Taxonomic analyses of members of the *Streptomyces cinnabarinus* cluster, description of *Streptomyces cinnabarigriseus* sp. nov. and *Streptomyces davaonensis* sp. nov.

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**Abstract**

Roseoflavin is the only known riboflavin (vitamin B2) analog with antibiotic properties. It is actively taken up by many microorganisms and targets flavinmononucleotide riboswitches and flavoproteins. It is described as the product of the tentatively named *Streptomyces davawensis* JCM 4913. Taxonomic analysis of this strain with a polyphasic approach showed that it is very closely related to *Streptomyces cinnabarinus* (DSM 40467). The two *Streptomyces* isolates were obtained from different geographical locations (the Philippines and the Kamchatka Peninsula, respectively), their genomes have been sequenced and the question was whether or not the two isolates were representatives of the same species. As we also worked with another isolate of *Streptomyces cinnabarinus* JS 360, the producer of the cinnabaramides, we wanted to clarify the taxonomic position of the three isolates by using a polyphasic approach. After analysis of the 16S rRNA gene sequence, we found in total 23 species of the genus *Streptomyces* that showed a similarity higher than 98.5% to the three strains. We showed that *S. davawensis* JCM 4913 and *S. cinnabarinus* DSM 40467 were very closely related but belong to two different species. Hence, we validate *S. davawensis* as *Streptomyces davawensis* sp. nov. with the type strain JCM 4913T (=DSM 101723T). In addition, the cinnabamide producer can be clearly differentiated from *S. davaonensis* and this isolate is described as *Streptomyces cinnabarigriseus* sp. nov. with strain JS360T (=NCIB 100590T=DSM 101724T) as the type strain.

*Streptomyces davawensis* DSM 101723T (=JCM 4913T) was first isolated from a soil sample collected in the Philippines within the framework of a screening program for novel antibiotics [1, 2]. The tentative specific epithet *davawensis* refers to the site of sampling, which was near Davao City [2]. *S. davawensis* produces a red compound, which exhibits antibiotic activity against a variety of Gram-positive bacteria [1]. Due to its red colour and its structural similarity to riboflavin (vitamin B2), the novel antibiotic was named roseoflavin [1] (Fig. S1, available in the online version of this article). Roseoflavin is the only known natural riboflavin analog with antibiotic properties and has been studied as a model compound for vitamin analogues [3]. Vitamin analogs such as roseoflavin represent a new class of antibiotics with novel target structures and could help to replenish the arsenal of antimicrobials urgently needed to fight multiresistant bacterial pathogens. Roseoflavin is taken up by many bacteria via riboflavin transporters [4–6]. Following uptake, roseoflavin is activated to roseoflavin mononucleotide (RoFMN) and roseoflavin adenine dinucleotide (RoFAD) by promiscuous flavokinases (EC 2.7.1.26)/FAD synthetases (EC 2.7.7.2) [1, 7]. Following uptake, roseoflavin is activated to roseoflavin mononucleotide (RoFMN) and roseoflavin adenine dinucleotide (RoFAD) by promiscuous flavokinases (EC 2.7.1.26)/FAD synthetases (EC 2.7.7.2) [1, 7]. RoFMN and RoFAD have different physicochemical properties.

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**Keywords:** *Streptomyces cinnabarinus* cluster; *Streptomyces cinnabarigriseus*; *Streptomyces davaonensis*; polyphasic taxonomy.

**Abbreviations:** ISP, International Streptomyces Project; SEM, scanning electron microscopy; TSB, tryptase soy broth. The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain DSM 101724 (=JS360) is JS360.sq J1=JS360MF536523. The Whole Genome Shotgun project have been deposited at GenBank/EMBL/DDBJ under the accession LMXA010000 (JS360). The versions described in this paper is version LMXA0100001=LMXA0100001. Three supplementary tables and three supplementary figures are available with the online version of this article.
properties when compared to the cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) and very likely reduce the activity of some (if not all) flavoproteins present within a cell [8, 9]. In addition, FMN riboswitches were found to be targets for RoFMN [9–14]. ‘S. davawensis’ carries a highly specialized FMN riboswitch which is not negatively affected by RoFMN and thus confers roseoflavin resistance to ‘S. davawensis’ [12]. Only three enzymes are necessary to convert the riboflavin-derived cofactor FMN into roseoflavin. The unique 8-demethyl-8-aminoriboflavin-5′-phosphate synthase RosB generates 8-demethyl-8-aminoriboflavin-5′-phosphate (AFP) from FMN [15–17]. An as yet unidentified enzyme dephosphorylates AFP to 8-demethyl-8-aminoriboflavin (AP) which is subsequently converted to roseoflavin by the S-adenosyl methionine-dependent N,N-8-demethyl-8-aminoriboflavin dimethyltransferase RosA [18, 19]. The sequence of the 4966 619 base pair linear chromosome of ‘S. davawensis’ and an 89331 base pair linear plasmid revealed the presence of 8616 predicted protein-coding genes, which strongly suggests that the same pathway as in ‘S. davawensis’ is used for roseoflavin biosynthesis.

