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https://doi.org/10.5109/24105

出版情報：九州大学大学院農学研究院紀要. 40 (1/2), pp.189-196, 1995-12. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 40 (1・2), 189-196 (1995)

Application of the Direct Colony TLC for Identification of Phytopathogenic Bacteria (III). Distinction of the Pseudomonads in the rRNA-homology Group II (*Burkholderia* Spp.)

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(Received July 31, 1995)

The comparison of chromatographic profiles of pseudomonads in the rRNA homology-group II (*Burkholderia* gen. nov.) was conducted by using the direct colony TLC method. The chromatograms of *Pseudomonas cepacia*, *P. caryophylli*, *P. glumae*, *P. gladioli*, *P. plantarii*, *P. andropogonis*, *P. solanacearum* and *P. rubrisubalbicans* resembled roughly each other. However, the profiles of three spots which were designated as S1, S2 and S3 were distinct at species level. The chromatograms of *P. andropogonis*, *P. solanacearum* and *P. rubrisubalbicans* were characteristic. Especially, the profile of *P. rubrisubalbicans* was quite unique in these pseudomonads. So far as the chromatograms for aminolipids concerned, some similarities were observed between *P. andropogonis* and *P. solanacearum*. However, *P. andropogonis* was distinguishable with the existence of single spot which is detectable at Rf 0.83 by spraying of the chromium-containing 55% sulfuric acid and heating at 110°C. The usefulness of the direct colony TLC method for rapid identification of phytopathogenic bacteria at species level was repeatedly verified.

INTRODUCTION

The direct colony thin layer chromatography was invented in 1986 (Matsuyama et *al.*, 1986) and firstly applied for rapid identification of phytopathogenic bacteria (Matsuyama, 1993a, Matsuyama et *al.*, 1993b, c, d, Matsuyama and Furuya, 1993e). The differentiation of the phytopathogenic bacteria at generic level was available among *Clavibacter*, *Agrobacterium* and others. Further, in genus *Erwinia*, the profiles of *E. chqsanthemi* and *E. carotovora* subsp. *carotovora* were clearly different. These differences at species level were also observed in genus *Pseudomonas*. These results indicated practical usefulness of this easy method for rapid identification of phytopathogenic bacteria. During these experiments, the existence of several groups which had similarities for chromatographic profiles in pseudomonads was found. These groups were Cepacia-type, Syringae-type and Solanacearum-type (Matsuyama and Furuya, 1993e).

Recent progress in rRNA-DNA hybridization analysis for bacterial taxonomy influenced largely on the taxonomy of pseudomonads (Palleroni *et al.*, 1973, De Vos and De Ley, 1983, Young *et al.*, 1992). A new genus *Burkholderia* and the transferring of some members of *Pseudomonas* to this genus were proposed, recently (Yabuuchi *et al.*, 1992).

In this report, the similarities and diversities of chromatographic profiles among the species of pseudomonads which were proposed to be transferred to a new genus

This study was supported in part by the Grant-in-aid from the Ministry of Education, Science and Culture of Japan (No. 06454064).

Burkholderia will be presented.

MATERIALS AND METHODS

Isolates tested

The isolates of Cepacia-type pseudomonads (*Burkholderia* gen. nov.) were tested in this experiment. To validate the results obtained, the type cultures from ATCC (American Type Culture Collection) were also used. The details of isolates were presented in Table 1.

Table 1. Isolates of pseudomonads used in this experiment.

Pseudomonas spp.	Isolates	Source
P. cepacia	356-5	NIAS
〃	ATCC 25416^T	ATCC
	243-4	NIAS
P. *caryophylli*	1192	
〃	1406	〃
P. gladioli pv. *gladioli*	1064	
〃	1065	〃
〃	251-17	〃
P. plantarii	AZ8201^T	〃
〃	MAFF 302484	NIAES
	MAFF 302387	〃
P. glumae	2	KNAS
	N7503	AKU
〃	8112	NIAS
P. andropogonis	MAFF 301006	NIAES
〃	MAFF 301129	
P. solanacearum	C319SR	KTES
〃	6211	NIAS
〃	ATCC 11696^T	ATCC
P. rubrisubalbicans	MAFF 301626	NIAES
〃	MAFF 301628	〃
Comamonas acidovorans	ATCC 15668^T	ATCC

NIAS : National Institute of Agricultural Sciences, Tokyo, Japan. Isolates from NIAS were stocked in duplicate at Kyushu University by Dr. S. Wakimoto after the consolidation of the Institutes of the Ministry of Agriculture, Forestry and Fisheries.

NIAES: National Institute of Agro-Environmental Sciences, Tsukuba, Japan.

