

Symposium Session V : Molecular Plant-Microbe Interactions I

SV-1

Pathogenesis-related genes and factors in plant pathogenic *Fusarium* spp.

Tsutomu Arie(1), Nobuhiro Nakamura, Masato Kawabe(2), and Tohru Teraoka (1)

(1)Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan; (2)present address: National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604, Japan.

Members of the filamentous ascomycete fungus genus *Fusarium* Link collectively represent the most important group of plant pathogens, causing a variety of wilts, blights, and root rots on virtually every economically important plant. Of equal concern is the health hazard posed to humans and other animals by mycotoxins produced by *Fusarium* spp. In spite of a number of studies, the mechanisms of infection, disease development, and symptom induction by *Fusarium* are poorly understood. Recently, several genes including *fnk1*, *FOWI*, *fga1*, *fgb1*, and *chsV*, and their products were demonstrated to play a role in virulence in *Fusarium* spp. (Di Pietro 2001; Inoue 2002; Jain 2002; Jain 2003; Madrid 2003) (Table 1).

Fusarium oxysporum Schlechtend.:Fr. causes soilborne vascular wilt diseases of various crops (Booth 1971). Our goal is to identify pathogenicity-related genes and factors in *F. oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder et H.N. Hans. (*FOL*), the tomato wilt pathogen, using mutants generated by restriction enzyme-mediated integration (REMI). REMI provides several advantages for functional genetic analyses in fungi including a several-fold increase in transformation frequency, the creation of random insertional mutations that are physically tagged, and the creation of a single genomic insertion that is stable and unrearranged (Riggle and Kumamoto 1998).

Here we report on *FPDI* which was identified from a reduced-pathogenicity mutant r120, *FCDI*, a gene adjacent to *FPDI* and encoding cellobiose: quinone oxidoreductase, and *AVRI*, an avirulence-determining locus identified from a mutant X-83.

1. *FPDI*

We selected a reduced-pathogenicity mutant (r120) of *FOL* race 2 (880621a-1, = JCM 12575) from ca. 1200 transformants generated by REMI. The gene tagged with the plasmid in the mutant was conceptually translated and predicted to encode a protein of 321 amino acids and was designated *FPDI*. BLAST searches against the GenBank DNA databases showed its partial similarity to a chloride conductance regulatory protein of *Xenopus laevis* (African clawed toed frog), suggesting that *FPDI* is a transmembrane protein. Although the function of *FPDI* has not been identified, it does play a role in pathogenicity of *F. oxysporum* f. sp. *lycopersici* because *FPDI*-deficient mutants reproduced the reduced-pathogenicity phenotype on tomato (Kawabe 2004).

2. *FCDI*

Adjacent to *FPDI*, a predicted ORF named *FCDI* was identified in the *FOL* race 2 (880621a-1) genome. *FCDI* was

predicted to encode a protein (*FCDI*) of 544 aa. which was similar (around 50% at the amino acid level) to the flavin-domain of cellobiose dehydrogenases (CDHs) produced by white rot fungi such as *Phanerochaete chrythosporium*, *Trametes versicolor* and *Humicola insolens* (Raices 1995; Dumonceaux 1998; Christensen 2001). CDH consists of a flavin-domain and a heme-domain connected via a linker. CDH oxidizes the reducing end of cellobiose, the component of plant cellulose, and is thought to play important roles in fungal cellulolysis (Henriksson 2000). The linker in CDH is degraded by proteases and the released flavin-domain functions as a cellobiose: quinone oxidoreductase (CBQ). *FCDI* of *FOL* is highly homologous to CBQ and *FCDI* is the first report of a gene encoding CBQ directly (Kawabe 2003). *FOL* 880621a-1 secreted CBQ into the culture medium when the fungus was grown without glucose but secretion was suppressed by addition of glucose and this response was correlated to *FCDI* expression in the fungus. Disruption of *FCDI* in 880621a-1 resulted in reduced CBQ activity in the culture filtrate. *FCDI*-disruptants had virulence phenotypes on tomato as equivalent to 880621a-1.