In search of relatives to ‘S. davawensis’ and S. cinnabarinus, we analysed the secondary metabolites from a variety of isolates which have been reported as S. cinnabarinus. One of the studied isolates was strain JS360 [21, 22]. This strain was isolated from a soil sample collected in Japan and was thought to be a variant or a very close relative to S. cinnabarinus based on its 16S rRNA gene sequence [21, 22]. Strain JS360 does not produce roseoflavin under the applied laboratory conditions but was described earlier to be a producer of the cinnabaramides A–G (Fig. S1). These compounds in turn were found in the course of a high throughput screening project of microbial extracts employing a human 20S proteasome inhibitory assay [21, 22]. Cinnabaramides inhibit NF-KB activation and are therefore proteasome inhibitors, which in general could be used for the treatment of allergies, asthma and cancer [23]. Moreover, the cinnabaramide structures closely resemble lactacycin and salinosporamide, and thus may be developed as anticancer drugs [21, 22]. Biosynthesis of the cinnabaramides was studied in detail previously [24]. We compared these three strains by using a polyphasic approach and found that ‘S. davawensis’ and S. cinnabarinus are closely related, with ‘S. davawensis’ being a separate species. Strain JS3601 in turn is different to S. cinnabarinus and thus represents a different species, for which the name Streptomyces cinnabarigriseus is proposed.

To analyse the morphological and physiological characteristics of the strains, different solid media [25] were used: yeast extract–malt extract agar (International Streptomyces Project 2; ISP2), oatmeal agar (ISP3), inorganic salt starch agar (ISP4), glycerol–asparagine agar (ISP5), peptone–yeast extract iron agar (ISP6) and tyrosine agar (ISP7). The corresponding cultures were incubated for 10 days at 30°C. Following incubation, the differences in growth, colony colour, morphology of the aerial mycelium and synthesis of soluble pigments between the strain ‘S. davawensis’ JS360 and its closest related type strains were analysed.

The utilisation of carbohydrates was investigated by employing microtitre plates [26] on ISP9 medium as described by Shirling and Gottlieb [25]. For analysis of the sodium chloride tolerance, a similar technique based on the method of Kutzner et al. [27] was used. The enzymatic activities were investigated by observing the production of significant fingerprints with the help of API ZYM strips [28]. The strains used for the comparative studies are listed in Table S1.

To analyse the structures of the mycelium and spores, the cultures were grown on a complex solid growth medium (DSMZ medium 65: glucose 4.0 g l−1, yeast extract 4.0 g l−1, malt extract 10.0 g l−1, CaCO3 2.0 g l−1, 15.0 g l−1 agar; pH 7.2). A section of the agar containing a bacterial lawn was fixed in glutaraldehyde according to the description of Wink et al. [29]. The samples were critical-point-dried and gold–palladium-sputtered, and the morphology of the spores was determined using a Zeiss Merlin field emission scanning electron microscope (SEM) with an Everhart-Thornley SE-detector and an Inlens-SEM detector in a 25:75% ratio applying the SEMSmart software version 5.05.

Biomass that was subjected to analyses of the quinone system and the polar lipids were produced by growing strains ‘S. davawensis’ (DSM 101723T) and S. cinnabarigriseus (DSM 101724T) for 48 h in M79 [30] at 28°C. The cells were harvested and lyophilized prior to analyses. Respiratory quinones of the strains were extracted, separated and identified as described previously [31], and analysed by using a JASCO X-LC system and a fluorescence detector. For instrument control, data acquisition and analysis, JASCO Chrompass software was employed. Quinones were analysed by employing an RP 18 column (250×4 mm inside diameter) and a running buffer containing acetonitrile and 2-isopropanol (65:35, vol/vol) at a flow rate of 1.3 ml min−1 and a temperature of 20°C. The quinones were detected at a wavelength of λ=269 nm.

Polar lipids extracted by the method of Minnikin et al. [32] were identified by two-dimensional thin-layer chromatography as described by Collins and Jones [33].

