北海道立農試集報 **42,** 29－42（1979）

Bacterial Stem Rot of Adzuki Bean (*Phaseolus radiatus* var. *aurea* Prain) Caused by *Pseudomonas adzukicola* A. Tanii et T. Baba nov. sp.

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Pseudomonas adzukicola A. Tanii et T. Baba nov. sp. によるアズキの茎腐細菌病

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Abstract

Since 1971, a new bacterial disease has been found on leaves, pods, stems and petioles of adzuki bean plants in the fields of southern areas of Hokkaido. This disease was characterized by a break in the stem where the petiole of primary or first trifoliate leaf is attached. From this symptom, bacterial stem rot of adzuki bean was proposed as disease name. The causal bacterium was aerobic, Gram-negative and motile with 1-3 polar flagella. It measured 0.8-2.3×0.5-0.9 (average, 1.4×0.6)μm. In agglutination test, antiserum prepared from heat-killed whole cells of isolate, DPA(L) 3, reacted not only to all the 15 isolates of the pathogen, but also to all other phytopathogenic pseudomonads tested. Agar gel diffusion test showed that all cultures of the pathogen were antigenically homogenous and possessed 2 common thermostable antigens, but one or all of them were shared by 8 isolates of *P. glycinea* except D(K)-1. The pathogen attacked kidney bean (cultivar. Taishōkintoki), cow pea and hyacinth bean, but not kidney bean (cultivar. Ōtebo), soybean, broad bean, pea and sword bean by artificial spray inoculation. Among the adzuki bean plants, large grain varieties had a tendency to be more susceptible than the small ones. Cultural and biochemical properties of the pathogen were similar to those of the halo blight group pathogens, especially to *P. glycinea* in respects of gelatin liquefaction, mannitol utilization and lipase activity.

However, the causal bacterium was not parasitic to soybean, and was characterized by such bacteriological characteristics as faint growth on NaCl-free beef-extract peptone medium, low-tolerance to NaCl, strong urea hydrolyzing ability and utilization of trehalose, D-tartaric acid and β-alanine. Therefore, it was given a specific rank, and the scientific name, *Pseudomonas adzukicola* A. Tanii et T. Baba nov. sp., was proposed.

Moreover, seed dressing at 0.3% of seed weight with dust formulation containing 3% Kasugamycin antibiotic and foliage application with wettable powder of copper fungicide soon after germination showed satisfactory control results.

Received 9 April 1979

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Introduction

In August 1971, there was a sudden occurrence of an unknown bacterial disease of adzuki bean (*Phaseolus radiatus* var. *aurea* Prain) in a field converted from paddy at Furano city of Kamikawa district in Hokkaido. The disease was characterized by a break in the stem where the petiole of primary or first trifoliate leaf is attached. The same disease was successfully found by either round inspections or diagnoses of diseased samples in various southern parts of Hokkaido; Oshima, Shiribeshi, Iburi, Ishikari and Sorachi districts. Despite the presence of bacterial exudation from the section of the lesions under microscopic observation, only moderate amount of yellow pigmented colonies appeared on beef-extract peptone agar containing 0.5% sodium chloride by conventional dilution plating and streaking, whereas uniform white colonies appeared in aboundance on King's agar accidentally used. The results of the investigations conducted on the bacterial and serological characteristics of the causal organism led us to conclude that this disease was a new one caused by a new species of *Pseudomonas*.

This report deals with the results of our identification work, including the chemical control methods and a few life cycle of this disease.

Preliminary reports of this disease have been already published[21,22].

Symptoms

This disease occurs in the seedling stage of adzuki bean plant. The lesions appear primarily as water-soaked small circular spots and streaks on blade and vine of the primary leaf(Plate I,1) or first trifoliate leaf without forming halo around them. They soon become reddish brown in color. These lesions gradually develop and enter the main stem through the petiole via leaf(Plate I, 3, 4). The diseased plant shows the damping-off appearence soon after germination when it has become progressively worse(Plate I, 2). When the degree of severity of the disease is slow in progress, the affected stems become easily broken by physical factors such as the late-August wind (Plate I, 5). Such symptom is known as stem girdle, or joint rot caused by *Xanthomonas phaseoli* and *Pseudomonas phaseolicola*[25]. In the later stage of growth, a large number of small necrotic spots are commonly found on mature leaves in severely infested fields. It also produce water-soaked spots on pods (Plate I,6) and streaks along the placenta-suture of pods (Plate I,7). Vascular bundle discoloration above the affected parts of the stems is not observed.

This is quite useful in the diagnosis of this disease because bacterial ooze is usually found on every diseased part.

Isolation of causal organism and inoculation test

Isolation was tried from freshly diseased lesions produced on various parts of adzuki bean plants collected from 9 localities during 1971-1972. King's B agar was used as standard medium for making isolation and for maintaining stock cultures. White round colonies producing pale yellow fluorescin consistently appeared in abundance on King's B agar plate from a suspension of a small piece of diseased tissues by dilution plating and streaking. This was not observed on beef-extract peptone agar plate containing 0.5% sodium chloride where Gram-negative fermentavive non-diffusible yellow

pigmented bacterium, namely *Erwinia herbicola* (Löhnis) Dye, was visible only in moderate amounts. Fifteen cultures listed in Table 1 were obtained from the lesions on leaves, stems and pods, respectively. After isolation, pathogenicity against adzuki bean, Hikari shōzu variety, of three trifoliate stage was immediately ascertained by spraying with the bacterial suspension (ca. 10^9 cells/ml) prepared from two-days old King's B agar slant cultures. The inoculated plants were kept under the green house without incubation in a moist chamber. After 2 weeks of inoculation, water-soaked spots and streaks were formed on the blades and vines of leaves, respectively. Their color soon changed into reddish brown and reached the main stems, invading the petioles via the leaves, as observed in natural infection. From the affected parts, white colonies identical with the original culture were reisolated by the use of King's B agar. No reisolation was made on the beef-extract peptone agar plate containing 0.5% sodium chloride. The check plants, which were treated in the same way as those of the inoculated ones except for the use of sterilized water as spray instead using bacterial suspension, did not show any symptom at all.

