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Streptomyces durocortorensis sp. nov., isolated from oak rhizosphere

Mathieu Cassarini^{1,*}, Caroline Rémond¹, Estelle Mühle², Dominique Clermont² and Ludovic Besaury¹

Abstract

Strain RHZ10^T was isolated from an oak rhizosphere sampled in Reims, France, and characterized to assess its taxonomy. Based on 16S rRNA gene sequence similarity, strain RHZ10^T was affiliated to the genus *Streptomyces* and the closest species were *Streptomyces anulatus* NRRL B-2000^T and *Streptomyces pratensis* ch24^T. Average nucleotide identity and digital DNA–DNA hybridization values were 77.3–92.4% and 23.0–50.9%, respectively, when compared to the type strains of fully sequenced related species having a 16S rRNA gene sequence similarity over 98%. These data suggested that strain RHZ10^T represented a novel species within the genus *Streptomyces*. The genome of RHZ10^T was 8.0 Mbp long, had 7894 predicted coding genes, and a G+C content of 71.7 mol%. Cultures of RHZ10^T on ISP 2 medium mostly led to the production a green pigmentation of the core of its colonies in the vegetative mycelium, surrounded by white pigmentation of the aerial mycelium. The main fatty acids of RHZ10^T were anteiso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0} and C_{16:0}. Polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, unidentified lipids, unidentified phospholipids, unidentified aminolipids and unidentified glycolipids. Its main quinones were MK-9(H₈) (69.3%), MK-9(H₄) (17.3%) and MK-9(H₆) (17.0%). Phylogenetic, physiological and chemotaxonomic studies clearly supported that strain RHZ10^T represents a novel species within the genus *Streptomyces*, for which the name *Streptomyces durocortorensis* sp. nov. is proposed and the type strain is RHZ10^T (=DSM 112634^T=LMG 32187^T=CIP 111907^T).

INTRODUCTION

The genus *Streptomyces* is a member of the phylum *Actinomycetota* [1], which encompasses Gram-positive filamentous bacteria having G+C contents generally over 50 mol%. The main morphological trait for this genus is the formation of long vegetative and aerial mycelia. During growth, elongation of the filaments occurs from the end of the hyphae [2]. *Streptomyces* species generally end up producing spores at maturity by septation of their aerial hyphae [3]. This genus is currently well documented, with almost 700 validated species discovered to date [4]. The great interest in this genus can be explained in part by the fact that it is known for being a great source of bioactive metabolites, often used as antibiotics [5]. It has been shown that *Streptomyces* species could use up to 15% of their genomes towards the production of secondary metabolites [6]. Members of the genus *Streptomyces* are found in a variety of environments, notably in soils, and can also be found in symbiosis with plants and animals [7]. They often display the ability to degrade plants, fungi and chitin [8]. As major plant endophytes, they are notably active in the rhizosphere [9]. Numerous *Streptomyces* species were discovered in rhizospheres of wild plants (nettle) [10], of agricultural crops (maize, wheat) [11, 12] or of trees (populous, paper mulberry) [13, 14]. In such environments, they play a major role in phosphate solubilization, nitrogen fixation and other nutrition processes [15]. Although they can coexist in symbiosis with plants [15, 16], they often display important lignocellulolytic enzyme activities that can be of great interest for industry [17–19]. They also contain high amounts of hexamenaquinones and octamenaquinones [20]. In this study, strain RHZ10^T was isolated on International *Streptomyces* Project ISP 2 medium, from an oak's rhizosphere, and its species affiliation was defined by a polyphasic taxonomy analysis.

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Keywords: novel species; rhizosphere; *Streptomyces*.

Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; FAME, fatty acid methyl ester; ISP, International *Streptomyces* Project; MLSA, multilocus sequence analysis; RAST, rapid annotation using subsystem technology.

Repositories: 16S rRNA gene and whole genome sequences were deposited at GenBank under the accession numbers MW582863.1 and JAFEU000000000.1, respectively.

Three supplementary tables and five supplementary figures are available with the online version of this article.

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ISOLATION

Samples were collected in a forest located in Reims, France (49° 15' 46" N, 4° 02' 05" E) in May 2019. Samples were dug in the rhizosphere of an oak, about 10 cm below the surface of its roots. The rich ISP 2 growth medium was used for its capacity to isolate micro-organisms from the phylum *Actinomycetota* [21]. One gram of soil was incubated in ISP 2 liquid medium, at 30 °C under agitation and pH 7, for a week. This culture was diluted from 10⁻³ to 10⁻⁷ in a sequential dilution and 100 µl was streaked on ISP 2 agar plates. The plates were incubated for 3 weeks at 30 °C until colonies with different morphological characteristics appeared. A colony which displayed green pigmentation, designated RHZ10^T, was selected for further study.

