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BIOCONTROL OF CASSAVA (MANIHOT ESCULENTA) ROOT ROTS BY FLUORESCENT PSEUDOMONAS

(Control biologique des pourritures racinaires du manioc (manihot esculenta Crantz) par des pseudomonas fluorescents)

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SUMMARY

Forty isolates of fluorescent *Pseudomonas* were isolated from the plants growing in 5 different ecosystems. Thirty-four of these isolates inhibited *Erwinia carotovora* pv. *carotovora*, in vitro, the causal agent of cassava stem rot. One month old plantlets, produced by rooting the shoots of a cultivar in distilled water, were inoculated with suspensions (1 x 10^9 cells/ml) of each *Pseudomonas*. Some isolates were able to increase root weight up to 96 per cent over uninoculated controls three months after planting when the inoculation was at planting, 15 and 30 days. Inoculated plants were free from symptoms of root pathogens and roots swelled earlier than controls. Microbial deterioration of bulked swollen roots was also reduced up to 60 per cent when roots were dip treated in a bacterial suspension (1 x 10 9 cells/ml) of the above isolates and stored for 15 days in polyethylene bags. Taxonomic studies showed that these bacterial isolates were either *Pseudomonas putida* (90 per cent) or *P. fluorescens* (10 per cent).

RESUME

Quarante isolats de Pseudomonasfluorescents ont été isolés à partir de la rhizosphère de plantes provenant de cinq écosystèmes différents. Trente quatre de ces isolats inhibent in vitro Erwinia carotovora pv. Carotovora, agent causal de la pourriture de la tige de manioc. Des jeunes plantes agées de 1 mois, produites par enracinement des pousses d'un cultivar dans de l'eau distillée, sont inoculées avec des suspensions (1 x 10^9 cellules/ml) de chaque Pseudomonas. Quelques isolats sont capables d'augmenter le poids racinaire jusqu'à 96 pour cent par rapport aux plantes témoins non inoculées. Ces résultats sont obtenus 3 mois après la plantation, lorsque l'inoculation a été réalisée au moment

de la plantation, 15 et 30 jours après. Les plantes inoculées sont indemnes de symptômes provoqués par les parasites racinaires et le renflement des racines se produit avant les témoins. La détérioration microbienne de la masse racinaire est également réduite jusqu'à 60 pour cent lorsque les racines sont traitées par trempage dans une suspension bactérienne (1.10 cellules/ml) des isolats précédemment cités puis placés dans des sacs de polyéthylène pendant 15 jours. Les études taxonomiques montrent que ces isolats bactériens sont soit Pseudomonas putida (90 pour cent) soit P. fluorescens 510 pour cent).

Roots rots are the most common and important production constraints among the pathological problems of cassava (3). They are caused by several species of fungi and bacteria (13), which are specific to the edalpho-climatic characteristics of each ecosystem (14).

Some cassava root pathogens have been controlled successfully by applying certain cultural practices, which in many cases are specific to one or more causal agents (15, 18). Their control has also been attempted through varietal resistance ; but due to the complex nature of these problems, selected clones have not performed universally well in all ecosystems (7). Resistance appears to be quite specific to each causal agent ; but as the problem are caused by more than one pathogen, the incorporation of such multiple resistance may been require a long time.

Several beneficial microorganisms have been reported to control root rots on crop species (1,9,19,20). The group of fluorescent *pseudomonas* appears to be the most promising (4,5) because of their nutritional versatility, ability to grow under a wide range of environmental conditions, and ability to colonize successfully the rhizosphere of many plant species (11). This paper reports the effectiveness of strains of *Pseudomonas putida* and *P. fluorescens* in controlling root rots of cassava.

MATERIALS AND METHODS

Strains of fluorescent pseudomonas were isolated from six cassava-growing areas of Colombia (CIAT, Palmira, Carimagua, Mondomo, Quilcace, Caidedonia and Popayan) with distinct edapho-climatic characteristics (Table 1). Samples of active rootlets from 5- to 9- month-old cassava plants were collected from different native clones in each location. Five grams of rootlet segments were washed in tap water and then in distilled water ($d\text{-}h_2O$) for 15 min. Root segments were placed in petri dishes with King B (KB) medium (10) for 24 h at 27°C. Bacterial isolates showing fluorescence on KB under ultraviolet light were purified from single isolated colonies after serial dilution seeding on KB medium.

