

# Mining Genomes of Three Marine Sponge-Associated Actinobacterial Isolates for Secondary Metabolism

Hannes Horn,<sup>a,b</sup> Ute Hentschel,<sup>a,b</sup> Usama Ramadan Abdelmohsen<sup>a\*</sup>

Department of Botany II, Julius-von-Sachs Institute for Biological Sciences, University of Würzburg, Würzburg, Germany<sup>a</sup>; Department of Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research, RD3 Marine Microbiology and Christian-Albrechts University of Kiel, Kiel, Germany<sup>b</sup>

\* Permanent address: Usama Ramadan Abdelmohsen, Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt.

**Here, we report the draft genome sequences of three actinobacterial isolates, *Micromonospora* sp. RV43, *Rubrobacter* sp. RV113, and *Nocardiopsis* sp. RV163 that had previously been isolated from Mediterranean sponges. The draft genomes were analyzed for the presence of gene clusters indicative of secondary metabolism using antiSMASH 3.0 and NapDos pipelines. Our findings demonstrated the chemical richness of sponge-associated actinomycetes and the efficacy of genome mining in exploring the genomic potential of sponge-derived actinomycetes.**

Received 14 August 2015 Accepted 17 August 2015 Published 1 October 2015

**Citation** Horn H, Hentschel U, Abdelmohsen UR. 2015. Mining genomes of three marine sponge-associated actinobacterial isolates for secondary metabolism. *Genome Announc* 3(5):e01106-15. doi:10.1128/genomeA.01106-15.

**Copyright** © 2015 Horn et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Usama Ramadan Abdelmohsen, usama.ramadan@uni-wuerzburg.de.

Actinomycetes are known for their unprecedented ability to produce novel lead compounds of clinical and pharmaceutical importance (1–4). Among the many actinobacterial genera, *Streptomyces*, *Micromonospora*, *Nocardiopsis*, and *Rhodococcus* are the most prolific producers of secondary metabolites, which display broad chemical diversity and diverse pharmaceutically and medically relevant bioactivities (5–8). Recent genomic sequencing data have revealed the presence of a plethora of putative biosynthetic gene clusters on the genomes of actinomycetes encoding for secondary metabolites that are not observed under standard fermentation conditions (9–13). In the present study, draft genomes of three actinobacterial isolates, *Micromonospora* sp. RV43, *Rubrobacter* sp. RV113, and *Nocardiopsis* sp. RV163 that had previously been cultivated from the Mediterranean sponges *Aplysina aerophoba* (RV43 and RV113) and *Dysidea avara* (RV163) (14), were established.

The genomic DNA of the isolates was extracted from 5-day-old ISP2 cultures. Paired-end, 2 × 250-bp libraries were prepared with the Nextera XT kit (Illumina, Inc.). Sequencing was performed on an Illumina MiSeq device. A total of 5,900,702 raw reads were produced for *Micromonospora* sp. RV43, 2,206,732 raw reads for *Rubrobacter* sp. RV113 and 4,851,980 raw reads were delivered for *Nocardiopsis* sp. RV163. Reads were adapter clipped, quality trimmed and length filtered (15). Initial contigs were generated using SPAdes (16) and only contigs ≥ 1000 bp were maintained. A further clean-up of contigs was performed using G+C-content, coverage, and taxonomic assignments (17). For *ab initio* gene prediction, prodigal was applied (18) and functional annotation of the predicted protein sequences was performed with the RAST webserver (19). Secondary metabolite gene clusters and possible encoded compounds were predicted with antiSMASH (20) and NapDos (21).

A number of 101 (RV43), 33 (RV113), and 82 (RV163) secondary metabolite gene clusters were detected with antiSMASH. For

strain RV43, 5 terpene clusters, 4 type 1 PKS clusters, 2 lantipeptides, 1 type 2 PKS cluster, 1 siderophore, 1 NRPS cluster, and 1 bacteriocin were found. For strain RV113, 3 terpene clusters, 1 fatty acid, and 1 mixed type 3 PKS-fatty acid cluster were found. The draft genome sequence of strain RV163 showed homologies to 7 NRPS clusters, 4 terpene gene clusters, 2 type 1 PKS clusters, 2 ectoines, 2 bacteriocins, 1 phenazine, 1 butyrolactone, 1 type 2 PKS, and 1 siderophore.

For *Micromonospora* sp. RV43, NapDoS predicted the presence of gene clusters encoding for compounds such as leinamycin, kirromycin, aclacinomycin, and tetronomycin. For *Nocardiopsis* sp. RV163, compounds such as alnumycin, avermectin, and neocarzinostatin were predicted. For *Rubrobacter* sp. RV113, only gene clusters encoding for fatty acids synthesis were found. These results highlight the genomic potential of at least two of three inspected isolates for natural products discovery.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project was deposited in DDBJ/ENA/GenBank under the accession numbers [LEKG00000000](https://www.ncbi.nlm.nih.gov/nuccore/LEKG00000000), [LEKH00000000](https://www.ncbi.nlm.nih.gov/nuccore/LEKH00000000), and [LEKI00000000](https://www.ncbi.nlm.nih.gov/nuccore/LEKI00000000). The versions described in this paper are the first versions [LEKG01000000](https://www.ncbi.nlm.nih.gov/nuccore/LEKG01000000), [LEKH01000000](https://www.ncbi.nlm.nih.gov/nuccore/LEKH01000000), and [LEKI01000000](https://www.ncbi.nlm.nih.gov/nuccore/LEKI01000000).

