

# 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.

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## Projects

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

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Studies of the biosynthetic potential of microbial symbionts of fungus-growing termites using *Ameycolatopsis* M39 as model organism

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## Abstract

Macrotermitinae termites have domesticated fungi of the genus *Termitomyces* as food for their colony, analogously to human farmers growing crops. Termites propagate the fungus by continuously blending foraged and predigested plant material with fungal mycelium and spores (fungus comb) within designated subterranean chambers. To test the hypothesis that the obligate fungal symbiont emits specific volatiles (odor) to orchestrate its life cycle and symbiotic relations, we determined the typical volatile emission of fungus comb biomass and *Termitomyces* nodules, revealing  $\alpha$ -pinene, camphene, and d-limonene as the most abundant terpenes. Genome mining of *Termitomyces* followed by gene expression studies and phylogenetic analysis of putative enzymes related to secondary metabolite production encoded by the genomes uncovered a conserved and specific biosynthetic repertoire across strains. Finally, we proved by heterologous expression and in vitro enzymatic assays that a highly expressed gene sequence encodes a rare bifunctional mono-/sesquiterpene cyclase able to produce the abundant comb volatiles camphene and d-limonene. **IMPORTANCE** The symbiosis between macrotermitinae termites and *Termitomyces* is obligate for both partners and is one of the most important contributors to biomass conversion in the Old World tropic's ecosystems. To date, research efforts have dominantly focused on acquiring a better understanding of the degradative capabilities of *Termitomyces* to sustain the obligate nutritional symbiosis, but our

knowledge of the small-molecule repertoire of the fungal cultivar mediating interspecies and interkingdom interactions has remained fragmented. Our omics-driven chemical, genomic, and phylogenetic study provides new insights into the volatilome and biosynthetic capabilities of the evolutionarily conserved fungal genus *Termitomyces*, which allows matching metabolites to genes and enzymes and, thus, opens a new source of unique and rare enzymatic transformations.

**Identifier**

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