Cloning and identification of the lobophorin biosynthetic gene cluster from marine *Streptomyces olivaceus* strain FXJ7.023

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Abstract: A full length about 105 kb gene cluster containing 35 open reading frames involved in the biosynthesis of lobophorins was cloned and sequenced from a fosmid genomic library of *Streptomyces olivaceus* strain FXJ7.023. The cluster was identified by genome wide annotation and analysis of secondary metabolite biosynthesis gene clusters by anti SMASH and knockout of loading module-contained region of polyketide skeleton synthesis gene (the starter of *lobS1*). Gene cluster comparative analysis suggested that the cluster encoded the complete genes for lobophorin polyketide assembly, modification, substrate catalysis, regulation, transportation and resistance, and shows great identity to the newest reported lobophorin biosynthetic gene cluster from *Streptomyces* sp. SCSIO 01127, but with a significant gene rearrangement in the PKS modules.

Keywords: Gene cluster; lobophorin; marine streptomycete; spirotetronate antibiotic

INTRODUCTION

Lobophorins are a group of spirotetronate antibiotics with a broad spectrum of bioactivities such as against pathogens, tumours and inflammation, and have a high chemical structure similarity with kijanimicin and tetrocarcins (Hirayama *et al*., 1980; Waitz *et al*., 1981; Demydchuk *et al*., 2008; Igarashi *et al*., 2011) and a lower similarity with chlorothricin (Schindler, 1975; Jia *et al*., 2006). There are three structurally distinct moieties in the former three chemicals including a polycyclic tetronolide core, a monosaccharide referred as D-tetronitrose (also named D-kijanose) and an oligosaccharide composed of alternating L-digitoxose and L-amicetose residues. Intriguingly, bioactivities of members of the spirotetronate antibiotics are directly proportional to the deoxysugars decorating their aglycone rings (Jia *et al*., 2006). Lobophotins have an aglycone same to KIJ, but show differences mainly in the functionalities of the glycosyl substitution at the C-9 (in the dashed ellipse) and C-17 positions (in the dashed box)(fig. 1).

Nowadays, seven members of the lobophorin family, lobophorins A-G, have been isolated from sea-derived actinomycetes such as strain CNB-837 (Jiang *et al*., 1999), strain AZS17 (Wei *et al*., 2011), *Streptomyces* sp. SCSIO 01127 (Niu *et al*., 2011) and *Streptomyces* sp. MS100061 (Chen *et al*., 2013). Structural analysis has revealed that each member of the lobophorin family has a little difference in specific bond forms and stereochemistry on their side chain glycosyl ofC-17 (Niu *et al*., 2011; Wei *et al*., 2011). Recent studies have demonstrated the lobophorin family members possesses different bioactivities, e.g., lobophorins A and B are potent inhibitors of topical PMA-induced edema (in our research, lobophorin A with the bioactivity against *Escherichia coli* while lobophorin B with the bioactivity against *Staphylococcus aureus*, respectively.),

lobophorins C and D display significant inhibitory effect on cancer cells, while lobophorin F displays both antibacterial activities and cytotoxic activities against tumor cell lines (Niu *et al*., 2011), and lobophorin G exhibited potent activities against BCG and B. subtilis and modera te activity against M. tuberculosis (Chen *et al*., 2013).

The medical importance of lobophorin analogues has fostered intensive research into their biogenesis, physiology and as well as characterization of the biosynthetic gene cluster for genetic manipulation. In this study, a full length of 105kb gene cluster that are responsible for biosynthesis of lobophorins A and B in a marine isolate *Streptomyces olivaceus* FXJ7.023 was cloned an identified. Gene cluster comparative analysis showed great identity on the levels of gene content and amino acid sequence to the newest reported lobophorin gene cluster from *Streptomyces* sp. SCSIO 01127 (Li *et al*., 2013), but with a gene rearrangement in the PKS modules. Both the two lobophorins gene clusters are all display a high similarity to kijanimicin and tetrocarcin A gene clusters on the levels of amino acid sequence, gene content and gene order and will provide insights into spirotetronate biosynthesis and demonstrate the possibility of applying combinatorial biosynthesis methods to the spirotetronate antibitic biosynthetic machinery to generate novel natural products with structural and bioactive diversity.

