Plant and Soil **145**: 51–63, 1992.

PLSO 9457

Isolation and characterization of fluorescent *Pseudomonas* associated with the roots of rice and banana grown in Sri Lanka

K. VLASSAK[1], L. VAN HOLM[2], L. DUCHATEAU[3], J. VANDERLEYDEN[4] and R. DE MOT[4,5]
[1]*CNPBS/EMBRAPA, Itaguai, Rio de Janeiro, Brazil,* [2]*Institute for Fundamental Studies, Kandy, Sri Lanka,* [3]*Prince Leopold Institute for Tropical Medicine, Antwerp, Belgium and* [4]*F.A. Janssens Laboratory of Genetics, Catholic University of Leuven, B-3001 Heverlee, Belgium.* [5]*Corresponding author*

Received 24 December 1991. Revised May 1992

Key words: antifungal activity, *Pseudomonas fluorescens*, *Pseudomonas putida*, rhizobacteria, tropical crops

Abstract

Bacterial populations in different parts of the rhizosphere of rice and banana in Sri Lanka were examined. On rice, the number of aerobic bacteria and the population of fluorescent bacteria were higher in the rhizoplane as compared to the exorhizosphere. However, the opposite was observed with banana. Percentage of fluorescent bacteria was significantly higher on banana (10.8%) than on rice from the wet and dry zones of Sri Lanka (4.3% and 2.7%, respectively). In the endorhizosphere fraction of rice, bacterial populations were very low. Fluorescent bacteria were absent.

Based on 33 phenotypical tests, 89 fluorescent isolates were grouped into 5 clusters. The three major clusters covered the isolates belonging to the *Pseudomonas fluorescens-putida* group, whereas the remaining small clusters contained other UV-fluorescent bacteria. SDS-PAGE of total cell proteins enabled classification of the isolates into one of 12 different protein-polymorphic types. Only a partial correlation was found between the latter classification and the phenotypical one. Cyanogenesis was observed with strains of *P. fluorescens* only. Isolates *P. fluorescens* RW9S1 and *P. cepacia* RW5P1 displayed a potent antagonism against several fungi.

Introduction

Several studies on bacterial populations within the root environment of plants have shown that the fluorescent *Pseudomonas* constitute a major group of rhizobacteria. Certain isolates of these fluorescent *Pseudomonas*, mainly *P. fluorescens* and *P. putida* strains, can stimulate the growth of several crops and thereby significantly increase their yield. These bacteria were termed plant growth-promoting rhizobacteria (PGPR) by Kloepper et al. (1980). Their beneficial effect is mainly exerted by antagonism of deleterious soil bacteria and phytopathogenic fungi (Kloepper et al., 1988; Lambert and Joos, 1989; Weller, 1988). In most cases, this biocontrol effect has been attributed to the production of antibiotics and/or siderophores in the rhizosphere of the *Pseudomonas*-colonized roots (Leong, 1986; Tomashow et al., 1990). Under certain conditions, suppression of plant pathogens is also brought about by in situ generation of hydrogen cyanide (Voisard et al., 1989).

A major hurdle for the development of commercial PGPR inocula remains their inconsistent performance in field trials. This has been attributed to a multitude of environmental variables introduced under field conditions, that were not

accounted for in the initial screenings (Kloepper et al., 1989; Lambert and Joos, 1989). This inconsistent performance is also due to the low competitiveness of the bacteria with endogenous rhizosphere microorganisms resulting in a poor colonization of the roots (Kloepper and Schroth, 1981; Kloepper et al., 1989; Schroth and Hancock, 1982).

Most research on the association of fluorescent *Pseudomonas* with roots was carried out with plants from temperate regions (De Freitas and Germida, 1990; Kleeberger et al., 1983; Kremer et al., 1990; Lambert et al., 1987, 1990; Miller et al., 1989; Van Outryve et al., 1988; Van Peer et al., 1990). Since much less data are available on the association of these bacteria with tropical plants, their occurrence in different parts of the rhizosphere of rice (*Oryza sativa*) and banana (*Musa* spp.) in Sri Lanka was examined. Representative fluorescent isolates were further characterized, including their capacity to produce antifungal compounds.

Methods

Plant material

Lowland rice plants at the grain-filling stage were sampled from 9 representative fields in the Anuradhapura district (dry zone) and from 10 fields in the Kurunegala district (wet zone) in Sri Lanka (Domross, 1974). At each site, 3 replicate plants were harvested. For banana, 16 root samples were collected from plants grown in home gardens in Peradeniya, Colombo and Galagadera (wet zone).

Isolation of fluorescent bacteria

For each root system, one sample was processed as described below. Root samples were shaken vigorously to remove loosely adhering soil. To isolate bacteria from the exorhizosphere, 1 g of firmly adhering soil was shaken in 100 mL of PBS solution (0.88% (w/v) NaCl, 2.9 m*M* KH_2PO_4, 7.1 m*M* K_2HPO_4, pH 7.2) for 2 h on a rotary shaker at 200 rpm. To obtain the rhizoplane population, the roots were thoroughly washed in running tap water and the root surface population was then extracted by shaking 1 g of washed roots in 100 mL of PBS for 2 h. Bacteria from the endorhizosphere (intercellular spaces accessible to microorganisms) were obtained from macerated roots (1 g) by shaking them in 100 mL PBS for 2 h after surface sterilization (immersion in 70% ethanol for 30 sec and in 4% NaOCl for 3 min). PBS-diluted extracts were plated in triplicate on trypticase soy agar (TSA) and King's B (KB) medium (Palleroni, 1986). After incubation for 24 h at 28°C, counts were made of the total aerobic bacteria on TSA and of the fluorescent bacteria on KB medium. From representative types of fluorescent colonies, bacteria were further purified on KB and stored in 25% glycerol at −80°C.

Prefixes in the respective strain codes refer to the origin of the isolate: banana (BW), rice from the dry (RD) or wet zone (RW). The rhizosphere fraction from which the strain was recovered is coded for by P (rhizoplane), S (exorhizosphere), or M (endorhizosphere).

Phenotypical characterization of the isolates

Fluorescent bacteria were identified through a number of standard microbiological and biochemical tests: Gram staining, growth at 41°C and 4°C, liquefaction of gelatin, assimilation of trehalose and *m*-inositol, and oxidase reaction (Palleroni, 1986). Additional tests (listed in Table 6) were carried out with the API20NE diagnostic strips (API System, La Balme Les Grottes, 38390 Montalieu Vercieu, France). Pectinolytic activity of the isolates on KB plates supplemented with 2.5% (w/v) pectin or polygalacturonic acid was detected as described by Hankin and Anagnostakis (1975). For siderophore production, the CAS plate assay of Schwyn and Neilands (1987) was used. Strains generating hydrogen cyanide from glycine-supplemented KB medium were identified according to Bakker and Schippers (1987). Isolates were screened for fungal antibiosis by the coinoculation method of Schippers et al. (1986) against *Rhizoctonia solani* MUCL 30157, *Pythium ultimum* MUCL 30159, *Fusarium graminearum* MUCL 30161 and *Pyrenophora tritici-repentis* MUCL 30217. Those showing strong inhibition and/or broad-spectrum activity were