



Genomics/technical resources

Draft genome sequences of three chemically rich actinomycetes isolated from Mediterranean sponges



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ABSTRACT

Metabolomic analysis has shown the chemical richness of the sponge-associated actinomycetes *Streptomyces* sp. SBT349, *Nonomureae* sp. SBT364, and *Nocardiopsis* sp. SBT366. The genomes of these actinomycetes were sequenced and the genomic potential for secondary metabolism was evaluated. Their draft genomes have sizes of 8.0, 10, and 5.8 Mb having 687, 367, and 179 contigs with a GC content of 71.6, 70.7, and 72.7%, respectively. Moreover, antiSMASH 3.0 predicted 108, 149, and 75 secondary metabolite gene clusters, respectively which highlight the metabolic capacity of the three actinomycete species to produce diverse classes of natural products.

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1. Short introduction

Actinomycetes harbor a wealth of natural products with structural complexity and diverse biological activities (Abdelmohsen et al., 2014a; Nett et al., 2009; Li and Vederas, 2009; Abdelmohsen et al., 2015). Genomic sequence data have revealed the presence of putatively silent biosynthetic gene clusters in the genomes of actinomycetes that encode for secondary metabolites, which are not seen under standard fermentation conditions (Cimermancic et al., 2014). The actinomycete isolates *Streptomyces* sp. SBT349, *Nonomureae* sp. SBT364, and *Nocardiopsis* sp. SBT366 were cultivated from marine sponges *Sarcotragus spinosulus*, *Sarcotragus foetidus*, and *Chondrilla nucula*, respectively. The strains have been deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ) with accession numbers DSM 100667 (SBT349), DSM 100666 (SBT364), and DSM 100668 (SBT366). The sponges were collected by SCUBA diving at 5–7 m depth from offshore Pollonia, Milos, Greece (N36.76612°; E24.51530°) in May 2013 under the umbrella of the EU-FP7 project entitled “SeaBioTech: From sea-bed to test-bed: harvesting the potential of marine biodiversity for industrial biotechnology” that aims to create innovative marine biodiscovery pipelines. Members of the genera *Streptomyces* and *Nocardiopsis* are widespread in terrestrial environments, including soil and plants and have also been isolated from the marine environment, i.e., from marine sponges (Abdelmohsen et al., 2010;

Vicente et al., 2013; Abdelmohsen et al., 2014b; Eltamany et al., 2014). We report here, to our knowledge for the first time, the isolation of members of the genus *Nonomureae* from marine environment. Among the 50 actinomycetes cultivated from the Milos collection, the organic extracts of isolates SBT349, SBT364, and SBT366 exhibited rich HPLC-peak profiles as well as diverse bioactivities including antioxidant, antitrypanosomal and anticancer, respectively (Cheng et al., 2015). These isolates were selected based on their HPLC-peak richness and bioactivity profile for further genomic sequencing.

2. Data description

Genomic DNA of the actinomycetes was extracted and prepared as described (Harjes et al., 2014). 250 bp paired-end sequencing was performed on a MiSeq benchtop sequencer (Illumina). Obtained reads were adapter trimmed as well as quality and length filtered using Trimmomatic 0.32 (Bolger et al., 2014). Assembly was performed using SPAdes 3.1.1 (Bankevich et al., 2012) and calculated contigs were manually filtered due to low coverage. Remaining contigs were extended and merged wherever possible using SSPACE 3.0. (Boetzer et al., 2011). The RAST webserver was used for annotation (Aziz et al., 2008) (Table 1).

The draft genomes were mined using antiSMASH 3.0 (“Antibiotic and Secondary Metabolites Analysis Shell”) (Weber et al., 2015) and NapDos (“The natural product domain seeker”) (Ziemert et al., 2012). Among the three genomes sequenced, *Streptomyces* sp. SBT349 displayed the most diverse antiSMASH read-out. A total of 108 potential secondary metabolite gene clusters were predicted, encoding for 23 type I polyketide synthases (PKS), 11 non-ribosomal peptide synthetases (NRPSs), 2 terpenes, 21 saccharides, 3 siderophores, 3 lantipeptides, 1 butyrolactone, 1 bacteriocin, 1 phenazine, 1 ladderane,

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Table 1

General features of *Streptomyces* sp. SBT349, *Nonomuraea* sp. SBT364, and *Nocardiopsis* sp. SBT366 genomes.