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MATERIALS AND METHODS

Strains, plasmids, and reagents

Streptomyces olivaceus strain FXJ7.023 was isolated from a sediment sample collected from the South China Sea (E115°06.314, N 19°53.468) at a depth of 1182 m during an open voyage in Sept. 2005 and was deposited in the China General Microbiological Culture Collection Center under the accession number CGMCC 4.7054.

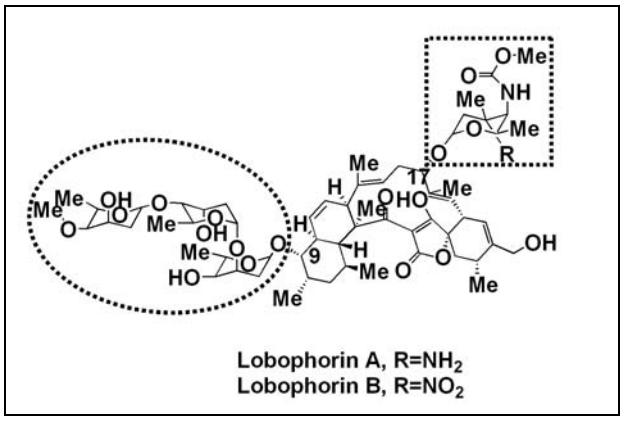
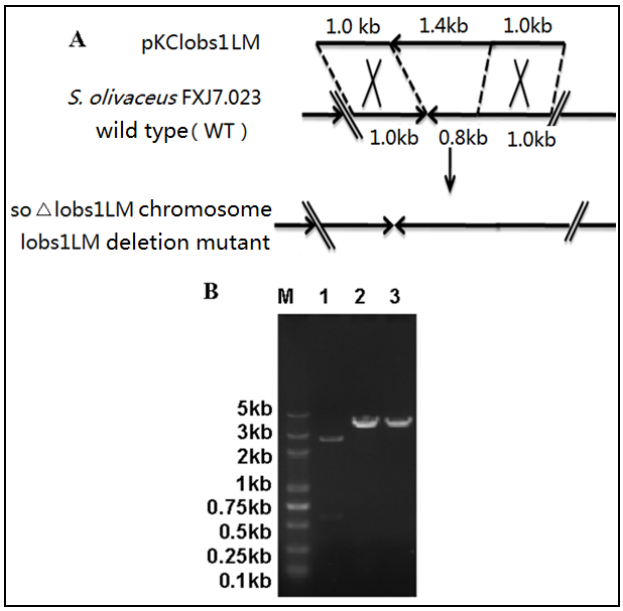


Fig. 1: Structures of lobophorins A and B.

The genomic DNA used for genome sequencing and genomic library construction was extracted directly from the expanded culture of ISP 2 liquid medium (Shirling and Gottlieb, 1966). The bacterial strain EPI300-T1^{R} and plasmid pCC2FOS contained in Copy Control™ HTP Fosmid Library Production Kit (Cat. No. CCFOS059) for construction of the genomic library were from Epicentre (Madison, USA), chemicals were from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cloning plasmids pMD18-T and pSIMPLE-18 *Eco*R V/BAP for construction of the sub-clone library and all following materials were purchased from Takara Biotechnology Company (Dalian, China): restriction enzymes, T4 DNA ligase, DNA end blunting kination ligation kit and PCR kit. Primers used for gene cloning and DNA sequencing were synthesized by Beijing Invitrogen Biotechnology Co., Ltd. DNA Gel Extraction Kit was from Axygen Biosciences, Inc (Hangzhou, China).

DNA isolation, manipulation and sequencing

Genomic DNA was isolated form *Streptomyces olivaceus* FXJ7.023 using the standard method described by Hopwood (Hopwood, 1995; Gao and Huang, 2009). Molecular manipulation was carried out according to standard methods described by Sambrook and Russel (Sambrook and Russel, 2001). Sequencing of plasmids and fosmids was performed on the 3730xl DNA analyzer at the Beijing Invitrogen Biotechnology Co., Ltd., using a shotgun cloning strategy. The pCC2/pEpiFOS forward and reverse primers (sequences in Copy Control protocol) were used to sequence the ends of each fosmid while the M13 primers were used to sequence the ends of sub clones. *S. olivaceus* FXJ7.023 draft genome sequencing was performed on the Illumina HiSeq2000 at the BGI Biotechnology Co., Ltd., using a proprietary reversible terminator-based strategy.



