フジこぶ病菌 (Erwinia herbicola pv. milletiae) のプラスミド

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短　報
Phytopathological Note

Plasmids of *Erwinia herbicola* pv. *milletiae**

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Key words : plasmid, *Erwinia herbicola* pv. *milletiae*, tumorigenicity.

The tumorigenicity of *Agrobacterium tumefaciens*[1] and *Pseudomonas syringae* pv. *savastanoi*[2] has been reported to be controlled by plasmid.

Erwinia herbicola pv. *milletiae* which incites galls on the stems of *Wistaria floribunda*[3,4] has been recorded only in Japan. The tumorigenicity of this bacterium is unstable and frequently lost during subculture. A cryptic plasmid of this bacterium was first reported by Tsuyumu *et al.*[5], and its molecular weight was estimated as 10-20 Mdal. The present study was undertaken to determine (I) whether or not *E. herbicola* pv. *milletiae* strains contain plasmids other than that reported by Tsuyumu *et al.* and (II) whether or not the loss of some plasmid is associated with loss of tumorigenicity or other traits.

E. herbicola pv. *milletiae* isolate EM 1, K-21 and Wist 801 were supplied by Drs. M. Goto and Y. Takikawa, Shizuoka University. Isolate E2 was supplied by Dr. S. Wakimoto, Kyushu University. Other 13 isolates were isolated from galls of *W. floribunda* and kept in our laboratory. Tumorigenicity was tested by inoculating bacteria on the stem of young seedlings of *W. floribunda*. A drop of the inoculum (conc. ca. 5×10^8 cells/ml) was placed at the stem and pricked several times with a needle. The inoculated stem was covered with polyethylene bag for 24 hr to keep high humidity. The plants were incubated in the greenhouse for about one month until gall formation. Isolate EM 1 and E2 did not cause galls, although small swellings developed at the inoculation sites. K-21 occasionally caused very small galls. All of the other strains incited galls about 8 mm in diameter after one month.

Plasmid DNA was prepared by the alkaline extraction method as described previously[6], and examined by electrophoresis in 0.7% agarose gel. The molecular weight of plasmid was estimated by measuring their electrophoretic mobilities in comparison with those of the standard plasmids, including pBR325 (3.6 Mdal), pAS8Tc^{s}repl::Tn7 (52 Mdal), and the large (124 Mdal) and small (29.8 Mdal) plasmid from *A. radiobactor* 84[7]. pAS8Tc^{s}repl::Tn7 was supplied by Dr. Sato, National Institute of Agrobiological

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1) Van Larebeke, N., Englers, G., Holsters, M., Van den Elsacher, S., Zaenen, I., Schilperoot, T. and Schell, J. (1974). Nature 252：169-170. 2) Comai, L. and Kosuge, T. (1980). J. Bacteriol. 143：950-957. 3) Kawakami, K. and Yoshida, S. (1920). Bot. Mag. Tokyo 30：110-115. 4) Goto, M., Takahashi, T. and Okajima, T. (1980). Ann. Phytopath. Soc. Japan. 46：185-192. 5) Tsuyumu, S., Shirata, A. and Goto, M. (1981). *Ibid.* 47：395 (Abstr.). 6) Kamiunten, H. and Wakimoto, S. (1983). *Ibid.* 49：633-638. 7) Merlo, D. J. and Nester, E. W. (1977). J. Bacteriol. 129：76-80.

Resources.

As shown in Fig. 1 and Table 1, six different plasmids, the molecular weight of which ranged from approximately 34 to 120 Mdal, were detected. The molecular weight of each plasmid was larger than that of a cryptic plasmid reported by Tsuyumu *et al.*[5]. One to three kinds of plasmid were contained in the isolates of *E. herbicola* pv. *milletiae* (Table 1). To detect the plasmid larger in size than those detected above, the methods of Currier and Morgan[8,9] and Kado and Lin[10] were also used. However, the plasmid having molecular weight larger than 120 Mdal could not be detected. The bacterial isolates were classified into 6 groups (I to VI) based on their plasmid patterns (Table 1). The highest number of the isolates belonged to the group IV. The 110 Mdal plasmid was detected in all isolates but its resolution was markedly different depending upon isolates, *viz.*, the tumorigenic isolates produced clear band, while non-tumorigenic isolates, EM 1 and E2, produced very faint band.

