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Secondary metabolite genome annotation for a new *Nonomuraea* sp.

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Abstract

Secondary metabolites produced by bacteria have potential to be developed as therapeutic compounds. Actinobacteria have long been known as the among precious sources of secondary metabolites. Responsible genes of the biosynthetic pathway for a particular secondary metabolite are often clustered. Such gene clusters were detected using antiSMASH web server in a rare Actinobacteria genus, a recently isolated strain of *Nonomuraea*, to predict the metabolic potential of bacteria for further analysis in the lab. The results demonstrated the capability of the bacteria in producing several different secondary metabolites such as terpenes, polyketide synthases and, Non-ribosomal Peptide Synthetases.

Keywords: Actinobacteria, *Nonomuraea*, Secondary metabolites, Genome mining, Functional annotation, Gene clusters

Introduction

Secondary metabolites produced by microorganisms include pigments, pharmaceutics, growth hormones, and other products which have been demonstrated to be useful in human health and industry (Singh et al., 2019). Actinobacteria are one of the most important bacteria used in the industrial production of various secondary metabolites that retain them among leading candidates in the drug discovery pipelines (O'Brien and Wright, 2011). Studies on the bacterial genomes have demonstrated that they have much more potential for producing secondary metabolites than what is perceived in laboratories (Schorn et al., 2016). *Nonomuraea* is a rare actinobacterial genus containing 72 species and 2 subspecies till now that is recognized as a promising resource for novel bioactive compounds (Sungthong and Nakaew, 2015). One of the most used tools for secondary metabolite gene clusters annotation is antiSMASH, which is an analysis shell for antibiotics and other secondary metabolites to identify secondary metabolites Biosynthetic Gene Clusters (BGCs) in three groups of bacteria, fungi, and plants (Blin et al., 2019). Finding such novel clusters in a newly identified strain will open new opportunities for the production of its valuable products with multidisciplinary usages. Most of the gene clusters for secondary metabolites of cultured Streptomyces are silent in laboratory growth conditions. Secondary metabolite analysis tools provides an overview of the existing secondary metabolite gene clusters in the bacteria rather than experimental analysing and can economizes the study by reducing laborious screening costs and time. Here, we used AntiSMASH to find out the possible capabilities of the strain *Nonomuraea* sp. UTMC 2524 in producing valuable new bioactive compounds with potential pharmaceutical applications.

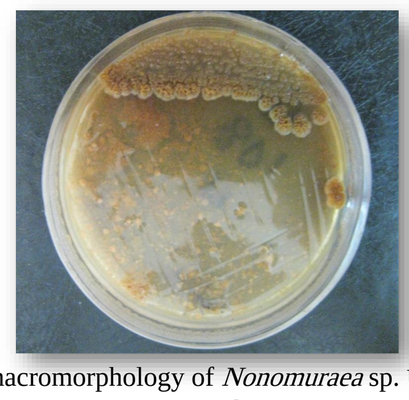

Figure 1. Mature macromorphology of *Nonomuraea* sp. UTMC 2524 On ISP2 solid medium

Materials and Methods

- Partial sequencing of 16S rDNA was performed for the identification of the isolated bacteria.
- Whole genome sequencing of the strain was obtained by PacBio sequencing service.
- To predict the potential of the bacteria in the biosynthesis of secondary metabolites, the whole genome sequence of the bacteria was analyzed using antiSMASH bacterial version 5.1.0 in FASTA format with default parameters including KnownClusterBlast, ActiveSiteFinder, and SubClusterBlast.
- The output was obtained and analyzed.

