

Xantomonas campestris pv. raphaniによるアブラナ科野菜斑点細菌病

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Bacterial Spot of Crucifers Caused by *Xanthomonas campestris* pv. *raphani*

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Abstract

A new bacterial disease has been observed on crucifers in Shizuoka, Aichi and Nagano Prefectures, Japan, since 1983. The disease appeared on the leaves as pin-point, water-soaked lesions which enlarged to form gray, necrotic spots. The causal bacterium was a gram negative, aerobic rod with single polar flagellum. It formed yellow slimy colonies, liquefied gelatin, produced hydrogen sulfide and hydrolized starch. It neither reduced nitrate to nitrite, nor produced arginine dihydrolase. The bacterium produced acid in the media added glucose, sucrose, lactose, starch, mannitol and dextrin, but not in those added ribose and dulcitol. On inoculation, the bacterium showed strong pathogenicity not only on several crucifers, with formation of numerous spots similar to natural infection, but also on tomato, physalis plant, cucumber and pumpkin. From these results, the causal bacterium was identified as *Xanthomonas campestris* pv. *raphani* (White 1930) Dye 1978. This is the first report of bacterial spot disease of crucifers in Japan.

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Key words : crucifers, a new bacterial disease, bacterial spot, *Xanthomonas campestris* pv. *raphani*.

INTRODUCTION

In 1983 and 1986, an unknown bacterial leaf spot disease was observed on cruciferous vegetables cultivated in Shizuoka, Aichi and Nagano Prefectures, Japan. The symptoms of the disease were similar to those of bacterial leaf spot of crucifers caused by *Pseudomonas syringae* pv. *maculicola*. Our preliminary study, however, indicated that the bacterium isolated from the diseased plants was a xanthomonad. This paper deals with the results of a taxonomic study of the causal bacterium of this disease.

MATERIALS AND METHODS

Bacteria. Twelve strains were used. Each strain was isolated from diseased leaves of cruciferous vegetables, including cabbage (*Brassica oleracea* L. var. *capitata*), chingensai (*B. rapa* L. var. *chinensis*, cv. Chingensai), turnip (*B. campestris* var. *rapifera* L.), Chinese cabbage (*B. campestris* L. var. *pekinensis*) and radish (*Raphanus sativus* L.). For comparison, many cultures of *Xanthomonas campestris* pv. *campestris*, *X. c.* pv. *vesicatoria*, *X. c.* pv. *physalidicola*, *X. c.* pv. *glycines* and *X. c.* pv. *cannabis* were obtained from the collection of phytopathogenic bacteria at Shizuoka University. Cultures of *X. c.* pv. *raphani* (PDDCC-1404, 1640) and *X. c.* pv. *armoraciae* (PDDCC-7, 19) were obtained from Plant Disease Division Culture Collection (Table 1). PDDCC-1404 and PDDCC-7 are the pathotype strains of the each pathovars, respectively. All bacteria were grown on PPGA medium (peptone, 5 g; glucose, 5 g; Na₂HPO₄ · 12H₂O, 3 g; KH₂PO₄, 0.5 g; NaCl, 3 g; agar, 18 g; 1 liter decoction of 200 g of potato)⁸⁾ and

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Table 1. Bacterial strains used in this study

Strains	Host	Location (prefecture or country)	Date isolated
Present isolates			
TMR-12, 13	Cabbage	Shizuoka	Dec. '83
TMR-46	Chingensai	Aichi	Jan. '86
TMR-61	Turnip	Aichi	Feb. '86
TMR-73	Turnip	Shizuoka	Jan. '85
TMR-74, 75	Radish	Shizuoka	Jan. '85
TMR-137, 138, 139, 140, 144	Chinese cabbage	Nagano	May '86
<i>Xanthomonas campestris</i>			
pv. <i>campestris</i>			
TMR-5, 15, 33, 41, 69, 179, 186, 193, 194, 208, 210	various cruciferous hosts	various places in Japan	'79~'86
pv. <i>raphani</i>			
PDDCC-1404	Radish	U.S.A.	'40
PDDCC-1640	Radish	U.S.A.	?
pv. <i>armoraciae</i>			
PDDCC-7	Candytuft	Tanzania	'54
PDDCC-19	Horse-radish	U.S.A.	'30
pv. <i>vesicatoria</i>			
TMR-191, 192, 207	Tomato	Nagano	'86
TMR-205, 206	Tomato	Yamanashi	'86
pv. <i>physalidicola</i>			
TMR-183, 184	Physalis	Shizuoka	'86
pv. <i>glycines</i>			
TMR-200	Soybean	Shizuoka	'86
pv. <i>cannabis</i>			
TMR-185	Hemp	Tochigi	'82

