUSC 135 ^T (KF195922) | 98.24 |
| <i>Streptomyces marokkonensis</i> Ap1 ^T (AJ965470) | 98.23 |
| <i>Streptomyces tritolerans</i> DAS 165 ^T (DQ345779.2) | 98.20 |
| <i>Streptomyces coelestis</i> AS 4.1594 ^T (AF503496) | 98.20 |
| <i>Streptomyces violaceoruber</i> CSSB 1016 ^T (AF503492) | 98.20 |
| <i>Streptomyces humiferus</i> DSM 43030 ^T (AF503491) | 98.20 |
| <i>Streptomyces violaceolatus</i> ATCC 19847 ^T (AF503497) | 98.19 |
| <i>Streptomyces intermedius</i> NBRC 13049 ^T (AB184277) | 98.16 |
| <i>Streptomyces gougerotii</i> NBRC 3198 ^T (AB184742) | 98.16 |

^b Sequence similarities were calculated using the method recommended by Meier-Kolthoff (1)

1. Meier-Kolthoff JP, Goker M, Sproer C, Klenk HP. When should a DDH experiment be mandatory in microbial taxonomy? Arch Microbiol. 2013b;195(6):413-8.

Table S2. Summary of *Streptomyces* genome sequenced in this work, including strain ID, genus, total genome size (in mega base pairs), GC content in %, number of assembled contigs (contiguous sequences); **for more details, see**

<https://www.ncbi.nlm.nih.gov/nuccore/1774106366>

| Strain ID | RB5 |
|--|--------------|
| Genus | Streptomyces |
| Total size [Mb] | ~7.9 |
| GC content [%] | 72.00 |
| Number of contigs | 107 |
| N50 [bp] | 161.565 |
| L50 | 13 |
| Total CDS | 7136 |
| Estimated completeness [%] | 100.00 |
| Estimated contamination | 1.32 |
| <pre> Assembly Date :: 2017 Assembly Method :: SPAdes v. 3.10.1 Genome Representation :: Full Expected Final Version :: Yes Genome Coverage :: 100.0x Sequencing Technology :: Illumina MiSeq Annotation Provider :: NCBI RefSeq Annotation Date :: 11/08/2019 04:06:55 Annotation Pipeline : NCBI Prokaryotic Genome Annotation Pipeline (PGAP) Annotation Method :: Best-placed reference protein set; GeneMarkS-2+ Annotation Software revision :: 4.10 Features Annotate: Gene; CDS; rRNA; tRNA; ncRNA; repeat_region Genes (total) :: 7,155 CDSs (total) :: 7,074 Genes (coding) :: 6,671 CDSs (with protein) :: 6,671 Genes (RNA) :: 81 rRNAs :: 5, 1, 2 (5S, 16S, 23S) complete rRNAs :: 3, 1, 1 (5S, 16S, 23S) partial rRNAs :: 2, 1 (5S, 23S) tRNAs :: 70 ncRNAs :: 3 Pseudo Genes (total) :: 403 CDSs (without protein) :: 403 Pseudo Genes (ambiguous residues) :: 0 of 403 Pseudo Genes (frameshifted) :: 55 of 403 Pseudo Genes (incomplete) :: 357 of 403 Pseudo Genes (internal stop) :: 13 of 403 Pseudo Genes (multiple problems) :: 22 of 403 CRISPR Arrays :: 2 </pre> | |

Table S3. Growth characteristics of strain RB5^T and the type strain of closest related *Streptomyces* species after 12 to 14 days of incubation at 28 °C. Strains: 1. RB5^T; 2. *S. polyrhachis* DSM42102^T; all data was acquired in this study. Morphological feature: G: growth, AM: aerial mycelium, SM: substrate mycelium, SP: soluble pigment, Colour coding (N.) corresponding to Baumann's Farbatlas 1 in parentheses.

