

A cryptic, chemically rich sponge symbiont identified by Raman microscopy, single-cell genomics, and biochemical studies

Franziska Hemmerling, ETH Zürich, Switzerland (1);

*Masato Kogawa, Waseda University, Tokyo/Japan (2); Masahito Hosokawa, (2);
Masahiro Ando, (2); Tetsushi Mori, Tokyo University, Japan; Jackson K. B. Cahn, (1);
Haruko Takeyama, (2); Jörn Piel, (1).*

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Uncultivated bacteria represent an underexplored source for the production of bioactive metabolites. The marine sponge *Theonella swinhoei* Y was previously found to be inhabited by members of the uncultivated bacterial candidate genus 'Entotheonella', "talented producers" of a wide range of natural products. In previous work, biosynthetic gene clusters for all polyketide and peptide families isolated from *T. swinhoei* Y were assigned to 'Entotheonella', with the single exception of the antifungal aurantoside-type polyketides. Since metagenomic sequencing of the sponge consistently failed to reveal the producer, uncultivated microbiota from *T. swinhoei* Y were encapsulated with a microfluidic device and subjected to Raman microscopy, permitting direct detection of aurantosides in individual bacterial multicellular filaments. Subsequent single-bacterial sequencing revealed the putative aurantoside biosynthetic gene cluster that was linked to aurantoside production via biochemical characterization of a late-step enzyme. The sponge symbiont belongs to a new Chloroflexi candidate genus named 'Poriflexus', with the aurantoside producer *Ca. 'Poriflexus aureus'* as its as-yet only representative. Its unusually large, 15 Mb genome harbors an impressive number of gene clusters from multiple natural product families. Our data suggest Raman microscopy and single-bacterial sequencing as a powerful complementary strategy to metagenomics to pinpoint uncultured producers. The identification of 'Entotheonella' and 'Poriflexus' supports the existence of diverse talented producer taxa in microbial dark matter that could be rich resources for novel drug leads.