Species-Specific Type II Restriction-Modification System of Xylella fastidiosa Temecula1

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The transformation efficiency of Xylella fastidiosa can be increased by interfering with restriction by the strain-specific type II system encoded by the PD1607 and PD1608 genes. Here, we report results for two strategies: in vitro methylation using M.SssI and isolation of DNA from an Escherichia coli strain expressing the methylase PD1607.

Xylella fastidiosa is a fastidious, xylem-limited, Gram-negative bacterium and the causative agent of Pierce’s disease (PD) of grapevines (1, 12). X. fastidiosa is transmitted from infected plants to susceptible plant species, like grapevines, by xylem-feeding insects. Once inside the grapevine xylem, X. fastidiosa impedes the flow of sap, thereby producing the characteristic symptoms of PD. Studies of the virulence and basic biology of X. fastidiosa have been facilitated by the availability of genetic and molecular tools (6, 8–10, 20, 22, 23). In each case, their successful use has depended on the ability to efficiently introduce DNA into X. fastidiosa.

In most bacteria, efficient acquisition of foreign DNA is limited by the presence of host-encoded restriction-modification (R-M) systems (14, 25). The restriction enzyme (REase) cleaves incoming DNA at specific sequences unless its recognition site has been modified by its cognate methyltransferase (MTase). R-M systems are divided into four categories (types I to IV) based on their mode of action and the distribution of the restriction, modification, and specificity functions within the enzyme subunits. Analysis of the X. fastidiosa Temecula1 genome has uncovered a number of potential R-M systems based on their overall sequence similarities to known systems (25, 28). According to the Restriction Enzyme Database (REBASE) (25), X. fastidiosa Temecula1 is predicted to encode four functional R-M systems, of the following types (with the following locus tags): type I (PD2070-PD2072 and PD2074-PD2076), type II (PD1607-PD1608), and type III (PD0833-PD0835). Restriction by these systems, particularly the type I systems, has been shown to have a major impact on the stable acquisition of foreign DNA by X. fastidiosa (9).

The focus of the manuscript is the type II R-M system encoded by the PD1607 and PD1608 genes (28). This system, which is present only in X. fastidiosa subsp. fastidiosa, does not impact the incidence of infection or the ability of X. fastidiosa to multiply within grapevines (17). The PD1607-PD1608 system is located within the prophage-like region Xpd8 (4) between the hypothetical protein PD1606 and tRNAAsn (Fig. 1). Analysis of their genetic organization suggests that the PD1606 and PD1607 genes are transcribed as a single polycistronic mRNA with the stop codon of the upstream REase overlapping the start codon of the downstream MTase. Based on naming conventions, the REase encoded by the PD1608 gene has been named XfaTORF1607P, and the MTase encoded by the PD1607 gene has been named M.XfaTORF1607P (REBASE) (25). For simplicity, we will refer to them as REase-PD1608 and MTase-PD1607, respectively. REase-PD1608 exhibits 58% amino acid identity to NspV from Nostoc sp. strain PCC7524 (27) and is predicted to recognize the sequence TTCGAA (REBASE) (25). The associated MTase-PD1607 exhibits 45% amino acid identity to M.NspV from Nostoc sp. strain PCC7524 (27). The similarity between the PD1607-PD1608 and NspV systems is further supported by the observation that X. fastidiosa Temecula1 genomic DNA is resistant to digestion with NspV (data not shown). The simplest interpretation for this result is that MTase-PD1607 methylates one of the bases within the sequence TTCGAA, thereby blocking cleavage by NspV.

To determine if X. fastidiosa transformation efficiency is lower for exogenous plasmids containing NspV sites, we conducted a series of experiments by using pBBR1MCS-5, a broad-host-range plasmid that contains a single NspV site and replicates in both E. coli and X. fastidiosa (15, 23). Plasmid DNA isolated from either X. fastidiosa or E. coli was introduced into X. fastidiosa electrocompetent cells as previously

