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Differences at Species Level and in Repertoires of Secondary Metabolite Biosynthetic Gene Clusters among *Streptomyces coelicolor* A3(2) and Type Strains of *S. coelicolor* and Its Taxonomic Neighbors

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Abstract: *Streptomyces coelicolor* A3(2) is used worldwide for genetic studies, and its complete genome sequence was published in 2002. However, as the whole genome of the type strain of *S. coelicolor* has not been analyzed, the relationship between *S. coelicolor* A3(2) and the type strain is not yet well known. To clarify differences in their biosynthetic potential, as well as their taxonomic positions, we sequenced whole genomes of *S. coelicolor* NBRC 12854^T and type strains of its closely related species—such as *Streptomyces daghestanicus*, *Streptomyces hydrogenans*, and *Streptomyces violascens*—via PacBio. Biosynthetic gene clusters for polyketides and non-ribosomal peptides were surveyed by antiSMASH, followed by bioinformatic analyses. Type strains of *Streptomyces albidoflavus*, *S. coelicolor*, *S. daghestanicus*, *S. hydrogenans*, and *S. violascens* shared the same 16S rDNA sequence, but *S. coelicolor* A3(2) did not. *S. coelicolor* A3(2) and *S. coelicolor* NBRC 12854^T can be classified as *Streptomyces anthocyanicus* and *S. albidoflavus*, respectively. In contrast, *S. daghestanicus*, *S. hydrogenans*, and *S. violascens* are independent species, despite their identical 16S rDNA sequences. *S. coelicolor* A3(2), *S. coelicolor* NBRC 12854^T, *S. daghestanicus* NBRC 12762^T, *S. hydrogenans* NBRC 13475^T, and *S. violascens* NBRC 12920^T each harbor specific polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) gene clusters in their genomes, whereas PKS and NRPS gene clusters are well conserved between *S. coelicolor* A3(2) and *S. anthocyanicus* JCM 5058^T, and between *S. coelicolor* NBRC 12854^T and *S. albidoflavus* DSM 40455^T, belonging to the same species. These results support our hypothesis that the repertoires of PKS and NRPS gene clusters are different between different species.

Keywords: biosynthetic gene; genome; non-ribosomal peptide; polyketide; *Streptomyces*

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1. Introduction

Actinomycetes are Gram-positive filamentous bacteria that are attracting attention as a source of bioactive secondary metabolites. Many pharmaceuticals, such as antibiotics, have been developed from these metabolites. The genus *Streptomyces* is a representative actinomycete, and includes approximately 673 species with validly published names at present. These members are a promising source of diverse bioactive compounds. In 1908, "*Streptothrix coelicolor*" (the former genus name for *Streptomyces*), which was characterized by its production of a blue pigment, was isolated by Müller [1,2]. The species name *Streptomyces coelicolor* was effectively published by Waksman and Henrici in 1948 [3], and validly approved in 1980 [4]. The type strain is Müller's strain, and has been added to the NBRC culture collection as NBRC 12854^T. On the other hand, *Streptomyces coelicolor* A3(2) is derived from Waksman's strain 3443. Stanier obtained several cultures from Waksman, which were classified as "*Actinomyces coelicolor*" based on cultural characteristics. Erikson obtained Waksman's strain 3443 from Stanier through van Niel, isolated a single spore, and designated it *Streptomyces coelicolor* A3(2) according to the current systematics for actinomycetes. Hopwood obtained this strain from Erikson as a starting strain for use

in genetic work [1]. Today, *S. coelicolor* A3(2) is used worldwide for genetic studies, and as a host for gene cloning experiments of streptomycetes [5–7]. According to a PubMed search, more than 3000 papers in which *S. coelicolor* A3(2) is used have been published to date. In 2002, the whole genome of the strain was sequenced, which revealed that it harbors a few dozen secondary metabolite biosynthetic gene clusters (smBGCs) in its genome. This number is more than that discovered by previous experiments based on activity-based screening for novel secondary metabolites. [8] Recent genome analyses have introduced a new approach called "genome mining" in the field of research for untapped secondary metabolites [9]. As the complete genome sequencing of *S. coelicolor* A3(2) had been published earlier, subsequent genome analyses of the genus *Streptomyces* have been targeted at strains belonging to species other than *S. coelicolor* [10]. Consequently, even the type strain of *S. coelicolor* has not been genome sequenced, although extensive whole-genome sequencing projects are ongoing [11]. The present study aims to provide the whole-genome sequence of the type strain, clarify the taxonomic relationship between *S. coelicolor* A3(2) and type strains of *S. coelicolor* and its closely related species, and reveal their potential to synthesize secondary metabolites such as polyketides and non-ribosomal peptides; it also aims to broaden knowledge about the potential of each strain as a source of diverse secondary metabolites, since these compounds are representative secondary metabolites in actinomycetes [10], diverse in bioactivities and structures, and often studied for evaluation as sources of new secondary metabolites [12–17]. This study posits that correct classification is significant in the pursuit of knowledge about the relationships between each species and their biosynthetic potential for secondary metabolites.

