Identification of Bacteria Living in the Vetiver Root by Molecular Approach


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Vetiver root, essential oil, endophytic bacteria, amplified 16S rRNA

Vetiveria zizanioides (L.) Nash (the Vetiver) is a graminaceous plant native to India, growing wild, half wild or cultivated in many tropical and subtropical areas. In particular, selected germlines of this plant species have long been cultivated for their odorous roots that contain the essential oil of Vetiver, used extensively in perfumery and cosmetics (Maffei, 2002).

Vetiver oil is one of the most complex mixtures of sesquiterpene alcohols and hydrocarbons, and also one of the most viscous oils with an extremely slow rate of volatility.

Electron microscope analysis of Vetiver cells of the root glands demonstrated the presence of intracellular bacteria into lysigen lacunae in association with essential oil. The close relationship between these bacteria and the essential oil stimulated the hypothesis of a direct involvement of the symbiotic bacteria in the essential oil metabolism (Massardo et al., 2004). In addition, recent data evidenced that a Vetiver cleansed of bacteria (presumed to be normally associated with field-grown Vetiver) produced only trace amounts of oil and strikingly different composition compared to the oils from non-cleansed Vetiver plants. Using a culture-based approach, a number of root-associated bacteria have been recently identified. Most of these microorganisms belong to the g subdivision of Proteobacteria. The objective of the present study was to analyze the root-associated community of Vetiveria zizanioides (L.) Nash by molecular approach. An amplified 16S rRNA pool was used to generate a library in pGEM®-T Easy Vector. 100 independent DNA clones were subjected to RLFP analysis (Restriction Fragment Length Polymorphisms) with Hinf1 and EcoRI endonucleases that cleave polymorphic sites in 16S rRNA genes, grouped on the basis of the restriction profile and finally sequenced. This analysis not only confirmed the presence of all cultivated strains, but it also demonstrated the existence of additional bacteria that eluded identification by culture-based approaches.

The present molecular approach led to identification of six additional taxa of Proteobacteria belonging to a, b, and g subdivisions, in addition to a single member of the Fibrobacter/Acidbacteria group. When the mix of amplified 16S rRNA samples from the isolates was enriched with the mix of the DNA clones, a pattern almost identical to that of the 16S rRNA pool from the Vetiver root was reproduced in SSCP (Single-Strand Conformational Polymorphism) experiments. This finding suggested that our analysis was nearly exhaustive. Work is in progress to analyze the capacity of those bacterial isolates to degrade the Vetiver essential oil.


The research was supported by Compagnia di San Paolo special grant “Iniziativa” to L. Del Giudice. P. Pontieri was supported by a postdoctoral grant from Istituto Banco di Napoli-Fondazione.