hydrobiology

MDPI

Article

Taxonogenomic Analysis of Marine-Derived *Streptomyces* sp. N11-50 and the Profile of NRPS and PKS Gene Clusters

Hisayuki Komaki [1,*], Yasuhiro Igarashi [2] and Tomohiko Tamura [1]

[1] Biological Resource Center, National Institute of Technology and Evaluation (NBRC), Chiba 292-0818, Japan; tamura-tomohiko@nite.go.jp
[2] Biotechnology Research Center, Department of Biotechnology, Toyama Prefectural University, Toyama 939-0398, Japan; yas@pu-toyama.ac.jp
* Correspondence: komaki-hisayuki@nite.go.jp

Abstract: *Streptomyces* sp. N11-50 was isolated from deep-sea water and found to produce diketopiperazine (DKP) compounds such as albonoursin and cyclo(Phe-Leu). This study aimed to reveal the potential to synthesize diverse nonribosomal peptide and polyketide compounds as the other secondary metabolites different from DKP after clarifying the taxonomic position. Strain N11-50 was identified as *Streptomyces albus*, as it showed 100% 16S rRNA gene sequence similarities and 95.5% DNA–DNA relatedness to *S. albus* NBRC 13014^T. We annotated the nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) gene clusters in the genome. Consequently, five NRPS, one hybrid PKS/NRPS, five type-I PKS and one type-II PKS gene clusters were observed, of which we predicted the products through bioinformatic analysis. These gene clusters were well conserved in already whole-genome sequence (WGS)-published strains belonging to *S. albus*. On the other hand, our taxonogenomic analysis revealed that three WGS-published *S. albus* strains were not *S. albus*. Two of the three should be classified as *Streptomyces albidoflavus*, and the remaining one was likely a new genomospecies. After reclassifying these appropriately, we demonstrated species-specific profiles of the NRPS and PKS gene clusters with little strain-level diversities.

Keywords: albonoursin; deep sea; diketopiperazine; genome; peptide; polyketide; reclassification; secondary metabolite; *Streptomyces albus*

Citation: Komaki, H.; Igarashi, Y.; Tamura, T. Taxonogenomic Analysis of Marine-Derived *Streptomyces* sp. N11-50 and the Profile of NRPS and PKS Gene Clusters. *Hydrobiology* **2023**, 2, 382–394. https://doi.org/10.3390/hydrobiology2020025

Academic Editor: Cláudia Pascoal

Received: 28 April 2023
Revised: 26 May 2023
Accepted: 30 May 2023
Published: 2 June 2023

1. Introduction

Secondary metabolites produced by actinomycetes are a promising source for pharmacological industries. Members of the genus *Streptomyces* are recognized as a rich source of structurally diverse secondary metabolites with useful bioactivities. Recent genome analyses revealed that each of the strains harbors a few dozen biosynthetic gene clusters (BGCs) for secondary metabolites in its genome. Secondary metabolites are classified according to their chemical structures and biosynthetic pathways. Polyketide and nonribosomal peptide compounds are major secondary metabolites in the genus *Streptomyces* because half to three quarters of the secondary metabolite-biosynthetic gene clusters (smBGCs) in a streptomycetal genome encode polyketide synthases (PKSs) and/or nonribosomal peptide synthetases (NRPSs) [1,2]. PKSs synthesize a polyketide chain from acyl-CoA molecules as the building blocks, whereas NRPSs do peptide chains from amino acids in a similar manner. These chains are modified through various mechanisms, such as reduction, cyclization, methylation, and epimerization, to yield the final products. Diversities in the chemical structures are due to the differences in the chain lengths and complex of various building blocks in addition to these modifications. Type-I PKSs and NRPSs are large modular enzymes with multiple catalytic domains. As chain elongations by these enzymes are based on the co-linearity rule of assembly lines [3], the chemical structures of the chains can be predicted through bioinformatic analysis of the domain organizations. In contrast, type-II and type-III PKSs iteratively catalyze polyketide chain elongations. Type-II PKSs are

composed of three small enzymes, a ketosynthase (KS)α, a KSβ (chain length factor) and an acyl carrier protein (ACP), and they are involved in synthesis of aromatic compounds. Type-III PKSs are standalone enzymes with a KS domain, and they are responsible for the synthesis of chalcone-like phenolic compounds.

The genus *Streptomyces* includes approximately 700 species with a validly published name [4]. Soil is known as the main habitat of the members. Therefore, researchers have extensively isolated many strains from terrestrial environments and screened them for new bioactive compounds for a long time. Consequently, it became harder to discover producers of new compounds from the environments because of frequent re-isolations of already reported compounds. Recently, marine environments are attracting attention to isolate new actinomycetes because they were not extensively searched for and include strains different from terrestrial ones. Members of genera such as *Salinispora* and "*Marinispora*" are known as marine actinomycete, which require seawater for growth and have marine chemotype signatures. It is reported that bioactive compounds from marine actinomycetes possess distinct chemical structures [5,6]. We have indeed discovered new compounds from marine-derived *Streptomyces* strains [7,8].

We recently isolated a *Streptomyces* strain, named N11-50, from deep-sea water, which produces two known diketopiperazine (DKP) compounds: albonoursin and cyclo (Phe-Leu) [7]. Albonoursin exhibits antimicrobial activities against *Bacillus* species and *Klebsiella pneumoniae*, an inhibitory effect on Ehrlich carcinoma in mice, antivirus effects on H1N1, and a pronuclear fusion inhibitory activity. *Actinomyces tumemacerans* 1NM1 P-42, *Streptomyces albulus* KO-23, marine-derived *Streptomyces* sp. FXJ7.328 and *Nocardiopsis alba* ATCC BAA-2165 have been reported as albonoursin-producers [9–12]. DKP compounds are synthesized by cyclodipeptide synthases (CDPSs), a family of tRNA-dependent peptide bond-forming enzymes [13,14]. We are investigating the taxonomic positions of the antibiotic producers that we isolated, identifying biosynthetic gene clusters for the antibiotics, and evaluating hidden potential on secondary metabolism focusing on PKS and NRPS pathways [15] to provide useful information for further screening and to deepen our knowledge on the relationship between each species and its secondary metabolism. Here, we examined taxonomic position and smBGCs encoding PKSs and NRPSs of strain N11-50 through whole-genome sequencing to demonstrate the relationships between the taxonomic species and the profiles of these gene clusters. As strain N11-50 was identified as *Streptomyces albus* and closely related to four whole-genome-sequence (WGS)-published *Streptomyces* strains, we included WGS-published *S. albus* strains, whose taxonomic positions have not been reviewed, and the four *Streptomyces* strains.

2. Materials and Methods

Streptomyces sp. N11-50 was isolated from deep-sea water collected in Toyama, Japan [7]. This strain, preserved in Toyama Prefectural University as TP-A0906, was deposited to the NBRC Culture Collection and is available as NBRC 113679. EzBioCloud was used to search for taxonomic neighbors based on 16S rRNA gene sequences [16]. Multilocus sequence analysis (MLSA) was conducted using the concatenated gene sequences of *atpD*, *gyrB*, *recA*, *rpoB* and *trpB*, as recommended by Rong and Huang [17]. Genomic DNA for whole-genome sequencing was prepared from cultured cells using the method of Saito and Kimura [18]. 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, MA, USA) with a SMRTbell Template Prep Kit 1.0 and a SMRTbell Damage Repair Kit (Pacific Biosciences, CA, USA), 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 were assembled using SMRT Link version 6.0 (Pacific Bioscience) and Prokka 1.13.3. The accession numbers of the draft genome sequence are BNEJ01000001–BNEJ01000031. Digital DNA–DNA hybridization (dDDH) was carried out using the Genome-to-Genome Distance Calculator (GGDC) [19]. The DDH estimate

(GLM-based) of Formula 2 (identities/HSP length), which is recommended in GGDC, was used as DNA–DNA relatedness. WGS-published *S. albus* strains were searched for on the NCBI website. Nucleotide BLAST (blastn) was used to search for WGS-published strains identified as *Streptomyces* sp. and showing >99.9% 16S rRNA gene sequence similarities to strain N11-50. Phylogenetic and phylogenomic trees were reconstructed using ClustalX 2.1 and the TYGS server [19], respectively. NRPS and PKS gene clusters in genomes were surveyed using antiSMASH [20], and then manually annotated as reported previously [15].

