Pseudomonas solanacearum biovar IIおよびIV系統によるジャガイモ青枯病の発生と病原細菌の温度要求性

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Prevalence and Temperature Requirements of Biovar II and IV Strains of *Pseudomonas solanacearum* from Potatoes

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片山克己*・木村貞夫*：*Pseudomonas solanacearum* Biovar II および
IV系統によるジャガイモ青枯病の発生と病原細菌の温度要求性

Abstract

Two hundred and nine strains of *Pseudomonas solanacearum* were obtained from potato fields in Nagasaki, Japan, at periodic intervals late in the growing season on the fall crop. Thirty seven strains were classified as biovar II, one as biovar III, and 171 as biovar IV. The percentage of biovar II strains increased in the latter period of the growing season. In static culture at 16.5 C, biovar II strains grew more rapidly than those of biovar IV. Biovar IV grew more at 35 C for four days than at 24 C for seven days and 16.5 C for 14 days. However, biovar II did not show clear difference of growth at 16.5 C for 14 days and 35 C for four days. There was no difference in the latent period between biovar II and IV strains in inoculation experiments. Biovar II and IV strains were considered to be race 3 and race 1, respectively. This is the first report of race 3 of *P. solanacearum* in Japan.

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Key Words : *Pseudomonas solanacearum*, potato, race, biovar, temperature.

Introduction

Bacterial wilt caused by *Pseudomonas solanacearum* is an important and widespread disease of potatoes (*Solanum tuberosum*) and many other crops in mild temperate, subtropical and tropical regions of the world[9]. Strains of *P. solanacearum* have been divided into three races, differing mainly in host ranges; Race 1 affects tobacco, tomato, many solanaceous and other weeds, and certain diploid bananas. Race 2 infects triploid bananas, *Heliconia*, or both, and race 3 affects mainly potatoes and tomatoes[2]. They have been also classified into four biovars according to their capacity to oxidize disaccharides and hexose alcohols[6]. Moreover, it has been proposed that the strains from mulberry isolated in China be designated race 4, biovar V[8]. Although the biovar classification is not always correlated with the races, biovar II has been considered to with race 3[1]. One of the characteristics of race 3 or biovar II strains from potatoes is a lower optimum growth temperature than strains of race 1[5,22]. Knowledge of races or strains is necessary to evaluate the pathogenic potential and ecological relationships of strains in a specific geographic area.

In Nagasaki, Japan, the potato has been cultivated continuously for over 20 years;

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during this period bacterial wilt has become one of the factors limiting production. Generally, spring and fall planting are standard in southwestern Japan; in general, this disease is more severe on fall than spring crops. On fall crops, bacterial wilt occurs in late September and continues to cause losses until early November when temperatures are relatively low. Therefore, there was a possibility that the strain infecting potatoes was favored by low temperatures. Race classification of strains that infect potatoes in Japan has not been studied previously. In previous studies *P. solanacearum* strains in Japan and Taiwan have been divided into many types on the basis of host range, utilization of carbon compounds, phage types, and bacteriocin production[10-15]. The objective of this research was to determine the biovars or races of *P. solanacearum* that infect potatoes in Nagasaki with emphasis on temperature relationships.

Materials and Methods

Isolation of the bacteria. On 7th, 15th and 25th of October 1981, wilted potato plants were obtained from three fields which were located in Aino, Nagasaki Prefecture. The soil type in this region is Light-colored Andosols and is moderately fine-textured. There were few root knot nematodes in the experimental fields. The cultivars were Tachibana, Dejima and Nishiyutaka that had been planted in late August. On each sampling, 8 to 40 diseased plants were collected from each field. After each sampling, the rest of plants showing symptoms of bacterial wilt were removed to avoid duplication in sampling. On the fourth of December, one tuber per plant from 50 plants in each field were sampled.

Isolations were made as follows: The stems or tubers of the potatoes were washed with tap water, and the surface was sterilized in a 1.0% of sodium hypochlorite. A segment including vascular tissue was placed in sterile water in a test tube. The bacterial suspension was streaked onto YPA medium (containing 5 g yeast extract, 10 g peptone, 15 g agar in one liter of distilled water, and adjusted to pH 6.9±1). The streaked plates were incubated for 48 hr at 33 C. Bacteria identified as *P. solanacearum* by the colony type[4] were restreaked, and maintained in sterilized water for further examinations.

Determination of biovars. Biovar determinations were completed as described by Hayward[6]. The basal medium contained 0.5 g K_2HPO_4, 0.5 g NH_4NO_3, 0.2 g $MgSO_4 \cdot 7H_2O$, 8 ml of 0.2% bromothymol blue, and 12 g agar in one liter of distilled water. The pH was adjusted to 6.8 before addition of the agar. Lactose or mannitol was added as sole carbon sources after autoclaving.

