Application of the Direct Colony TLC for Identification of Phytopathogenic Bacteria (II) Chromatographic Profile of Erwinia and Pseudomonas spp.

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Application of the Direct Colony TLC for Identification of Phytopathogenic Bacteria (II) Chromatographic Profile of *Erwinia* and *Pseudomonas* spp.

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A rapid identification of phytopathogenic bacteria was available at species level by the direct colony thin-layer chromatography. Distinct difference was observed in chromatographic profile between *Erwinia chrysanthemi* and *E. carotovora* subsp. *carotovora*. In the case of *Erwinia carotovora* subsp. *carotovora*, a characteristic spot at Rf 0.57 appeared concomitantly with a common spot at 0.62. The chromatograms of *Pseudomonas* spp. were divided into three major types. The chromatograms of *P. gladioli* pv. *gladioli*, *P. glumae*, *P. plantarii*, *P. caryophylli* and *P. cepacia* resembled each other. This chromatographic profile was clearly different from those of other pseudomonads and designated as a Cepacia-type. In the case of *P. solanacearum*, a characteristic spot appeared at Rf 0.54 and this profile was named as a Solanacearum-type. The profile of remaining pseudomonads was simple and similar each other, and was designated as a Syringae-type. A practical usefulness of this easy method was certified also in this experiment.

INTRODUCTION

In 1986, an easy method for identification of lipids from bacterial whole cells was invented by Matsuyama et ***al.*** and used for the lipid-analyses of *Serratia* spp. (Matsuyama et ***al.,*** 1986, 1987). This method is one-dimentional TLC but involves two steps of the development with two kinds of developing-solvent systems. The first development with chloroform-methanol (CM, 2:1, v/v) for short time (10min) is conducted for the extraction of lipids from bacterial whole cells which were pasted directly on silica gel TLC plate. The second development with chloroform-methanol-water (CMW, 60:25:4, v/v/v) was conducted for the separation of lipids extracted by the first development with CM solvent-system. Recently, the authors applied this method for the distinction of the phytopathogenic bacteria (Matusyama et ***al.,*** 1993a, b, c) and verified its practical usefulness for a rapid identification of bacteria. In these former experiments, we found that this procedure was avairable for the distinction of bacteria at generic level and in some cases at species level. To certify these results, the comparison with more isolates was conducted.

MATERIALS AND METHODS

Bacterial strains used

Fifty-nine isolates of *Erwinia* and ***Pseudomonas*** spp. which were originally donated

Table 1. *Erwinia* species used in this experiment.

Erwinia spp.	Isolates	Source
E. chrysanthemi pv. *chrysanthemi*	*Ku8601* L1	AKU
〃 〃	Ichihara l-l	TUA
〃 pv. zeae	Corn 801	s u
〃 pv. unidentified	Ech 44	AKU
E. carotovora subsp. *carotovora*	EH8519	NIAS
〃	N7101	〃
〃	473-1	LSPPM
〃	489-4	〃
〃	493-l	〃
〃	645ar	IU
E. herbicola pv. *milletiae*	1	NIAS

NIAS: National Institute of Agricultural Sciences, Tokyo, Japan.
(This Institute was reconstructed partly to NIAES)
NIAES: National Institute of Agro-Environmental Sciences, Tsukuba, Japan.
AKU: Faculty of Agriculture, Kyushu University, Fukuoka, Japan.
TUA: Tokyo University of Agriculture, Tokyo, Japan.
SU: Faculty of Agriculture, Shizuoka University, Shizuoka, Japan.
LSPPM: Laboratory of Seed and Post-harvest Disease, Plant Pathology and Microbiology Division, Department of Agriculture, Bangkok, Thailand.
IU: Faculty of Agriculture, Iwate University, Morioka, Japan.

from various institutes and have been kept in author's laboratory were used in this experiment (Table 1, 2).

Culture medium

King B medium (EIKEN Chemical Co.) was used in this experiment. The ingredient of this medium was as follows : 20.0g peptone, 1.5g K_2HPO_4, 1.5g $MgSO_4 \cdot 7H_2O$, 15.0g agar, 1l of 1% glycerol solution, pH7.2.

