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Variation of *Erwinia carotovora* subsp. *carotovora* isolated from Korea

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Twenty three Korean strains of soft rot pathogen, *Erwinia carotovora* subsp. *carotovora* (Ecc), isolated from various kinds of plants were investigated for their bacteriological, pathological and genetic properties. On the basis of bacteriological tests, the strains were grouped into 6 biovars. Intermediate strains between *E. carotovora* subsp. *carotovora* and subsp. *atroseptica* were found (biovar B and L). The strains were divided into two pathovars based on their pathogenicity to tomato plant, onion, cucumber and potato. All the Korean strains of Ecc caused soft rot on the test plants (pathovar I), except for strain Ecc5/95 which was non pathogenic to onion (pathovar II). ERIC-PCR revealed a greater genotypic diversity with 18 profiles. Two RFLP groups were recognized when RFLP analysis was carried out for a *pel* gene by digesting the amplified fragments with *Sau*3A. Although all strains of biovar E were shown to belong to the same RFLP group, ERIC and RFLP profiles were not directly related to biovar and pathovar.

INTRODUCTION

Pectolytic erwinia cause soft rot diseases on a wide range of plants. The species *Erwinia carotovora* has been divided into four subspecies *atroseptica* (Eca), *carotovora* (Ecc), *betavasculorum* (Ecb) and *wasabiae* (Ecw) as stated by Darrasse *et al.* (1994). Eca is usually restricted to potato disease under cool temperate climate (Perombelon and Kelman, 1980). Ecb and Ecw cause soft rot of sugar beet and Japanese horseradish, respectively (Thomson *et al.*, 1981; Goto and Matsumoto, 1987). Among the different subspecies of erwinia, Ecc strains have a world wide distribution with a wide host range. An understanding of the diversity of different strains of Ecc could be useful for formulating disease management strategies.

In the previous paper, we reported that the genetic diversity of Ecc isolated from various sources in Thailand appears to be related to their host plants and biovars in considering enterobacterial repetitive intergenic consensus (ERIC) and restriction fragment length polymorphism (RFLP) of a *pel* gene analyses (Seo *et al.*, 2000).

The present study has been undertaken to characterize the Korean strains of Ecc in

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respect of biovar, pathovar, RFLP and ERIC profiles.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Twenty three Korean strains of Ecc (Table 3) and four type strains of *E. carotovora* subsp. *carotovora* ATCC15713^T, *E. carotovora* subsp. *atroseptica* ATCC 33260^T, *E. carotovora* subsp. *betavasculorum* ATCC 43762^T and *E. carotovora* subsp. *wasabiae* ATCC 43316^T were used in this study. All the strains were stored at -70 °C in skim milk and cultured on MGY agar (mannitol 10.0 g, L-glutamic aicd 2.0 g, KH_2PO_4 0.5 g, NaCl 0.2 g, $MgSO_4.7H_2O$ 0.2 g, yeast extract 0.25 g, agar 15.0 g, distilled water 1 liter, pH7.0) at 28 °C for 2 days.

Biovar determination

The growth of the strains were tested at 36 °C to determine the biovar of the 23 Korean strains of Ecc. A series of biochemical tests including reducing substances from sucrose, casein hydrolysis, indole production, production of acid from palatinose, sorbitol and α-methyl glucoside was performed as described by Schaad (1980).

Pathovar determination

Pathogenicity of the strains was tested by inoculation to tomato plant (*Lycopersicon esculentum* Mill; Oogatafukuju), onion (*Allium cepa* L.; Satsuki), cucumber (*Cucumis sativus* L.; Nashio) and potato (*Solanum tuberosum*; May-Queen). Tomato plants were grown for 3–4 weeks in plastic pots of 8 cm diameter containing sterilized soil. Inoculation was done with bacterial suspension (conc. ca 10^9–10^{10} cells/ml) by the needle pricking method. Inoculated and non-inoculated control plants were incubated in a moist chamber (RH 95% and temperature 28 °C) and were observed for development of symptoms for 2–3 days after inoculation. Cucumber and potato were cut into slices while onion was cut into small pieces and the surfaces were disinfested by 70% ethyl alcohol. The slices or pieces were inoculated by the needle pricking method and put into Petri-dishes and incubated at 28 °C for 2–3 days.

