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Identification of the Endophytic Bacterial Isolates and their *in vitro* and *in vivo* Antagonism against *Ralstonia solanacearum*

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Seventy-three endophytic bacterial isolates obtained from the rootstocks of *Solanum* sp., widely distributed in Hanoi, Vietnam, were applied to Vitek32 system for their identification. Out of these, 29 isolates were tentatively identified as *Chryseobacterium indologenes, Pseudomonas fluorescens, Chryseomonas luteola, Bacillus pumilis, B. megaterium, B. sphaericus, Sphingomonas paucimobilis, Enterobacter cloacae, Pantoea agglomerans,* and *Staphylococcus auricularis* with the similarity ranging from 97 to 99% to the species covered in the system. Eight isolates with similarity less than 97% were also tentatively put into the relevant genus. However, the remaining 36 isolates could not be identified by this system. Thirty-seven identifiable isolates were evaluated for *in vitro* antagonism against *Ralstonia solanacearum* and disease suppression of bacterial wilt of tobacco. Thirteen isolates formed growth inhibition zones around their colonies placed on lawn of *R. solanacearum* as an indicator. The variation of anti-*R. solanacearum* activity spectrum within the endophytic bacterial species and isolates suggests that there could be different antibacterial substances participated in the antagonism. Most of the endophytic bacterial isolates have not significantly shown disease suppression. There was no significant correlation between antibiotic activity *in vitro* and disease suppression. For example, the tobacco plants treated with the isolate KuTox708, which was the least inhibitory to *R. solanacearum in vitro*, were significantly protected from bacterial wilt infection.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is a soil-borne plant disease of substantial economic importance because it is endemic in most tropical and subtropical countries. Moreover, its wide host range is ceaselessly increasing and the means of general control of the disease are still limited. Some success has been achieved by using bacterial tolerant commercial cultivars of tobacco and groundnut. However, due to the oligogenic nature of the plant resistance and to the great variation in aggressiveness of bacterial isolates from different locations around the world, alternative control measures such as biological control have been investigated with an increased interest recently.

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Biological control of bacterial wilt of different crops has been reported. Kempe and Sequeira (1983) have tested avirulent strains of *Pseudomonas solanacearum* to induce cross protection in potato against virulent strains of the pathogen. In addition, Chen *et al.* (1984) and Trigalet *et al.* (1994) reported the efficacy of an avirulent bacteriocin–producing strain of *P. solanacearum* on the control of bacterial wilt of tobacco and tomato. Furthermore, antagonistic rhizosphere bacteria such as *Bacillus* sp. (Celino and Gottlieb, 1952), *P. fluorescens* (Aspiras and de la Cruz, 1986; Anuratha and Gnanamanickam, 1990) and *P. glumae* (Furuya *et al.*, 1991) have also been used for the suppression of bacterial wilt of tomato, potato, eggplant and banana. However, the biocontrol potential of bacterial wilt of tobacco by endophytic bacteria has not been intensively investigated.

Endophytic bacteria are bacteria living in plant tissues without doing substantive harm or gaining benefit other than securing residency (Kado, 1992). As cited extensively by Kobayashi and Palumbo (2000), endophytic bacteria have been isolated from both monocotyledonous and dicotyledonous plants that range from woody tree species to herbaceous crop plants. In the previous study (Long *et al.*, 2003), *Solanum* sp. was found to be a good source of endophytic bacteria, because many bacterial endophytes of gram positive and gram negative have been isolated from the rootstocks. In this study, identification of the endophytic bacterial isolates from *Solanum* sp. was conducted and their capacity for inhibiting growth of *R. solanacearum in vitro* and suppressing bacterial wilt of tobacco *in vivo* were also investigated.