2. Materials and Methods

EzBioCloud [18] was used to search for taxonomic neighbors based on 16S rDNA sequences. Multilocus sequence analysis (MLSA) was conducted using DNA sequences of five housekeeping genes—*atpD*, *gyrB*, *recA*, *rpoB*, and *trpB*—as established in the genus *Streptomyces* [19]. The accession numbers of used gene sequences for MLSA are listed in Table 1. The phylogenetic trees were reconstructed using ClustalX 2.1 [20]. *S. coelicolor* NBRC 12854^{T}, *Streptomyces daghestanicus* NBRC 12762^{T}, *Streptomyces hydrogenans* NBRC 13475^{T}, and *Streptomyces violascens* NBRC 12920^{T} were distributed from the NBRC Culture Collection; their genomic DNA was prepared from cultured cells via the method of Saito and Kimura [21]. Subsequently, library preparation and whole-genome de novo sequencing were performed by the Kazusa DNA Research Institute using a single-molecule real-time (SMRT) strategy. Sequencing was performed using the BluePippin system (Sage Science) with a SMRTbell Template Prep Kit 1.0 and a SMRTbell Damage Repair Kit (Pacific Bioscience), via the Sequel system with Sequel SMRT cell 1M versions 2 and 3, Sequel Sequencing Kits 2.1 and 3.0, a Sequel Binding Kit 2.0, and a Sequel Binding and Internal Ctrl Kit 3.0 (Pacific Biosciences). The resulting reads for each strain were assembled using SMRT Link version 6.0 (Pacific Bioscience) and Prokka 1.13.3. The assembled genome sequences were deposited to DDBJ under the following accession numbers: *S. coelicolor* NBRC 12854^{T}, BNDZ00000000.1; *S. daghestanicus* NBRC 12762^{T}, BNDX00000000.1; *S. hydrogenans* NBRC 13475^{T}, BNDW00000000.1; and *S. violascens* NBRC 12920^{T}, BNDY00000000.1. DNA–DNA relatedness was digitally calculated using Formula 2 of the Genome-to-Genome Distance Calculator (GGDC) [22]. Polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) gene clusters in the genomes were surveyed using antiSMASH [23], and then manually analyzed as reported previously [15].

Table 1. Accession numbers of housekeeping gene sequences used in MLSA.

Strain	*atpD*	*gyrB*	*recA*	*rpoB*	*trpB*
S. albus NBRC 13014^T	BBQG01000033	BBQG01000007	BBQG01000035	BBQG01000012	BBQG01000017
"S. abyssomicinicus" CHI39^T	BBZI01000006	BBZI01000009	BBZI01000014	BBZI01000006	BBZI01000008
S. albidoflavus DSM 40455^T	FJ406416	FJ406427	FJ406438	FJ406449	FJ406460
S. althioticus NRRL B-3981^T	KT384460	KT384809	KT385157	KT388779	KT389129
S. ambofaciens NRRL B-2516^T	KT384462	KT384811	KT385159	KT388781	KT389131
S. anthocyanicus NRRL B-24292^T	KT384465	KT384814	KT385162	KT388784	KT389134
S. ardesiacus NRRL B-1773^T	KT384534	KT384883	KT385231	KT388853	KT389203
S. arenae NRRL ISP 5293^T	KT384470	KT384819	KT385167	KT388789	KT389139
S. cadmiisoli ZFG47^T	CP030073	CP030073	CP030073	CP030073	CP030073
S. carpinensis NRRL B-16921^T	KT384503	KT384852	KT385200	KT388822	KT389172
S. cinerochromogenes NRRL B-16928^T	KT384514	KT384863	KT385211	KT388833	KT389183
S. coelicoflavus NRRL B-16363^T	KT384524	KT384873	KT385221	KT388843	KT389193
S. coelicolor NBRC 12854^T	BNDZ01000005	BNDZ01000005	BNDZ01000003	BNDZ01000005	BNDZ01000005
S. coelicolor A3(2)	AL939123	AL939118	AL939125	AL939121	AL939121
S. coeruleorubidus NRRL B-2569^T	KT384528	KT384877	KT385225	KT388847	KT389197
S. collinus NRRL B-5412^T	KT384529	KT384878	KT385226	KT388848	KT389198
S. daghestanicus NRRL B-5418^T	KJ137021	KJ137038	KJ137055	KJ996779	KJ137089
S. diastaticus NBRC 13412^T	BLLN01000005	BLLN01000002_	BLLN01000005	BLLN01000002	BLLN01000003
S. eurythermus NRRL ISP-5014^T	KT384544	KT384893	KT385242	KT388863	KT389213