3. Results

3.1. Taxonomic Position of Streptomyces sp. N11-50 and Related WGS-Published Strains

Streptomyces sp. N11-50 showed 100% 16S rRNA gene sequence similarity to *Streptomyces albus* NBRC 13014^{T} as the closest species. The second most similar species was observed to be *Streptomyces reniochalinae*, but the value is 98.6%, which is less than the cut-off (99.0%) for species delineation recognized in actinomycetes [21]. This suggests that the strain differs from the other species, except for *S. albus*.

The WGSs of 21 *S. albus* strains are published in GenBank/ENA/DDBJ at present. Among them, the taxonomic positions of eighteen strains are already reported, but the remaining three *S. albus* strains, G153, INA 01303 and NRRL B-2238, have not been studied [22]. Additionally, the WGSs of four strains showing >99.9% 16S rRNA gene sequence similarities to *S. albus* N11-50, such as *Streptomyces* sp. NRRL F-5639, *Streptomyces* sp. NRRL F-5917, *Streptomyces* sp. HPH0547 and *Streptomyces* sp. PHES57 51, are also published under the accession numbers JOGK01000000, JOHQ01000000, ATCE01000000 and JAINRF01000000, respectively. These four strains have not been classified at the species level. Thus, we included the three *S. albus* strains and four *Streptomyces* strains as well as *Streptomyces* N11-50 in our analysis. Strains G153, INA 01303 and NRRL B-2238 showed 100%, 99.7% and 100% rRNA gene sequence similarities to the type strain of *Streptomyces albidoflavus*. *Streptomyces koyangensis* was the next closest species, with 99.4–99.3% similarities. Type strains of the other species, including *S. albus*, did not show >99.0% similarities to the three strains. A phylogenetic tree of these members, in addition to strain N11-50, based on 16S rRNA gene sequences was reconstructed with type strains showing >99.0% sequence similarities (Figure 1). *Streptomyces violascens* strains ATCC 27968 and NBRC 12920^{T} were included in the tree because we noticed that *S. violascens* ATCC 27968 was closely related to strain INA 01303 but was not the type strain. *Streptomyces* sp. N11-50, *Streptomyces* sp. NRRL F-5639, *Streptomyces* sp. NRRL F-5917, *Streptomyces* sp. HPH0547 and *Streptomyces* sp. PHES57 51 formed a clade with *S. albus* NBRC 13014^{T}. In contrast, G153 and NRRL B-2238 were not included in the clade but formed a clade with *S. albidoflavus.* Similarly, INA 01303 formed a clade with *S. violascens* ATCC 27968, which is not closely related to the type strain of *S. violascens*. These results suggest that strains G153, NRRL B-2238 and INA 01303 were incorrectly identified as *S. albus*. These species names registered in the databases must be properly curated.

Streptomyces strains are unable to be classified at the species level through only 16S rRNA gene sequence analysis [23]. MLSA [24] and/or dDDH [23] are recommended for the molecular classification. We therefore sequenced the whole genome of *Streptomyces* sp. N11-50 to classify the strain. The genome size and G + C content were 8.29 Mb and 72.8%, respectively. The genome size was 0.7 Mb larger than that of *Streptomyces albus* NBRC 13014^{T} (7.59 Mb) whereas their G + C contents were almost the same value (*S. albus* NBRC 13014^{T}, 72.7%).

We reconstructed MLSA-based phylogenetic and phylogenomic trees. Their phylogenetic relationships were similar to those in the tree based on the 16S rRNA gene sequences (Figures 2 and 3). We estimated the evolutionary distance in MLSA and DNA-DNA relatedness in dDDH (Table 1). *Streptomyces* sp. N11-50, *Streptomyces* sp. HPH0547, *Streptomyces* sp. PHES57 51, *Streptomyces* sp. NRRL F-5639 and *Streptomyces* sp. NRRL F-5917 showed an evolutionary distance of <0.001 and DNA-DNA relatedness of >90% to the type strain of *S. albus*. As the thresholds of MLSA evolutionary distance and DNA-DNA relatedness

for species delineation are 0.007 and 70%, respectively, these five strains were identified as *S. albus*. In contrast, as these values of strains G153, NRRL B-2238 and INA 01303 to the type strain of *S. albus* were >0.15 and <24%, respectively, these strains were not identified as *S. albus.* Strains G153 and NRRL B-2238 were identified as *S. albidoflavus* because their evolutionary distances and DNA-DNA relatedness to the type strain were 0.001–0.002 and 91.7–92.1, respectively. On the other hand, although *S. albus* INA 01303 and *S. violascens* ATCC 27968 belong to the same genomospecies based on the evolutionary distance (0.003) and DNA-DNA relatedness (93.5%), they were considered as a putative new species, because *S. violascens* ATCC 27968 is not the type strain of *S. violascens* and they could not be classified as any known species.

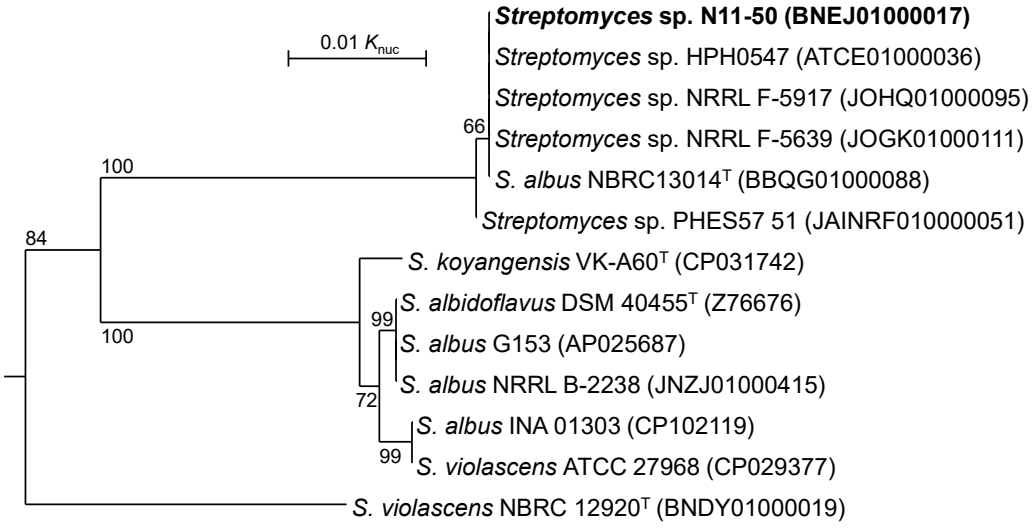


Figure 1. Phylogenetic tree based on 16S rRNA gene sequences. Numbers on the branches are the confidence limits estimated through bootstrap analysis with 1000 replicates. Values above 50% are indicated at branching points. *Embleya scabrispora* NBRC 100760^T (AB249946) was used as an outgroup (not shown) to show the root.