Attribute	<i>Streptomyces</i> sp. SBT349	<i>Nonomuraea</i> sp. SBT364	<i>Nocardiopsis</i> sp. SBT366
Assembly size (bp)	8,013,004	9,992,837	5,790,753
Contigs	687	367	179
Contig N50	19,800	50,206	60,060
GC content %	71.67	70.74	72.72
Predicted ORFs	6939	9338	5123
tRNA	54	57	57
rRNA	7	7	8

and 1 linaridin, as well as 26 unidentified putative clusters (Table 2). antiSMASH results showed that strain *Streptomyces* sp. SBT349 has the highest potential in comparison to the two other strains to produce type I polyketides and non-ribosomal peptides which are the major classes of pharmacologically active natural products as well as the potential to produce linaridins which are post-translationally modified peptides with interesting biological properties. Furthermore, NaPDos predicted the presence of natural products such as nystatin, rapamycin, rifamycin, epothilone, and tetronomycin. For *Nonomureae* sp. SBT364, NaPDos predicted the presence of gene clusters encoding for rifamycin, avermectin, avilamycin, concanamycin, and tetronomycin. Thirdly, for *Nocardiopsis* sp. SBT366, gene clusters encoding for pikromycin, alnumycin, amphoterin, and mycinamicin were predicted. In summary, sequencing genomes of three sponge-associated actinomycete *Streptomyces* sp. SBT349, *Nonomureae* sp. SBT364, and *Nocardiopsis* sp. SBT366 provided new insights into the genomic underpinnings of actinomycete secondary metabolism, which may deliver novel chemical scaffolds with interesting biological activities for the drug discovery pipeline. Future work will include bioassay-guided isolation of the

Table 2

Number of predicted secondary metabolite biosynthetic gene clusters (antiSMASH 3.0).

	<i>Streptomyces</i> sp. SBT349	<i>Nonomuraea</i> sp. SBT364	<i>Nocardiopsis</i> sp. SBT366
Bacteriocin	1	2	2
Butyrolactone	1	–	–
Butyrolactone-CF_fatty_acid	–	–	1
CF_fatty_acid	2	3	1
CF_putative	26	72	34
CF_saccharide	21	43	24
Ectoine	1	–	–
Ectoine-CF_saccharide	–	–	1
Ladderane	–	1	–
Ladderane-acylpolyene	1	1	–
Ladderane-CF_fatty_acid-NRPS	–	1	–
Lantipeptide	1	2	1
Linaridin	1	–	–
Linaridin-CF_saccharide	–	1	–
NRPS	11	8	4
NRPS-CF_saccharide	–	–	1
Other	3	–	–
Phenazine	1	–	–
Phosphonate	2	–	–
Siderophore	3	1	1
Terpene	2	4	2
Thiopeptide	–	–	1
Thiopeptide-lantipeptide-terpene	1	–	–
Type 1 PKS	23	7	1
Type 1 PKS-CF_fatty acid	1	–	–
Type 1 PKS-NRPS	1	1	–
Type 1 PKS-other	–	1	–
Type 2 PKS	–	–	1
Type 3 PKS	1	1	–
Type 3 PKS-lantipeptide-CF_fatty acid	1	–	–
Type 3 PKS-terpene	1	–	–
Overall	108	149	75

Table 3

Minimum information about the genome sequence (MIGS).

Item	<i>Streptomyces</i> sp. SBT349	<i>Nonomuraea</i> sp. SBT364	<i>Nocardiopsis</i> sp. SBT366
Investigation		Bacteria_archaea	
Project name		SeaBioTech	
Country		Milos, Greece	
Latitude and longitude		N36.76612° E24.51530°	
Depth		5–7 m	
Collection date		May-2013	
Biome		ENVO:01000047	
Feature		ENVO:00000130	
Material		ENVO:01000161	
Material		Sponge sample	
Specific host	1088795	1162770	220712
Habitat: temperature		20 °C	
Habitat: salinity		Not applicable	
Sequencing method		Illumina MiSeq	
Genome coverage	100×	115×	146×
Assembly method		SPAdes 3.1.1, SSPACE 3.0	
Estimated size	8,013,004	9,992,837	5,790,753
Finishing_strategy		Draft	
GenBank_locus	LAVK01000000	LAVL01000000	LAVM01000000
Ref_biomaterial		Include publication if used elsewhere	
Isol_growth_condt		24604655	
Rel_to_oxygen		Not applicable	

bioactive natural products based on the genomic information gained from this study. Minimum Information about the Genome Sequence (MIGS) is provided in Table 3.

3. Nucleotide sequence accession number

The whole-genome shotgun (WGS) projects were deposited at GenBank under the Bioproject ID PRJNA280805 with the accession numbers LAVK00000000, LAVL00000000 and LAVM00000000. The versions described here are LAVK01000000, LAVL01000000 and LAVM01000000.

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