To analyse the whole-cell diaminopimelic acid isomers and sugars, the samples were prepared as described by Hasegawa et al. [34]. For extraction of the whole-cell fatty acids, cells were cultured under aerobic conditions for 3 days and 28°C in 5 ml trypticase soy broth (TSB). The cells were harvested by centrifugation (1800 r.p.m., 5 min). Wet cell pellets were placed into reaction tubes, the cellular fatty acids were extracted, methylated and separated by gas chromatography using an Agilent Technologies 6890N instrument. The
instrument was fitted with an auto sampler and a 7683 injector according to the standard protocol of the Microbial Identification System Sherlock software version 6.1 (method TSBA40) [35].

The genomic DNA for the phylogenetic analysis of the Streptomyces species was isolated using the MasterPure Yeast DNA Purification Kit (Epiconcentre). For DNA isolation, the cells were grown in complex medium (DSMZ medium 65) for 7 days at 30 °C.

The 16S rRNA genes of S. davawensis DSM 101723T and S. cinnabararigriseus DSM 101724T were amplified by PCR and sequenced by the Sanger sequencing method using primers 27F and 1492R [36]. To clarify the evolutionary distance between the strains and to determine their phylogenetic relationship to other Streptomyces type strains, detailed phylogenetic analyses on nearly full-length 16S rRNA gene sequences were performed in ARB release 5.2 [37] using the All-Species Living Tree Project (LTP) [38] database (release LTPs123, September 2015). All sequences not included in the LTP database were aligned according to the SILVA seed alignment using the SILVA Incremental Aligner (SINA) version 1.2.9 (www.arb-silva.de) [39] before the sequences were implemented into the ARB database. The alignment of all sequences of Streptomycescaceae type strains was manually corrected including secondary structure information.

Phylogenetic trees were reconstructed with the maximum-likelihood method using PhyML and RAxML version 7.0.4 [40] with the general time-resolved model (GTR) [52]. Gene sequences were derived by genome BLAST (GenBank, NCBI) from the genome sequences. Nucleotide sequences of the ATP synthase F1, β-subunit (atpD, 495 nt), the RNA polymerase, β-subunit (rpoB, 420 nt), the recombinase A (recA, 504 nt), the DNA gyrase, β-subunit (gyrB, 540 nt) and the tryptophan synthase, β-subunit (trpB, 567 nt). The analysis was performed in MEGA7 version 7.10.2 [51]. Gene sequences were derived by genome BLAST (GenBank, NCBI) from the genome sequences. Nucleotide sequences were aligned according to the respective amino acid sequence based-alignment and concatenated in the following order: atpD, rpoB, recA, gyrB and trpB. Phylogenetic trees were reconstructed with the maximum-likelihood method using the general time-resolved model (GTR) as implemented in the analysis.

Phylogenetic analyses were furthermore performed by a multilocus sequence analysis (MLSA) scheme established for the genus Streptomyces [47–50]. Partial nucleotide sequences of the ATP synthase F1, β-subunit (atpD, 495 nt), the RNA polymerase, β-subunit (rpoB, 420 nt), the recombinase A (recA, 504 nt), the DNA gyrase, β-subunit (gyrB, 540 nt) and the tryptophan synthase, β-subunit (trpB, 567 nt). The analysis was performed in MEGA7 version 7.10.2 [51]. Gene sequences were derived by genome BLAST (GenBank, NCBI) from the genome sequences. Nucleotide sequences were aligned according to the respective amino acid sequence based-alignment and concatenated in the following order: atpD, rpoB, recA, gyrB and trpB. Phylogenetic trees were reconstructed with the maximum-likelihood method using the general time-resolved model (GTR) as implemented in the analysis.

Another tool for the phylogenetic description of bacteria is the analysis of ribosomal proteins with the help of the matrix-assisted laser-desorption/ionization time-of-flight spectrometry (MALDI-TOF MS). Sample preparation was done according to the ethanol/formic acid extraction protocol described by protocol 3 of Schumann and Maier [54]. About 10 mg biomass from a liquid culture (cultivated in the complex medium DSMZ 65 at 30 °C for 7 days) was suspended in 300 µl H2O and homogenised carefully. Subsequently, 900 µl ethanol was added to the resulting suspension. The cells were collected after a centrifugation step and suspended in 50 µl of 70% formic acid. After the addition of 50 µl acetonitrile, the suspension was mixed and
centrifuged. The supernatant was removed immediately and aliquots of 1.5 µl were placed on each spot of a stainless steel target plate. After air drying 1.5 µl matrix solution (saturated solution of α-cyano-hydroxy-cinnamic acid in 50 % aqueous acetonitrile containing 2.5 % trifluoro acetic acid) was added to each spot. Mass spectrometric analysis was conducted using a Microflex L20 mass spectrometer (Bruker Daltonics) with a N₂ laser unit. The spectra were recorded in linear positive mode and the acceleration voltage was 20 kV. In sum, a spectrum was collected out of 250 shots on a spot. To analyse the data, a mass range of 2000-20 000 m/z was observed. For internal calibration, the Bacterial Test Standard #255343 (Bruker Daltonics) was used.

