life

MDPI

Article

Taxonomic Positions and Secondary Metabolite-Biosynthetic Gene Clusters of Akazaoxime- and Levantilide-Producers

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

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

Abstract: *Micromonospora* sp. AKA109 is a producer of akazaoxime and A-76356, whereas *Micromonospora* sp. AKA38 is that of levantilide C. We aimed to clarify their taxonomic positions and identify biosynthetic gene clusters (BGCs) of these compounds. In 16S rRNA gene and DNA gyrase subunit B gene (*gyrB*) sequence analyses, strains AKA109 and AKA38 were the most closely related to *Micromonospora humidisoli* MMS20-R2-29^T and *Micromonospora schwarzwaldensis* HKI0641^T, respectively. Although *Micromonospora* sp. AKA109 was identified as *M. humidisoli* by the *gyrB* sequence similarity and DNA–DNA relatedness based on whole genome sequences, *Micromonospora* sp. AKA38 was classified to a new genomospecies. *M. humidisoli* AKA109 harbored six type-I polyketide synthase (PKS), one type-II PKS, one type-III PKS, three non-ribosomal peptide synthetase (NRPS) and three hybrid PKS/NRPS gene clusters, among which the BGC of akazaoxime and A-76356 was identified. These gene clusters are conserved in *M. humidisoli* MMS20-R2-29^T. *Micromonospora* sp. AKA38 harbored two type-I PKS, one of which was responsible for levantilide C, one type-II PKS, one type-III PKS, two NRPS and five hybrid PKS/NRPS gene clusters. We predicted products derived from these gene clusters through bioinformatic analyses. Consequently, these two strains are revealed to be promising sources for diverse non-ribosomal peptide and polyketide compounds.

Keywords: akazaoxime; A-76356; biosynthesis; classification; levantilide; *Micromonospora*; non-ribosomal peptide; polyketide

check for updates

Citation: Komaki, H.; Tamura, T.; Igarashi, Y. Taxonomic Positions and Secondary Metabolite-Biosynthetic Gene Clusters of Akazaoxime- and Levantilide-Producers. *Life* **2023**, *13*, 542. https://doi.org/10.3390/life13020542

Academic Editors: Kuei-Hung Lai, Ho-Cheng Wu and Mohamed El-Shazly

Received: 15 December 2022
Revised: 8 February 2023
Accepted: 10 February 2023
Published: 15 February 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Actinomycetes are Gram stain-positive and filamentous bacteria with high G + C contents in genomic DNAs. They are well known as a promising source for pharmacologically useful bioactive substances with diverse chemistries, from which many pharmaceuticals were developed and are clinically used [1]. The genus *Streptomyces* is the representative of actinomycetes, and its main habitat is soil. However, soil environments are extensively searched for novel actinomycetes, and consequently, it is getting harder to isolate novel actinomycetal strains from the same environments. In contrast, marine environments are attracting attention as rich sources of underexplored actinomycetes. Indeed, we have discovered new and diverse bioactive secondary metabolites from marine actinomycetes [2–9]. *Micromonospora* strains are frequently isolated from marine environments. Many bioactive substances are reported from this genus [10,11]. We previously isolated *Micromonospora* sp. AKA109 and *Micromonospora* sp. AKA38 from deep sea water. From *Micromonospora* sp. AKA109, a new compound named akazaoxime (**1**, Figure 1) was discovered, along with a known compound, A-76356 (**2**, Figure 1). Akazaoxime and A-76356 are enteromycin-class antibiotics. Incorporation experiments of labelled precursors suggested these two compounds are biosynthesized from glycine, leucin and propionate. Akazaoxime exhibits antibacterial activity to Gram-positive *Kocuria rhizophila*, whereas A-76356 is active against filamentous fungi such as the plant pathogen *Glomerella cingulata* [12]. *Micromonospora* sp. AKA38 produces levantilide C (**3**, Figure 1), which is a 20-membered

macrolide and exhibits antiproliferative activities against several tumor cell lines [13]. Biosynthetic gene clusters (BGCs) of these compounds have not been identified yet, although identification of BGCs plays an important role in developments in combinatorial biosynthesis and synthetic biology.

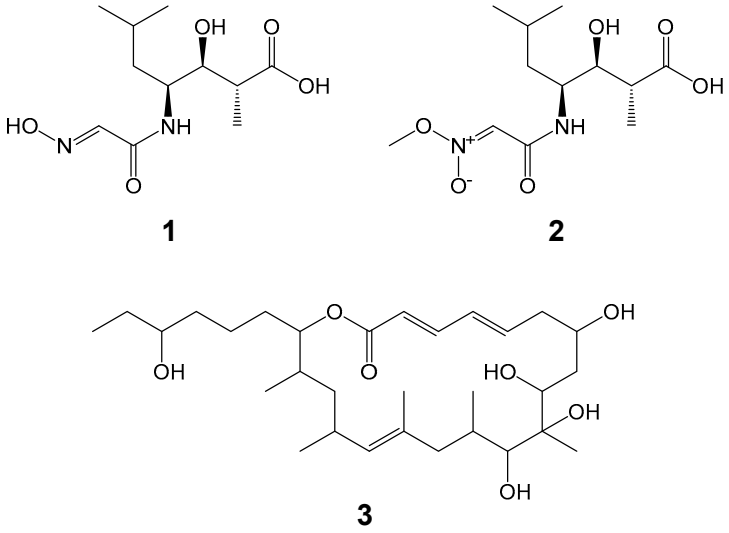

Figure 1. Chemical structures of akazaoxime (**1**), A-76356 (**2**) and levantilide C (**3**).

Polyketides such as macrolide backbones are biosynthesized by the assemblage of acyl-CoAs as building blocks. The assembly is catalyzed by polyketide synthases (PKSs). PKSs are classified by three types. Type-I PKSs are large modular enzymes composed of multiple catalytic domains. Polyketide chains are synthesized according to the co-linearity rule of assembly lines. Such a mechanism shows similarity to that in the biosynthesis of non-ribosomal peptides by non-ribosomal peptide synthetases (NRPSs), which is based on assembly of amino acids as building blocks. NRPSs as well as type-I PKSs are large and modular enzymes with multiple catalytic domains, and they accord to the co-linearity rule [14,15]. Polyketide chains for macrolide compounds are synthesized by type-I PKSs. Backbones synthesized by type-I PKSs and/or NRPSs can be predicted from their domain organizations by bioinformatic analysis [14,15]. In contrast, type-II PKSs are composed of three monofunctional enzymes, ketosynthase α (KSα), KSβ (chain length factor), and acyl carrier protein (ACP). Differently from type-I PKSs, these three enzymes iteratively catalyze multiple chain elongation steps. The main products of type-II PKSs are aromatic compounds [16]. Type-III PKSs are not multimodular or composed of abovementioned three enzymes, but stand alone with a KS domain and iteratively catalyze the assembly of the acyl-CoA unit [17]. Genome analyses revealed that half to three quarters of the secondary metabolite-BGCs in each actinomycetal genome are associated with PKSs or NRPSs. This suggests that polyketides, non-ribosomal peptides, and their hybrid compounds, which are derived from hybrid PKS/NRPS gene clusters, are main secondary metabolites in actinomycetes [18].

In the present study, we classified *Micromonospora* sp. AKA109 and *Micromonospora* sp. AKA38 at species level. Next, we identified BGCs for akazaoxime/A-76356 and levantilide C through analysis of PKS and NRPS gene clusters in their genomes. The analysis revealed the potential of the two strains to act as producers of diverse polyketide- and nonribosomal peptide-compounds. These results are useful to elucidate potential products of each strain.