Bacterial growth temperatures. Eighteen strains of biovar II and 14 of biovar IV that were obtained from the three fields in October were examined for growth in static culture. The cultures were grown on YPA slants for 48 hr at 33 C, then suspensions in sterile distilled water were prepared from these cultures. One drop of bacterial suspension was added to 10 ml per test tube of Stanier *et al.* 's medium[20] containing 0.25% sucrose. Each strain was grown in triplicate in static condition for 14 days at 16.5 C, for seven days at 24 C, and for four days at 35 C. Absorbance at 540 nm was determined with a spectrophotometer (UVIDEC 320, Japan Spectroscopic Co., LTD.).

Inoculation test. To compare biovars II and IV with respect to virulence and the time before development of symptoms, a greenhouse inoculation test was completed using a root cutting method. Potatoes (cultivar: Dejima) were grown in sterilized soil in 16 cm diameter plastic pots. Four bacterial strains (No. 3-23 and 4-19 of biovar II, No. 2-7 and 3-2 of biovar IV) were examined. Inocula were prepared in shake culture in YP broth medium (components similar to YPA but without agar) and adjusted 10^7 cells/ml with sterile water. When the plants were about 20 cm high, roots were cut with a knife to a depth of 10 cm along two sides and then 20 ml of inoculum was poured over the cut roots. Ten plants were inoculated for each isolate, and 10 plants served as non-inoculated controls. The temperature of the soil in the pots was ranged from 14 to 28 C, with an average of 21 C for 22 days after inoculation.

Soil temperature. The soil temperature of the potato field was determined at 10 cm below the surface of row with a soil thermometer from September to November.

Results

Relationship of isolation date to biovars

The number of plants infected with *P. solanacearum* from which isolations were made was 43 on 7 th of October, 82 on 15 th of October, and 75 on 25 th of October. Nine cultures were obtained from tubers on the fourth of December. Following Hayward's classification[6], strains were divided into three of four biovars based on pH change in cultures after incubation for three and seven days. Of the 209 strains obtained from

Table 1. The number of strains isolated in October and December and the percentage classified as to biovar

Field No.	Cultivar	Sampling Date	Biovar II		Biovar III		Biovar IV		Total Number
			Number	%	Number	%	Number	%	
1	Tachibana	7 Oct.	0	0	0	0	8	100	8
		15 Oct.	3	13	1	4	19	83	23
		25 Oct.	9	56	0	0	7	44	16
		4 Dec.	2	40	0	0	3	60	5
2	Dejima	7 Oct.	2	12	0	0	15	88	17
		15 Oct.	7	20	0	0	28	80	35
		25 Oct.	9	23	0	0	30	77	39
		4 Dec.	1	50	0	0	1	50	2
3	Nishiyutaka	7 Oct.	0	0	0	0	18	100	18
		15 Oct.	0	0	0	0	23	100	23
		25 Oct.	4	20	0	0	16	80	20
		4 Dec.	0	0	0	0	2	100	2
	Total	7 Oct.	2	5	0	0	41	95	43
		15 Oct.	10	12	1	1	71	87	82
		25 Oct.	22	29	0	0	53	71	75
		4 Dec.	3	33	0	0	6	67	9

the potato plants and tubers, 37 were classified in biovar II, one in biovar III and 171 in biovar IV. Table 1 shows that the percentage of biovar II strains increased in the latter period of growing season, and this increase was noted among strains from each field. Three to ten colonies from each culture obtained from each ten plants and five tubers were examined with respect to biovar. All from a given source were shown to be of the same biovar.

Growth temperatures for different biovars

The growth of 32 isolates of *P. solanacearum* in the static culture are given in Fig. 1, in which each biovar is arranged in order of growth at 35 C. More strains of biovar II grew rapidly at 16.5 C than those of biovar IV; however, most biovar II strains grew less than biovar IV at 35 C. There were some exceptions, the growth of biovar II strains at 16.5 C for 14 days and 24 C for seven days was not very different from growth at 35 C for four days, but the growth of biovar IV at 35 C for four days was better than at 16.5 C for 14 days and 24 C for seven days. The difference in sampling date did not apparently influence the growth of each strains.