S. flaveolus NRRL B-1334^T	KT384550	KT384899	KT385248	KT388869	KT389219
S. flavofungini JCM 4753^T	JAEKOZ01000002	JAEKOZ01000001	JAEKOZ010000015	JAEKOZ010000008	JAEKOZ010000005
S. fragilis NBRC 12862^T	BEVZ01000004	BEVZ01000008	BEVZ01000002	BEVZ01000004	BEVZ01000003
S. griseoflavus NRRL B-5312^T	KT384578	KT384927	KT385276	KT388897	KT389247
S. griseoincarnatus NRRL B-5313^T	KT384580	KT384929	KT385278	KT388899	KT389249
S. griseorubens NRRL B-3982^T	KT384583	KT384932	KT385281	KT388903	KT389252
S. hawaiiensis NRRL B-1988^T	KT384592	KT384941	KT385290	KT388912	KT389261
S. hyderabadensis JCM 17657^T	JAIOJZ010000004	JAIOJZ010000004	JAIOJZ010000060	JAIOJZ010000079	JAIOJZ010000001
S. hydrogenans NBRC 13475^T	BNDW01000004	BNDW01000040	BNDW01000004	BNDW01000004	BNDW01000019
S. iakyrus NRRL B-3317^T	KT384600	KT384949	KT385298	KT388920	KT389269
S. indiaensis NRRL B-24311^T	KT384601	KT384950	KT385300	KT388921	KT389270
S. intermedius NRRL B-2670^T	KT384602	KT384951	KT385301	KT388922	KT389271
S. janthinus NRRL B-3365^T	KT384604	KT384953	KT385303	KT388924	KT389273
S. koyangensis JCM 14915^T	LC381971	LC381972	LC381973	LC381974	LC413709
S. lavenduligriseus NRRL ISP-5487^T	JOBD01000030	JOBD01000019	JOBD01000017	JOBD01000005	JOBD01000013
S. levis NRRL B-16370^T	KT384621	KT384970	KT385320	KT388941	KT389290
S. lienomycini NRRL B-16371^T	KT384622	KT384971	KT385321	KT388942	KT389291
S. lomondensis NRRL 3252^T	KT384626	KT384975	KT385326	KT388946	KT389295
S. luteogriseus NRRL B-12422^T	KT384632	KT384981	KT385332	KT388952	KT389301
S. massasporeus NRRL B-3300^T	KT384636	KT384985	KT385336	KT388956	KT389305
S. mutabilis NRRL ISP-5169^T	KT384652	KT385002	KT385353	KT388972	KT389321
S. nigra 452^T	CP029043	CP029043	CP029043	CP029043	CP029043
S. nogalater NRRL ISP-5546^T	KT384664	KT385014	KT385365	KT388984	KT389333
S. olivaceus NRRL B-1224^T	KT384667	KT385017	KT385368	KT388987	KT389336
S. pactum ATCC 27456^T	JACYXC010000001	JACYXC010000001	JACYXC010000001	JACYXC010000001	JACYXC010000001
S. paradoxus NRRL B-3457^T	KT384674	KT385024	KT385375	KT388994	KT389343
S. parvulus NRRL B-1628^T	KJ196367	KJ196369	KJ196371	KJ196373	KJ196375
S. purpurascens NRRL B-12230^T	KT384696	KT385046	KT385397	KT389017	KT389365
S. rochei NRRL B-2410^T	KT384704	KT385054	KT385405	KT389025	KT389373
S. roseoviolaceus NRRL B-12177^T	KT384710	KT385060	KT385411	KT389031	KT389379
S. rubrogriseus NRRL B-24295^T	KT384715	KT385065	KT385416	KT389036	KT389384
S. spinoverrucosus NRRL B-16932^T	KT384725	KT385074	KT385426	KT844525 *	KT389394
S. tendae NRRL B-2313^T	KT384733	KT385082	KT385434	KT389053	KT389402
S. tibetensis XZ 46^T	SZVR01000021	SZVR01000021	SZVR01000011	SZVR01000023	SZVR01000005
S. tricolor NBRC 103112^T	LC634004	LC634005	LC634006	LC634007	LC634008
S. tuirus NRRL B-3631^T	KT384742	KT385090	KT385444	KT389062	KT389411
S. venetus CMU-AB225^T	LC381976	LC381977	LC381978	LC381979	LC381980
S. violaceochromogenes NRRL B-5427^T	KT384748	KT385096	KT385450	KT389068	KT389417
S. violaceoruber NBRC 12826^T	LC634009	LC634010	LC634011	LC634012	LC634013
S. violaceorubidus NRRL B-16381^T	JODM01000021	JODM01000004	JODM01000011	JODM01000001	JODM01000015
S. violaceus JCM 4533^T	LC381981	LC381982	LC381983	LC381984	LC381985
S. violarus JCM 4534^T	BMUP01000003	BMUP01000013	BMUP01000001	BMUP01000012	BMUP01000009
S. violascens NRRL B-2700^T	KT384752	KT385100	KT385454	KT389072	KT389421
S. viridochromogenes NRRL B-1511^T	KT384756	KT385104	KT385458	KT389076	KT389425
S. viridodiastaticus NRRL B-5622^T	KT384757	KT385105	KT385459	KT389077	KT389426