2. Materials and Methods

Micromonospora strains AKA109 and AKA38 were isolated from deep sea water collected in Shizuoka, Japan, maintained as TP-A0907 and TP-A0908, respectively, in Toyama Prefectural University, and have been deposited to and are available from the NBRC culture collection as NBRC 113680 and NBRC 113681, respectively. The 16S rRNA genes were

amplified by PCR using 9F and 1541R primers. The amplicons were sequenced by the method described in our previous report [19]. Type strains showing high 16S rRNA gene sequence similarities to AKA109 and AKA38 were searched using the EzBioCloud web server [20]. Phylogenetic trees based on 16S rRNA gene and DNA gyrase subunit B gene (*gyrB*) sequences were reconstructed by the neighbor-joining method using ClustalX 2.1. Whole genomes were sequenced using PacBio, as reported [21]. Draft genome sequences of strains AKA109 and AKA38 were deposited to DDBJ under the accession numbers of BNEH01000001–BNEH01000007 and BNEI01000001–BNEI01000011, respectively. A phylogenomic tree was reconstructed using the TYSG server [22]. DNA–DNA relatedness was calculated by digital DNA–DNA hybridization (DDH) using the Genome-to-Genome Distance Calculator 2.1 (GGDC) [23], and DDH estimates by the Formula 2 were employed. PKS and NRPS gene clusters in the whole genome were searched, and their domains were determined using antiSMASH [24]. The products were predicted by reviewing module numbers and domain organizations in PKSs and NRPSs, the substrates of acyltransferase (AT) and adenylation (A) domains, and orthologs searched by BLAST, in addition to results of ClusterBlast in antiSMASH.

3. Results

3.1. Classification of Micromonospora Strains AKA109 and AKA38

In the 16S rRNA gene sequence analysis, *Micromonospora* sp. AKA109 showed 100% similarity to *Micromonospora humidisoli* MMS20-R2-29^T, whereas *Micromonospora* sp. AKA38 showed 99.9% similarity to *Micromonospora schwarzwaldensis* HKI0641^T as the closest. In the phylogenetic tree shown in Figure 2, strain AKA109 formed an independent clade with *M. humidisoli* MMS20-R2-29^T, whereas strain AKA38 did that with *M. schwarzwaldensis* HKI0641^T.

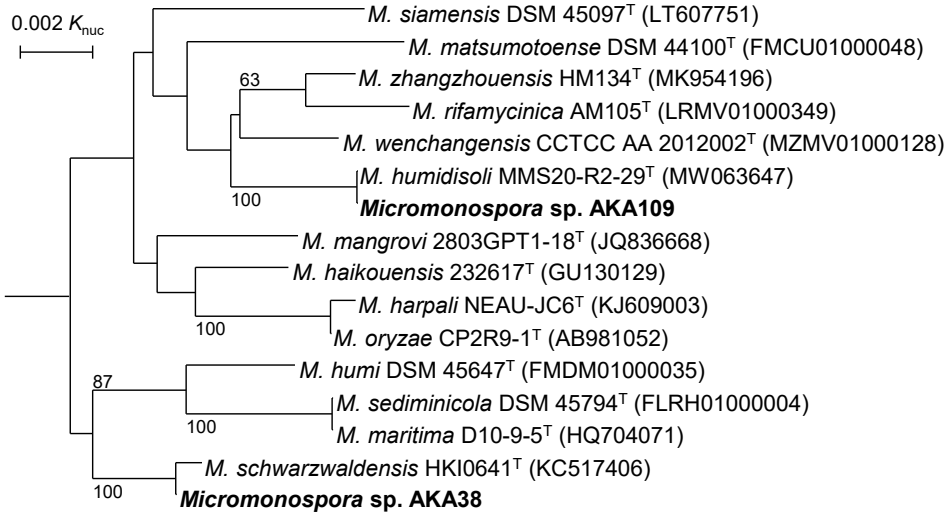

Figure 2. Phylogenetic tree based on 16S rRNA gene sequences. Type strains of species showing sequence simiralities of >99.0% to *Micromonospora* sp. AKA109 and/or *Micromonospora* AKA38 are included in this tree. Numbers on the branches are the confidence limits estimated by bootstrap analysis with 1000 replicates, and values above 50% are indicated at branching points. *Phytohabitans suffuscus* K07-0523^T (AB490769) was used as an outgroup (not shown).

We next reconstructed a phylogenetic tree based on *gyrB* sequences, as shown in Figure 3, since *gyrB* sequences are recognized to be more suitable than 16S rRNA gene sequences for phylogenetic classification and identification [25]. In this tree, *M. humidisoli* and *M. schwarzwaldensis* were also phylogenetically the closest species of strains AKA109 and AKA38, respectively. The *gyrB* sequence similarity between *Micromonospora* sp. AKA109 and *M. humidisoli* MMS20-R2-29^T was 99.0%. Since 98.5% in *gyrB* sequence similarity is recognized to correspond to 70% in DNA–DNA relatedness [25,26], *Micromonospora* sp.

AKA109 is likely *M. humidisoli*. In contrast, the *gyrB* sequence similarity between *Micromonospora* sp. AKA38 and *M. schwarzwaldensis* HKI0641^T was 97.4%, which is much below than 98.5%; therefore, *Micromonospora* sp. AKA38 is considered an independent new genomospecies.

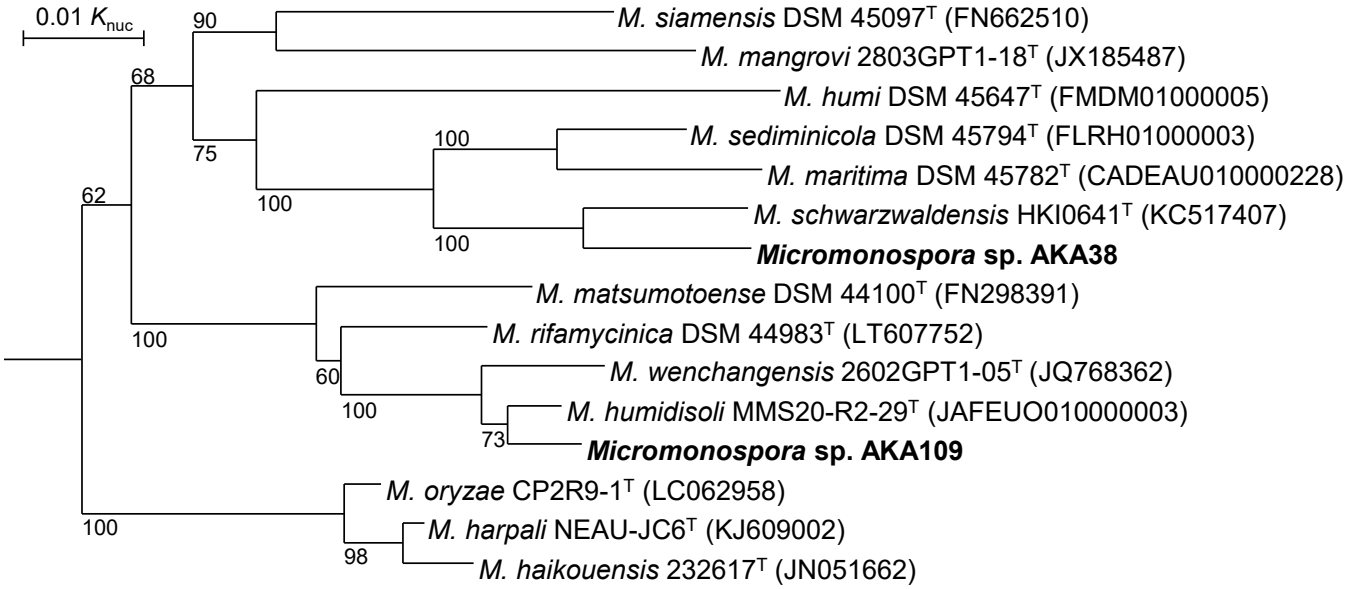

Figure 3. Phylogenetic tree based on *gyrB* sequences. Type strains of species shown in Figure 2 are included in this tree. Numbers on the branches are the confidence limits estimated by bootstrap analysis with 1000 replicates, and values above 50% are indicated at branching points. *P. suffuscus* NBRC 105367^T (AP022871) was used as an outgroup (not shown).

Additionally, a phylogenomic tree was reconstructed with type strains whose whole genome sequences are published (Figure 4). The phylogenetic relationships well correlated to those in phylogenetic trees of Figures 1 and 2. DNA–DNA relatedness, estimated by digital DDH, between *Micromonospora* sp. AKA109 and *M. humidisoli* MMS20-R2-29^T was 93.5%. As this value is much higher than 70%, which is the established cut-off for species delineation [27–29], strain AKA109 was identified to be *M. humidisoli*. In contrast, DNA–DNA relatedness between *Micromonospora* sp. AKA38 and the other strains shown in Figure 4 were less than 41.4%. This result also shows *Micromonospora* sp. AKA38 to be an independent genomospecies.