Using FlexAnalysis software (version 3.4, Bruker Daltonics), the MALDI-TOF MS spectra were smoothed, baseline-corrected and re-calibrated. A score-orientated dendrogram was calculated by using BioTyper software (version 3.1, Bruker Daltonics). MALDI-TOF MS provided complementary evidence to the phylogenetic relationship between S. davawensis DSM 42467 and other strains used for the characterization of S. cinnabarigriseus (=JCM 4913). S. cinnabarigriseus was able to utilize cellulose but not D-xylene. In contrast to S. davawensis and S. cinnabarigriseus, S. atriruber was not able to use sucrose but did use xylose. Growth on mannose, fructose and rhamnose was also much better in this species. S. cinnabarigriseus was the only species of the closely related group (more than 98.5 % homology in 16Sr RNA) that expressed the combination of a reddish substrate mycelium and grey spore mass; only Streptomyces griseoruber (DSM 40281) showed some similarities in colour, but this strain is not producing any soluble pigment on the ISP media. All other Streptomyces species of this group are having brown or beige substrate mycelium. A comparison of S. davawensis and S. cinnabarigriseus with Streptomyces atriruber (DSM 41860), a closely related species, showed grey spore mass in S. atriruber on some of the ISP media, whereas the other two produced white to cream aerial mycelia.

Glucose, arabinose, sucrose, inositol, mannose, fructose, rhamnose and raffinose were utilized as a carbon source by strains S. cinnabarinus DSM 40467, S. davawensis DSM 101723 and S. cinnabarigriseus DSM 101724. Strain DSM 40467 was able to utilize cellulose but not D-xylene (Table 1) whereas S. cinnabarinus DSM 40467 and S. davawensis DSM 101724 did not grow on cellulose (but did on D-xylene). In contrast to S. davawensis and S. cinnabarinus, S. atriruber was not able to use sucrose but did use xylose. Growth on mannose, fructose and rhamnose was also much better in this species. S. cinnabarigriseus was the only species in the group with high homology that was able to use all carbohydrates that were tested.

Enzymatic activities in S. cinnabarinus, S. davawensis and S. cinnabarigriseus and other Streptomyces strains and isolates were monitored by using the API ZYM test system (Table 1). S. cinnabarinus, S. davawensis and S. cinnabarigriseus showed similar enzymatic patterns, although some differences were observed. α-Galactosidase appeared to be present in S. cinnabarigriseus only, N-acetyl-β-glucosaminidase was found in S. davawensis only and α-fucosidase was found in S. cinnabaricus only. S. atriruber and S. cinnabarinus were able to form a melanoid pigment, but ‘S. davawensis’ was not. In the S. cinnabarigriseus group, besides this strain, S. griseoruber, S. ciscaucasicus, S. cellosomaticus and S. avermitilis were also able to produce a melanoid pigment.

The menaquinone profile of ‘S. davawensis’ DSM 101723 contained MK-9(H4), MK-9(H6) and MK-9(H2) (52 : 26 : 22). The strain exhibited a complex polar lipid profile, containing the major polar lipids diphosphatidylglycerol and phosphatidylethanolamine, minor amounts of phosphatidylglycerol, phosphatidylinerine and phosphatidylinositol, three unidentified phospholipids, one unidentified aminolipid, and two hydrophobic and two hydrophilic yet unidentified lipids (Fig. 3a).
The menaquinone profile of *S. cinnabarigriseus* DSM 101724\(^T\) consisted of MK-9(H6) and MK-9(H8) (72 : 28). It exhibited a polar lipid profile that contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, four unidentified phospholipids and three unidentified aminolipids (Fig. S2).