* As the sequence of *S. spinoverrucosus* NRRL B-16932^T is not published in GenBank, that of *S. spinoverrucosus* DSM 41648^T is alternatively used.

3. Results

3.1. Taxnomic Positions of S. coelicolor and Related Strains

S. coelicolor NBRC 12854^{T} shares the same 16S rDNA sequences as those of the type strains of *Streptomyces albidoflavus*, *S. daghestanicus*, *S. hydrogenans*, and *S. violascens,* but the similarity between *S. coelicolor* strains NBRC 12854^{T} and A3(2) is only 97.8%. However, *S. coelicolor* A3(2) showed sequence similarities of 100% to the type strains of *Streptomyces anthocyanicus*, *Streptomyces violaceoruber,* and *Streptomyces tricolor*. These two *S. coelicolor* strains are phylogenetically distant, as shown in Figure 1.

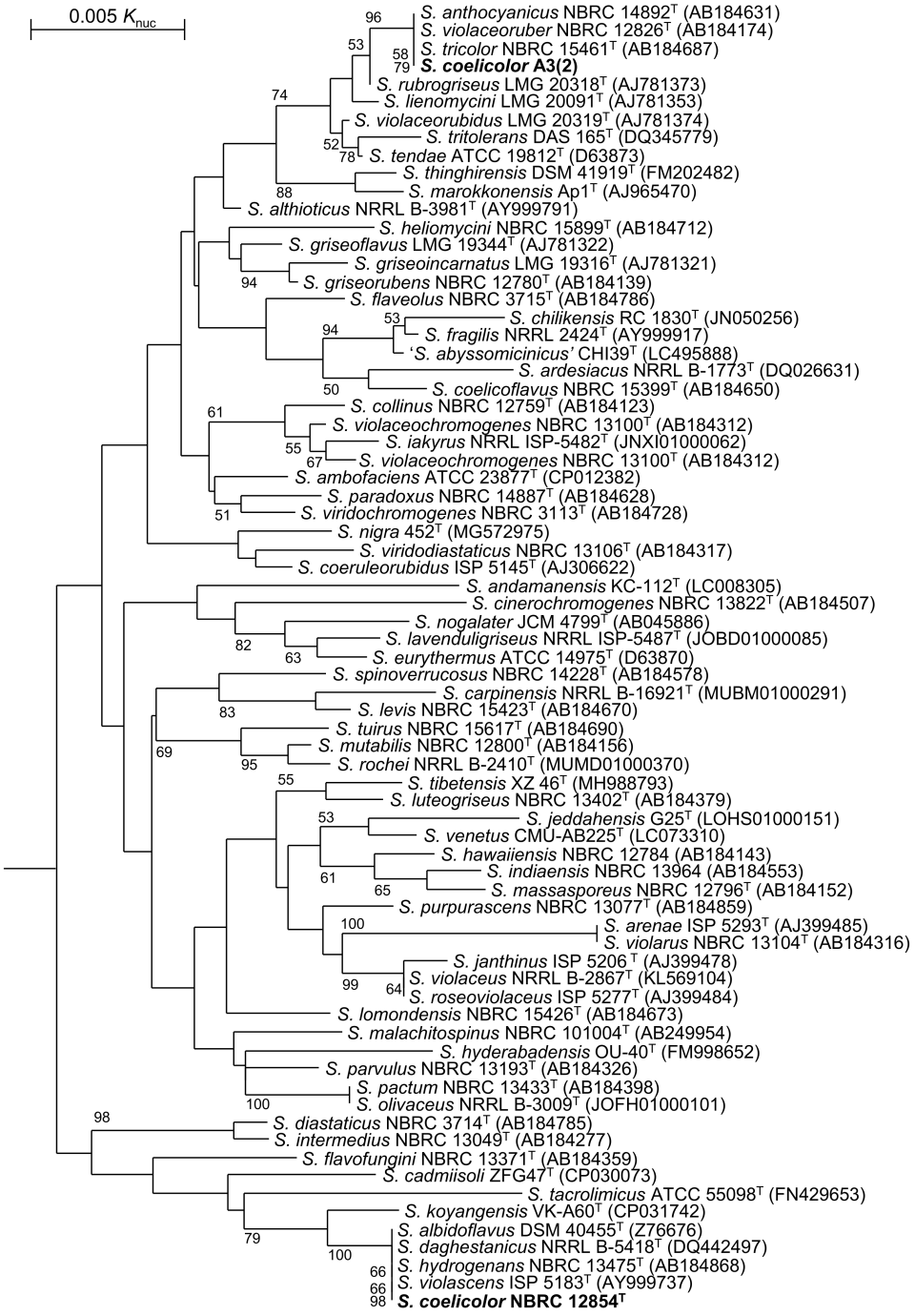

Figure 1. Phylogenetic tree based on the 16S rDNA sequences. Numbers on the branches represent the confidence limits estimated by a bootstrap analysis with 1000 replicates; values above 50% are at the branching points. *Streptomyces albus* NBRC 13014^{T} (AB184257) was used as an outgroup.

We also reconstructed a phylogenetic tree based on MLSA because it is often used to elucidate more precise phylogenetic relationships in the genus *Streptomyces* [19]. As shown in Figure 2, *S. coelicolor* NBRC 12854^T formed a clade with *S. albidoflavus*, but the clade did not include *S. daghestanicus*, *S. hydrogenans*, or *S. violascens*. In contrast, *S. coelicolor* A3(2) formed a clade with *S. anthocyanicus*, *S. violaceoruber*, and *S. tricolor*. These results also support the hypothesis that the two *S. coelicolor* strains are phylogenetically distant.