A: Mutation strategies of *lobS1* loading module. B: Analysis of *lobS1* loading module deletion by PCR (lane 1, wild type stain; lane 2 and lane 3: *lobS1* loading module deleted mutants; M: DNA marker).

Fig. 2: Delete mutation of *lobS1* loading module.

Genomic library and sub clone library construction and screening

In order to get the full length sequence of predicted lobophorin biosynthetic gene cluster, a genomic library of *S. olivaceus* FXJ7.023 was constructed in Copy Control pCC2FOS (Kim *et al*., 1995) according to the manufacturer's instructions, which contained more than 6000 clones with approximate 40 kb inserted genomic DNA fragments. The genomic library was screened by colony PCR with primer pairs that were designed based on the nucleic acid sequences of LOBKS1 (has 73% identity on the level of amino acid sequence) according to the predictive analysis of draft genome sequencing results, and end sequences of positive clone was sequenced in order to confirm that the clone was located in lobophorin cluster. The next round screening was performed with primer pairs that were designed based on the end sequences of the last round of screening results, until six positive clones overlap one by one were obtained. One μg fosmid DNA was fragmented by an ultrasonic cell disruptor under the condition of 200 watts for 1 second on ice. DNA fragments in a size range from 4 to 8 kb were recovered by AxyPrep DNA gel extraction kit. After end blunted and 5'-phosphorylated, the fragments were cloned into pSIMPLE-18 *Eco*R V/BAP vector to construct sub clone libraries. PCR screening was performed by sequence-specific primers which were

designed based on the end sequences of each gap, and the positive clones were then sequenced with M13-47F/M13-48R primers and the specific primers.

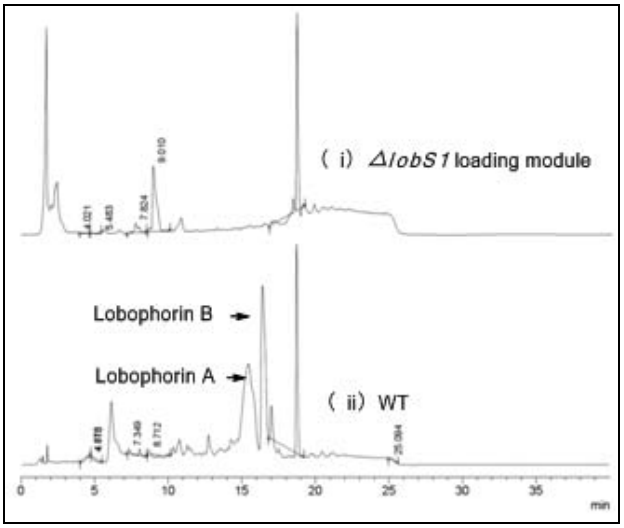


Fig. 3: HPLC metabolite profiling of (i) *lobS1* loading module knockout mutation and (ii) wild type *S. olivaceus* FXJ7.023.

Lobophorin biosynthetic gene cluster analysis

A 151 kb contiguous DNA segment was obtained by sub cloned library sequencing and was deposited in Gen Bank under the accession number JX306680. The sequence was uploaded to the anti SMASH website (http://antiSMASH.secondarymetabolites.org) (Medema *et al*., 2011) to determine the gene content and gene order. Open reading frames (ORF) proposed for biosynthetic pathway of lobophorin skeleton, post modification and involved in forming the spirotetronate (which is a characteristic structural motif of all spirotetronate members) were deduced based on functional gene analysis by sequence homology comparison using BlastP with its homologous clusters found by antiSMASH. Comparative analysis and global rearrangement structure of the two-lobophorin biosynthetic gene clusters were conducted using mauve software (Darling *et al*. 2010).

Inactivation of the lobophorin biosynthetic gene cluster

A 2394 bp DNA fragment (from 20931 nt to 23325 nt of JX306680) encoding the loading module of LOBS1 within the 6073 bp DNA fragment (from 19201nt to 25274nt of JX306680, including the upstream sequence and partial coding region of LOBS1) was replaced by an artificial sequence containing TCP830 promoter followed by full-length kanamycin resistance protein-coding region (no. AAF85969.1), which was constructed by PCR and inserted in to pKC1139 plasmid and then transformed into *E. coli* ET12567. The constructs for gene inactivation were then introduced into *S. olivaceus* FXJ 7.023 by conjugal transfer following the procedure of Hopwood (Hopwood, 1995). The disruption was confirmed by PCR and then by HPLC for the abolishment of lobophorins production.