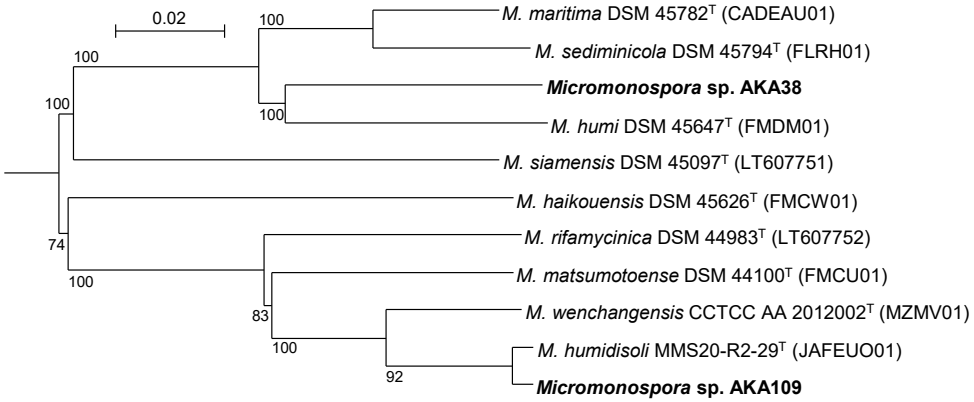

Figure 4. Phylogenomic tree reconstituted using the TYGS server. *P. suffuscus* NBRC 105367^T (AP022871) was used as an outgroup (not shown) to show the root. The numbers in parentheses are accession numbers of WGS Projects or whole genome sequences in GenBank. Type strains of species shown in Figure 2 whose whole genome sequences are published are included in this tree.

3.2. PKS and NRPS Gene Clusters in the Whole Genome of M. humidisoli AKA109

Six type-I PKS, one type-II PKS, one type-III PKS, three NRPS and three hybrid PKS/NRPS gene clusters were encoded in the genome of *Micromonospora* sp. AKA109. Type-I PKS gene cluster 1 (*t1pks-1*) encoded three PKSs, whose domain organization was almost identical to those (KS AT_m ACP KS AT_m DH KR ACP KS AT_m DH KR ACP, KS AT_m DH KR ACP KS AT_m DH <u>KR</u> ACP, KS $AT_{\underline{m}/mm/\underline{em}}$ DH KR ACP TD) of camporidine-, argimycin- and streptazone-BGCs [30–32]. However, *t1pks-1* lacked the KR domain (underlined in the previous brackets) present in CamD, ArpII and StzC. Although the substrate of the last AT domain in *t1pks-1* was methylmalonyl-CoA, those in ArpIII and StzB are malonyl-CoA. Thus, product(s) of *t1pks-1* may resemble camporidine, argimycin or streptazone, but will be different from these. PKSs encoded in *t1pks-2*, *t1pks-3* and *t1pks-4* did not show high sequence similarities to PKSs whose products have been identified. Thus, the products of these PKS gene clusters were not predicted. The domain organization, KS/AT/KR/DH, of the PKS encoded by TPA0907_18690 in *t1pks-3* is well known as that of iterative PKSs for enediyne syntheses. Hence, the products of *t1pks-3* may include an enediyne moiety. *T1pks-5* encoded five PKSs. These PKSs showed high similarities to those in the marinolactam-BGC (*mrl*) [33]. Their domain organization was identical to that of *mrl* except for the presence of a DH domain in the first module of MrlB, which is absent in that of TPA0907_35890. Therefore, we annotated this cluster to be responsible for a marinolactum congener. As genes in *t1pks-6* showed high similarities to those in the amycomicin-BGC, the product was predicted to be amycomicin. Products of type-II PKS gene cluster 1 (*t2pks-1*) were predicted to be an aromatic compound. Type-III PKS gene cluster 1 (*t3pks-1*) showed similarity to *agq*, which is the BGC of alkyl-*O*-dihydrogeranyl-methoxyhydroquinone [34]. Three NRPS gene clusters (*nrps-1*, *nrps-2*, and *nrps-3*) did not show high similarities to those whose products are elucidated, suggesting that they are orphan gene clusters. Although the product of *nrps-2* was unpredictable because its NRPS was not multimodular, those of *nrps-1* and *nrps-3* were predicted as dipeptide and tetrapeptide, respectively, as shown in Table 1. Hybrid PKS/NRPS gene clusters 1 and 2 (*pks/nrps-1* and *pks/nrps-2*) were orphan. The domain organization of *pks/nrps-1* was unusual, because thioesterase (TE) domain is not present at the terminal, but as the first domain. Hence, it is doubtful that the cluster works to synthesize hybrid polyketide/non-ribosomal peptide compounds. The product derived from *pks/nrps-2* was predicted to be a hybrid polyketide/non-ribosomal peptide compound including Asn and Ser residues.

Table 1. PKS and NRPS gene clusters in the whole genome of *M. humidisoli* AKA109.

Cluster	Locus Tag (TPA0907)	Domain Organization	Product Predicted
t1pks-1	_14850 _14840 _14830	KS AT_m ACP KS AT_m DH KR ACP KS AT_m DH KR ACP KS AT_m DH KR ACP KS AT_m DH ACP KS AT_{mm} DH KR ACP TD	New analog(s) of camporidine, argimycin, streptazone
t1pks-2	_16830 _16820 _16810	KS AT_m ACP ACP ACP KR KS AT_{mm} ACP	Unknown
t1pks-3	_18400 _18690	KS AT DH KR ACP KS AT_m KR DH	Compound with an enediyne moiety
t1pks-4	_47680	KS AT_m DH ER KR ACP	Unknown

Table 1. *Cont.*

Cluster	Locus Tag (TPA0907)	Domain Organization	Product Predicted
t1pks-5 (*mrl*)	_35900	KS AT_m DH KR ACP	Marinolactam congener
	_35890	KS AT_m KR ACP KS AT_m KR ACP	
	_35880	KS AT_{mm} KR ACP	
	_35870	KS AT_m DH KR ACP KS AT_m DH KR ACP KS AT_m DH KR ACP TE	
	_35750	ACP KS AT_m DH KR ACP KS AT_{mm} DH KR ACP KS AT_{mm} DH KR ACP KS AT_m DH KR ACP	
t1pks-6	_29310	KS AT_m DH ER KR ACP	Amycomicin
t2pks-1	_20160	KSα	Aromatic polyketide
	_20170	KSβ (CLF)	
	_20190	ACP	
t3pks-1 * (*aqq*)	_59200	KS	Alkyl-*O*-dihydrogeranyl-methoxyhydroquinone
nrps-1	_47220	C A_{phe} PCP	Phe-x
	_47230	C A PCP	
	_47240	C	
nrps-2	_47680	A PCP C	Unknown
nrps-3	_56920	C A PCP C	Tetrapeptide including Cys and Glu
	_56930	A_{cys} PCP	
	_56940	A PCP C	
	_56970 [C]	C A_{glu} PCP E C	
pks/nrps-1	_15480	TE A PCP KS AT_m KR ACP	Unknown
pks/nrps-2 *	_28040	A PCP KS	x-x-y-mal-Asn-Ser
	_28030	TE	
	_28010	A PCP C PCP	
	_28000	KS AT_m KR DH ACP	
	_27970	C A_{asn} PCP	
	_27960	C A_{ser} PCP TE	
pks/nrps-3	_56660	A PCP	Akazaoxime and A-76356
	_56670	KS AT_m KR ACP	
	_56710	ACP	
	_56840 [C]	C A_{leu} PCP	

[C], encoded in the complementary strand; *, conserved between strains AKA109 and AKA38; A, adenylation domain; ACP, acyl carrier protein; AT, acyltransferase domain; AT_m, AT for malonyl-CoA, AT_{mm}, AT for methylmalonyl-CoA; $AT_{em/mx}$, AT for ethylmalonyl-CoA or methoxymalonyl CoA; C, condensation domain; CLF, chain length factor; CoL, CoA ligase domain; DH, dehydratase domain; Cyc, cyclase domain; E, epimerization domain; ER, enoylreductase domain; KR, ketoreductase domain; KS, ketosynthase domain; mal, residue derived from malonyl-CoA; MT, methyltransferase domain; *nrps*, PCP, peptidyl carrier protein; *nrps*, NRPS gene; *pks/nrps*, hybrid PKS/NRPS gene; pk, residue derived from a single module of type-I PKS; TD, termination domain; TE, thioesterase domain, *t1pks*, type-I PKS gene; *t2pks*, type-II PKS gene; *t3pks*, type-III PKS gene; x, unidentified amino acid residue; y, unknown unit by lack of A domain in the module. Amino acids incorporated by A domains are indicated as 3-letter abbreviations in subscript just after A.

