Identification of the XorII methyltransferase gene and a vsr homolog from Xanthomonas oryzae pv. oryzae

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Abstract The gene encoding the XorII methyltransferase (M·XorII) was cloned from Xanthomonas oryzae pv. oryzae and characterized in Escherichia coli. The M·XorII activity was localized to a 3.1 kb BamHI–BstXI fragment, which contained two open reading frames (ORFs) of 1272 nucleotides (424 amino acids) and 408 nucleotides (136 amino acids). Ten polypeptide domains conserved in other M·5 cytosine methyltransferases (MTases) were identified in the deduced amino acid sequence of the 1272 ORF. E. coli Mrr + strains were transformed poorly by plasmids containing the XorII MTase gene, indicating the presence of at least one M·5CG in the recognition sequence for M·XorII (CGATCG). The 408 nucleotide ORF was 36% identical at the amino acid level to sequences of the E. coli dcm-vsr gene, which is required for very short patch repair. X. oryzae pv. oryzae genomic DNA that is resistant to digestion by PvuI and XorII hybridizes with a 7.0 kb fragment containing the XorII MTase gene and vsr homolog, whereas DNA from strains that lack M·XorII activity do not hybridize with the fragment.

Key words Restriction-modification · Cytosine methyltransferase · vsr · Very short patch repair · Xanthomonas oryzae pv. oryzae

Introduction

Xanthomonas oryzae pv. oryzae, which causes bacterial blight, is a major pathogen of rice found in most rice-producing areas of the world. Control of bacterial blight by the development and cultivation of resistant rice cultivars has been frustrated by the rapid appearance of new, virulent strains of the pathogen (Mew 1987). In addition to this pathogenic variability, a high degree of genetic variability has been demonstrated in the genome of X. oryzae pv. oryzae (Leach et al. 1990, 1992). We are characterizing factors that might promote or prevent the generation of genomic and possibly pathogenic diversity, such as mobile DNA elements (Leach et al. 1992; Yun 1991) and, in this study, restriction-modification (R-M) systems. Bacterial R-M systems may impede genomic diversity by protecting cells from invasion by foreign DNA or through the correction of mismatch errors (Endlich and Linn 1985; Hennecke et al. 1991; Krüger and Bickle 1983; Levin 1986). An understanding of R-M systems and mobile elements and their distribution in X. oryzae pv. oryzae populations may provide clues as to how these factors might influence the diversity of this pathogen.

The presence of type II R-M systems (Wilson 1988) in X. oryzae pv. oryzae (G+C about 64.5%; Vera Cruz et al. 1984) was implied by previous studies in which genomic DNAs from some strains were shown to be resistant to digestion by endonucleases such as PstI, XorII, and XbaI, all of which are inhibited by methylation of a cytosine residue in their respective recognition sequences (McClelland and Nelson 1992). Two sequence-specific type II endonucleases, XorI and XorII have been purified from X. oryzae pv. oryzae (Wang et al. 1980). The XorII endonuclease recognizes the sequence CGATCG, and activity of the enzyme is inhibited by the presence of a 5-methylcytosine in the 3' position or a hydroxymethylcytosine at the 5' position or when the adenine residue is methylated (McClelland and Nelson 1992; Wang et al. 1980).

Of the other R-M systems that have the same recognition sequence as XorII (BspC1, NblI, PvuI, RshI, Rspl, EcIJI, and XrII), only the genes of the PvuI system have been cloned and only the sequence of the PvuI methyltransferase (MTase) has been reported (McClelland and Nelson 1992; Kessler and Manta 1990; Smith et al. ...
In this communication, we describe the identification and characterization of the XorII MTase gene (xorIIM) and a csr-like gene from *X. oryzae* pv. *oryzae*.

Materials and methods

Bacterial strains, plasmids, and culture media

Strains of *X. oryzae* pv. *oryzae* were obtained from K. S. Jin, Agricultural Sciences Institute, Rural Development Administration, Suwon, Korea; T. W. Miew, International Rice Research Institute, Los Baños, the Philippines; and from a collection maintained in the Dept. of Plant Pathology, Kansas State University. A 5-aza-cytidine-resistant mutant of the *X. oryzae* pv. *oryzae* strain PX099, designated as PX099A, was used as a recipient for plasmids containing xorIIM. *X. oryzae* pv. *oryzae* strains were cultured in peptone-sucrose broth (PSB; Tsuchiya et al. 1982) or nutrient broth (NB; Difco Laboratories, Detroit, Mich., USA) at 28°C with shaking at 200 rpm. *Escherichia coli* strains JH132 (Mrr+, Mcr+, Dam+) and JH132MCR (Mcr-, Mrr-, Dam+) were used without further purification for routine digestion experiments. When required, cesium chloride (CsCl) gradient centrifugation was used for subcloning in pUFRO27 (plasmid, Km^r) were used for genomic library construction. Five micrograms of cosmid DNA was digested with 20 Units of XorII for 3 h at 37°C. After precipitation with ethanol, the digested DNA was used directly (without ligation) to transform *E. coli* strain JW89011 by electroporation. Kanamycin-resistant clones were individually screened by digestion of the cosmid DNA with *Pvu*I and XorII. Clones with cosmids or genomic DNA resistant to digestion by *Pvu*I and XorII were selected as candidates likely to contain xorIIM.