The major cellular fatty acids (see Table S3) of *S. davawensis* DSM 101723\(^T\) were 15:0 iso (11.6 %), 15:0 anteiso (13.1 %), 16:0 iso (13.4 %), 16:1 cis9 (2.0 %), 16:0 (5.0 %), 16:0 9 methyl (9.9 %), 17:1 anteiso C (4.3 %), 17:0 iso (14.0 %) and 17:0 anteiso (19.8 %). The comparison with the most correlated strains showed a high amount of 15:0 iso, 17:0 anteiso and 16:0 9 methyl and a low amount of 15:0 anteiso. The cell wall of *S. cinnabarigriseus* DSM 101724\(^T\) contained 14:0 iso (3.7 %), 15:0 iso (17.7 %), 15:0 anteiso (15.4 %), 16:1 isoH (3.4 %), 16:0 iso (17.1 %), 16:1 cis9 (7.9 %), 16:0 (12.3 %), 16:0 9 methyl (3.6 %), 17:1 anteiso C (1.8 %), 17:0 iso (5.5 %) and 17:0 anteiso (6.3 %). Here, the amount of 15:0 iso, 16:0, 16:1 cis9 and 17:0 iso was higher, but that of 15:0 anteiso, 16:1 cis9 and 17:0 anteiso was lower in comparison with the related type strains.

Pairwise 16S rRNA gene sequence similarity analysis showed that *S. davawensis* DSM 101723\(^T\) and *S. cinnabarins* DSM 40467\(^T\) shared 99.0 % 16S rRNA gene sequence similarity with each other and 98.5 % 16S rRNA gene sequence similarity to strain JS360\(^T\). Phylogenetic analysis based on 16S rRNA gene sequences including all *Streptomyces* type strains indicated that several other *Streptomyces* type strains also shared high 16S rRNA gene sequence similarities with the three strains investigated here and clustered in between the three strains in phylogenetic trees based on all current *Streptomyces* type strains (Fig. 2). While *S. davawensis* DSM 101723\(^T\) and *S. cinnabarins* DSM 40467\(^T\) shared highest 16S rRNA gene sequence similarity with each other, strain JS360\(^T\) shared highest 16S rRNA gene sequence similarity with the type strains of *S. griseoru- biginosus* (DSM 40469\(^T\)), *S. avermitilis* (DSM 46492\(^T\)) and *S. olivochromogenes* (DSM 40451\(^T\)) (all 98.8 %) (see Fig. 1. Scanning electron micrographs displaying the mycelia morphology of a variety of *Streptomyces* strains a, b: *S. cinnabarins* DSM 40467\(^T\), c, d: *S. davawensis* JCM 4913 (=DSM 101723\(^T\)), e, f: *S. cinnabarigriseus* JS360\(^T\) (=DSM 101724\(^T\)). Cultivation was carried out on ISP 3 medium at 28 °C for 14 days. (Bar: a, b, c, d, f: 1 µm; e: 2 µm).
Table 1. Physiological properties of: 1, Streptomyces davawensis JCM 4913=DSM 101723\(^T\); 2, Streptomyces cinnabarinus DSM 40467\(^T\); 3, Streptomyces cinnabarinus JS60=DSM 101724\(^T\); 4, Streptomyces avermitilis DSM 46492\(^T\); 5, Streptomyces flavoviridis DSM 41479\(^T\); 6, Streptomyces novaeaeareae DSM 40358\(^T\); 7, Streptomyces albogriseus DSM 40043\(^T\); 8, Streptomyces cellostatisicus DSM 40189\(^T\); 9, Streptomyces bobbi DSM 40065\(^T\); 10, Streptomyces gailaua DSM 40481\(^T\); 11, Streptomyces griseochromogenes DSM 40499\(^T\); 12, Streptomyces pseudovenezuelae DSM 40212\(^T\); 13, Streptomyces peucetius DSM 41896\(^T\); 14, Streptomyces atriruber DSM 41860\(^T\); 15, Streptomyces resistomyceticus DSM 40133\(^T\); 16, Streptomyces yokosukaeus DSM 40224\(^T\); 17, Streptomyces olivochromogenes DSM 40451\(^T\); 18, Streptomyces orchidis DSM 40340\(^T\); 19, Streptomyces longwoodensis DSM 41677\(^T\); 20, Streptomyces curvus DSM 40107\(^T\); 21, Streptomyces antibioticus DSM 40234\(^T\); 22, S. canus DSM 40017\(^T\); 23, S. cicaucusicus DSM 40275\(^T\); 24, Streptomyces griseoflavus DSM 40469\(^T\); 25, Streptomyces phaeopurpureus DSM 40125\(^T\); and 26, Streptomyces griseonibryus DSM 40281\(^T\); all data from this study.

| 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
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|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

API ZYM tests:

- Phosphatase alkaline
- Esterase (C4)
- Esterase lipase (C8)
- Lipase (C14)
- Leucine arylamidase
- Valine arylamidase
- Cystine arylamidase
- Trypsin
- Chymotrypsin
- Phosphatase acid
- Naphthol-AS-Bl-phosphohydrodase
- α-Galactosidase
- β-Galactosidase
- α-Glucosidase
- β-Glucosidase
- N-acetyl-β-glucosaminidase
- α-Mannosidase
- α-Fucosidase
- Carbon utilization:
  - Glucose
  - Arabinose
  - Sucrose
  - Xylose
  - Inositol
  - Mannose
  - Fructose
  - Rhamnose
  - Raffinose
  - Cellulose
Fig. 2. Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic position of *S. cinna-barisisus* DSM 101724T, *S. davaonensis* DSM 101723T and *S. cinnabarinus* DSM 40467T among each other and to type strains of next closest related *Streptomyces* species. The phylogenetic tree was generated with ARB in the LTPs database release LTPs123 using RAxML with GTR-GAMMA and rapid bootstrap analysis (100 replications). Only part of the original tree is depicted here including the three
Table 2). In the 16S rRNA gene sequence-based phylogenetic tree ‘S. davawensis’ DSM 101723T formed a distinct cluster (supported by a bootstrap value of 80 %) with the type strain of S. atriruber (DSM 41860T), while S. cinnabarinus DSM 40467T clustered with other type strains (Fig. 2). S. davawensis DSM 101723T and the type strain of S. atriruber shared 98.8 % 16S rRNA gene sequence similarity.

Phylogenetic trees based on nucleotide (Fig. 3a) and amino acid sequences (Fig. 3b) of genes shared by the investigated strains showed that ‘S. davawensis’ DSM 101723T and S. cinnabarinus DSM 40467T clustered together while strain JS360T clustered with S. griseoruber DSM 40281T. Those phylogenetic relationships were also confirmed by the five-gene-based MLSA analysis at the level of concatenated nucleotide (Fig. 3c) or amino acid sequences (Fig. 3d).

The GGD of the WGS sequences of ‘S. davawensis’ DSM 101723T and S. cinnabarinus DSM 40467T was 54.7 % and the two strains shared an ANI of 92.1 % (reciprocal 91.8 %). Furthermore, they shared a GGD of 26.4 and 27.2 % and an ANI of 92.1 % (reciprocal 91.8 %). Table 2 shows pairwise 16S rRNA gene sequence similarities (calculated in the LTP database of ARB-SILVA), genome-to-genome distances (calculated with the GGDC) and mean ANI values (determined in EzGenome), MLSA distances (pairwise distance of concatenated partial atpD-gyrB-recA-rpoB-trpB gene sequences calculated in MEGA 7 using Kimura’s two-parameter model for distance calculation).

### Table 2. Comparative genotypic analysis of S. cinnabarigriseus JS360T (=DSM 101724T) and S. davaonensis JCM 4913T (=DSM 101723T) with each other and with closest related type strains (>98.65 % 16S rRNA gene sequence similarity)

<table>
<thead>
<tr>
<th>Next closest related species (type strain 16S rRNA gene sequence numbers are given)</th>
<th>16S rRNA gene sequence similarity</th>
<th>GGD</th>
<th>GGD range</th>
<th>ANI</th>
<th>MLSA distance</th>
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<tr>
<td>S. cellostaticus DSM 40189T</td>
<td>99.9</td>
<td>26.2</td>
<td>[23.8–28.7 %]</td>
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<tr>
<td>S. griseoruber DSM 40281T</td>
<td>99.4</td>
<td>50.1</td>
<td>[47.5–52.8 %]</td>
<td>92.1507 (91.7644)</td>
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<td>S. yokosukaei DSM 40224T</td>
<td>98.9</td>
<td>25.3</td>
<td>[23–27.8 %]</td>
<td>80.8914 % (80.5128 %)</td>
<td>0.068</td>
</tr>
<tr>
<td>S. canus DSM 40017T</td>
<td>98.9</td>
<td>26.6</td>
<td>[24.3–29.1 %]</td>
<td>82.1035 % (81.6348 %)</td>
<td>0.065</td>
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<tr>
<td>S. ciscaucasicus DSM 40275T</td>
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<td>26.4</td>
<td>[24–28.9 %]</td>
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</tr>
<tr>
<td>S. adustus DSM 46492T</td>
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</tr>
<tr>
<td>S. avermitilis DSM 46492T</td>
<td>98.8</td>
<td>25.1</td>
<td>[22.8–27.6 %]</td>
<td>80.5072 % (80.723 %)</td>
<td>0.078</td>
</tr>
<tr>
<td>S. rhizophaerhabitans</td>
<td>98.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>S. olivochromogenes DSM 40451T</td>
<td>98.8</td>
<td>26.9</td>
<td>[24.5–29.4 %]</td>
<td>82.3222 % (82.7291 %)</td>
<td>0.074</td>
</tr>
<tr>
<td>S. griseorubignus DSM 40469T</td>
<td>98.8</td>
<td>29.7</td>
<td>[24.7–29.5 %]</td>
<td>82.5249 % (82.3482 %)</td>
<td>0.061</td>
</tr>
<tr>
<td>S. antibioticus DSM 40234T</td>
<td>98.7</td>
<td>27.1</td>
<td>[24.7–29.5 %]</td>
<td>82.5249 % (82.3482 %)</td>
<td>0.061</td>
</tr>
<tr>
<td>S. phaeoverticorum DSM 40125T</td>
<td>98.7</td>
<td>27.3</td>
<td>[24.9–29.8 %]</td>
<td>82.695 % (82.8268 %)</td>
<td>0.074</td>
</tr>
<tr>
<td>S. davaonensis JCM 4913T</td>
<td>98.5</td>
<td>26.4</td>
<td>[24–28.9 %]</td>
<td>81.7213 % (81.7699 %)</td>
<td>0.066</td>
</tr>
<tr>
<td>S. cinnabarinus NRRL B-12382T</td>
<td>98.5</td>
<td>27.2</td>
<td>[24.8–29.7 %]</td>
<td>81.7219 % (81.6201 %)</td>
<td>0.063</td>
</tr>
</tbody>
</table>