Molecular analyses

Genomic DNA was extracted according to the method of Sambrook *et al.* (1989). ERIC-PCR was achieved by using primers ERIC1R and ERIC2 which are specific to the conserved repetitive sequences in bacteria (Mcmanus and Jones, 1995). Amplification was performed in a total volume of 50 μl containing 67 mM Tris-HCl (pH 8.8), 2.0 mM $MgCl_2$, 0.125 mM each of dATP, dCTP, dGTP and dTTP, 2.0 units of *Taq*DNA polymerase (TOYOBO, Japan), 50 pmol each primer, and 1 μl of a 50 ng/ml solution of purified DNA. PCR reactions were performed in a thermal cycler (Astec, Japan) under the following conditions; 94 °C for 4 min for the first cycle, 30 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min, and a final cycle of 72 °C for 10 min.

RFLP-PCR was done using primers Y1 and Y2, specific for *E. carotovora* (Helias *et al.*, 1998). The volume and content of amplification reactions followed as described above. Amplification was done in the thermal cycler under the following conditions;

35 cycles of 30 sec at 94 °C, 30 sec at 65 °C, and 45 sec at 72 °C. The amplification cycles were preceded by a denaturation step of 5 min at 95 °C and followed by an elongation step of 10 min at 72 °C. RFLP analysis of pectate lyase gene was done as follows; the amplified DNA was ethanol precipitated and resuspended in 50 μl of 10 mM Tris–HCl (pH 8.0), 1 mM EDTA. Aliquots of the concentrated DNA were digested with *Sau*3A at 37 °C for 2 h in a 15 μl volume according to the manufacturer's recommendations.

RESULTS AND DISCUSSION

Erwinia carotovora subsp. *carotovora* strains isolated from various plants in Korea had differences in their bacteriological properties, and were grouped into 6 biovars (Table 1 and 3). Bacteriological characteristics of the strains such as ability to grow at 36 °C, to produce reducing substances from sucrose and acids from α-methyl glucoside and palatinose are considered to be useful to distinguish Ecc and Eca (Lelliot and Dickey, 1984; Schaad, 1980). Two biovars, B and L, showed the similar properties to Eca (Table 1). The variations of biovars within Korean strains of Ecc were far greater than those of biovars within Thai strains. A little variations were also observed in the biovars of Japanese strains (Karnjanarat *et al.*, 1987). The results of the present study are in agreement with Tanii (1984) who observed variations of Japanese strains of Ecc in their ability to produce reducing substances from sucrose and acid production from α-methyl glucoside.

The Korean strains of Ecc were divided into two pathovars, pathovar I and II (Table 2 and 3), on the basis of their ability to cause soft rot on the test plants. All Ecc strains

Table 1. Bacteriological properties of Korean strains of *Erwinia carotovora* subsp. *carotovora*

Charater	Biovars						Ecc[a]	Eca[a]
	B	D	E	F	G	L		
Growth at 36 °C	+	+	+	+	+	+	+	–
Reducing substances from sucrose	+	–	–	–	–	+	–	+
Casein hydrolysis	+	–	+	–	+	–	+	+
Indole production	–	–	+	+	–	–	–	–
Acid production from								
Palatinose	+	–	–	–	–	+	w[b]	+
Sorbitol	+	–	–	–	–	+	–	–
α-methyl glucoside	+	–	–	–	–	–	–	+
No. Korean strains (23)	5	4	5	1	6	2		

[a] Type strains of *E. carotovora* subsp. *carotovora* and subsp. *atroseptica*, respectively
[b] w; weak positive

