Identification of polyketide synthase gene clusters in a phage P1-derived artificial chromosome library of a Philippine strain of *Streptomyces* sp. PCS3-D2

Aileen Bayot Custodio^a and Edwin Plata Alcantara^{a*}

^aNational Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Laguna, Philippines 4031

Received 5th January 2019 / Accepted 11th March 2019

Abstract. A phage P1-derived artificial chromosome (PAC) library was constructed from genomic DNA of *Streptomyces* sp. PCS3-D2. Polymerase chain reaction (PCR) screening of the PAC library revealed two clones, PAC16D and P222O, which were positively identified to harbor polyketide synthase (PKS) Type I and PKS Type III gene clusters, respectively. Restriction enzyme digestion showed that PAC16D and PAC222O contained a 130 kb and a 140 kb insert, respectively. Results of sequencing and bioinformatics analyses revealed that PAC16D comprised of a full-length PKS type I bafilomycin gene cluster while PAC222O harbored truncated siderophore and putative gene clusters as well as a complete PKS III biosynthetic gene cluster. The PKS III gene cluster had three genes similar to alkyl resorcinol biosynthetic genes, however majority of the novel gene cluster had little similarity to known PKS Type III gene clusters. The successful cloning and identification of these gene clusters from *Streptomyces* sp. PCS3-D2 serve as the jump off point to further genetic manipulation in order to produce the insecticidal natural product in a heterologous host.

Keywords: polyketide synthase gene clusters, *Streptomyces* sp. PCS3-D2, phage P1-derived artificial chromosome (PAC) library, natural products

INTRODUCTION

Actinobacteria are ubiquitous Gram-positive, filamentous bacteria that could be easily isolated from various soil and marine environments. They are well known prolific producers of bioactive compounds including natural insecticides (Kirst, 2010). The use of natural products as insecticides for crop protection is encouraging. Recent reports indicate that the use of natural products and natural product-derived insecticides continue to increase (Dayan *et al.*, 2009). In 2012, natural products and natural product-inspired products comprised approximately 30% of the global agrochemical sales, while an additional 22% of synthetic agrochemical compounds could have

been inspired by natural products under different circumstances (Gerwick and Sparks, 2014).

The strain *Streptomyces* sp. PCS3-D2 produces insecticidal compounds active against important agronomic insect pests (Bayot-Custodio *et al.*, 2014). The development of potentially beneficial natural products from this strain is hindered by the lack of proper genetic characterization which when ignored could lead to large missed opportunity for developing effective solutions for mitigating problems in insect resistance to insecticides. Recent statistics showed that at least 30 cases of insecticide resistance have been reported for the Philippines (www.pesticide

* Author for correspondence: Edwin Plata Alcantara, National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Laguna, Philippines 4031. Email - epalcantara@up.edu.ph

resistance.org).

The bottom-up approach for natural product discovery utilizes genome sequencing and bioinformatics to identify gene clusters predicted to synthesize important bioactive compounds. Upon identifying a target gene cluster, genetic manipulation techniques are employed to activate the gene clusters either in native or heterologous hosts for the production of the corresponding natural product (Luo *et al.*, 2014). Given that the size of some gene clusters can exceed 100 kb, artificial chromosome-based vectors have been constructed for the maintenance and genetic manipulation of natural producers in *Streptomyces* (Alduina and Gallo, 2012).

Phage P1-derived artificial chromosomes (PACs) can carry large 100–300 kb DNA fragments in *E. coli* cells (Shizuya *et al.*, 1992). However, because *E. coli* is not suitable to heterologously express high GC% actinomycete genes, shuttle vectors have been designed to allow library construction and manipulation in *E. coli*, while the expression of actinomycete genes are done in *Streptomyces* host strains. An example of this is the pESAC13 vector, which contains the phage P1 origin of replication, phiC31 integrase gene and a phiC31 attP site, an oriT site that allows transfer into *Streptomyces* by conjugation (Sosio *et al.*, 2000) and a *Bam*HI cloning site for the insertion of large DNA fragments (Jones *et al.*, 2013).

In this paper, we first report the construction of a phage P1-derived artificial chromosome library and the *in silico* characterization of two cloned polyketide gene clusters from a Philippine strain of *Streptomyces* sp. PCS3-D2 using a bioinformatics approach.