### Table 2. Comparative genotypic analysis of Streptomyces davawensis JCM 4913T

<table>
<thead>
<tr>
<th>Next closest related species (type strain 16S rRNA gene sequence numbers are given)</th>
<th>16S rRNA gene sequence similarity</th>
<th>GGDC</th>
<th>GGDC range</th>
<th>ANI</th>
<th>MLSA distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cinnabaritus NRRL B-12382T</td>
<td>99.1</td>
<td>54.7</td>
<td>[52–57.4 %]</td>
<td>93.5228 % (93.3171 %)</td>
<td>0.017</td>
</tr>
<tr>
<td>S. adustus</td>
<td>98.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>S. pseudovenezuelae DSM 40212T</td>
<td>98.9</td>
<td>27.1</td>
<td>[24.8–29.6 %]</td>
<td>82.8886 % (82.5892 %)</td>
<td>0.054</td>
</tr>
<tr>
<td>S. cellostaticus DSM 40189T</td>
<td>98.8</td>
<td>26.4</td>
<td>[24.1–28.9 %]</td>
<td>82.1932 % (81.885 %)</td>
<td>0.083</td>
</tr>
<tr>
<td>S. avermitilis DSM 46492T</td>
<td>98.8</td>
<td>25.9</td>
<td>[23.5–28.3 %]</td>
<td>81.6708 % (81.7138 %)</td>
<td>0.072</td>
</tr>
<tr>
<td>S. novocaesareae NRRL B-1267T</td>
<td>98.8</td>
<td>21.7</td>
<td>[19.4–24.1 %]</td>
<td>73.7378 % (73.7177 %)</td>
<td>0.158</td>
</tr>
<tr>
<td>S. phaeoverticicus</td>
<td>98.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>S. atriruber NRRL B-24165T</td>
<td>98.8</td>
<td>23.1</td>
<td>[20.8–25.5 %]</td>
<td>78.7158 % (78.2872 %)</td>
<td>0.095</td>
</tr>
<tr>
<td>S. flavoviridans NRRL B-16367T</td>
<td>98.7</td>
<td>26.8</td>
<td>[24.5–29.2 %]</td>
<td>82.4787 % (82.3041 %)</td>
<td>0.068</td>
</tr>
<tr>
<td>S. cinnabarigriseus DSM 101724T (=JS360T)</td>
<td>98.5</td>
<td>27.2</td>
<td>[24.8–29.7 %]</td>
<td>81.7699 % (81.7213 %)</td>
<td>0.066</td>
</tr>
</tbody>
</table>

ND, not determined.
ANI of 81.769 % (81.7 %) and an ANI of 81.6 % (reciprocal 81.7 %) with S. griseoruber DSM 40281T and a GGD of 26.2 % and an ANI of 81.6 % (reciprocal 81.2 %) with S. cellostaticus DSM 40189T, the two closest related species, respectively. All further GGD and ANI values to next related type strains were in the same range or lower; all values were clearly below the proposed species cut-off values [55–57]. The species differentiation was furthermore confirmed by the high pairwise distances obtained from concatenated sequences of the MLSA approach. Based on the comparison of DDH values and MLSA-based genetic distances [48–50] calculated, two strains of two different species shared a pairwise nucleotide distance based on the MLSA approach of >0.007. None of the pairwise nucleotide differences were below that value (Table 2).