Table 2. Differentiation of Korean strains of *Erwinia carotovora* subsp. *carotovora* into pathovar

Pathovar	Pathogenicity to				No. of strains
	Onion	Tomato	Cucumber	Potato	
I	+	+	+	+	22
II	–	+	+	+	1

Table 3. Groupings of *Erwinia carotovora* subsp. *carotovora* strains by biovar, pathovar, RFLP of a *pel* gene and ERIC analyses

Strain	Source	Isolated from	Year isolated	Biovar	Pathovar	RFLP[a]	ERIC[b]
Ecc1/95	Pyungchang,Korea	Wasabi	1995	E	I	1	3
Ecc2/95	Inje	Chicory	〃	B	I	2	1
Ecc3/95	〃	Potato	〃	B	I	2	2
Ecc4/95	Wanju	Chinese cabbage	〃	F	I	1	11
Ecc5/95	Chunchon	〃	〃	E	II	1	5
Ecc6/95	Pyungchang	Potato	〃	E	I	1	3
Ecc1/96	Bonghwa	Chinese cabbage	1996	G	I	2	4
Ecc2/96	Hoengke	〃	〃	B	I	2	6
Ecc3/96	Chunchon	〃	〃	D	I	2	7
Ecc4/96	Pyungchang	〃	〃	D	I	2	8
Ecc5/96	Hongchon	〃	〃	L	I	2	1
Ecc6/96	Chunchon	Wasabi	〃	D	I	2	9
Ecc7/96	〃	Onion	〃	B	I	2	1
Ecc8/96	Hongchon	Crisphead lettuce	〃	D	I	2	12
Ecc9/96	Hongchon	Radish	〃	G	I	2	15
Ecc11/96	Pyungchang	Potato	〃	L	I	2	1
Ecc12/96	〃	Cucumber	〃	B	I	2	16
Ecc13/96	〃	Pumpkin	〃	G	I	2	13
Ecc14/96	Hongchon	Potato	〃	G	I	2	10
Ecc1/97	Chunchon	Pepper	1997	E	I	1	14
Ecc2/97	Kangsung	Cala	〃	G	I	1	17
Ecc3/97	Milyang	Potato	〃	G	I	2	4
Ecc1/98	Kangsung	Cactus	1998	E	I	1	18

[a] RFLP, restriction fragment length polymorphism of a *pel* gene
[b] ERIC, enterobacterial repetitive intergenic consensus

caused soft rot in tomato plant, onion, cucumber and potato (pathovar I), except for strain Ecc5/95 that showed no pathogenicity to onion (pathovar II). Variations of pathovars within Ecc strains of Korea were less than those within Japanese strains (Karnjanarat *et al.*, 1987)

The Korean strains of Ecc were separated into 18 profiles based on the fingerprints of ERIC-PCR (Fig. 1 and Table 3). These profiles showed that the Korean strains have wider diversity than Thai strains (Seo *et al.*, 2000).

All products obtained by PCR for *pel* gene were of the expected size (434 bp) except *E. carotovora* subsp. *betavasculorum* in which no fragment was found. An RFLP analysis by digesting the amplified fragments with *Sau*3A was undertaken and revealed two RFLP groups (Fig. 2 and Table 3). In the previous study, most of the Thai strains of Ecc were shown to belong to the RFLP group 1 (Seo *et al.*, 2000). In contrast, the current study indicated that most of the Korean strains of Ecc belongs to the RFLP group 2. In general, the profiles obtained from the ERIC and RFLP analyses of the Korean strains of Ecc have no direct correlation to the biovar and pathovar, although all strains of biovar E belong to the same RFLP group 1.