Inoculation test

Wilt symptoms in inoculated potato plants appeared seven to ten days of inoculation,

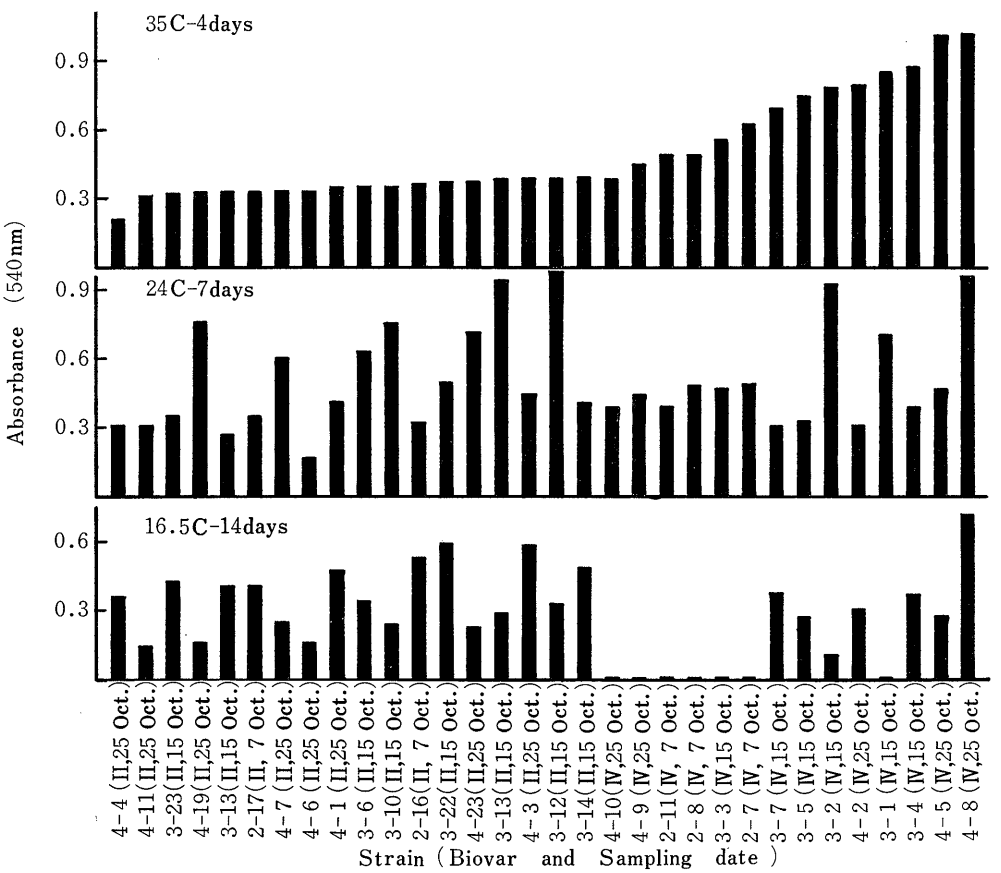

Fig. 1. Growth of biovar II and IV strains of *P. solanacearum* at three temperatures in Stanier *et al.*'s medium. Each biovar was arranged in ascending order of absorbance at 35 C.

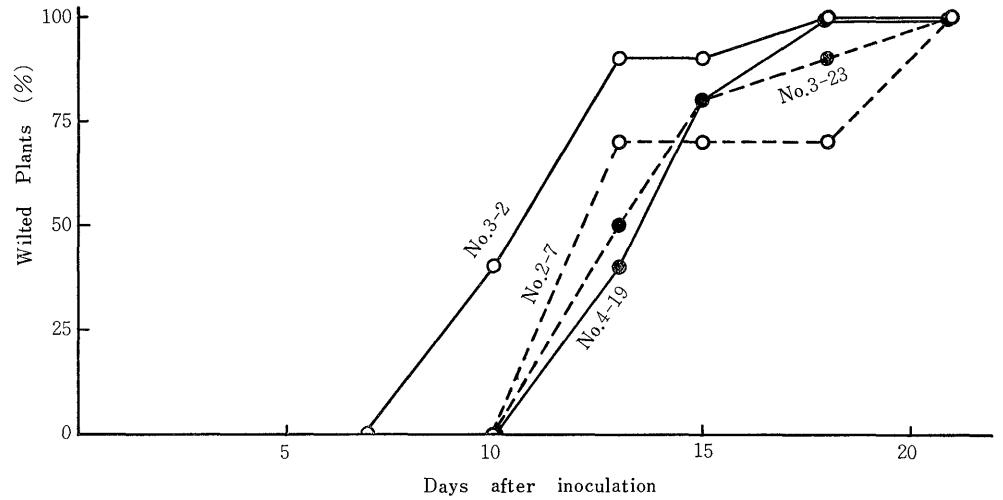

Fig. 2. Disease development in potato plants after inoculation with two strains of Biovar II (—●—) and two of biovar IV (—○—) of *P. solanacearum*.

