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Pseudomonas syringae pv. *solidagae* pv. nov., the Causal Agent of Bacterial Leaf Spot of Tall Goldenrod *Solidago altissima* L.

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ABSTRACT

A new bacterial disease of tall goldenrod (*Solidago altissima* L., "Seitaka-awadachiso" in Japanese), one of the most serious weeds in non-agricultural land, was discovered in Ibaraki Prefecture, Japan. Characterized by angular or round, dark brown necrotic spots on leaves, this disease resulted in defoliation and terminal dieback of the plants in severe cases. The disease was named "bacterial leaf spot". The causal bacterium was identified as *Pseudomonas syringae* based on its bacteriological properties including those determined by LOPAT tests. The present bacterium was pathogenic to tall goldenrod alone but not to many other tested plants including weeds, flowers, trees and crops. In addition, *P. syringae* pv. *syringae* and other pathovars did not show any pathogenicity to tall goldenrod. Because no pathovars of *P. syringae* pathogenic to tall goldenrod have been reported, the present bacterium was concluded to be a new pathovar of *P. syringae*. We propose the name *P. syringae* pv. *solidagae* pv. nov. , and strain Sei 1 (MAFF 810053) is designated as the pathotype strain and has been deposited in the MAFF collection with two reference strains (MAFF 810054 and MAFF81055).

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Key words : new bacterial disease, new pathovar, bacterial leaf spot of *Solidago altissima* L., *Pseudomonas syringae* pv. *solidagae*.

INTRODUCTION

Tall goldenrod *Solidago altissima* L. (Seitaka-awadachiso in Japanese), which originated in North America, has become a very serious weed throughout non-agricultural land in Japan. In June 1998, an unknown disease of tall goldenrod causing dark brown leaf spots was observed in Ibaraki Prefecture, Japan. Our preliminary studies[7] suggested that the disease might be a new disease caused by a new pathovar of *P. syringae* van Hall 1902.

In this paper, taxonomic studies and pathogenicity tests of the causal agent are described, and we propose a new disease name, bacterial leaf spot of tall goldenrod and a new name of the pathogen, *Pseudomonas syringae* pv. *solidagae* pv. nov.

MATERIALS AND METHODS

Bacteria The bacterial strains used in this study are listed in Table 1. Ten strains (Sei 1 to Sei 10) were isolated from the necrotic lesions that appeared on leaves of tall goldenrod grown in non-agricultural land located in Tsukuba and Ushiku cities, Ibaraki Prefecture.

Several strains of *P. syringae* pv. *syringae* having a broad host range, *P. syringae* pv. *phaseolicola* pathogenic to the weed Kudzu (*Pueraria lobata*), and several other pathovars, including pv. *mori*, pv. *pisi*, pv. *sesami*, pv. *morspurunorum*, were used for pathogenicity tests for tall goldenrod plants.

Pathogenicity tests Bacterial strains were grown on modified LB agar medium (peptone 10 g, yeast extract 5 g, NaCl 10 g, agar 15 g and 1 liter distilled water) for 1 to 2 days and the concentration was adjusted to *ca.* 10^9 cfu/ml. Tween 20 (0.05%) was added to the resulting bacterial suspensions. Potted young seedlings were inoculated with the bacteria by either a spray method or a needle method using a bundle of ten needles. The inoculated plants were placed in a moist chamber (22-23°C) for 1 day and then moved to a greenhouse (*ca.* 25°C).

Seedlings of tall goldenrod grown from seeds collected in autumn were used for the pathogenicity tests. Most weed plant seedlings were supplied by the Upland Weed Laboratory, National Agricultural Research Center (NARC). In some cases, young seedlings growing in fields were used. Plant species were selected mainly from

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Table 1. Bacterial strains used in this study

Present bacterium	Year isolated	Locality	Original host
Sei 1 (MAFF 810053)	1998	Tsukuba-A, Ibaraki	*Solidago altissima* L.
Sei 2	1998	do.	do.
Sei 3	1998	do.	do.
Sei 4 (MAFF 810054)	1998	Ushiku, Ibaraki	do.
Sei 5	1998	do.	do.
Sei 6	1998	do.	do.
Sei 7 (MAFF 810055)	1998	Tsukuba-B, Ibaraki	do.
Sei 8	1998	do.	do.
Sei 9	1998	do.	do.
Sei 10	1998	do.	do.