Table 1. Evolutionary distance in MLSA and DNA-DNA relatedness in dDDH.

Strain	Evolutionary Distance in MLSA												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1. *S. albidoflavus* DSM 40455^T	-	0.001	0.002	0.011	0.010	0.122	0.008	0.153	0.154	0.153	0.154	0.155	0.153
2. *S. albus* G153	91.7	-	0.003	0.011	0.010	0.121	0.008	0.154	0.155	0.154	0.155	0.156	0.154
3. *S. albus* NRRL B-2238	92.1	91.8	-	0.010	0.010	0.124	0.008	0.154	0.154	0.154	0.154	0.156	0.154
4. *S. albus* INA 01303	64.6	64.8	65.7	-	0.003	0.125	0.013	0.156	0.156	0.156	0.156	0.157	0.156
5. *S. violascens* ATCC 27968	65.7	65.8	66.7	93.5	-	0.126	0.012	0.156	0.156	0.156	0.156	0.157	0.156
6. *S. violascens* NBRC 12920^T	22.7	22.8	24.4	22.6	22.6	-	0.124	0.165	0.166	0.165	0.166	0.167	0.165
7. *S. koyangensis* VK-A60^T	64.6	64.5	64.8	61.1	61.9	22.8	-	0.156	0.156	0.156	0.156	0.157	0.156
8. *S. albus* NBRC 13014^T	21.4	21.6	23.3	21.3	21.4	21.8	21.5	-	0.000	0.000	0.001	0.001	0.000
9. *Streptomyces* sp. N11-50	21.4	21.6	23.4	21.3	21.5	21.7	21.5	95.5	-	0.000	0.001	0.001	0.000
10. *Streptomyces* sp. HPH0547	21.5	21.7	23.4	21.5	21.5	21.8	21.5	96.0	95.4	-	0.001	0.001	0.000
11. *Streptomyces* sp. PHES57 51	21.3	21.6	23.2	21.3	21.5	21.8	21.4	90.1	89.6	89.9	-	0.001	0.001
12. *Streptomyces* sp. NRRL F-5639	21.4	21.6	23.3	21.4	21.5	21.7	21.5	92.4	91.5	92.0	89.6	-	0.001
13. *Streptomyces* sp. NRRL F-5917	21.4	21.6	23.2	21.4	21.5	21.7	21.6	91.9	91.0	91.3	89.4	92.8	-
	DNA-DNA Relatedness												

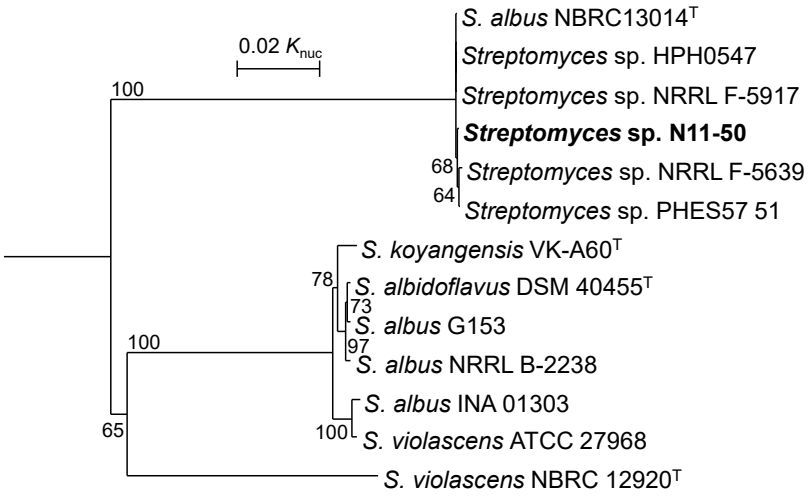


Figure 2. Phylogenetic tree based on MLSA. Numbers on the branches are the confidence limits estimated through bootstrap analysis with 1000 replicates and values above 50% are indicated at branching points. *Embleya scabrispora* DSM 41855^T was used as an outgroup (not shown) to show the root. Accession numbers of used *atpD* (485 bp), *gryB* (377 bp), *recA* (504 bp), *rpoB* (540 bp) and *trpB* (510 bp) sequences are as follows: *S. albidoflavus* DSM 40455^T, FJ406416, FJ406427, FJ406438, FJ406449, FJ406460; *S. albus* G153, AP025687; *S. albus* INA 01303, CP102119; *S. albus* NBRC 13014^T, BBQG01000033, BBQG01000013, BBQG01000035, BBQG01000012, BBQG01000017; *S. albus* NRRL B-2238, JNZJ01000642, JNZJ01000486, JNZJ01002092, JNZJ01000037, JNZJ01002932; *S. koyangensis* VK-A60^T, CP031742; *Streptomyces* sp. HPH0547, ATCE01000035, ATCE01000059, ATCE01000036, ATCE01000063, ATCE01000026; *Streptomyces* sp. NRRL F-5639, JOGK01000004, JOGK01000037, JOGK01000028, JOGK01000009, JOGK01000011; *Streptomyces* sp. NRRL F-5917, JOHQ01000026, JOHQ01000037, JOHQ01000048, JOHQ01000040, JOHQ01000004; *Streptomyces* sp. PHES57 51, JAINRF010000011, JAINRF010000031, JAINRF010000026, JAINRF010000019, JAINRF010000006; *S. violascens* ATCC 27968; CP029377; *S. violascens* NBRC 12920^T, BNDY01000017, BNDY01000008, BNDY01000017, BNDY01000020, BNDY01000002; *E. scabrispora* DSM 41855^T; KB889571, KB889730, KB889690, KB889574, KB889675.

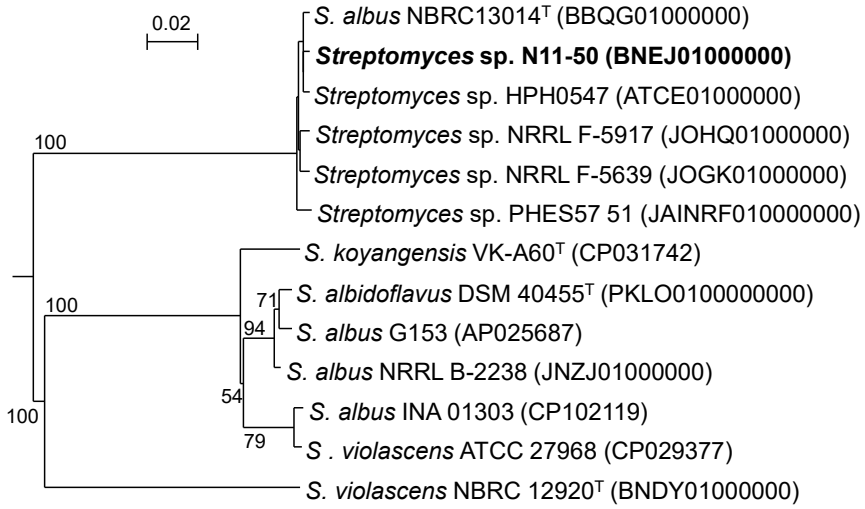


Figure 3. Phylogenomic tree reconstructed with the TYGS server. Tree inferred with FastME 2.1.6.1 [25] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5. The numbers above branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 63.4%. *E. scabrispora* DSM 41855^T was used as the outgroup (not shown) to show the root.