MATERIALS AND METHODS

Endophytic bacteria

Endophytic bacteria were isolated from *Solanum* sp. which is widely distributed in Hanoi, Vietnam (Long *et al.*, 2003). Seventy–three endophytic bacterial isolates (Table 1) was used in this experiment. Each isolate was cultured on the slant of YPDA medium (yeast peptone dextrose agar medium; 0.6 g peptone, 3 g dextrose, 3 g yeast extract and 15 g agar/l, pH 7.2) at 30 °C for 2 to 3 days, suspended in sterile distilled water at the concentration of ca. 10^9cfu/ml in test tubes, and preserved at room temperature. For long–term preservation, the bacteria were lyophilized with 10% skim–milk containing 0.05% L–glutamic acid and kept at 4 °C. Each isolate was preliminarily grown on the YPDA slant at 30 °C for 48 hr, suspended in 10 ml of sterile distilled water, added to 200 ml of YP broth in Sakaguchi flask, and shaken at 30 °C for 48 hr. The bacterium was collected by centrifugation at 6,000×*g* for 20 min, and resuspended in sterile distilled water at the concentration of ca. 10^9cfu/ml. The bacterial suspension was then used for protecting tobacco plants from the infection with *R. solanacearum*.

Ralstonia solanacearum

R. solanacerum C319 isolated from *Nicotiana tabacum* in Kagoshima Tobacco Experimental Station was used in this study. The strain was maintained in sterile distilled water at room temperature. Stock suspension was spread on TTC medium (triphenyl tetrazolium chloride medium; 1 g casamino acid, 10 g peptone, 5 g glucose, 17 g agar/l and 5 ml of a 1% stock solution of 2–3–5 triphenyl tetrazolium chloride) and incubated at 30 °C for 48 hr in order to select virulent colonies. Typical virulent colony was selected and

Table 1. Origin of the endophytic bacterial isolates from the rootstocks of *Solanum* sp.

Isolates	*Solanum* sp. sampling		Isolates	*Solanum* sp. sampling		Isolates	*Solanum* sp. sampling	
	Location[a)]	Date		Location	Date		Location	Date
KuTox101	Thanh Xuan	01/10/2002	KuTox508	Thanh Tri	22/10/2002	KuTox901	Gia Lam	21/09/2002
KuTox102	Thanh Xuan	01/10/2002	KuTox509	Thanh Tri	22/10/2002	KuTox902	Gia Lam	21/09/2002
KuTox201	Thanh Tri	22/10/2002	KuTox510	Thanh Tri	22/10/2002	KuTox903	Gia Lam	21/09/2002
KuTox202	Thanh Tri	22/10/2002	KuTox511	Thanh Tri	22/10/2002	KuTox904	Gia Lam	21/09/2002
KuTox203	Thanh Tri	22/10/2002	KuTox512	Thanh Tri	22/10/2002	KuTox905	Gia Lam	21/09/2002
KuTox204	Thanh Tri	22/10/2002	KuTox513	Thanh Tri	22/10/2002	KuTox1001	Gia Lam	23/05/2002
KuTox301	Thanh Tri	22/10/2002	KuTox514	Thanh Tri	22/10/2002	KuTox1002	Gia Lam	23/05/2002
KuTox302	Thanh Tri	22/10/2002	KuTox601	Thanh Tri	22/10/2002	KuTox1003	Gia Lam	23/05/2002
KuTox303	Thanh Tri	22/10/2002	KuTox602	Thanh Tri	22/10/2002	KuTox1101	Gia Lam	23/05/2002
KuTox304	Thanh Tri	22/10/2002	KuTox701	Thanh Tri	22/10/2002	KuTox1102	Gia Lam	23/05/2002
KuTox305	Thanh Tri	22/10/2002	KuTox702	Thanh Tri	22/10/2002	KuTox1103	Gia Lam	23/05/2002
KuTox306	Thanh Tri	22/10/2002	KuTox703	Thanh Tri	22/10/2002	KuTox1201	Gia Lam	23/05/2002
KuTox307	Thanh Tri	22/10/2002	KuTox704	Thanh Tri	22/10/2002	KuTox1202	Gia Lam	23/05/2002
KuTox308	Thanh Tri	22/10/2002	KuTox705	Thanh Tri	22/10/2002	KuTox1203	Gia Lam	23/05/2002
KuTox401	Thanh Tri	22/10/2002	KuTox706	Thanh Tri	22/10/2002	KuTox1301	Gia Lam	23/05/2002
KuTox402	Thanh Tri	22/10/2002	KuTox707	Thanh Tri	22/10/2002	KuTox1302	Gia Lam	23/05/2002
KuTox403	Thanh Tri	22/10/2002	KuTox708	Thanh Tri	22/10/2002	KuTox1303	Gia Lam	23/05/2002
KuTox404	Thanh Tri	22/10/2002	KuTox709	Thanh Tri	22/10/2002	KuTox1401	Gia Lam	23/05/2002
KuTox501	Thanh Tri	22/10/2002	KuTox710	Thanh Tri	22/10/2002	KuTox1402	Gia Lam	23/05/2002
KuTox502	Thanh Tri	22/10/2002	KuTox711	Thanh Tri	22/10/2002	KuTox1403	Gia Lam	23/05/2002
KuTox503	Thanh Tri	22/10/2002	KuTox712	Thanh Tri	22/10/2002	KuTox1501	Gia Lam	23/05/2002
KuTox504	Thanh Tri	22/10/2002	KuTox713	Thanh Tri	22/10/2002	KuTox1502	Gia Lam	23/05/2002
KuTox505	Thanh Tri	22/10/2002	KuTox801	Gia Lam	21/09/2002	KuTox1503	Gia Lam	23/05/2002
KuTox506	Thanh Tri	22/10/2002	KuTox802	Gia Lam	21/09/2002	KuTox1601	Gia Lam	23/05/2002
KuTox507	Thanh Tri	22/10/2002						

