

## **DIRECT INVOLVEMENT OF THE ROOT ASSOCIATED MICROORGANISMS INTO ESSENTIAL OIL METABOLISM IN *VETIVERIA ZIZANIOIDES* (L.) NASH**

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The species *Vetiveria zizanioides* (L.) Nash is a perennial graminaceous plant native to north India. Selected germlines of this plant species have long been cultivated for their odorous roots that contain the essential oil of Vetiver which consists of a complex mixture of sesquiterpene hydrocarbons and alcohols mostly used as a basic material for perfumery and cosmetics (Maffei 2002).

Electron microscope analysis of the root cells of *Vetiveria zizanioides* (L.) Nash revealed the occurrence of bacteria as well as electrodense crystals of essential oils in the external layers of cortical parenchymatous cells up to those close to the endodermis (Massardo et al. 2004). The close relationship between bacteria and the essential oil stimulated the hypothesis of a direct involvement of those endophytic bacteria in the essential oil metabolism.

The aim of the present study was to analyze the root-associated bacterial community of *V. zizanioides* by using culture-based and culture independent approaches, and to investigate the possible involvement of the root-associated microorganisms with the essential oil metabolism. Planting in Campania Region of the species *V. zizanioides* native to Thailand and isolation of endophytic bacteria were performed as previously described (Pontieri et al. 2005). Independent bacterial isolates from the microbial community inside cell roots were molecularly controlled by analyzing the differences in ribosomal 16S DNA. The bona fide endophytic bacterial species of *V. zizanioides* roots included the cultivable *Pseudomonas*, *Kluyvera*, *Enterobacter*, *Serratia* and *Arthrobacter* strain species. The ability of the bacterial strain isolates to grow using the essential oil as a sole source was analyzed investigating the eventual modification in the oil molecular structure caused by the bacterial growth *in vitro*. To this purpose, bacterial strain isolates were individually cultivated in SRM-oil medium (NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 1g/l, KCl 0.2 g/l, MgSO<sub>4</sub> 0.2 g/l, replacing glucose with a commercial DMSO-dissolved Vetiver oil). Successively, the Vetiver oil constituents (Massardo et

al. 2006) were extracted from the exhausted growth medium by means of Stirr Bar Sorptive Extraction (Gerstel Twister) and subjected to cryodesorption followed by quantitative analysis by Gas Chromatography-Mass Spectrometry (GC-MS). Here we show that bacteria living inside the Vetiver roots are capable of oil biotransformation. These results open the intriguing and immediate possibility to biotransform the molecular structure of the Vetiver oil either *in vivo* by acting on the modulation of the plant root bacterial colonization/presence, or *in vitro* by means of bioconversion processes.

- M. Maffei (2002). "Vetiveria. The genus *Vetiveria*" (ed. M. Maffei), 1-18. Taylor & Francis, London and New York.

- D.R. Massardo et al. (2004) SIGA–XLVIII Annual Congress.

- P. Pontieri et al. (2005) SIGA–XLIX Annual Congress.

- D.R. Massardo et al. (2006) Vetiver oil production correlates with early root growth. *Biochemical Systematics and Ecology*. 34: 376-382.

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