16S rRNA GENE PHYLOGENY

DNA was extracted using QIAamp DNA Micro Kit (Qiagen). It was firstly amplified with primers 8F (5'-AGAGTTTGATC-CTGGCTCAG-3') and 1392R (5'-ACGGGCGGTGTGTAC-3') as previously described [22]. The partial 16S rRNA sequence was amplified using traditional 8F Sanger primers. The 16S rRNA gene (1459 bp, partial sequence) was also extracted bioinformatically from the genome of strain RHZ10^T with a local BLAST [23] using the 16S rRNA sequences from the SILVA database [24]. The extracted 16S rRNA sequence, which fully matched a part of the sequenced genome, was deposited in the GenBank database (accession number: MW582863.1). The 16S rRNA gene sequence of strain RHZ10^T was compared with those of type strains available from the EzBioCloud website [25]. Phylogenetic trees were built based on the 16S rRNA gene sequences having a pairwise similarity score above 98.65% according to the EzBioCloud results. *Kitasatospora setae* KM-6054^T (NR_112082.2) was used as an outgroup. Phylogenetic trees were reconstructed using the MEGA-X software [26] after alignment by the ClustalW program [27]. They were inferred using the neighbour-joining and maximum-likelihood methods, with a bootstrap analysis based on 1000 replicates to estimate the stability of the grouping for each tree.

Results from the EzBioCloud showed that strain RHZ10^T had 16S rRNA sequence similarity above 98.65% to 45 strains. Strain RHZ10^T fully matched *Streptomyces anulatus* NRRL B-2000^T and *Streptomyces pratensis* ch24^T (100%). However, the two 16S rRNA gene phylogenetic trees (Figs 1 and S1) showed that strain RHZ10^T did not fully group with *S. anulatus* NRRL B-2000^T or *Streptomyces pratensis* ch24^T, showing variations between the two sequences.

GENOME FEATURES

DNA of strain RHZ10^T was purified using a PureLink Genomic DNA Mini Kit (Invitrogen) and quantified by an ND-1000 NanoDrop spectrophotometer. Its purity was assessed by electrophoresis on a 0.8% agarose gel (w/v) in Tris–acetate–EDTA buffer. It was sequenced by the Mutualized Microbiology Platform (P2M) at the Institut Pasteur (Paris, France). DNA whole-genome shotgun sequencing libraries were prepared using the Nextera XT kit (Illumina), and 2×150 bp paired-end sequencing was performed using an Illumina NextSeq500 instrument. Gene annotation was performed using the RAST server (Rapid Annotation using Subsystem Technology) [28] and the genome sequence was then deposited in the GenBank database under the accession number JAFEUF000000000.1.

The size of the genomic DNA of RHZ10^T was 7959 868 bp. This was in accordance with the genome sizes of its closest relatives (6646 162–9703 912 bp) (Table 1). The 71.7 mol% G+C content of RHZ10^T was consistent with the average G+C content of species of the genus *Streptomyces* (66–78%) [3].

Digital DNA–DNA hybridization (dDDH) was used to further clarify the genetic relationship between strain RHZ10^T and the related type strains. dDDH values were calculated using the Genome-to-Genome Distance Calculator 2.1 server (<http://ggdc.dsmz.de>; formula 2), according to the method described by Meier-Kolthoff *et al.* [29]. Average nucleotide identity (ANI) analyses were carried out using the JSpeciesWS server (<https://jspecies.ribohost.com/jspeciesws>) [30]. Those scores were calculated to compare RHZ10^T with its closest relatives. Therefore, the strains used were those previously shown with a 16S rRNA gene sequence similarity above 98.65%, and for which a complete genome was available.

Overall, dDDH values ranged from 23.0 to 50.9% (Table 1). The highest values were obtained for *Streptomyces rubiginosohelvolus* JCM 4415^T (50.9%), *Streptomyces pluricolorescens* JCM 4602^T (50.8%), *Streptomyces globisporus* C-1027^T (50.5%), *Streptomyces parvus* NRRL B-1455^T (48.9%), *Streptomyces sindenensis* JCM 4164^T (47.1%) and *Streptomyces badius* JCM 4350^T (46.9%). All these values were far below the threshold value of 70%, which is recommended as cutoff to differentiate bacterial species [29, 31].

ANIb scores (ANI analysis with a sequence pairing based on BLAST+) ranged from 77.3 to 92.4% (Table 1). The most closely related type strain genomes were those of *S. globisporus* C-1027^T (92.48%), *S. rubiginosohelvolus* JCM 4415^T (92.35%), *S. pluricolorescens* JCM 4602^T (92.00%), *S. parvus* NRRL B-1455^T (91.82%), *S. sindenensis* JCM 4164^T (91.58%) and *S. badius* JCM 4350^T (91.29%). This was clearly below the species-delineating consensus threshold (95–96%) [32]. Therefore, the ANI and dDDH values supported the conclusion that strain RHZ10^T represented a distinct species in the genus *Streptomyces*.