[a)] Districts in Hanoi, Vietnam

grown in PS broth (potato semi-synthetic broth; 5 g peptone, 15 g sucrose, 2 g $Na_2HPO_4 \cdot 12H_2O$, 0.5 g $Ca(NO_3)_2 \cdot 4\ H_2O$ and decoction of 300 g potato/l, pH 7.0) with rotary shaking at 30 °C for 48 hr. Cells were collected by centrifugation at 6,500 × *g* for 20 min and resuspended in sterile distilled water to produce concentration of ca. 10^9cfu/ml to be used as inoculum. Five hundred ml of this suspension was drenched in each planter containing 7 kg of steam-sterilized soil.

Identification of the endophytic bacterial isolates using Vitek32 system

Seventy-three endophytic bacterial isolates were applied to automatic Vitek32 system for their identification. Each identification Vitek card comprises 30 wells which contain biochemical substrates in a dehydrated form. No additional reagents are needed, thus eliminating any risk of omission or error. Vitek identification database covers over 300 species encountered clinically and in the industrial field (Vitek®, bioMérieux Inc.). In this experiment, gram negative identification + card (GNI+) and *Bacillus* card were used. The results were expressed in terms of percentage of similarity to the species covered in the database of the system. Only tentatively identified isolates were used in further studies such as *in vitro* antagonism and pot assay.

In vitro antagonism assay

To detect the antibacterial activity of the tentatively identified isolates, the plate chloroform method (Chen *et al.*, 1984; Wakimoto *et al.*, 1986) was used. A fresh culture (1–2 days) of the producer (endophytic bacterial isolate) from YPDA slant was transferred to the centre of YPDA plate. The plates were then incubated at 30 °C for 2–3 days. After the bacteria formed colonies several mm in diameter, the plate was turned upside down. A sheet of filter paper was placed in the Petri plate lid and 2 ml of chloroform was added to it and kept at room temperature for 3 hr. After complete evaporation of chloroform, the indicator bacterial suspension (conc. ca. 10^8cfu/ml, 0.5 ml) was mixed with 5 ml of 0.5% plain agar medium (melted and kept at 50 °C) and overlaid on the plate and incubated at 30 °C for 2 days. If an inhibition zone appeared, its semi-diameter was measured. The experiment was conducted in duplicate.

Tobacco plants

Tobacco seeds (*N. tabacum* L cv. Xanthi-nc) were grown in the greenhouse conditions at temperature 20–30 °C and watered daily. Three week-old plants were then transplanted onto cell trays. Two weeks after transplanting, healthy and uniform plants were selected for the experiment.

