Streptomyces poriferae sp. nov., A Novel *Streptomyces* Species from Marine Sponges That Produce Metabolites That Inhibit the Growth of Methicillin-Resistant *Staphylococcus aureus*(MRSA)

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Antimicrobial resistance of pathogens is a growing public health threat.[1] Because the efficacy of our current antibiotics to treat infections and disease is declining, there is a societal need to identify new antibiotic compounds with unique mechanisms of action. Microorganisms have historically been a rich source of therapeutic drugs, including antibiotics, anticancer, and antifungal compounds. *Streptomyces* species are well-known microbial producers of medically relevant antimicrobial compounds that are important for bacterial survival in natural environments.[2] Given the species' history of antimicrobial metabolite production, the discovery and characterization of novel *Streptomyces* species are a target of new antimicrobial research.[3] In this study, two marine sponge-derived *Streptomyces* isolates, P01-B04^T and P01-F02, were characterized and screened for their ability to produce antimicrobial compounds.

To address this objective, phylogenetic analysis using 16S rRNA gene sequences and genome-based analyses, including average nucleotide identity and DNA-DNA hybridization comparisons, were used to confirm the novelty of the *Streptomyces* isolates and distinguish them from their closest relatives. Bioinformatic analyses using draft genome sequences of each isolate were also performed to further explore the biosynthetic potential of the novel isolates by mining genomes for biosynthetic gene clusters (BGCs) that can encode natural products like antibiotics. Draft genome sequences were uploaded to the pipeline antiSMASH[4] and manually compared to predict the number and types of BGCs present in each genome. Additionally, to characterize the antimicrobial potential of strains P01-B04T and P01-F02, each isolate was grown in various growth media and screened for activity against bacterial and fungal pathogens.

Phylogenetic analyses showed that the ANI and DDH values between the novel isolates and their closest *Streptomyces* relatives were below the species threshold values confirming that the two isolates represent novel species. Additionally, the two isolates showed nearly identical 16S rRNA gene sequences (99.93%), and ANI and DDH relatedness values were determined to be 99.96% and 99.6%, respectively. These data suggest that these isolates are affiliated with the same species, which is hereby named *Streptomyces poriferae*. The antimicrobial activity assays demonstrated that supernatants from the S. poriferae isolates inhibited the growth of Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* and plant pathogens (Table 1). The bioactivity of the isolates was dependent on the duration of incubation and the media used. Furthermore, genome analyses revealed that the isolates harbored on average 30 BGCs, many of which were predicted to be uniquely present in these *S. poriferae* strains.

These results suggest that strains P01-B04^T (the type of strain for this novel species) and P01-F02 produce many bioactive metabolites that may contribute to the chemical ecology of their host sponges, as well as have potential clinical efficacy against a multidrug-resistant human pathogen. These newly discovered antibiotics may have applications in human medicine, veterinary medicine, and agriculture. Ongoing work to determine the structure of these antibiotics will allow assessment of their clinical potential.

Table 1. Summary of antimicrobial activity of novel *Streptomyces poriferae* isolates, P01-B04^T and P01-F02, against bacterial and fungal strains. The fermentation media used for initial culture of isolates is shown and the degree of inhibition of tester strains. "+++" ≥ 10 mm zone of inhibition (ZOI); "++" 6-9 mm ZOI; "+" ≤ 5 mm ZOI.

Isolate:	Activity against:	Fermentation media:	Degree of inhibition
P01-F02	*Curtobacterium flaccumfaciens subsp. flaccumfaciens* CV3	YEME	++
	Clavibacter michiganensis 89C-4	YEME	++
P01-B04	*Staphylococcus aureus* Xen29	YEME	++
	Staphylococcus aureus MRSA30	YEME	++
	Curtobacterium flaccumfaciens subsp. flaccumfaciens CV3	YEME	++
	Micrococcus luteus ATCC 10240	YEME, GYE, MF, TSB, ISP-2	+++
	Clavibacter michiganensis 89C-4	YEME	++

Statement of Research Advisor

Dory Fawwal has made very significant contributions in our understanding of the potential of members of this novel species *Streptomyces poriferae* to produce bioactive metabolites. The production of antibiotics by these bacteria, which Dory has demonstrated under lab conditions, may indicate that these bioactive secondary metabolites are important for the chemical ecology of the marine sponges that host these *Streptomyces* bacteria. Dory has therefore earned authorship on a manuscript that has been submitted for publication in the journal, **Systematic and Applied Microbiology**, which is the best indicator of her important contributions to this research project.
-Mark Liles, Biological Sciences

References

[1] Andersson, D.I., Balaban, N.Q., Baquero, F., Courvalin, P., Glaser, P., Gophna, U., Kishony, R., Molin, S. and Tønjum, T., 2020. Antibiotic resistance: turning evolutionary principles into clinical reality. *FEMS Microbiology Reviews*, 44 (2), pp.171-188.

[2] de Lima Procópio, R. E., da Silva, I. R., Martins, M. K., de Azevedo, J. L., & de Araújo, J. M. (2012). Antibiotics produced by *Streptomyces. The Brazilian Journal of Infectious Diseases,* 16(5), 466–471.

[3] Valli, S., Suvathi, S. S., Aysha, O. S., Nirmala, P., Vinoth, K. P., & Reena, A.(2012). Antimicrobial potential of Actinomycetes species isolated from marine environment. *Asian Pacific Journal of Tropical Biomedicine*, 2(6), 469–473.

[4] Blin, K., Wolf, T., Chevrette, M. G., Lu, X., Schwalen, C. J., Kautsar, S. A., ... & Dickschat, J.S. (2017). antiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. *Nucleic acids research*, 45(W1), W36-W41.