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# The role of siderophores in potato tuber yield increase by Pseudomonas putida in a short rotation of potato

P.A.H.M. BAKKER[1], J.G. LAMERS[2], A.W. BAKKER[1], J.D. MARUGG[3], P.J. WEISBEEK[3] and B. SCHIPPERS[1]

[1] Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, the Netherlands
[2] Research Station for Arable Farming and Field Production of Vegetables (PAGV), Edelheltweg 1, 8200 AK Lelystad, the Netherlands
[3] Department of Molecular Cell Biology, State University of Utrecht, Padualaan 8, 3584 CH Utrecht, the Netherlands

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**Abstract**

The effect of treatment of potato seed tubers with *Pseudomonas putida* isolate WCS358 on tuber yield was studied in different crop rotations at the Experimental Farm 'De Schreef', near Lelystad. With untreated plants, tuber yield in a 1:3 (short) rotation compared to yield in a 1:6 (long) rotation of potato was decreased by 11% at 86 days (seed tuber harvest) and by 14% at 130 days (ware potato harvest) after seeding. Seed tuber treatment with the wild-type isolate WCS358 increased tuber yield with 13% in a short rotation of potato 86 days after seeding, whereas a siderophore-negative Tn*5* transposon mutant of this isolate had no effect on tuber yield. Seed tuber treatment with the wild-type isolate or the siderophore-negative mutant in a long rotation of potato had no effect on tuber yield. At 130 days after seeding no effect of any of the seed tuber treatments was found in both short and long rotations of potato.

Root colonization by siderophore-producing Tn5 transposon mutants of WCS358 was decreased at the end of the growing season. No difference in root colonization between siderophore-producing and siderophore-negative Tn*5* transposon mutants was found at 130 days after seeding.

Siderophore production seems to be a prerequisite in potato tuber yield increase by WCS358 under field conditions. This is the first time that the involvement of siderophores in growth stimulation has been demonstrated in the field.

*Additional keywords:* Tn*5* transposon mutants, root colonization.

## Introduction

Potato growth stimulation after seed tuber treatment with selected fluorescent pseudomonads under field conditions has been reported several times (Burr et al., 1978; Kloepper et al., 1980a; Geels and Schippers, 1983c; Geels et al., 1986). In the Netherlands this growth stimulation is effective only in crops liable to a decrease in yield with increasing cropping frequency (short rotations of potato) and not in long rotations of potato (Schippers et al., 1985). This was demonstrated in pot experiments (Geels and Schippers, 1983b) and under field conditions (Geels and Schippers, 1983c; Geels et al., 1986).

Siderophore-mediated competition for $Fe^{3+}$ ions plays an important role in growth stimulation by fluorescent pseudomonads in pot experiments (Kloepper et al., 1980b).

The purpose of the present study was to assess the role of siderophores in potato tuber yield increase by *Pseudomonas putida* isolate WCS358 under field conditions, using a siderophore-negative Tn*5* transposon mutant of this isolate (Marugg et al., 1985). Root colonization by WCS358 throughout the growing season was studied using siderophore-producing Tn*5* transposon mutants of this isolate which were resistant to kanamycin and streptomycin.

## Material and methods

*Pseudomonas putida isolate.* The unchanged *P. putida* isolate WCS358 was used as the wild-type isolate. Several properties of this isolate have been studied extensively (Geels and Schippers, 1983a,b; Marugg et al., 1985; Geels et al., 1986; Van der Hofstad et al., 1986; De Weger et al., 1986). JM217 is a siderophore-negative Tn*5* transposon mutant of WCS358 (Marugg et al., 1985). C173 and B243 are Tn*5* transposon mutants of WCS358 which do not differ from the wild-type with regard to siderophore production and specific growth rate in King's medium B (KB; King et al., 1954) and minimal medium (Evans et al., 1970) with 11 mM glucose as the carbon source. Their resistance against kanamycin and streptomycin is stable for at least nine generations (P.A.H.M. Bakker en B. Schippers, unpublished results). The bacteria were grown from freeze-dried cultures. WCS358 was grown on KB and the Tn*5* transposon mutants on KB supplemented with 50 mg kanamycin sulfate $l^{-1}$.

