ISOLATION AND CHARACTERISATION OF SUGARCANE RHIZOBACTERIA AND THEIR EFFECT ON NEMATODES

J L VOGEL[1], J BALANDREAU[2], T VAN ANTWERPEN[1], P DANA[1] and P CADET[1]

[1]*South African Sugar Association Experiment Station, Private Bag X02, Mount Edgecombe, 4300, South Africa.*
[2]*Ecologie Microbienne UMR5557 CNRS-Université Claude Bernard Lyon I, 43 Boulevard du 11 Novembre 1918, F-69622 Villeurbanne cedex, France*
E-mail: xpatpc1@sugar.org.za

Abstract

A survey showed that species of *Burkholderia* were frequently associated with the roots of sugarcane. Based on amplified ribosomal DNA restriction analysis the isolates of these bacteria belonged to *Burkholderia* groups A,B,C,F and G. Data collected from field trials revealed a succession of species during the growth of the crop. Strains belonging to the *B. cepacia* complex were dominant during the early stages of growth but, in time, were replaced by isolates of the *B. tropicalis* and *B. caribensis* groups. In a series of *in vitro* bioassays it was found that a large proportion of the *Burkholderia* isolates tested paralysed juveniles of a root-knot nematode, *Meloidogyne* sp.

Keywords: Burkholderia, Pseudomonas, nematode biocontrol, sugarcane, rhizosphere.

Introduction

Root feeding nematodes cause a considerable loss in yield of sugarcane in South Africa. Some species cause more damage than others and yield loss appears to be related to the proportions of pathogenic (*Meloidogyne* and *Xiphinema*) to less pathogenic species (*Helicotylenchus*) within the nematode community (Cadet *et al*., 2002). There is a possibility that this balance is controlled by the composition of the bacterial flora associated with sugarcane roots. Soil microorganisms naturally regulate the activity of pathogens, leading to suppressiveness (Rouxel, 1991). The widespread soil bacteria, *Burkholderia* and fluorescent *Pseudomonas*, are very efficient root colonizers even in the presence of indigenous microorganisnms (Schroth and Hancock, 1982). They promote plant growth by preventing or minimising the deleterious effects of pathogenic organisms (including nematodes) through the synthesis of antibiotics, toxins and enzymes. A *B. cepacia* strain (Bc-2) has been shown to inhibit egg hatch and mobility of second-stage juveniles of *M. incognita in vitro* (Meyer *et al*., 2000) and *in vivo* (Meyer *et al.,* 2001). Moreover, it was found that *B. cepacia* and *B. gladioli* were the dominant bacteria obtained from plants antagonistic to *M. incognita* and the soybean cyst nematode, *Heterodera glycines,* but were not present in soybean which is susceptible to both nematodes (Kloepper *et al*., 1992). Reports have also shown that treatment of soils with chitin and powdered pine bark could control plant-parasitic nematodes in cotton and suppress *H. glycines*. It was noted that numbers of *B. cepacia* were higher in the amended soils compared with non-amended soils (Hallmann *et al.*1999, Kokalis-Burelle and Rodríguez-Kábana, 1994).

The objective of the project was to isolate and genotypically characterise *Burkholderia* and fluorescent *Pseudomonas* strains from sugarcane roots and to assess their effect on the juveniles of *Meloidogyne.*

Materials and Methods

Sample sites
Sugarcane root samples were collected from a transect along a cane row with contrasting levels of pathogenic (*Xiphinema elongatum*) and less-pathogenic (*Helicotylenchus dihystera*) nematodes and from sites on the KwaZulu Natal North Coast region where bacterial populations could be influenced by different management practices. These included two variety trials on the La Mercy farm, an organic amendment trial near Tinley Manor and a green manure trial at the Kearsney Research Station farm near Statnger. Bacterial isolates were obtained on semi-selective media PCAT (*Burkholderia*) and S1 (*Pseudomonas* fluorescents).