Pot assay for bacterial wilt suppression

Tobacco plants were removed from the cell trays and rinsed with tap water to remove loosely adhering soil. Tobacco root was dipped in endophytic bacterial suspension for 24 hr. Seedlings mock-inoculated with distilled water served as the check. Treated plants were transplanted into the soil infested with *R. solanacearum* C319 and kept in an air-conditioned greenhouse with daily irrigation. Each treatment contains 10 plants and the experiment was repeated twice. The disease severity was recorded at 5 day-intervals for 45 days using the following index: 0: no symptom; 1: 1–20% of the foliage wilted; 2: 21–40% of the foliage wilted; 3: 41–60% of the foliage wilted; 4: more than 61% of the foliage wilted and 5: entire plant wilted or dead.

Data analysis

Data from greenhouse experiments were analyzed using an analysis of variance (ANOVA) with StatView software (SAS Institute, 1992–1998) and the protected least significant difference (PLSD) was determined. Differences with $P \leq 0.05$ were considered to be significant. All treatments in which disease ratings were significantly ($P \leq 0.05$) lower than the check was considered to be effective.

RESULTS

Identification of the endophytic bacterial isolates by Vitek32 system

Twenty-nine isolates was tentatively identified as *Chryseobacterium indologenes, P. fluorescens, Chryseomonas luteola, Bacillus pumilis, B. megaterium, B. sphaericus, Sphingomonas paucimobilis, Enterobacter cloacae, Pantoea agglomerans,* and *Staphylococcus auricularis* with a range of similarity from 97% to 99% (Table 2). In addition, 8 isolates were tentatively identified to belong to the relevant genera with the

Table 2. Identification of the endophytic bacterial isolates by using Vitek32 system (bioMérieux).

Species	Isolates	Similarity (%)[a)]	Species	Isolates	Similarity (%)
Chryseobacterium indologenes	KuTox301	99	*Sphingomonas paucimobilis*	KuTox501	98
	KuTox302	98		KuTox706	99
	KuTox402	99		KuTox707	99
	KuTox403	99		KuTox709	99
	KuTox701	99	*Enterobacter cloacae*	KuTox201	99
	KuTox702	99		KuTox203	99
Chryseobacterium sp.	KuTox503	96		KuTox904	99
	KuTox401	92		KuTox905	98
	KuTox308	92	*Pantoea agglomerans*	KuTox801	99
	KuTox703	92		KuTox802	98
Pseudomonas fluorescens	KuTox1001	98		KuTox903	99
	KuTox1203	98		KuTox1002	98
	KuTox1303	98		KuTox1202	98
Pseudomonas sp.	KuTox1201	96		KuTox1503	97
	KuTox1101	94		KuTox1601	98
Chryseomonas luteola	KuTox1003	97	*Pantoea* sp.	KuTox901	96
Bacillus pumilis	KuTox102	99		KuTox1102	93
Bacillus megaterium	KuTox202	99	*Staphylococcus auricularis*	KuTox708	97
Bacillus sphaericus	KuTox514	99			

[a)] Percentage of similarity between the endophytic bacterial isolates with the species covered in the Vitek32 system's database

similarity less than 97% to the species covered in the database. However, 36 isolates could not be identified by this system.

In vitro antagonism against *R. solanacearum* C319

Antibacterial activity against *R. solanacearum* of 37 endophytic bacterial isolates identified by the Vitek32 system to the species or genus level was investigated. Our result indicates that the anti-*R. solanacearum* activity spectrum of these isolates was greatly different depending upon the endophytic bacterial isolates (Table 3). Thirteen isolates were antagonistic against *R. solanacearum in vitro*. Three isolates (KuTox1201, KuTox905 and KuTox801) tentatively identified as *Pseudomonas* sp., *E. cloacae* and *P. agglomerans* have shown strong antagonistic activity (Fig. 1). However, 24 isolates belonging to *C. indologenes*, *Chryseobacterium* sp., *S. paucimobilis*, *B. megaterium*, *P. agglomerans*, *E. cloacae* and *B. sphaericus* did not inhibit the growth of *R. solanacearum*.