*Bacterization of seed tubers.* Pregerminated potato *(Solanum tuberosum* L. cv. Bintje) seed tubers were treated in the laboratory with pseudomonads according to the method described by Geels et al. (1986). After treatment the tubers were stored at 4 °C until they were seeded. The number of colony forming units (cfu) on the treated tubers was determined 4 h after seed tuber treatment and 6 days after storage according to the method described by Geels and Schippers (1983a).

*Field plots.* Plots were established in two different potato rotation fields at the Experimental Farm 'De Schreef' in 1985: a 1:6 rotation (2a) in which potatoes are cropped once every six years and a 1:3 rotation (3c) in which potatoes are cropped once every three years. In rotation 2a the previous crops were summer barley, peas, winter wheat, flax and grass seed, respectively, while in rotation 3c they were summer barley and grass seed (Hoekstra, 1981). In both rotations grass seed was the last crop before the experiment was started. Characteristics of the soil are described by Hoekstra (1981) and Geels et al. (1986).

*Effects of bacterization on tuber yield.* In the two rotations seed tubers treated with 1% (w/v) sodium carboxymethylcellulose (CMC), WCS358 or JM217 were seeded on 6 May 1985 in different plots using a randomized block design. The design of the plots was as described by Geels et al. (1986). Each plot contained 20 treated plants divided over four rows and was guarded by 14 non-treated plants. Each treatment was repeated in four plots. Tubers were harvested at 86 (seed potatoes) and 130 days (ware potatoes) after seeding. Tuber fresh weight was determined after removal of adhering soil.

*Bacterial colonization of the roots.* In the 1:3 rotation, seed tubers treated with a bacterial suspension containing a mixture of $5.10^8$ cfu of C173 and $5.10^8$ cfu of B243 $ml^{-1}$, were seeded in a plot containing 80 plants divided over four rows. The root systems of 16 plants were dug up 26, 57 and 104 days after seeding. The roots were collected in cooled boxes and stored at 0 °C. After storage for 12 hours, 1 -cm-long root pieces were selected at random at three depths from the stem base (0-5, 20 and 40 cm). At 130 days after seeding, roots of five plants treated with C173 and B243 and roots of five plants treated with JM217 in a 1:3 rotation were collected. Root pieces of 1 cm length were selected at random at 0-5 cm from the stem base.

The root pieces were shaken vigorously for 30 s in glass test tubes containing 2.5 g 3-mm diameter glass beads and 2 ml sterile 0.1% (w/v) proteose peptone in distilled water. Suspensions were dilution plated on tryptic soy agar (TSA) and KB (Geels et al., 1986) for determining the number of cfu of the total aerobic bacterial population and the total population of fluorescent *Pseudomonas* spp., respectively. The number of cfu of Tn5 transposon mutants of WCS358 was determined by dilution plating on KB supplemented with 200 mg kanamycin sulfate and 200 mg streptomycin sulfate per liter.

*Statistics.* Results were analysed by analysis of variance, when necessary after log or arcsine transformation. The Student's t-test was used to calculate minimum significant difference (MSD). In case of heterogeneity of variances or nonnormal distribution the Kruskal-Wallis test was used followed by nonparametric multiple comparisons by STP (Sokal and Rohlf, 1981).

## Results

*Pseudomonads on treated tubers during storage.* The number of cfu of WCS358, JM217 and C173 + B342 on the periderm of treated seed potatoes decreased, but not significantly, during storage at 4 °C for 6 days (Table 1).