Characterisation of the bacteria
The *Burkholderia* isolates and 37 reference strains belonging to known species were classified into genotypic groups using amplified ribosomal DNA restriction analysis (ARDRA), using 10U of the restriction enzymes AluI and HaeIII. The length polymorphism of restriction fragments was characterised following electrophoresis.

Bioassays against Meloidogyne *juveniles*
In vitro bioassays were conducted to assess the effect of the bacterial isolates on the survival and movement of *Meloidogyne* juveniles. Eggs were extracted from galled roots of a nematode-susceptible tomato variety (*Lycopersicon esculentum* cv. Red Kaki). The egg suspension was incubated at room temperature. Hatched, second-stage juveniles were collected after 2 days and rinsed with sterile distilled water. Approximately 50 juveniles in 500µl sterile distilled water were added to each well of a 24-well microplate. At the same time a 700µl bacterial suspension, which had been multiplied in TSB at 30°C for 1 day, was added to each well. The behaviour of the juveniles was observed under the microscope at 3, 6 and 9 hours and recorded as a positive reaction if most of the juveniles showed paralysis.

Results and Discussion

The survey showed that species of *Burkholderia* represented 2-7% of cultivable bacteria isolated from the soil and 17-25% of cultivable bacteria isolated from roots. This indicates the close association between *Burkholderia* species and sugarcane and their ability to colonise sugarcane roots in the presence of other indigenous microorganisms. In contrast fluorescent *Pseudomonas* spp were either not present or represented less than 1% of the total cultivable bacteria isolated from the soil and roots.

A total of 980 *Burkholderia* isolates were collected and classified into genotypic groups according to the 37 reference strains of known species. These reference strains could be classified into eight groups. All *B. cepacia* complex strains belonged to Group A, the plant-associated reference strains belonged to Groups B, F and G and the plant and animal pathogens belonged to groups C, D and E. Based on ARDRA, the sugarcane root isolates belonged to groups A,B,C, F and G.

Overall, the majority (30%) of the root isolates belonged to Group F that contains the reference strains of *B. caribensis* and *B. fungorum*. Subsequent restriction digestions indicated that the isolates are probably *B. caribensis*, an exopolysaccharide producing bacterium forming microaggregates in vertisols (Achouak *et al.,* 1999).

The next most common group (27%) was Group G which also contains strains BM273F and BM16. Both are nitrogen-fixing endophytes isolated from maize in Mexico in an area where the traditional cultivation has been maintained for thousands of years, using old Indian varieties and no agrochemicals (Estrada *et al*., 2002).

Twenty percent of the isolates belonged to Group A which contains all *B. cepacia* complex representative strains. This complex is a group of 10 very closely related species which are common in the rhizosphere and tissues of several plants. Two of these species (*B. cepacia* genomovar III and *B. multivorans*) are involved in a dramatic health deterioration in patients affected by cystic fibrosis, the most common genetic disease of caucasians. Genomovar III is common in the rhizosphere (Balandreau *et al*., 2001).

In the transect at the La Mercy farm, the cane roots at 4 months were colonised predominantly by the *cepacia* complex strains (29%) and the *B. tropicalis* group (29%). However, at 9 and 19 months there were fewer *cepacia* complex strains (<9%) and more *B. tropicalis* (36-43%) and *B. caribensis/fungorum* (36-39%). In one of the variety trials at La Mercy, the cane roots at four months were colonised predominantly by *cepacia* complex strains (82%), but at 11 months this group was virtually non-existent (2%) and had been replaced by isolates belonging to the *B. caribensis/fungorum* group (68%).