We considered *pks/nrps-3* to be the BGC for akazaoxime and A-76356, according to its domain organization and the biosynthetic pathway revealed by incorporation of labeled precursors [12]. These two compounds have been reported to be synthesized from glycine, leucine, and propionate. Similarly, *pks/nrps-3* encodes two NRPS and one PKS, which incorporate two amino acids and one acyl-CoA, respectively, to the product. One of the amino acids was predicted to be leucine, although the other was bioinformatically not. Presence of a KR domain in the PKS well accounts for hydration of the keto group derived from carboxyl group of leucine. The cluster encoded a diiron oxygenase and a nitronate *O*-methyltransferase, which are essential to form aldoxime functionality and an

O-methyl nitronic acid moiety [35]. We predicted the biosynthetic pathway of akazaoxime and A-76356, as shown Figure 5. A glycine molecule is loaded on the NRPS encoded by TPA0907_56660. Its amino group is converted to an aldoxime functionality through an intermediate by the diiron oxygenase, as reported in the biosynthesis of althiomycin [35,36]. If the methyltransferase encoded by TPA0907_56720 acts the intermediate, the amino group is converted to *O*-methyl nitronic acid moiety, as reported in the biosynthesis of enteromycin carboxamide [35]. To the modified glycine molecules, leucine and methylmalonyl-CoA are bound by the other NRPS (TPA0907_56840) and the PKS (TPA0907_56670). Finally, the chains are released from the PKS to yield akazaoxime (**1**) and A-76356 (**2**), respectively.

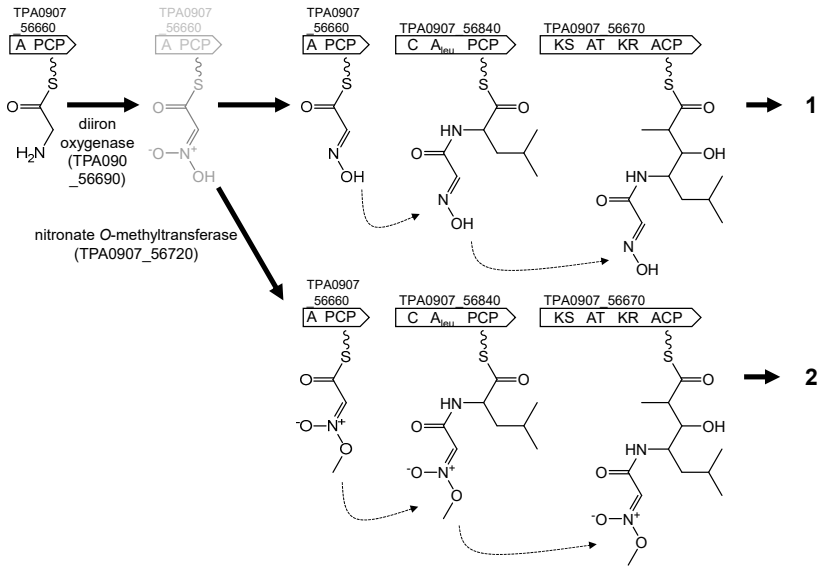

Figure 5. Putative biosynthetic pathways for akazaoxime (**1**) and A-76356 (**2**). An intermediate converted by the diiron oxygenase is shown in gray.

3.3. PKS and NRPS Gene Clusters in the Whole Genome of Micromonospora sp. AKA38

Micromonospora sp. AKA38 harbored two type-I PKS, one type-II PKS, one type-III PKS, two NRPS and five hybrid PKS/NRPS gene clusters in its genome, as listed in Table 2.

T1pks-8 is a large type-I PKS gene cluster encoding 13 PKSs, which form 33 modules. The product was predicted to be quinolidomicin based on the domain organization and similarities to quinolidomicin's PKSs (QmnA1 to QmnA13) [37]. The gene cluster is widely distributed in the genus *Micromonospora* [38]. The product of *t2pks-2* could not be predicted because the type-II PKSs did not show high sequence similarities to enzymes for the reported compounds. In most type-II PKS gene clusters, an ACP is encoded downstream of KSβ (CLF), but the ACP of *t2pks-2* is upstream of KSα and includes a cyclase domain. Two gene clusters, *t3pks-1* and *pks/nrps-2*, asterisked in the tables, were orthologs of those present in *M. humidisoli* AKA109. The other gene clusters, such as *nrps-4*, *nrps-5*, *pks/nrps-4*, *pks/nrps-5*, *pks/nrps-6* and *pks/nrps-7*, were orphan, and their products were predicted as shown in Table 2. In *pks/nrps-7*, two type-I PKSs whose domain organizations are KS-AT-KR-DH and KS-AT-ACP, respectively and one type-III PKS were encoded in addition to NRPSs. The domain pair, KR-DH, observed in one of the type-I PKSs is known to be specific for PksE. Therefore, the product of *pks/nrps-7* will include an enediyne moiety [39].

Table 2. PKS and NRPS gene clusters in the whole genome of *Micromonospora* sp. AKA38.

Gene Cluster	Locus Tag (TPA0908)	Domain Organization	Product Predicted
t1pks-7	_40860	AT_{mm} ACP KS AT_m KR ACP KS AT_m DH ER KR ACP KS AT_{mm} DH ER KR ACP KS AT_{mm} DH ER KR ACP	Levantilide C
	_40870	KS AT_{mm} DH KR ACP KS AT_{mm} DH ER KR ACP KS AT_{mm} KR ACP	
	_40880	KS AT_m DH KR ACP KS AT_m KR ACP KS AT_m DH KR ACP KS AT_m DH KR ACP TE	
t1pks-8 (*qmn*)	_45370	CoL ACP KS AT_m DH KR ACP KS AT_{mm} DH ER KR ACP	Quinolidomicin
	_45410	KS AT_m DH KR ACP	
	_45420	KS AT_m KR ACP KS AT_m KR ACP KS AT_m KR ACP	
	_45440	KS AT_m DH ER KR ACP KS AT_{mm} DH ER KR ACP KS AT_m DH ER KR ACP KS AT_m KR ACP	
	_45450	KS AT KR ACP KS AT_m DH KR ACP KS AT_m DH KR ACP KS AT_m DH KR ACP KS AT_m KR ACP KS AT_m KR ACP	
	_45460	KS AT_m KR ACP KS AT_{mm} DH KR ACP	
	_45470	KS AT_m DH KR ACP KS AT_{mm} KR ACP KS AT_m KR ACP	
	_45480	KS AT_{mm} KR ACP KS AT_{mm} DH ER KR ACP	
	_45490	KS AT_m KR ACP KS AT_{mm} KR ACP KS AT_{mm} KR ACP	
	_45500	KS AT_m DH KR ACP KS AT_{mm} DH KR ACP	
	_45510	KS AT_{mm} KR ACP	
	_45520	KS AT_{mm} KR ACP KS AT_m KR ACP	
	_45530	KS AT_m KR ACP TE	
t2pks-2	_49930	ACP Cyc	Unknown
	_49910	KSα	
	_49900	KSβ (CLF)	
t3pks-1 * (*aqq*)	_06420	KS	Alkyl-*O*-dihydrogeranyl-methoxyhydroquinone
nrps-4	_34180	A_{thr} MT PCP C A_{pro} PCP C PCP C PCP TE	Val-Thr-Leu-Pro-Leu-mThr-Pro-y-y
	_34160	TE	
	_34150	A_{val} PCP	
	_34130	A	
	_34100	C A_{thr} PCP C A_{leu} PCP C A_{pro} PCP C A_{leu} PCP C	
nrps-5	_34870 [C]	C A PCP C A PCP C A_{asn} PCP TE	x-Asn-x-Thr-Asn-x-x-Thr-x-x-x-Asn
	_34920	A PCP C A_{asn} PCP C A PCP	
	_35060	C A_{thr} PCP C A_{asn} PCP C A PCP C A PCP C A_{thr} PCP C A PCP	
	_35080	TE	
pks/nrps-2 *	_42740	A PCP KS	x-x-y-mal-Asn-Ser
	_42750	TE	
	_42770	A PCP C PCP	
	_42780	KS AT_m KR DH ACP	
	_42810	C A_{asn} PCP	
	_42820	C A_{ser} PCP TE	
pks/nrps-4	_08330	C A_{asn} PCP KS AT_m ACP C A PCP	Asn-mal-x-Ala-Glu-y
	_08340	A_{ala} PCP C	
	_08370	A_{glu} PCP C PCP	