Suppression of bacterial wilt of tobacco by the endophytic bacterial isolates

Thirty-seven previously identified isolates were evaluated for bacterial wilt suppression of tobacco plant under greenhouse conditions (Table 4). Most of these isolates did not reduce significantly ($P \leq 0.05$) the disease severity of tobacco 45 days after inoculation. Although several isolates such as KuTox1201, KuTox801 and KuTox905 exhibited a high extent of antagonistic activity against *R. solanacearum in vitro*, significant disease suppression was not found in this study. However, the isolate KuTox708 showing a low degree of antagonistic activity was remarkably suppressed the disease when compared

with the check (Fig. 2).

Table 3. Growth inhibition of *Ralstonia solanacearum* C319 by the endophytic bacterial isolates.

Species	Isolates	Width of inhibition zone (mm)[a]	Species	Isolates	Width of inhibition zone (mm)
Chryseobacterium indologenes	KuTox301	–	*Sphingomonas paucimobilis*	KuTox501	–
	KuTox302	–		KuTox706	–
	KuTox402	–		KuTox707	–
	KuTox403	–		KuTox709	–
	KuTox701	–	*Enterobacter cloacae*	KuTox201	–
	KuTox702	–		KuTox203	–
Chryseobacterium sp.	KuTox503	–		KuTox904	+
	KuTox401	–		KuTox905	+++
	KuTox308	–	*Pantoea agglomerans*	KuTox801	+++
	KuTox703	–		KuTox802	++
Pseudomonas fluorescens	KuTox1001	+		KuTox903	–
	KuTox1203	+		KuTox1002	–
	KuTox1303	+		KuTox1202	–
Pseudomonas sp.	KuTox1201	+++		KuTox1503	–
	KuTox1101	+		KuTox1601	–
Chryseomonas luteola	KuTox1003	+	*Pantoea* sp.	KuTox901	–
Bacillus pumilis	KuTox102	(+)		KuTox1102	+
Bacillus megaterium	KuTox202	–	*Staphylococcus auricularis*	KuTox708	(+)
Bacillus sphaericus	KuTox514	–			

[a] Inhibition zone semi-diameter was measured after 24 hr of incubation at 30 °C; Inhibition zone semi-diameter index: +++, >10 mm; ++, 5–10 mm; +, <5 mm; (+), doubtful; –, no zone of inhibition.

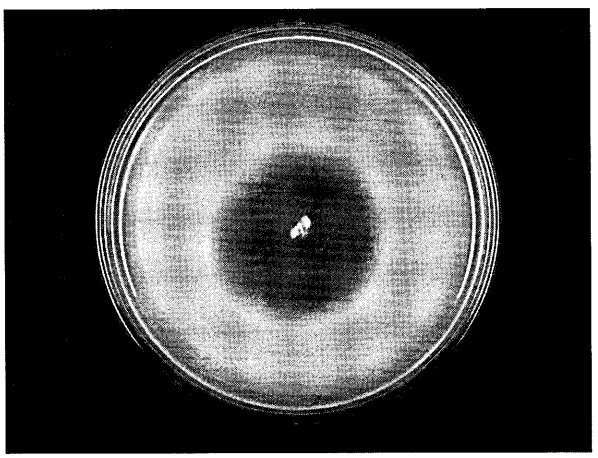

Fig. 1. Growth inhibition zone formed by the endophytic bacterial isolate KuTox905 on the lawn of *Ralstonia solanacearum* C319.

Table 4. Protection efficacy of bacterial wilt of tobacco by the endophytic bacterial isolates.