*Effects of bacterization on tuber yield.* Potato tuber yields at 86 and 130 days after seeding are presented in Table 2. In the control treatments a yield reduction of 11% was observed in the 1:3 rotation compared to the yield in the 1:6 rotation, at 86 days after seeding. Seed tuber treatment with WCS358 in the 1:3 rotation increased tuber

Table 1. Number of cfu of pseudomonads in suspensions used for treatment of seed potatoes and on periderm of seed tubers treated with WCS358, JM217 or C173 + B243, 4 h after treatment and 6 days after storage at 4 °C.

| Treatment | Number of cfu $ml^{-1}$ suspension | Number of cfu $cm^{-2}$ of tuber periderm | |
|---|---|---|---|
| | | 4 h | 6 days |
| WCS358 | $1.4 .10^9$ | $2.5 .10^3$ | $1.5 .10^3$ |
| JM217 | $7.3 .10^8$ | $1.6 .10^4$ | $7.2 .10^3$ |
| C173/B243 | $8.7 .10^8$ | $4.7 .10^3$ | $3.9 .10^3$ |

Table 2. Tuber fresh weight 86 (seed potatoes) and 130 days (ware potatoes) after seeding in 1:3 and 1:6 rotations of potato field plots after different seed tuber treatments.

| Treatment | Tuber fresh weight kg are$^{-1}$) | |
|---|---|---|
| | 86 days[2] | 130 days[3] |
| **3C (1:3 rotation)** | | |
| control[1] | 334 a[4] | 535 a |
| WCS358 | 372 b | 551 a |
| JM217 | 339 a | 534 a |
| **2A (1:6 rotation)** | | |
| control | 376 b | 625 b |
| WCS358 | 377 b | 634 b |
| JM217 | 374 b | 641 b |

[1] Control: 1% CMC.
[2] MSD = 28.
[3] MSD = 65.
[4] Values with the same letter are not significantly different at p = 0.05, based on Student's t-test.

yield significantly to the level of the control in the 1:6 rotation, whereas the siderophore-negative mutant JM217 had no effect. Seed tuber treatment with WCS358 or JM217 had no effect on tuber yield in the 1:6 rotation. At 130 days after seeding a decrease of 14% in tuber yield was observed in the 1:3 rotation compared to the 1:6 rotation. In both rotations seed tuber treatment with WCS358 or JM217 had no effect on final tuber yield.

*Bacterial root colonization.* Data on bacterial root colonization are presented in Fig. 1. The number of cfu of the total aerobic bacteria population, as determined on TSA, did not differ at different depths but was decreased significantly at day 104. The total population of fluorescent pseudomonads was similar at different depths and did not change throughout the growing season. The number of cfu of Tn*5* transposon mutants was different at different depths and in time. At day 26 the population at 20 and 40 cm depth was significantly smaller than the population at 0-5 cm and after 104 days the population at 20 cm depth was significantly smaller than the population at 0-5 cm depth. The population at 0-5 cm decreased significantly in time.

As a consequence of the changes in numbers of cfu of the bacterial populations the Tn*5* transposon mutants of WCS358, expressed as percentage of the total population of fluorescent *Pseudomonas* spp., decreased significantly in time at 20 and 40 cm depth (Table 3). The relative density of the total population of fluorescent *Pseudomonas* spp., however, expressed as a percentage of the total population of aerobic bacteria, had increased significantly at day 104 (Table 3).

At 130 days after seeding no differences in numbers of cfu of JM217 ($2.0 \cdot 10^4$ cfu $g^{-1}$ root fresh weight) and C173 + B243 ($6.5 \cdot 10^3$ cfu $g^{-1}$ root fresh weight) were observed at a depth of 0-5 cm. The number of cfu on lower root segments could not be determined because of disintegration of the root system by this time.