Table 2. *Cont.*

Gene Cluster	Locus Tag (TPA0908)	Domain Organization	Product Predicted
pks/nrps-5	_34600	TE	x-x-x-y-mal-Ser
	_34620	A PCP	
	_34650	A PCP	
	_34660	KS	
	_34670	A PCP C PCP	
	_34690	KS AT_m KR ACP	
	_34690	C A_{ser} PCP	
	_34730	PCP	
pks/nrps-6	_35130	A_{thr} PCP	x-Thr-x-Asn-pk
	_35200	A PCP C A_{asn} PCP	
	_35210	ACP	
	_35230 [C]	KS AT DH KR ACP	
	_35250 [C]	C A PCP C	
pks/nrps-7	_54560 [C]	C	Ala-Val-enediyne-Val-mal-Ser-x-x with an aromatic moiety
	_54550 [C]	A PCP	
	_54470 [C]	PCP C	
	_54430 [C]	A	
	_54260	A_{ala} PCP C A_{val} PCP	
	_54200	KS (type III PKS)	
	_54120	KS AT_m KR DH	
	_54020 [C]	PCP TE	
	_54000	C A_{val} PCP	
	_53990	KS AT_m ACP	
	_53970	A_{ser}	

Footnotes are the same as those of Table 1.

We annotated *t1pks-7* as the BGC of levantilide C, according to its domain organization and the chemical structure. The cluster encoded three PKSs including a loading module and eleven modules to incorporate acyl-CoAs in the polyketide chain, as shown in Figure 6. The chemical structure predicted by the domain organization well matched to that of levantilide C. DH and ER domains in module 3 and the DH domain in module 8 would be inactive considering the actual chemical structure of levantilide C. A hydroxyl group is present at C-10 in levantilide C, and it does not form by polyketide biosynthesis. Because a cytochrome P450 is encoded near the PKSs in the gene cluster as TPA0908_40790, the hydroxyl group is likely introduced by the cytochrome P450.

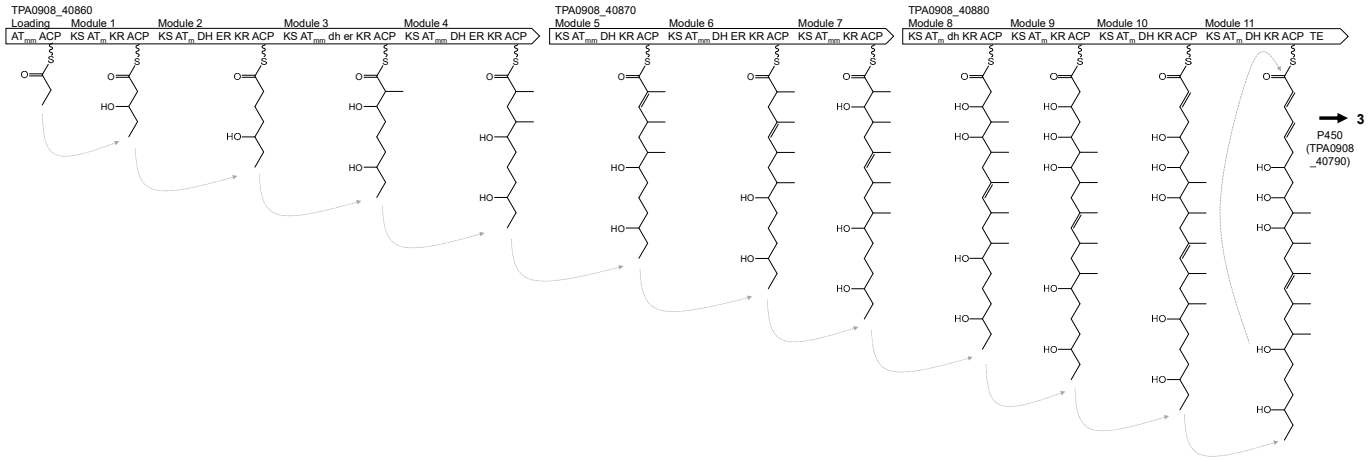

Figure 6. Proposed biosynthetic pathway of levantilide C (**3**). Abbreviations of domains are the same as those in Table 1. dh, inactive DH; er, inactive ER.

3.4. Specificity of the PKS and NRPS Gene Clusters in Each Strain

We conducted a BLAST search to investigate whether the gene clusters identified in this study are specific in each strain or present in the other strains. All the PKSs and NRPSs of *M. humidisoli* AKA109 were also present in *M. humidisoli* MMS20-R2-29^T (Table 3). As the TPA0907_16820 homolog in *M. humidisoli* MMS20-R2-29^T is not well sequenced, it was not hit in the search. Although a homolog of TPA0907_20190 was also present in *M. humidisoli* MMS20-R2-29^T, it is not described in the table because its sequence identity/similarity were lower (99/98 in%) than those of *Micromonospora* sp. RL09-050-HVF-A.

Table 3. The closest homolog or ortholog of PKSs and NRPSs encoded by the gene clusters of *M. humidisoli* AKA109 and *Micromonospora* sp. AKA38.

Cluster	Locus Tag (TPA090)	BLAST Top Hit		
		I/S (%) [1]	Locus Tag or Gene (Accession No.)	Origin
t1pks-1	7_14850	99/99	JQN84_27510	*M. humidisoli* MMS20-R2-29^T
	7_14840	99/99	JQN84_31080	*M. humidisoli* MMS20-R2-29^T
	7_14830	99/99	JQN84_29090	*M. humidisoli* MMS20-R2-29^T
t1pks-2	7_16830	90/92	J7462_RS07410	*Micromonospora* sp. RL09-050-HVF-A
	7_16820	99/99	JQN84_30180	*M. humidisoli* MMS20-R2-29^T
	7_16810	100/100	JQN84_30185	*M. humidisoli* MMS20-R2-29^T
t1pks-3	7_18400	99/100	JQN84_22230	*M. humidisoli* MMS20-R2-29^T
	7_18690	99/100	JQN84_22370	*M. humidisoli* MMS20-R2-29^T
t1pks-4	7_47680	99/99	JQN84_24840	*M. humidisoli* MMS20-R2-29^T
t1pks-5(*mrl*)	7_35900	99/99	JQN84_05260	*M. humidisoli* MMS20-R2-29^T
	7_35890	99/99	JQN84_05265	*M. humidisoli* MMS20-R2-29^T
	7_35880	99/99	JQN84_05270	*M. humidisoli* MMS20-R2-29^T
	7_35870	99/99	JQN84_05275	*M. humidisoli* MMS20-R2-29^T
	7_35750	99/99	JQN84_05335	*M. humidisoli* MMS20-R2-29^T
t1pks-6	7_29310	99/100	JQN84_14785	*M. humidisoli* MMS20-R2-29^T
t2pks-1	7_20160	99/100	JQN84_23105	*M. humidisoli* MMS20-R2-29^T
	7_20170	99/99	JQN84_23110	*M. humidisoli* MMS20-R2-29^T
	7_20190	99/100	J7462_05705	*Micromonospora* sp. RL09-050-HVF-A
t3pks-1 * (*aqq*)	7_59200	100/100	JQN84_06220	*M. humidisoli* MMS20-R2-29^T
nrps-1	7_47220	99/99	JQN84_30545	*M. humidisoli* MMS20-R2-29^T
	7_47230	99/99	JQN84_30550	*M. humidisoli* MMS20-R2-29^T
	7_47240	99/100	JQN84_30555	*M. humidisoli* MMS20-R2-29^T
nrps-2	7_47680	99/99	JQN84_14135	*M. humidisoli* MMS20-R2-29^T
nrps-3	7_56920	99/100	JQN84_29450	*M. humidisoli* MMS20-R2-29^T
	7_56930	99/99	JQN84_29445	*M. humidisoli* MMS20-R2-29^T
	7_56940	99/99	JQN84_29440	*M. humidisoli* MMS20-R2-29^T
	7_56970	99/99	JQN84_29425	*M. humidisoli* MMS20-R2-29^T
pks/nrps-1	7_15480	99/99	JQN84_27845	*M. humidisoli* MMS20-R2-29^T
pks/nrps-2 *	7_28040	99/99	JQN84_25460	*M. humidisoli* MMS20-R2-29^T
	7_28030	99/99	JQN84_25465	*M. humidisoli* MMS20-R2-29^T
	7_28010	99/99	JQN84_25475	*M. humidisoli* MMS20-R2-29^T
	7_28000	99/99	JQN84_25480	*M. humidisoli* MMS20-R2-29^T
	7_27970	98/98	JQN84_25495	*M. humidisoli* MMS20-R2-29^T
	7_27960	99/99	JQN84_25500	*M. humidisoli* MMS20-R2-29^T