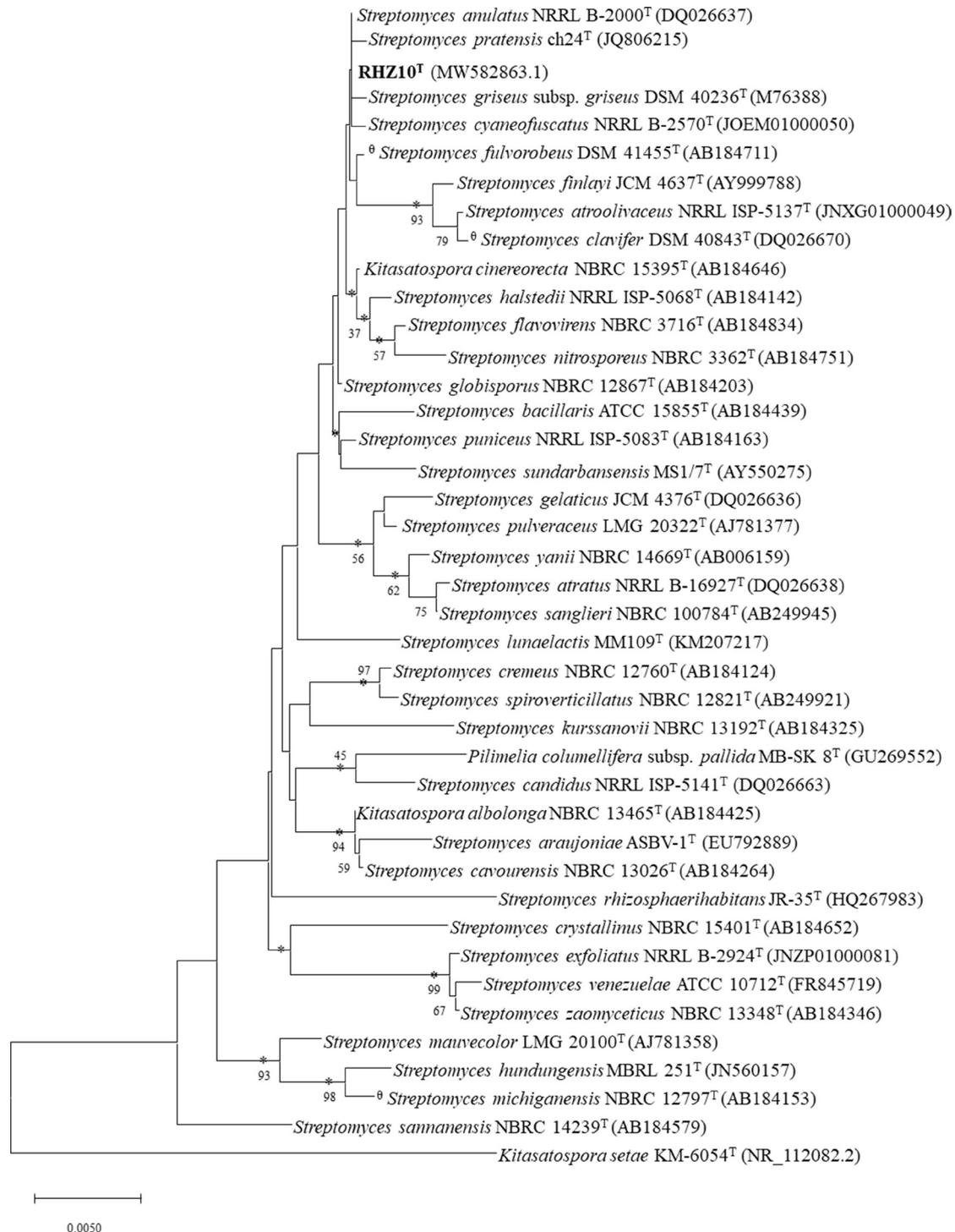


Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences showing the relationships between strain RHZ10^T and its closest relatives. Numbers next to nodes represent percentage confidence levels from 1000 replicate bootstrap samplings over 50%. GenBank accession numbers are given in brackets. Bar, 0.005 substitutions per nucleotide position. * Signifies that every descendant from a branch were found grouped together with the maximum-likelihood method (Fig. S1, available in the online version of this article). Strains that were in the dendrogram at the end of a same branch were removed for clarity, such branches are indicated with θ. Species with full similarity that were removed are as follows: *Streptomyces clavifer* DSM 40843^T (DQ026670)=*Streptomyces mutomycini* NRRL B-65393^T (MAPV01000102; bootstrap value: 94); *Streptomyces fulvorobeus* DSM 41455^T (AB184711)=*Streptomyces fulvissimus* DSM 40593^T (CP005080)=*Streptomyces microflavus* JCM 4496^T (AB184284; bootstrap value: 53); *Streptomyces michiganensis* NBRC 12797^T (AB184153)=*Streptomyces xanthochromogenes* NRRL B-5410^T (DQ442559; bootstrap value: 98); *Streptomyces parvus* NRRL B-1455^T (AB184756)=*Streptomyces badius* JCM 4350^T (AY999783)=*Streptomyces globisporus* NBRC 12867^T (AB184203)=*Streptomyces pluricolorascens* JCM 4602^T (AB184162)=*Streptomyces rubiginosohelvolus* JCM 4415^T (AB184240)=*Streptomyces sindenensis* JCM 4164^T (AB184759; bootstrap value: 30).

