POSTER SUMMARY

THE APPLICATION OF FLUORESCENT SINGLE-STRAND CONFORMATION POLYMORPHISM (F-SSCP) TO ASSESS THE DIVERSITY OF *BURKHOLDERIA* SPECIES IN SUGARCANE RHIZOSPHERE

OMARJEE J[1] and BALANDREAU J[2]

[1]*South African Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa*
jehhan.omarjee@sugar.org.za
[2]*Ecologie Microbienne, UMR5557 CNRS-Université Lyon 1, Bât. Gregor Mendel, 43, Boulevard du 11 Novembre, 69622 Villeurbanne cedex, France.* balandreau@univ-lyon1.fr

Abstract

The genus *Burkholderia* is the dominant component of the sugarcane rhizosphere microbial community. The *Burkholderia* population is complex and assessments performed on these populations do not always reveal specific members. Moreover, current cultivation based methods are limited because they do not assess the non-culturable fraction of rhizosphere microbiota. The aim of this work was to develop root DNA-based PCR fluorescent single-strand conformation polymorphism (F-SSCP), a molecular method for studying the diversity of microbial communities, for analysis of *Burkholderia* diversity in root samples. Species-specific primers for *Burkholderia* were developed, based on a small region of the 16S rRNA gene sequence. F-SSCP analyses of the PCR products obtained showed that there were sufficient differences in migration to distinguish all 39 *Burkholderia* species tested. For microbial rhizosphere community studies, six-month-old variety N29 from the existing *Burkholderia* field trial at Zinkwazi was used. Roots from plots inoculated with *Burkholderia* isolates N8.2 and LM1-376.8 and from a corresponding control plot were sampled. Total genomic DNA extracted from roots was subjected to PCR and assessed using F-SSCP. F-SSCP profiles on amplicons generated from root DNA were aligned with *Burkholderia* reference species profiles. The root DNA exclusively revealed profiles affiliated with profiles of *Burkholderia* reference species, and demonstrated that the F-SSCP method befits the study of diversity in *Burkholderia* spp. in natural samples. The dominant *Burkholderia* spp. in the root samples were *B. caledonica*, *B. saccharri*, *B. cepacia*, *B. graminis* and *B. ambifaria*. No differences in profiles between control and *Burkholderia* inoculated plots were distinguished.

Keywords: F-SSCP, *Burkholderia* spp., sugarcane rhizosphere

Introduction

Fluorescent Single Strand Conformation Polymorphism (F-SSCP) is based on the principle that the electrophoretic mobility of a single stranded DNA molecule, in a non-denaturing gel, is related to its structure (conformation) and size. In solution, the single stranded molecules take on secondary and tertiary conformations as a result of base pairing between nucleotides within each strand. The conformations are dependent on the length of the strand and the location and number of regions of base pairing. They are also highly dependent on the primary sequence of the molecule, and therefore a mutation at a single nucleotide position in the primary sequence can change the conformation of the molecule (Duthoit *et al*, 2003).

The objective of this study was to assess if the technique can be used for the study of functional bacterial groups in the rhizosphere of sugarcane. If successful, further studies can be conducted to measure the effect of different sugarcane management regimes, adverse environmental conditions and the application of agricultural chemicals, on soil bacterial communities.

Materials and Methods

DNA region of interest (H17)

Balandreau *et al*, 2004 showed that a small region of the 16S, corresponding to nucleotides 454 to 477 (*E. coli* numbering), had a *Burkholderia* species-specific sequence outside the *Burkholderia cepacia* complex. Using this 200 bp region, it seemed possible to study the diversity of *Burkholderia* species (and other bacteria) with F-SSCP in a given niche such as the rhizosphere and plant tissues of sugarcane.

Burkholderia *reference strains and root samples*

Reference strains of 39 known *Burkholderia* species were used for initial analysis. For microbial rhizosphere community studies, root samples from six-month-old sugarcane of variety N29 from an existing *Burkholderia* field trial at Zinkwazi in KwaZulu-Natal were used. The trial was designed to determine the effects of inoculated *Burkholderia* on the growth of sugarcane and their effect on the parasitic nematode community. An ADE-4 analysis showed that the bacterial isolates N8.2 (ARDRA group A) and LM1-376.8 (identified as a nitrogen fixing *B. tropica*) significantly reduced the numbers of *Pratylenchus* and *Meloidogyne* spp. in sett and shoot roots. From these results, it was decided to sample roots from plots inoculated with N8.2 and LM1-376.8 and from a corresponding control plot.