Causal organism

The following descriptions are based on the study of 15 isolates listed in Table 1. All methods used in this study are in accordance with those described in the Manual of Microbiological Methods[26] and in A Guide to the Identification of Genera of Bacteria[19], unless otherwise noted. Sodium chloride was taken away from all media used for testing various bacteriological characters except for the test of tolerance to sodium chloride.

Morphology :

Bacterial cells grown on King's B agar[11] slant for 48 hours at 25°C appeared as short rods with rounded ends, usually single or occasionally in pairs. The organism was Gram-negative, non-spore-forming and motile with 1-3 polar flagella. It measured 0.8-2.3×0.5-0.9(average, 1.4×0.6)μm and capsules were not observed.

Poly-β-hydroxybutyrate inclusion was not observed by staining with ethanolic solution of sudan black B[8].

Cultural characters :

After a week incubation on beef-extract agar plate and slant containing 0.2 or 0.5% sodium chloride, no growth was observed in all isolates. However, very slight growth appeared in heavily inoculated media. Four to five days old colonies on King's B agar plate, were 3 to 4 mm. in diameter, white, circular, smooth, glistening, with edges entire and butyrous in consistency. Sodium chloride-free beef-extract peptone broth became slightly turbid without pellicle or ring with the medium unchanged in color.

In peptone (polypeptone) water without sodium chloride, slight growth which was similar to that of beef-extract peptone broth was observed, however, growth was moderate when proteose-peptone was used. In litmus milk, growth was slow and no visible change in the milk occurred until after 3 weeks. Litmus turned blue in color and was slightly reduced from the bottom. In Uschinsky's and Fermi's solutions, faint turbidity was observed and the media turned slightly pale yellowish green after 3 weeks culture. There was no growth in Cohn's solution.

Physiological and biochemical characters :

All isolates were aerobic and decomposed glucose by oxidation in Hugh-Leifson medium[10], but did not have the ability to ferment it. Pale yellowish green fluorescent pigment was pro-

duced in King's B agar after 2-3 days and was blue in color under UV light (2537 Å). All tested isolates produced a hypersensitive reaction to tobacco leaves by using Klement's method[12]. Levan formation was observed on beef-extract peptone agar plate containing 5% sucrose[14]. Negative reactions were obtained in indole production, hydrogen sulfide production and reduction of nitrate to nitrite. Gluconate test was negative by the use of Haynes' method modified by Graham and Dowson[7]. All cultures failed to liquefy gelatin after 3 weeks incubation and to produce arginine dihydrolase in Thornley's 2A medium[24]. Methyl red and Voges-Proskauer tests were negative. Ammonia was confirmed in peptone water by Nessler's reagent. All isolates were positive for catalase. Aesculin was not hydrolyzed in liquid medium by using the Sneath's method[18]. Margarine and Tween 80 were also not hydrolyzed by using the medium of Lelliott *et al*[14] and Sierra's method[17], respectively. All cultures were negative for diastatic action and for tyrosinase production by using the medium described by Lelliott *et al*[14]. Lecithinase was not produced in nutrient agar containing egg yolk[14]. Urea was strongly hydrolyzed 2 hours after inoculation in Christensen's medium. Sodium malonate was utilized. All cultures failed to assimilate L-valine and L-lysine, but β-alanine and asparagine were assimilated as a sole source of carbon and nitrogen by using the standard mineral base of Stanier *et al* modified by Misaghi and Grogan[15]. Nitrate respiration was negative by the use of the method described by Komagata *et al*[13]. Kovacs' oxidase, cytochrome oxidase and phenylalanine tests were negative. In basal inorganic medium of Ayers *et al*, containing bromthymol blue as indicator and sugars in concentration of 1%, all cultures except isolate AF(P) 1 produced acid but not gas from xylose, glucose, mannose sucrose, trehalose, inositol, glycerol, mannitol, raffinose and levulose. Neither acid nor gas was produced from lactose, maltose, salicine, starch, inuline, L-sorbose, dultitol, erithritol and ethanol. Isolate AF(P) 1 did not produce acid from inositol. In the mineral base of Koser's medium containing bromthymol blue as indicator and organic compounds in concentration of 0.2%, D-tartric acid, citrate, succinate and DL-acetic acid were used by all cultures as a sole carbon source with an alkaline reaction, but not L-tartrate, urate, hipprate, alginate and DL-lactate. For the test of tolerance to sodium chloride, 3 isolates, AF(P) 1, APA(S) 4 and KPA(P) 4, were used. Isolate AF(P) 1 did not grow in proteose-peptone water containing 0.5% sodium chloride, and APA(S) 4 and KPA(P) 4 did not grow under the concentration of 0.2%. The optimun temperatures for growth of all cultures were laid between 25° and 27°C. The maximun temperature was between 30° and 35°C, the minimun was between 3° and 5°C. They could not grow at 2-3° or 35°C. All isolates did not produce potato rot by the method of Lelliott *et al*[14]. Sodium polypectate was liquefied at pH4.9-5.1, but not at pH8.3-8.5 by using Hildebrand's method[9].