3. *AVRI*

REMI of *FOL* race 1 (NBRC 6531) generated a transformant X-83 which had a dramatic change in host range. X-83 became pathogenic on tomato cultivars (cvs.) resistant to *FOL* race 1 (carrying a resistant gene *I*), showing the same trait of *FOL* race 2 (Fig. 1A). This result may be explained by the gene-for-gene hypothesis (Flor 1955) in which a putative avirulence gene (*AVRI*) is knocked out in X-83. Plasmid rescue recovered a 7 kb genome DNA sequence of *FOL* NBRC 6531 including the transformation vector-integration site in X-83. In this sequence, we found a locus of 1089 bp in size including the plasmid-integration site and terminal inverted-repeats (TIRs; 26 and 27 bp) at both ends (Fig. 1B) (Nakamura 2004). Insertional mutants of the locus in NBRC 6531 reproduced the same phenotype of X-83, confirming that the locus determines the avirulence in NBRC 6531. BLAST searches of the GenBank DNA Databases revealed that part of the locus had a very high similarity (98% at the nucleotide level) to a 265 bp region of the cucumber mosaic virus (CMV) coat protein (CP) RNA. The CMV CP-homologues existed in inverted-repeats (Fig. 1B). This is the first report of a plant virus genome present in an eukaryotic genome. Moreover, this is the first demonstration of race evolution through inactivation of an avirulence locus in *F. oxysporum*.

Acknowledgment

This work was supported by a Grant-in-Aid for Scientific

Research from Japan Society for the Promotion of Science for TA.

