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Metabolomic and transcriptomic analysis of the defensive role of Actinobacteria within the fungus-growing termite system

Fungiculture in Macrotermitinae evolved 30 Mio. years ago in Sub-Saharan Africa. Fungus farming is accomplished through an intricate mode of substrate incorporation and intensive maintenance of the fungus comb, in which the symbiotic fungus and the associated microbial community reside to degrade the pre-digested plant material. Fungal fruiting bodies are ultimately eaten by the termites to secure insect nutrition.

Once the termite colony dies, when comb material deteriorates or is incubated without termites, fruiting bodies of species of the ascomycete genus *Pseudoxylaria* appear and rapidly overgrow the comb material. While *Termitomyces* sp. is an obligatory mutualist the termites feed on, the ecological role of *Pseudoxylaria* sp. in fungus-growing termite nests is still under debate. Overall, the fungus comb harbors a complex microbial community that is governed by intricate relationships (e.g. synergistic, antagonistic, cheating, co-existing) and only the concerted actions of the community ensure the emergent properties that vary significantly from the combined effects of individual microorganisms. Secreted microbial community.

Our general scientific activity focuses on the biochemical analysis of the secreted metabolites, which governs the multilateral symbiotic interactions between termites, fungal cultivar, fungal competitors and

associated bacteria. In the outlined project, we want to biochemically analyse the defensive role of isolated Actinobacteria and their interaction with the co-evolved garden weed *Pseudoxylaria* to gain insights how the complex termite-fungus symbiosis has remained sustainable over evolutionary time. We hypothesize that Actinobacteria provide a third line of defence against invading species by secretion of antimicrobial secondary metabolites (in addition to immunity provided by the insects and the mutualist *Termitomyces*). To investigate the hypothesis, we will analyse the response mechanism of Actinobacteria upon the presence of mutualists or competitors on a genetic and metabolomic level.

Publications

Kreuzenbeck NB, Seibel E, Schwitalla JW, Fricke J, Conlon BH, Schmidt S, Hammerbacher A, Köllner TG, Poulsen M, Hoffmeister D, Beemelmanns C (2022) Comparative Genomic and Metabolomic Analysis of Termitomyces Species Provides Insights into the Terpenome of the Fungal Cultivar and the Characteristic Odor of the Fungus Garden of Macrotermes natalensis Termites. *mSystems*, e0121421. <u>PubMed</u>

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