TABLE 1 : Climatological characteristics at sites where fluorescent pseudomonads were collected

Site		Altitude (Mosl)	Mean Temperature (C)	Rainfall 2 (mm)
CIAT	(IV) 1	1,000	27.8	960
Quilcace	(IV)	900	27.2	1.090
Carimagua	(II)	200	26.2	2.560
Caicedonia	(IV)	1,350	22.3	1.860
Popayan	(V)	1.800	18.0	3.460
Mondomo	(V)	1.580	20.0	3.120

1 CIAT identification zone (2,3)

2 Total rainfall during the actual growing cycle.

Pure isolates were tested for their inhibition of *Erwinia carotovora* pv. *carotovora* (Ecc), which causes cassava bacterial stem rot (12). Ecc suspension (1 ml of a 9 x 10^8 cells/ml) was seeded on nutrient agar (NA) medium in Petri dishes. Sterile paper filter discs (5 mm in diameter) were then soaked in a suspension (1 x 10^9 cells/ml) of each isolated *pseudomonas* strain, settled on the inocculated petri dish and incubated for 24 h at 28°C. The inhibition ability/ strain to Ecc was rated according to the diameter of the halo showing on the medium as negative (-), slight (+ = up to 5 mm), moderate (++ = from 5 to 10 mm), and strong (+++ = more than 10 mm). A similar system was used to evaluate the inhibitory effect of fluorescent *pseudomonas* on strains of the following bacterial species : *Xanthosomonas campestris* pv. *cassavae*, *X. campestris* pv. *manihotis* and *P. solanacearum*. Inhibition of the following cassava fungal pathogens was also tested : *Fusarium oxysporium*, *F. solani*, *Diplodia manihotis*, *Phytophthora drechsleri* and *Pythium* sp. In the case of fungal species, isolates were grown on potato-dextrose-agar (PDA) for 8 days at 27°C. Five-mm- diameter discs of mycelia were placed in the center of a petri dish and surrounded with discs similar to those used to evaluate inhibition of bacterial pathogens. At least 6 discs inoculated with each bacterial strain were used to rate the inhibitory effect on each fungal isolate.

Strains showing inhibitory effect on Ecc were used to inoculate 10-cm high cassava (hybrid CM 523-7) plantlets obtained by the shoot rooting system (6). Ten plantlets per strain were inoculated by dipping their roots for 15 min in a 24-h-old bacterial suspension (1 x 10 cells/ml). Inoculated plants were then planted in plastic pots containing sandy soil ; placed in a greenhouse at 24°C (±9°C), 70 per cent RH and 15,000 lux/12-h daily photoperiod ; and watered daily with tap water. They were harvested 2 months after inoculation; and top and root growth compared.

Strain F-44, which showed the best Ecc inhibition and satisfactory root promotion on hybrid CM 523-7, was used to inoculate cuttings and plantlets of the same hybrid planted in non sterile sandy soil in plastic pots, (7.5 cm diameter x 9 cm high). The cuttings and plantlets (only the root system) were inoculated by dipping them for 15 min in a suspension of 1 x 10^9 cells/ml of the fluorescent *pseudomonas* strain. Pots were maintained under the greenhouse conditions described above.

Strain F-44 was also used to inoculate 20-cm cuttings of the hybrid CM 523-7 with bacterial suspensions at dilutions of 1 x 10^7, 2.8 x 10^8, 8.2 x 10^9, 1.1 x 10^9 and 8.3 x 10 cells/ml. Cuttings were dipped into each bacterial suspension for 15 minutes before planting and maintained as previously described.

Cuttings of CM 523-7 were also inoculated at planting (dip and soil inoculation) and 15 and 30 days afterward with bacterial suspensions of 1 x 10^9 cells/ml of strain

F-44. Dip inoculations was as described above, and soil inoculations at 15 and 30 days of planting were by pouring 20 cm of the suspension around the cutting base. Plants were grown in soil in pots as previously described and maintained in the greenhouse as above.