Fermentation, isolation and chemical identification of lobophorins A and B

Strain FXJ7.023 was cultured using 140×500-ml shake flasks each containing 100 ml of ISP 2 agar medium, which were incubated for 10 d at 28°C. After cultivation, the solid culture was fully mashed and extracted three times with 14 L ethanol. The organic portion was then concentrated *in vacuo* to remove the solvent. The crude extract was applied to silica gel column chromatography using gradient of $CHCl_3$/MeOH to obtain the crude products. Further purification was applied using Sephadex LH-20 (MeOH) column and RP-HPLC (Shimadzu SPD-M20A with Xbridge ODS 10×150-mm column). Compound identification was carried out by HRESI-MS (Waters Xevo G2 QTOF mass spectrometer) and NMR (Bruker AV 600 MHz) analyses.

RESULTS

Chemical identification of lobophorins A and B

Chromatography and preparative high-pressure liquid chromatography (HPLC) yielded compounds 1 (49.1 mg) and 2 (62.3 mg) from strain FXJ7.023. The two compounds were obtained as pale yellow amorphous solid, and showed UV absorbance and HRESI-MS spectra as follows: Compound 1: $[\alpha]_D^{25}$=-155°(c0.2, MeOH); UV λ_{max} (MeOH:H_2O 70:30) 240, 266, 280(sh) nm; HEESI-MS m/z 1157.6390 $[M+H]^+$ (calcd. for 1157.6372); Compound 2: $[\alpha]_D^{25}$=-127°(c0.2, MeOH); UV λ_{max} (MeOH:H_2O 70:30) 240, 266, 280(sh) nm; HRESI-MS m/z 1204.6396 $[M+NH_4]^+$ (calcd. for 1204.6380). The ^1H and ^{13}C NMR data of compound 1 matched those of lobophorin A (Jiang *et al*. 1999). According to these chemical data and from a biogenetic perspective, compound 1 was identified as lobophorin A and compound 2 as lobophorin B (Jiang *et al*. 1999).

Cloning of lobophorin gene cluster

During bioinformatic analysis of the *S. olivaceus* FXJ7.023 draft genome sequence using antiSMASH, seven scaffolds with high similarities to the gene clusters of kijanimicin and tetrocarcin A were detected, suggesting a spirotetronate antibiotic biosynthesis gene cluster lying in the genome. Six positive fosmid clones embodying the seven scaffolds with six gaps were obtained. Four gaps were contained in two of the six positive clones and the other two gaps existed in the other four clones. The latter two gaps were filled by PCR while the former four gaps lied in the two fosmids were sewed up by subclone sequencing. Forty five positive subclones were obtained and sequenced. The results of sequence re-assembly and re-annotation showed that a 151 kb contiguous DNA sequence containing a 105 kb spirotetronate antibiotic biosynthesis gene cluster was obtained (GenBank: JX306680).

Table 1: ORFs in the *lob, kij* and *tca* biosynthetic gene clusters and their deduced functions