Table 1. Information and comparison of the whole characterized genome of RHZ10^T with those of its closest strains according to their 16S rRNA sequences

| Strain | dDDH with strain RHZ10 ^T (%) | ANI with strain RHZ10 ^T (%) | Size (bp) | DNA G+C content (mol%) | Accession no. |
|---|--|---|-----------|---------------------------|-------------------|
| RHZ10^T | * | * | 7959868 | 71.7 | JAFEUF000000000.1 |
| <i>Streptomyces anulatus</i> ATCC 11523 ^T | 43.30 | 90.43 | 8759578 | 71.7 | CM003601.1 |
| <i>Streptomyces atroolivaceus</i> NRRL ISP-5137 ^T | 26.40 | 80.96 | 8216207 | 70.7 | JNXG000000000.1 |
| <i>Streptomyces bacillaris</i> ATCC 15855 ^T | 33.80 | 86.37 | 7888441 | 72.0 | CP029378.1 |
| <i>Streptomyces badius</i> JCM 4350 ^T | 46.90 | 91.29 | 7515187 | 71.4 | BMSZ000000000.1 |
| <i>Streptomyces candidus</i> JCM 4629 ^T | 23.20 | 77.88 | 7307831 | 71.2 | BNBN000000000.1 |
| <i>Streptomyces cavourensis</i> DSM 41795 ^T | 33.80 | 86.43 | 7670394 | 72.1 | GCA_006788935.1 |
| <i>Streptomyces clavifer</i> DSM 40843 ^T | 26.90 | 81.67 | 7703094 | 71.1 | JAGINS000000000.1 |
| <i>Streptomyces cyaneofuscatus</i> NRRL B-2570 ^T | 34.30 | 87.06 | 7899007 | 71.6 | JOEM000000000.1 |
| <i>Streptomyces exfoliatus</i> NRRL B-2924 ^T | 23.50 | 78.15 | 7874957 | 71.9 | JNZP000000000.1 |
| <i>Streptomyces finlayi</i> JCM 4637 ^T | 23.00 | 77.32 | 9487627 | 71.3 | BMVC000000000.1 |
| <i>Streptomyces fulvissimus</i> DSM 40593 ^T | 34.80 | 87.37 | 7905758 | 71.5 | CP005080.1 |
| <i>Streptomyces fulvorobeus</i> DSM 41455 ^T | 27.10 | 82.10 | 6646162 | 70.8 | JACCCF000000000.1 |
| <i>Streptomyces gelaticus</i> JCM 4376 ^T | 26.60 | 81.17 | 7755498 | 70.6 | BMTF000000000.1 |
| <i>Streptomyces globisporus</i> C-1027 ^T | 50.50 | 92.48 | 7608611 | 71.6 | CP013738.1 |
| <i>Streptomyces griseus</i> subsp. <i>griseus</i> DSM 40236 ^T | 41.70 | 89.19 | 8630192 | 72.2 | FNTW01000004.1 |
| <i>Streptomyces halstedii</i> NRRL ISP-5068 ^T | 26.80 | 81.40 | 7740838 | 71.9 | JOAZ000000000.1 |
| <i>Streptomyces lunaelactis</i> MM109 ^T | 24.20 | 78.89 | 8570191 | 69.8 | CP026305.1 |
| <i>Streptomyces microflavus</i> JCM 4496 ^T | 34.70 | 87.02 | 8517311 | 71.2 | BMUG000000000.1 |
| <i>Streptomyces mutomycini</i> NRRL B-65393 ^T | 26.80 | 81.29 | 7070041 | 70.6 | MAPV01000010.1 |
| <i>Streptomyces nitrosporeus</i> NBRC 3362 ^T | 26.40 | 80.87 | 7581562 | 72.2 | CP023702.1 |
| <i>Streptomyces parvus</i> NRRL B-1455 ^T | 48.90 | 91.82 | 7985796 | 71.6 | VXCD01000100.1 |
| <i>Streptomyces pluricolorascens</i> JCM 4602 ^T | 50.80 | 92.0 | 7332794 | 71.7 | BMUW000000000.1 |