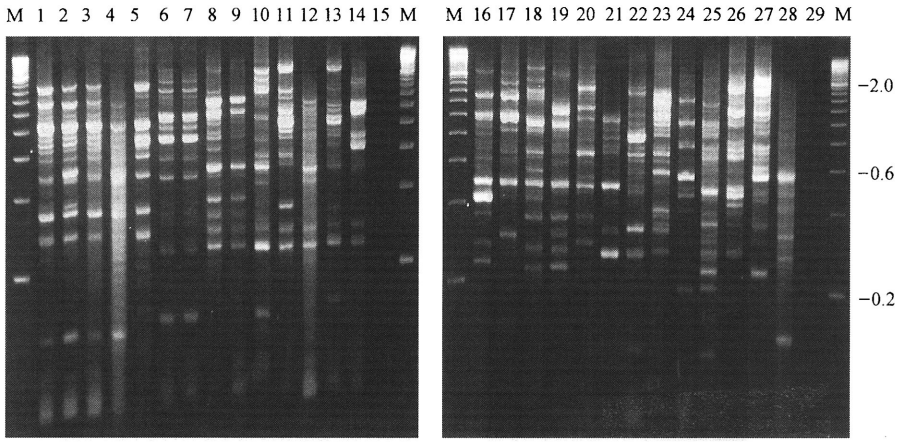

Fig. 1. ERIC–PCR patterns generated from purified DNA of *E. carotovora*. PCR products were separated on a 2.0% agarose gel, stained with ethidium bromide, and photographed under UV illumination. Lanes labeled M were run with the DNA size standard (kb). Lane 1, Ecc2/95; Lane 2, Ecc5/96; Lane 3, Ecc7/96; Lane 4, Ecc11/96; Lane 5, Ecc3/95, Lane 6, Ecc6/95; Lane 7, Ecc1/95; Lane 8, Ecc3/97; Lane 9, Ecc1/96; Lane 10, Ecc5/95; Lane 11, Ecc2/96; Lane 12, Ecc3/96; Lane 13, Ecc4/96; Lane 14, Ecc6/96; Lane 15 and 29, the same PCR mixture but lacking template DNA (control); Lane 16, Ecc14/96; Lane 17, Ecc4/95; Lane 18, Ecc8/96; Lane 19, Ecc13/96; Lane 20, Ecc1/97; Lane 21, Ecc9/96; Lane 22, Ecc12/96; Lane 23, Ecc2/97; Lane 24, Ecc1/98; Lane 25, ATCC15713^T (*E. carotovora* subsp. *carotovora*); Lane 26, ATCC33260^T (*E. carotovora* subsp. *atroseptica*); Lane 27, ATCC43762^T (*E. carotovora* subsp. *betavasculorum*); Lane 28, ATCC43316^T (*E. carotovora* subsp. *wasabiae*)

REFERENCES

Darrasse, A., S. Priou, A. Kotoujansky and Y. Bertheau 1994 PCR and restriction fragment length polymorphism of a *pel* gene as a tool to identify *Erwinia carotovora* in relation to potato diseases. *Appl. Environ. Microbiol.*, **60**(5): 1437–1443

Goto, M and K. Matsumoto 1987 *Erwinia carotovora* subsp. *wasabiae* subsp. nov. isolated from diseased rhizomes and fibrous roots of Japanese horseradish (*Eutrema wasabi* Maxim.). *Int. J. Syst. Bacteriol.*, **37**: 130–135

Helias, V., A. L. Roux, Y. Bertheau, D. Andrivon, J. Gauthier and B. Jouan 1998 Characterization of *Erwinia carotovora* subspecies and detection of *Erwinia carotovora* subsp. *atroseptica* in potato plants, soil and water extracts with PCR–based methods. *Eur. J. Plant Pathol.*, **104**: 685–699

Karnjanarat, S., K. Tsuchiya, N. Matsuyama and S. Wakimoto 1987 Physiological, biochemical and pathological differentiation among strains of *Erwinia carotovora* isolated from Japan and Thailand. *Ann. Phytopathol. Soc. Japan*, **53**: 460–469