Culture conditions

Each bacterial isolate was cultured routinely at 30°C for 3 days on a slant of the medium stated above.

Thin-layer chromatography

The procedures of the direct colony thin-layer chromatography with CMW solvent-system and conditions for practice were shown elsewhere (Matsuyama *el* al., 1993b, c).

One loopful bacterial colony was taken from the slants stated above and pasted directly on the origins of the silica gel G TLC plate (Merck Co., Si60, 20 x **20** cm or 10 x **20** cm, **0.25 mm** in thickness) and dried completely. The silica gel plate was developed with chloroform-methanol (CM, 2:1, v/v) in well-moistured rectungular glass tank. This first development was performed for only 10 min until the solvent front will reach 6 cm line from the origin spots. By this first development for a short time, the extraction of lipids from whole bacterial cells was carried out. Then, the plate was dried well and the bacterial cells pasted on the plate were scraped out. The plate was

Table 2. *Pseudomonas* species used in this experiment.

Pseudomonas spp.	Isolates	Source
P. aeruginosa	IAM1054	NIAS
P. aureofaciens	IAM1001	〃
P. avenue	H8206	HNAES
	H8210	〃
	H8505	〃
P. azotoformans	IAM1603	NIAS
P. caryophylli	NIAS1192	〃
	NIAS1406	〃
P. cepacia	243-4	〃
	256-3	〃
	356-5	〃
P. conjac	b-1	〃
P. cruciviae	IAM1048	〃
P. fragi	IAM1650	〃
P. gladioli pv. *gladioli*	NIAS1064	〃
〃	NIAS1065	〃
	E-14	AKU
	251-17	NIAS
	251-20	〃
P. glumae	2	KNAES
	Kyu82-34-2	〃
	N7503	NIAS
P. jaegeri	IAM1008	〃
P. marginalis pv. *marginalis*		〃
P. melanogenum	IAM1554	〃
P. mildenbergii	IAM1505	〃
P. ovalis	IAM1002	〃
P. plantarii (Type strain)	AZ8201	HNAES
P. polycolar	IF03918	NIAS
P. putida	IAM1506	〃
P. rugosa	Pl-15-1	〃
P. solanacearum	6509	〃
	6511	〃
	6515	〃
	6277	〃
	Ku7502-1	AKU
	C319-SR	KTES
	BY-4	〃
P. syringae pv. *aptata*	MAFF301008	NIAS
P. s. pv. *atrofaciens*		〃
P. s. pv. *eriobotryae*	I	〃
P. s. pv. *mori*		〃
P. s. pv. *striafaciens*		〃
P. s. pv. *syringae* (*oryzicola*)		〃
P. s. pv. *syringae*	1	〃
P. s. pv. *tabaci*	Ku7103	〃
	MAFF301074	〃
P. vendrelli		〃

NIAS, AKU: See the note of Table 1.
KNAES: Kyushu National Agricultural Experiment Station, Kumamoto, Japan.
HNAES: Hokuriku National Agricultural Experiment Station, Takada, Japan.
KTES: Kagoshima Tobacco Experiment Station, Kagoshima, Japan.

developed once more at the same direction with other solvent-system, chloroform-methanol-water (CMW, 60:25:4, v/v/v> for ca. 1.5 hr. Whole processes were conducted at 25°C in the incubator. Then, the plate was dried well and sprayed with ninhydrin (Ninhydrin spray, Tokyo Kasei Chemical Co.). Spots appeared on the plate by heating at 100°C for 10 min were recorded by a photograph and photocopy (Canon Co. FC-311).

