Homologous Sequences with a Plasmid pJTPS1 Are Contained in *Pseudomonas solanacearum* Isolated from Tobacco in Japan

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Bacterial wilt caused by *Pseudomonas solanacearum* (Smith) Smith is one of the most destructive diseases in solanaceous plant fields. We have previously reported that a circular double-stranded DNA plasmid pJTPS1 carrying unique restriction endonuclease sites existed in *P. solanacearum* M4S⁹⁾, a spontaneous non-pathogenic mutant from *P. solanacearum* U-7¹¹⁾. This plasmid was thought to be excised from genomic DNA or megaplasmid DNA in the parental pathogenic strain of U-7⁹⁾ and autonomously replicated by an unknown mechanism. We showed the evidences that the presence of a small plasmid pJTPS1 correlated to the shift of the phenotypic changes (pathogenicity, colony morphology, extracellular polysaccharide (EPS) production and endoglucanase (EG) activity) in the spontaneous mutation of *P. solanacearum* and it was postulated that a small plasmid pJTPS1 carried non-pathogenicity factor for bacterial wilt disease⁶⁾. However, it has not been elucidated whether the homologous sequences with pJTPS1 generally exists in the genome or megaplasmid of pathogenic isolates of *P. solanacearum*.

It has been reported that there are five bacterial groups in *P. solanacearum* isolated from eggplant in Japan based on the different aggressiveness to eggplant and its stocks⁸⁾. Biovar is another standard to classify strains of *P. solanacearum*⁴⁾ by the ability to utilize sugars. However, the bacterial group or biovar of tobacco isolates in Japan have not been well characterized.

This paper describes 1) the investigation of bacterial groups and biovars of *P. solanacearum* isolated from tobacco in Japan, and 2) the existence of the homologous sequences with pJTPS1 in a variety of strains of the bacterium.

Strains of *P. solanacearum* were isolated from diseased plants or soil of tobacco fields by growing on the modified GS medium^{2,12)}. Isolated bacteria (186 strains) were routinely grown on Kelman's TZC medium⁹⁾ at 30°C for 3 days and stored in sterilized distilled water at room temperature. Bacterial group of these strains was investigated according to the method of Ozaki and Kimura⁸⁾. Biovar of these strains was investigated according to the method of Hayward⁴⁾. The number of strains isolated from different geographical areas and their bacterial groups are listed in Table 1. The largest population belonged to bacterial group II (41.9%) followed to III, IV, V and I (35.5%, 17.7%, 3.8% and 1.1%, respectively). One hundred seventy-seven out of 186 strains belonged to biovar III and the rest to biovar II. No strain belonged to biovar I, IV or V. There was no distinct relationship between the areas of isolation and bacterial groups or biovars.

Fifty-five strains of *P. solanacearum* were chosen for RFLP analysis based upon geographical

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Table 1. The bacterial groups of *P. solanacearum* isolated from tobacco and tobacco fields in Japan

Area	Total number	Bacterial group				
		I	II	III	IV	V
Northern Japan	45	1	22	10	8	4
Eastern Japan	74	0	33	25	13	3
Western Japan	24	0	11	11	2	0
Southern Japan	43	1	12	20	10	0
Total	186	2 (1.1%)	78 (41.9%)	66 (35.5%)	33 (17.7%)	7 (3.8%)

Table 2. DNA : DNA hybridization analysis of strains of *P. solanacearum* with pJTSP1 as a probe, the location of their isolation and their bacterial group

# of strain	Prefecture	Bacterial group	RFLP type*	# of strain	Prefecture	Bacterial group	RFLP type
U-7	Tochigi	III	I	186	Toyama	IV	I
213	Akita	II	I	232	Ishikawa	II	I
322	Akita	III	I	191	Ishikawa	III	I
212	Akita	IV	I	23	Fukui	IV	I
324	Akita	V	I	207	Shiga	II	I
250	Iwate	II	I	113	Hyogo	II	I
248	Yamagata	II	I	160	Okayama	II	I
158	Yamagata	IV	I	204	Tottori	III	II
230	Miyagi	II	I	118	Shimane	III	I
195	Fukushima	II	I	197	Hiroshima	II	I
193	Fukushima	III	I	117	Hiroshima	III	II
194	Fukushima	IV	I	199	Yamaguchi	III	—
284	Fukushima	V	I	20	Yamaguchi	IV	I
10	Tochigi	I	I	116	Tokushima	II	I
164	Tochigi	II	I	157	Tokushima	III	II
21	Tochigi	III	I	189	Kagawa	II	I
226	Tochigi	IV	I	114	Ehime	II	I
162	Ibaraki	II	I	154	Kouchi	II	—
276	Ibaraki	V	I	111	Kouchi	III	I
275	Ibaraki	III	I	161	Kouchi	IV	—
166	Ibaraki	IV	I	224	Nagasaki	I	II
168	Chiba	II	I	142	Nagasaki	III	—
155	Shizuoka	III	II	123	Kumamoto	II	I
214	Niigata	II	I	180	Kumamoto	III	—
215	Niigata	IV	II	130	Kumamoto	IV	I
247	Niigata	V	I	125	Miyazaki	III	I
183	Nagano	II	I	108	Kagoshima	III	—
234	Toyama	II	I				