Table 3. *Cont.*

Cluster	Locus Tag (TPA090)	BLAST Top Hit		
		I/S (%) [1]	Locus Tag or Gene (Accession No.)	Origin
pks/nrps-3	7_56660	99/99	JQN84_29575	*M. humidisoli* MMS20-R2-29^T
	7_56670	99/99	JQN84_29570	*M. humidisoli* MMS20-R2-29^T
	7_56710	100/100	JQN84_29550	*M. humidisoli* MMS20-R2-29^T
	7_56840	99/99	JQN84_29485	*M. humidisoli* MMS20-R2-29^T
t1pks-7	8_40860	59/69	C8E87_8689	*Actinoplanes brasiliensis* DSM 43805^T
	8_40870	56/67	M4V62_39485	*Streptomyces durmitorensis* MS405
	8_40880	54/66	SBI_01382	"*Streptomyces bingchenggensis*" BCW-1
t1pks-8 (*qmn*)	8_45370	98/98	C8054_25705	*Micromonospora* sp. RP3T
	8_45410	96/96	C8054_25725	*Micromonospora* sp. RP3T
	8_45420	95/96	C8054_25730	*Micromonospora* sp. RP3T
	8_45440	91/93	H1D33_RS20350	*M. ferruginea* 28ISP2-46
	8_45450	91/93	H1D33_20360	*M. ferruginea* 28ISP2-46
	8_45460	97/98	C8054_27580	*Micromonospora* sp. RP3T
	8_45470	98/98	C8054_27585	*Micromonospora* sp. RP3T
	8_45480	97/97	C8054_27590	*Micromonospora* sp. RP3T
	8_45490	94/95	H1D33_20380	*M. ferruginea* 28ISP2-46
	8_45500	97/98	C8054_11295	*Micromonospora* sp. RP3T
	8_45510	99/99	C8054_11300	*Micromonospora* sp. RP3T
	8_45520	96/96	C8054_11305	*Micromonospora* sp. RP3T
	8_45530	96/97	C8054_11310	*Micromonospora* sp. RP3T
t2pks-2	8_49930	98/99	C8054_23750	*Micromonospora* sp. RP3T
	8_49910	99/99	CO540_02355	*Micromonospora* sp. WMMA2032
	8_49900	99/99	C8054_23735	*Micromonospora* sp. RP3T
t3pks-1 *(*aqq*)	8_06420	99/98	C8054_27190	*Micromonospora* sp. RP3T
nrps-4	8_34180	55/66	ADL15_RS07780	"*Actinoplanes awajinensis* subsp. *mycoplanecinus*" NRRL B-16712
	8_34160	63/73	*bnvE* (QVQ62850)	*Streptomyces* sp. UTZ13
	8_34150	55/65	HUV60_15065	*Streptomyces* sp. KMM 9044
	8_34130	53/66	Raf01_61150	*Rugosimonospora africana* NBRC 104875^T
	8_34100	51/64	HUV60_15130	*Streptomyces* sp. KMM 9044
nrps-5	8_34870 [C]	42/58	KA716_28265	*Gloeotrichia echinulata* DEX184
	8_34920	44/57	HRW08_08145	*Streptomyces lunaelactis* MM15
	8_35060	55/67	SAMN05216553_119106	*Lentzea fradiae* CGMCC 4.3506^T
	8_35080	54/68	DMC61_21850	*Amycolatopsis* sp. WAC 04169
pks/nrps-2 *	8_42740	99/99	C8054_04550	*Micromonospora* sp. RP3T
	8_42750	97/97	C8054_04555	*Micromonospora* sp. RP3T
	8_42770	98/98	C8054_04565	*Micromonospora* sp. RP3T
	8_42780	99/99	C8054_04570	*Micromonospora* sp. RP3T
	8_42810	96/96	C8054_04585	*Micromonospora* sp. RP3T
	8_42820	99/99	C8054_04590	*Micromonospora* sp. RP3T
pks/nrps-4	8_08330	87/88	GA0070213_12115	*M. humi* DSM 45647^T
	8_08340	86/88	CO540_09565	*Micromonospora* sp. WMMA2032
	8_08370	87/90	CO540_09580	*Micromonospora* sp. WMMA2032

Table 3. *Cont.*

Cluster	Locus Tag (TPA090)	BLAST Top Hit		
		I/S (%) [1]	Locus Tag or Gene (Accession No.)	Origin
pks/nrps-5	8_34600	98/98	C8054_08855	*Micromonospora* sp. RP3T
	8_34620	98/98	C8054_08865	*Micromonospora* sp. RP3T
	8_34650	96/96	C8054_08880	*Micromonospora* sp. RP3T
	8_34660	95/96	C8054_08885	*Micromonospora* sp. RP3T
	8_34670	97/98	C8054_08890	*Micromonospora* sp. RP3T
	8_34690	97/97	C8054_08900	*Micromonospora* sp. RP3T
	8_34690	97/97	C8054_08905	*Micromonospora* sp. RP3T
	8_34730	99/98	C8054_08920	*Micromonospora* sp. RP3T
pks/nrps-6	8_35130	52/59	GCM10011578_091720	*Streptomyces fuscichromogenes* CGMCC 4.7110^T
	8_35200	59/69	MXD61_11230	*Frankia* sp. AgPm24
	8_35210	55/70	LX86_002128	*Lentzea aerocolonigenes* DSM 40034^T
	8_35230	64/73	SAMN05216215_102899	*Saccharopolyspora shandongensis* CGMCC 4.3530^T
	8_35250	56/68	SAMN05216215_102897	*S. shandongensis* CGMCC 4.3530^T
pks/nrps-7	8_54560	63/76	Psuf_070260	*Phytohabitans suffuscus* NBRC 105367^T
	8_54550	71/82	Psuf_070270	*P. suffuscus* NBRC 105367^T
	8_54470	57/69	FHG89_16340	*M. orduensis* S2509
	8_54430	89/94	DER29_6205	*Micromonospora* sp. M71_S20
	8_54260	94/95	C8054_02625	*Micromonospora* sp. RP3T
	8_54200	89/92	DLJ59_18505	*M. inaquosa* LB39^T
	8_54120	93/95	C8054_02645	*Micromonospora* sp. RP3T
	8_54020	98/98	C8054_02695	*Micromonospora* sp. RP3T
	8_54000	96/97	C8054_02705	*Micromonospora* sp. RP3T
	8_53990	98/98	C8054_02710	*Micromonospora* sp. RP3T
	8_53970	98/98	C8054_02715	*Micromonospora* sp. RP3T

[1] Similarity/identity in amino acid sequences. [C], encoded in the complementary strand; *, conserved between strains AKA109 and AKA38.

Among eleven gene clusters of *Micromonospora* sp. AKA38, seven (*t1pks-8*, *t2pks-2*, *t3pks-1*, *pks/nrps-2*, *pks/nrps-4*, *pks/nrps-5* and *pks/nrps-7*) were present in other strains with high sequence identity/similarity, although TPA0908_54560, TPA0908_54550 and TPA0908_54470 in *pks/nrps-7* were not observed, suggesting *pks/nrps-7* orthologs in other strains may be partial or not completely sequenced. Except for *pks/nrps-4*, the closest genes were present in *Micromonospora* sp. RP3T and their identity/similarity values were quite high. In contrast, four gene clusters, *t1pks-7*, *nrps-4*, *nrps-5* and *pks/nrps-6*, were not present in other strains because their BLAST top hits showed low identity/similarity values. This suggests that they are novel and specific to strain AKA38.