Taxonomic studies of the isolated strains were carried out including the following tests : cultural growth on KB, PDA and NA media ; morphological observations through an electron microscope (JEOL 100 SX) of 24-h-old cells washed in bidistilled water and stained with phosphotunstic acid 2 per cent, pH 7.0 (2) ; and physiological and biochemical tests related to this group of bacterial species following common bacteriological procedures (2).

All controls used in this study were treated with sterile distilled water following procedures described for each treatment or test.

RESULTS AND DISCUSSION

A total of 136 isolates of fluorescent *pseudomonas* were isolated, 46 (34 per cent) of which were able to inhibit Ecc in vitro. A high percentage (73 per cent) of isolates from Caicedonia inhibited Ecc (Table 2), which is endemic but rarely severe in cassava plantings there. Thus inhibitory fluorescent *pseudomonas* may an important role in preventing Ecc outbreaks. The inhibitory effect of fluorescent *pseudomonas* was also shown against several bacterial and fungal pathogens of cassava and other crop species (Table 3). This effect was quite specific to tested pathogens, but some biotypes induced inhibition to a wide range of pathogens, both bacterial and fungal. Their beneficial action in the rhizosphere should be related to the ability of inhibiting a large number of pathogens. These results are in accordance with WELLER (21) and HOWELL et al. (18), working with other strains known to inhibit fungal pathogens of other crops.

Four groups of strains of fluorescent *pseudomonas* were distinguished in relation to their effect on cassava clone CM 523-7, compared to d-h 0 control, three months after the bacterial inoculation under greenhouse conditions : (a) strains that reduced total plant weight (root and top plant weights) significantly (at 1 pour cent levels) ; (b) strains with no significant influence on plant growth (although treated plants appeared more vigorous than controls) ; (c) strains that induced a significant (at 1 per cent levels) increase in the root weight of treated plants ; and (d) strains that increased both root and aerial plant weight significantly (at 5 per cent levels) (Fig. 1). Such results are in accordance with those of SUSLOW and SCHROTH (17) on sugar beets, as well as those of KLOEPPER et al. (11) and BURR et al. (5) on potatoes. However, the collected strains

TABLE 2 : Source, number of isolates collected and number of isolates of fluorescent pseudomonads that inhibit Erwinia carotovora pv. carotovora (Ecc) in vitro.

	Number of Isolates Inhibiting		
Source	N. of Isolates Collected	E. carotovora pv. carotovora	Collection N of Selected Isolates
CIAT	33	16	44[a]
Popayan	18	7	72[a]
Quilcacé	35	4	65 71[a]
Carimagua	25	7	88
Caicedonia	13	9	84
Mondomo	12	3	

[a] Isolates with strong inhibiting effect on Ecc and greater root growth on plants of the clone CM 523-7 when inoculated for 15 minutes in a bacterial suspension of 1 x 10 9 cells/ml.

TABLE 3 : In vitro inhibition induced to several bacterial and fungal pathogens by some fluorescent pseudomonad strains

Fluorescent Pseudomonad Strains	Bacterial and Fungal Pathogens*								
	a	b	c	d	e	f	g	h	i
C-4a	++**	+++	-	+	++	++	+++	++	+++
C-5b	+	+++	+	+	+	++	++	++	+++
C-6	+	+++	-	-	-	++	++	+	+++
R-1	++	++	-	-	-	+	+++	+++	++
C-4b	++	+++	-	-	+	+	++	+++	+++
C-88	++	+++	++	+	+	+++	+	+++	+
C-3b	++	++	-	-	+	+	+	+++	+++
C-7a	++	+++	+++	-	+	++	++	++	+
C-7d	+	+++	+++	-	++	++	++	+++	++
C-7c	+++	+++	+++	-	+++	+++	+++	+++	+++
C-5a	++	+++	++	+++	+++	++	+++	+++	+++
C-7b	+++	++	+++	-	+	++	+++	++	++
F-44	+++	+++	++	++	+++	+++	+++	++	++
F-64	++	++	++	++	+	+++	+++	++	+++
F-61	++	++	++	-	-	++	++	++	+++
F-71	+	++	++	-	-	++	+++	++	++
F-56	++	++	+	++	++	++	++	+++	++
F-87	+	++	-	+++	++	++	++	++	++

* a = Erwinia carotovora pv. carotovora (CIAT 1144) ; b = Xanthomonas campestries pv. manihotis (CIAT 1111) ; c = Diplodia manihotis (MS 081) ; d = Fusarium oxysporum (MS 079) ; e = Pythium sp. (MS 080) ; f = Xanthomonas campestris pv. cassavae (CIAT 1148) ; g = Xanthomonas campestris pv. oryzae (CIAT 1186) ; h = Pseudomonas solanacearum (CIAT 1057) ; i = Brown blotch bacterial pathogen (CIAT 1187).