maintained at 4°C for routine work.

Inoculation tests. Inoculations were carried out the following cruciferous plants: horse-radish (*Armoracia rusticana* L.), Chinese cabbage (*B. campestris* L. var. *pekinensis*, cv. Musouhakusai), cauliflower (*B. oleracea* L. var. *botrytis*, cv. Hakurakuhanayasai), cabbage (*B. oleracea* L. var. *capitata*, cv. Kosuikanran), broccoli (*B. oleracea* L. var. *italica*, cv. Ryokuyou), turnip (*B. campestris* var. *rapifera* L., cv. Wasekabu), chingensai (*B. rapa* L. var. *chinensis*, cv. Chingensai), tatsuai (*B. rapa* L. var. *narinosa*, cv. Tatsuai) and radish (*Raphanus sativus* L., cv. Taibyousoubutori). Inoculations of plants other than crucifers were done on tomato (*Lycopersicon esculentum* Mill., cv. Ponteloz), physalis (*Physalis alkekengi* L. var. *franchetii* Hort.), pepper (*Capsicum annuum* L. var. *grossum* Mill., cv. Sweet pepper), soybean (*Glycine max* Merrill, cv. Sapporo-midori), kidney bean (*Phaseolus vulgaris* L. var. *humilis* Alef., cv. Kentucky wonder), cucumber (*Cucumis sativus* L., cv. Suyou), pumpkin (*Cucurbita moschata* Duch., cv. Shintosa-ichigou), lettuce (*Lactuca sativa* L. var. *capitata*, cv. Greatlake 54), shungiku (*Chrysanthemum coronarium* L., cv. Taiyoushugiku), sugar beet (*Beta vulgaris* L. var. *rapa* Dumort, cv. Kabemegamono), oat (*Avena sativa* L., cv. Aogari-enbaku), peach (*Prunus persica* Batsch) and mulberry (*Morus bombycis* Koidz). For inoculation tests, plants were grown in pots of 15 cm diameter for 4 to 6 weeks in the greenhouse at ca. 20°C. Peach and mulberry trees were inoculated on young shoots and leaves of field grown trees. Inoculations were made either by spraying bacterial suspension (ca. 10^{8-9} cfu/ml) over mature leaves (after wounding with single needle at several points per leaf) or by puncturing young shoots and leaves with a needle through drops of bacterial suspension placed on the surface. The inoculated plants were kept humid overnight by holding them in a mist chamber or by covering them with polyethylene bags.

Bacteriological properties. Morphological and physiological properties were tested using the methods already described⁸⁾. DNase and phosphatase activity were tested using the methods described by Cowan⁴⁾. Acid production from carbohydrate was tested using the medium C of Dye⁵⁾, and utilization of organic acids and amino acids were tested using either the medium OY of Dye⁵⁾ or the medium of Ayers *et al.*¹⁾ as a basal medium, respectively.

RESULTS

Symptoms

Lesions on the leaves appeared as pin-point spots with water-soaked appearance. These spots gradually enlarged to form round dark-green spots 2-3 mm in diameter, and later turned to brown or gray, sunken and necrotic lesions which were surrounded by narrow water-soaked zones. No halo was observed around them (Plate I-1). Few lesions were observed on the stems or petioles. The lesions frequently coalesced each other to form large irregular blotches 5-6 mm in diameter, but were not limited by veins. Where the lesions were numerous along the veins and midrib of a growing leaf, considerable distortion of the leaf blade occurred (Plate I-2). Under microscopic observation, exudation of bacteria from diseased parenchymatous tissues was recognized, but not from vascular systems.