Continued

Table 1. Continued

| Strain | dDDH with strain RHZ10 ^T (%) | ANI with strain RHZ10 ^T (%) | Size (bp) | DNA G+C content (mol%) | Accession no. |
|---|--|---|-----------|---------------------------|----------------|
| <i>Streptomyces puniceus</i> NRRL ISP-5083 ^T | 38.70 | 89.08 | 8179821 | 72.5 | JOBQ00000000.1 |
| <i>Streptomyces rubiginosohelvolus</i> JCM 4415 ^T | 50.90 | 92.35 | 7548572 | 71.7 | BMTW00000000.1 |
| <i>Streptomyces sindenensis</i> JCM 4164 ^T | 47.10 | 91.58 | 7564582 | 71.8 | BMSG00000000.1 |
| <i>Streptomyces spiroverticillatus</i> JCM 4609 ^T | 23.00 | 77.76 | 9703912 | 71.3 | BMUX00000000.1 |
| <i>Streptomyces xanthochromogenes</i> JCM 4612 ^T | 23.60 | 78.11 | 8774150 | 71.1 | BMUZ00000000.1 |
| <i>Streptomyces zaomyceticus</i> JCM 4864 ^T | 23.60 | 78.40 | 8311400 | 72.1 | BNBZ00000000.1 |

A phylogenomic tree using a genome-BLAST distance calculation [33] was built with the Type (Strain) Genome Server (DSMZ) (<https://tygs.dsmz.de>) with automatically selected strains. That phylogenomic tree (Fig. S2) confirmed that RHZ10^T was clearly isolated within the genus *Streptomyces*.

Moreover, two multilocus sequence analysis (MLSA) studies were carried out. MLSA techniques are extremely valuable in determining species-level relationships within the genus *Streptomyces* [34]. Two phylogenetic trees, based on the concatenation of either six genes (Fig. 2) or 89 housekeeping genes (Fig. S3), were built to estimate the taxonomic position of strain RHZ10^T.

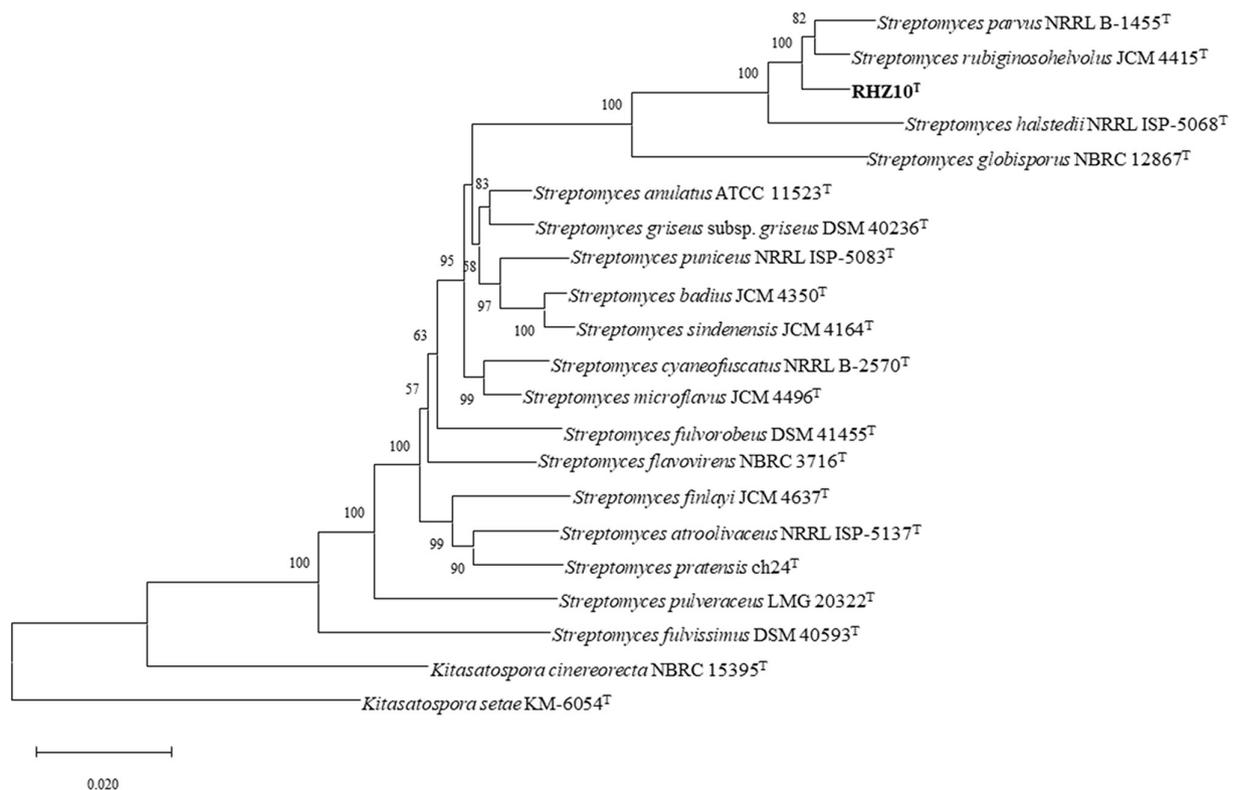


Fig. 2. Neighbour-joining phylogenetic tree showing the relationships between strain RHZ10^T and its closest relatives based on the concatenated sequences of five housekeeping genes and the 16S rRNA gene. The housekeeping genes are *atpD*, *gyrB*, *rpoB*, *recA*, and *trpB*. Sequence accession numbers are available in Table S1. Numbers represent percentage confidence levels from 1000 replicate bootstrap samplings. Bar, 0.02 substitutions per nucleotide position.

