한국식물병리학회지 12(2) : 162-168(1996)
Korean J. Plant Pathol. 12(2) : 162-168(1996)

Restriction Fragment Length Polymorphisms of Genomic DNA in Strains of *Xanthomonas campestris* pv. *vesicatoria* from Different Geographic Areas

Hee Jeong Chung, Tae Seon Lee, Young Jin Koh*, Ill Sup Nou[1] and Byung Kook Hwang[2]
Department of Agricultural Biology, Sunchon National University, Sunchon 540-742, Korea
[1]College of Education, Sunchon National University, Sunchon 540-742, Korea
[2]Department of Agricultural Biology, College of Natural Resources, Korea University, Seoul 136-701, Korea

지리적 기원이 다른 고추 더뎅이병균 균주 Genomic DNA의 RFLP 분석

정희정 · 이태선 · 고영진* · 노일섭[1] · 황병국[2]
순천대학교 농과대학 농생물학과, [1]순천대학교 사범대학, [2]고려대학교 자연자원대학 농생물학과

ABSTRACT : Genetic variation of 44 strains of *Xanthomonas campestris* pv. *vesicatoria* collected from the various pepper-growing areas in Korea, and the United States, Taiwan, Australia, and Argentina was analyzed using restriction fragment length polymorphism (RFLP) of their genomic DNA. The *X. campestris* pv. *vesicatoria* strains were classified into 11 RFLP groups based on the dendrogram generated by cluster analysis of RFLP profiles of the genomic DNA. The strains from foreign countries formed discrete genomic DNA RFLP groups for each country. Among the foreign strains, the United States strain 87-77 was genetically closely related with some of the Korean strains. All of the 39 Korean strains tested were classified into 6 genomic DNA RFLP groups and most of them fell into a narrow cluster distinctly separated from the foreign strains except the United States strain. However, strain Ms93-1 collected in Masan was distinctly separated from the other Korean strains. The existence of genetically distinct subgroups leads to the conclusion that there might already occur genetic variation in the strains of *X. campestris* pv. *vesicatoria* in each different geographic area in Korea.

***Key words* : pepper, bacterial spot, RFLP, genomic DNA, *Xanthomonas campestris* pv. *vesicatoria*.**

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye is one of the most important diseases of pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) in the world. The disease causes severe defoliation of the pepper plants, and results in reduced yield when temperatures and rainfall are high (7). Control of bacterial spot has depended on bactericides or resistant varieties, but appearance of a bactericide-resistant strain or a new pathotype of *X. campestris* pv. *vesicatoria* has hampered efforts to manage the disease (1). Therefore, monitoring of genetic differentiation in the populations of the bacterium is very important to develop effective control strategy of the bacterial spot.

DNA restriction fragment length polymorphism (RFLP) is one of the molecular markers convenient to characterize the nature of DNAs responsible for the genetic differentiation of plant pathogens. RFLP of plasmid and genomic DNA has been intensively used for analysis of clonal population structure or genetic diversity of various pathovars of *X. campestris* (2, 11, 16, 17, 24). Recently, RFLP analyses of genomic and plasmid DNA have been used to differentiate several groups of xanthomonads, including some strains of *X. campestris* pv. *vesicatoria* (9, 10). Hwang *et al.* (6) also investigated the genetic relatedness among

*Corresponding author.

plasmids in strains of *X. campestris* pv. *vesicatoria* from different geographic areas using RFLPs of plasmid DNA.

In the present study, we investigated genetic variation of genomic DNA in Korean strains of *X. campestris* pv. *vesicatoria* from intensively pepper-growing areas in Korea, and compared the polymorphisms of genomic DNA in the Korean strains with those from diverse geographic origins worldwide.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The sources and origins of 44 strains of *X. campestris* pv. *vesicatoria* used in this study are shown in Table 1. Korean strains were isolated in 1991 and 1992 from infected pepper plants growing in various pepper-growing areas of Korea, identified as *X. campestris* pv. *vesicatoria*, and confirmed to be pathogenic on pepper leaves (20). Isolation of the bacteria was carried out on a semiselective Tween medium (10 g peptone, 10 g potassium bromide, 0.25 g calcium chloride, 0.3 g boric acid, 10 ml Tween 80, 50 mg cycloheximide, 65 mg cephalexin, 12 mg 5-fluorouracil and 0.4 mg tobramycin in 1000 ml deionized water) (13). Other strains were provided by Dr. R. E. Stall, Department of Plant Pathology, University of Florida, Gainesville, FL, USA.