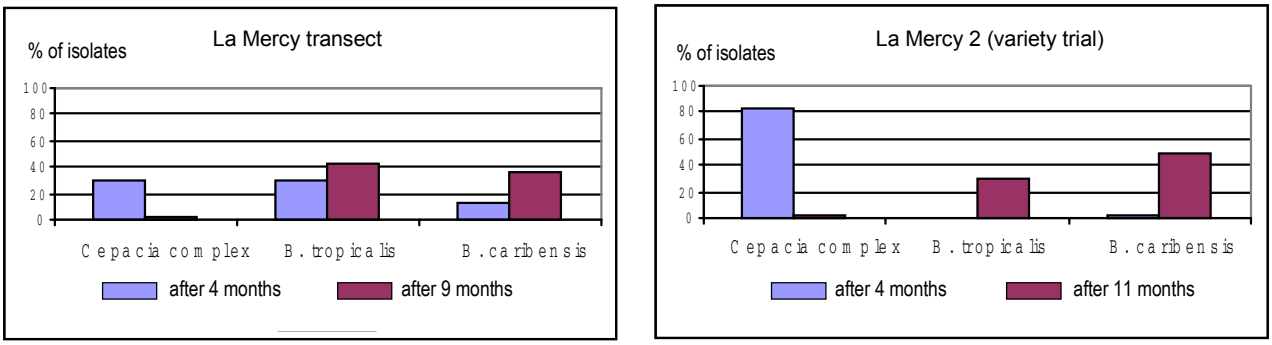

Figure 1. Effect of sugarcane age on the proportions of three groups of *Burkholderia* along the La Mercy transect and in the LM2 variety trial.

A total of 505 *Burkholderia* strains, including members from each genotypic group, and 88 *Pseudomonas* strains were included in the *in vitro* bioassays to determine their effects on the survival and movement of *Meloidogyne* juveniles. Sixty-eight percent of isolates belonging to the *cepacia* complex and between 17% and 29% of *Burkholderia* spp belonging to the other groups paralysed *Meloidogyne* juveniles for up to 9 hours. Only 6% of the *Pseudomonas* isolates paralysed the juveniles. Several of the isolates that inhibited movement of the *Meloidogyne* juveniles also inhibited growth of smut sporidia (*Ustilago scitaminea*) and a stalk rot fungus, *Fusarium* sp (van Antwerpen, unpublished). Examples are given in Table 1.

These preliminary results are indicative of the potential of many *Burkholderia* strains. Obtaining a bacterial inoculant combining an anti-nematode effect with the ability to increase plant growth (possibly through atmospheric nitrogen fixation) or with the ability to control bacterial or fungal diseases of sugarcane is the objective of the next phase of this research.

Table 1. Properties of interest displayed by some *Burkholderia* isolates obtained from the rhizosphere of sugarcane. Strain LM1-376.8 is capable of fixing atmospheric nitrogen.

Isolates	Origin	Taxon	Meloidogyne paralysis	Smut inhibition	Fusarium inhibition
T16.1B	*Xiphinema*-rich point along La Mercy transect	A	+++	+	+++
OAF.2B	Filtercake plot, organic amendment trial (Tinley Manor)	A	+++	+++	+++
OAC.1B	Control plot in organic amendment trial (Tinley Manor)	C	+++	+++	+
GMCP.4B	Cowpea residue enriched soil, green manure trial (Kearsney)	A	+++	+++	+++
N8.1	Variety trial (var N8, resistant to nematodes)	A	+++	+++	+++
TR30.6B	*Helicotylenchus*-rich point along La Mercy transect	F	+++	+++	+++
TR30.8B	*Helicotylenchus*-rich point along La Mercy transect	F	+++	+++	+
LM1-N16.8	Variety trial, var N16 (resistant to *Meloidogyne,* susceptible to *Helicotylenchus*)	F	+++	+++	+++
T37.1B	*Helicotylenchus*-rich point along La Mercy transect	F	0	0	0
T37.3B	*Helicotylenchus*-rich point along La Mercy transect	G	0	0	0
T37.5B	*Helicotylenchus*-rich point along La Mercy transect	C	0	0	0
T39.8B	*Helicotylenchus*-rich point along La Mercy transect of the transect (La Mercy1)	B	0	0	0
T11.1B	*Xiphinema*-rich point along La Mercy transect of the transect (La Mercy1)	A	0	0	0
LM1-376.8	Variety trial, var NCo376 (resistant to *Meloidogyne,* susceptible to *Helicotylenchus*)	Gc	+++	0	0

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