The tree based on six genes used five housekeeping genes (*atpD*, *gyrB*, *rpoB*, *recA*, *trpB*) and the 16S rRNA sequence [35]. Every accession number of these housekeeping genes can be found in Table S1. The 89 housekeeping genes tree was made using the automated Multi-Locus Species Tree (MLST) server (<https://automl.st.ziemertlab.com/>) [36]. The first 50 type strains given as pre-results by autoMLST were used to build this tree. The 89 housekeeping genes are listed in Table S2. They were inferred as previously described using the neighbour-joining method.

The two MLSA phylogenetic trees (Figs 2 and S3) also confirmed the positioning of strain RHZ10^T within the genus *Streptomyces*. However, differences in their closest phylogenetic neighbours were noted. The same observation was made for the trees presented previously.

The genomic sequence of RHZ10^T was submitted to the antiSMASH 6.0 server [37] to identify genes potentially coding for bioactive secondary metabolites. The *in silico* analysis of the secondary metabolites by antiSMASH predicted the presence of 48 clusters. The most predicted types of secondary metabolites were saccharides (eight clusters) and type I polyketide synthases (seven clusters). Four clusters were predicted with a similarity of 100% to known gene clusters producing either a polyketide, a terpene (geosmin), ectoine or desferrioxaminB.

The genome sequence was annotated using the RAST server (<https://rast.nmpdr.org>) [28]. According to the annotation using the RAST server, strain RHZ10^T had 7894 coding genes. Comparisons with genomes of *S. rubiginosohelvolus* JCM 4415^T, *S. globisporus* C-1027^T, *S. sindenensis* JCM 4164^T, *S. anulatus* ATCC 11523^T, *S. parvus* NRRL B-1455^T and *S. pluricolorescens* JCM 4602^T were performed. The presence of many genes dedicated to L-arabinose utilization, glycogen metabolism and isochromate synthesis could be detected in RHZ10^T. The absence of many genes involved in the metabolism of mannose as well as in the use of fucose and lactate were also noted.

Moreover, when compared to the other strains, RHZ10^T had two additional genes: a beta-hexosaminidase (EC 3.2.1.52) and a periplasmic alpha-amylase (EC 3.2.1.1), for chitin and maltose/maltodextrine utilization, respectively. The compared strains had many additional enzymes dedicated to amino acid biosynthesis which were not present in RHZ10^T, i.e. a cysteine synthase (EC 2.5.1.47) and a serine acetyltransferase (EC 2.3.1.30). Moreover, RHZ10^T lacked an isocitrate lyase (EC 4.1.3.1) found in *S. rubiginosohelvolus* JCM 4415^T and an enzyme involved in the type II fatty acid biosynthesis pathway (FASII; EC 1.1.1.100) found in *S. pluricolorescens* JCM 4602^T. It also lacked many enzymes involved in thiazole-oxazole-modified microcin and lanthione synthesis found both in *S. globisporus* C-1027^T and *S. parvus* NRRL B-1455^T, and many enzymes involved in L-fucose utilization in *S. sindenensis* JCM 4164^T.

PHYSIOLOGY AND CHEMOTAXONOMY

RHZ10^T tested Gram-stain-positive and showed aerobic growth. Cell morphology of colonies grown for 14 days on ISP 2 agar plates at 30 °C was examined under a light microscope (Olympus BH-2) and with a scanning electron microscope (JEOL, 5400 LV) after gold-palladium coating (JEOL JFC-1100 ion sputter apparatus). RHZ10^T formed straight hyphae that were 0.45–0.65 µm wide with occasional single ramifications on their side. Spores were short cylinders of length 0.5–1.0 µm (Fig. S4).