FIG. 1. Chromosomal region surrounding the X. fastidiosa type II R-M system. The orientations and locations of the putative open reading frames (ORFs; arrows) and tRNA* (triangle) are indicated. The PD1608 gene is predicted to encode a subtype P type II REase; the PD1607 gene is predicted to encode a subtype gamma type II MTase (REBASE) (25). The PD1608-PD1607 operon is located within prophage-like region Xpd8 (4) and adjacent to tRNA* (the PD1605 ORF contains a frameshift mutation, which disrupts the putative integrase of Xpd8 (4). PD1606 extends toward and overlaps the 3′ end of PD1607 by 143 bp. The hypothetical protein encoded by the PD1606 gene shows some similarity in its N terminus (4e-10) to proteins belonging to the bacteriophage P4-like integrase subfamily (cd00801 sequence cluster) (19). However, PD1606 does not resemble PD1605 or other X. fastidiosa integrases.
FIG. 2. Transformation efficiency of plasmids into *X. fastidiosa*. Efficiency of transformation was calculated as the number of transformants per microgram of DNA. The amount of DNA was quantified by measuring absorbance at 260 nm by using a NanoDrop spectrophotometer (Thermo Scientific). The means of the results for three independent experiments and their standard errors are indicated. (A) *X. fastidiosa*-isolated pBBR1MCS-5. (B) DH5α-isolated pBBR1MCS-5 alone. (C) DH5α-isolated pBBR1MCS-5 with TRI. (D) DH5α-isolated pAM232 with TRI. (E) DH5α-isolated pBBR1MCS-5 treated with M.SssI according to manufacturer’s instructions before coelectroporation with TRI. (F) EAM1-isolated pBBR1MCS-5 with TRI.

Strains

| Strain, plasmid or oligonucleotide | Description | Reference or source |
|------------------------------------|-------------|-------------------|
| *X. fastidiosa* Temecula1          | *X. fastidiosa* subsp. *fastidiosa*; PD isolate ATCC 700964 | 8 |
| *Escherichia coli*                 |             | lab collection    |
| DH5α                               | supE44 ΔlacU169 (φ80 lacZΔM15) recA1 endA1 hsdR17 thi-1 gyrA96 relA1 | 8 |
| DH5α*pir                           | DH5α lysogenized with λ pir bacteriophage | Lab collection |
| EAM1                               | DH5α derivative; Sp r Str; att*P*K022-; (P_lacO-1-PD1607) | This study |
| Plasmids                           |             |                   |
| pAH69                              | Ap r; CRIM helper plasmid; oriR101 Int*HK022 | 11 |
| pAH144                             | Sp r Str; R6K γ ori att*P*K022 plasmid dependent upon pirγ in host | 11 |
| pAM217                             | Ap r; PD1607 in pJET1.2/blunt | This study |
| pAM218                             | Ap r; PD1607 from pAM217 in pZE12 | This study |
| pAM224                             | Sp r Str; P_lacO-1-PD1607 from pAM218 in pAH144 | This study |
| pAM232                             | Gm r; disrupted NspV site on pBBR1MCS-5 by filling-in with Klenow | 15 |
| pBBR1MCS-5                         | Gm r; pBBR1 replicon; broad-host-range cloning vector | Fermentas |
| pJET1.2/blunt                      | Ap r; blunt PCR cloning vector | 18 |
| pZE12                              | Ap r; ColE origin; P_lacO-1 promoter | 18 |

Oligonucleotides

| Oligonucleotides | Description | Reference or source |
|------------------|-------------|-------------------|
| PD1607-Kpn       | 5′-GGTACCCTGTAAGAAGAAAGAAAGAG-3′ (KpnI site is underlined) | 16 |
| PD1607-Xba       | 5′-TCTAGACTATGCAGCGCAGCCTTTGCGG-3′ (XbaI site is underlined) | 16 |
Another strategy for preventing restriction involves in vivo methylation of DNA by passage through an E. coli strain expressing the appropriate MTase (3, 21). The vectors, intermediate plasmids, strains, and oligonucleotides that we used in this construction are listed in Table 1. To create this strain, the PD1607 gene was amplified by PCR using oligonucleotides PD1607-Kpn and PD1607-Xba and placed downstream of the IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible P1lacO-1 promoter in pZE12 (18). The resulting fusion was integrated into the attHK site in DH5α by using the CRIM system (11), generating EAM1. Following transformation of EAM1 with pBBR1MCS-5, expression of the MTase-PD1607 was induced by growth in LB containing 1 mM IPTG and 10 μg/ml gentamicin. As shown in Fig. 3, EAM1-isolated pBBR1MCS-5 was resistant to cleavage by NspV but not by either SalI or SphI. Furthermore, its transformation efficiency was 10-fold higher than that of the DH5α-isolated plasmid (Fig. 2, column C versus F).

Concluding remarks. Comparative genomics has revealed that X. fastidiosa strains are unusually rich in R-M systems (14, 25, 28) and that some systems, like PD1607-PD1608, are found only in specific X. fastidiosa subspecies. Strain-specific R-M systems, which influence intraspecies genetic exchange, are thought to contribute to the genetic isolation necessary for pathogens to successfully colonize a new host (13, 14). At a practical level, these systems present obstacles for genetic manipulations that require the transformation of X. fastidiosa with DNA from a foreign source. In this paper, we described two different methods for overcoming restriction by REase-PD1608 that involve prior methylation of the DNA. Similar strategies could be developed to overcome the restriction by species-specific REase in other X. fastidiosa subspecies.

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