Gene[a]	Size[e]	Gene[b]	Identity[f]	Gene[c]	Identity[f]	Gene[d]	Identity[f]	Deduced function
						Tca T1		Glycosyltransferase Tca T1
				KijD6	69	TcaC		Flavoprotein oxidoreductase
?		lobR5						LysR regulatory protein
?		lobR4						Putative regulatory protein
?		lobT2						Forkhead-associated protein
lobR1	824	lobR3	99	KijD1	75	TcaR1	70	TetR type regulatory protein
lobD8	608	lobS11	99	KijD1	86	TcaB5	81	Sugar 5-epimerase
lobD7	1013	lobS10	99	KijD1	85	TcaB4	78	Sugar 3-ketoreductase/ oxidoreductase
						TcaM		O-acyltransferase
lobD6	917	lobS9	98	KijD5	80	TcaB1	80	Sugar nucleotidyltransferase
lobD5	1034	lobS8	99	KijD4	83	TcaB2	81	Sugar 4,6-dehydratase
lobD4	1352	lobS7	99	KijD3	77	TcaB10	71	FAD-dependent oxidoreductase
lobD3	1121	lobS6	98	KijD2	86	TcaB8	82	Sugar 3-aminotransferase
lobD2	1244	lobS5	99	KijD1	82	TcaB9	78	Sugar 3-C-methyl transferase
lobD1	455	lobU2	97			TcaU4	81	Unknown
lobS1	19091	lobA5	97	KijS1	73	TcaA1	63	Type I polyketide synthase
lobS2	22349	lobA4	95	KijS2	74	TcaA2	66	Type I polyketide synthase
lobS3	5447	lobA3	94	KijS3	72	TcaA4	51	Type I polyketide synthase
lobS5	4772	lobA2	95	KijS5	68	TcaA5	63	Type I polyketide synthase
lobA	1478	lobP3	98	KijA	76	TcaE1	70	FAD-dependent oxidoreductase
lobB	1058	lobC4	98	KijB	84	TcaD4	75	3-oxoacyl- (acyl carrier protein)
lobC	1865	lobC3	98	KijC	78	TcaD1	70	Acetyl-transferase FkbH like protein
lobC0	227	lobC2	100	KijD	75	TcaD2	63	Acyl carrier protein
lobE	1682	locC1	98	KijE	82	TcaD3	76	2-oxoacid dehydrogenase
lobR2	569	lobR2	99	KijC5	84	TcaR2	73	TetR family transcriptional regulator
lobC4	1202	lobG3	99	KijC4	72	TcaT3	75	Glycosyltransferase, MGT family
lobC3	1250	lobG2	99	KijC3	80	TcaT4	73	Glycosyltransferase, MGT family
				KijC2				Sugar 4-ketoreductase
				KijC1				Glycosyltransferase, MGT family
lobB3	1520	lobP2	99	KijB3	79	TcaE2	69	FAD linked oxidase domain protein
lobB2	761	lobB	99	KijB2	76	TcaF	74	Thioesterase
lobB1	1361	lobS4	98	KijB1	79	TcaB3	68	Sugar 2,3-dehydratase
lobS4	12296	lobA1	93	KijS4	78	TcaA3	72	Type I polyketide synthase
lobA0	1175	lobG1	99	KijD9	76	TcaT2	75	Glycosyltransferase, MGT family
lobA9	878	lobS3	99	KijD8	75	TcaB11	74	SAM-dependent methyltransferase
lobA8	1154	lobS2	98	KijD7	83	TcaB7	78	Sugar 4-aminotransferase
lobA7	818	lobS1	100	KijA1	79			Sugar-O-methyltransferase
lobA6	980	lobU1	100	KijA2	84			Aryl-alcohol dehydrogenase
lobA5	1178	lobP1	99	KijA3	70	TcaB6		P450 oxycytochrome P450
				KijA4				Glycosyltransferase, MGT family
lobA4	1493	lobT1	99	KijA5	72	TcaG	63	Efflux permease
				KijA6				
				KijA7				
lobR3	614	lobR1	96	KijA8	75			TetR family transcriptional regulator
lobA3	782	orf(-1)	97					FkbM family methyltransferase
lobA2	1196	orf(-2)	98					Macrolide glycosyltransferase, MGT family
lobR4	2546							Protein kinase/ transcriptional regulator

[a]lobophorin gene cluster from *S. olivaceus* FXJ7.023 (GenBank: JX306680); [b] lobophorin gene cluster from *S.* sp. SCSIO 01127 (GenBank: KC013978); [c] kijanimicin gene cluster (GenBank: EU301739); [d] Tetrocarcin A gene cluster (GenBank: EU443633); [e] bp, in nucleotide sequence; [f] %, amino acids compared with sequences from *S. olivaceus* FXJ7.023.

Metabolite profiling analysis of lobS1 loading module knockout mutation

Results of PCR products sequencing of the *lobS1* loading module from knockout mutant strain (*lobS1*) suggesting that the 800bp DNA fragment was replaced by kanamycin resistance gene (fig. 2B). Fermentation, isolation and chemical preparation of wild type strain *S. olivaceus* FXJ7.023 and it *lobS1* loading module deleted mutant strain (*lobS1*) were performed under the same conditions, followed by HPLC detection.