All strains of *X. campestris* pv. *vesicatoria* were maintained routinely on yeast-nutrient agar (5 g yeast extract, 8 g nutrient broth, 15 g agar, 1000 ml deionized water) at 28°C and *Escherichia coli* NM522 was maintained on LB (Luria-Bertani) agar at 37°C. All bacteria were also stored for long periods as turbid suspensions in 15% glycerol at −70°C.

Extraction of genomic DNA and purification. Genomic DNA for hybridization experiments was extracted from *X. campestris* pv. *vesicatoria* by a modification of alkaline lysis extraction procedure (12, 21). To extract genomic DNA, *X. campestris* pv. *vesicatoria* was cultured in 5 ml yeast-nutrient broth for 12 hr at 28°C, and then harvested by centrifugation for 5 min at 3,500 rpm. Lysis of bacterial cell pellets harvested was performed at room temperature for 20 min in a mixture of 50 mM glucose, 25 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 2 mg/ml freshly mixed lysozyme (total volume, 3.3 ml). To each DNA sample, sodium dodecyl sulfate (SDS; 334 μl of a 10% solution) was added. After incubation for 10 min at 50°C, 268 μl of RNase A

Table 1. Strains of *Xanthomonas campestris* pv. *vesicatoria* used in the study

Strain	Geographic origin			Host of isolation
	Area	Province	Country	
79-2, Bv5-4a			Argentina	Tomato
89-8			Australia	Pepper
87-48			Taiwan	Pepper
87-77			U.S.A.	Tomato
Hs1	Hoengsung	Kangwon	Korea	Pepper
As5	Ansung	Kyunggi	Korea	Pepper
Hs93-5	Hwasung	Kyunggi	Korea	Pepper
Ic1	Inchun	Kyunggi	Korea	Pepper
Ky1	Koyang	Kyunggi	Korea	Pepper
Sw4	Suwon	Kyunggi	Korea	Pepper
Uj1	Uijeongbu	Kyunggi	Korea	Pepper
Cj1	Chungju	Chungbuk	Korea	Pepper
Jc93-5	Jinchon	Chungbuk	Korea	Pepper
Mw4	Miwon	Chungbuk	Korea	Pepper
Ad93-2	Andong	Kyungbuk	Korea	Pepper
Yd1	Youngduk	Kyungbuk	Korea	Pepper
Kc1~Kc3, Kc6~Kc9, Kc14, Kc15, Kc17, Kc23, Kc25	Kochang	Chonbuk	Korea	Pepper
Kj93-1	Kimje	Chonbuk	Korea	Pepper
Bs5	Pusan	Kyungnam	Korea	Pepper
Cr93-1	Changryung	Kyungnam	Korea	Pepper
Ds2, Ds5, Ds7~Ds10, Ds13, Ds14, Ds16~Ds18	Dukso	Kyungnam	Korea	Pepper
Ms93-1	Masan	Kyungnam	Korea	Pepper

(2.5 mg/ml in 10 mM Tris-HCl, pH 7.6) was added, and the mixture was incubated at 37°C for 1 hr. Then, 340 μl of 0.5 M EDTA was added, and the mixture was incubated for 10 min at 50°C. Pronase (140 μl of a 10 mg/ml solution in 10 mM Tris-HCl, pH 7.6) was added, and the mixture was incubated for 6 hr at 37°C. Each sample was then extracted with one volume of phenol saturated with 0.1 M Tris-HCl (pH 8.0), one volume of a 1 : 1 phenol and chloroform-isoamyl alcohol (24 : 1, v/v) mixture, and one volume of chloroform-isoamyl alcohol. The DNA was precipitated with 1/10 volume of 3 M sodium acetate and two volumes of 99.9% ethanol, and redissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Purified genomic DNA was digested to completion with *Eco*RI, *Eco*RV, *Hin*dIII, *Sal*I, *Pst*I, and *Bgl*II according to the manufacturer's instructions (Bethesda Research Laboratories, Inc., Gaithersburg, MD, USA). The DNA fragments were separated by 0.8% agarose gel electrophoresis in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0). They were visualized with ultraviolet light after staining in ethidium bromide. Sizes of DNA fragments were estimated using lambda phage DNA digested with *Hin*dIII as a molecular size standard.