Further investigations on the relationship between the 110 Mdal plasmid and tumorigenicity was carried out. Isolate M8501, M8505 and M8508 kept on the slant of YPA

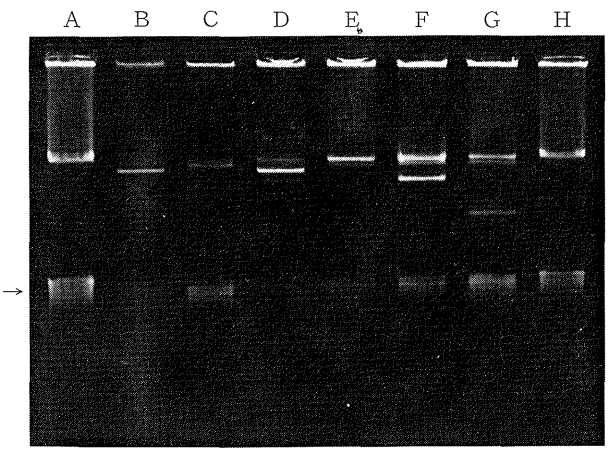

Fig. 1. Agarose gel electrophoresis of plasmid DNA isolated from *Erwinia herbicola* pv. *milletiae*.
(A) M 8505, (B) EM 1, (C) E 2, (D) K-21, (E) Wist 801, (F) M 8501, (G) M 8508, (H) M 8505.
Arrow : chromosomal DNA

Table 1. Plasmids of *Erwwinia herbicola* pv. *milletiae*

Bacterial isolates	Tumorigenicity	Molecular weight of plasmids ($\times 10^6$)	No. of plasmids	Grouping[a]	No. of isolates belonging to each group
EM 1	−	80,110,120	3	I	1
E 2	−	100,110,	2	II	2
K-21	±	80,110	2	III	1
Wist 801	+	100,110	2	(II)	
M 8501	+	70,100,110	3	IV	6
M 8508	+	34,100,110	3	V	4
M 8505	+	110	1	VI	2

a) Grouping by plasmid pattern.

8) Currier, T. C. and Morgan, M. K. (1982). J. Bacteriol. 150 : 251-259. 9) Currier, T. C. and Morgan, M. K. (1983). Can. J. Microbiol. 29 : 84-89. 10) Kado, C. I. and Lin, S. T. (1981). J. Bacteriol 145 : 1365-1373.

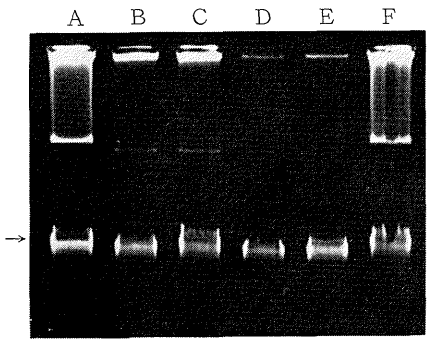

Fig. 2. Agarose gel electrophoretic profiles of plasmid DNA isolated from M 8505 and its derivative isolates obtained by the treatment with acridine orange.
(A) M 8505, (B) M 8505^{d} 1, (C) M 8505^{d} 2, (D) M 8505P^{-} 1, (E) M 8505P^{-} 2, (F) M 8505. Arrow: chromosomal DNA

medium (yeast extract 5 g, peptone 10 g, NaCl 5 g, agar 15 g, per liter of distilled water, pH 7.0) for about 6 months at room temperature were streaked onto the plate of YPA medium. Ten colonies from each isolates were picked up and cultured for testing tumorigenicity. All colonies obtained from each isolate lost tumorigenicity but all of these non-tumorigenic mutants still maintained the 110 Mdal and other plasmids. Thus it may be concluded that the loss of tumorigenicity by prolonged cultivation was not associated with any detectable alteration in the contents or molecular size of plasmids.