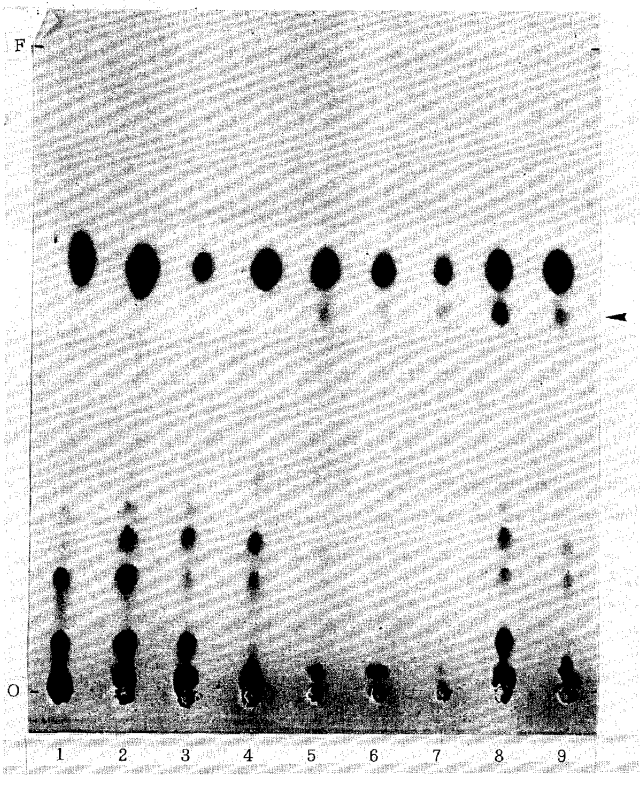

Fig. 1. TLC chromatogram of lipids from phytopathogenic bacteria.

1:	*Erwinia chrysanthemi*	pv. *chrysanthemi*	Ku8601 L1
2:	〃	〃	Ichihara l-l
3:	〃	pv. zeae	Corn 801
4:	〃	pv. unidentified	Ech 44
5:	*E. carotovora*	subsp. *carotovora*	*489-4*
6:	〃		*493-l*
7:	〃		N7101
8:	〃		645ar
9:	〃		473-l

Each spot was visualized by ninhydin with successive heating at 100°C for 10 min.
Arrow-head at right-side indicates a benchmark of *E. carotovora* subsp. *carotovora*.
F: Solvent front, 0: Origin

RESULTS AND DISCUSSION

The results were shown in Fig. 1, 2, 3. The chromatographic profiles of phytopathogenic bacteria were different at species level.

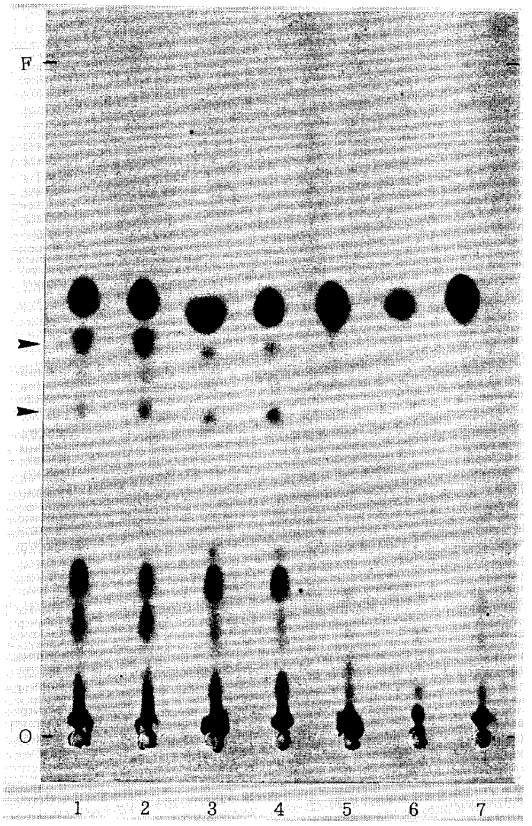

Fig. 2. TLC chromatogram of lipids from phytopathogenic bacteria.