Isolates	Mean of disease severity[a)]		Isolates	Mean of disease severity	
	Treatment	Check		Treatment	Check
KuTox301	2.2	3.1	KuTox708	1.5*	3.1
KuTox302	2.0	2.0	KuTox501	1.8	2.8
KuTox402	2.7	3.1	KuTox706	2.0	2.3
KuTox403	2.8	3.1	KuTox707	4.5	4.7
KuTox701	2.2	2.0	KuTox709	1.3	2.3
KuTox702	3.0	2.8	KuTox201	2.0	2.3
KuTox503	3.0	3.6	KuTox203	3.5	2.7
KuTox401	2.3	2.0	KuTox904	1.0	1.5
KuTox308	2.7	3.6	KuTox905	1.2	2.3
KuTox703	4.0	2.0	KuTox801	1.8	1.9
KuTox1001	2.1	1.9	KuTox802	0.6	1.5
KuTox1203	1.9	2.3	KuTox903	2.6	2.3
KuTox1303	0.9	1.7	KuTox1002	1.9	3.0
KuTox1201	2.0	2.3	KuTox1202	1.8	2.7
KuTox1101	1.3	2.5	KuTox1503	1.8	2.7
KuTox1003	1.3	2.0	KuTox1601	1.8	2.7
KuTox102	2.7	2.1	KuTox901	1.5	2.3
KuTox202	1.6	2.3	KuTox1102	2.8	2.7
KuTox514	1.5	2.7			

[a)] Disease severity was rated using a 0 (no disease) to 5 (entire plant wilted) rating scale 45 days after inoculation.

* Significantly different from the check (Determined by the Fisher's PLSD test $P \leq 0.05$).

Fig. 2. Suppression of bacterial wilt of tobacco by the endophytic bacterial isolate KuTox708.

DISCUSSION

Endophytic bacteria have been found to be associated with the root of numerous plant species, with most being members of common bacterial genera such as *Pseudomonas, Bacillus, Corynebacterium* (Lalande *et al.,* 1989; Gardner *et al.,* 1982; Jacobs *et al.,* 1985), *Burkholderia, Enterobacter* (McInroy and Kloepper, 1995; Gardner *et al.,* 1982). In our previous paper (Long *et al.,* 2003), diversity in endophytic bacterial population in the root of *Solanum* sp. was clarified. For a rapid identification of the endophytic bacterial isolates, Vitek32 system was used in this study. The endophytic bacterial isolates from *Solanum* sp. were tentatively identified as *Bacillus, Pseudomonas, Enterobacter, Pantoea, Sphingomonas, Chryseomonas, Staphylococcus* and *Chryseobacterium* which is the most prominent genus. Study for more accurate identification of the endophytic bacterial isolates including 36 unidentifiable isolates is in progress.

Among 37 tentatively identified isolates of endophytic bacteria, 13 isolates exhibited growth inhibition of *R. solanacearum in vitro*. In particular, the identified isolates of *Pseudomonas* sp. that has been known to be one of the most antibiotic producers in the rhizosphere (Weller and Cook, 1983; Brisbane and Rovira, 1988) showed anti-*R. solanacearum* activity. The inhibitory spectrum against *R. solanacearum* varied depending upon isolates of endophytic bacteria, suggesting that various antibiotics may be involved in the formation of inhibition zones.

Antibiotic production could play a significant role in disease suppression, since there was relationship between the level of *in vitro* antibiosis and *in vivo* suppression of bacterial wilt (Chen *et al.,* 1984; Trigalet *et al.,* 1994). In this study, however, the isolate with strong antagonistic activity against *R. solanacearum in vitro* has shown a low level of disease suppression in tobacco plant under greenhouse conditions. On the other hand, although KuTox708 did not produce a clear inhibition zone against the pathogen *in vitro*, the isolate protected tobacco plants from bacterial wilt caused by *R. solanacearum*. Significant correlation between *in vitro* antagonism and disease suppression *in vivo* was not found in this study. The role of antibiotic produced by the endophytic bacterial isolates seems to be slight in the mechanisms of disease suppression. Kempe *et al.* demonstrated that the induced resistance by pre-treatment with pseudomonads may be involved in the mechanism of suppression of bacterial wilt of potato. The disease suppression shown in this experiment may be also the result of systemic resistance induced by KuTox708. Further studies are needed to clarify the mechanisms of disease suppression in detail.