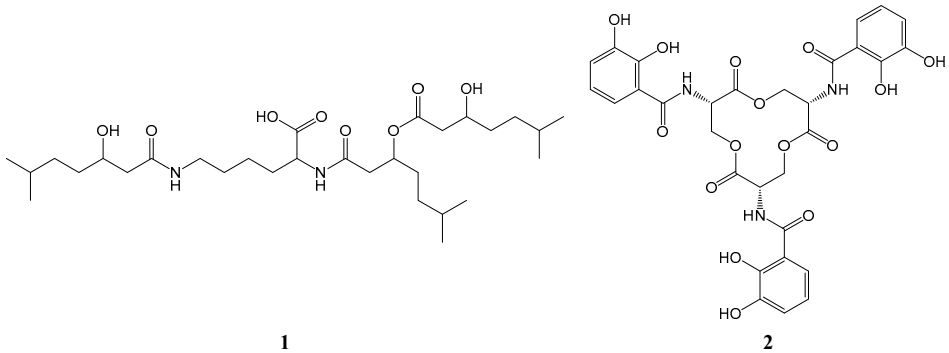
3.2. NRPS and Hybrid PKS/NRPS Gene Clusters in S. albus N11-50

S. albus N11-50 harbored five NRPS gene clusters and one hybrid PKS/NRPS gene cluster, as recorded in Table 2. NRPS gene cluster 2 (*nrps-2*) and *nrps-3* were identified as BGCs of dudomycin (**1**) [26] and enteromycin (**2**) [27], respectively (Figure 4), according to the domain organizations identical to theirs. In contrast, the others were orphan, whose products have not been identified. Therefore, we bioinformatically predicted their products. The product of *nrps-1* was predicted to be a tripeptide compound derived from threonine, valine and serine residues. *Nrps-4* seemed to synthesize a compound derived from dipeptide, but the amino acid residues could not be predicted. The product of *nrps-5* was predicted to be a tetrapeptide with one serine and two cysteine residues. Hybrid PKS/NRPS gene cluster-1 (*pks/nrps-1*) includes four PKS modules and two NRPS modules, one of which was responsible for incorporating asparagine residue. Thus, its product was predicted to be a tetraketide compound with asparagine residue.

Table 2. NRPSs or PKSs in the NRPS and hybrid PKS/NRPS gene clusters of *S. albus* N11-50.

Gene Cluster	ORF	Domain Organization	Putative Product
nrps-1	TPA0909_06090	A_{val}/PCP-C	Thr-Val-Ser-Y
	TPA0909_06100	A_{ser}/PCP-C/PCP-TE	
	TPA0909_06130	A_{thr}/PCP	
nrps-2	TPA0909_10490	C/A/PCP-TE	dudomycin (**1**)
nrps-3	TPA0909_28110	$A_{diOH-Bz}$	enterobactin (**2**)
	TPA0909_28090	PCP	
	TPA0909_28080	C/A_{ser}/PCP-TE	
nrps-4	TPA0909_28620	A/PCP-TD	dipeptide
	TPA0909_28750	A	
nrps-5	TPA0909_30010	C/A_{cys}/MT/PCP-TE	X-Ser-Cys-mCys
	TPA0909_30080	A	
	TPA0909_30100	PCP-C/A/PCP-C/A_{cys}/PCP	
pks/nrps-1	TPA0909_66390	A_{asn}/PCP	tetraketide with Asn
	TPA0909_66410	C/PCP	
	TPA0909_66430	AT_{mm}/ACP-KS/AT/KR/ACP	
	TPA0909_66440	KS/AT_m/KR/ACP-KS/AT_m/ACP	

A, adenylation; ACP, acyl carrier protein; AT, acyltransferase; AT_m, AT for malonyl-CoA; AT_{mm}, AT for methyl malonyl-CoA; C, condensation; diOH-Bz, dihydroxy benzoate; KR, ketoreductase; KS, ketosynthase; m, methyl; MT, methyltransferase; *nrps*, NRPS gene cluster; PCP, peptidyl carrier protein; *pks/nrps*, hybrid PKS/NRPS gene cluster; TD, termination; TE, thioesterase; X, unidentified amino acid residue; Y, unknown residue due to lack of A domain. Amino acids incorporated by A domains are indicated as 3-letter abbreviations in subscript just after A. Chemical structures of **1** and **2** are shown in Figure 4.



1 **2**

Figure 4. Chemical structures of dudomycin (**1**) and enteromycin (**2**) shown in Table 2.

3.3. Type-I and Type-II PKS Gene Clusters in S. albus N11-50

S. albus N11-50 harbored five type-I PKS and one type-II PKS gene clusters, as recorded in Table 3. Although *t1pks-2* was identified as a BGC of tambjamine BE-18591 (**3**), the others

were orphan. The product of *t1pks-1* could not be predicted because *t1pks-1* included only one PKS module and the ORF did not show high sequence similarities to PKSs with the identified products. *T1pks-3* was predicted to be a BGC for an ibomycin congener based on its domain organization, which resembles that of ibomycin (**4a**) [28]. The polyketide chain synthesized by PKSs of *t1pks-3* (**4b**) was predicted, as shown in Figure 5. *T1pks-4* was predicted to synthesize a congener of lactomycins (**5a**) [29] and phoslactomycin (**5b**) [30] with a polyketide backbone, shown as **5c** in Figure 5, and based on their similar domain organizations. *T1pks-5* was predicted to synthesize an enediyne compound because the domain organization is KS-AT-KR-DH-ACP, which is specific to PksE, responsible for synthesis of enediyne moiety [31]. Type-II PKS gene cluster-1 (*t2pks-1*) was predicted to synthesize an aromatic compound like xantholipin (**6**) because its KSα and KSβ (CLF) showed 93% (89%) and 85% (87%) amino acid sequence similarities (identities) to those of xantholipin [32], respectively.

Table 3. PKSs or NRPSs in the PKS gene clusters of *S. albus* N11-50.

Gene Cluster	ORF (TPA0909) [1]	Domain Organization	Putative Product
t1pks-1	_14380	ACP	unknown
	_14390	KS/AT/ACP	
	_14420	ACP	
t1pks-2	_21420	ACP	tambjamine BE-18591 (**3**)
	_21430	KS	
	_21460 [2]	KS/KS	
	_21470	ACP	
	_21480	ACP/ACP/AmT	
	_21500	TE	
t1pks-3	_24630	KS/AT_{mm}/ACP-KS/AT/KR/ACP-KS/AT_{mm}/KR/ACP	ibomycin congener derived from polyketide chain shown as **4b**
	_24640	KS/AT_{m}/KR/ACP-KS/AT_{mm}/KR/ACP-KS/AT_{mm}/KR/ACP -KS/AT_{m}/DH/KR/ACP	
	_24650	KS/AT_{m}/DH/KR/ACP-KS/AT_{mm}/KR/ACP	
	_24660	KS/AT_{mm}/KR/ACP-KS/AT_{m}/KR/ACP-KS/AT_{mm}/KR/ACP	
	_24670	KS/AT_{m}/KR/ACP-KS/AT_{m}/KR/ACP-KS/AT_{m}/DH/ACP -KS/AT_{mm}/KR/ACP-KS/AT_{m}/DH/ER/KR/ACP	
	_24680	KS/AT_{m}/KR/ACP-KS/AT_{m}/KR/ACP	
	_24690	KS/AT_{mm}/DH/KR/ACP-KS/AT_{m}/DH/KR/ACP-TE	
t1pks-4	_28890	AT/ACP-KS/AT_{m}/DH/KR/ACP	congener of lactomycins and phoslactomycin derived from **5c**
	_28880	KS/AT_{m}/DH/KR/ACP-KS/AT/KR/ACP	
	_28870	KS/AT_{m}/KR/ACP	
	_28860	KS/AT_{m}/KR/ACP-TE	
	_28850	KS/AT_{mm}/DH/ER/KR/ACP	
	_28840 [2]	KS/AT_{em}/KR/ACP	
t1pks-5	_29480	KS/AT_{m}/KR/DH/ACP	enediyne
t2pks-1	_14620	ACP	aromatic compound like xantholipin (**6**)
	_14680	KSα	
	_14690	KSβ (CLF)	

[1] shown by locus tag such as TPA0909_14380; [2] encoded in the complementary strand. Abbreviations are as follows: AmT, aminotransferase; AT_{em}, AT for ethylmalonyl-CoA; CLF, chain length factor; DH, dehydratase; ER, enoyl reductase; *t1pks*, type-I PKS gene cluster; *t2pks*, type-II PKS gene cluster. The other abbreviations are the same as those in Table 2. Chemical structures of **3** to **6** are shown in Figure 5.