ATCC : American Type Culture Collection.

KTES : Kagoshima Tobacco Experiment Station, Kagoshima, Japan.

AKU : Faculty of Agriculture, Kyushu University, Fukuoka, Japan.

KNAS : Kyushu National Agricultural Experiment Station, Kumamoto, Japan.

Type culture: Type culture was indicated at the shoulder of isolate number as a small T.

Culture of bacteria and methods of the direct colony thin-layer chromatography

Isolates were cultured on the slant of King's B medium at 30°C for 3 days unless otherwise stated. One loopful bacterial cells of each bacterial isolate was pasted directly on the origin of pre-coated silica gel TLC plate (Merck Co., Si 60, 0.25mm in thickness) and completely dried in the autodesiccator for ca.1 hour and/or by hair-drier. At first, the TLC plate was developed with chloroform-methanol(2:1, v/v) for 10 min. After the drying, the bacterial cells pasted were scraped off and the plate was developed to the same direction with chloroform-methanol-water (60:25:4, v/v/v) for 1.5 hr. The developments were carried out in the incubator at 25°C. After the development, the TLC plate was dried well, sprayed with ninhydrin and kept at 100°C for the detection of aminolipids. Detection by spraying of chromium-containing sulfuric acid (0.6% $K_2Cr_2O_7$ in 55% H_2SO_4) and heating at 110°C was conducted, also, for visualizing the benchmark spots of some pseudomonads. A frequent renewal of the TLC solvents in the glass vessels was carried out for a well reproducibility. The chromatograms were recorded by a photocopy. Other techniques have been presented in the former reports (Matsuyama et al., 1993b, c, d, Matsuyama & Furuya, 1993e).

RESULTS AND DISCUSSION

The chromatographic profiles of the species in rRNA homology group II *(Pseudomonas cepacia, P. caryophylli, P. gladioli* pv. *gladioli, P. glumae, P. plantarii, P. rubrisubalbicans, P. andropogonis* and *P. solanacearum)* were compared to verify the practical usefulness of this convenient measure, the direct colony TLC, for the rapid identification of phytopathogenic bacteria at species level. In this rRNA homology group II in pseudomonads, the chromatographic differences at species level were obviously observed. Especially, the benchmark spots Sl, S2 and S3 (Fig. 1) under the common spot (Rf 0.62) represented well the characteristics of each species. The existence and the relative size of Sl, S2 and S3 spots seemed to be species specific and the characteristics appeared at high reproducibility if King's B medium was used (Fig. 1). In the case of *P. cepacia,* spot Sl was larger than S2 and S3, and spot S2 was sometimes quite faint. While, *S2* spot was larger than S1 and S3 in case of *P. caryophylli* (Fig. 4). The chromatograms of *P. glumae, P. gladioli* and *P. plantarii* resembled each other. Especially, chromatograms of *P. glumae* and *P. gladioli* were not distinguishable, easily. However, a small difference between the chromatograms of these two species was sometimes observed for the ratio in size of spots S1 and S3 (Matsuyama and Furuya, 1993e). The size of spot S3 in *P. plantarii* is larger than S1 and S2 (Matsuyama *et al.*, 1993d, Matsuyama and Furuya,1993e). This characteristic of *P. plantarii* was always observed on the chromatograms of three isolates used.

The chromatograms of *P. andropogonis* and *P. solanacearum* are roughly similar but different in detail(Fig. 2, 3). The spot at Rf 0.83 which was detectable by the spraying of the chromium-containing 55% sulfuric acid and heating at 110°C existed on the chromatogram of *P. andropogonis* and not on that of *P.* solanacearum.

In the case of *P. rubrisubalbicans*, the profile was quite different from those of other