Serological characters:

Slide-agglutination and Ouchterlony double gel-diffusion tests were employed for the determination of serological reaction. Selected pathogenic isolate, DPA(L) 3, used for antisera production was grown in King's B liquid medium, incubated on shaker at 25°C for 24 hours. Bacterial cells were harvested and washed twice with physiological saline by centrifugation. In the slide-agglutination test, antiserum of 1 : 800 titer was prepared by subcutaneously injecting a rabbit 3 times at the interval of 2 weeks with heat-killed (120°C, 10 min.) whole cells emulsified with Freund's incomplete adjuvant. For use, the antiserum was diluted 1 : 10 with physiological saline presented by adding sodium azide at concentration of 0.1%. As shown in Table 2, agglutination reactions have shown that this antiserum reacted with the living cell antigens of all phytopathogenic pseudomonads examined as well as the causal pathogen. In gel-diffusion test, the same antiserum was used in the agglutination test and a new one was made in like manner except for the use of living cell antigen. The antisera, diluted 1 :

2 with physiological saline containing 0.1% sodium azide, were placed into the central well and either heat-killed (100°C, 30 min.) whole cell antigens or untreated living cell of various *Pseudomonas* spp. were placed in the surrounding wells. The petri dishes were kept under room temperature and observations were made within 7 days. As shown in Plate II and III, Ouchterlony double gel-diffusion test showed that all isolates of the causal organism were antigenically homogeneous and possessed 4 antigens which were thermostable when heated at 100°C, 30 min. or 120°C, 10 min. Two, out of 4 antigens, were observed as clear precipitation bands in the side of antigen well and were species specific. But one all of them were shared by *P. glycinea* except isolate D(K)-1(Plate III, 2, 3).

Host range

Out of fifteen isolates listed in Table 1, five (SPA(S) 4, DPA(L) 3, K 1, A1and D 7) were used in order to determine the host range. The inoculum was prepared by suspending a 48 hours old King's B agar slant culture in 10 ml. of sterile water and adjusted to a concentration of ca. 10^8 cells/ml. The bacterial suspension was sprayed on various leguminous plants with a fine atomizer. The inoculated plants were incubated in a moist chamber of 25°C for 48 hours. After incubation, they were kept in the green house. After 2 weeks of spray inoculation, an area of water-soaked appearence was visible on the leaf blade and vine of adzuki bean varieties. These symptoms were exactly like those which were observed in the field cases. Among the adzuki bean varieties, Akatsuki-dainagon, Wase-tairiyu No. 1, Akane-dainagon and Hikari-shōzu were most susceptible, and Takara-shōzu and Takahashi-wase were moderate, whereas Kotobuki-shōzu, Chagara-wase, Shinashichi, Chūiku No. 2 and Sōbetsu-zairai were resistant. These facts were considered to indicate that large grain varieties were more susceptible than small grains. The Taishōkintoki variety of kidney bean (*Phaseolus vulgaris* L.) formed both small reddish brown spots on leaf blade and reddish necrotic streaks on vine, causing the malformation of leaflet. However, the Ōtebo variety of kidney bean was not attacked. Although small necrotic lesions were also observed on leaves of cow pea (*Vigna sinensis* (L.) Endl.) and hyacinth bean (*Dolichos lablab* L.), negative results occurred when inoculations were made on soybean (*Glycine max* (L.) Merrill), broad bean (*Vicia faba L.),* pea *(Pisum sativum* L.) and sword bean (*Canavalis gladiate* DC.).

Dissemination and control methods

This disease was caused from seedling infection which originated from seed transmission at germinating period. When the adzuki bean seeds obtained from diseased plants were planted in the field and in the green house, symptoms similar to those with natural infection reappeared. The seeds soaked in the suspension of the causal organism also showed the same symptoms as those of the naturally infected plants. On the other hand, it was considered that this disease was not soil-borne because negative infection was obtained from the most susceptible adzuki bean variety, Wase-tairiyu No. 1, grown in a plot where the soil collected from the heavily infested field in the previous year was applied before planting. Furthermore, secondary spread from plants to plants occurred. In order to establish the suitable control methods, dust coating treatment for seeds and foliage application were done by the use of several fungicides in the spot field or in the Tokachi Agri. Exp. Sta. field in 1973-1977. With respect to seed treatments, dust formulation containing Kasugamycin antibiotic (KSM), which consists of KSM+bis (dimethylthiocarbamoyl) disulfide (TMTD) +0, 0-diethyl 0-2, 4-di-

chlorophenyl phosphororthioate (ECP), 3+47+25% active ingredient, gave the most effective result among the fungicides used. This was applied by dusting just before planting at the rate of 0.3%. As for the foliage application, the satisfactory control effect was obtained by spraying twice or 3 time at the interval of about 10 days with wettable powder of copper fungicides soon after germination.