** Average of 5 replicates/pathogen. Inhibition effect : - = negative ; + = slight up to 5 mm ; ++ = moderate (from 5 to 10 mm) ; +++ = strong (more than 10 mm).

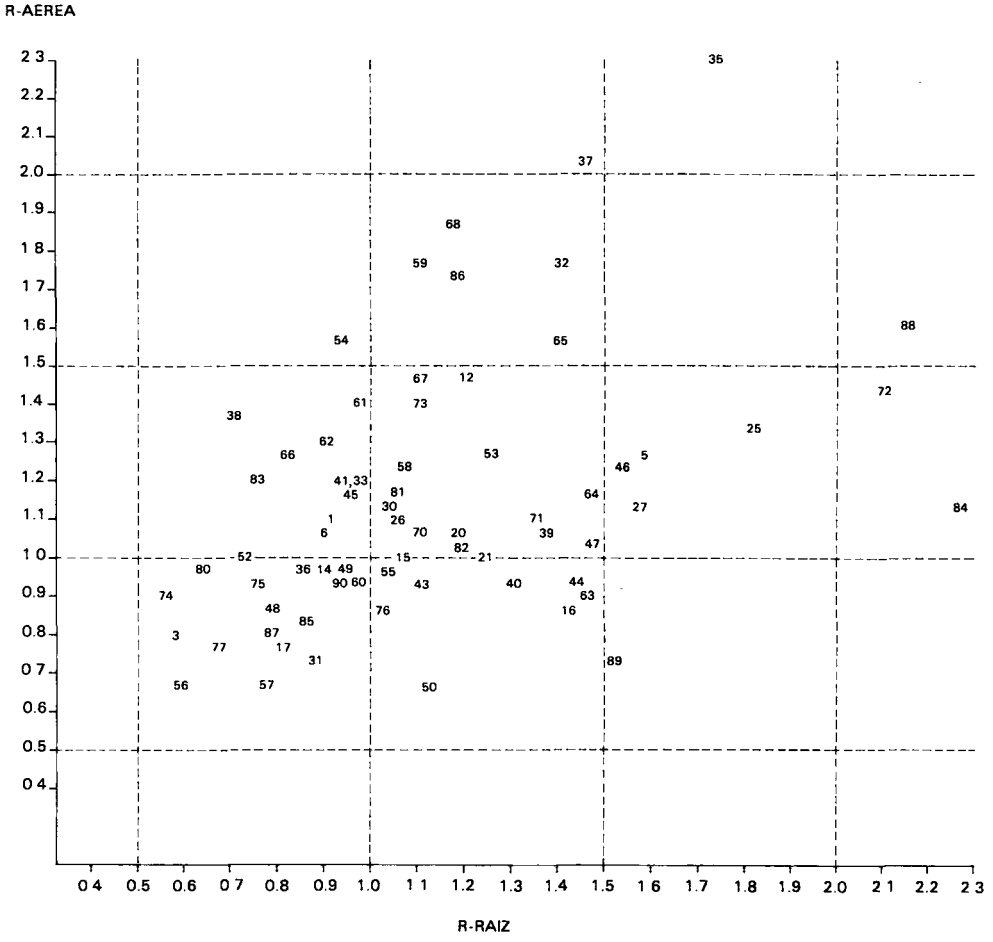

Fig. 1. Effect of fluorescent pseudomonads on cassava plantlets (Clone CM 523-7) two months after inoculation and in relation to controls (10).

of fluorescent *pseudomonas* did not significantly increase the top weight of inoculated plantlets. It appears that their effect is restricted to the root system.