MATERIALS AND METHODS

Genome mining of *Streptomyces* sp. PCS3-D2 for biosynthetic gene clusters. The previously annotated genome sequence of *Streptomyces* sp. PCS3-D2 (Bayot-Custodio *et al.*, 2014) was re-analyzed using the antiSMASH 4.0 software (Blin *et al.*, 2017) to determine both the presence and location of biosynthetic gene clusters in the JDUZ01 genome sequence.

Cell culture for construction of phage P1 artificial chromosome (PAC) library. For preparing mycelia for PAC library construction, *Streptomyces* sp. PCS3-D2 was grown in YMB (100 mL) that was inoculated with mycelia from a 10 mL TSB starter culture. The mycelia were grown for 72 hours with shaking at ambient temperature and centrifuged (4°C, 12000 rpm, 15 min) to remove the supernatant. The mycelial pellets (approximately 7 mL of concentrated mycelia) were flash frozen in liquid nitrogen and shipped in dry ice to BioS&T (Montreal, Canada) for custom construction of a phage P1 artificial chromosome (PAC) library using a modified *E. coli-Streptomyces* Artificial Chromosome (ESAC) vector (Fig. 1). The constructed *Streptomyces* sp. PCS3-D2 PAC library generated a total of 2,688 clones with an average insert size of 125 kb.

Figure 1. Vector map of PAC pESAC13.

Screening of PAC Library for PKS I and PKS III gene clusters. PCR primers (Table 1) were designed to screen the *Streptomyces* sp. PCS3-D2 PAC library for clones containing PKS I and PKS III gene clusters. These PCR primers were initially tested using *Streptomyces* sp. PCS3-D2 genomic DNA as template for PCR. PKS I-specific primers were expected to amplify a 700 bp amplicon corresponding to the 5-aminolevulinate synthase gene of the bafilomycin gene cluster. On the other hand, a 450 bp PCR amplicon was expected to be amplified using PKS III-specific primers corresponding to the stilbene synthase

gene. Each PCR amplicon was later sequenced to verify that the desired region of the gene clusters was amplified.

The *Streptomyces* sp. PCS3-D2 PAC library was PCR screened by BioS&T (Montreal, Canada) using the designed PKS I and PKS III-specific primers. The positively identified clones were

subjected to restriction enzyme digestion using *NotI* to estimate the size of the cloned inserts. Positively identified clones were subjected to next generation sequencing to determine if they contained the full-length PKS I and PKS III gene cluster.

Table 1. Bacterial strains, vectors and PCR primers used in the study.

Items	Description	Reference
<i>Streptomyces</i> sp. PCS3-D2	Wildtype strain producing insecticidal compounds	This work
<i>Escherichia coli</i> DH10B	Cloning strain; F- mcrA Δ (mrr-hsdRMS-mcrBC) ϕ 80lacZ Δ M15 Δ lacX74 recA1 endA1 araD139 Δ (ara, leu)7697 galU galK λ - rpsL nupG	Invitrogen
pESAC13 Vector	PAC vector (P1-phage replicon) for genomic library construction; RP conjugative, Φ C31 integrative tsr, neo, oriT, attP and int from Φ C31, P1 rep, sacB	Sosio <i>et al.</i> , 2000 Genbank No. LM999999.1
BafBIII-F	TCCGAGATCAGCGTGTGGTGC	This work
BafBIII -R	GCSGTGGTGAAGATGAAGGRGC	This work
STS-F	TTCCCGCCCCACCGCCATTCACG	This work
STS-R	ACACRGCTCGGTGGACAGCAGCAG	This work

Sequencing, assembly, annotation and analysis of PAC clones.

To ensure that the positive PAC clones contained the entire PKS I and PKS III gene clusters, they were subjected to Ion Torrent single ended sequencing with >200 coverage depth using the Ion PGM™ System by BioS&T (Montreal, Canada). The resulting raw sequence reads were quality checked using FastQC (Andrews, 2018). The sequences were processed by trimming low quality ends using the Trim Ends algorithm of the Geneious R11 software (Kearse *et al.*, 2012). The sequence reads were also screened against the UniVec database to trim off contaminating vector and adapter sequences at the 5' and 3' ends. The filtered and trimmed sequences were subjected to reference guided mapping and assembly using the Geneious R11 software. The Bowtie2 version 2.3.0. plugin (Langmead and Salzberg, 2012) was used in reference guided assembly to align the sequencing reads to the reference sequence. The reference sequence used was scaffold 1 (scf1) of *Streptomyces* sp. PCS3-D2 JDUZ01 genome sequence. The consensus sequence from reference guided mapping was annotated using the Prokka Galaxy Tool Version: 1.12.0 (Seemann, 2014). The annotated gene clusters were then analyzed using antiSMASH 4.0 for the identification, annotation and analysis of secondary metabolite biosynthesis gene clusters (BGC).