## ACKNOWLEDGMENTS

We gratefully acknowledge C. Gernert for technical assistance in the laboratory. We thank the Group of Molecular Biodiversity/Zoology 3 for library preparation and the Department of Human Biology for access to the sequencing device (both University of Würzburg).

Financial support was provided by the DFG (SFB 630 TP A5) to U.H.

## REFERENCES

- Abdelmohsen UR, Bayer K, Hentschel U. 2014. Diversity, abundance and natural products of marine sponge-associated actinomycetes. *Nat Prod Rep* 31:381–399. <http://dx.doi.org/10.1039/C3NP70111E>.
- Li JW, Vederas JC. 2009. Drug discovery and natural products: end of an era or an endless frontier? *Science* 325:161–165. <http://dx.doi.org/10.1126/science.1168243>.

3. Eltamany EE, Abdelmohsen UR, Ibrahim AK, Hassanean HA, Hentschel U, Ahmed SA. 2014. New antibacterial xanthone from the marine sponge-derived *Micrococcus* sp. EG45. *Bioorg Med Chem Lett* 24: 4939–4942. <http://dx.doi.org/10.1016/j.bmcl.2014.09.040>.
4. Grkovic T, Abdelmohsen UR, Othman EM, Stopper H, Edrada-Ebel R, Hentschel U, Quinn RJ. 2014. Two new antioxidant actinosporin analogues from the calcium alginate beads culture of sponge-associated *Actinokineospora* sp. strain EG49. *Bioorg Med Chem Lett* 24:5089–5092. <http://dx.doi.org/10.1016/j.bmcl.2014.08.068>.
5. Reimer A, Blohm A, Quack T, Grevelding CG, Kozjak-Pavlovic V, Rudel T, Hentschel U, Abdelmohsen UR. 20 May 2015. Inhibitory activities of the marine streptomycete-derived compound SF2446A2 against *Chlamydia trachomatis* and *Schistosoma mansoni*. *J Antibiot*. <http://dx.doi.org/10.1038/ja.2015.54>.
6. Dashti Y, Grkovic T, Abdelmohsen UR, Hentschel U, Quinn RJ. 2014. Production of induced secondary metabolites by a co-culture of sponge-associated actinomycetes, *Actinokineospora* sp. EG49 and *Nocardioopsis* sp. RV163. *Mar Drugs* 12:3046–3059. <http://dx.doi.org/10.3390/md12053046>.
7. Abdelmohsen UR, Yang C, Horn H, Hajjar D, Ravasi T, Hentschel U. 2014. Actinomycetes from Red Sea sponges: sources for chemical and phylogenetic diversity. *Mar Drugs* 12:2771–2789. <http://dx.doi.org/10.3390/md12052771>.
8. Abdelmohsen UR, Szesny M, Othman EM, Schirmeister T, Grond S, Stopper H, Hentschel U. 2012. Antioxidant and anti-protease activities of diazepinomicin from the sponge-associated *Micromonospora* strain RV115. *Mar Drugs* 10:2208–2221. <http://dx.doi.org/10.3390/md10102208>.
9. Harjes J, Ryu T, Abdelmohsen UR, Moitinho-Silva L, Horn H, Ravasi T, Hentschel U. 2014. Draft genome sequence of the antitrypanosomally active sponge-associated bacterium *Actinokineospora* sp. strain EG49. *Genome Announc* 2(2):e00160-14. <http://dx.doi.org/10.1128/genomeA.00160-14>.
10. Abdelmohsen UR, Grkovic T, Balasubramanian S, Kamel MS, Quinn RJ, Hentschel U. 2015. Elicitation of secondary metabolism in actinomycetes. *Biotechnol Adv* 33:798–811. <http://dx.doi.org/10.1016/j.biotechadv.2015.06.003>.
11. Challis GL. 2008. Mining microbial genomes for new natural products and biosynthetic pathways. *Microbiology* 154:1555–1569. <http://dx.doi.org/10.1099/mic.0.2008/018523-0>.
12. Jensen PR, Chavarria KL, Fenical W, Moore BS, Ziemert N. 2014. Challenges and triumphs to genomics-based natural product discovery. *J Ind Microbiol Biotechnol* 41:203–209. <http://dx.doi.org/10.1007/s10295-013-1353-8>.
13. Nett M, Ikeda H, Moore BS. 2009. Genomic basis for natural product biosynthetic diversity in the actinomycetes. *Nat Prod Rep* 26:1362–1384. <http://dx.doi.org/10.1039/b817069j>.
14. Abdelmohsen UR, Pimentel-Elardo SM, Hanora A, Radwan M, Abou-El-Ela SH, Ahmed S, Hentschel U. 2010. Isolation, phylogenetic analysis and anti-infective activity screening of marine sponge-associated actinomycetes. *Mar Drugs* 8:399–412. <http://dx.doi.org/10.3390/md8030399>.
15. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <http://dx.doi.org/10.1093/bioinformatics/btu170>.
16. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <http://dx.doi.org/10.1089/cmb.2012.0021>.
17. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* 10:421. <http://dx.doi.org/10.1186/1471-2105-10-421>.
18. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <http://dx.doi.org/10.1186/1471-2105-11-119>.
19. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
20. Weber T, Blin K, Duddela S, Krug D, Kim HU, Brucoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res* 43: W237–W243. <http://dx.doi.org/10.1093/nar/gkv437>.
21. Ziemert N, Podell S, Penn K, Badger JH, Allen E, Jensen PR. 2012. The natural product domain seeker NaPDoS: a phylogeny based bioinformatic tool to classify secondary metabolite gene diversity. *PLoS One* 7:e34064. <http://dx.doi.org/10.1371/journal.pone.0034064>.