P. syringae pathovar (pv.)	Original host
pv. *syringae* MAFF 301429	*Prunus persica* var. *vulgaris* (Peach)
MAFF 301861	*Syringa vulgaris* L. (Lilac)
MAFF 730120	*Allium fistulosum* (Welsh onion)
R168	*Prunus* spp. (Cherries)
pv. *phaseolicola* Kuz-1	*Pueraria lobata* (Kudzu)
pv. *mori* S6804	*Morus* spp. (Mulberry)
SM4	*Morus* spp. (Mulberry)
pv. *sesami* Goma1	*Sesamum indicum* (Sesame)
pv. *pisi* MAFF 301208	*Pisum sativum* (Pea)
MAFF 301214	*Pisum sativum* (Pea)
pv. *morsprunorum* R58	*Prunus* spp. (Peaches)

a) MAFF : Collection of National Institute of Agrobiological Sciences, Tsukuba Ibaraki. Strains R168 and R58 were supplied by K. Takanashi.

weeds and flowers, particularly belonging to the Compositae because *Solidago altissima* L belongs to this family.

Bacteriological properties Bacteriological characteristics were examined using mainly the protocol described by Tominaga[11]. Utilization of carbohydrates was determined using Ayers' medium[1]. An API 20 NE kit (BIO MERIEUX S.A. Company) was used according to the manufacture's protocol.

Ice nucleation activity was determined using a droplet freezing method on chromium plating copper plates as described by Takahashi[10]. Ten droplets (10 μl) were placed on a controlled-temperature (−5°C) plate. When all droplets freeze within 60 sec, the strain is judged to be positive for ice nucleation activity.

RESULTS

Symptoms

Naturally infected tall goldenrod plants were observed in non-agricultural land in Tsukuba and Ushiku cities, Ibaraki Prefecture, Japan in June 1998, and again in late May and June of 1999 and 2000. This disease was characterized by the appearance of angular or round, dark brown necrotic spots 1–5 mm in diameter on leaves (Plate I). Necrotic spots on leaves enlarged, fused with each other, resulting in eventural defoliation and terminal dieback in severe cases. In midsummer (August), however, leaves regrown on the diseased plants were not usually infected.

Bacteriological properties

The present strains isolated from necrotic spots of tall goldenrod plants were gram-negative rods that were 0.3–0.5 by 1.0–2.5 μm. On nutrient agar plates, the colonies were white, convex and circular with smooth surfaces and entire margins. Motile cells had one to five polar flagella. They produced a water-soluble fluorescent pigment on King's B medium and did not grow at 40°C. Therefore, these strains were thought to belong to the genus *Pseudomonas*. LOPAT tests[5] were as follows : levan production (−), oxidase activity (−), potato rot ability (−), arginine dihydrolase (−), and tobacco hypersensitivity (+). Supplemental tests in LOPAT were as follows : gluconate oxidation (−), nitrate reduction (−) and sucrose utilization (+). From these results, these strains belong to group Ib of *P. syringae*. In addition, API 20NE tests had a profile index of 0447451, the same as that of 10 pathovars of *P. syringae* in the diagnostic tables of the API20NE kit described by Nishiyama[6]. In addition, ice nucleation activity was positive in all strains, like many pathovars of *P. syringae*. From these results, we identified the present strains as *P. syringae*.

Table 2. Pathogenicity tests of the present strains

Plants inoculated (No. of species)	Present strains[a]		*P. syringae* strains[b]	
	Spray	Needle	Spray	Needle
Solidago altissima L.	+++[c]	+++	−	−
Group A[d] (29)	−	−	NT	NT
Group B[d] (10)	−	N	NT	NT
Group C[d] (8)	−	W	NT	NT

a) Ten strains (Sei 1-10) in Table 1 were used to test pathogenicity on *Solidago altissima* L., but only strain Sei 1 was used to test the other plants.

b) Eleven strains (six pathovars) of *P. syringae* (Table 1) were used to test pathogenicity on *Solidago altissima* L, but not to test other plants.

c) +++ : Plants had typical disease symptoms, many dark brown necrotic spots on leaves after spray inoculation, and enlarging dark brown necrosis around inoculated sites after needle inoculation method. − : no disease symptoms developed. N : Dark brown necrosis appeared within inoculated sites but did not enlarge. W : White necrosis appeared within inoculated sites. NT : Not tested.

d) Scientific names and English or Japanese common names (parenthesis) of plants belonging to each group (A, B, C) are as follows :

Group A :

Trees : *Morus bombycis* Koidz. (mulberry), *Broussonetia kazinoki* Sieb. (kozo, paper mulberry), *Broussonetia papyrifera* (L.) Vent. (kajino-ki, paper mulberry), *Prunus mume* Sieb. et Zucc. (Japanese apricot), *Malus pumila* var. *domestica* (apple), *Acer* spp. (maple).