Discussion

From the bacteriological properties, the present causal organism is identified as a menber of the fluorescent group of the genus *Pseudomonas*. With regards to the bacterial pathogen which attack the adzuki bean plant under natural conditions, the only known bacterium that cause bacterial brown spot[20] is *P. syringae* van Hall. Comparing *P. syringae* and the present causal organism, it was considered not to be of the same species because they differed in respects of growth on nutrient agar, levan formation, gelatin liquefaction, tolerance to sodium chloride, utilization of several carbon compounds and the symptoms observed on the adzuki bean plant. The other *Pseudomonas* spp. known to attack legumenous plants, like *P. andropogonis* (Smith) Stapp (*P. stizolobii* (Wolf) Stapp[8]), *P. solanacearum* (Smith) Smith, *P. cichorii* (Swingle) Stapp, *P. viridiflava* (Burkholder) Dowson, *P. pisi* Sackett, *P. phaseolicola* (Burkholder) Dowson, *P. glycinea* Coerper, *P. fabae* (Yu) Burkholder, *P. radiciperda* (Zhavoronkova) Săvulescu *P. astragali* (Takimoto) Săvulescu, *P. seminum* Cayley, *P. viciae* Uyeda and *P. medicaginis* Sackett are noted according to the descriptions of Bergey's Manual of Determinative Bacteriology, 7th *ed*[2]. In addition to them, *P. marginalis* (Brown) Stevens and *P. tabaci* (Wolf&Foster) Stevens have been reported as the pathogens of bacterial stem rot of pea[5] and wildfire of soybean[1], respectively. Of these, *P. andropogonis* and *P. solanacearum* are differentiated from the adzuki bean pathogen, as they do not produce the fluorescent pigment. *P. cichorii* and *P. marginalis* are also considered different because both belong to oxidase positive group. *P. viridiflava* differs in its bacteriological characters such as sucrose utilization and potato rotting ability. The present organism is also distinguished from *P. pisi* and *P. tabaci* with the same reasons as those in comparing it with *P. syringae*. However, we are not able to compare with the remaining *Pseudomonas* spp. except *P. phaseolicola* and *P. glycinea* because their descriptions are quite inadequate. Dye *et al.*[4]. suggested that these nomenspecies should not be retained for the following reasons: they are inadequately described and/or there are no cultures available for further study. Furthrmore, cultures and/or descriptions suggest inappropriate classification. As a matter of fact, the present organism resembles the bean halo blight group pathogens[16], *P. phaseolicola* and *P. glycinea*, considering their bacteriological properties and pathogenicity. It has been known under special conditions that *P. phaseolicola* attacks the adzuki bean plants in the field and *P. glycinea* is pathogenic against adzuki bean plants by spray inoculation[23]. The discrepancies of the 3 organisms, including *P. mori* (Boyer & Lambert) Stevens, are given in Table 3 for comparison. Among the bean halo blight group organisms, *P. glycinea* resembles more the others in gelatin liquefaction, mannitol utilization and lipase activity (tween 80). The serological reaction using gel-diffusion has also shown that *P. glycinea* except isolate D(K)-1possess common thermostable antigens with the writers' organism. However, the writers' isolates are not parasitic to soybean and are characterized by such bacteriological characteristics as faint growth on sodium chloride-free beef-extract peptone medium, low-tolerance to sodium chloride, strong urea hydrolyzing ability of the same degree as that of *Proteus vulgaris* and utilization of trehalose, D-tartaric acid and β-alanine. It should therefore be given a specific rank, and the scientific name, *Pseudomonas adzukicola* A. Tanii et T. Baba nov. sp., is proposed. Moreover, by using the LOPAT characteristics[14], the writers' pathogen is in

group Ia. At present, the nomenspecies which belong to group Ia have included in the taxospecies, *P. syringae*, according to 8th *ed.* of Bergey's manual[3].

The results of round inspections and diagnoses have shown that this disease is distributed in the southern areas of Hokkaido and is especially severe in Iburi district, but not in the eastern part where is the largest producing area of beans. One reason for this may be attributed to the wide introduction of bean seeds from various localities due to the adjustment of rice production since 1971. Another reason is considered that since Tokacki district is the largest bean producing area, there is no need to introduce seeds from other areas. However, this might also be due to the small grain varieties which are mainly cultivated in the area. As for the chemical control of this disease, the seed treatment by dust formulation containing KSM antibiotic and the foliage application of copper fungicides, which were the same as those being used against halo blight[2,3], gave satisfactory results. However, the best control method is the use of disease-free seeds because this disease is seed-borne rather than soil-borne as previously described.

Acknowledgment

The authors thank Dr. T.Nakayama, the ex-directer of Tokachi Agricultural Experiment Station, for reading the manuscript.

Table 1. Source of bacterial isolates

Isoiate No.	Tissue	Locality	Date
AF(S)3	stem	Furano	1971
AF(P)1	pod	do	do
DPA(L)3	leaf	Date	do
DPA(S)3	stem	do	do
D 7	do	do	1972
KPA(P)4	pod	Kyōwa	1971
K 1	stem	do	1972
APA(S)4	do	Abuta	1971
A 1	do	Azuma	1972
SPA(S)4	do	Sōbetsu	1971
S 3	do	do	1972
PD-1	do	Ōno	do
PK-2	pod	Kameda	do
PI-2	do	Iwamizawa	do
PI-4	do	do	do

Table 2. Agglutination of bacteria with anti-DPA(L)3 serum

Bacteria	No. of isolates tested	Response[a)]
The present causal organism	15	+
Pseudomonas aptata	6	+
P. cichorii	1	+
P. coronafaciens	1	+
P. glycinea	10	+
P. lachrymans	3	+
P. marginalis	4	+
P. mori	3	+
P. phaseolicola	13	+
P. syringae	4	+
P. tabaci	2	+
P. fluorescens	1	−
P. gelidicola	1	−
P. septirivora	1	−
P. riboflavina	1	−
P. segnis	1	−
P. xanthe	1	−
Corynebacterium michiganense	1	−

a) +=positive reaction, −=negative reaction.