Lelliot, R. A. and R. S. Dickey 1984 Genus VII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209^{AL}, p.469–476. In: Krieg NR and Holt JG (eds) Bergey's manual of systematic bacteriology, vol. 1. Williams and Wilkins Co., Baltimore

Mcmanus, P. S. and A. L. Jones 1995 Genetic fingerprinting of *Erwinia amylovora* strains isolated from tree–fruit crops and *Rubus* spp.. *Phytopathology*, **85**: 1547–1553

Perombelon, M. C. M. and A. Kelman 1980 Ecology of the soft rot erwinias. *Ann. Rev. Phytopathol.*, **18**: 361–387

Sambrook, J., E. F. Fritsch and T. Maniatis 1989 *Molecular clonig*: a laboratory manual, 2nd edn. Cold

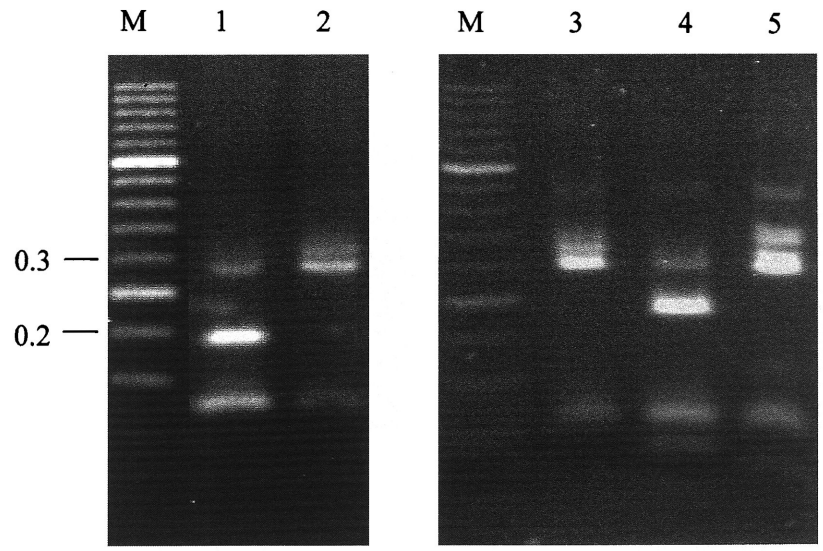

Fig. 2. RFLP analysis of the amplified fragments of a *pelY* gene. The DNA products were digested with restriction enzyme *Sau*3A and separated on a 2.5% agarose gel, stained with ethidium bromide, and photographed under UV illumination. Lanes labeled M were run with the DNA size standard (kb). Lane 1, RFLP pattern 1; Lane 2, RFLP pattern 2; Lane 3, ATCC15713^T (*E. carotovora* subsp. *carotovora*); Lane 4, ATCC33260^T (*E. carotovora* subsp. *atroseptica*); Lane 5, ATCC43316^T (*E. carotovora* subsp. *wasabiae*)

Spring Harbor, N. Y.

Schaad, N. W. 1980 Initial identification of common genera. *In* laboratory guide for identification of plant pathogenic bacteria. Edited by Schaad, N. W. Bact. Comm. Amer. Phytopath. Soc. Minn.

Seo, S. T., N. Furuya and Y. Takanami 2000 Genetic diversity in Thai strains of *Erwinia carotovora* subsp. *carotovora* based on ERIC profiles and RFLP of a *pel* gene. *J. Fac. Agr. Kyushu Univ.*, **45**(1): 15–19

Tanii, A. 1984 Studies on the blackleg disease of potato in Hokkaido. Hokkaido Pref. Agr. Exp. Sta. No. 45, p 104

Thomson, S. V., D. C. Hildebrand and M. N. Schroth 1981 Identification and nutritional differentiation of the erwinia sugar beet pathogen from members of *Erwinia carotovora* and *Erwinia chrysanthemi*. *Phytopathology*, **71**: 1037–1042