Hybridization probes. Genomic DNA probes were prepared from the strain 81-23 of *X. campestris* pv. *vesicatoria* obtained from Dr. R. E. Stall, Department of Plant Pathology, University of Florida, Gainesville, FL, USA. Genomic DNA of the strain 81-23 isolated by a modification of alkaline lysis extraction procedure (12, 21) was purified and completely digested with *Eco*RI, ligated into *Eco*RI-digested pUC119, followed by transformation into *E. coli* strain NM522 as described by Maniatis *et al.* (12). The transformed *E. coli* colonies were selected from LB medium supplemented with ampicillin at 50 μg/ml. Ampicillin-resistant colonies appearing were selected arbitrarily on isopropylthio-β-D-galactoside (IPTG) plus 5-bromo-4-chloro-3-indoly-β-D-galactoside (X-gal) plates. In addition to the DNA fragments cloned from genomic library of *X. campestris* pv. *vesicatoria*, 26S rRNA gene (4.4 kb) originated from pRR217 (7.8 kb) rDNA genes was also used as a hybridization probe (15).

Genomic DNA hybridization. Genomic DNA fragments for use as hybridization probes were separated from vector (pUC119) sequences by restriction enzyme (*Eco*RI), and fractionated by electrophoresis in 0.8% agarose gels in TBE buffer. The DNA fragments were recovered from agarose using a Geneclean II kit (BIO 101, Inc.). Genomic DNA from each of *X. campestris* pv. *vesicatoria* strains digested with several restriction enzymes was electrophoresed in 0.8% agarose gel in TBE buffer and transferred onto a nylon membrane (BM Inc.) using a vacuum blotting apparatus (Hoefer Scientific Instruments). Blotted nylon membranes were dried under vacuum at 80°C for 1 hr and UV-crosslinked for 1 min. Prehybridizations, hybridizations, and detection procedures were performed by DIG-ELISA methods according to the manufacturer's instructions (BM Inc.).

RFLP data analysis. Hybridization patterns of genomic DNAs digested with each of the restriction enzymes used were compared for genetic analysis among the strains of *X. campestris* pv. *vesicatoria*. Presence (coded 1) or absence (coded 0) of each fragment was recorded. The binomial data matrix was analyzed according to the NTSYS computer program, version 1.80 (19). A dendrogram was produced by a UPGMA clustering of the similarity matrix calculated by Jaccard's formula (18, 22, 23).

RESULTS

Southern hybridization of genomic DNA. A library of *Eco*RI-digested genomic DNA from *X. campestris* pv. *vesicatoria* was made in pUC119. Among the twenty *Eco*RI-fragments cloned into the vector pUC119, four *Eco*RI-fragments (0.35 kb, 1.2 kb, 2.2 kb, and 2.4 kb) hybridizing to multiple bands were selected as hybridization probes against genomic DNA digests from 44 strains of *X. campestris* pv. *vesicatoria*.

Four *Eco*RI-fragments were hybridized to 2~3 restriction fragments of the genomic DNAs from 44 strains of *X. campestris* pv. *vesicatoria* digested with several enzymes, respectively (Table 2). Probe 26S rRNA also produced 6~8 hybridizing fragments of the genomic DNAs from 44 strains of *X. campestris* pv. *vesicatoria* digested with 4 different restriction enzymes, respectively.

Southern blot hybridization patterns of genomic DNAs from 20 representative strains of *X. campestris* pv. *vesicatoria* were shown in Fig. 1. The 0.35 kb *Eco*RI-DNA probe (Dsc40) and 1.2 kb *Eco*RI-DNA probe (81c5) hybridized 1~3 bands in genomic DNA of most strains digested with *Pst*I and *Hin*dIII, respectively. Probe 26S rRNA hybridized 2~8 bands in genomic DNA digested with *Eco*RV and *Bgl*II, respec-

tively. Significant variation in hybridization patterns was observed among foreign strains. Australian strain 89-8 had no homology to the probes Dsc40 and 81c5, but hybridized strongly to the probe 26S rRNA. Taiwanese strain 87-48 and Argentine strains 79-2 and Bv5-4a shared a band of 0.8 kb, which hybridized intensively to the probe Dsc40, but there was no homology between Taiwanese and Argentine strains in the hybridization patterns to the probe 26S rRNA. The United States strain 87-77 had homology with most Korean strains to the probes used. However, minor variation in hybridization patterns was observed among Korean strains except Ms93-1. Ms93-1 had no homology to the other Korean strains in hybridization patterns to the 4 probes.