4. Discussion

Many strains found as producers of new bioactive substances have not been classified yet at species level. Consequently, relationships between products and taxonomic positions of the producer are not well understood. In this study, we classified *Micromonospora* sp. AKA109, a producer of akazaoxime and A-76356, to *M. humidisoli* [40]. In contrast, *Micromonospora* sp. AKA38, a producer of levantilide C, was revealed to be a novel genomospecies. If *Micromonospora* sp. AKA38 is characterized in detail [41], it can be proposed as a new *Micromonospora* species because it was not classified to known species. *M. humidisoli* is very recently proposed, and its type strain, MMS20-R2-29^T, was isolated from riverside soil. It is explained that its growth occurs in the presence of 0–2% NaCl, with optimal growth at 0% NaCl [40]. In contrast, strain AKA109 was isolated from deep sea water with a higher

salt concentration. To the best of our knowledge, this is the first report on marine-derived *M. humidisoli*.

Recently, genome mining has often been used when searching for new compounds. However, if researchers find an unknown BGC that appears novel by genome mining, it may be a BGC for known compounds, because many BGCs of known compounds have not been identified, and consequently, they are considered BGCs for new compounds. Thus, BGCs of known compounds need to be identified for more effective genome mining if the BGCs have not been unidentified. We here identified the BGC of akazaoxime and A-76356, and that of levantilide C from *Micromonospora* sp. AKA109 and *Micromonospora* sp. AKA38, respectively. This is the first report on the BGCs and biosynthetic pathways of these compounds.

Micromonospora sp. AKA109, classified to *M. humidisoli*, harbored fourteen PKS and NRPS gene clusters, all of which are also present in *M. humidisoli* MMS20-R2-29^{T}. This well supports our idea that members of the same species possess similar sets of PKS and NRPS gene clusters [42–44]. *Micromonospora* sp. AKA38, classified as a new genomospecies, harbored eleven PKS and NRPS gene clusters. Although seven of them were present in other strains, such as *Micromonospora* sp. RP3T and *Micromonospora* sp. WMMA2032, the remaining four are not found in any other strains. If a strain is taxonomically novel at the species level, it may possess new PKS and/or NRPS gene clusters.

Although PKS and NRPS gene clusters found from our two strains include BGCs of known compounds such as amycomicin, alkyl-*O*-dihydrogeranyl-methoxyhydroquinone and quinolidomicin, and congeners of known compounds, they include many orphan and unknown clusters. Their products were predicted to be novel at present. Thus, these two strains are expected to produce new and diverse polyketide and non-ribosomal peptide compounds.

Except for PKS and NRPS gene clusters, eleven putative secondary metabolite-biosynthetic gene clusters are present in each genome of *M. humidisoli* AKA109 and *Micromonospora* sp. AKA38 (Tables S1 and S2). The products, except for SapB, desferrioxamine, *N*-acetylglutaminylglutamine amide (NAGGN) and class II lanthipeptides of *Micromonospora* sp. AKA38, could not be predicted because there is less information on these types of gene clusters. SapB, desferrioxamine, NAGGN, three terpene, and one hybrid oligosaccharide/terpene gene cluster are conserved in the two strains. SapB, desferrioxamine and NAGGN are known as common secondary metabolites in actinomycetes. The numbers of gene clusters shown in Tables S1 and S2 did not exceed those of the PKS and NRPS gene clusters (Tables 1 and 2). This supports the assertion that polyketides and non-ribosomal peptides are major and diverse secondary metabolites, as previously reported [18].

5. Conclusions

We sequenced whole genomes of an akazaoxime- and A-76356-producer, *Micromonospora* sp. AKA109, and a levantilide C-producer, *Micromonospora* sp. AKA38. *Micromonospora* sp. AKA109 was identified as *M. humidisoli,* whereas *Micromonospora* sp. AKA38 was revealed to be a new genomospecies. Akazaoxime- and A-76356-BGC and levantilide C-one were identified from whole genome sequences of these two strains, respectively. *M. humidisoli* AKA109 harbored fourteen PKS and NRPS gene clusters, all of which were conserved in the type strain of *M. humidisoli*. *Micromonospora* sp. AKA38 harbored eleven PKS and NRPS gene clusters. Our bioinformatic analysis suggested their potential to synthesis diverse non-ribosomal peptides and polyketides.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life13020542/s1, Table S1: Secondary metabolite-biosynthetic gene clusters, except for PKS and NRPS gene clusters, of *M. humidisoli* AKA109; Table S2: Secondary metabolite-biosynthetic gene clusters, except for PKS and NRPS gene clusters, of *Micromonospora* sp. AKA38.

Author Contributions: Conceptualization, H.K. and Y.I., methodology, T.T., resources, Y.I., data curation, H.K., writing—original draft preparation, H.K., writing—review and editing, Y.I., project administration, T.T. and Y.I., 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 *Micromonospora* sp. AKA109 and *Micromonospora* sp. AKA38 have been deposited at GenBank under the accession numbers BNEH00000000 and BNEI00000000, respectively. BioProject accession numbers are PRJDB9818 and PRJDB9819. BioSample accession numbers are SAMD00228008 and SAMD00228009.

Acknowledgments: We thank Shinpei Ino and Takahiro Matsuyama for genome DNA preparation and Aya Uohara for registering whole genome sequences in DDBJ.

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

References

1. Berdy, J. Bioactive microbial metabolites. *J. Antibiot.* **2005**, *58*, 1–26. [CrossRef] [PubMed]
2. Harunari, E.; Imada, C.; Igarashi, Y.; Fukuda, T.; Terahara, T.; Kobayashi, T. Hyaluromycin, a new hyaluronidase inhibitor of polyketide origin from marine *Streptomyces* sp. *Mar. Drugs* **2014**, *12*, 491–507. [CrossRef] [PubMed]
3. Igarashi, Y.; Ikeda, M.; Miyanaga, S.; Kasai, H.; Shizuri, Y.; Matsuura, N. Two butenolides with PPARα agonistic activity from a marine-derived *Streptomyces*. *J. Antibiot.* **2015**, *68*, 345–347. [CrossRef] [PubMed]
4. Igarashi, Y.; Shimasaki, R.; Miyanaga, S.; Oku, N.; Onaka, H.; Sakurai, H.; Saiki, I.; Kitani, S.; Nihira, T.; Wimonsiravude, W.; et al. Rakicidin D, an inhibitor of tumor cell invasion from marine-derived *Streptomyces* sp. *J. Antibiot.* **2010**, *63*, 563–565. [CrossRef] [PubMed]
5. 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] [PubMed]
6. Karim, M.R.U.; In, Y.; Zhou, T.; Harunari, E.; Oku, N.; Igarashi, Y. Nyuzenamides A and B: Bicyclic peptides with antifungal and cytotoxic activity from a marine-derived *Streptomyces* sp. *Org. Lett.* **2021**, *23*, 2109–2113. [CrossRef] [PubMed]
7. Kim, Y.; Ogura, H.; Akasaka, K.; Oikawa, T.; Matsuura, N.; Imada, C.; Yasuda, H.; Igarashi, Y. Nocapyrones: Alpha- and gamma-pyrones from a marine-derived *Nocardiopsis* sp. *Mar. Drugs* **2014**, *12*, 4110–4125. [CrossRef]
8. Yang, T.; Yamada, K.; Zhou, T.; Harunari, E.; Igarashi, Y.; Terahara, T.; Kobayashi, T.; Imada, C. Akazamicin, a cytotoxic aromatic polyketide from marine-derived *Nonomuraea* sp. *J. Antibiot.* **2019**, *72*, 202–209. [CrossRef]
9. Zhang, Z.; Zhou, T.; Yang, T.; Fukaya, K.; Harunari, E.; Saito, S.; Yamada, K.; Imada, C.; Urabe, D.; Igarashi, Y. Nomimicins B-D, new tetronate-class polyketides from a marine-derived actinomycete of the genus *Actinomadura*. *Beilstein J. Org. Chem.* **2021**, *17*, 2194–2202. [CrossRef]
10. Qi, S.; Gui, M.; Li, H.; Yu, C.; Li, H.; Zeng, Z.; Sun, P. Secondary metabolites from marine *Micromonospora*: Chemistry and bioactivities. *Chem. Biodivers* **2020**, *17*, e2000024. [CrossRef]
11. Yan, S.; Zeng, M.; Wang, H.; Zhang, H. *Micromonospora*: A prolific source of bioactive secondary metabolites with therapeutic potential. *J. Med. Chem.* **2022**, *65*, 8735–8771. [CrossRef] [PubMed]
12. Igarashi, Y.; Matsuyuki, Y.; Yamada, M.; Fujihara, N.; Harunari, E.; Oku, N.; Karim, M.R.U.; Yang, T.; Yamada, K.; Imada, C.; et al. Structure determination, biosynthetic origin, and total synthesis of akazaoxime, an enteromycin-class metabolite from a marine-derived actinomycete of the genus *Micromonospora*. *J. Org. Chem.* **2021**, *86*, 6528–6537. [CrossRef]
13. Fei, P.; Chuan-Xi, W.; Yang, X.; Hong-Lei, J.; Lu-Jie, C.; Uribe, P.; Bull, A.T.; Goodfellow, M.; Hong, J.; Yun-Yang, L. A new 20-membered macrolide produced by a marine-derived *Micromonospora* strain. *Nat. Prod. Res.* **2013**, *27*, 1366–1371. [CrossRef]
14. 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]
15. Schwarzer, D.; Marahiel, M.A. Multimodular biocatalysts for natural product assembly. *Naturwissenschaften* **2001**, *88*, 93–101. [CrossRef] [PubMed]
16. Zhan, J. Biosynthesis of bacterial aromatic polyketides. *Curr. Top Med. Chem.* **2009**, *9*, 1598–1610. [CrossRef] [PubMed]
17. Katsuyama, Y.; Ohnishi, Y. Type III polyketide synthases in microorganisms. *Methods Enzym.* **2012**, *515*, 359–377.