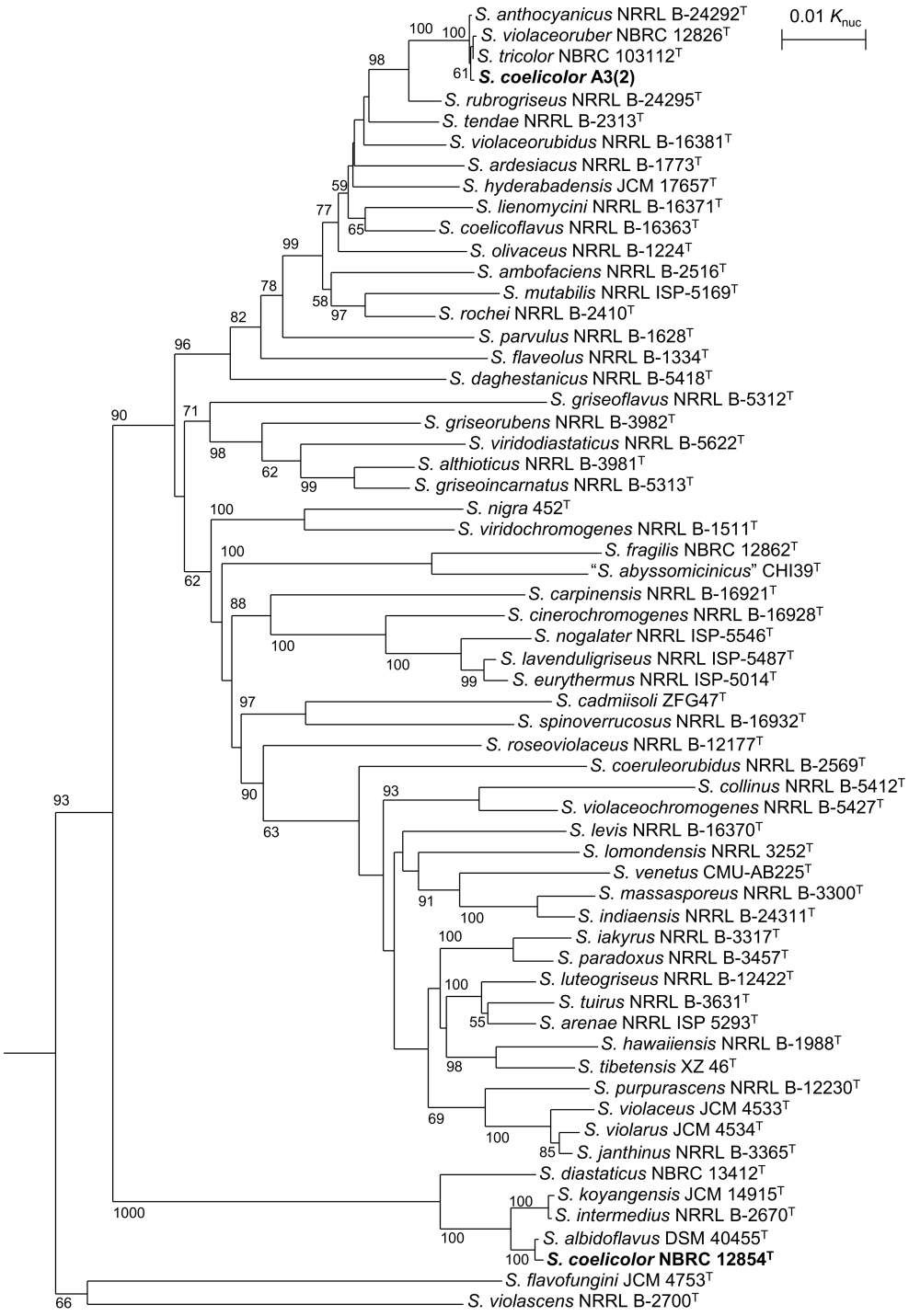

Figure 2. Phylogenetic tree based on MLSA. Numbers on the branches represent the confidence limits estimated by a bootstrap analysis with 1000 replicates; values above 50% are at the branching points. *S. albus* NBRC 13014^T was used as an outgroup.

To identify species, we conducted digital DNA–DNA hybridization (DDH). DNA–DNA relatedness of 70% is recognized to be the cutoff of species delineation [22,24]. The relatedness of strain A3(2) to the type strains of *S. coelicolor* and *S. anthocyanicus* was 23.1% and 94.2%, respectively, suggesting that strain A3(2) is not *S. coelicolor* but *S. anthocyanicus*. In contrast, as DNA–DNA relatedness between the type strains of *S. coelicolor* and *S. albidoflavus* was 91.8%; thus, these two species are synonymous, as reported in [25]. The type strains of *S. coelicolor*/*S. albidoflavus S. daghestanicus*, *S. hydrogenans,* and *S. violascens* did not show DNA–DNA relatedness of > 70% with one another, suggesting each to be an independent species (Table 2).

Table 2. DNA–DNA relatedness.

	Strain	DDH Estimate (%)						
		1	**2**	**3**	**4**	**5**	**6**	**7**
1.	*S. coelicolor* NBRC 12854^T	-	91.8	23.2	23.2	22.7	22.7	23.1
2.	*S. albidoflavus* DSM 40455^T (1)		-	23.0	23.1	22.7	22.7	22.7
3.	*S. daghestanicus* NBRC 12762^T			-	23.0	22.7	28.0	28.1
4.	*S. hydrogenans* NBRC 13475^T				-	23.4	22.8	22.9
5.	*S. violascens* NBRC 12920^T					-	22.6	22.8
6.	*S. anthocyanicus* JCM 5058^T (2)						-	94.2
7.	*S. coelicolor* A3(2) (3)							-

The accession numbers of the used genome sequences are as follows: (1) PKLO00000000.1; (2) BMWI00000000.1; (3) AL645882. The other accession numbers are shown in the Materials and Methods.

3.2. PKS and NRPS Gene Clusters in Genomes

As shown in Figure 3, *S. coelicolor* A3(2) possessed eight PKS and four NRPS gene clusters, [8] whereas *S. anthocyanicus* JCM 5058^T harbored six PKS and four NRPS gene clusters. Ten gene clusters (closed circle)—excluding the PKS gene clusters for coelimycin and arsono-polyketide (open circle)—were conserved between *S. coelicolor* A3(2) and *S. anthocyanicus* JCM 5058^T, which were classified as the same species, as stated in the previous section. *S. coelicolor* NBRC 12854^T had three PKS, six NRPS, and four hybrid PKS/NRPS gene clusters, whereas *S. albidoflavus* DSM 40455^T had two PKS, six NRPS, and four hybrid PKS/NRPS gene clusters. Twelve (closed circle) gene clusters—excluding *fdm* (fredericamycin) (open circle) [26]—were conserved between *S. coelicolor* NBRC 12854^T and *S. albidoflavus* DSM 40455^T, since *S. coelicolor* and *S. albidoflavus* are synonymous species. In contrast, among the twelve gene clusters of *S. coelicolor* A3(2), seven PKS and four NRPS gene clusters—excluding *rpp* [27,28]—were not present in the genome of *S. coelicolor* NBRC 12854^T. *S. daghestanicus* NBRC 12762^T encoded four PKS, one NRPS, and four hybrid PKS/NRPS gene clusters in its genome. Six gene clusters—excluding *pfa* (polyunsaturated fatty acid), *ant* (antimycin) [29], and *rpp*—were specific to this strain among the strains studied here. *S. hydrogenans* NBRC 13475^T possessed seven PKS, two NRPS, and three hybrid PKS/NRPS gene clusters. Ten gene clusters—excluding *pfa* and *whi* (spore pigment) [8,30]—were specific to this strain. *S. violascens* NBRC 12920^T harbored six PKS, six NRPS, and one hybrid PKS/NRPS gene clusters. Twelve gene clusters—excluding the NRPS gene cluster for coelichelin—were specific to this strain. Although *S. coelicolor* NBRC 12854^T, *S. daghestanicus* NBRC 12762^T, *S. hydrogenans* NBRC 13475^T, and *S. violascens* NBRC 12920^T are phylogenetically close (Figure 1), most of their PKS and NRPS gene clusters were not conserved between the different species. In the seven strains, *t1pks-1* to *-5*, one *t2pks* named *tjh*, one *t3pks* named *plh*, *nrps-1* to *-10*, and *pks/nrps-1* to *-7* were orphan, and their products have not been experimentally clarified and, thus, could not be identified by our bioinformatic analysis.