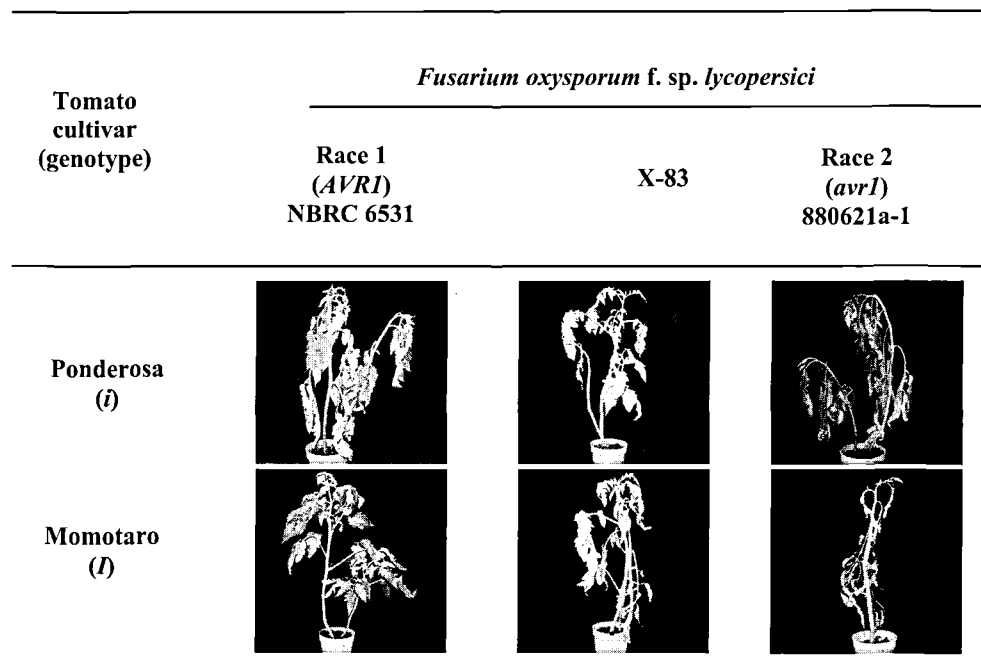
References

- Arie T, Gouthu S, Shimazaki S, Kamakura T, Kimura M, Inoue M, Takio K, Ozaki A, Yoneyama K, Yamaguchi I (1998) *Ann Phytopathol Soc Jpn* 64: 7-15
- Bai GH, Desjardins AE, Plattner RD (2002) *Mycopathologia* 153: 91-8
- Booth C (1971) In: The genus *Fusarium*, CMI
- Caracuel Z, Casanova C, Roncero MI, Di Pietro A, Ramos J (2003) *Eukaryot Cell* 2: 1246-52
- Caracuel Z, Roncero MI, Espeso EA, Gonzalez-Verdejo CI, Garcia-Maceira FI, Di Pietro A (2003) *Mol Microbiol* 48:765-79
- Catlett NL, Yoder OC, Turgeon BG (2003) *Eukaryot Cell* 2: 1151-61
- Christensen S, Brown KM, Brown SH, Schulein M (2001) *Enzyme Microb Technol* 28: 744-53
- Di Pietro A, Roncero GIM (1998) *Mol Plant-Microbe Interact* 11: 91-8
- Di Pietro A, Garcia-Maceira FI, Meglecz E, Roncero GIM (2001a) *Mol Microbiol* 39: 1140-52
- Di Pietro A, Huertas-Gonzalez MD, Gutierrez-Corona JF, Martinez-Cadena G, Meglecz E, Roncero GIM (2001b) *Mol Plant-Microbe Interact* 14: 653-662
- Dumoncaux TJ, Bartholomew KA, Charles TC, Moukha SM, Archibald FS (1998) *Gene* 210:211-9
- Flor HH (1955) *Phytopathology* 45: 680-5
- Garcia-Maceira FI, Di Pietro A, Huertas-Gonzalez MD, Ruiz-Roldan MC, Roncero MI (2001) *Appl Environ Microbiol* 67: 2191-6
- Garcia-Maceira FI, Di Pietro A, Roncero MI (2000) *Mol Plant-Microbe Interact* 13: 359-65
- Gomez-Gomez E, Roncero GIM, Di Pietro A, Hera C (2001) *Curr Genet* 40: 268-75
- Gomez-Gomez E, Ruiz-Roldan MC, Di Pietro A, Roncero GIM, Hera C (2002) *Fungal Genet Biol* 35: 213-22
- Guo W, Gonzalez-Candelas L, Kolattukudy PE (1996) *Arch Biochem Biophys* 332: 305-12
- Henriksson G, Johansson G, Pettersson G (2000) *J Biotechnol* 78: 93-113
- Hou Z, Xue C, Peng Y, Katan T, Kistler HC, Xu JR (2002) *Mol Plant-Microbe Interact* 15: 1119-27
- Inoue I, Namiki F, Tsuge T (2002) *Plant Cell* 14: 1869-83
- Iyama M, Kaneko I, Yamaguchi I, Teraoka T, Arie T (2002) *Ann Phytopathol Soc Jpn* 68: 181-2 (Abstr in Japanese)
- Iyama M, Kaneko I, Teraoka T, Arie T (2003) *Ann Phytopathol Soc Jpn* 69: 240-1(Abstr in Japanese)
- Jain S, Akiyama K, Mae K, Ohguchi T, Takata R (2002) *Curr Genet* 41: 407-13
- Jain S, Akiyama K, Kan T, Ohguchi T, Takata R (2003) *Curr Genet* 43: 79-86
- Jenczmionka NJ, Maier FJ, Losch AP, Schafer W (2003) *Curr Genet* 43: 87-95
- Kawabe M, Nakamura N, Teraoka T, Arie T (2003) *Ann Phytopathol Soc Jpn* 69: 240 (Abstr in Japanese)
- Kawabe M, Mizutani K, Yoshida T, Teraoka T, Yoneyama K, Yamaguchi I, Arie T (2004) *J Gen Plant Pathol* 70: 16-20
- Madrid MP, Di Pietro A, Roncero MI (2003) *Mol Microbiol* 47: 257-66
- Namiki F, Matsunaga M, Okuda M, Inoue I, Nishi K, Fujita Y, Tsuge T (2001) *Mol Plant-Microbe Interact* 14: 580-4
- Narasimhan ML, Lee H, Damsz B, Singh NK, Ibeas JI, Matsumoto TK, Woloshuk CP, Bressan RA (2003) *Plant J* 36: 390-400
- Nakamura N, Kawabe M, Teraoka T, Arie T (2004) *Ann Phytopathol Soc Jpn* 70: in press (Abstr in Japanese)
- Ospina-Giraldo MD, Mullins E, Kang S (2003) *Curr Genet* 44: 49-57
- Raices M, Paifer E, Cremata J, Montesino R, Stahlberg J, Divne C, Szabo IJ, Henriksson G, Johansson G, Pettersson G (1995) *FEBS Lett* 369: 233-8
- Riggle PJ, Kumamoto CA (1998) *Curr Opin Microbiol* 1: 395-9
- Rolodan-Arjona T, Perez-Espinosa A, Ruiz-Rubio, M (1999) *Mol Plant-Microbe Interact* 12: 852-61
- Ruiz-Roldan MC, Di Pietro A, Huertas-Gonzalez MD, Roncero MI (1999) *Mol Gen Genet* 261: 530-6
- Stahl DJ, Schafer W (1999) *Plant Cell* 4: 621-9
- Temporini ED, VanEtten HD (2004) *Curr Genet* 46: 29-36
- Yoshida T, Arie T, Kawabe M, Takahasih M, Nomura Y, Yoneyama K, Yamaguchi I (1999) In: Book of abstracts of 9th Internat Congr of Mol Plant-Microbe Interact pp. 110 (Abstr)

Table 1 Pathogenicity-related genes and proteins reported in *Fusarium* spp.