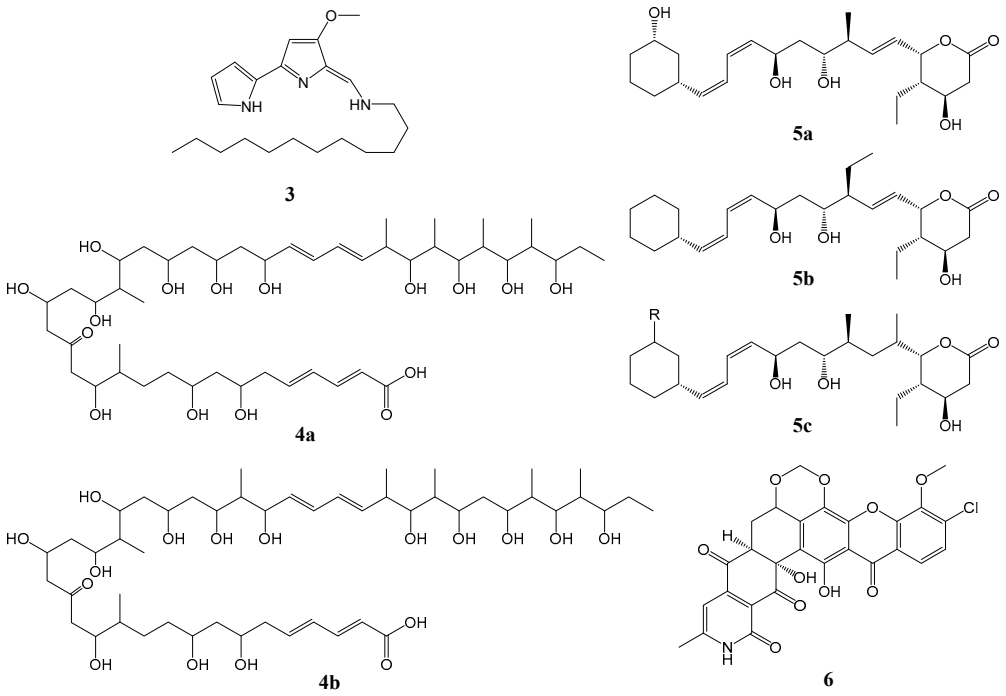


Figure 5. Chemical structures of putative products in Table 3. **3**, tambjamine BE-18591; **4a**, polyketide chain synthesized by PKSs for ibomycin; **4b**, predicted polyketide chain synthesized by PKSs of *t1pks-3*; **5a**, lactomycin C; **5b**, polyketide backbone synthesized by PnA to PnF (PKSs for phoslactomycin) before post-PKS processing; **5c**, predicted backbone synthesized by PKS of *t1pks-4*. R = H or OH; **6**, xantholipin.

3.4. Distribution of NRPS and PKS Gene Clusters Found in S. albus N11-50

We investigated whether the twelve NRPS and PKS gene clusters of *S. albus* N11-50 are conserved in WGS-published *S. albus* strains that have been confirmed to be *S. albus* in the report of Vela Gurovic et al. [22], three WGS-published *S. albus* strains that were reclassified as other species and four WGS-published *Streptomyces* strains that were identified as *S. albus* in Section 3.1. All the NRPS and PKS gene clusters were conserved in *S. albus* strains except for the type strain, which lacked *nrps-3*, as shown in Table 4. *S. albus* NRRL F-5917 harbored an extra NRPS, as stated in the footnote of Table 4. In contrast, strains G153, NRRL B-2238 and INA 01303, which are registered as *S. albus* but revealed not to be *S. albus*, did not harbor these gene clusters.

Table 4. Distribution of NRPS and PKS gene clusters found in *S. albus* N11-50.

Strain	*nrps-*					*pks/nrps -1*	*t1pks-*					*t2pks -1*
	1	*2*	*3*	*4*	*5*		*1*	*2*	*3*	*4*	*5*	
S. albus N11-50 [1]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus NBRC 13014^{T} [2]	+	+	-	+	+	+	+	+	+	+	+	+
S. albus CAS922 [3]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus DSM 40763 [3]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus NRRL B-1335 [3]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus NRRL B-1685 [3]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus NRRL B-2362 [3]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus NRRL B-2465 [3]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus NRRL B-12378 [3]	+	+	+	+	+	+	+	+	+	+	+	+
S. albidoflavus G153 [4]	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. *Cont.*

Strain	*nrps-*					*pks/nrps -1*	*t1pks-*					*t2pks -1*
	1	*2*	*3*	*4*	*5*		*1*	*2*	*3*	*4*	*5*	
S. albidoflavus NRRL B-2238 [4]	-	-	-	-	-	-	-	-	-	-	-	-
Streptomyces sp. INA 01303 [5]	-	-	-	-	-	-	-	-	-	-	-	-
S. albus NRRL F-5639 [1]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus NRRL F-5917 [1]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus HPH0547 [1]	+	+	+	+	+	+	+	+	+	+	+	+
S. albus PHES57 51 [1]	+	+	+	+	+	+	+	+	+	+	+	+

[1] classified as *S. albus* according to the results of Section 3.1 in this study; [2] The same result was also obtained from *S. albus* NRRL B-1811^T; [3] Vela Gurovic et al. have confirmed to be *S. albus* [22]; [4] published as *S. albus* in GenBank but classified as *S. albidoflavus* in this study; [5] published as *S. albus* but classified as a new genomospecies in this study. +, present; -, not observed. *Streptomyces* sp. NRRL F-5917 possesses an extra NRPS gene cluster composed of IF56_RS0123365 (domain organization, A_{ile}/PCP-C), IF56_RS0123365 (A_{phe}/PCP), IF56_RS0123325 (C) and IF56_RS0123295 (A_{thr}/PCP). IF56_RS0123365 is encoded in the complementary strand. NRPS and PKS gene clusters of *S. albidoflavus* G153 and *Streptomyces* sp. INA 01303 are shown in Figure 6b.