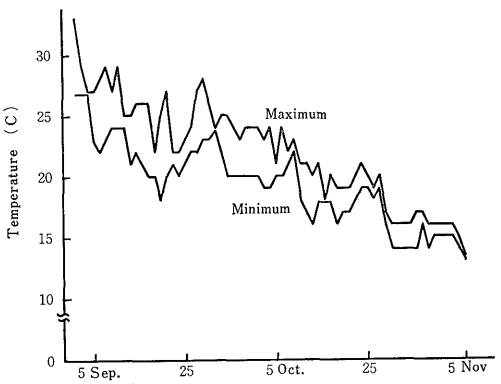

Fig. 3. Soil temperatures in a potato field in Aino, Nagasaki Pref. at 10 cm below the surface from September to November in 1981.

and all plants were wilted after 18 to 21 days (Fig. 2). No differences were observed between biovar II and IV strains in virulence and the time before symptoms developed. This indicates that the latent period of biovar II strains was not different from that of biovar IV strains under the temperature conditions of this test.

Soil temperature

The soil temperatures at 10 cm below the surface from September to November are shown in Fig. 3. The maximum temperature was more than 30 C, minimum was more than 25 C at the planting time, and temperatures decreased to below 20 C in late October.

Discussion

Biovar II and IV strains of *P. solanacearum* were mainly detected in fields where potato has been grown twice a year for 10 to 20 years. An increase in biovar II strains was observed late in the fall cropping period. Biovar II strains grew as well or more rapidly than biovar IV strains at low (16.5 C) and intermediate temperatures (24 C). At the high temperature (35 C), biovar II grew less rapidly than biovar IV. Since there was no difference in the latent period between biovar II and IV strains, the results obtained in the temperature study may provide an explanation for the increase of biovar

II strains in the latter period. Biovar II is probably less active than biovar IV under high temperature conditions in the field, and the increase of biovar II strains may reflect the decrease of temperature at the end of growing season. Ciampi and Sequeira (1980) reported that growth *in vitro* is not related to the ability of different strains to induce symptoms at low temperature[3]. However, our data would support the view that less rapid growth of biovar II strains at high temperatures may explain why so few biovar II strains were obtained in early October. The soil temperature measurements indicated that temperatures did not exceed 30 C in October. On the assumption that growth of *P. solanacearum* in the rhizosphere[21] occurs after rooting of potato, the soil temperature favoring symptom development may be correlated with temperatures favoring the bacterial growth in static culture.

Bacterial wilt of potato can occur in low temperature or high altitude regions[9,16,18,19]. Furthermore, certain potato strains with characteristics of race 3 had a lower optimum temperature than other strains[1,5,22]. The biovar II and IV strains isolated in this study can be considered to be race 3 and race 1, respectively, based on criteria defined by Buddenhagen and Kelman (1962)[2]. This is the first report of race 3 of *P. solanacearum* in Japan.

It appears that two biologically different strains, biovar II and IV, exist in Nagasaki, Japan. Biovar II may attack potatoes under relatively low temperature conditions. Therefore, the damage by *P. solanacearum* on potatoes in fall plantings is greater than would be expected if only biovar IV was present. Although some differences between biovars have been reported[7,17], natures of strains infested potatoes in Japan were not known very well. Further studies on the host range and distribution of biovar II strains in Japan as well as their biological characteristics are necessary.

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和 文 摘 要

Pseudomonas solanacearum Biovar II および IV 系統によるジャガイモ青枯病の発生と病原細菌の温度要求性

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長崎県内のジャガイモ栽培ほ場で秋作後期，定期的に罹病植物体から *Pseudomonas solanacearum* を分離し，その biovar を調べた。分離した209菌株のうち37菌株は biovar II，1菌株が biovar III，171菌株は biovar IV であったが，発病後期になるほど biovar II 系統の分離比率が増加する傾向が認められた。静置培養条件下で両系統の増殖を比較した結果，16.5 C では biovar IV 系統に比べて biovar II 系統の増殖が速かった。また，biovar IV 系統の 35 C，4 日間の増殖は 24 C，7 日間および 16.5 C，14日間のそれに比較して良好であったのに対して，biovar II 系統では 16.5 C，14日間培養と 35 C，4 日間培養との間に増殖量の明瞭な差は認められなかった。なお，接種試験による病徴発現までの潜伏期間も biovar II 系統と biovar IV 系統の間には差異が認められなかった。*P. solanacearum* にはレース1のほか主にジャガイモを侵し，低温でも病原性を示すレース3が存在し，かつ，レース3は biovar II に当ると報告されているが，本試験で分離された biovar II 系統は，我国で未報告のレース3に相当するものと考えられる。