Soil inoculation was highly successful. When soils were inoculated before planting, root weight increased considerably (Table 4). Root weight also increased when cuttings and plantlets were root-inoculated, but significative differences were not found. On the other hand, the weight of the aerial part of plants did not increase when the cuttings and soils were inoculated (Table 4). All inoculated plants showed a healthy and very-developed root system when compared with untreated controls . A high population of fluorescent pseudomonads was reisolated from inoculated plants, whereas on control roots, profuse pathogenic fungal growth was noted.

A significant (1 per cent level) increase in root weight was obtained, regardless of the concentrations of bacterial cells (Table 5). The ability of these bacterial isolates to colonize the substrate early may play an important role on final effects. On the other hand, 95 per cent of root weight increase was obtained when cuttings were inoculated before and after rooting (at 15 and 30 days after planting). Root weight also increased when cuttings and soils were inoculated before planting and when plants were inoculated at 15 or 30 days after planting (Table 6). Generally, these results indicate that for more beneficial effects, additional innoculations after planting might be required to obtain a significant increase in root weight. However, the sucess of inoculations may be a function of the complex environment of the rhyzosphere.

Taxonomic studies showed that around 94 per cent of the isolates collected belonged to *P. putida* and the rest to *P. fluorescens*. Other studies (5,8,16,17,21) also report these bacterial species to be the most commonly found in the rhizosphere of other crops.

The above results suggest that there is a group of fluorescent pseudomonad strains that promote root development in cassava. It appears that there is no nutritional effect ; if there is, it is very low. Some isolates inhibit detrimental rhizosphere microorganisms in vitro. Results thus far are very encouraging, and biological control for the root rots commonly found in any cassava plantation appears very promising.

TABLE 4. Weight of the roots and aerial part two months after inoculation of cuttings of clone CM 523-7 with isolate N.44 of Pseudomonas putida. Cuttings of the same size, weight and thickness were used for all treatments. A bacterial suspension of approximately 10^9 cells/ml was used.

Inoculation Systems	Root wt (g)* At 2 months	Wt (g) of aerial part At 2 months
Of cuttings**	5.84 b (8.2)****	8.73 [a]
Soil***	8.49 a (57.3)	9.20 [a]
Control	5.40 b	9.28 [a]

* Data represent an average of 20 plants/inoculation system.

** Inoculation of the cuttings was done by immersion of the cutting material.

*** Soil was inoculated by pouring 10ml of inoculum/plant 15 days after planting the cuttings.

**** Increase in the percentage with respect to the control. Figures followed by the same lettter(s) do not differ significantly according to the Duncan Multiple Range Test.

TABLE 5. Weight of the root system of the aerial part of the plants of clone CM 523-7 inoculated with strain N.44 Pseudomonas putida at different concentrations.

Inoculum concentration* (N. cells/ml)	Root Wt. (g)	Wt. of Aerial Part (g)
1.0×10^7	6.28 a** (46.4)***	12.20 a
2.8×10^8	6.05 a (41.1)	10.65 a
8.2×10^8	6.36 a (48.2)	11.90 a
1.1×10^9	6.38 a (48.8)	12.59 a
8.3×10^9	6.45 a (50.3)	11.62 a
Control	4.29 b	9.84 a

* Cuttings were inoculated before planting by imersion in the inoculum for 15 minutes.

** Figures followed by the same letter(s) are not significantly different at the 0.05 level of the Duncan Multiple Range Test. Data correspond to the average of 20 inoculated stakes/treatment.

*** Percentage increase with respect to the control.

TABLE 6. Weight of the roots, the aerial part and the total plant from clone CM 523-7 two months after inoculation with the isolate N. 44 Pseudomonas putida (1 X 10^9 cells/ml).

Treatments (inoculations)*	Root wt. (g)	Percentage Increase with Respect to the Control	Wt.of Aerial Part (g)
At planting and at 15 and 30 days	9.27 a**	94.7b	12.27a
At planting and at 15 days	7.06 ab	48.4 12.64 a	
At planting	6.14 b	28.9	12.58 a
Control	4.76 c		10.74 ab

* Inoculation was done by immersion of the stakes in the inoculum for 15 minutes before planting and pouring the inoculum over the soil around the stakes at different periods.

** The data correspond to averages of 10 plants/treatment. Figures followed by the same letter(s) are not significantly different according to the Duncan Multiple Range Test.

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