Fermentation, isolation and chemical preparation of wild type strain *S. olivaceus* FXJ7.023 and *lobS1* loading module knockout mutant strain (*lobS1*) were performed under the same conditions, followed by HPLC detection. The result of metabolite profiling analysis showed that mutant did not produce lobophorins anymore, while lobophorins A and B were still detected in the wild type strain, confirming that *lobS1* involved in the biosynthesis of lobophorins (fig. 3).

DISCUSSION

Gene clusters online blast results by anti SMASH showed that the gene clusters of lobophorin, kijanimicin and tetrocadin A shared a high similarity in gene composition, order and orientation of the open reading frames (ORFs), except for a few genes. Thirty-six ORFs were identified within the 105 kb region of lobophorin (*lob*) gene cluster from *S. olivaceus* FXJ7.023. These genes and their proposed functions are listed in table 1. The 36 genes are involved in substrates catalysis, polyketide assembly, post-modification, pathway regulation, transportation and resistance of lobophorins, among which the five type I polyketide synthase genes, four glycosyltransferase genes, four TetR family transcriptional regulator genes, one efflux permease gene, two aminotransferase genes, two methyl transferase genes and seventeen genes involved in deoxysugar biosynthesis and modification all have high identities to the *lob* gene cluster from *Streptomyces* sp. SCSIO 01127 (from 93 to 100%) and relatively high identities to the gene clusters *kij* (from 68 to 86%) and *tca* (from 63 to 82%), implying similar polyketide assembly, glycosyltransfer reactions and tailoring steps, which insure *S. olivaceus* FXJ7.023 to produce their analogue. On the basis of sequence analysis, five modular polyketide synthase (PKS) genes, *lobSs1 to lobS5*, covering about 64 kb DNA fragment in the lobophorin cluster from *S. olivaceus* FXJ7.023 were predicted. These genes encode a PKS complex with 12 modules comprised of a loading module (module L) and 11 extender modules (modules 1 to 11) and show great identity to the newest reported lobophorin gene cluster from *S.* sp. SCSIO 01127, but with a significant gene rearrangement in the PKS modules of *lobS2* (corresponding to *lobA4* of the gene cluster from *S.* sp. SCSIO 01127). Just like the PKS gene organization in *kij* and *tca*, both *lobS4* from *S. olivaceus* FXJ7.023 and *lobA1* from *S.* sp. SCSIO 01127 are separated away from the other four PKS genes by 14.7 kb. The order and domain organization of *lobS1* to *lobS5* (named *lobA1* to *lobA5* in the homologous gene cluster from *S.* sp. SCSIO 01127) suggests that *lobS1* encodes the loading module followed by three extender modules (modules 1 to 3), *lobS2* encodes the following four modules (modules 4 to 7), *lobS3* encodes module 8, *lobS4* encodes modules 9 and 10, and *lobS5* encodes module 11, just like their homologous genes from *S.* sp. SCSIO 01127. Acyltransferase (AT) domain analysis based on sequence comparison indicates that *lob* AT domains have the same substrate specificity with *kij* AT domains, i.e. modules 1, 3, 5 and 8-10 are specific for methylmalonyl-CoA, modules L, 2, 4, 6, 7 and 11 are specific for malonyl-CoA. The two *lob*, *kij* and *tca* gene clusters have the same substrate specificity at the AT domains of modules 1, 3, 5 and 8-10 (for methylmalonyl-CoA) and modules 4, 6, 7 and 11 (for malonyl-CoA), and this is consistent with the overall structures of lobophorins, kijanimicin and tetrocarcins and supports the idea that the PKS system of lobophorins may have the same ancestor with those of kijanimicin and tetrocacin. Lobophorins contain four deoxysugars, including three L-digitoxose and one D-kijanose, which have also been found in the KIJ and TCA structures, forming the sugar side chains at the C-9 and C-17 positions, respectively. Deoxysugars are regarded as important structural components of natural products which frequently have essential effects for biological activities (Oh *et al*. 2007), and D-kijanose is thought to be one of the most highly functionalized sugars found in nature (Bruender *et al*. 2010a; Bruender *et al*. 2010b; Bruender and Holden 2012). Unfortunately, the biosynthetic pathway of D-kijanose is still unclear because it is likely to involve unusual chemistry. TDP-2, 6-dideoxy-3, 4-diketo-D-glucose is the common precursor substrate for L-digitoxose and D-kijanose biosynthesis which is catalyzed from glucose-1-phosphate by *lobD6*, *lobD5* and *lobB1* (*lobS9*, *lobS8* and *lobS4* in *S.* sp. SCSIO 01127) in turn. At least three genes including *lobD7*, *lobD8* and some unknown genes are proposed to be responsible for the biosynthesis of L-digitoxose from TDP-2, 6-dideoxy-3, 4-diketo-D-glucose, and at least six genes, *lobD3*, *lobD2*, *lobD4*, *lobA8*, *lobA9* and *lobB3* for D-kijanose in *S. olivaceus* FXJ7.023.