Studies using MALDI-TOF MS (results see Fig. S3) support the view that strain DSM 101723T is closely related to the type strain of S. cinnabarinus DSM 40467T. Strain DSM 101724T is closely related to S. griseoruber DSM 40281T.  

**DESCRIPTION OF STREPTOMYCES DAVAONENSIS SP. NOV.**  

*S. davaonensis* (da.v.a.o.nen’sis. N.L. masc. adj. davaonensis pertaining to the sampling site Davao City in the Philippines).

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**Fig. 3.** Phylogenetic relationships of strains *S. cinnabarinus* DSM 101724T, *S. davaonensis* DSM 101723T and *S. cinnabarinus* DSM 40467T with each other and to the next closest related type strains based on nucleotide (a) and amino acid sequences (b) of the shared core genomes as well as nucleotide (c) and amino acid sequences (d) of the five concatenated protein-coding genes atpD, gyrB*, recA, rpoB, and trpB. Gene sequences of *Streptomyces galilaeus* AS 4.1320T and *Streptomyces cyaneus* AS 4.1671T were taken from Guo et al. (2009). All other sequences were obtained from genome sequences. All genome accession numbers are given in Table S1. Bars, 0.01 nucleotide or amino acid sequence exchange per sequence position.
This species is a non-motile, Gram-positive aerobic actinomycete which forms an aerial mycelium with spore chains on ISP3, ISP4, ISP5 and ISP7 media. The spores form fragmented, almost parallel laying spore chains comprised of slightly rough spores. The colour of the substrate mycelium differs between sand yellow (ISP2), light pink (ISP3), red orange (ISP4), black red (ISP5, 7) and greenish brown (ISP6). Formation of a reddish pigment is observed on ISP 3 medium and an olive brown pigment on ISP6 and also on ISP7. Growth is detected at sodium chloride concentration of 0 to 5 % (w/v) and temperature between 15 to 37 °C. The utilization of D-glucose, D-arabinose, sucrose, D-xylene, inositol, D-mannose, D-fructose, L-rhamnose and raffinose, but not cellulose, is observed. The strain is melanin-negative. A positive enzymatic reaction can be determined (using API ZYM strips) for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, phosphatase acid, naphtol-AS-BI-phosphohydrolase, alpha glucosidase, beta glucosidase, alpha fucosidase and N-acetyl-beta-glucosaminidase, aspartate aminotransferase, endogalactosidase, beta galactosidase, alpha glucosidase, beta glucuronidase and alpha fucosidase activities are not detected.

The cell wall contains L- diaminopimelic acid and 15 : 0 iso, 15: 0 anteiso, 16 : 0 iso, 16 : 0 : 9 methyl, 17 : 1 anteiso C, 17 : 0 iso and 17: 0 anteiso as major fatty acids. The menaquinone profile of S. cinnabarigriseus consists of MK-9(H6) and MK-9(H8) (72 : 28).

The type strain is JS360T (=DSM 101724T=NCCB100590T).

DESCRIPTION OF STREPTOMYCES CINNABARIGRISEUS SP. NOV.

S. cinnabarigriseus (cin.na.ba.ri.gri’seu.us. L. fem. n. cinnabar-ris cinnabar; L. adj. griseus grey; N.L. masc. adj. cinnabrigri-seus referring to a brick-red colour of the mycelium and the grey spore mass).

Aerobic, Gram-positive, non-motile actinomycete which forms a highly branched and long substrate mycelium and aerial hyphae which differentiate into spore chains which appear to dry and fragment. Growth is detected at 15–40 °C and 0–7.5 % NaCl. Able to utilise D-glucose, D-arabinose, sucrose, D-xylene, inositol, D-mannose, D-fructose, L-rhamnose and raffinose, but not cellulose. Melanin-positive and shows the following enzymatic activities: phosphatase alkaline, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, phosphatase acid, naphtol-AS-BI-phosphohydrolase, alpha galactosidase, beta galactosidase, alpha glucosidase, beta glucosidase and alpha mannosidase. The cells are weakly positive with regard to trypsin and negative with regard to chymotrypsin, beta glucuronidase, N-acetyl-beta-glucosaminidase and alpha fucosidase. The cell wall contains L- diaminopimelic acid and 14 : 0 iso, 15 : 0 iso, 15: 0 anteiso, 16 : 0 iso, 16 : 1 cis9, 16 : 0, 16 : 0 : 9 methyl, 17 : 1 anteiso C, 17 : 0 iso and 17: 0 anteiso as major fatty acids. The

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References


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