Gene (protein)	Pathogenicity of gene-disruptant	Reference
<i>F. oxysporum</i>		
<i>pg1</i> (endopolygalacturonase)	→	Arie (1998); Di Pietro (1998)
<i>xyl1</i> (xylanase)	?	Ruiz-Roldan (1999)
<i>xyl3</i> (xylanase)	→	Ruiz-Roldan (1999); Gomez-Gomez (2002)
<i>pl1</i> (pectate lyase)	?	Huertas-Conzalez (1999)
<i>FoTom1</i> (tomatinase)	?	Roldan-Arjona (1999)
<i>fap1</i> (aspartic proteinase)	→	Yoshida (1999)
<i>pgx4</i> (exopolygalacturonase)	→	Garcia-Macera (2000)
<i>pg5</i> (endopolygalacturonase)	→	Garcia-Macera (2001)
<i>fmk1</i> (MAPK)	↓	Di Pietro (2001a); Ortoneda (2004)
<i>prt1</i> (subtilisin-like proteinase)	→	Di Pietro (2001b)
<i>ARG1</i> (argininosuccinate lyase)	↓	Namiki (2001)
<i>FOW1</i> (mitochondrial carrier protein?)	↓	Inoue (2002)
<i>xyl5</i> (xylanase)	→	Gomez-Gomez (2001)
<i>xyl4</i> (xylanase)	→	Gomez-Gomez (2002)
<i>fga1</i> (G protein alpha subunit)	↓	Jain (2002)
<i>chsV</i> (class V chitin synthase)	↓	Madrid (2003)
<i>PacC</i> (pH signalling transcription factor)	↑	Caracuel (2003)
<i>fgb1</i> (G protein beta subunit)	↓	Jain (2003)
<i>SNF1</i> (sucrose non-fermenting 1, control CWDEs)	↑	Ospina-Giraldo (2003)
<i>PIR2</i> (cell wall glycoprotein of <i>S. cerevisiae</i>)	OE↑	Narasimhan (2003)
<i>FPD1</i> (membrane protein?)	(↓)	Kawabe (2004)
<i>FCD1</i> (cellobiose:quinone oxydoreductase)	→	Kawabe (2003)
<i>AVR1</i>	↑	Nakamura (2004)
<i>F. sacchari</i> (<i>Gibberella fujikuroi</i> MPB)		
<i>ggb1</i> (G protein beta subunit)	↓	Iyama (2002)
<i>gfk1</i> (MAPK)	↓	Iyama (2003)
<i>Fusarium verticillioides</i> (<i>Gibberella moniliformis</i>)		
histidine kinase	↓	Catlett (2003)
<i>F. graminearum</i> (<i>Gibberella zeae</i>)		
<i>Tri5</i> (trichodiene synthase)	(↓)	Bai (2002)
<i>MGV1</i> (MAPK)	↓	Hou (2002)
<i>Gpmk1</i> (MAPK)	↓	Jenczmionka (2003)
<i>F. solani</i> (<i>Nectria haematococca</i>)		
cutinase	→	Stahl (1992); Crowhurst (1997)
<i>pelD</i> (pectate lyase)	?	Guo (1996)
PEP cluster (pea pathogenicity cluster)	?	Temporini (2004)

A



B

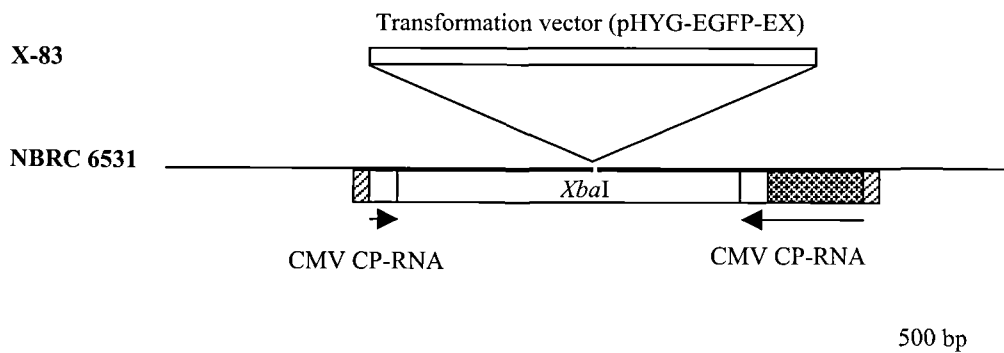


Fig. 1 A *Fusarium oxysporum* f. sp. *lycopersici* race-cultivar relationship explained by the gene-for-gene model (Flor 1955). X-83, a REMI-transformant derived from NBRC 6531, shows the same reaction of race 2, suggesting that the avirulence locus (*AVRI*) is knocked out in X-83. **S**, susceptible (compatible reaction, infection); **R**, resistant (incompatible reaction, noninfection).

B Map of NBRC 6531 genome around the transformation vector-integration site (*Xba* I) in X-83. ▨, terminal inverted repeat (TIR); ▤, region homologous with partial coat protein (CP)-RNA of many CMV isolates; □, regions homologous with partial CP-RNA of a CMV banana isolate. CMV CP-homologues exist in inverted-repeats adjacent to the TIRs.