

Novel insertion sequence-like elements in phytoplasma strains of the aster yellows group are putative new members of the IS3 family

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Abstract

Novel insertion sequence (IS)-like elements were isolated and characterized from phytoplasma strains in the aster yellows (AY) group (16SrI). The IS-like elements were cloned from phytoplasma strains AY1 and NJAY or PCR-amplified from 15 additional strains representing nine subgroups in the AY group using primers based on sequences of the putative transposases (Tpases). All IS-like elements contained sequences encoding similar Tpases of 321 amino acids (320 for strain CPh). Substantial amino acid sequence variability suggested multiple species of Tpases or IS-like elements exist in the AY phytoplasma group. These Tpases have an identical DDE motif that is most similar to the DDE consensus of Tpases in the IS3 family.

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1. Introduction

Phytoplasmas are plant pathogenic *Mollicutes* that cannot be cultured in vitro. To date, several hundred strains representing 15 16S rDNA RFLP or more than 20 phylogenetic groups have been identified by molecular means [1–3]. The aster yellows (AY) phytoplasma group (16SrI) strains are associated with more than 100 economically important diseases worldwide and represents the most diverse group known [4–6]. Currently, the AY group is differentiated into 16 subgroups on the basis of RFLP analysis of 16S rRNA gene sequences [6]. Moreover, there is considerable genetic variation among strains in some subgroups. Earlier studies on

the genomic organization revealed extensive chromosomal rearrangements among strains in a given subgroup, and the presence of multiple uncharacterized repetitive sequences in chromosomes of strains in the AY phytoplasma group [4]. Often, genetic variations among strains coincide with the pathogenic variations. The primary mechanism that is attributable to genetic variations among phytoplasmas is unknown.

Insertion sequences (ISs) are a group of small, mobile genetic elements. In prokaryotes, IS elements are known to effect chromosomal rearrangements and the expression of neighboring genes. Genomic mutation caused by IS elements is an important mechanism for generating genetic diversity. The presence of insertion sequence elements in the chromosomes of phytoplasmas had not been investigated and verified until the recent report of an IS-like element in peanut witches'-broom (PnWB) phytoplasma (GenBank accession no. AY270153).

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Typical IS elements are between 800 and 2500 bp long and contain two important components, a gene encoding a transposase (Tpase) (typically 250–400 amino acids long) and inverted repeat (IR) structures (usually 10–40 bp long) at the termini. In prokaryotes, more than 500 such elements have been identified and classified into at least 17 families on the basis of one or more of the following criteria: (i) IS open reading frame (ORF) organization, (ii) conserved signature DDE motifs, an essential part of the catalytic domain among Tpsases, (iii) similarity of IR sequences, and (iv) length of target site duplications [7]. In the present study, we report the discovery of several distinct but closely related IS-like elements in the AY group phytoplasmas. These IS-like elements encode Tpsases that share conserved signature motifs and sequence similarity with those of the IS3 family [7]. A preliminary synoptic report has been published in abstract form [8].

2. Materials and methods

2.1. Phytoplasma strains and DNA extraction

Twenty three phytoplasma strains representing nine subgroups in the aster yellows phytoplasma group (16Sr I) [9] and strain Mexican periwinkle virescence (MPV), belonging to group 16Sr XIII [2], were used

in this study (Table 1). Two symptom type variants of strain IA4 were also included in this study. Strain variant IA4Ph exhibited typical phyllody (the development of floral parts into green leafy structures) or virescence (the development of green flowers) symptoms, while strain variant IA4W exhibited small normal flower structure with normal pigment. Total nucleic acid was extracted from phytoplasma infected tissues according to previously described methods [5,6].

2.2. Cloning and sequencing of IS-like elements

Plasmid probe pAY27, previously constructed based on a cloned DNA fragment from AY phytoplasma strain AY1 (subgroup 16SrI-B), was shown to hybridize to multiple regions of genomic DNA from AY phytoplasma strains [4]. This probe was sequenced and a BLAST search of the NCBI protein database was performed to search for the presence of a putative IS. Primers used in this study for PCR amplification and sequencing are as follows: AYIS-F0 (5'-gcggtcatcgtaaaatcactga-3'), AYIS-F01A (5'-ataaaaatccccagttgcgc-3'), AYIS-F01 (5'-ccaacaaaatcgcatataagc-3'), AYIS(rt)F1 (5'-acaccaaattatcgcttccc-3'), AYIS-F3 (5'-gggtacttaataatagtgtaagg-3'), AYIS-R1 (5'-ggggagtttgaaataag-3'), AYIS-R02 (5'-taaaaaagggttagcgcgcaa-3'), AYIS(rt)R1 (5'-ccttggtcggaatgaatgata-3'), AYIS-R3 (5'-gcgttatc-

Table 1
Phytoplasma strains analyzed in this study

Disease caused, strain	Sources	16Sr subgroup
Chrysanthemum yellows, CHRY	Marguerite, Germany	16SrI-A
Hydrangea phyllody, HYDP	Hydrangea, Belgium	16SrI-A
Iowa aster yellows, IA4Ph and IA4W	Periwinkle, Iowa	16SrI-A
New Jersey aster yellows, NJAY	Lettuce, New Jersey	16SrI-A
Oklahoma aster yellows, OKAY1Ph	Lettuce, Oklahoma	16SrI-A
Onion yellows, OnionD3, OnionD8	Onion, Texas	16SrI-A
Plantago virescence, PVM	Plantago, Germany	16SrI-A
Tomato big bud, BB	Tomato, Arkansas	16SrI-A
Hydrangea phyllody, HyPH	Hydrangea, Italy	16SrI-B
Maryland aster yellows, AY1	Periwinkle, Maryland	16SrI-B
Oklahoma aster yellows, OKAY9B	Carrot, Oklahoma	16SrI-B
Primrose virescence, PRIVC	Primrose, Germany	16SrI-B
Severe aster yellows, SAY	Celery, California	16SrI-B
Erigeron yellows, ErY	Horseweed, Maryland	16SrI-B
Maize bushy stunt, MBS	Corn, Mexico	16SrI-B
Onion yellows, OY1, OY2, OY5, OY6, OY7, OY8, OY9	Onion, Japan	16SrI-B
Clover phyllody, CPH	Red clover, Canada	16SrI-C
Clover phyllody, KVG=KV	Clover, Germany	16SrI-C
Paulownia witches'-broom, PaWB	Paulownia, Taiwan	16SrI-D
Blueberry stunt, BBS3	Blueberry, Michigan	16SrI-E
Apricot chlorotic leaf roll, ACLR (AY)	Apricot, Spain	16SrI-F
Strawberry multiplier, STRAWB2	Strawberry, Florida	16SrI-K
Aster yellows, AV2192	China aster, Germany	16SrI-L
Aster yellows, AVUT	China aster, Germany	16SrI-M
Peanut witches'-broom, PnWB	Peanut, Taiwan	16SrII-A
Mexican periwinkle virescence, MPV	Periwinkle, Mexico	16SrXIII-A

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