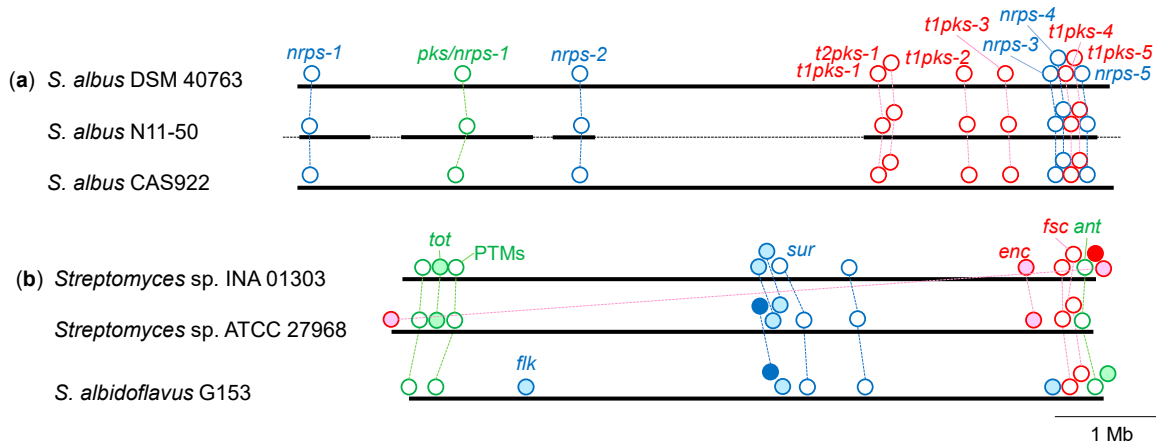


Figure 6. NRPS and PKS gene clusters in chromosomal DNAs. Chromosomal DNAs are indicated by black and bold horizontal lines. For *S. albus* N11-50, only contig sequences with these gene clusters are shown. The alignments and directions are unclear because its WGS sequence is incomplete drafts. Light-gray horizontal dashed lines under the black and bold lines indicate putative chromosomes. Red: PKS gene cluster; blue: NRPS gene cluster; green: hybrid PKS/NRPS gene cluster; *t1pks*: type-I PKS gene cluster; *t2pks*: type-II PKS gene cluster. Details of gene clusters in (**a**) are shown in Tables 2 and 3. Orphan gene clusters of the other strains were not numbered but gene clusters whose products were predicted are indicated with the gene name or product. Gene clusters specific in *Streptomyces* sp. INA 01303/ATCC 27968 or *S. albidoflavus* are shown as circles filled with a light color (**b**). The same gene clusters are as connected by dashed lines. Gene clusters specific to a strain are shown by circles filled a dark color. All the gene clusters of *S. albidoflavus* G153 were conserved in *S. albidoflavus* DSM 40455^T but the data is not indicated here because the WGS of *S. albidoflavus* DSM 40455^T is draft composed of 66 contig sequences. *ant*, antimycin [33]; *enc*, enterocin [34]; *fdm*, fredericamycin [35]; *flk*, cyclofaulknamycin [36]; *fsc*, candicidin [37]; PTMs, polycyclic tetramate macrolactams [38]; *sur*, surugamide [39]; *tot*, totopotensamides [40].

The positions of these gene clusters in each chromosome were shown in a diagram using the *S. albus* strains DSM 40763, N11-50 and CAS922 as examples (Figure 6a) because the strains DSM 40763 and CAS922 have been confirmed to be *S. albus* [22], and their WGSs are complete. Although WGS of *S. albus* N11-50 is composed of 31 contig sequences, it is less incomplete than the other WGS-published *S. albus* strains. Similarly, strains INA 01303 and ATCC 27968, which were identified as the same new genomospecies in this study, shared twelve gene clusters different from those of *S. albus*, although INA 01301 and ATCC 27968 harbored one extra PKS gene cluster (filled in red) and one extra NRPS gene cluster

(filled in blue), respectively, and the positions of a PKS gene cluster differed between the strains (Figure 6b, upper). In contrast, only seven to eight of the gene clusters of the two strains were conserved in *S. albidoflavus*, and five to six and four gene clusters were specific (filled in a light color) to a putative new species INA 01303/ATCC 27968 (indicated as *Streptomyces* sp. in Figure 6b) and *S. albidoflavus*, respectively, although these two species showed approximately 65% DNA–DNA relatedness (Table 1) and are taxonomically close.

4. Discussion

Our isolate, N11-50, was identified as *S. albus*, and its genome encoded twelve NRPS and PKS gene clusters. These twelve gene clusters were well conserved in WGS-published *S. albus* strains. Strain diversity within *S. albus* was low in profile of these gene clusters because the strain diversity observed here is only the lack of *nrps-3* in the type strains of *S. albus* and the presence of an extra NRPS gene cluster in *S. albus* NRRL F-5917. Except for the extra gene clusters, these conserved NRPS and PKS gene clusters in principle limit the structural diversity in nonribosomal peptide- and polyketide-skeletons that can be synthesized by *S. albus* to twelve, as elucidated in this study. However, *S. albus* can be expected to produce new nonribosomal peptide- and polyketide-compounds because nine clusters, except for *nrps-2*, *nrps-3* and *t1pks-2*, were orphan and seemed to be BGCs for the unknown compounds. Seipke reported that smBGCs are diverse among the strains within *S. albus* [41], but the author studied not *S. albus* but *S. albidoflavus* J1074 and its phylogenetically close and unidentified strains, which were likely neither *S. albus* nor *S. albidoflavus* [42]. He did not include type strains. Therefore, his report unfortunately did not actually examine strain-level diversity within *S. albus*. Very recently, Vela Gurovic et al. reported core secondary metabolome in *S. albus*, where ten NRPS and PKS gene clusters were described [22]. However, data from these gene clusters were nothing more than a result through only antiSMASH analysis, and the authors did not carefully review and annotate the gene clusters. Although one PKS gene cluster was reasonably annotated to be a BGC of xantholipin, ibomycin-BGC was mistakenly annotated as a hybrid oligosaccharide/T1PKS gene cluster. The metabolites of the other PKS and NRPS gene clusters were unassigned at all. Two type-I PKS gene clusters were inappropriately assigned to hybrid with other types of gene clusters. It was not described what extra gene clusters are. In contrast, we carefully and manually annotated the NRPS and PKS gene clusters, predicted the chemical structures of the products, as shown in Tables 2 and 3 and Figures 4 and 5, and then investigated the strain-level diversity of the NRPS and PKS gene clusters within *S. albus*. Thus, this is the first report that studied the strain-level diversity on NRPS and PKS gene clusters, in fact. Additionally, we investigated the taxonomic positions of three WGS-published *S. albus* strains, which have not been studied by Vela Gurovic et al. [22]. We do not include the taxonomic positions of the other WGS-published *S. albus* strains reported by Vela Gurovic et al. [22] in our present study. Consequently, it is revealed that *S. albus* G153 and NRRL B-2238 are *S. albidoflavus*, whereas *S. albus* INA 01303 is a new genomospecies with *S. violascens* ATCC 27968. If we did not confirm the taxonomic positions of these WGS-published strains, we might have concluded that the NRPS and PKS gene clusters are diverse among strains even within a single species. Hence, species names need to be updated according to the latest criteria for classification.

We also revealed that strains NRRL F-5639, NRRL F-5917, HPH0547 and PHES57 51, which are registered as *Streptomyces* sp., belong to *S. albus*. Recent availability of type strains' WGSs enabled us to conduct taxonogenomic classification easier than before. On the other hand, many WGSs were not complete but draft sequences with several dozen to thousands of contig sequences due to short-read sequencing. Although they can be used for dDDH, they are not appropriate for the analysis of PKS and NRPS gene clusters because many gene clusters are not completely sequenced but fragmentated into several contigs. In contrast, since we completely sequenced all the twelve gene clusters, this study does not include such issues. As used in this study, long-read sequencing such as PacBio would be better to analyze BGCs encoding large modular enzymes.

We analyzed NRPS and NRPS gene clusters in strains of not only *S. albus* but also *S. albidoflavus* and a putative new genomospecies with a complete WGS available. Our present study demonstrated that strains belonging the same species share the same or similar sets of NRPS and PKS gene clusters, and that strains classified as different species do not share similar sets of these gene clusters even if the strains are phylogenetically and/or taxonomically close, as shown in Figure 6. These results strongly support our idea that has been proposed in our previous studies [42]. Many researchers seem to believe that there is no correlation between taxonomic species and secondary metabolites. By accumulating and publishing more examples from *Streptomyces* strains with an updated species name, it can be further clarified that our idea is widely applicable to the genus *Streptomyces*.