1:	***Pseudomonas***	***gladioli*** pv. ***gladioli***	NIAS1064
2:	〃	〃	NIAS1065
3:	〃	*glumae*	N7503
4:	〃	〃	2
5:	〃	***syringae*** pv. syringae	1
6:	〃	***conjac***	b-1
7:	***JJ***	***syringae*** pv. ***tabaci***	***Ku7103***

Each spot was visualized by ninhydrin with successive heating at 100°C for 10 min.
Arrow-heads at left-side indicate benchmarks of a Cepacia-type pseudomonads.
F: Solvent front, 0: Origin

In the case of *Erwinia* spp., the distinct diversity of chromatograms was observed between *Erwinia carotouora* subsp. *carotouora* and *E. chrysanthemi* at species level. This result agreed well with author's results by gas-liquid chromatography (Kori *et al.*, 1992). A characteristic spot at Rf 0.57 (arrow-head at right margin of Fig. 1) was always detected on the chromatogram of *Erwinia carotouora* subsp. *carotovora*. While, no differences were observed between *E. chrysanthemi* and *E. herbicola* pv. *milletiae*, or

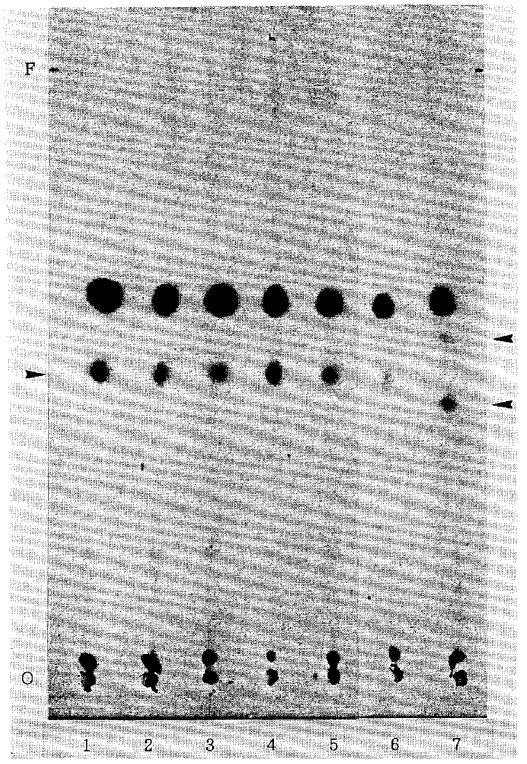

Fig. 3. TLC chromatogram of lipids from phytopathogenic bacteria.

1: ***Pseudomonas solanacearum*** 6515
2: 〃 6511
3: 〃 6509
4: 〃 Ku7502-1
5: 〃 6277
6: 〃 C319-SR
7: 〃 *plantarii* ***AZ8201*** (Type strain)

Each spot was visualized by ninhydrin with successive heating at 100°C for 10 min.
Arrow-head at left-side indicates the benchmark spot of ***P. solanacearum*** and two arrow-heads at right-side indicate those of a Cepacia-type pseudomonads.
F: Solvent front, 0: Origin

among the pathovars of ***E. chrysanthemi***. The distinction of pseudomonads at species level was available among some species. As can be seen in Fig. 2 and 3, the chromatograms of ***P. glumae, P. gladioli pv. gladioli, P. plantarii, P. cepacia*** and ***P. caryophylli*** were similar each other and different from those of other pseudomonads. This type of the chromatogram was designated as a Cepacia-type. In the case of *P. solanacearum*, a distinct spot was detected at Rf 0.54 (arrow-head at left margin of Fig. 3). This spot will be a benchmark of *P.* ***solanacearum*** and this chromatographic profile was designated as a Solanacearum-type. Most chromatograms of pseudomonads other than the species stated above were quite simple and only a common major spot at Rf 0.62 was observed except for minor spots below ca. Rf 0.30. This type of the chromatogram was designated as a Syringae-type. In former experiment with other solvent-system, chloroform-methanol-5M ammonia (CMA, 60:25:4, v/v / v), V-shaped abnormal spots appeared in the case of *P. solanacearum*. Such troubles disappeared by the substitutions of solvent-system CMW for CMA and KB medium for PSA (potato semi-synthetic medium). The complete drying of bacterial cells which were pasted on the TLC plate with hair-drier followed by keeping in desiccator gave a preferable result, especially in the case of *P.* ***solanacearum*** and *P.* avenue.

This direct colony thin-layer chromatography was easy to practice and showed well reproducibility. Although this method is not always applicable to the distinction of all phytopathogenic bacteria at species level, it will be useful mean for a rapid identification and systematic classification of phytopathogenic bacteria.

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