Inoculation tests

The present bacterium showed strong pathogenicity on all cruciferous plants except radish and horse-radish. On the former, only radish strains produced leaf spots, and on the latter, none of the present strains showed pathogenic reactions. Symptoms firstly appeared on the leaves about 7 days after inoculation, as pin-point, water-soaked spots. These spots enlarged to form round, dark-green lesions 2-3 mm in diameter within 1 week, and later they turned to gray necrotic lesions with narrow water-soaked area around them. No halo was observed (Plate I-3,4). The present bacterium also showed pathogenicity

Table 2. Comparison of host range of the present isolates and other *Xanthomonas campestris* pathovars

Plants tested	Present isolates	XCR ^{a)}	XCC ^{b)}	XCA ^{c)}	XCV ^{d)}	XCP ^{e)}
Radish	V ^{f)}	+	(+)	—	—	—
Turnip	+	+	(+)	—	—	—
Cabbage	+	+	(+)	—	—	—
Chinese cabbage	+	+	(+)	—	—	—
Cauliflower	+	+	(+)	—	—	—
Broccoli	+	+	(+)	—	—	—
Chingensai	+	+	(+)	—	—	—
Tatsuai	+	+	(+)	—	—	—
Horse-radish	—	—	—	+	—	—
Tomato	+	+	—	—	+	—
Physalis	+	+	—	—	—	+
Pepper	—	—	—	—	—	—
Soybean	—	—	—	—	—	—
Kidney bean	—	—	—	—	—	—
Cucumber	+	+	—	—	—	—
Pumpkin	+	+	—	—	—	—
Lettuce	—	—	—	—	—	—
Shungiku	—	—	—	—	—	—
Sugar beet	—	—	—	—	—	—
Oat	—	—	—	—	—	—
Peach	—	NT	NT	NT	NT	NT
Mulberry	—	NT	NT	NT	NT	NT

+, Leaf spot symptom; (+), Vascular infection; —, No symptom; NT, Not tested.

a) *X. campestris* pv. *rappani*; b) pv. *campestris*; c) pv. *armoraciae*; d) pv. *vesicatoria*; e) pv. *physalidicola*; f) Radish isolates producing leaf spot symptom and others no symptom.

on tomato, physalis, cucumber and pumpkin, producing brown or gray necrotic lesions on their leaves (Plate I-5,6), but did not produce any symptoms on the other plant species tested. Among the other bacteria, *X. c. pv. campestris* strains produced typical black rot lesions on all cruciferous plants except for horse-radish. *X. c. pv. armoraciae* strains produced leaf spot symptoms only on horse-radish. Reference strains of *X. c. pv. raphani* showed the same pathogenic reaction as the present bacterium. *X. c. pv. vesicatoria* and *pv. physalidicola* had pathogenicity only on tomato and physalis, respectively (Table 2).

Bacteriological properties

The cells of the bacterium were gram-negative, nonsporeforming aerobic rods, and motile by means of single polar flagellum. On nutrient agar plates, the colonies were yellow, slimy, convex, transparent and circular with a smooth surface and entire margin. They measured about 4-5 mm in diameter on nutrient agar after 5 days incubation at 28°C. No production of water-soluble fluorescent or other pigment was recognized on King's B medium. The present bacterium showed positive reactions in the following tests: oxidative metabolism of glucose, hydrolysis of starch, Tween 80, aesculin and casein, gelatin liquefaction, production of H₂S, ammonia and reducing substances from sucrose, mucoid colony formation on the medium with sucrose, activity of catalase, tyrosinase, lecithinase, pectinase, DNase and phosphatase, potato soft rot, acid production from glucose, ribose, sucrose, lactose, maltose, trehalose, cellobiose, melibiose, raffinose, galactose, mannose, fructose, xylose, L-arabinose, dextrin, starch, mannitol and glycerol, utilization of propionate, citrate, formate, fumarate, lactate, malonate, succinate and saccharate. Negative reactions were obtained in the following tests: oxidation of gluconate, production of indole and acetoin, activity of oxidase, arginine dihydrolase and urease, nitrate respiration, nitrate reduction, methyl-red test, tobacco hypersensitive reaction, growth in 3% NaCl, acid production from rhamnose, dulcitol, sorbitol, inositol, ethanol, adonitol, salicin, inulin, erythritol, α -methyl-D-glucoside and propylene glycol, utilization of galacturonate, gluconate, tartrate, oxalate, benzoate, glycerate, L-arginine, triacetin and DL-phenylalanine. In purple milk reaction, hydrolysis of casein and alkali-production were observed.