Table 2. Hybridization probes and restriction enzymes used for digestion of genomic DNA of *Xanthomonas campestris* pv. *vesicatoria*, with number of hybridizing DNA fragments for each probe

Hybridization probe		Restriction enzyme	No. of hybridizing DNA fragments
Name	Fragment size (kb)		
Dsc40	0.35	*Pst*I	3
		*Hin*fI	3
81c5	1.2	*Hin*dIII	3
		*Eco*RI	2
Dsc14	2.2	*Eco*RV	3
Dsc11	2.4	*Eco*RI	2
26S rRNA	4.4	*Eco*RV	8
		*Bgl*II	6
		*Hin*fI	6
		*Sal*I	6

Cluster analysis. Cluster analysis based on the RFLPs of genomic DNA generated a dendrogram of relationships between strains of *X. campestris* pv. *vesicatoria* (Fig. 2). The 44 strains of *X. campestris* pv. *vesicatoria* were classified into 11 genomic DNA RFLP groups (Table 3). The strains from foreign countries formed discrete genomic DNA RFLP groups for

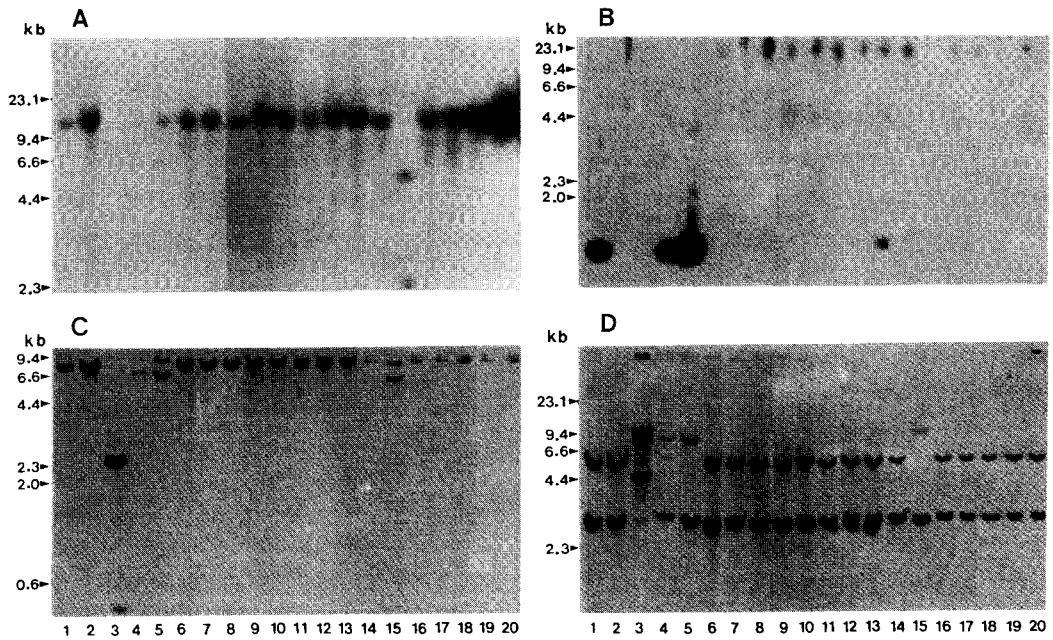

Fig. 1. Southern blot hybridization of 4 different enzyme-digested genomic DNAs from 20 representative strains of *Xanthomonas campestris* pv. *vesicatoria* with 4 different probes, respectively. (A) Hybridization profiles of *Hin*dIII-digested genomic DNA with the probe 81c5, (B) Hybridization profiles of *Pst*I-digested genomic DNA with the probe Dsc40, (C) Hybridization profiles of *Bgl*II-digested genomic DNA with the probe 26S rRNA, (D) Hybridization profiles of *Eco*RV-digested genomic DNA with the probe 26S rRNA. Lane 1 : strain 87-48 from Taiwan; lane 2 : 87-77 from USA; lane 3 : 89-8 from Australia; lane 4 : 79-2 from Argentina; lane 5 : Bv5-4a from Argentina; lane 6 : Ds6; lane 7 : Ds7; lane 8 : Kc2; lane 9 : Kc25; lane 10 : Hs1; lane 11 : Ic1; lane 12 : Ky1; lane 13 : Yd1; lane 14 : Uj1; lane 15 : Ms 93-1; lane 16 : Bs5; lane 7 : Cr93-1; lane 18 : Cj1; lane 19 : Hs93-5; lane 20 : Kj93-1.

each country with a wide cluster. The thirty-nine Korean strains were classified into 6 genomic DNA RFLP groups. All Korean strains (groups III~VII) except Ms 93-1 (group VIII) fell into a narrow cluster, distinctly separated from the foreign strains except the United States strain 87-77. However, the United States strain 87-77 clustered with some of the Korean strains together. Of the 39 Korean strains, one strain, Ms93-1 collected in Masan, was distinctly separated from the other Korean strains.