DNA isolation and manipulation

Isolation of genomic DNA from *X. oryzae* pv. *oryzae* and cosmids or plasmid DNA from *E. coli* or *X. oryzae* pv. *oryzae* were as described previously (Leach et al. 1990; Morellé 1989). The DNA was used without further purification for routine digestion experiments. When required, cesium chloride (CsCl) gradient centrifugation or Geneclean (Bio 101, La Jolla, Calif., USA) were used to purify genomic or plasmid DNA. Restriction endonucleases, nick-translant kits, and carbenicillin was used at 100 μg/ml.

Genomic library construction

Genomic DNA from *X. oryzae* pv. *oryzae* strain JW89011 was digested partially with *Sau*3A (0.006–0.01 Units/μg DNA) for 1 h and fractionated in a 0.5% agarose gel. Fragments larger than 25 kb were extracted from the agarose by electroelution. The *X. oryzae* pv. *oryzae* genomic DNA fragments were ligated to the BamHI-digested, phosphatase-treated cosmid vector pUFRO34, packaged into lambda phage in vitro (Gigapak Plus packaging kit, Stratagene), and transduced into *E. coli* strain JW89011. Eleven hundred kanamycin-resistant clones (average insert size of 32 kb) were selected; screening this number of clones would yield any single-copy gene with a probability of 99%, if the *X. oryzae* pv. *oryzae* genome size is assumed to approximate that of *Pseudomonas fluorescens* (Trevisan) Migula (7.4×10^6 kb; Bak et al. 1970).

Selection of clones containing xorIIM

Cosmid DNAs were isolated from pooled genomic pUFRO34 library clones and purified by CsCl gradient centrifugation. Five micrograms of cosmid DNA was digested with 20 Units of XorII for 3 h at 37°C. After precipitation with ethanol, the digested DNA was used directly (without ligation) to transform *E. coli* strain JW89011 by electroporation. Kanamycin-resistant clones were individually screened by digestion of the cosmid DNA with *Pvu*I and XorII. Clones with cosmids or genomic DNA resistant to digestion by *Pvu*I and XorII were selected as candidates likely to contain xorIIM.

DNA sequence analysis

Sequencing was performed by the dideoxy chain-termination method (Sanger et al. 1977) from double-stranded templates with a Sequenase version 2.0 kit (United States Biochemical, Cleveland, Ohio, USA). In addition to T3 and T7 primers, oligonucleotide primers derived from previously sequenced regions in the cloned gene were used in the sequencing reaction. To reduce compressions, terminal deoxynucleotidyl transferase (TdT; BRL) was added to each termination reaction (Li and Schweiger 1993). For DNA or protein sequence analysis, SeqAid II version 3.81 (Rhoads and Roufa 1991, Kansas State University, Manhattan, Kan., USA), Microgenie (Beckman, Palo Alto, Calif., USA), and Blast (Altschul et al. 1990) programs were used.

Results

Characterization of *X. oryzae* pv. *oryzae* genomic DNA

Genomic DNA isolated from *X. oryzae* pv. *oryzae* strain JW89011 was resistant to digestion by XorII, PvuI, PstI, and XbaI, but was sensitive to digestion with other type II endonucleases such as *Eco*RI, *Bam*HI, *Cla*I, *Hpa*II, *Ava*I, *Pvu*I, and *Hae*II (Fig. 1). All of the ineffective endonucleases tested are sensitive to methylation at either cytosine or adenine residues within the recognition sequences (McClelland and Nelson 1992). Treatment of pBluescriptII KS+ DNA with crude protein extracts from the *X. oryzae* pv. *oryzae* strain JW89011 resulted in fragments of 1.7, 1.0, and 0.2 kb, the sizes expected for XorII-specific endonuclease activity, indicating that the XorII R-M system is the major system present in the strain (data not shown).