Table 3. Comparative characteristics[a)] of bean halo blight group pathogens (*P. phaseolicola*, *P. glycinea* and *P. mori*) and the present causal organism

Characters \ Organisms	Bean halo blight group pathogens[23)]			The present causal organism
	P. phaseolicola	*P. glycinea*	*P. mori*	
Beef-extract peptone agar (0.5% NaCl)	Growth	Growth	Growth	No
Gelatin liquefaction	+	−	−	−
Tolerance to NaCl	> 3 %	> 3 %	> 3 %	< 0.2−0.5%
Urease production (after 2 hrs.)	−	−	−	+
Lipase production (Tween 80)	+ or −	−	+	−
Acid from mannitol	−	+	+	+
Acid from inositol	−	+ or −	+	+ or (−)
Acid from sorbitol	−	−	+	−
Acid from trehalose	−	−	−	+
D-tartaric acid	−	−	−	+
β-alanine	−	−	−	+

a) +=positive reaction, or can be utilized for growth, −=negative reaction, or can not be utilized for growth, (−)=one isolate AF(P)1 negative reaction.

Literature cited

1) Allington, W.H. 1945. Wildfire disease of soybean. Phytopathology 35 : 857-869.
2) Burkholder, W.H. 1957. Genus *Pseudomonas*. In Bergey's manual of determinative bacteriology. 7th *ed*. (Breed, R.S. *et al. eds.*). Williams and Wilkins Co., Baltimore. pp.88-152.
3) Doudoroff, M. and Palleroni, N.J. 1974. Genus *Pseudomonas*. *Ibid.* 8th *ed.* (Buchanan, R.E. *et al. eds.*). pp.217-243.
4) Dye, D.W. *et al.* 1974. The problem of nomenclature of the plant pathogenic pseudomonads. Rev. Plant Pathol. 53 : 953-962.
5) Goto, M. and Okabe, N. 1958. Bacterial plant diseases in Japan IX. 1. Bacterial stem rot of pea. Bull. Agric. Fac. Shizuoka Univ. 8 : 33-40.
6) Goto, M. and Starr, M.P. 1971. A comparative study of *Pseudomonas andropogonis, P. stizolobii* and *P. albopreciptans*. Ann. Phytopath. Soc. Japan 37 : 233-241.
7) Grahan, D.C. and Dowson, W.J. 1960. The coliform bacteria associated with potato black-leg and other soft rots. II. Biochemical characteristics of low and high temperature strains. Ann. appl. Biol. 48 : 58-64.
8) Hayward, A.C., Forsyth, W.G.C. and Roberts, J.B. 1959. Synthesis and breakdown of poly-β-hydroxybutyric acid by bacteria. J. gen. Microbiol. 20 : 510-518.
9) Hildebrand, D.C. 1971. Pectate and pectin gels for differentiation of *Pseudomonas* sp. and other bacterial plant pathogens. Phytopathology 61 : 1430-1436.
10) Hugh, R. and Leifson, E. 1953. The taxonomic significance of fermentative versus oxidative of carbohydrate by various gram-negative bacteria. J. Bact. 66 : 24-26.
11) King, E.O., Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein J. Lab. Clin. Med. 44 : 301-307.
12) Klement, Z. 1963. Rapid detection of the pathogenicity of phytopathogenic pseudomonads. Nature, Lond. 165 : 299-300.
13) Komagata, K., Iizuka, H. and Takahashi, H. 1965. Taxonomic evaluation of nitrate respiration and carbohydrate fermentation in aerobic bacteria. J. gen. appl. Microbiol. 11 : 191-201.
14) Lelliott, R.A., Billing, E. and Hayward, A.G. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. appl. Bact. 29 : 470-489.
15) Misaghi, I. and Grogan, R.G. 1969. Nutritional and biochemical comparison of plant-pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59 : 1436-1450.
16) Schroth, M.N., Vitanza, V.B. and Hildebrand, D.C. 1971. Pathogenic and nutritional variation in the halo blight group of fluorescent pseudomonads of bean, *Ibid.* 61 : 852-857.
17) Sierra, G. 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observation on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhock 23 : 15-22.
18) Sneath, P.H.A. 1966. Identification methods applied to *Chromobacterium*. In Identification Methods for Microbiologists, part A (Gibbs, B.M. and Skinner, F.A. *eds.*). Acadmic Press, London and New York. pp. 15-20.
19) Skerman, V.B.D. 1967. A Guide to the Identification of the Genera of Bacteria. 2nd *ed.* Williams and Wilkins Co., Baltimore. pp. 213-286.
20) Tanii, A. and Baba, T. Bacterial plant diseases in Hokkaido. I. Bacterial brown spot of adzuki bean caused by *Pseudomonas syringae* van Hall. Bull. Hokkaido Prefect. Agric. Exp. Stn. 23 :90-96.
21) Tanii, A. and Baba, T. 1974. Species name of the causal pathogen causing bacterial stem rot of adzuki bean. Ann. Phytopath. Soc. Japan 39 : 202-203 (Abstr.).
22) Tanii, A., Narita, T., Akai, J. and Baba, T. 1973. Bacterial stem rot of adzuki bean and its causal bacterium. *Ibid.* 39 : 154 (Abstr.).
23) Tanii, A., Takakuwa, M., Baba, T. and Takita, T. 1976. Studies on halo blight of beans (*Phaseolus vulgaris*) caused by *Pseudomonas phaseolicola* (Burkholder) Dowson. Misc. Pub. Hokkaido Prefect. Tokachi Agric. Exp. Stn. No. 6 : 1-66.
24) Thornley, M.J. 1960. The differentiation of *Pseudomonas* from other gram-negative bacteria on the basis of arginine metabolism. J. appl. Bact. 23 : 37-52.
25) Zaumeyer, W.J. and Thomas, H.R. 1957. A monographic study of bean diseases and methods of their control. Tech. Bull. U.S. Dept. Agr. No. 868 : 65-84.
26) Society of American Bacteriologists. 1957. Manual of Microbiological Methods. McGraw-Hill book Co., New York. pp. 140-168.