Flowers : *Callistephus chinensis* (L.) Nees. (aster), *Salvia coccinea* L. (salvia), *Tagetes erecta* L. (marigold), *Viola*×*wittrockiana* Hort. (garden pansy), *Fragaria*×*ananassa* Duch. (strawberry), *Dianthus* spp. (carnation), *Cirsium* spp. (German thistle).

Weeds : *Fatoua villosa* (Thunb.) Nakai (kuwa-kusa), *Pueraria lobata* (Willd.) Ohwi (kudzu), *Stellaria media* (L.) Villars (chickwood), *Acalypha australis* L. (copperleaf, enoki-gusa), *Solanum nigrum* L. (black nightshade), *Persicaria longiseta* (De Bruyn) Kitag. (polygonum), *Persicaria vulgaris* Webb et Miq. (lady's thumb), *Portulaca oleracea* L. (common purslane), *Cyperus microiria* Steud. (galingale), *Digitaria ciliaris* (Retz.) Koeler (crabgrass), *Cyperus iria* L. (rice flatsedge), *Setaria viridis* (L.) Beauv. (green foxtail), *Echinochloa crus-galli* (L.) Beauv. var. *crus-galli* (barnyardgrass), *Glechoma hederacea* L. var. *grandis* (A. Gray) Kudo (ground ivy), *Commelina communis* L. (dayflower).

Crops : *Oryza sativa* L. (rice), *Glycine max* (L.) Merr. (soybean).

Group B :

Flowers : *Chrysanthemum morifolium* Ramat. (chrysanthemum), *Antirrhinum majus* L. (snapdoragon), *Hydrangea* spp. (hortensia), *Calendula officinalis* L. (pot marygold).

Weeds : *Glycine soja* Sieb. et Zucc. (tsurumame), *Erigeron canadensis* L. (norseweed), *Cayratia japonica* (Thunb.) Gagn. (sorrel vine, yabukarashi), *Taraxacum officinale* Weber (dandelion).

Crops : *Sesamum indicum* L. (sesame), *Chrysanthemum coronarium* L. var. *spatiosum* (garland chrysanthemum).

Group C :

Weeds : *Abutilon theophrasti* Medic. (Indian mallow), *Eclipta prostrata* (L.) L. (American false, takasaburou), *Chenopodium album* L. (common lambsquerters), *Chenopodium album* L. var. *centrorubrum* Makino (wild spinach), *Amaranthus viridis* L. (slender amaranth), *Eleusine indica* (L.) Gaertn. (goosegrass), *Amaranthus patulus* Bertoloni (redshank), *Amaranthus lividus* L. (amaranth).

The present strains were further characterized as follows : anaerobic growth under nitrate (−), catalase activity (+), OF test (O), gelatin hydrolysis (−), H_2S production (−), salt tolerance (2%+, 5%−), pH growth (6.0-9.0), growth under 0.01% lysozyme (−), VP (−), MR (+), casein hydrolysis (+), starch decomposition (−), DNase activity (−), tyrosine decomposition (−), citric acid utilization (+) ; positive utilization from D-glucose, D-fructose, D-xylose, L-arabinose, D-galactose, D-mannose, L-rhamnose (8 str./10 str.), sucrose, D-melibiose (9 str./10 str.), D-mannitol, sorbitol and inositol ; Negative utilization of D-cellobiose, maltose, D-trehalose, lactose, salicin, dulcitol, β-alanine, betaine or D-raffinose.

Pathogenicity tests

Ten strains (Sei 1 to 10) listed in Table 1 were used to inoculate to seedlings of tall goldenrod. All strains that were spray inoculated caused typical symptoms, many dark brown necrotic leaf spots similar to those of diseased plants observed in the field. Needle inoculation of these strains also caused dark brown necrosis within inoculated sites, and the necrosis then enlarging outside these spots. A high population of these bacteria were recovered from these lesions.