5. Conclusions

An albonoursin- and cyclo(Phe-Leu)-producing *Streptomyces* sp. N11-50 was classified as *S. albus* and revealed to possess twelve NRPS and PKS gene clusters. These gene clusters were well conserved in the WGS-published strains that belonged to *S. albus*. Our taxonogenomic analysis revealed that *S. albus* G153 and NRRL B-2238, *Streptomyces* sp. HPH0547, PHES57 51, NRRL F-5639 and NRRL F-5917, and *S. albus* INA 01303 and *S. violascens* ATCC 27968 were *S. albidoflavus*, *S. albus*, and a new genomospecies, respectively. By reclassifying the WGS-published strain appropriately, the species-specific profiles of the NRPS and PKS gene clusters, with little strain-level diversities, were clearly demonstrated.

Author Contributions: Conceptualization, H.K. and Y.I.; methodology, T.T.; investigation, H.K.; resources, Y.I.; data curation, H.K.; writing—original draft preparation, H.K.; writing—review and editing, Y.I. and T.T.; supervision, Y.I.; project administration, T.T.; funding acquisition, T.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported in part by a commissioned project from the Japan Patent Office.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The whole genome shotgun project of *Streptomyces* sp. N11-50 has been deposited at GenBank under the accession numbers BNEJ01000001-BNEJ01000031. The BioProject and BioSample accession numbers are PRJDB9820 and SAMD00228010, respectively.

Acknowledgments: We are grateful to Shinpei Ino and Takahiro Matsuyama for genome DNA preparation, and to Aya Uohara for depositing the whole-genome sequence to DDBJ.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Baltz, R.H. Gifted Microbes for Genome Mining and Natural Product Discovery. *J. Ind. Microbiol. Biotechnol.* **2017**, *44*, 573–588. [CrossRef] [PubMed]
2. Nett, M.; Ikeda, H.; Moore, B.S. Genomic Basis for Natural Product Biosynthetic Diversity in the Actinomycetes. *Nat. Prod. Rep.* **2009**, *26*, 1362–1384. [CrossRef] [PubMed]
3. Fischbach, M.A.; Walsh, C.T. Assembly-Line Enzymology for Polyketide and Nonribosomal Peptide Antibiotics: Logic, Machinery, and Mechanisms. *Chem. Rev.* **2006**, *106*, 3468–3496. [CrossRef] [PubMed]
4. Komaki, H. Recent Progress of Reclassification of the Genus *Streptomyces*. *Microorganisms* **2023**, *11*, 831. [CrossRef] [PubMed]
5. Manivasagan, P.; Venkatesan, J.; Sivakumar, K.; Kim, S. Pharmaceutically Active Secondary Metabolites of Marine Actinobacteria. *Microbiol. Res.* **2014**, *169*, 262–278. [CrossRef]
6. Subramani, R.; Aalbersberg, W. Marine Actinomycetes: An Ongoing Source of Novel Bioactive Metabolites. *Microbiol. Res.* **2012**, *167*, 571–580. [CrossRef]
7. Harunari, E.; Ogino, K.; Kanaki, S.; Kumagai, T.; Igarashi, Y. Isolation and Metabolites Analysis of Actinomycetes from Deep-Sea Water in Toyama Bay. *Deep Ocean Water Res.* **2021**, *22*, 49–57.
8. Igarashi, Y.; Zhou, T.; Sato, S.; Matsumoto, T.; Yu, L.; Oku, N. Akaeolide, a Carbocyclic Polyketide from Marine-Derived *Streptomyces*. *Org. Lett.* **2013**, *15*, 5678–5681. [CrossRef]
9. Fukushima, K.; Yazawa, K.; Arai, T. Biological Activities of Albonoursin. *J. Antibiot.* **1973**, *26*, 175–176. [CrossRef]