Explanation of plates

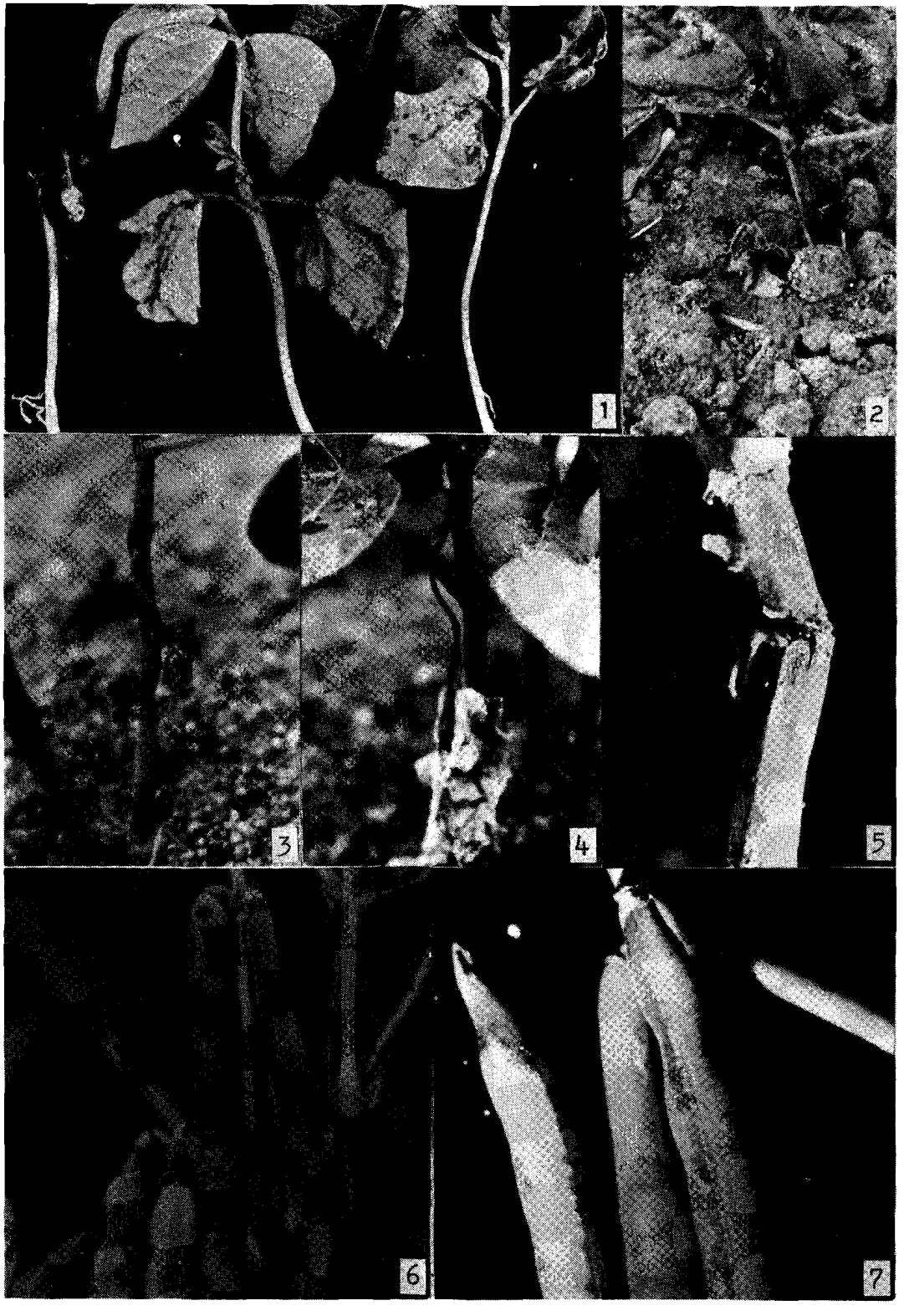
Plate I : Symptoms of bacterial stem rot of adzuki bean plant produced by natural infection.