All of the isolates of *E. herbicola* pv. *milletiae* tested in this study contained the 110 Mdal plasmid. Therefore, this indigenous plasmid was expected to play a important genetic role in this bacterium. To detect the genetic functions of the 110 Mdal plasmid of M8505, curing of this plasmid was performed according to the method of Comai and Kosuge[2]. Two hundred and thirty one colonies isolated from the cultures of the bacteria treated with acridine orange (150 μg/ml) were used for testing their plasmid patterns.

As shown in Fig. 2, two derivative isolates (M8505P^{-} 1 and M8505P^{-} 2) lost the 110 Mdal plasmid, and other two derivative isolates (M8505^{d} 1 and M8505^{d} 2) appeared to have undergone a small deletion. Change of M8505 in phenotypes correlating with the loss or deletion of 110 Mdal plasmid was examined. No difference between parental isolate and four derivative isolates was detected in basic physiological and biochemical characteristics [growth factor requirement, Hugh and Leifson's test, H_2S production, reaction in litmus milk, coagulation of litmus milk, methyl red test, production of acetoin, catalase, oxidase, urease, phenylalanine deaminase, nitrate reduction, gelatin liquefaction, production of indole in the minimal medium containing tryptophan, resistance to α-methyltryptophan and antibiotics, growth in 5% NaCl], and utilization of carbohydrates such as malonate, xylose, arabinose, rhamnose, glucose, fructose, galactose, mannose, sucrose, lactose, salicin, galacturonic acid, citric acid, lactic acid, formic acid and tartaric acid.

It had been reported that yellow pigmentation of *E. herbicola* is controlled by plasmid[11]. Isolate M8505 lost yellow pigmentation at a frequency range of 2×10^{-2}-4×10^{-3} when grown at elevated temperature (37 C). In our experiment, however, all of the

11) Gantotti, B. V. and Beer, S. V. (1982). J. Bacteriol. 151 : 1627-1629. 12) Gantotti, B. V., Kindle, K. L. and Beer, S. V. (1981). Current Microbiology 6 : 377-381. 13) Fredericq, P. (1957). Ann. Rev. Microbiol. 11 : 7-22.

pigmentless mutants contained 110 Mdal plasmid.

The involvement of the plasmid (96 Mdal) in bacteriocin production has been reported in *E. herbicola*[12]. To determine if the isolates of *E. herbicola* pv. *milletiae* produce bacteriocin, bioassays were performed according to Fredericq's method[13]. Isolates M8501, M8505, M8508, EM 1, E2, K-21 and Wist 801 did not produce antibacterial substance against *Escherichia coli*, *E. carotovora* subsp. *carotovora*, *Pseudomonas solanacearum*, *Xanthomonas campestris* pv. *oryzae*, *X. campestris* pv. *citri*, *Corynebacterium michiganense* pv. *michiganense* and *A. tumefaciens*.

Further studies on the functions of the 110 Mdal plasmid are required.

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和 文 摘 要

上運天　博：フジこぶ病菌（*Erwinia herbicola* pv. *milletiae*）のプラスミド

供試したフジこぶ病菌16菌株は分子量約 34～120 Mdal のプラスミドをそれぞれ 1～3 個有しており，それらの菌株はプラスミドパターンから 6 群に分けることができた。また，全供試菌株が持っている 110 Mdal プラスミドと菌株のこぶ形成能との関係を調べた。その結果，長期保存中にこぶ形成能を完全に失った菌株も同プラスミドを保持しており，こぶ形成能との明らかな関連性は認められなかった。この 110 Mdal プラスミドの機能を調べる目的で M8505 株からアクリジンオレンジ処理（150 μg/ml）による除去を試みた。その結果，231コロニー中，2 コロニーからは完全に脱落し，他の 2 コロニーのプラスミドからは一部が欠失していた。これら 4 菌株と親株の細菌学的諸性質34項目について比較検討したが差は認められず，その機能については不明である。

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