Results

The bioactive strain was identified as a member of *Nonomuraea* using partial sequencing of 16S rDNA. The strain *Nonomuraea* sp. UTMC 2524 contained 28 gene regions containing 37 major BGCs putative secondary metabolites. Among the identified BGCs, clusters including one Lanthipeptide, one terpene, one type III polyketide synthase, one non-ribosomal peptide synthase, one ectoine, and one Lasso peptide all had 100% similarity with their closest known compounds in MiBIG database (Table 1). However, the rest of the 31 clusters produced 8 types of different compounds with 2-92% similarity to the known secondary metabolites. This strain has the potential to produce a diverse group of secondary metabolites including nine terpenes, four Nonribosomal Peptide Synthetases (NRPS), four siderophore cluster, four t1pks (Type 1 polyketide synthase), three t2pks (Type 2 polyketide synthase), two butyrolactone cluster, two NAPAA (non-alpha poly-amino acids like e-polylysin, two RiPP-like (unspecified ribosomally synthesized and post-translationally modified peptide product) cluster, two melanin cluster, one lantipeptide class III, one t3pks (Type 3 polyketide synthase), one ectoine cluster, one linaridin cluster, one lasso peptide cluster (Table 2).

Table 1. AntiSMASH output showing the secondary metabolite gene clusters in *Nonomuraea* sp. UTMC 2524 with 100% similarity with known clusters in MiBIG database.

Region	Type	From	To	Most similar known cluster		Similarity
Region 2	lanthipeptide-class-iii , RiPP-like	1,271,146	1,297,152	informatipeptin	RiPP:Lanthipeptide	100%
Region 6	terpene	2,613,222	2,634,381	geosmin	Terpene	100%
Region 8	T3PKS	2,781,505	2,822,719	alkylresorcinol	Polyketide	100%
Region 14	ectoine	8,068,272	8,078,676	ectoine	Other	100%
Region 21	NRPS , melanin	9,798,392	9,863,215	scabichelin	NRP	100%
Region 24	lassopeptide	10,025,099	10,047,708	citrulassin D	RiPP	100%

Table 2. secondary metabolite gene clusters in *Nonomuraea* sp. UTMC 2524 predicted using AntiSMASH.

The product type	Description	Frequency
terpene	**Terpene**	9
Siderophore	**Siderophore cluster**	4
NRPS	**Non-ribosomal peptide synthetase cluster**	4
T1PKS	**Type 1 polyketide synthase**	4
T2PKS	**Type 2 polyketide synthase**	3
butyrolactone	**Butyrolactone cluster**	2
RiPP-like	**Other unspecified ribosomally synthesized and post-translationally modified peptide product (RiPP) cluster**	2
NAPAA	**non-alpha poly-amino acids like e-polylysin**	2
melanin	**Melanin cluster**	2
T3PKS	**Type 3 polyketide synthase**	1
ectoine	**Ectoine cluster**	1
linaridin	**Linaridin cluster**	1
lassopeptide	**Lasso peptide cluster**	1
lantipeptide class III	**Class III lanthipeptide clusters like labyrinthopeptin (FN178622)**	1

Discussion, Conclusion and Suggestions

By analysing the existing gene clusters in the studied strain, we can conclude that the number of 37 clusters and their diversity, rank this strain at the level of *Streptomyces* strains which are well known for their potent secondary metabolite synthetic machine. Approximately 6.4% of total DNA in Streptomyces species make up the gene clusters for secondary metabolites. The revealed type of the known compounds helps in dereplication of its crude metabolites. In addition, the gene sequence coding the unknown compounds can be subjected to bioinformatics analysis to predict the possible chemical structure.

The complementary experimental studies should be accomplished to confirm the expression possibility of computationally predicted clusters. Using non-native transcriptional activators is an efficient method for the expression of biosynthetic pathways of desirable secondary metabolites in actinobacteria. Another method is synthetic biology as a powerful approach to discover, engineer, and design the biosynthetic pathway of actinobacterial secondary metabolites. The investigated strain in this study contains 9 gene clusters for terpene biosynthesis, more than all the other secondary metabolite gene clusters that existed in this strain. Terpenes render a major and variety of activities in pharmaceutical uses such as antioxidant, anticancer, antiviral, antiseptic, and antidiabetic properties. Polyketide synthetases, the second major secondary metabolite produced by the studied strain, are used as antimicrobial, anticancer, hypocholestrolemics, and immunosuppressant agents. The strain *Nonomuraea* sp. UTMC 2524 is a suitable candidate to be subjected to induction of its new secondary metabolites.

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