*RFLP type

I : At least three *Pst*I fragments (3.8, 1.1 and 0.5 kb) hybridized with pJTSP1II : At least four *Pst*I fragments (3.8, 1.1, 0.5 and 1.2 kb) hybridized with pJTSP1

— : No fragment hybridized with pJTSP1

distribution and bacterial groups. By using total DNA isolated from these strains and U-7 as a standard, DNA : DNA blot hybridization analysis was performed with pJTSP1 digested with *Pst*I as a probe. The total DNAs, including genome, megaplasmid(s) and small plasmid(s), of these strains were isolated by the procedure described by Xu *et al.*¹³⁾ except that isopycnic centrifugation in CsCl-ethidium bromide was omitted. After the treatment with RNaseI (Sigma), 5 μ g of the total DNA was digested with *Pst*I and subjected to electrophoresis on a 0.7% agarose gel in TBE buffer. For DNA labeling, blotting and

Fig. 1. DNA : DNA blot hybridization analysis of total DNA from *Pseudomonas solanacearum* with pJTPS1 as a probe. The lanes consist of pJTPS1 (lane A), total DNA extracted from *P. solanacearum* U-7 [RFLP type I] (lane B) and a strain number 157 [RFLP type II] (lane C). The numbers on the left denote the size (in kb) of the DNA fragments.

DNA hybridization, non radioactive DNA Labeling and Detection Kit™ (Boehringer Mannheim Yamanouchi Inc.) and Southern Light™ (Tropix Inc.) were used according to the manufacture's specification. U-7 had at least five *Pst*I fragments hybridizing to pJTPS1 and the three *Pst*I fragments positioned at 3.8, 1.1 and 0.5 kb were identical to those of pJTPS1⁷⁾ (Fig. 1, lane B). RFLP analysis of these DNAs with a plasmid, pJTPS1, as a probe were shown in Table 2. Forty-nine strains out of 55 had homologous sequences with pJTPS1. Furthermore, 43 strains including U-7 had at least three *Pst*I fragments (3.8, 1.1 and 0.5 kb) hybridized with pJTPS1 (RFLP type I). Six strains had an additional *Pst*I fragment (1.2 kb) hybridized with pJTPS1 (RFLP type II) (Fig. 1, lane C). Six strains had no homologous sequences with pJTPS1 and all of them were isolated in western Japan. There was no clear correlation between the locations of isolation and bacterial groups by RFLP analysis. These data suggest that homologous sequences with pJTPS1 commonly exist in *P. solanacearum*, and most of these sequences are same in length. *P. solanacearum* of RFLP type II may have the duplicate homologous sequences with pJTPS1.

In this paper, we have demonstrated that *P. solanacearum* isolated from tobacco in Japan commonly possesses the homologous sequences with pJTPS1 regardless of the locations of isolation and their bacterial groups. pJTPS1 is thought to carry non-pathogenicity factor which is involved in phenotype changes of *P. solanacearum*. Gene(s) on pJTPS1 may be free from the control and/or regulation mechanism which suppresses the expression of the corresponding gene(s) on the genome or megaplasmid, ultimately resulting in the phenotypic change of wild type⁶⁾. Therefore, almost all strains of *P. solanacearum* may change their phenotypes (pathogenicity, colony morphology, EPS production, EG activity etc.) by the same mechanism as M4S. It still may be possible that the strains possessing no homologous sequences with pJTPS1 change their phenotypes, because the gene(s) or region involving in phenotypic change is not only pJTPS1 but also *phcA*¹⁾ and *epsR*³⁾. It is interesting to investigate what kind of phenotypic change will occur in these strains, if they may be mutated by pJTPS1. Studies are under way to determine the entire DNA sequence of pJTPS1 and to characterize the role of the genes responsible for spontaneous mutation in pathogenicity.

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和 文 摘 要

根岸秀明・田中 博・前田初枝・山田哲治：日本各地のタバコから分離した*Pseudomonas solanacearum*におけるプラスミドpJTSP1 相同性配列の存在

日本各地のタバコから 186 菌株の *P. solanacearum* を分離した。これらの菌株について、ナスとナス台木に対する病原性をもとにした菌群、生理型 (biovar) を調べた。菌群は II 型が最も多く次いで III, IV, I の順であった。生理型はほとんどの菌株が III 型であり、そのほかわずかに II 型が存在した。分離された地域と菌群および生理型には明確な関係は認められなかった。これらの菌株から菌群と分離地域が異なる 55 菌株を選び、全 DNA を抽出し、非病原性菌株 M4S 由来のプラスミド pJTSP1 をプローブとした DNA:DNA プロットハイブリダイゼーションを行ったところ、49 菌株が pJTSP1 と相同性を示し、そのうち 43 菌株は M4S の親株 U-7 と同様に、少なくとも 3 本の *Pst*I 消化 DNA 断片 (3.8, 1.1 and 0.5 kb) が pJTSP1 と相同性を示した。これら 3 本の DNA 断片に加えて 1.2 kb の pJTSP1 と相同性を示す *Pst*I 消化断片を持つものが 6 菌株存在した。pJTSP1 に対する相同性部位の有無に関する RFLP 解析では、地域と菌群との明確な関係は認められず、pJTSP1 との相同性部位は病原性の *P. solanacearum* に広く全般的に存在していた。

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