From bacteria to bivalves to birds: Inter-kingdom interactions in a marine symbiosis

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Lucinid bivalves, as one of the most abundant marine chemosynthetic taxa, form a close nutritional association with sulphur-oxidizing γ-Proteobacteria housed in their gills. The clams provide the symbiont with access to electron donors and acceptors for autotrophic CO_2 fixation and the generation of bacterial biomass. In exchange, a portion of the carbon fixed by the symbiont is used for host energy and biosynthesis.

In the intertidal flats off the coast of Mauritania, lucinids cause toxic effects in intertidally foraging shorebirds and this is hypothesized to be due to sulphur granules stored intracellularly in the bacterial symbionts. However, up until now, the effects of the biotic habitat and interactions with other organisms in the host's environment on the symbiosis have been largely ignored. Shallow-water symbioses such as the lucinid clams offer the unique opportunity for integrating research on the hosts, their associated microbes, and their natural environment.

Here, we describe a new lucinid species and its bacterial symbionts from intertidal seagrass beds in Mauritania. Multiplexed amplicon sequencing and fluorescence-*in-situ*-hybridization (FISH) determined the bacterial community of the lucinid reproductive stages. The lack of the host-specific bacterial symbiont argues for an environmental symbiont acquisition in a later life stage of the bivalve.

A potential secondary symbiont could be detected in the gills of this new lucinid species using metagenomic high-throughput sequencing as well as FISH. As a member of the γ-Proteobacteria (genus *Shewanella*) it forms a distinct, basal monophyletic clade (based on the 16S rRNA gene) with a sequence identity of 95.5% to its sister clade. The metabolic capabilities of this new clade remain to be studied as well as the effect that it might have on the ecology of chemosynthetic bivalves in Mauritania but there is evidence that *Shewanella* sp. is able to use sulfides stored by the primary symbionts.

The abundance of lucinids is usually higher in seagrass sediments, possibly due to mutualistic interactions between chemosynthetic clams and seagrasses. However, it remains unclear what potential role the bacterial symbionts play in this tripartite symbiosis. By using a meta-transcriptomic approach as well as FISH we aim to identify potential links between the seagrass day-night cycle and symbiont activity.