Physiological parameters were assessed on ISP 2 agar medium. Growth was assessed visually by the appearance of colonies after 3 days of incubation. Temperature range for growth was determined at 4, 15, 20, 25, 30, 37, 45 and 45 °C. Growth of RHZ10^T was only obtained between 20 and 30 °C. The pH range for growth was tested between pH 4.0 to 12.0 with intervals of 1.0 pH unit. This showed that RHZ10^T grew well in neutral and high alkali conditions (from pH 6.0 to 12.0), with optimal growth at pH 7. Growth results depending on pH were in part confirmed with the Biolog GEN III MicroPlate assay [38], as growth was witnessed at pH 6 but not at pH 5. NaCl tolerance was determined using ISP 2 agar medium supplemented with final NaCl concentrations of 0.25, 0.5, 0.75, 1.0, 1.5 and 3.0% (w/v). Growth with NaCl concentrations at 1, 4 and 8% are also parameters tested using the Biolog Gen III MicroPlates [38]. According to these results, RHZ10^T did not show any sensitivity to NaCl concentrations up to 3%.

After 6 days of growth on ISP 2 agar, all colonies showed a green core at the centre of the colonies in the vegetative mycelium, surrounded by white hypha on their side (aerial mycelium). Pigmentation was visible at each pH tested, except at pH 6, where colonies became yellow. This pigmentation also appeared with NaCl concentrations from 0.25 to 1.5%. Pigmentation was lost on ISP 2 medium at 0% NaCl and 3% NaCl. Its closest related strains *S. rubiginosohelvolus* JCM 4415^T, *S. globisporus* C-1027^T, *S. sindenensis* JCM 4164^T, *S. anulatus* ATCC 11523^T, *S. parvus* NRRL B-1455^T and *S. pluricolorescens* JCM 4602^T are known to be able to produce a yellow pigment on a variety of culture media [39–41]. However, when grown on other culture media, RHZ10^T was shown to produce other types of pigmentation. Yellow pigmentation was released in the supernatant of liquid Luria Bertani (NaCl, 10 g l⁻¹; tryptone, 10 g l⁻¹; autolytic yeast extract, 5 g l⁻¹; pH 7.0) and P1 media (soy flour, 25 g l⁻¹; glucose, 20 g l⁻¹; soluble starch, 10 g l⁻¹; yeast extract, 4 g l⁻¹; NaCl, 2 g l⁻¹; meat extract, 1 g l⁻¹; K₂HPO₄, 0.05 g l⁻¹; pH 7.3), for instance, while pellets were light yellow and brown, respectively.

The Biolog Gen III MicroPlates [38] allowed us to assess the assimilation of 71 carbon sources and 23 chemical sensitivities. Growth on L-arabinose and D-xylose was assessed visually in liquid cultures set at 3.33 mM in minimal M9 medium (NH₄Cl,

1 g l⁻¹; Na₂HPO₄·2H₂O, 6 g l⁻¹; KH₂PO₄, 3 g l⁻¹; NaCl, 0.5 g l⁻¹; pH 7.2) and incubated for a week at 30 °C in an incubator shaker. The classical H₂O₂ catalase test protocol was performed for catalase enzyme detection [42]. These tests showed that strain RHZ10^T had the ability to grow on a high variety of compounds. From all the sugars tested, only raffinose led to no growth. RHZ10^T could grow on a wide range of amino acids, hexose acids, carboxylic acids, esters and fatty acids. In comparison, *S. rubiginosohelvolus* JCM 4415^T, *S. globisporus* C-1027^T, *S. sindenensis* JCM 4164^T, *S. anulatus* ATCC 11523^T, *S. parvus* NRRL B-1455^T and *S. pluricologrescens* JCM 4602^T are all known to be unable to grow on sucrose and *myo*-inositol [39–41], while RHZ10^T could (Table S3).

The chemotaxonomic studies regarding fatty acids methyl esters (FAMES), polar lipids and respiratory quinones were done by DSMZ (Braunschweig, Germany) from cultures grown for 6 days at 30 °C on ISP 2 agar plates. FAMES were extracted from 40 mg cells scraped from agar plates. FAMES were then obtained according to previous protocols slightly modified using saponification, methylation then extraction [43, 44]. The characteristic branched FAMES found were iso-C_{14:0} (5.22%), iso-C_{15:0} (4.4%), anteiso-C_{15:0} (35.3%), C_{16:0} (11.07%), iso-C_{16:0} (18.6%), iso-C_{17:0} (4.1%) and anteiso-C_{17:0} (14.3%), which are consistent with patterns of known species of the genus *Streptomyces* [3]. The other fatty acids found were anteiso-C_{13:0} (0.23%), C_{14:0} (0.44%), C_{15:0} (0.98%), C_{17:0} (0.71%), iso-C_{17:1} ω9c (0.43%), anteiso-C_{17:1} ω9c (0.45%), cyclo-C_{17:0} (0.53%), C_{18:0} (0.34%), iso-C_{18:0} (0.44%) and iso-C_{18:1} ω9c (0.46%).

Polar lipids were extracted from 200 mg freeze-dried cells with chloroform, methanol and 0.3% NaCl in water. They were then separated with 2D silica gel chromatography, using chloroform–methanol–water and chloroform–methanol–acetic acid–water as first and second solvents, respectively. Detection of total lipids and specific functional groups were made using various spray reagents as mentioned by the DSMZ service [45]. RHZ10^T had mostly lipids, noticeable amounts of phospholipids and aminolipids, as well as a few phosphatidylethanolamines, diphosphatidylglycerols and glycolipids (Fig. S5).

