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Plasmids of *Pseudomonas glumae**

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Plasmids associated with pathogenicity were reported to be detected in some plant pathogenic bacteria such as *Agrobacterium tumefaciens*[1], *Pseudomonas savastanoi*[2] and *Pseudomonas syringae* pv. *atropurpurea*[3].

Pseudomonas glumae is the pathogenic bacterium causing bacterial grain rot and bacterial seedling rot of rice[4-6]. Pathogenicity of this bacterium, however, is known to be unstable and often lost during subculture. It is suspected, therefore, that the pathogenicity might be encoded by plasmid DNA. In order to investigate whether some plasmids are associated with pathogenicity or not, plasmids of the pathogenic isolates of *P. glumae* were compared with those of nonpathogenic isolates. Pathogenicity of the bacteria against rice seedlings was tested by dipping grains to the inoculum suspension (conc. ca 10^8 cells/ml) just before germination and that against grains was tested by dipping ears at the heading stage of rice[7]. All isolates, including both pathogenic and nonpathogenic, showed positive reaction producing a precipitin band with antisera of *P. glumae* isolate N7501 in gel diffusion test.

Plasmid DNA was isolated by the alkaline extraction method as described previously[8]. The plasmid DNA isolated was subjected to electrophoresis in 0.7% agarose gel at 7 V/cm for 3 hr. The molecular weight of plasmid was estimated by measuring their electrophoretic mobilities relative to the standard plasmids. Molecular weight standards included pBR 325 from *E. coli* (3.6 Mdal), and the large (124 Mdal) and small (29.8 Mdal) plasmids from *Agrobacterium radiobactor* 84[9].

As shown in Fig. 1 and Table 1, both pathogenic and nonpathogenic bacteria contained 2 to 6 plasmids, the molecular weight of which ranged from approximately 8 to 135 megadalton. The electrophoresis patterns were different in each isolate and the

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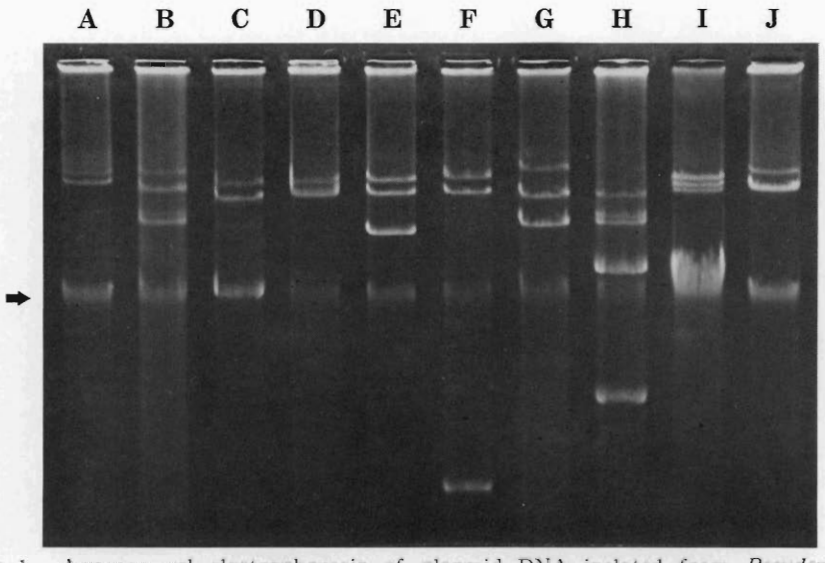

Fig. 1. Agarose gel electrophoresis of plasmid DNA isolated from *Pseudomonas glumae*. (A) P1-22-1, (B) N7401, (C) YN7805, (D) 8020, (E) 750, (F) Ku8121, (G) N7501, (H) N7505, (I) So 1, (J) Ku8101. Arrow : chromosomal DNA

Table 1. Plasmids of *Pseudomonas glumae*

Bacterial isolate	Pathogenicity	Molecular weight of plasmid ($\times 10^6$)	No. of plasmids
P1-22-1	−	100, 124	2
N7401	−	56, 63, 93, 124	4
YN7805	−	55, 85, 110	3
8020	−	85, 110	2
750	−	49, 85, 110	3
Ku8121	+	8, 85, 124	3
N7501	+	55, 85, 135	3
N7505	+	13, 30, 55, 63, 85, 135	6
So 1	+	90, 100, 110	3
Ku8101	+	93, 130	2

plasmid related to the pathogenicity was not found. Plasmid of 85 megadalton was detected irrespective of pathogenicity in six isolates out of 10 tested. Furthermore, the pathogenic isolate N7501 and nonpathogenic isolate N7501-av obtained from N7501 during subculture were found to be the same in plasmid pattern. These results suggest that the plasmids did not directly participate in the pathogenicity of *P. glumae*.

To detect the plasmids larger than 300 megadalton in molecular weight, Currier and Morgan's "in-well lysis" technique[10,11] was used, because larger plasmids are not detectable by the above alkaline extraction method. The cells were picked up with a tooth

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pick from a fresh colony and suspended in 20 μl of Tris-borate buffer (0.89 M Tris, 0.89 M boric acid, 25 mM EDTA). The bacterial suspension was transferred to a well in a horizontal plate prepared with 0.7% agarose gel. Two microliters of lysing mixture (20% glycerol, 5 M urea, 0.25 M EDTA, 10% SDS, 0.05% bromophenol blue, pH 7.0) was added to the bacterial suspension, and the solution was mixed by five strokes with plastic pipette tip used for delivering the lysing mixture. Electrophoresis was performed for 1 hr at 2 mA and for 3 hr at 5 V/cm. No plasmid whose molecular weight was greater than those isolated by alkaline extraction method was detected.

Curing test was performed according to the method of Comai and Kosuge[2]. Single coloies of Ku8101 and N7501 were isolated from cultures treated with acridine orange (50, 100, 150, 200 μg/ml) and ethidium bromide (50, 100 μg/ml). In each treatment, about 100 to 300 colonies were tested by the "in-well lysis" technique for detecting plasmid. All colonies tested, however, still retained their plasmids.

和 文 摘 要

上運天 博・山口純一郎・脇本 哲 ：イネもみ枯細菌病菌のプラスミド

イネもみ枯細菌病菌は継代培養中に容易にその病原性を失うことが知られており，病原性とプラスミドとの関連性を検討した。幼苗と籾に病原性を示す5菌株と継代保存中に両者に対する病原性を失った5菌株からアルカリ法によりプラスミドを分離し，0.7%アガロースゲル電気泳動によりそのパターンを比較した。その結果，全供試菌株はそれぞれ分子量約 8-135 Mdal のプラスミドを2～6個有していたが，それらのプラスミドパターンは菌株毎に異っており，病原性との関連性は認められなかった。また，N7501 菌株とそれから病原性を失った N7501-av 菌株のプラスミドパターンは全く同一であった。これらの結果から，イネもみ枯細菌病菌の病原性発現に対してプラスミドは直接的には関与していないものと考えられる。さらに大型プラスミドをも検討したが，その存在は認められなかった。

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