The properties of the present bacterium which differ from those and other pathovars of *X. campestris* are summarized in Table 3.

Table 3. Differences in biochemical and physiological properties of strains of the unidentified crucifer pathogen and selected *Xanthomonas campestris* pathovars

Properties	Present isolates	XCR ^{a)}	XCCam ^{b)}	XCV ^{c)}	XCA ^{d)}	XCP ^{e)}	XCG ^{f)}	XCCan ^{g)}
Tween 80 hydrolysis	+	+	+	—	V ^{k)}	—	+	+
Lecithinase	+	+	+	—	V ^{k)}	—	+	+
Potato soft rot	+	+	+	+	+	—	+	+
Tobacco H.R.	—	—	—	—	—	+	—	—
Casein hydrolysis	+	+	+ ^{j)}	+	+	+	+	+
Pectinase	+	+	+	+	+	—	—	+
Utilization of								
Lactose ^{h)}	+	+	—	—	V ^{l)}	—	+	+
Mannitol ^{h)}	+	+	+	+	+	—	+	+
Saccharate ⁱ⁾	+	+	+	+	+	—	—	+
Propionate ⁱ⁾	+	+	+	+	+	+	—	+
Glycerate ⁱ⁾	—	—	+	—	V ^{k)}	+	+	—

+, Positive reaction; —, Negative reaction; V, Variable.

a) *X. campestris* pv. *raphani*; b) pv. *campestris*; c) pv. *vesicatoria*; d) pv. *armoraciae*; e) pv. *physalidicola*; f) pv. *glycines*; g) pv. *cannabis*; h) In the medium C of Dye; i) In the medium of Ayers *et al.*; j) Formation of white zone around transparent zone; k) PDDCC-7 showing positive reaction and other negative; l) PDDCC-19 showing positive reaction and other negative.

DISCUSSION

The causal agent of this disease is a gram-negative, aerobic short rod bacterium with single polar flagellum. It produces water-insoluble yellow pigment and slime. Therefore, it was assigned to the genus *Xanthomonas*. The bacterium showed positive reactions in liquefaction of gelatin, hydrolysis of casein, production of hydrogen sulfide and acid production from L-arabinose, glucose, mannose, galactose, trehalose and cellobiose. These results indicate that the bacterium should be included into the *X. campestris* group^{3,6)}.

The bacterium showed the same reactions as the pathotype strain of *X. c. pv. raphani* in all bacteriological properties and pathogenic traits, but different reactions from other *X. campestris* pathovars.

X. c. pv. raphani was firstly reported by White¹⁰⁾ in U.S.A. as the pathogen causing bacterial leaf spot of radish and turnip in 1930. The bacteriological properties of the present bacterium coincided with those described by White. He reported that *X. c. pv. raphani* produced necrotic lesions on leaves, petioles and stems of radish and turnip, and had pathogenicity to cabbage, cauliflower, kale, Brussels sprouts, tobacco, pepper, and tomato plants. Although kale and Brussels sprouts were not included in our inoculation tests, radish, turnip, cabbage, and cauliflower together with other four species of crucifers were shown to be host plants of our present bacterium. In addition, the symptoms of the infected plants coincided with description and figures reported by White. On the other hand, pepper was not infected by either the present bacterium or the reference strains of *X. c. pv. raphani*. It was assumed that there were some differences in the inoculation conditions between our tests and White's tests, or that there were differences in susceptibility among the varieties of pepper plants tested.