Strain Sei 1 was then used to inoculate to the species of

weeds, flowers, trees and crops shown in Table 2. All these plants failed to develop symptoms after spray inoculation. After needle inoculation, however, plants designated as group B developed a dark brown necrosis only within the inoculated sites, but the necrotic spot did not enlarge as in tall goldenrod. In addition, plants designated as group C developed a "white necrosis" within the inoculated circles. The necrosis induced by needle inoculation was judged to be a "resistant reaction" induced after inoculation with a high population of bacteria.

Thus, the present strains had a marked pathogenicity only on tall goldenrod plants.

DISCUSSION

We found a new bacterial disease of a very serious weed in Japan. The finding should be significant in the biological control of the weed. Many species of fungi, such as *Colletotrichum* spp., *Fusarium* spp. and *Alternaria* spp., have been evaluated for their ability to control weed growth. In contrast, only a few species of bacteria have been examined. Currently, *Xanthomonas campestris* pv. *poae*, which is pathogenic to annual bluegrass, a serious weed in bermudagrass golf greens, has already been evaluated and applied for practical use[3]. *Pseudomonas syringae* pv. *tagetis* also has great potential as a biocontrol agent against several composite weeds (Canada thistle, common ragweed, *etc.*) and is being evaluated in the United States[4]. In addition, *P. syringae* pv. *phaseolicola* was tested as a control against the serious weed Kudzu (*Pueraria lobata*)[12]. Our discovery of a new bacterial pathogen against tall goldenrod will contribute to the development of this research field.

The present strains isolated as the pathogen causing an unknown disease of tall goldenrod were identified as *P. syringae* based on their major bacteriological properties. LOPAT and supplemented LOPAT tests revealed that the present strains belonged to group Ib of *P. syringae*. In addition, the following data also supported this identification: the API 20NE profile index (0447451) of the present strains is composed of many *P. syringae* pathovars, such as *P. syringae* pv. *oryzae*, pv. *aptata*, pv. *atropurpurea*, pv. *lachrimans* and pv. *tabaci*[6]. The ice nucleation activity present in these strains is also common in many pathovars of *P. syringae*. Thus, the present strains were determined to be unknown pathovars of *P. syringae*.

P. syringae is composed of more than 50 pathovars based on their host specificity. The present strains were pathogenic only to tall goldenrod. No pathovars of *P. syringae* pathogenic to tall goldenrod have been reported. Therefore, the findings strongly suggest that the present bacterium may be a new pathovar of *P. syringae*. To exclude the possibility of the present strains being known pathovars of *P. syringae*, strains of several pathovars, such as pv. *syringae*, which has a broad host range, the pv. *phaseolicola* kudzu strain, pathogenic on the weed, kudzu, and other pathovars were randomly selected for pathogenicity tests. These known pathovars were not pathogenic to tall goldenrod plants. On the other hand, *P. syringae* pv. *tagetis* caused characteristic symptoms (apical chlorosis) against several composite weeds and flowers (marigold, Canada thistle, common ragweed, *etc.*)[2,8,9]. The present strains did not induce such symptoms on any composite weeds, including marigold and German thistles. Based on these results, we conclude that the present bacterium is a new pathovar of *Pseudomonas syringae*, and *Pseudomonas syringae* pv. *solidagae* pv. nov. is proposed.

DESCRIPTION

***Pseudomonas syringae* pv. *solidagae* pv. nov.**

Gram-negative rods, 0.3–0.5 by 1.0–2.5 μm. Motile by means of one to five polar flagella. Aerobic growth. Colonies on nutrient agar plates are rounded, convex and smooth with entire margins. A diffusible fluorescent yellowish green pigment is produced on King's B medium. Growth at 40°C is negative. Positive for the following reactions: tobacco hypersensitivity, oxidative metabolism of glucose, catalase activity, MR test and casein hydrosis. Negative for the following reactions: levan production, Kovacs' oxidase activity, potato rotting ability, arginine dihydrolase, gluconate oxidation, nitrate reduction, anaerobic growth under nitrate, indole production, gelatin hydrolysis, H_2S production and VP test. Positive utilization of D-glucose, D-fructose, D-xylose, L-arabinose, D-galactose, D-mannose, L-rhamnose (8 str./10 str.), sucrose, D-melibiose (9 str./10 str.), D-mannitol, sorbitol and inositol; negative utilization of D-cellobiose, maltose, D-trehalose, lactose, salicin, dulcitol, β-alanine, betaine and D-raffinose. Positive for ice nucleation activity. Plant pathogen causing leaf spots, shoot blight and dieback on tall goldenrod (*Solidago altissima* L., "Seitaka-awadachiso" in Japanese). The pathotype strain is Sei-1 (=MAFF 810053).