RESULTS

Genome mining of *Streptomyces* sp. PCS3-D2 for biosynthetic gene clusters. The antiSMASH 4.0 software enables bacterial genome mining for biosynthetic gene clusters that code for chemical classes of secondary metabolites (Blin *et al.*, 2017). This is done by comparing each gene product in the uploaded DNA sequence against a manually curated collection of profile hidden Markov models (pHMMs) which describe key biosynthetic enzymes of the secondary metabolite classes. The key enzymes encoded in each gene cluster are assigned to secondary metabolite-specific clusters of orthologous groups (smCOGs). Depending on the class of secondary metabolite gene cluster detected (i.e. multimodular polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs), etc.), further detailed analyses are performed (Blin *et al.*, 2013). KnownClusterBlast is also integrated in antiSMASH which allows the identification of gene clusters against a repository of known and experimentally characterized secondary metabolite biosynthesis gene clusters called MIBiG (Minimum Information on Biosynthetic Gene Cluster) for comparative gene cluster analysis (Blin *et al.*, 2017).

The antiSMASH 4.0 analysis revealed that JDUZ01 genome sequence has a total of 26 BGCs which includes polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS), NRPS hybrids, terpenes, siderophores, bacteriocin, butyrolactone, lantipeptides, ectoine, nucleoside and other BCGs as summarized in Table 2. The % similarity from antiSMASH results was based on how similar the query sequences were with known and characterized gene clusters in the

MIBiG repository. Among the PKS gene clusters, two were identified as type I PKS: cluster 14 which has high similarity (100%) to the bafilomycin gene cluster and cluster 3 that has low homology (11%) to PKS-Cf saccharide herboxidiene BGC. Cluster 19, which is probably involved in the synthesis of spore pigments, was classified as a type II PKS, while cluster 13 has strong similarity (100%) to type III PKS alkyl resorcinol gene cluster.

Table 2. List of predicted secondary metabolite biosynthetic gene clusters in *Streptomyces* sp. PCS3-D2 based on antiSMASH 4.0 analysis.

Cluster	Type	Predicted Products	% Similarity
1	Bacteriocin	Unknown	-
2	Terpene	Unknown	-
3	Type I PKS-Cf saccharide	Herboxidiene	11%
4	Terpene-NRPS	Hopene	61%
5	NRPS	Streptolydigin	10%
6	NRPS	Bleomycin	6%
7	NRPS	Streptothricin	87%
8	Ectoine	Ectoine	100%
9	Thiopeptide-Terpene-NRPS	Coelichelin	100%
10	Terpene	2-methylisoborneol	100%
11	Melanin-Terpene	Istamycin	4%
12	Siderophore	Kedarcidin	1%
13	Type III PKS	Alkylresorcinol	100%
14	Type I PKS	Bafilomycin	100%
15	Butyrolactone	Neocarzinostatin	6%
16	Lantipeptide	Galbonolides	6%
17	Lantipeptide	Venezuelin	100%
18	NRPS	Tambromycin	100%
19	Type II PKS	Spore pigment	66%
20	NRPS	Unknown	-
21	Terpene	Carotenoid	63%
22	Siderophore	Desferrioxamine B	100%
23	Nucleoside	Unknown	-
24	Siderophore	Unknown	-
25	NRPS	Unknown	-
26	NRPS	Enduracidin	6%

PCR screening of *Streptomyces* sp. PCS3-D2 PAC library. The PKS I primers amplified a 700 bp fragment of the 5-aminolevulinate synthase gene of the bafilomycin gene cluster (BafBIII) while PKS III primers amplified a 450 bp portion of the stilbene synthase gene (STS) which represents PKS III gene clusters. PCR-based screening of the PAC library identified a clone in plate 1-6D (designated as PAC16D) to harbor the

PKS I gene cluster and another in plate 2-22O (designated as PAC222O) to contain the PKS III gene cluster. Figure 2a illustrates the PCR screening of PAC16D amplifying a 700 bp PKS I gene product while PAC222O produced the expected 450 bp PKS III band. A 130 kb and 140 kb digest were observed from the *NotI*-treated PAC16D and PAC222O, respectively (Figure 2b).