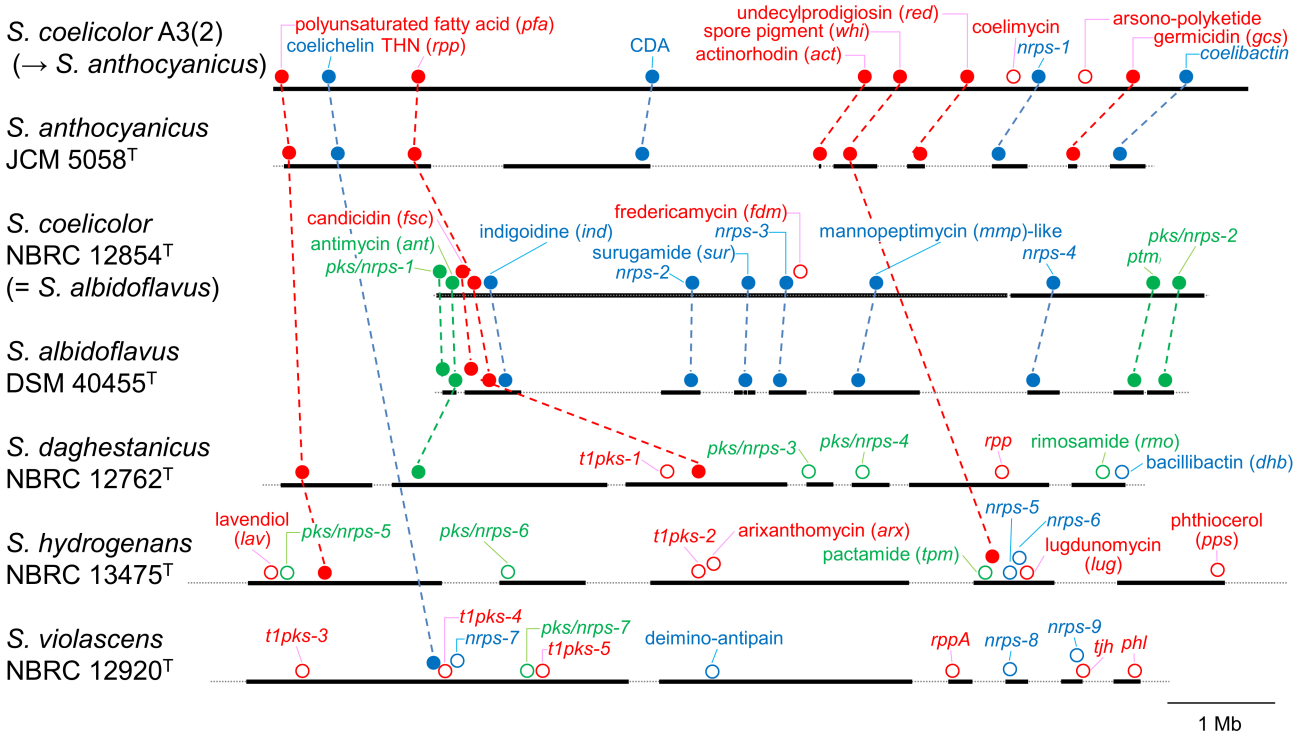

Figure 3. PKS and NRPS gene clusters in genomes. Only chromosome or scaffold sequences encoding these gene clusters are indicated by black and bold horizontal lines. Alignments and directions of the scaffold sequences in six strains—excluding *S. coelicolor* A3(2)—are unclear, because their whole-genome sequences are incomplete drafts. The parts that look like gaps, where no PKS and NRPS gene clusters are present, are equally spaced in the figures of *S. daghestanicus* NBRC 12762^T, *S. hydrogenans* NBRC 13475^T, and *S. violascens* NBRC 12920^T, because the lengths are unpredictable; however, the parts of *S. anthocyanicus* JCM 5058^T and *S. albidoflavus* DSM 40455^T are not, because they are aligned according to locations of PKS and NRPS gene clusters in the *S. coelicolor* A3(2) chromosome and *S. coelicolor* NBRC 12854^T draft genome sequences, respectively. Light-gray horizontal dashed lines under the black and bold lines indicate putative whole genomes, such as chromosomes. Red: PKS gene cluster; blue: NRPS gene cluster; green: hybrid PKS/NRPS gene cluster; CDA: calcium-dependent antibiotics; THN: 1,3,6,8-tetrahydroxynaphthalene; *ptm*: polycyclic tetramate macrolactam biosynthetic gene cluster; *t1pks*: type-I PKS gene cluster; *t2pks*: type-II PKS gene cluster; *t3pks*: type-III PKS gene cluster. Gene clusters are shown as the products and/or gene names. As *t1pks-1* to *-5*, *tjh*, *phl*, *nrps-1* to *-9*, and *pks/nrps-1* to *-7* are orphan, their products are unclear. Gene clusters shown as closed circles are present in other strain(s) studied in this study, as connected by dashed lines. Gene clusters specific to each strain are shown by open circles. *rppA*, *gcs*, and *phl* are *t3pks*; *act*, *whi*, *fdm*, *arx*, *lug*, and *tjh* are *t2pks*; the other named PKS gene clusters are *t1pks*. All of the gene clusters shown here are completely sequenced in these draft genome sequences. →, *S. coelicolor* A3(2) is reclassified to *S. anthocyanicus*; =, *S. coelicolor* is a synonym of *S. albidoflavus*.