The above-mentioned results lead us to identify the present bacterium as *X. c. pv. raphani* (White 1930) Dye 1978. White¹⁰⁾ originally named this bacterium as *Bacterium vesicatorium* var. *raphani* because he attached much importance to the fact that it produced leaf spot symptom on tomato. However, from our study it was revealed that *X. c. pv. vesicatoria* could be obviously distinguished from *X. c. pv. raphani* by their host ranges and some bacteriological properties.

Until now, the disease caused by *X. campestris* pv. *raphani* had not been reported in Japan. The presence of the disease in other countries was, however, reported by Takimoto in 1927, who named it as hanten-saikinbyou in Japanese (bacterial spot)⁹⁾. To avoid of confusion this disease and the disease caused by *P. syringae* pv. *maculicola*, the above name was proposed for the present bacterium.

As the result of comparative studies, it was found that *X. c. pv. raphani* and other pathovars of *X. campestris* group were apparently distinguishable as shown in Table 3. The differences indicated that those properties might be useful tools for the distinguishing the pathovars of *X. campestris*. From the result of inoculation tests, it was also found that *X. c. pv. raphani* had new host plants such as physalis plant, cucumber and pumpkin in addition to already reported host plants. This implies that this bacterium has a wide host range including families of crucifer, solanaceae and cucurbitaceae.

In 1983, Black and Machmud²⁾ reported that *X. c. pv. armoraciae* and pv. *raphani* were identical and should be considered as a single pathovar, *X. c. pv. armoraciae*⁷⁾, which had priority over pv. *raphani*. They mentioned that the leaf spot disease of crucifers caused by xanthomonad had been observed on cabbage since 1977 in U.S.A. Comparative studies of the causal bacterium, *X. c. pv. armoraciae* and pv. *raphani*, indicated that there was no difference among them in pathogenicity, serological and phage tests. However, our data demonstrated that *X. c. pv. raphani* and pv. *armoraciae* including pathotype strains could be apparently distinguished on the basis of their bacteriological properties and host ranges. White¹⁰⁾ also reported that the two bacteria could be distinguished by the difference of their host ranges, based on their cross inoculation tests. Therefore, we propose that each of two existing pathovar names should be retained.

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和 文 摘 要

田村勝徳・瀧川雄一・露無慎二・後藤正夫：*Xanthomonas campestris* pv. *raphani* によるアブラナ科野菜斑点細菌病

1983年から静岡県、愛知県および長野県でアブラナ科野菜に斑点細菌病害の発生を認めた。病徴は最初葉に針頭大の暗緑色水浸状斑点として現れ、その後次第に拡大して直径2~3mmの灰白色壊死斑となった。分離された病原細菌はグラム陰性好気性の極毛桿菌で、黄色の粘濁集落を形成し、ゼラチン液化、硫化水素産生、デンプン加水分解は陽性、硝酸還元、アルギニンジヒドロラーゼ産生は陰性であった。グルコース、スクロース、ラクトース、デンプン、マンニトールおよびデキストリンから酸を産生したが、リボースおよびズルシトールからは産生しなかった。接種試験により数種アブラナ科植物の他、トマト、ホウズキ、キュウリおよびカボチャに病原性を示し、いずれも葉に多数の病斑を生じた。これらの結果より、本病原細菌を *Xanthomonas campestris* pv. *raphani* (White 1930) Dye 1978 と同定した。本病の病名をアブラナ科野菜斑点細菌病 (bacterial spot) としたい。

Explanation of plate

Plate I

- 1-2. A radish leaf (1) and a Chinese cabbage leaf (2) naturally infected by *Xanthomonas campestris* pv. *raphani*.
- 3-6. Symptoms of radish (3), cauliflower (4), tomato (5) and cucumber (6) artificially inoculated with *X. campestris* pv. *raphani* strain.

Plate I