Figure 2. PCR screening of the *Streptomyces* sp. PCS3-D2 PAC library for (A) PKS I (PAC16D) and PKS III (PAC222O) gene clusters and (B) PAC clone insert verification by *NotI* restriction enzyme digestion. Lane 1: 130 kb PAC16D insert; Lane 2: 140 kb PAC222O insert; M: molecular weight markers.

PAC clone sequencing and bioinformatics analysis. Genome sequence analysis of JDUZ01 scf1 showed that it contained 14 gene clusters as illustrated in Figure 3. PKS III and PKS I BGCs were determined to be located near the 3' end of JDUZ01 scf 1. The PKS III gene cluster was estimated to be located at 1,457,140 to 1,492,845 bp, having a total size of approximately 35.7 kb. On the other hand, the PKS I gene cluster was located at 1,621,560 bp to 1,713,412 bp with a total length of about 91.8 kb.

Next Generation Sequencing generated 246,357 and 181,775 raw read sequences for PAC16D and PAC222O, respectively. Based on the assembly results, PAC222O had an insert length of about 132 kb which spanned a portion of the neighboring siderophore gene cluster, full length PKS III gene cluster and a putative gene cluster downstream of PKS III (Figure 3, green line). This shows that PAC222O contained the full length PKS III gene cluster.

On the other hand, PAC16D had a 130 kb insert which included a portion of a putative gene cluster upstream of the PKS I gene cluster as well as the full length PKS I bafilomycin gene cluster (Figure 3, red line). This proves that PAC16D also contains the entire PKS I gene cluster.

Bioinformatics analysis using the antiSMASH 4.0 software revealed that PAC16D had high similarity with the PKS I bafilomycin gene cluster. PAC16D contained the modular polyketide synthases which were responsible for the synthesis of bafilomycin's polyketide backbone, bafilomycin regulatory genes, and other genes responsible for the production and conversion of bafilomycin derivatives (Figure 4a). PAC16D was also compared with known bafilomycin gene clusters from *Streptomyces griseus* DSM 2608, *S. lohii* JCM 14114 and *Kitasatospora setae* KM-6054. Pairwise alignment revealed that PAC16D had 83.9% similarity with *S. lohii* JCM 14114, 81.6% with *S. griseus* DSM 2608 and 77.2% with *K. setae* KM-6054 bafilomycin gene clusters. This proves that PAC16D contains the full length PKS I bafilomycin gene cluster. The best way to confirm that PAC16D contains a bafilomycin gene cluster that is capable of producing bafilomycin is to transform the PAC16D clone into a *Streptomyces* heterologous host and check for the production of bafilomycin by HPLC or LC-MS.

PAC222O, on the other hand contained three gene clusters: a truncated siderophore gene cluster (red box), the PKS III gene cluster (tan box) and a putative gene cluster (navy blue box) (Figure 4b). The truncated siderophore gene cluster was about 12 kb in length and included nine genes (Figure 4c). Downstream from the siderophore gene cluster was the full-length PKS III gene cluster (Figure 4d). A neighboring putative gene cluster that overlapped with the 3' end of the PKS III gene cluster was also observed in PAC222O. The PKS III gene cluster has three genes that has high similarity (100%) with *S. griseus* subsp. *griseus* NBRC 13350 alkyl resorcinol genes (monooxygenase, methyltransferase and stilbene synthase genes) (red box). This proves that PAC222O contains a PKS III alkyl resorcinol gene cluster. However, the genes surrounding the PAC222O PKS III gene cluster itself were not similar with other known alkyl resorcinol gene clusters and could therefore be a novel producer of resorcinol derivatives.

Figure 3. Mapping of biosynthetic gene clusters in JDUZ01 scaffold 1 of *Streptomyces* sp. PCS3-D2. The green line represents the location of the PAC222O insert while the red line represents the insert of PAC16D. (Cf-saccharide: Putative saccharide cluster; NRPS: Nonribosomal peptide synthetase; PKS I: Polyketide synthase Type I; PKS III: Polyketide synthase Type III).