Four genes, *lobA0, lobA2, lobC3* and *lobC4* in the *lob* gene cluster from *S. olivaceus* FXJ7.023 were identified as encoding the glycosyltransferases (GTs) (table 1). Sequence comparison of these GTs reveals that LobA0 (LobG1 in *S.* sp. SCSIO 01127) is significantly different from the other GTs in the gene cluster, and shows high similarities to KijD7 (76%) and TcaT2 (75%) in amino acids, indicating that it catalyzes attachment of D-kijanose or its precursor to the C-17 hydroxyl group of lobophorin skeletal structure. In the two lobophorin gene clusters, the remaining GTs are similar to each other (50-51% pairwise

identity) as well as to the TcaT3, TcaT4, KijC4 and KijC3, strongly suggesting that they are digitoxosyltransferases responsible for construction the C-9 oligosaccharide to LOB skeletal structure. Unfortunately there is no predicted gene showing functional similarity to *kijC2*, which involves in ketoreduction, this may attribute to complement effect of isoenzyme genes in the genome.

Similar to the structural genes and tailoring gene, three genes in the *lob* gene cluster from *S. olivaceus* FXJ7.023, the three putative TetR transcriptional regulator genes *lobR1, lobR2*, and *lobR3* all show high similarity to their equivalents from *lob* (in *S.* sp. SCSIO 01127), *kij* and *tca* gene clusters, while *lobR4* belongs to LuxR family and has no counterparts in the other three gene clusters. These genes are likely involved in lobophorins biosynthesis pathway-specific regulation in response to environmental or cellular changes. The existence of more regulators in the two *lob* gene clusters suggest that *lob* might be under stricter regulation. The secondary protein structure of LobA4 (LobT1 in *S.* sp. SCSIO 01127), predicted by predict protein online (Rost *et al*., 2004), contains 13 strong transmembrane helices, suggesting that LobA4 is a transmembrane protein. PSI-BLAST analysis (Altschul, 1999) of LobA4 reveals high similarity to efflux permeases KijA5 and Tca G (table 1) as well as numerous antibiotic efflux transporters. It is therefore expected that LobA4 serves as the efflux pump for lobophorins as part of a self resistance mechanism.

CONCLUSION

In this study, we cloned a full length of 108 kb gene cluster with 35 open reading frames (ORFs) which encode the complete biosynthesis, modification, transportation and resistance genes of lobophorins A and B from a marine isolate Streptomyces sp. FXJ7.023, by subclone sequencing and genome walking PCR. Results of gene cluster comparative analysis suggested a high similarity to kijanimicin and tetrocarcin A gene clusters on the levels of amino acid sequence, gene content and gene order.

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REFERENCES

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.,* **25**(17): 3389-402.

Bruender NA and Holden HM (2012). Probing the catalytic mechanism of a C-3'-methyltransferase involved in the biosynthesis of D-tetronitrose. *Protein Sci.,* **21**: 876-886.

Bruender NA, Thoden JB and Holden HM (2010a). X-ray Structure of KijD3, a Key Enzyme Involved in the Biosynthesis of D-Kijanose. *Biochemistry-Us,* **49**: 3517-3524.

Bruender NA, Thoden JB, Kaur M, Avey MK and Holden HM (2010b). Molecular architecture of a C-3 '-Methyltransferase involved in the biosynthesis of D-tetronitrose. *Biochemistry-Us,* **49**: 5891-5898.

Chen CX, Wang J, Guo H, Hou WY, Yang N, Ren B, Liu M, Dai HQ, Liu X, Song FH and Zhang LX (2013). Three antimycobacterial metabolites identified from a marine-derived Streptomyces sp. MS100061. *Appl. Microbiol. Biotechnol.,* **97**(9): 3885-3892.