3.3. Prediction of Products Synthesized by Orphan PKS and NRPS Gene Clusters

According to the co-linearity of the assembly lines [31], the backbones of polyketides and non-ribosomal peptides can be bioinformatically predicted from the sequences of the PKS and NRPS gene clusters. Based on the module numbers, substrates of adenylation (A) domains, and domain organizations [15], we predicted the products of orphan PKS and NRPS gene clusters in *S. coelicolor* A3(2), *S. coelicolor* NBRC 12854^T, *S. daghestanicus* NBRC 12762^T, *S. hydrogenans* NBRC 13475^T, and *S. violascens* NBRC 12920^T. Each strain possesses 1–9 orphan PKS and/or NRPS gene clusters. These products are diverse, as shown in Table 3, although some products could not be predicted because they are not multimodular. In addition to the known secondary metabolites shown in Figure 3, these strains show the potential to produce novel polyketides and non-ribosomal peptides.

Table 3. PKSs and NRPSs in the orphan gene clusters and the predicted products.

Strain	Gene Cluster	ORF (Locus Tag)	Domain Organization	Predicted Product
S. coelicolor A3(2)	*nrps-1*	SCO6431 SCO6432	A/T-C/T C/A_{cys}/T-Te	Tripeptide (x-y-cys)
S. coelicolor NBRC 12854^T	*nrps-2*	ScoT_42860	A/T/E-TD	Unknown
	nrps-3	ScoT_34810	C/A/T-C/A/T-C/A/T-Te	Tripeptide
	nrps-4	ScoT_12950 ScoT_12970	A_{phe}/T-C/A/T/E-C/A_{val}/T C/A/T/E-C/A_{thr}/T-C/A_{thr}/T/E	Hexapeptide (phe-x-val-x-thr-thr)
	pks/nrps-1	ScoT_62740 ScoT_62750 ScoT_62760 ScoT_62780	A_{gly}/T-KS/DH/ACP-KS/AT_m DH/KR/ACP-KS/ACP- KS/KR/ACP KS/DH/ACP-KS/AT_m DH/KR/ACP-AmT	Hexaketide including gly
	pks/nrps-2	ScoT_02700 ScoT_02710 ScoT_02720	A/T KS/ACP-C/Aala/T/C-Te C/A_{thr}/T-C/T/E-C/A_{val}/T-C	Pentapeptide with polyketide moiety (thr-y-val-x-pk-ala)
S. daghestanicus NBRC 12762^T	*t1pks-1*	Sdagh_09690 Sdagh_09680 Sdagh_09670	CoL/KR/ACP- KS/AT_{mm}/KR/ACP- KS/AT_{mm}/DH/KR/ACP KS/AT_m/DH/KR/ACP KS/AT_m/ACP	Tetraketide with a starter
	pks/nrps-3	Sdagh_50890 Sdagh_50900 Sdagh_50940 Sdagh_50980	C A/KR/ACP A T	Unknown
	pks/nrps-4	Sdagh_52260 Sdagh_52060 Sdagh_51920 Sdagh_51910 Sdagh_51900	T-C/A_{cys}/MT/T KS (type-III PKS) A/T/E-C/A/T/E-C/A/T/E- C/A/T/E-C/A_{thr}/T/E C/A/T/E C/A/T-C/A/T-C/A/T-X/A-Te	Undecapeptide including cys, thr, and a polyketide moiety
S. hydrogenans NBRC 13475^T	*t1pks-2*	Shyd_35460	KS/AT_{mm}/ACP- KS/AT_{mm}/DH/ER/KR/ACP	Unknown
	nrps-5	Shyd_62130 Shyd_62150 Shyd_62160	A_{phe}/MT/T C/A_{val}/T-C/A_{gly}/T/E C/A/T-Te	Tetrapeptide (methyl phe-val-gly-x)
	nrps-6	Shyd_62460 Shyd_62450	A/MT/T-C/A/T-C/A_{val}/T C/A/T-C/A_{thr}/T-Te	Pentapeptide (methyl x-x-val-x-thr)
	pks/nrps-5	Shyd_13440 Shyd_13450 Shyd_13460 Shyd_13470 Shyd_13490 Shyd_13520 Shyd_13530	A_{val}/T C/T-C T KS/ACP-TD T FkbH A	Dipeptide with a polyketide moiety
	pks/nrps-6	Shyd_48540 Shyd_48670 Shyd_48680	A_{val}/T-C Atyr/T KS/AT_m/ACP	Dipeptide with a polyketide moiety (val-tyr-pk)
S. violascens NBRC 12920^T	*t1pks-3*	Sviol_48010 Sviol_48000 Sviol_47990	KS/AT_m/ACP-KR KS/AT_m ACP	Unknown
	t1pls-4	Sviol_60680	KS/AT/DH/MT/ER/KR/ACP	Unknown
	t1pks-5	Sviol_68600	KS/AT/DH/KR/ACP	Unknown

Table 3. *Cont.*

Strain	Gene Cluster	ORF (Locus Tag)	Domain Organization	Predicted Product
	tjh (t2pks)	Sviol_35480 Sviol_35470 Sviol_35460 Sviol_35440	KSα KSβ (CLF) ACP KR	Unidentified aromatic polyketide
	phlD	Sviol_40150	KS (type-III PKS)	Unidentified polyketide
	nrps-7	Sviol_61030	C/A_{thr}/T-Te	Unknown including thr
	nrps-8	Sviol_25110	C/A_{phe}/T/E-C/A_{phe}/T-C/A/T/T-Te	Tripeptide (phe-phe-x)
	nrps-9	Sviol_35280 Sviol_35230 Sviol_35220	A/T-C T A	Dipeptide
	nrps-10	Sviol_76120 Sviol_76140 Sviol_76160	A/KR/ACP A_{cys}/T-C/A/T C/Aala/T/E-C/A/T-C/A/T/E-C/A/T-Te	Heptapeptide including cys and ala
	pks/nrps-7	Sviol_68100 Sviol_68090 Sviol_68080 Sviol_68070 Sviol_68030 Sviol_68020 Sviol_67990 Sviol_67980	A/T C/A/T-C/T KS/DH/ACP-TD A AT_m TE ACP KS	Tripeptide with a polyketide moiety