Figure 4. antiSMASH prediction of biosynthetic gene clusters of *Streptomyces* sp. PCS3-D2 PAC clones (A) PAC16D and (B) PAC222O; (C) truncated siderophore gene cluster in PAC222O; and (D) segment of PAC222O showing the PKS III gene cluster and an overlapping putative gene cluster. The red box represents genes with high similarity to alkyl resorcinol biosynthetic genes.

DISCUSSION

In this study, genome mining and construction of a PAC library was carried out in order to gain insight into possible ways of exploiting the diversity of BGCs present in *Streptomyces* sp. PCS3-D2. The whole genome sequence of *Streptomyces*

sp. PCS3-D2 served as a blueprint to identify which gene clusters should be prioritized for screening in the PAC library. Indeed, more than twenty-five BGCs were discovered some of which might be responsible for production of novel insecticidal secondary metabolites in *Streptomyces* sp. PCS3-D2. The discrepancy in the present number of predicted BGCs as compared to our

previous results (Bayot-Custodio *et al.*, 2014) might be due to modifications made in the antiSMASH 4.0 software (Blin *et al.*, 2017) used in this study.

The PKS I and PKS III BGCs were screened out from the PAC library because from the list of predicted BGCs (Table 2), we infer that there was a high possibility that these two types of PKS might be responsible for production of insecticidal compound in *Streptomyces* sp. PCS3-D2. Several variants of bafilomycin have been reported to have insecticidal activity (Kretschmer *et al.*, 1985). To the best of our knowledge, insecticidal alkyl resorcinol has not been reported from any *Streptomyces* strains although insecticidal counterpart had been reported from plant origin (Kwon *et al.*, 1996). Other previously discovered insecticides from Actinomycetes are also produced by PKS gene clusters (Waldron *et al.*, 2000; Ikeda *et al.*, 1999; Sun *et al.*, 2003). The PKS type II gene cluster in *Streptomyces* sp. PCS3-D2 was identified to be involved in spore pigment synthesis and was therefore not included in the PCR screening.

Closer analysis of the PKS III BGC showed that only three of the PKS III genes (monooxygenase, methyltransferase and stilbene synthase) had high similarity to the alkyl resorcinol biosynthesis genes. The rest of the PKS III gene cluster did not show similarity to any known PKS Type III gene cluster, making it a potentially novel PKS Type III gene cluster.

Screening of the *Streptomyces* sp. PCS3-D2 PAC library resulted to the identification of desired clones with insert size up to 140 kb. Cloning of large DNA fragments of Actinomycetes BGCs with sizes up to 150 kb has been reported previously (Alduina *et al.*, 2005; Dai *et al.*, 2011). Because the complete PKS I and PKS III gene clusters were present in PAC16D and PAC222O, they are good candidates for heterologous expression to increase yield of insecticidal compound.

CONCLUSION

Genome mining facilitated the identification of 26 biosynthetic gene clusters including type I and type III polyketide synthases (PKSs) which

corresponds to a bafilomycin and alkyl resorcinol gene cluster, respectively. These two PKS gene clusters were successfully screened out from a constructed PAC library of *Streptomyces* sp. PCS3-D2. Heterologous expression will allow confirmation of insecticidal activity being conferred to *Streptomyces* sp. PCS3-D2 by either or both these two PKS gene clusters.

ACKNOWLEDGEMENT

The authors thank the Department of Agriculture—Philippine Agriculture and Fisheries Biotechnology Program (DA-BIOTECH) for funding this research. We also thank Bea Crisostomo, Felaine Anne Sumang and Rachelle Leah Melendrez for their assistance with this project. The *Streptomyces* sp. PCS3-D2 isolate was kindly provided by Teofila Zulaybar (Antibiotics Laboratory, BIOTECH, University of the Philippines Los Baños).