Finally, the respiratory quinones were extracted from 100 mg freeze-dried cells with a methanol and hexane mix first, and then with hexane, according to previously described methods [46, 47]. Analyses were done by HPLC with a diode-array detector and mass spectrometry. The detected quinones were MK-9(H₆) (69.3%), MK-9(H₈) (17.0%), MK-9(H₄) (17.3%), MK-8(H₆) (1.6%), MK-9(H₂) (1.3%), MK-8(H₄) (1.1%) and MK-9(H₁₀) (0.9%). Menaquinones were present as respiratory quinones.

DESCRIPTION OF *STREPTOMYCES DUROCORTORENSIS* SP. NOV.

Streptomyces durocortorensis (du.ro.cor.to.ren'sis, L. masc. adj. *durocortorensis*, of Durocortorum, the Latin name of Reims).

Gram-positive, aerobic and catalase-positive actinomycete. Hyphae are straight with occasional single ramifications on their side. Hyphae are 0.45–0.65 μm wide. Spores are short cylinders of length 0.5–1.0 μm. Green pigmentation in the vegetative mycelium surrounded by white pigmentation in the aerial mycelium is visible on solid ISP 2 medium at 30 °C and pH 7–10 at 6 days of culture. Yellow pigmentation is visible at 30 °C and pH 6 at 6 days of culture. On ISP 2 medium under agitation and pH 7, grows between 20 and 30 °C with an optimum at 25 °C. On ISP 2 medium and agitation at 30 °C, grows at pH 6–12, with optimum growth at pH 7. When cultivated at pH 7 and 30 °C on agitated ISP 2 medium, grows with NaCl concentrations ranging from 0 to 3% (w/v). Grows on 3-methyl-glucose, *N*-acetyl neuraminic acid, *N*-acetyl-*D*-galactosamine, *N*-acetyl-*D*-glucosamine, *N*-acetyl-β-*D*-mannosamine, *L*-alanine, γ-amino-butyric acid, *D*-arabitol, *L*-arginine, *D*-aspartic acid, *L*-aspartic acid, bromo-succinic acid, cellobiose, dextrin, *D*-fructose, *D*-fructose-6-PO₄, *D*-fucose, *L*-fucose, *D*-galactose, *D*-galacturonic acid, gelatin, *D*-gluconic acid, α-*D*-glucose, *D*-glucose-6-PO₄, glucuronamide, *D*-glucuronic-acid, *L*-glutamic acid, glycerol, glycyl-*L*-proline, *L*-histidine, α-hydroxy-butyric acid, β-hydroxy-*D,L*-butyric acid, inosine, *myo*-inositol, α-keto-butyric acid, α-keto-glutaric acid, *L*-lactic acid, *D*-lactic acid methyl ester, lactose, *D*-malic acid, *L*-malic acid, maltose, *D*-mannitol, *D*-mannose, melibiose, methyl β-*D*-glucoside, mucic acid, pectin, *p*-hydroxy-phenylacetic acid, propionic acid, *L*-pyroglutamic acid, quinic acid, *L*-rhamnose, *D*-saccharic acid, *D*-salicin, *L*-serine, *D*-sorbitol, stachyose, sucrose, trehalose, turanose, Tween 40 and *D*-xylose. Unable to grow on *L*-arabinose, aztreonam, citric acid, formic acid, fusidic acid, *L*-galactonic acid lactone, guanidine HCl, lincomycin, lithium chloride, methyl pyruvate, minocycline, nalidixic acid, Niaproof 4, potassium tellurite, raffinose, rifamycin SV, *D*-serine, *L*-serine, sodium bromate, sodium butyrate, tetrazolium blue, tetrazolium violet, troleandomycin and vancomycin. Most abundant FAMES are anteiso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0} and C_{16:0}. Detected polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, unidentified lipids, unidentified phospholipids, unidentified aminolipids and unidentified glycolipids. MK-9 menaquinones are the predominant type of quinones, with more than half being MK-9(H₆).

The type strain, RHZ10^T (=DSM 112634^T=LMG 32187^T=CIP 111907^T), was isolated from an oak's rhizosphere in Reims, France. Genome size is 7959686 bp with G+C content of 71.7 mol%.

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Author contributions

Formal analysis, investigation, visualization and writing (original draft): M.C. Project administration: L.B. Data curation: E.M. and D.C. Funding acquisition and supervision: C.R. and L.B. Conceptualization: M.C., C.R. and L.B. Methodology: C.R., E.M., D.C. and L.B. Validation and writing (review and editing): M.C., C.R., E.M., D.C. and L.B.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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