DNA extraction

Burkholderia reference strains were prepared for fluorescent-based PCR by extracting genomic DNA as described by Yabuuchi *et al*. (1992). Genomic DNA from sugarcane roots was extracted using a QIAgen DNeasy Plant mini kit.

Fluorescent-based PCR

The DNA region of interest (H17) was amplified with upstream and downstream primers, which were fluorescent labelled at their 5’ end with HEX or FAM (Proligo Primers and Probes, France). The final PCR reaction volume was 50 µl, containing 25 ng of genomic DNA, 100 ng/µl of each primer, 2.5 mM dNTP and 2.5 U/µl Pfu *Taq* DNA polymerase (Stratagene). The reaction conditions were 2 minutes at 94°C, followed by 25 cycles of 30 seconds at 94°C, 30 seconds at 61°C, 30 seconds at 72°C and a final elongation step of 10 minutes at 72°C.

F-SSCP (capillary electrophoresis)

Prior to analysis, the fluorescent labelled PCR products were diluted 200-fold with Milli-Q water. One micro-litre of the diluted product was mixed with 18.8 µl of Formamide and 0.2 µl of standard GS400 Rox, denatured at 95°C for 5 minutes and chilled on ice. Capillary electrophoresis was performed on the ABI Genetic Analyser 3100 (Applied Biosystems). F-SSCP analysis was performed automatically at a running temperature of 26°C under 12 kv for 30 minutes. A laser induced fluorescent detector was used, which emitted light for FAM

(488 nm) and HEX (515 nm). The data was compiled by data collection software and analysed by Gene-Scan software (Applied Biosystems).

Results and Discussion

Results confirmed that all reference species of *Burkholderia* could be differentiated using F-SSCP, suggesting that identification in a soil or root sample was possible.

F-SSCP profiles of the roots of sugarcane obtained from the *Burkholderia* field trial were not as complex as expected. The profiles were simple and it was not difficult to differentiate between the bacterial communities in the rhizosphere. To identify the dominant bacterial genera in the rhizosphere, clones of the amplified root DNA was prepared. The clones were analysed for F-SSCP and aligned with the rhizosphere profiles to establish whether they were true clones. The clones were sequenced and identified as the genus *Burkholderia*. It was confirmed that *Burkholderia* was the dominant bacterial genus present in the rhizosphere.

To identify the *Burkholderia* population in a root sample, the profiles were compared with *Burkholderia* reference species previously analysed. It was concluded that *B. caledonica*, *B. saccharri*, *B. cepacia*, *B. graminis* and *B. ambifaria* were the dominant species found in the root samples. Furthermore, all root samples obtained from all the plots had a similar profile. This could be due to many factors, one being that drought may have had an impact on the diversity of the microbial rhizosphere community.

The use of F-SSCP also provided the opportunity to determine whether the *Burkholderia* isolates that were inoculated at the planting of the *Burkholderia* field trial, were still present after six months. This was achieved by aligning the profiles of inoculated *Burkholderia* species with those profiles obtained from roots sampled from the respective inoculated plots. The inoculated *Burkholderia* species were not found in the root profile, suggesting that they may have been eliminated by drought or by other resident *Burkholderia* colonisers.

F-SSCP also identified several *Burkholderia* species that have nitrogen fixing and nematicidal. Profiles obtained from reference species of *Burkholderia* were compared with profiles obtained from unknown isolates. The technique allowed for quick and efficient identification as compared with sequencing.

Should this technique be implemented at SASRI, the following assessments could be made:

- Detection and quantification of beneficial fungi and bacteria, mycorrhizae, pathogenic fungi and bacteria in field situations.
- Identification and quantification of nematodes species in soil.
- Detection and identification of endophytic bacteria or fungi in sugarcane plant tissues.

REFERENCES

Balandreau J, Omarjee J and Guyon S (2004). The use of variable regions of 16S for *Burkholderia* taxonomy and detection. *J Microbiol Meth* (in press).

Duthoit F, Godon JJ and Montel M (2003). Bacterial community dynamics during production of registered designation of origin salers cheese as evaluated by 16S rRNA gene single strand conformation polymorphism analysis. *Appl Env Microbiol* 69: 3840-3848.

Yabuuchi E, Kosako Y, Oyaizu H, Yano I, Hotta H, Hashimoto Y, Ezaki T and Arakawa M (1992). Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes, 1981) comb. nov. *Microbiol Immunol* 36: 1251-1275.