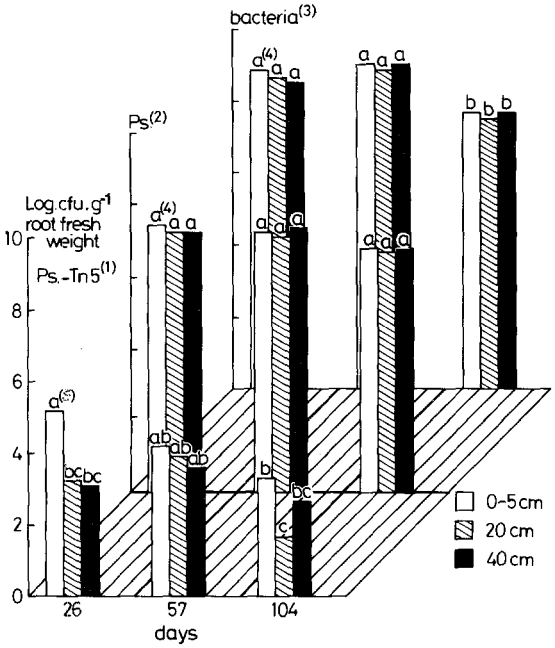


Fig. 1. Bacterial colonization of potato roots in a 1:3 rotation field plot after seed tuber treatment with kanamycin- and streptomycin-resistant Tn*5* mutants of *P. putida* isolate WCS358. Bacterial population densities were determined at 26, 57 and 104 days after seeding at different depths from the stem base.

(1) Ps.–Tn*5*; Tn*5* transposon mutants (C173 + B243) of WCS358.

(2) Ps.; total population of fluorescent pseudomonads.

(3) Bacteria; total population of aerobic bacteria.

(4) and (5) Bars with the same letter are not significantly different at $p = 0.05$, based on Student's t-test (4) or on nonparametric multiple comparisons by STP (5).

## Discussion

In this study the prerequisite of siderophore production for potato yield increases after seed tuber treatment with *P. putida* is demonstrated for the first time in the field. Whereas WCS358 increased tuber yield in the 1:3 rotation 86 days after seeding, the siderophore-negative Tn*5* transposon mutant of this isolate (JM217) had no effect. This agrees with observations in pot experiments in which WCS358 stimulated root development in short rotation soil, whereas JM217 had no such effect (P.A.H.M. Bakker and B. Schippers, unpublished results). Absence of growth stimulation by JM217 is obviously not due to a decreased root colonizing ability, since root colonization by JM217 was not impaired compared to that by siderophore-producing mutants of WCS358 (C173 + B243). This rootcolonizing ability of JM217 under the $Fe^{3+}$-limited conditions in the field, despite its inability to produce its siderophore, can be explained by observations that the mutant is capable to use siderophores produced by other micro-organisms in the rhizosphere (P.A.H.M. Bakker and B. Schippers, unpublished results).

Table 3. Bacterial colonization of potato roots in a 1:3 rotation field plot after seed tuber treatment with kanamycin- and streptomycin-resistant Tn*5* transposon mutants of *P.putida* isolate WCS358. The introduced bacteria (Tn5 mutants) are expressed as a percentage of the total population of fluorescent pseudomonads and the total population of fluorescent pseudomonads is expressed as a percentage of the total population of aerobic bacteria.

| Population fraction[1] | Depth from stem base (cm) | Days after seeding | | |
|---|---|---|---|---|
| | | day 26 | day 57 | day 104 |
| $\frac{\text{Ps.–Tn5}}{\text{Ps}} \times 100$ | 0-5 | 0.95 a[2] | 0.20 ab | 0.24 ab |
| | 20 | 0.20 ab | 0.48 ab | 0.06 b |
| | 40 | 1.44 a | 0.14 b | 0.06 b |
| $\frac{\text{Ps}}{\text{bacteria}} \times 100$ | 0-5 | 3.65 a | 1.39 a | 16.95 b |
| | 20 | 4.42 a | 2.69 a | 20.02 b |
| | 40 | 4.93 a | 2.92 a | 14.19 b |

[1] For explanation of notations of bacterial populations, see Fig. 1.
[2] Per fraction values with the same letter are not significantly different at $p=0.05$, based on Student's t-test.