A: adenylation; ACP: acyl carrier protein; AmT: aminotransferase; AT: acyltransferase; AT_m: AT for malonyl-CoA: AT_{mm}: AT for methyl malonyl-CoA; C: condensation; CLF: chain length factor; CoL: CoA ligase; DH: dehydratase; E: epimerization; ER: enoyl reductase; KR: ketoreductase; KS: ketosynthase; MT: methyltransferase; pk: polyketide; T: thiolation; TD: termination; Te: thioesterase: x: unidentified amino acid residue; X: unknown domain: y: unknown unit by lack of A domain in the module. Amino acids incorporated by A domains are indicated as three-letter abbreviations in subscripts just after A.

4. Discussion

S. coelicolor A3(2) is the best studied strain as a model microorganism of actinomycetes. It has been extensively used for elucidating mechanisms in morphological differentiation [32], physiological features [33,34], metabolisms [35,36], and genetic regulation [37–39] in the genus *Streptomyces*. The genetics of actinomycetes were especially advanced with the strain [7]. Its wild strain and derived strains have contributed to various biotechnological fields. It is notable that these strains are used as the hosts for heterogeneous secondary metabolite production [40–44]. In contrast to these applications, the classification of *S. coelicolor* A3(2) has been paid less attention. Hatano et al. [1] reclassified strain A3(2) from *S. coelicolor* to *S. violaceoruber*; however, this reclassification is not widely known. In addition to many recent reports [45–48], even GenBank still classifies strain A3(2) as *S. coelicolor* [49,50]. In this study, we showed that strain A3(2) can be reclassified to *S. anthocyanicus* based on DNA–DNA relatedness, because the whole-genome sequence of the type strain of *S. violaceoruber* has not yet been published. Very recently, Komaki proposed that *S. anthocyanicus* and *Streptomyces tricolor* are later heterotypic synonyms of *S. violaceoruber*, in a paper submitted to the *International Journal of Systematic and Evolutionary Microbiology* that is now under review. Therefore, there is no contradiction between the report by Hatano et al. and our present results.

Rong et al. reclassified *S. coelicolor* to *S. albidoflavus* based on multilocus sequence analysis, DDH experiments, and phenotypic comparisons [25]; our digital DDH supported this. Even if it is recognized that strain A3(2) and the type strain of *S. coelicolor* belong to different species, many researchers may think that *S. coelicolor* A3(2) and the type strain of *S. coelicolor* harbor a similar set of smBGCs, because they believe that there is no correlation between taxonomic species and secondary metabolites. Our present study provides the

evidence to deny this possibility, and rather supports our hypothesis that strains belonging to different species harbor different repertoires of PKS and NRPS gene clusters—even if they are phylogenetically close [15,51–54]. *S. coelicolor* A3(2) and the type strains of *S. anthocyanicus*, *S. violaceoruber,* and S. *tricolor* share the same 16S rDNA sequence, and these four strains can be reclassified to the same species. In contrast, the type strains of *S. albidoflavus*/*S. coelicolor*, *S. daghestanicus*, *S. hydrogenans,* and *S. violascens* also share the same 16S rDNA sequence, but these four are independent species. It is often reported that the resolution of 16S rDNA sequences is so low that we are unable to classify *Streptomyces* members at the species level by only analyzing 16S rDNA sequences [15,55,56]. The present study suggests that each species harbors specific smBGCs. These gene clusters might have moved via repeated and/or complicated horizontal gene transfers (HGTs) between various *Streptomyces* strains. On the other hand, HGTs between strains belonging to the same species may not be as frequent, because strains within the same species—such as strains A3(2) and JCM 5058^{T} of *S. anthocyanicus,* or strains NBRC 12854 and DSM 40455^{T} of *S. albidoflavus*—harbor a similar set of PKS and NRPS gene clusters. In contrast, it has been reported that a part of the secondary metabolism is related more to strain ecology than to the phylogeny of a strain [57]. It is still unclear whether the presence of PKS and NRPS gene clusters in genomes indeed changes based on the ecology of strains. Our genome analysis of the type strains of the four species revealed the presence of many orphan PKS and NRPS pathways whose metabolites have not yet been identified; their presence suggests the potential for producing novel polyketides and non-ribosomal peptides through further research. Appropriate classification at the species level is significant in the search for bioactive secondary metabolites in the post-genomic era, and to deepen our understanding of the relationship between taxonomical species and smBGCs.

5. Conclusions

We examined the taxonomic relationships between *S. coelicolor* A3(2) and the type strains of *S. coelicolor* and its phylogenetically close species. We suggest that *S. coelicolor* A3(2) should be reclassified to *S. anthocyanicus*, which is a synonym of *S. violaceoruber*, whereas *S. coelicolor* is a synonym of *S. albidoflavus*, as reported by Rong et al. [25]. Analysis of PKS and NRPS gene clusters in the genomes revealed that the repertoires of these gene clusters are quite different between *S. coelicolor* A3(2) and *S. coelicolor* NBRC 12854^{T}, although these strains were once classified as *S. coelicolor*. Although the type strains of *S. albidoflavus*/*S. coelicolor*, *S. daghestanicus*, *S. hydrogenans,* and *S. violascens* share the same 16S rDNA sequence, they are independent species, and most of their PKS and NRPS gene clusters differ between the species. This is the first report on a genomic study for the type strains of *S. coelicolor* and its closely related species.

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