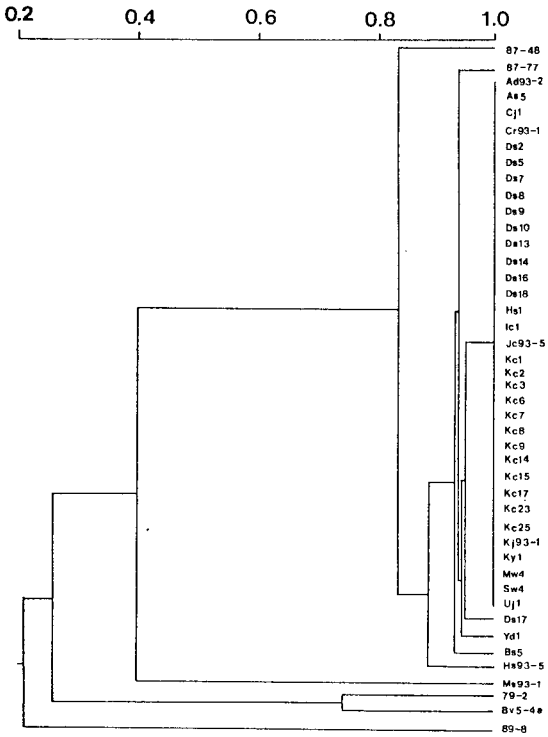

Fig. 2. Computer-generated dendrogram of the 44 strains of *Xanthomonas campestris* pv. *vesicatoria* produced from the similarity matrix using UPGMA cluster analysis from pooled restriction fragment length polymorphism profiles of genomic DNA.

DISCUSSION

RFLPs are very useful tools for studying bacterial genetics because they are precise, selectively neutral, relatively easy to assay, and can provide a virtually unlimited supply of genetic markers. Intraspecific variation in RFLPs of plasmid and genomic DNA has been reported by several authors for several pathovars of *X. campestris* (2, 9, 10, 16, 24). Hwang *et al.* (6) classified 77 strains of *X. campestris* pv. *vesicatoria* of diverse geographic origins into 14 plasmid DNA RFLP groups based on the restriction endonuclease digestion patterns of their plasmid DNA. Such a variation of RFLPs in strains of *X. campestris* pv. *vesicatoria* would probably result from length alteration or mutation of base sequences of restriction sites (5, 6, 14). In this study, the forty-four strains of diverse geographic origins were classified into 11 RFLP groups based on the dendrogram generated by cluster analysis of RFLP profiles of the genomic DNA. In particular, the strains from foreign countries formed discrete genomic DNA RFLP groups for each country. Since the strains belonging to each of the RFLP groups had identical restriction sites of genomic DNA in common,

Table 3. Grouping of 44 geographic strains of *Xanthomonas campestris* pv. *vesicatoria* based on the Southern blot hybridization profiles of genomic DNA

RFLP-group	Strains
I	87-48 (Taiwan)
II	87-77 (USA)
III	Ad93-2, As5, Cj1, Cr93-1, Ds2, Ds5, Ds7, Ds8, Ds9, Ds10, Ds13, Ds14, Ds16, Ds18, Hs1, Ic1, Jc93-5, Kc1, Kc2, Kc3, Kc6, Kc7, Ks8, Ks9, Kc14, Kc15, Kc17, Kc23, Kc25, Kj93-1, Ky1, Mw4, Sw4, Uj1
IV	Ds17
V	Yd1
VI	Bs5
VII	Hs93-5
VIII	Ms93-1
IX	79-2 (Argentina)

genomic DNA sequences in each of foreign strains of *X. campestris* pv. *vesicatoria* have been highly conserved and stable in each geographically separated area.