REFERENCES

Anuratha, C. S. and S. S. Gnanamanickam 1990 Biological control of bacterial wilt caused by *Pseudomonas solanacearum* in India with antagonistic bacteria. *Plant Soil,* **124**: 109–116

Aspiras, R. B. and A. de la Cruz 1985 Potential biological control of bacterial wilt in tomato and potato with *Bacillus polymyxa* Fu6 and *Pseudomonas fluorescens. In* "Bacterial Wilt Disease in Asia and the South Pacific", ACIAR Proceedings No. 13 ed. by G. J. Persley, pp. 89–92

Brisbane, P. G. and A. D. Rovira 1988 Mechanisms of inhibition of *Gaeumannomyces graminis* var. *tritici* by fluorescent pseudomonads. *Plant Pathol.*, **37**: 104–111

Celino, M. S. and D. Gottlieb 1952 Control of bacterial wilt of tomato by *Bacillus polymyxa.*

Phytopathology, **42**: 4

Chen, W. Y. and E. Echandi 1984 Effects of avirulent bacteriocin-producing strains of *Pseudomonas solanacearum* on the control of bacterial wilt of tobacco. *Plant Pathol.*, **33**: 245–253

Furuya, N., Y. Kushima, K. Tsuchiya, N. Matsuyama and S. Wakimoto 1991 Protection of tomato seedlings by pre-treatment with *Pseudomonas glumae* from infection with *Pseudomonas solanacearum* and its mechanisms. *Ann. Phytopath. Soc. Jpn.*, **57**(3): 363–370

Gardner, J. M., A. W. Feldman and R. M. Zablotowicz 1982 Identity and behavior of xylem-residing bacteria in rough lemon roots of Florida citrus trees. *Appl. Environ. Microbiol.*, **43**: 1335–1342

Hallmann, J., A. Quadt-Hallmann, W. F. Mahaffee and J. W. Kloepper 1997 Bacterial endophytes in agricultural crops. *Can. J. Microbiol.*, **43**: 895–914

Jacobs, M. J., W. M. Bugbee and D. A. Gabrielson 1985 Enumeration, location, and characterization of endophytic bacteria within sugar beet roots. *Can. J. Bot.*, **63**: 1261–1265

Kado, C. I. 1992 Plant Pathogenic Bacteria. *In* "The prokaryotes". ed. by A. Balows, H. G. Trüper, M. Dworkin, W. Harder and K. H. Schleifer. Springer-Verlag, New York, pp. 660–662

Kempe, J. and L. Sequeira 1983 Biological control of bacterial wilt of potatoes: attempts to induce resistance by treating tubers with bacteria. *Plant Dis.*, **67**: 499–503

Kobayashi, D. Y. and J. D. Palumbo 2000 Bacterial endophytes and their effects on plants and uses in agriculture. *In* "Microbial endophytes" ed. by C. W. Bacon and J. F. White, Marcel Dekker Inc., New York, pp. 199–233

Lalande, R., N. Bissonnette, D. Coutlee and H. Antoun 1989 Identification of rhizobacteria from maize and determination of their plant-growth promoting potential. *Plant Soil*, **115**: 7–11

Long, H. H., N. Furuya, D. Kurose, M. Takeshita and Y. Takanami 2003 Isolation of endophytic bacteria from *Solanum* sp. and their antibacterial activity against plant pathogenic bacteria. *J. Fac. Agr., Kyushu Univ.*, **48**(1–2): 21–28

McInroy, J. A. and J. W. Kloepper 1995 Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant Soil*, **173**: 337–342

Trigalet, A., P. Frey and D. Trigalet-Demery 1994 Biological control of bacterial wilt caused by *Pseudomonas solanacearum*: State of the art and understanding. *In* "Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum*" ed. by A. C. Hayward and G. L. Hartman. CABI, pp. 225–231

Wakimoto, S., K. Hirayae, K. Tsuchiya, Y. Kushima, N. Furuya and N. Matsuyama 1986 Production of antibiotics by plant pathogenic Pseudomonads. *Ann. Phytopath. Soc. Jpn.*, **52**: 835–842

Weller, D. M. and R. J. Cook 1983 Suppression of take-all of wheat by seed treatment with fluorescent pseudomonads. *Phytopathology*, **73**: 463–469