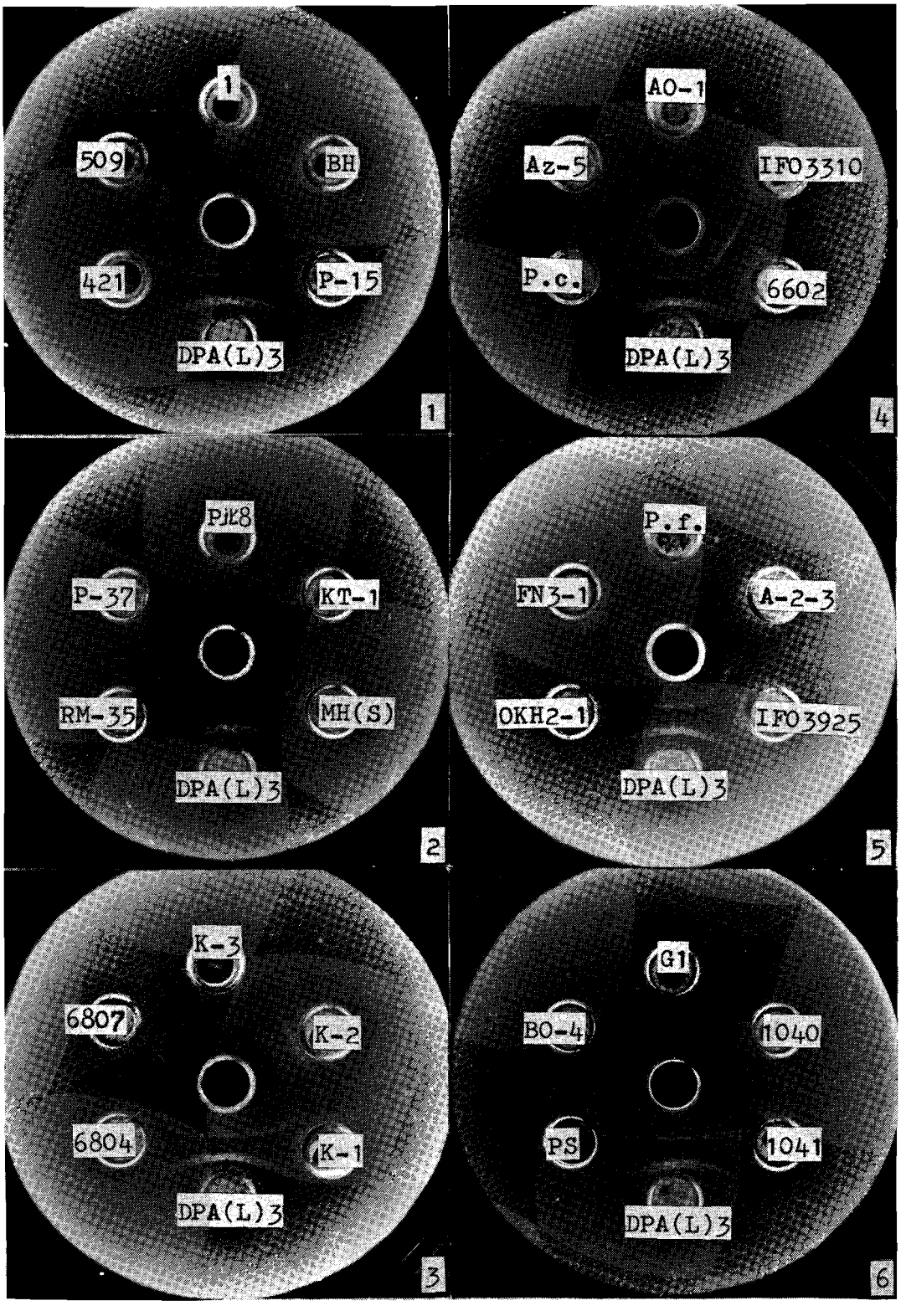
1. Water-soaked small circular spots and streaks on leaf blades and vines of the primary leaves.
2. Damping-off symptom.
3,4. Lesion entered the main stem through the petiole via primary and first trifoliate leaf, respectively.
5. Stem girdle symptom.
6. Water-soaked spots on pods.
7. Water-soaked streak along the placenta-suture of pod.

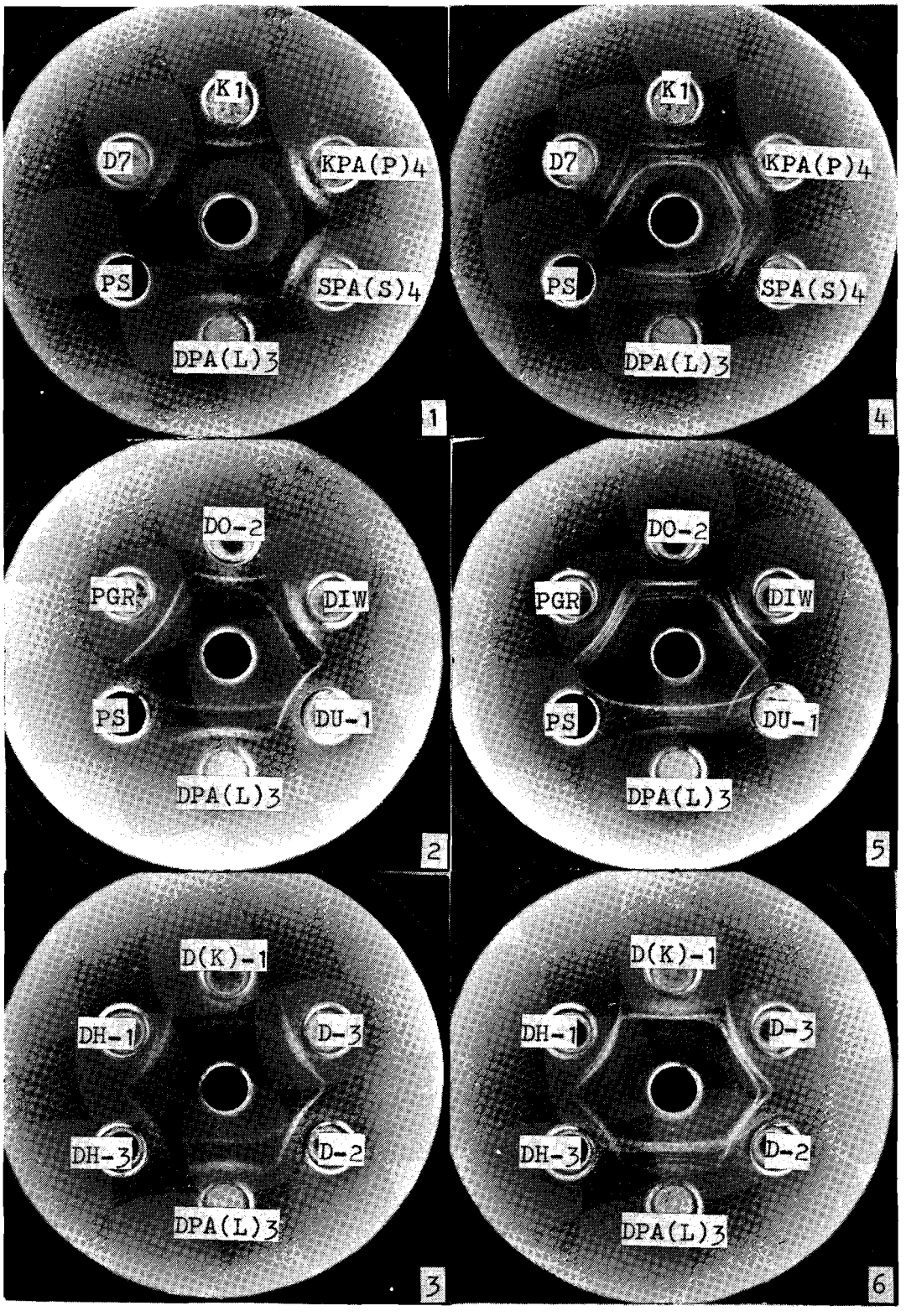
Plate II, III : Precipitin patterns in agar gel diffusion method.