REFERENCES

- Alduina R., Giardina A., Gallo G., Renzone G., Ferraro C., Contino A., Scaloni A., Donadio S., & Puglia A. 2005. Expression in *Streptomyces lividans* of *Nonomuraea* genes cloned in an artificial chromosome. *Applied Microbiology and Biotechnology* 68 (5): 656–662.
- Alduina R. & Gallo G. 2012. Artificial chromosomes to explore and exploit biosynthetic capabilities of Actinomycetes. *Journal of Biomedicine and Biotechnology*.
- Andrews, S. 2018. FastQC: A quality control tool for high throughput sequence data <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>. Accessed February 10 2018.
- Bayot-Custodio A. N., Alcantara E. P., & Zulaybar T. O. 2014. Draft genome sequence of insecticidal *Streptomyces* sp. strain PCS3-D2, isolated from mangrove soil in the Philippines. *Genome Announcement* 2(3): e00448-14.
- Blin, K., Medema, M. H., Kazempour D., Fischbach, M. A., Breitling, R., Takano E., & Weber T. 2013. antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. *Nucleic Acids Research* 41 (W1): W204–W212.
- Blin, K., Wolf, T., Chevrette, M. G., Lu, X., Schwalen, C. J., Kautsar, S. A., Suarez Duran, H. G., De Los Santos, E. L. C., Kim, H. U., Nave, M., Dickschat, J. S., Mitchell, D. A., Shelest, E., Breitling, R., Takano, E., Lee, S. Y., Weber, T., & Medema, M. H. 2017. AntiSMASH 4.0- improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Research* 45(W1): W36–W41.

- Dai S., Ouyang Y., Wang G., & Li X. 2011. *Streptomyces autolyticus* JX-47 Large-insert bacterial artificial chromosome library construction and identification of clones covering geldanamycin biosynthesis gene cluster. *Current Microbiology* 63(1): 68–74.
- Dayan F., Cantrell C., & Duke S. 2009. Natural products in crop protection. *Bioorganic & Medicinal Chemistry* 17(12): 4022–4034.
- Gerwick B. C. & Sparks T. C. 2014. Natural products for pest control: an analysis of their role, value and future. *Pest Management Science* 70: 1169–1185.
- Ikeda H., Nonomiya T., Usami M., Ohta T., & Ōmura S. 1999. Organization of the biosynthetic gene cluster for the polyketide anthelmintic macrolide avermectin in *Streptomyces avermitilis*. *Proceedings of the National Academy of Sciences USA*. 96(17): 9509–95.
- Jones A. C., Gust B., Kulik A., Heide L., Buttner M. J., & Bibb M. J. 2013. Phage P1-derived artificial chromosomes facilitate heterologous expression of the FK506 gene cluster. *PLoS ONE* 8(7): e69319.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1647–1649.
- Kirst H. 2010. The spinosyn family of insecticides: realizing the potential of natural products research. *Journal of Antibiotics* 63: 101–111.
- Kretschmer, A., Dorgerloh, M., Deeg, M., & Hagenmaier, H. 1985. The structures of novel insecticidal macrolides: bafilomycins D and E, and oxohygrolicidin. *Agricultural and Biological Chemistry* 49(8): 2509–2511.
- Kwon M., Ahn Y. J., Yoo J. K., & Choi B. R. 1996. Potent insecticidal activity of extracts from Ginkgo biloba leaves against *Nilaparvata lugens* (Homoptera: Delphacidae). *Applied Entomology and Zoology* 31: 162–166.
- Langmead B. & Salzberg S. 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9: 357–359.
- Luo Y., Cobb R. E., & Zhao H. 2014. Recent advances in natural product discovery. *Current Opinion in Biotechnology* 30: 230–237.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30(14): 2068–2069.
- Shen, B. 2003. Polyketide biosynthesis beyond the type I, II and III polyketide synthase paradigms. *Current Opinion in Chemical Biology* 7: 285–295.
- Shizuya H., Birren B., Kim U. J., Mancino V., Slepak T., Tachiiri Y., & Simon M. 1992. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *Proceedings of the National Academy of Sciences USA* 89(18): 8794–8797.
- Sosio M., Giusino F., Cappellano C., Bossi E., Puglia A. M., & Donadio S. 2000. Artificial chromosomes for antibiotic-producing actinomycetes. *Nature Biotechnology* 18(3): 343–345, 2000.
- Sun Y., Zhou X., Bao K., Zhang G. *et al.* 2002. *Streptomyces nanchangensis*, a producer of the insecticidal polyether-antibiotic nanchangmycin and the antiparasitic macrolide meilingmycin, contains multiple polyketide gene clusters. *Microbiology* 36: 1–71.
- Waldron, C., Madduri, K., Crawford, K. *et al.* 2000. A cluster of genes for the biosynthesis of spinosyns, novel macrolide insect control agents produced by *Saccharopolyspora spinosa*. *Antonie Van Leeuwenhoek* 78: 385–390.