18. 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]
19. Komaki, H.; Ichikawa, N.; Oguchi, A.; Hamada, M.; Harunari, E.; Kodani, S.; Fujita, N.; Igarashi, Y. Draft genome sequence of *Streptomyces* sp. TP-A0867, an alchivemycin producer. *Stand Genomic. Sci.* **2016**, *11*, 85. [CrossRef] [PubMed]
20. 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] [PubMed]
21. Komaki, H.; Igarashi, Y.; Tamura, T. Taxonomic positions of a nyuzenamide-producer and its closely related strains. *Microorganisms* **2022**, *10*, 349. [CrossRef]
22. Meier-Kolthoff, J.P.; Göker, M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat. Commun.* **2019**, *10*, 2182. [CrossRef]
23. Meier-Kolthoff, J.P.; Auch, A.F.; Klenk, H.P.; Göker, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinform.* **2013**, *14*, 60. [CrossRef]
24. Blin, K.; Shaw, S.; Steinke, K.; Villebro, R.; Ziemert, N.; Lee, S.Y.; Medema, M.H.; Weber, T. antiSMASH 5.0: Updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res.* **2019**, *47*, W81–W87. [CrossRef] [PubMed]
25. Hatano, K.; Nishii, T.; Kasai, H. Taxonomic re-evaluation of whorl-forming *Streptomyces* (formerly *Streptoverticillium*) species by using phenotypes, DNA-DNA hybridization and sequences of *gyrB*, and proposal of *Streptomyces luteireticuli* (*ex* Katoh and Arai 1957) corrig., sp. nov., nom. rev. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 1519–1529. [CrossRef] [PubMed]
26. Kasai, H.; Tamura, T.; Harayama, S. Intrageneric relationships among *Micromonospora* species deduced from gyrB-based phylogeny and DNA relatedness. *Int. J. Syst. Evol. Microbiol.* **2000**, *50*, 127–134. [CrossRef] [PubMed]
27. 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] [PubMed]
28. Stackebrandt, E.; Ebers, J. Taxonomic parameters revisited: Tarnished gold standards. *Microbiol. Today* **2006**, *33*, 152–155.
29. Wayne, L.G.; Brenner, D.J.; Colwell, R.R.; Grimont, P.A.D.; Kandler, O.; Krichevsky, M.I.; Moore, B.S.; Moore, W.E.; Murray, R.G.E.; Stackebrandt, E.; et al. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* **1987**, *37*, 463–464. [CrossRef]
30. Hong, S.; Ban, Y.H.; Byun, W.S.; Kim, D.; Jang, Y.; An, J.S.; Shin, B.; Lee, S.K.; Shin, J.; Yoon, Y.J.; et al. Camporidines A and B: Antimetastatic and anti-inflammatory polyketide alkaloids from a gut bacterium of *Camponotus kiusiuensis*. *J. Nat. Prod.* **2019**, *82*, 903–910. [CrossRef] [PubMed]
31. Ohno, S.; Katsuyama, Y.; Tajima, Y.; Izumikawa, M.; Takagi, M.; Fujie, M.; Satoh, N.; Shin-ya, K.; Ohnishi, Y. Identification and characterization of the streptazone E biosynthetic gene cluster in *Streptomyces* sp. MSC090213JE08. *ChemBioChem* **2015**, *16*, 2385–2391. [CrossRef]
32. Ye, S.; Molloy, B.; Brana, A.F.; Zabala, D.; Olano, C.; Cortes, J.; Moris, F.; Salas, J.A.; Mendez, C. Identification by genome mining of a type I polyketide gene gluster from *Streptomyces argillaceus* involved in the biosynthesis of pyridine and piperidine alkaloids argimycins P. *Front. Microbiol.* **2017**, *8*, 194. [CrossRef]
33. Liang, M.; Liu, L.; Xu, F.; Zeng, X.; Wang, R.; Yang, J.; Wang, W.; Karthik, L.; Liu, J.; Yang, Z.; et al. Activating cryptic biosynthetic gene cluster through a CRISPR-Cas12a-mediated direct cloning approach. *Nucleic Acids Res.* **2022**, *50*, 3581–3592. [CrossRef] [PubMed]
34. Awakawa, T.; Fujita, N.; Hayakawa, M.; Ohnishi, Y.; Horinouchi, S. Characterization of the biosynthesis gene cluster for alkyl-*O*-dihydrogeranyl-methoxyhydroquinones in *Actinoplanes missouriensis*. *ChemBioChem* **2011**, *12*, 439–448. [CrossRef] [PubMed]
35. He, H.Y.; Ryan, K.S. Glycine-derived nitronates bifurcate to *O*-methylation or denitrification in bacteria. *Nat. Chem.* **2021**, *13*, 599–606. [CrossRef]
36. Cortina, N.S.; Revermann, O.; Krug, D.; Muller, R. Identification and characterization of the althiomycin biosynthetic gene cluster in *Myxococcus xanthus* DK897. *ChemBioChem* **2011**, *12*, 1411–1416. [CrossRef] [PubMed]
37. Hashimoto, T.; Hashimoto, J.; Kozone, I.; Amagai, K.; Kawahara, T.; Takahashi, S.; Ikeda, H.; Shin-ya, K. Biosynthesis of quinolidomicin, the largest known macrolide of terrestrial origin: Identification and heterologous expression of a biosynthetic gene cluster over 200 kb. *Org. Lett.* **2018**, *20*, 7996–7999. [CrossRef] [PubMed]
38. Komaki, H.; Ichikawa, N.; Hosoyama, A.; Hamada, M.; Igarashi, Y. In silico analysis of PKS and NRPS gene clusters in arisostatin- and kosinostatin-producers and description of *Micromonospora okii* sp. nov. *Antibiotics* **2021**, *10*, 1447. [CrossRef]
39. Horsman, G.P.; Van Lanen, S.G.; Shen, B. Iterative type I polyketide synthases for enediyne core biosynthesis. *Methods Enzymol.* **2009**, *459*, 97–112.
40. Lee, D.H.; Ra, J.S.; Kim, M.J.; Kim, S.B. *Micromonospora antibiotica* sp. nov. and *Micromonospora humidisoli* sp. nov., two new actinobacterial species exhibiting antimicrobial potential. *Int. J. Syst. Evol. Microbiol.* **2022**, *72*, 005522. [CrossRef]
41. 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]
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]

43. Komaki, H.; Tamura, T. Reclassification of *Streptomyces diastaticus* subsp. *ardesiacus*, *Streptomyces gougerotii* and *Streptomyces rutgersensis*. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 4291–4297. [CrossRef]
44. Komaki, H.; Tamura, T. 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. *Appl. Microbiol.* **2021**, *1*, 573–585. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.