Central well contains antiserum prepared from heat-killed (120°C, 10 min.) (Plate II, 1-6) or living cells (Plate III, 1-6) of DPA(L) 3 isolate and outer ones heat-killed (100°C, 30 min.) (Plate II, 1-6 and III, 1-3) or living cells antigens (Plate III, 3-6).

DPA(L) 3, K 1, D 7, KPA(P) 4 and SPA(S) 4 ; the present causal organism, 1, BH, P-15, 421, 509, P 北 8, KT-1, MH(S), RM-35, P-37, K-1, K-2 and K-3 ; *P. phaseolicola,* 6804 and 6807 ; *P. mori,* AO-1 and AZ-5 ; *P. syringae,* IFO 3310 ; *P. coronafaciens,* 6602 ; *P. tabaci,* P.c. ; *P. cichorii,* P.f. ; *P. fluorescens,* A-2-3 and IFO 3925 ; *P. marginalis,* FN 3-1 and OKH 2-1 ; *P.* sp. (isolated from rotting onion bulbs), G 1 and BO-4 ; *P. alboprecipitans,* 1040 and 1041 ; *E. coli,* DO-2, DIW, DU-1, PGR, D(K)-1, D-2, D-3, DH-1 and DH-3 ; *P. glycinea,* PS ; physiological saline.


1
2
3
4
5
6
7


1
509
BH
421
P-15
DPA(L)3
1
AO-1
Az-5
IFO3310
P.c.
6602
DPA(L)3
4
Pil8
P-37
KT-1
RM-35
MH(S)
DPA(L)3
2
P.f.
FN3-1
A-2-3
OKH2-1
IFO3925
DPA(L)3
5
K-3
6807
K-2
6804
K-1
DPA(L)3
3
G1
BO-4
1040
PS
1041
DPA(L)3
6


K1
D7
KPA(P)4
PS
SPA(S)4
DPA(L)3
1
K1
D7
KPA(P)4
PS
SPA(S)4
DPA(L)3
4
DO-2
PGR
DIW
PS
DU-1
DPA(L)3
2
DO-2
PGR
DIW
PS
DU-1
DPA(L)3
5
D(K)-1
DH-1
D-3
DH-3
D-2
DPA(L)3
3
D(K)-1
DH-1
D-3
DH-3
D-2
DPA(L)3
6

Pseudomonas adzukicola A. Tanii et T. Baba nov. sp. によるアズキの茎腐細菌病

谷井昭夫・馬場徹代

1971年以来，北海道の富良野市以南の地方でアズキに一種の細菌病の発生が認められていた。本病は初生葉および第一複葉の葉身と葉脈に斑点，条斑を形成し，それらは葉柄を経由して主茎に達し，主茎は折れ易くなる。この病徴から病名を茎腐細菌病（bacterial stem rot）とした。病原細菌は好気性桿菌，グラム染色陰性，大きさ0.8−2.3×0.5−0.9（平均1.4×0.6）μm，運動性で1～3本の極性鞭毛を有する。本病原細菌 DPA(L) 3菌株の加熱菌体抗血清は凝集反応で供試した全ての植物病原 *Pseudomonas* 属細菌と反応した。寒天ゲル内二重拡散法では，本病原細菌は種特異的な2個の耐熱性抗原を所有していたが，それらは D(K)-1 菌株を除く8株の *P. glycinea* にも共通であった。本病原細菌は人為噴霧接種でアズキをはじめ，インゲン（大正金時），ササゲ，フジマメに病原性があったが，インゲン（大手亡），ダイズ，ソラマメ，エンドウ，ナタマメには寄生しなかった。アズキ品種では大粒品種が小粒品種に比較して感受性の高い傾向にあった。本病原細菌の細菌学的性質は bean halo blight グループの病原細菌に似ており，特にゼラチン液化，マンニットールの利用，リパーゼ活性の点から *P. glycinea* に近かった。しかしながら，本病原細菌はダイズに病原性なく，食塩を含まない肉エキス・ペプトン培地での貧育，低耐塩性，尿素分解能，トレハロース，D-酒石酸，β-アラニンの利用能の点で特徴があった。それ故，本病原細菌に *Pseudomonas adzukicola* A. Tanii et T. Baba の学名を与えた。なお，本病の防除法として，カスガマイシンを3％含む粉衣剤による0.3%種子粉衣と発芽直後からの銅水和剤による茎葉散布は有効であった。