Among the foreign strains, however, some strains were genetically closely related with Korean strains. Especially the United States strain 87-77 had high homology to some of the Korean strains in genomic DNA RFLPs. This result suggests that these strains may have originated from a common ancestral strain of *X. campestris* pv. *vesicatoria*. The concurrence of race 3 of *X. campestris* pv. *vesicatoria* in Korea in recent years (8), which was detected only in the United States in late 1980s (3, 4), reveals that some of the Korean strains of *X. campestris* pv. *vesicatoria* might have been introduced from the United States.

All tested Korean strains were classified into 6 genomic DNA RFLP groups. This suggests that distinct subgroups exist in the strains of *X. campestris* pv. *vesicatoria* in Korea. Furthermore, one strain, Ms93-1 collected in Masan, was distinctly separated from the other strains, suggesting that genomic DNA of Ms93-1 may be genetically very distant from that of the others. The appearance of genetically separated strains leads to the conclusion that there might already occur genetic differentiation in the strains of *X. campestris* pv. *vesicatoria* in each different geographic area in Korea.

Korean strains were clustered and genetically most distant from the non-Korean strains according to cluster analysis of plasmid DNA RFLPs in *X. campestris* pv. *vesicatoria* by Hwang *et al.* (6). This result suggests that plasmid DNA of the Korean strains may be very different genetically from those of the others. In the present study, however, some of the Korean strains showed genomic DNA RFLPs homologous to the United States strain 87-77 and Taiwanese strain 87-48, indicating that RFLP profiles of genomic DNA do not necessarily coincide with those of plasmid DNA in the strains of *X. campestris* pv. *vesicatoria*, as previously observed by Xu and Gonzalez (24) in strains of *X. campestris* pv. *oryzae* in the United States. It is possible, therefore, that *X. campestris* pv. *vesicatoria* may have separate phylogenies for plasmid and choromosomal DNA within a particular population.

요 약

우리 나라의 주요 고추 재배지와 미국, 대만, 호주, 아르헨티나에서 수집된 44개 고추 더뎅이병균(*Xanthomonas campestris* pv. *vesicatoria*) 균주간의 유전적 변이를 genomic DNA의 restriction fragment length polymorphism(RFLP)에 의해 분석하였다. Genomic DNA RFLP profiles을 cluster 분석하여 얻은 dendrogram에서 지리적 기원이 다른 44개 균주들은 11개 RFLP 그룹으로 분류되었다. 외국 균주들은 genomic DNA의 RFLP 분석에 의해 모두 각각 다른 RFLP 그룹으로 분류되었다. 외국 균주들 중에서 미국 균주는 우리 나라 일부 균주들과 밀접한 유전적 관련성을 가지고 함께 cluster를 이루었는데, 이것은 이 균주들이 동일한 고추 더뎅이병균의 조상 균주 집단에서 유래했으리라는 것을 시사해 준다. 우리 나라 균주들은 6개의 RFLP 그룹으로 분류되었다. 대부분의 우리 나라 균주들은 가까운 cluster를 이루며 미국 균주를 제외한 외국 균주들과 뚜렷하게 구분되었다. 그러나 우리 나라 균주들 중에서 마산에서 수집된 Ms93-1은 다른 우리 나라 균주들과 뚜렷하게 구분되었다. 유전적으로 격리된 균주의 출현은 우리 나라에서 지리적 기원이 다른 고추 더뎅이병균 균주 사이에 이미 발생한 다양한 유전적 분화의 결과라고 추론된다.

ACKNOWLEDGEMENT

This study was supported in part by funds from the Korea Science and Engineering Foundation, grant No. 93-04-00-08. We also thank Dr. R. E. Stall for providing *Xanthomonas campestris* pv. *vesicatoria* strains of different geographic origins.

REFERENCES

1. Dahlbeck, D. and Stall. R. E. 1979. Mutations for change of race in cultures of *Xanthomonas vesicatoria*. *Phytopathology* 69 : 634-636.
2. Gabriel, D. W., Hunter, J. E., Kingsley, M. T., Miller, J. W. and Lazo, G. R. 1988. Clonal population structure of *Xanthomonas campestris* and genetic diversity among citrus canker strains. *Mol. Plant-Microbe Interact.* 1 : 59-65.
3. Hibberd, A. M., Bassett, M. J. and Stall, R. E. 1987. Allelism tests of three dominant genes for hypersensitive resistance to bacterial spot of pepper. *Phytopathology* 77 : 1304-1307.
4. Hibberd, A. M., Stall, R. E. and Bassett, M. J. 1987. Different phenotypes associated with incompatible races and resistance genes in bacterial spot disease of pepper. *Plant Disease* 71 : 1075-1078.
5. Hwang, B. K., De Cock, A. W. A. M., Bahnweg, G.,

Prell, H. H. and Heitefuss, R. 1991. Restriction fragment length polymorphisms of mitochondrial DNA among *Phytophthora capsici* isolates from pepper (*Capsicum annuum*). *System. Appl. Microbiol.* 14 : 111-116.

