

**Title:** Effects of sample handling and cultivation bias on the specificity of bacterial communities in keratose marine sponges

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## Abstract

Complex and distinct bacterial communities inhabit marine sponges and are believed to be essential to host survival, but our present-day inability to domesticate sponge symbionts in the laboratory hinders our access to the full metabolic breadth of these microbial consortia. We address bacterial cultivation bias in marine sponges using a procedure that enables direct comparison between cultured and uncultured symbiont community structures. Bacterial community profiling of the sympatric keratose species *Sarcotragus spinosulus* and *Ircinia variabilis* (Dictyoceratida, Irciniidae) was performed by PCR-DGGE and 454-pyrosequencing of 16S rRNA gene fragments. Whereas cultivation-independent methods revealed species-specific bacterial community structures in these hosts, cultivation-dependent methods resulted in equivalent community assemblages from both species. Between 15 and 18 bacterial phyla were found in *S. spinosulus* and *I. variabilis* using cultivation-independent methods. However, *Alphaproteobacteria* and *Gammaproteobacteria* dominated the cultivation-dependent bacterial community. While cultivation-independent methods revealed about 200 and 220 operational taxonomic units (OTUs, 97% gene similarity) in *S. spinosulus* and *I. variabilis*, respectively, only 33 and 39 OTUs were found in these species via culturing. Nevertheless, around 50% of all cultured OTUs escaped detection by cultivation-independent methods, indicating that standard cultivation makes otherwise host-specific bacterial communities similar by selectively enriching for rarer and generalist symbionts. This study sheds new light on the diversity spectrum encompassed by cultivated and non-cultivated sponge-associated bacteria. Moreover, it highlights the need to develop alternative culturing technologies to capture the dominant sponge symbiont fraction that currently remains recalcitrant to laboratory manipulation.

**Keywords:** holobiont, microbial cultivation, microbial diversity, pyrosequencing, symbiosis

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