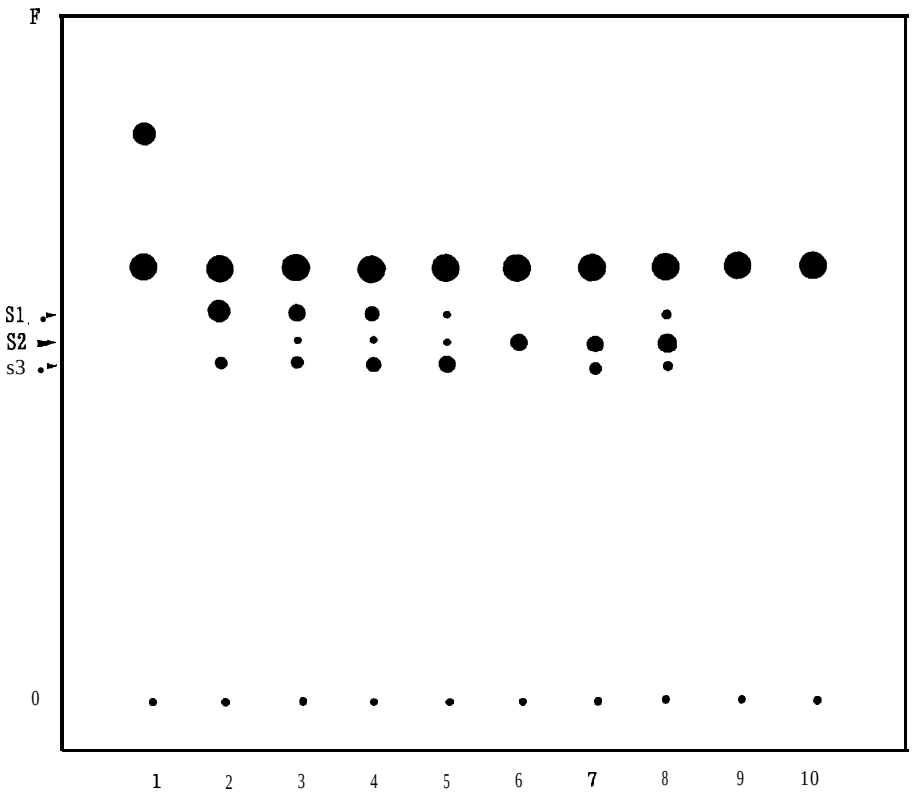

Fig. 1. Diagrammatic representation of TLC chromatogram of lipids from phytopathogenic bacteria.
1: *Pseudomonas rubrisubalbicans*, 2: *P. cepacia*, 3: *P. gladioli*, 4: *P. glumae*, 5: *P. plantarii*, 6: *P. solanacearum*, 7: *P. andropogonis*, 8: *P. caryophylli*, 9: *P. syringae*, 10: *P. avenae*
F: Solvent front, O: Origin
Arrow heads indicate S 1, S2 and S3 spots, respectively.

members of the homology group II. As can be seen in Fig. 1, 2, the spot at Rf. 0.71 appeared and the benchmark spots S1, S2 and S3 were absent. This individuality of *P. rubrisubalbicans* has been reported at the fatty acid analysis (Young *et al.*, 1992).

In this experiment, a practical usefulness of this direct colony TLC method for rapid identification of the phytopathogenic bacteria was repeatedly certified. Further, this novel technique gave lots of informations for the systematic bacteriology. Especially, the data on isolates of the homology group II in genus *Pseudomonas* will be meaningful for the discussion on the transferring of these pseudomonads as the members of *Burkholderia* gen. nov.

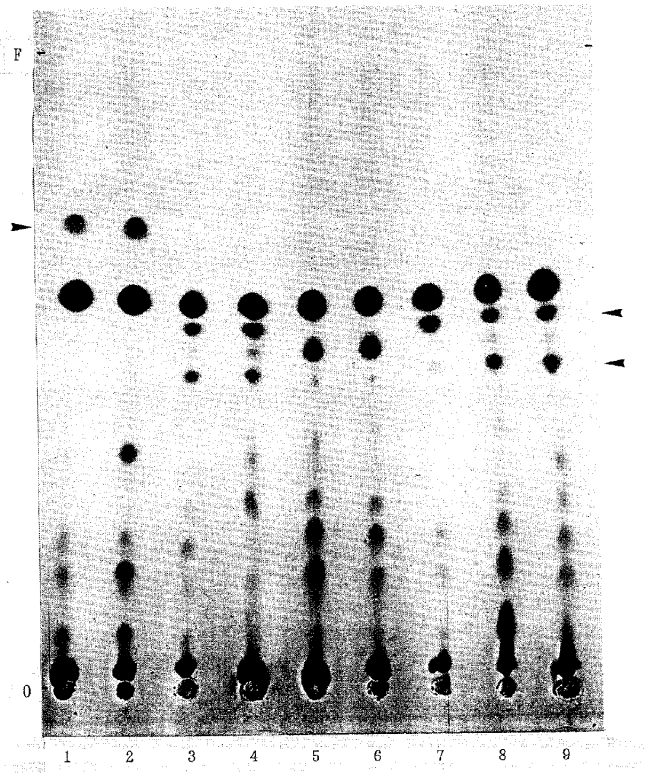

Fig. 2. TLC chromatogram of lipids from phytopathogenic bacteria

1:	*Pseudomonas*	〃 *rubrisubalbicans*	MAFF 301628
2:	〃	*gladioli*	MAFF 301626
3:	〃	pv. gladioli	251-17
4:	〃	〃 〃	1065
5:	〃	*andropogonis*	MAFF 301006
6:	〃	〃	MAFF 301129
7:	〃	*cepacia*	356-3
8:	〃	*glumae*	2
9:	〃	〃	N 7503

F: Solvent front, 0: Origin

Arrow head of left-side represents benchmark of *P. rubrisubalbicans* and those of right-side represent benchmarks S1 and S3.

