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Microbes at work in perfumery: the microbial community of vetiver root and its involvement in essential oil biogenesis†

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Background: the Vetiver Plant and the Essential Oil

Vetiveria zizanioides (L.) Nash (vetiver) is a perennial graminaceous plant growing wild, half-wild or cultivated in many tropical and subtropical areas.[1] It is cultivated for its unique ability among grasses to produce in the root an essential oil, a complex mixture of sesquiterpene alcohols and hydrocarbons, which are mostly used as a basic material for perfumery and cosmetics. Because of this complexity, the oil is difficult to reproduce with synthetic aromatic chemical formulations. Moreover, differences in the quality of the oil may depend on genetic, environmental and technological factors.[1,2] The biological activity of the vetiver oil is also important. Termicidal, insecticidal, antimicrobial and antioxidant activities of vetiver oil have been described.[3,4] Finally, vetiver, which also has a feed value,[5] has been extensively used for land protection purposes as a barrier against erosion and for the restoration of contaminated land.[6]

Vetiver oil is produced in secretory cells localized in the first cortical layer outside the endodermis of mature roots.[1,7] Thus, while most essential oils are extracted from aerial tissues of dicotyledonous plants, vetiver oil is distilled from the roots of this monocotyledonous plant. The terpene oils in aerial tissues are often found as a complex mixture of different terpene compounds, including monoterpenes and sesquiterpenes, arising from complex interactions between the action of the cytosolic (mevalonate) and plastidic (2-C-methylerythritol-4-phosphate) pathways, and the oils accumulate as extracellular exudates or in specialized glands (lactifiers) or oil bodies (associated with trichomes).[8] Much less is known about the biosynthesis, regulation and localization of terpenes synthesized in roots.

The Microbial Community of the Vetiver Root

The idea that vetiver root bacteria may be involved in essential oil biogenesis was stimulated by light and transmission electron microscopy studies demonstrating the presence of bacteria in the cortical parenchymatous essential oil-producing vetiver cells and in the lysigen lacunae in close association with the essential oil. In addition, axenic vetiver cultured *in vitro* produced only trace amounts of oil, with a strikingly different composition compared to the oils from *in vivo* vetiver plants.[9–12]

In a recent paper, a number of bacterial species were isolated from surface-sterilized roots of 12 month-old vetiver plants.[12] Among cultivated microorganisms, a total of 10 taxa were represented, including four strains belonging to the family Pseudomonadaceae (VET-3, VET-4, VET-5 and VET-8), four to the Enterobacteriaceae (VET-2, VET-7, VET-37 and VET-40), one to the Aeromonadaceae (VET-1) and one to the Micrococcaceae (VET-35). Some of them, *Pseudomonas* sp. VET-3, VET-4, VET-5 and VET-8, were taxonomically related to *Pseudomonas* spp. previously found associated with roots in a variety of plants belonging to the division Magnoliophyta, *Brassica napus* (oilseed rape), *Arabidopsis thaliana* and others. Other isolates, VET-7 and VET-37, occupied taxonomic positions very close to *Enterobacter (Pantoea) agglomerans* and *Enterobacter ludwigii*, respectively. Phylogenetic analysis located VET-2 in the *Serratia liquefaciens* complex, while both phylogenetic and biochemical data demonstrated that VET-40 was a non-pigmented *Serratia marcescens* strain, and assigned both VET-7 and VET-37 to the genus *Enterobacter*. Biochemical data and 16S rRNA sequence analysis identified VET-1 as *Aeromonas caviae*. Lastly, phylogenetic data located VET-35 close to *Arthrobacter nitroguajacolicus*.

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The complexity of the vetiver root-associated microbial community was also analysed using culture-independent methods [12]. In addition to most of cultivated species, this approach led to detection of: (a) uncultured α-proteobacteria related to *Afipia* genospecies 14 (Bradyrhizobiaceae); (b) β-Proteobacteria belonging to the genus *Duganella*; (c) uncultivated β-Proteobacteria belonging to the family Rhodocyclaceae, which were also found in the trembling aspen rhizosphere; (d) β-Proteobacteria distantly related to the genus *Rubrivivax (*Burkholderiales); (e) γ-Proteobacteria closely related to *Pseudomonas corrugata* SB4, an endophytic bacterium capable of growing on 4-chloroaniline; and (f) bacteria belonging to the Fibrobacteres/Acidobacteria group.

Role of Vetiver Root Bacteria in Essential Oil Biogenesis

Owing to the close association of bacteria with vetiver root cells producing essential oil, the ability of the root-associated bacteria to grow using vetiver oil as a sole carbon source was tested. Enterobacteriaceae strains VET-2, VET-7, VET-37 and VET-40 grew well in a mineral medium supplemented with vetiver oil as a sole carbon source (SMR–oil medium) with generation times (μ) in the range 0.22–0.23; *Arthrobacter* sp. strain VET-35 also demonstrated ability to grow in this medium (μ = 0.19). On the other hand, Pseudomonadaceae strains exhibited poor growth (VET-5, μ = 0.14; VET-8, μ = 0.14; and VET-3, μ = 0.09), whereas VET-4 and *Aeromonas* sp. VET-1 were unable to grow.[12]

The ability of several root-associated bacteria to grow using the essential oil as carbon source led the present authors to investigate the modification in the oil molecular structure caused by bacterial growth *in vitro*. To this purpose, VET-2, VET-3, VET-5, VET-7, VET-8, VET-35, VET-37 and VET-40 were individually cultivated in SRM–oil medium for 40 h. Then the vetiver oil constituents were extracted from the exhausted growth medium and subjected to quantitative analysis by gas chromatography–mass spectrometry (GC–MS). Interestingly, each given microorganism specifically metabolized the raw vetiver oil by releasing into the medium a large number of compounds, some of which were absent (or present in very low amounts) in the raw oil but are typically found in other commercial vetiver oils.[12]

Bacteria were also incubated in the presence of individual compounds of the vetiver oil, for instance (+)-cuparene, one of the main components of raw vetiver oil. Interestingly, only VET-7 and VET-35 were able to metabolize (+)-cuparene, which was almost completely biotransformed by VET-7, yielding an array of compounds characteristic of vetiver oil as well as new, interesting compounds. Moreover, when bacteria were fed on β-caryophyllene, the main sesquiterpene produced by axenic vetiver, they oxidized it to β-caryophyllene oxide, and some of them (in particular VET-7, VET-8 and VET-40) produced typical vetiver oil constituents that were not produced by axenic roots.[12]

Root-associated bacteria were also able to induce host plant gene expression. Indeed, most of the root isolates induced transcription of sesquiterpene synthase-encoding gene *Vet733* in the host axenic vetiver. This sesquiterpene synthase produces a mixture of at least seven different sesquiterpene hydrocarbons that usually do not appear in vetiver essential oil.[13] Interestingly, the highest *Vet733* gene expression was found in axenic vetiver plants colonized with VET-7, VET-8 and VET-35, which were also the most active in β-caryophyllene metabolism.[12]

Perspectives

The results of these studies shed new light on the ecological significance of the association between vetiver and its root-associated bacterial community and open the intriguing and immediate possibility of manoeuvring the molecular structure of the vetiver oil, either *in vivo*, by acting on the bacterial colonization of the plant root, or *in vitro*, by means of strain-dependent bioconversion processes. The ability of the isolated bacterial strains to produce arrays of new interesting compounds is of relevance for biological struggle strategies and will find many industrial applications. Moreover, the knowledge gained on the vetiver system can be translated to other plants of relevant interest in both the biotechnological and the agronomic fields for their specific activities and products.

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