Darling AE, Mau B and Perna NT (2010). Progressive Mauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One.* **5**(6): e11147.

Demydchuk Y, Sun YH, Hong H, Staunton J, Spencer JB and Leadlay PF (2008). Analysis of the tetronomycin gene cluster: Insights into the biosynthesis of a polyether tetronate antibiotic. *Chembiochem.,* **9**:1136-1145.

Gao P and Huang Y (2009). Detection, Distribution and organohalogen compound discovery implications of the reduced flavin adenine dinucleotide-dependent halogenase gene in major filamentous actinomycete taxonomic groups. *Appl. Environ. Microb.,* **75**: 4813-4820.

Hirayama N, Kasai M, Shirahata K, Ohashi Y and Sasada Y (1980). The structure of tetronolide, the aglycone of anti-tumor antibiotic tetrocarcin. *Tetrahedron Lett.,* **21**: 2559-2560.

Hopwood DA (1995). Genetic manipulation of streptomyces polyketide synthase genes for novel secondary metabolite production. *Fems. Microbiol. Rev.,* **16**: 233-234.

Igarashi Y, Ogura H, Furihata K, Oku N, Indananda C and Thamchaipenet A (2011). Maklamicin, an antibacterial polyketide from an endophytic Micromonospora sp. *J. Nat. Prod.,* **74**: 670-674.

Jia XY, Tian ZH, Shao L, Qu XD, Zhao QF, Tang J, Tang GL and Liu W (2006) Genetic characterization of the chlorothricin gene cluster as a model for spirotetronate antibiotic biosynthesis. *Chem. Biol.,* **13**: 575-585.

Jiang ZD, Jensen PR and Fenical W (1999). Lobophorins A and B, new anti-inflammatory macro lides produced by a tropical marine bacterium. *Bioorg. Med. Chem. Lett.,* **9**: 2003-2006.

Kim UJ, Shizuya H, Sainz J, Garnes J, Pulst SM, deJong P and Simon MI (1995). Construction and utility of a human chromosome 22-specific Fosmid library. *Genet Anal-Biomol. E.,* **12**: 81-84.

Li S, Xiao J, Zhu Y, Zhang G, Yang C, Zhang H, Ma L and Zhang C (2013) Dissecting glycosylation steps in lobophorin biosynthesis implies an iterative glycosyltransferase. *Org Lett.,* **15**(6): 1374-1377.

Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E and Breitling R (2011). anti SMASH: Rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids. Res.,* **39**: W339-W346.

Niu SW, Li SM, Chen YC, Tian XP, Zhang HB, Zhang GT, Zhang WM, Yang XH, Zhang S, Ju JH and Zhang CS (2011). Lobophorins E and F, new spirotetronate antibiotics from a South China Sea-derived Streptomyces sp. SCSIO 01127. *J. Antibiot.,* **64**: 711-716.

Oh TJ, Mo SJ, Yoon YJ and Sohng JK (2007). Discovery and molecular engineering of sugar-containing natural product Biosynthetic pathways in actinomycetes. *J. Microbiol. Biotechn.,* **17**: 1909-1921.

Rost B, Yachdav G and Liu J (2004). The predict protein server. *Nucleic Acids. Res.,* **32**: W321-326.

Schindler PW. (1975) Chlorothricin, an inhibitor of porcine-heart malate dehydrogenases, discriminating between mitochondrial and cytoplasmic isoenzyme. *Eur. J. Biochem.,* **51**: 579-585.

Sambrook J and Russell DW (2001). Molecular cloning: A laboratory manual. Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press.

Shirling EB and Gottlieb D (1966). Methods for characterization of Streptomyces species. *Int. J. Syst. Bacteriol.,* **16**: 313-340.

Waitz JA, Horan AC, Kalyanpur M, Lee BK, Loebenberg D, Marquez JA, Miller G and Patel MG (1981). Kijanimicin (Sch-25663), a Novel Antibiotic Produced by Actinomadura-Kijaniata Scc-1256 - Fermentation, Isolation, Characterization and Biological properties. *J. Antibiot.,* **34**: 1101-1106.

Wei RB, Xi T, Li J, Wang P, Li FC, Lin YC and Qin S (2011). Lobophorin C and D, New Kijanimicin Derivatives from a Marine Sponge-Associated Actinomycetal Strain AZS17. *Mar. Drugs,* **9**: 359-368.