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Plate I

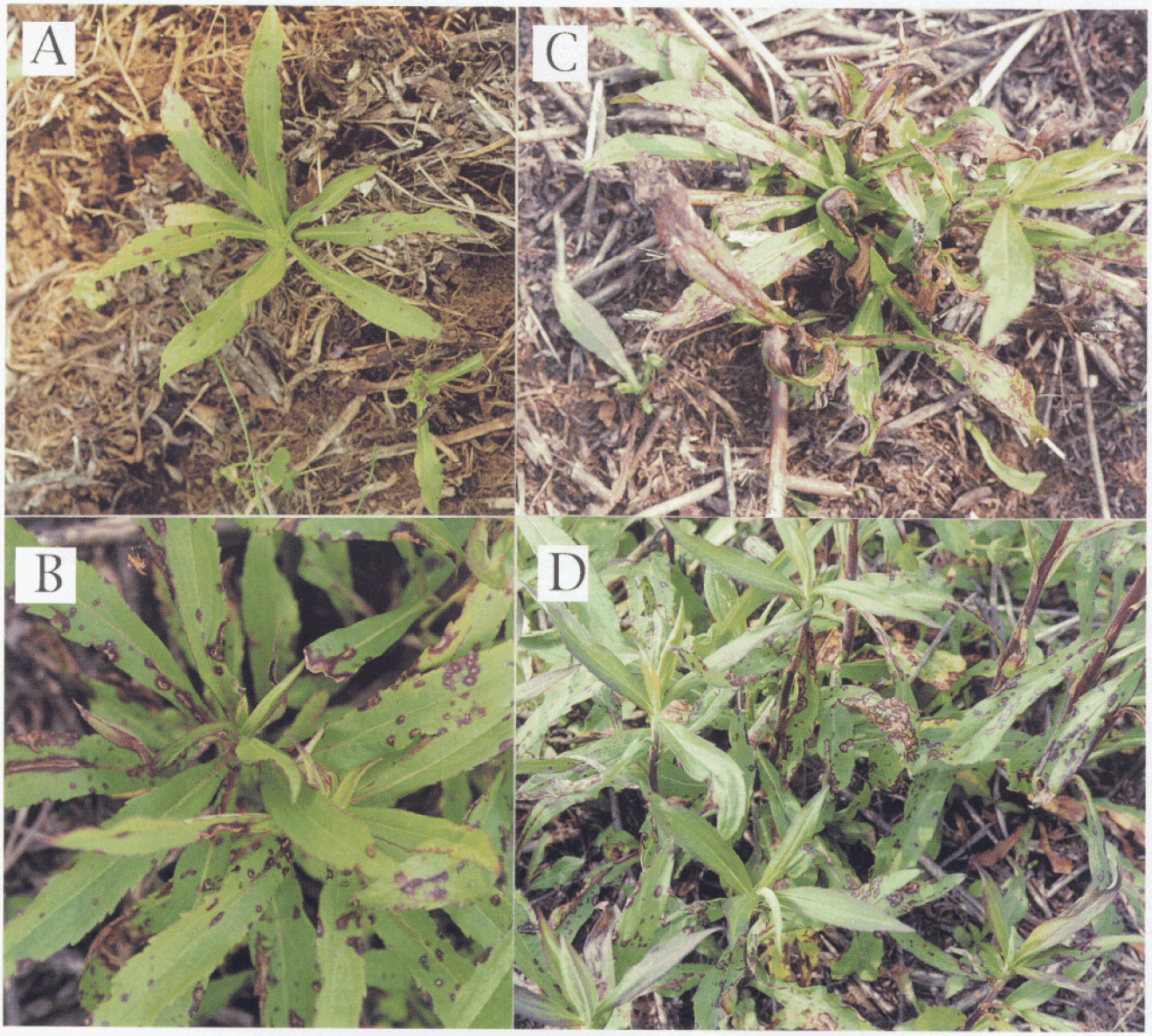

Plate I

Naturally occurring symptoms of bacterial leaf spot of tall goldenrod, *Solidago altissima* L. A, early appearance of necrotic spots on leaves, B, typical leaf spot symptoms, C, severe symptoms with coalescing leaf spots and withered leaves, D, many diseased plants.