6. Hwang, B. K., Lee, J. T., Hwang, B. G. and Koh, Y. J. 1995. Restriction fragment length polymorphism analyses of the plasmid DNAs in strains of *Xanthomonas campestris* pv. *vesicatoria* from different geographic areas. *J. Phytopathology* 143 : 185-191.
7. Jones, J. B., Pohronezny, K. L., Stall, R. E. and Jones, J. P. 1986. Survival of *Xanthomonas campestris* pv. *vesicatoria* in Florida on tomato crop residue, weeds, seeds, and volunteer tomato plants. *Phytopathology* 76 : 430-434.
8. Kim, B. S., Kwon, Y. S. and Hur, J. M. 1990. Differentiation and distribution of pathotypes of *Xanthomonas campestris* pv. *vesicatoria* pathogenic on pepper in Korea. *Korean J. Plant Pathol.* 6(2) : 245-249.
9. Lazo, G. R. and Gabriel, D. W. 1987. Conservation of plasmid DNA sequences and pathovar identification of strains of *Xanthomonas campestris. Phytopathology* 77 : 448-453.
10. Lazo, G. R., Roffey, R. and Gabriel, D. W. 1987. Pathovars of *Xanthomonas campestris* are distinguishable by restriction fragment length polymorphisms. *Int. J. Syst. Bacteriol.* 37 : 214-221.
11. Leach, J. E., White, F. F., Rhoads, M. L. and Leung, H. 1990. A repetitive DNA sequence differentiates *Xanthomonas campestris* pv. *oryzae* from other pathovars of *X. campestris. Mol. Plant-Microbe Interact.* 3 : 238-246.
12. Maniatis, T., Fritsch, E. F. and Sambrook, J. 1982. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545pp.
13. McGuire, R. G., Jones, J. B. and Sasser, M. 1986. Tween media for semiselective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. *Plant Disease* 70 : 887-891.
14. Nei, M. and Tajima, F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* 97 : 145-163.
15. Oono, K. and Sugiura, M. 1978. Isolation of rRNA genes in rice. *Jpn J. Genet.* 53 : 444.
16. Pruvost, O., Hartung, J. S., Civerolo, E. L., Dubois, C. and Perrier, X. 1992. Plasmid DNA fingerprints distinguish pathotypes of *Xanthomonas campestris* pv. *citri*, the causal agent of citrus bacterial canker disease. *Phytopathology* 82 : 485-490.
17. Qhobela, M., Leach, J. E., Claflin, L. E. and Pearson, D. L. 1991. Characterization of strains of *Xanthomonas campestris* pv. *holcicola* by PAGE of membrane proteins and by REA and RFLP analysis of genomic DNA. *Plant Disease* 75(1) : 32-36.
18. Rohlf, F. J. 1975. Note on algorithm 81: Dendrogram plot. *Computer J.* 18 : 90-92.
19. Rohlf, F. J. 1993. NTSYS-pc, *Numerical Taxonomy and Multivariate Analysis System.* State Univ. of New York, Stony Brook.
20. Schaad, N. W. 1980. *Laboratory Guide for Identification of Plant Pathogenic Bacteria.* Bacteriology Committee of American Phytopathological Society. St. Paul, Minnesota. 72pp.
21. Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual,* Second edition. Cold Spring Harbor Press, Cold Spring Harbor, NY.
22. Sneath, P. H. A. and Sokal, R. R. 1973. *Numerical Taxonomy.* Freeman, San Francisco.
23. Sokal, R. R. and Sneath, P. H. A. 1963. *Principles of Numerical Taxonomy.* Freeman, San Francisco. 359pp.
24. Xu, G.-W. and Gonzalez, C. F. 1991. Plasmid, genomic, and bacteriocin diversity in U.S. strains of *Xanthomonas campestris* pv. *oryzae. Phytopathology* 81 : 628-631.