10. Kanzaki, H.; Imura, D.; Sashida, R.; Kobayashi, A.; Kawazu, K. Effective Production of Dehydro Cyclic Dipeptide Albonoursin Exhibiting Pronuclear Fusion Inhibitory Activity. I. Taxonomy and Fermentation. *J. Antibiot.* **1999**, *52*, 1017–1022. [CrossRef]
11. Wang, P.; Xi, L.; Liu, P.; Wang, Y.; Wang, W.; Huang, Y.; Zhu, W. Diketopiperazine Derivatives from the Marine-Derived Actinomycete *Streptomyces* sp. FXJ7.328. *Mar. Drugs* **2013**, *11*, 1035–1049. [CrossRef] [PubMed]
12. Li, Y.; Lai, Y.; Lu, Y.; Yang, Y.; Chen, S. Analysis of the Biosynthesis of Antibacterial Cyclic Dipeptides in *Nocardiopsis alba*. *Arch. Microbiol.* **2014**, *196*, 765–774. [CrossRef] [PubMed]
13. Gondry, M.; Sauguet, L.; Belin, P.; Thai, R.; Amouroux, R.; Tellier, C.; Tuphile, K.; Jacquet, M.; Braud, S.; Courcon, M.; et al. Cyclodipeptide Synthases Are a Family of tRNA-Dependent Peptide Bond-Forming Enzymes. *Nat. Chem. Biol.* **2009**, *5*, 414–420. [CrossRef] [PubMed]
14. Lautru, S.; Gondry, M.; Genet, R.; Pernodet, J.L. The Albonoursin Gene Cluster of *S. noursei* Biosynthesis of Diketopiperazine Metabolites Independent of Nonribosomal Peptide Synthetases. *Chem. Biol.* **2002**, *9*, 1355–1364. [CrossRef]
15. Komaki, H.; Tamura, T.; Igarashi, Y. Classification and Secondary Metabolite-Biosynthetic Gene Clusters of Marine *Streptomyces* Strains Including a Lobophorin- and Divergolide-Producer. *Hydrobiology* **2023**, *2*, 151–161. [CrossRef]
16. Yoon, S.; Ha, S.; Kwon, S.; Lim, J.; Kim, Y.; Seo, H.; Chun, J. Introducing EzBioCloud: A Taxonomically United Database of 16S rRNA Gene Sequences and Whole-Genome Assemblies. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 1613–1617. [CrossRef]
17. Rong, X.; Huang, Y. Taxonomic Evaluation of the *Streptomyces hygroscopicus* Clade Using Multilocus Sequence Analysis and DNA-DNA Hybridization, Validating the MLSA Scheme for Systematics of the Whole Genus. *Syst. Appl. Microbiol.* **2012**, *35*, 7–18. [CrossRef]
18. Saito, H.; Miura, K. Preparation of Transforming Deoxyribonucleic Acid by Phenol Treatment. *Biochim. Biophys. Acta* **1963**, *72*, 619–629. [CrossRef]
19. Meier-Kolthoff, J.P.; Carbasse, J.S.; Peinado-Olarte, R.L.; Göker, M. TYGS and LPSN: A Database Tandem for Fast and Reliable Genome-Based Classification and Nomenclature of Prokaryotes. *Nucleic Acids Res.* **2021**, *50*, D801–D807. [CrossRef]
20. Blin, K.; Shaw, S.; Kloosterman, A.M.; Charlop-Powers, Z.; van Wezel, G.P.; Medema, M.H.; Weber, T. antiSMASH 6.0: Improving Cluster Detection and Comparison Capabilities. *Nucleic Acids Res.* **2021**, *49*, W29–W35. [CrossRef]
21. Meier-Kolthoff, J.P.; Goker, M.; Sproer, C.; Klenk, H.P. When Should a DDH Experiment Be Mandatory in Microbial Taxonomy? *Arch. Microbiol.* **2013**, *195*, 413–418. [CrossRef]
22. Vela Gurovic, M.S.; Díaz, M.L.; Gallo, C.A.; Dietrich, J. Phylogenomics, CAZyome and Core Secondary Metabolome of *Streptomyces albus* Species. *Mol. Genet. Genom.* **2021**, *296*, 1299–1311. [CrossRef]
23. Chun, J.; Oren, A.; Ventosa, A.; Christensen, H.; Arahal, D.R.; da Costa, M.S.; Rooney, A.P.; Yi, H.; Xu, X.-W.; De Meyer, S.; et al. Proposed Minimal Standards for the Use of Genome Data for the Taxonomy of Prokaryotes. *Int. J. Syst. Evol. Microbiol.* **2018**, *68*, 461–466. [CrossRef] [PubMed]
24. Glaeser, S.P.; Kämpfer, P. Multilocus Sequence Analysis (MLSA) in Prokaryotic Taxonomy. *Syst. Appl. Microbiol.* **2015**, *38*, 237–245. [CrossRef] [PubMed]
25. Lefort, V.; Desper, R.; Gascuel, O. FastME 2.0: A Comprehensive, Accurate, and Fast Distance-Based Phylogeny Inference Program. *Mol. Biol. Evol.* **2015**, *32*, 2798–2800. [CrossRef] [PubMed]
26. Lasch, C.; Stierhof, M.; Estevez, M.R.; Myronovskyi, M.; Zapp, J.; Luzhetskyy, A. Dudomycins: New Secondary Metabolites Produced after Heterologous Expression of an NRPS Cluster from *Streptomyces albus* ssp. chlorinus NRRL B-24108. *Microorganisms* **2020**, *8*, 1800. [CrossRef] [PubMed]
27. Patzer, S.I.; Braun, V. Gene Cluster Involved in the Biosynthesis of Griseobactin, a Catechol-Peptide Siderophore of *Streptomyces* sp. ATCC 700974. *J. Bacteriol.* **2010**, *192*, 426–435. [CrossRef]
28. Robbins, N.; Spitzer, M.; Wang, W.; Waglechner, N.; Patel, D.J.; O'Brien, J.S.; Ejim, L.; Ejim, O.; Tyers, M.; Wright, G.D. Discovery of Ibomycin, a Complex Macrolactone that Exerts Antifungal Activity by Impeding Endocytic Trafficking and Membrane Function. *Cell Chem. Biol.* **2016**, *23*, 1383–1394. [CrossRef]
29. Sun, Y.; Carandang, R.R.; Harada, Y.; Okada, S.; Yoshitake, K.; Asakawa, S.; Nogi, Y.; Matsunaga, S.; Takada, K. Lactomycins A–C, Dephosphorylated Phoslactomycin Derivatives that Inhibit Cathepsin B, from the Marine-derived *Streptomyces* sp. ACT232. *Mar. Drugs* **2018**, *16*, 70. [CrossRef]
30. Geyer, K.; Sundaram, S.; Sušnik, P.; Koert, U.; Erb, T.J. Understanding Substrate Selectivity of Phoslactomycin Polyketide Synthase by Using Reconstituted In Vitro Systems. *ChemBioChem* **2020**, *21*, 2080–2085. [CrossRef]
31. Horsman, G.P.; Van Lanen, S.G.; Shen, B. Iterative Type I Polyketide Synthases for Enediyne Core Biosynthesis. *Methods Enzymol.* **2009**, *459*, 97–112. [CrossRef]
32. Zhang, W.; Wang, L.; Kong, L.; Wang, T.; Chu, Y.; Deng, Z.; You, D. Unveiling the Post-PKS Redox Tailoring Steps in Biosynthesis of the Type II Polyketide Antitumor Antibiotic Xantholipin. *Chem. Biol.* **2012**, *19*, 422–432. [CrossRef] [PubMed]
33. Yan, Y.; Zhang, L.; Ito, T.; Qu, X.; Asakawa, Y.; Awakawa, T.; Abe, I.; Liu, W. Biosynthetic Pathway for High Structural Diversity of a Common Dilactone Core in Antimycin Production. *Org. Lett.* **2012**, *14*, 4142–4145. [CrossRef] [PubMed]
34. Piel, J.; Hertweck, C.; Shipley, P.R.; Hunt, D.M.; Newman, M.S.; Moore, B.S. Cloning, Sequencing and Analysis of the Enterocin Biosynthesis Gene Cluster from the Marine Isolate '*Streptomyces maritimus*': Evidence for the Derailment of an Aromatic Polyketide Synthase. *Chem. Biol.* **2000**, *7*, 943–955. [CrossRef]

35. Rodriguez Estévez, M.; Myronovskyi, M.; Rosenkränzer, B.; Paululat, T.; Petzke, L.; Ristau, J.; Luzhetskyy, A. Novel Fredericamycin Variant Overproduced by a Streptomycin-Resistant *Streptomyces albus* subsp. chlorinus Strain. *Mar. Drugs* **2020**, *18*, 284. [CrossRef]
36. Horbal, L.; Stierhof, M.; Palusczak, A.; Eckert, N.; Zapp, J.; Luzhetskyy, A. Cyclofaulknamycin with the Rare Amino Acid D-capreomycidine Isolated from a Well-Characterized *Streptomyces albus* Strain. *Microorganisms* **2021**, *9*, 1609. [CrossRef] [PubMed]
37. Chen, S.; Huang, X.; Zhou, X.; Bai, L.; He, J.; Jeong, K.J.; Lee, S.Y.; Deng, Z. Organizational and Mutational Analysis of a Complete FR-008/Candicidin Gene Cluster Encoding a Structurally Related Polyene Complex. *Chem. Biol.* **2003**, *10*, 1065–1076. [CrossRef] [PubMed]
38. Luo, Y.; Huang, H.; Liang, J.; Wang, M.; Lu, L.; Shao, Z.; Cobb, R.E.; Zhao, H. Activation and Characterization of a Cryptic Polycyclic Tetramate Macrolactam Biosynthetic Gene Cluster. *Nat. Commun.* **2013**, *4*, 2894. [CrossRef]
39. Xu, F.; Nazari, B.; Moon, K.; Bushin, L.B.; Seyedsayamdost, M.R. Discovery of a Cryptic Antifungal Compound from *Streptomyces albus* J1074 Using High-Throughput Elicitor Screens. *J. Am. Chem. Soc.* **2017**, *139*, 9203–9212. [CrossRef]
40. Chen, R.; Zhang, Q.; Tan, B.; Zheng, L.; Li, H.; Zhu, Y.; Zhang, C. Genome Mining and Activation of a Silent PKS/NRPS Gene Cluster Direct the Production of Totopotensamides. *Org. Lett.* **2017**, *19*, 5697–5700. [CrossRef] [PubMed]
41. Seipke, R.F. Strain-Level Diversity of Secondary Metabolism in *Streptomyces albus*. *PLoS ONE* **2015**, *10*, e0116457. [CrossRef] [PubMed]
42. Komaki, H.; Sakurai, K.; Hosoyama, A.; Kimura, A.; Igarashi, Y.; Tamura, T. Diversity of Nonribosomal Peptide Synthetase and Polyketide Synthase Gene Clusters among Taxonomically Close *Streptomyces* Strains. *Sci. Rep.* **2018**, *8*, 6888. [CrossRef] [PubMed]