No effect of seed tuber treatment with *P. putida* isolate WCS358 was observed at ware potato harvest (130 days after seeding) in the present study, whereas a 13% increase in tuber yield was observed at 86 days after seeding. These results are in agreement with the obervations by Geels et al. (1986) that treatment of seed tubers with pseudomonads was mainly effective until seed tuber harvest. Significant increases in yields of ware potatoes (12%) were only observed in 1981 (Geels et al., 1986). A possible explanation for the disappearance of growth stimulation in the present study is the concurrent reduction of the population densities of the introduced bacterial isolate on the roots. Recently, a role for HCN-producing pseudomonads in yield decreases in short rotations of potato was suggested (Schippers et al. 1986 a,b). In the present study the total population of fluorescent pseudomonads, expressed as a percentage of the total aerobic bacteria population, was shown to increase significantly with time (Table 3). This could imply that the relative numbers of HCN-producing pseudomonads increase at the end of the growing season, thereby counteracting the yield increase induced by WCS358 earlier in the season.

The use of kanamycin- and streptomycin-resistant Tn5 transposon mutants for studying root colonization in the field proved to be successful. The mutants could be recovered throughout the growing season and changes in their numbers could be detected easily. The resistance against kanamycin and streptomycin is stable under in vitro conditions. The used mutants are comparable to the wild-type isolate with regard to their specific growth rate, indicating that the fitness of the mutants is similar to that of the wild-type isolate.

The decreasing root colonization by WCS358 seems to interfere with the possible application of seed tuber bacterization in potato production. Our research will therefore focus on the root-colonizing ability of growth-stimulating pseudomonads. However, we need to know more about properties involved in the process of root colo-

nization. The availability of Tn5 mutants defective in different properties will be very useful in these studies.

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**Samenvatting**

*De rol van sideroforen bij verhoging van de aardappelopbrengst door Pseudomonas putida in nauwe aardappelrotaties*

De invloed van een behandeling van aardappelpootgoed met *Pseudomonas putida* isolaat WCS358 op de knolopbrengst werd onderzocht in verschillende gewasrotaties op een proefveld van proefboerderij 'De Schreef', Flevopolder. In de controlebehandelingen werd in een nauwe aardappelrotatie (1:3) een reductie van 11% in opbrengst van pootaardappelen (86 dagen na het poten) geconstateerd ten opzichte van een ruime aardappelrotatie (1:6); 130 dagen na het poten werd een vermindering met 14% gevonden in de opbrengst van consumptieaardappelen.

Pootgoedbehandeling met het siderofoorproducerende isolaat WCS358 verhoogde de opbrengst van pootaardappelen in de 1:3-rotatie met 13%. Een Tn5-transposonmutant van dit isolaat die het vermogen sideroforen te produceren had verloren, had geen effect op de opbrengst. In de 1:6-rotatie had behandeling van pootgoed met WCS358 geen effect op de opbrengst van pootaardappelen.

Zowel in de nauwe (1:3) als in de ruimte (1:6) rotatie werd (130 dagen na het poten), geen effect van behandeling van pootgoed met WCS358 op de opbrengst van consumptieaardappelen gevonden.

Wortelkolonisatie door siderofoorproducerende Tn5-transposonmutanten van WCS358 nam aan het eind van het seizoen af. Er werd, 130 dagen na het poten, geen verschil in wortelkolonisatie geconstateerd tussen siderofoorproducerende en siderofoornegatieve Tn5-transposonmutanten.

Siderofoorproduktie blijkt een voorwaarde te zijn voor verhoging van de knolopbrengst door WCS358 onder veldomstandigheden. De verhoging van de knolopbrengst treedt alleen op in de nauwe aardappelrotatie. Dit is de eerste keer dat de betrokkenheid van sideroforen bij groeistimulatie onder veldomstandigheden is aangetoond.

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