| | | 1 | 2 |
|----------------|----|------------------------------------|------------------------|
| ISP1 | G | Good | Good |
| | AM | None | None |
| | SM | Light green , (No.577-580 NE,1) | Cream white (No.4) |
| | SP | None | None |
| ISP2 | G | Good | Good |
| | AM | White | None |
| | SM | Light green, white (No.577-580 NE) | Cream white (No.4) |
| | SP | None | None |
| ISP3 | G | Good | Good |
| | AM | None | Very poor, white |
| | SM | Light green, white (No.577-580 NE) | Cream white (No.4) |
| | SP | None | None |
| ISP4 | G | Good | Good |
| | AM | None | None |
| | SM | Light green, (No.577-580 NE) | White |
| | SP | None | None |
| ISP5 | G | Good | Good |
| | AM | Poor on margin, white | Very poor, white |
| | SM | Light green (No.577-580 NE) | White |
| | SP | None | None |
| ISP6 | G | Good | Good |
| | AM | White | None |
| | SM | Dark green (No.590-593) | Yellowish |
| | SP | None | None |
| ISP7 | G | Good | Weak |
| | AM | None | Very poor, white |
| | SM | Light green , (No.577-580 NE) | Orange (No.4) |
| | SP | None | None |
| medium with | G | Good | Good |
| | AM | None | None |
| | SM | Beige | Cream white (No.4) |
| | SP | None | Reddish brown (No.173) |
| medium without | G | Good | Good |
| | AM | None | None |
| | SM | Beige | Cream white (No.4) |
| | SP | None | None |

Table S4. Antibiotic susceptibility test of strains RB5^T and *S. polyrhachis* DSM 42102^T after 12 days of incubation at 28 °C. The diameter of the inhibition zone is given in mm.

| Antibiotic | Conc. | 1 | 2 |
|-------------------|--------------|----------|----------|
| Amikacin | 30 µg | 50 | 24 |
| Ampicillin | 10 µg | 24 | 0 |
| Azlocillin | 75 mg | 10 | 0 |
| Bacitracin | 130 µg | 40 | 30 |
| Cephalothin | 30 µg | 11 | 0 |
| Imipenem | 10 µg | 60 | 21 |
| Mezlocillin | 75 µg | 24 | 0 |
| Penicillin G | 10 units | 0 | 0 |
| Piperacillin | 100 µg | 20 | 0 |
| Polymyxin B | 30 units | 12 | 0 |
| Vancomycin | 30 µg | 32 | 23 |
| Chloramphenicol | 30 µg | 37 | 20 |
| Chlortetracycline | 30 µg | 24 | 26 |
| Doxycycline | 30 µg | 28 | 24 |
| Erythromycin | 15 µg | 24 | 40 |
| Gentamicin | 10 µg | 19 | 9 |
| Kanamycin | 30 µg | 46 | 20 |
| Lincomycin | 2 µg | 8 | 0 |
| Oxytetracycline | 30 µg | 17 | 15 |
| Rifampin | 5 µg | 36 | 35 |
| Streptomycin | 10 µg | 37 | 15 |
| Tetracycline | 30 µg | 16 | 40 |
| Ciprofloxacin | 5 µg | 24 | 30 |
| Norfloxacin | 10 µg | 10 | 12 |
| Novobiocin | 5 µg | 10 | 25 |
| Nalidixic acid | 30 µg | 10 | 0 |

Table S5. Cellular fatty acid compositions of strain RB5^T and close related type strains of *Streptomyces polyrhachis*. Strains: 1. RB5^T; 2. *S. polyrhachis* DSM 42102^T. Amounts of fatty acids below 1.0% were not listed or marked with tr = traces. All data were acquired in this study.

| Fatty acids | 1 | 2 |
|---|----------|----------|
| Saturated fatty acids | | |
| 14:0 | tr | tr |
| 15:0 | 6.6 | tr |
| 16:0 | tr | 2.7 |
| Unsaturated fatty acids | | |
| 16:1 cis 9 | tr | tr |
| <i>iso</i> -16:1 ω 6 <i>cis</i> | 2.1 | 7.1 |
| <i>anteiso</i> -17:1 ω 7 <i>cis</i> | tr | tr |
| Sum 17:1 ISO ω 7 <i>cis</i> /17:1 ISO ω 9 <i>cis</i> | - | - |
| Branched fatty acids | | |
| <i>iso</i> -14:0 | 13.0 | 11.2 |
| <i>iso</i> -15:0 | 4.1 | 4.1 |
| <i>iso</i> -16:0 | 19.4 | 38.9 |
| <i>iso</i> -17:0 | tr | tr |
| <i>anteiso</i> -15:0 | 44.8 | 21.9 |
| <i>anteiso</i> -17:0 | 3.9 | 8.4 |