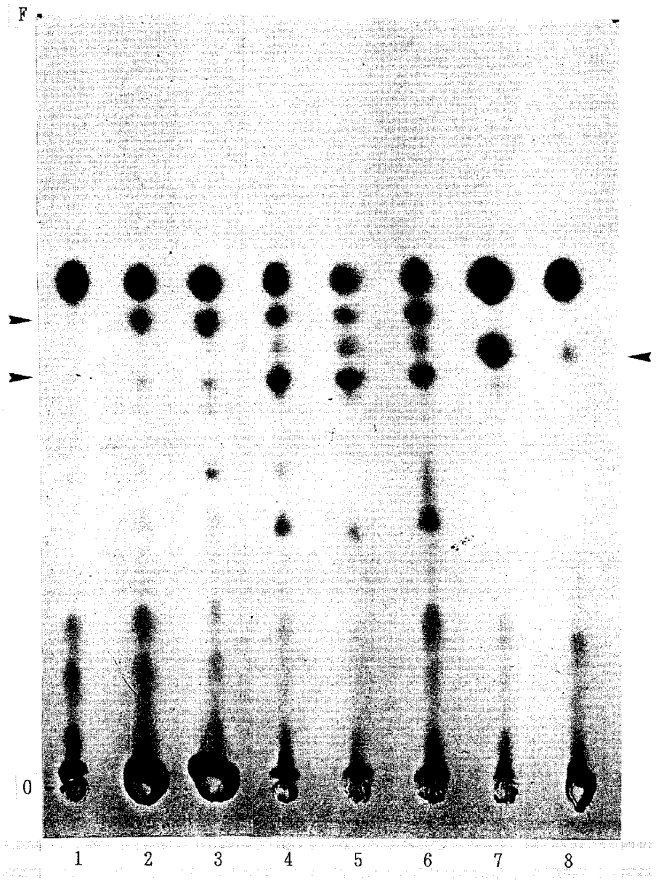

Fig. 3. TLC chromatogram of lipids from phytopathogenic bacteria.

1:	*Comamonas acidovorans*	ATCC 15668'
2:	*Pseudomonas cepacia*	*356-3*
3:	〃 〃	ATCC 25416'
4:	〃 *gladioli* pv. *gladioli*	251-17
5:	〃 〃	〃
6:	〃 *glumae*	*2*
7:	〃 *solanacearum*	C319SR
8:	〃 〃	6211

F: Solvent front, 0: Origin

Arrow heads of left-side represent benchmarks S1 and S3 spots and that of right-side represents benchmark S2 spot.

4: Three days culture, 5: Twelve days culture.

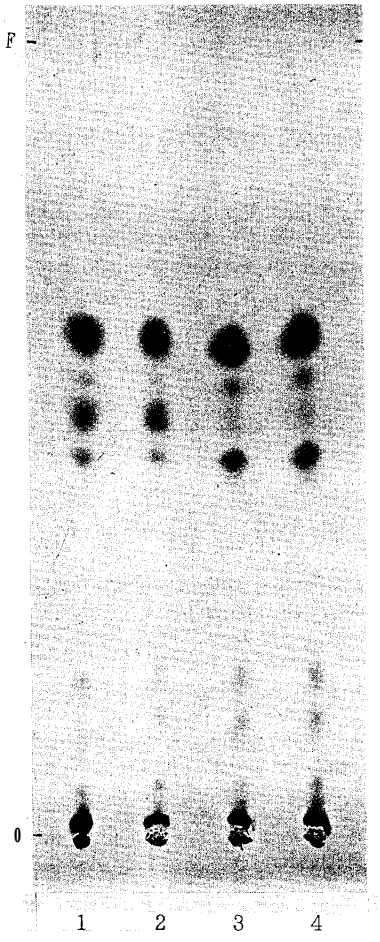

Fig. 4. TLC chromatogram of lipids from phytopathogenic bacteria.
1: Pseudomonas *caryophylli* 1192
2: 〃 〃 1406
3: 〃 *plantarii* AZ8201^T
4: 〃 〃 〃
F: Solvent front, 0: Origin

ACKNOWLEDGEMENT

The author is indebted to the staff of the Institute of the Biological Resources, Tsukuba and Dr. N. Furuya of author's laboratory for their kind supply of the phytopathogenic bacteria.

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