Phylogenetic Analysis of the pPT23A Plasmid Family of \textit{Pseudomonas syringae}

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Introduction

Many \textit{Pseudomonas syringae} (Ps.) strains contain native plasmids (collectively termed the pPT23A plasmid family) that cross-hybridize with replication sequences from pPT23A of \textit{Ps. pv. tomato} PT23. Plasmid members of the pPT23A family have been shown to encode determinants of importance in host-pathogen interactions such as the coronatine-biosynthesis locus which increases virulence and the avirulence genes \textit{avrD}, \textit{avrPphC}, and \textit{avrPphF} which affect host range (see references in 1). Additional sequences known to be encoded on plasmids of the pPT23A family include the \textit{stbCBAD} locus involved in plasmid stability, copper-resistance determinants and the streptomycin-resistance transposon Tn5393, and insertion sequence elements including IS51, IS801, IS870, and IS1240 (1). A common feature of all of these determinants is that functional loci encoding these traits are typically limited in distribution among small groups of \textit{Ps.} pathovars.

We are interested in the evolution of the pPT23A plasmid family in \textit{Ps.} including determining the range of pathovars encompassed by particular plasmid lineages, characterizing genes encoded on these plasmids and delineating the importance of horizontal transfer in their biology. In order to further understand the biology of the pPT23A plasmid family, it was desirable to study loci, which are widely distributed among \textit{Ps.} pathovars and might encode traits of general importance. We chose the \textit{rulAB} and \textit{repA} loci which, according to recent studies, fulfilled these requirements. The \textit{rulAB} determinant confers a phenotype of tolerance to ultraviolet radiation, a trait which is important for phyllosphere colonization, and the \textit{repA} gene encodes the major replication gene of these plasmids. The goals of this study were to perform comparative sequence analyses of the \textit{rulA} and \textit{repA} loci from pPT23A-like plasmids isolated from \textit{Ps. pv. syringae} and among a number of other pathovars.

Materials and Methods

The following bacterial strains were utilized in this study: \textit{Ps. pv. apii} 1089-5, \textit{Ps. pv. glycinea} race 6, \textit{Ps. pv. lachrymans} 1188-1, \textit{Ps. pv. maculicola} 438, \textit{Ps. pv. phaseolicola} 1302A, \textit{Ps. pv. pisi} 1086-2, \textit{Ps. pv. savastanoi} 0886-21, \textit{Ps. pv. syringae} 4981, 5D425, 7B12, 8B48, A2, B76, B86-17, BBS32-5, HS191, \textit{Ps. pv. tomato} B120, DC3000, OK-1, PT14, and PT23. The \textit{rulA} gene was
amplified using primers \textit{ru12} (5'-CGTTAACTGTACGTCCATACAG-3') and \textit{ru14} (5'-CGAA TTGCAA TCGACCAG-3'), and the \textit{repA} gene was amplified using primers 532 (5'-GAACGGTGACCTATGG-3') and 1588 (5'-CTCCAGCTTGCGGCCC-3'). Following amplification, the PCR products were ligated directly into the pCR2.1 vector (Invitrogen). Nucleotide sequencing was done using the Big Dye kit (ABI) following the instructions of the manufacturer; sequence reactions were run at the Genetic Technologies Center, Texas A&M University. The entire \textit{ruIA} gene was sequenced and approximately 300 bp from the 5' and 3' ends of the \textit{repA} gene were sequenced. The genetic relationships among \textit{Ps.} host strains was determined using arbitrarily-primed PCR using the IS50 primer or the extragenic repetitive consensus (ERIC) primer set.

\textbf{Results}

We determined the sequence of the \textit{ruIA} gene from 14 strains representing seven \textit{Ps.} pathovars. At the nucleotide level, intrapathovar divergence (within \textit{pv. syringae}) from 5.4 to 8.0\% and interpathovar divergence as high as 12.5\% was observed when the \textit{ruIA} sequences were compared to \textit{ruIA} from \textit{Ps. pv. syringae} A2. An alignment of the deduced amino acid sequences of \textit{ruIA} indicated that 32 of the 141 amino acids (22.7\%) were polymorphic among the strains. A matrix of amino acid substitutions among the strains showed that the minimum and maximum number of amino acid differences observed among single strain pairs was 1 (0.7\%) and 14 (9.9\%), respectively. We utilized the ProtPars program of Phylip to phylogenetically analyze the 14 derived amino acid sequences using the sequence of \textit{rumA}, a homolog from plasmid R391 of \textit{Klebsiella pneumoniae} (GenBank accession U13633) as the outgroup. The ProtPars analysis yielded a cladogram, which readily differentiated the sequences into several subgroups (Fig. 1). Three of the subgroups (\textit{Ps. pv. tomato} OK-1 and PT14, \textit{Ps. pv. syringae} A2 and 8B48, \textit{Ps. pv. syringae} B86-17 and BBS32-5), contained strains that were isolated from the same plant host. In two other cases, however, subgroups contained strains from different pathovars.

Results from the analyses of the \textit{repA} sequences were similar to those seen with \textit{ruIA}. \textit{repA} sequences from the A plasmids (80 to 100 kb) from \textit{Ps. pv. tomato} PT23, OK-1, DC3000, and \textit{Ps. pv. apii} 1089-5 formed a well-defined group. \textit{repA} sequences from \textit{Ps. pv. syringae} plasmids were included in either of two groups. A final group included the \textit{repA} sequence from the A plasmid of \textit{Ps. pv. tomato} B120 and the C plasmid (60 to 80 kb) of \textit{Ps. pv. glycinea} Race 6. Analyses of genetic fingerprints of the host strains obtained by using arbitrarily-primed PCR suggested that individual members of the pPT23A plasmid family have had long associations with their respective \textit{Ps.} host (data not shown).