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Extrachromosomal DNA isolated from tomato big bud and *Candidatus* Phytoplasma australiense phytoplasma strains

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Abstract

The nucleotide sequences of two extrachromosomal elements from tomato big bud (TBB) and one extrachromosomal element from *Candidatus* Phytoplasma australiense (*Ca.* P. australiense) phytoplasmas were determined. Both TBB plasmids (3319 and 4092 bp) contained an open reading frame (~570 bp) with homology to the rolling circle replication initiator protein (Rep). This gene was shorter than the *rep* genes identified from other phytoplasma plasmids, geminiviruses and bacterial plasmids. Both TBB extrachromosomal DNAs (eDNAs) encoded a putative DNA primase (*dnaG*) gene, a chromosomal gene required for DNA replication and which contains the conserved topoisomerase/primase domain. We speculate that the replication mechanism for the TBB phytoplasma eDNA involves the *dnaG* gene instead of the *rep* gene. The *Ca.* P. australiense eDNA (3773 bp) was shown to be circular and contained four open reading frames. The *rep* gene was encoded on ORF 1 and had homology to both plasmid (pLS1) and geminivirus-like domains.

Keywords: Phytoplasma; Extrachromosomal DNA; Tomato big bud; *Candidatus* Phytoplasma australiense; Rep and DnaG proteins

1. Introduction

Extrachromosomal DNA (eDNA) elements play important roles in the pathogenicity, host specificity and virulence of many plant pathogenic bacteria (Panopoulos and Peet, 1985; Vivian et al., 2001). They are mobile genetic elements that can replicate autonomously. Plasmids as a form of eDNA can incorporate genetic material from their host by recombination or transposition, and transfer it to a new host either by conjugation, mobilisation, transformation, or transduction (del Solar et al., 1996). A large majority of small, multicopy plasmids of Gram-positive bacteria replicate using the rolling circle replication (RCR) mechanism (Khan, 2000). This mechanism involves the generation of a site-specific nick by the plasmid-encoded replication initiator protein (Rep) that has a nucleotydil-transferase activity. The nascent strand is extended at the 3′ hydroxyl end by a DNA polymerase (Khan, 1997). RCR mechanism is also used

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by families of prokaryotic and eukaryotic single stranded (ss) DNA viruses such as geminiviruses (Stenger et al., 1991).

Phytoplasmas associated with numerous plant diseases in northern Australia have been extensively studied and documented (Collmer, 1998; Davis et al., 1997; Davis et al., 2003; De La Rue et al., 1999; Gibb et al., 1995; Padovan and Gibb, 2001; Schneider et al., 1999b; Tran-Nguyen et al., 2000). Two phytoplasmas, tomato big bud (TBB) and *Candidatus* Phytoplasma australiense (*Ca.* P. australiense) are widespread in Australia. The TBB phytoplasma has a wide host range including papaya, sesame, eggplant, and passionfruit (Davis et al., 1997; Padovan and Gibb, 2001; Schneider et al., 1999b). The *Ca.* P. australiense phytoplasma has a more limited host range of crop and non-crop host species (Davis et al., 1997; Davis et al., 2003; Padovan et al., 2000; Schneider et al., 1999b; Streten et al., 2005).

To date, eDNAs have been isolated from phytoplasmas associated with maize bushy stunt (Davis et al., 1988); phyllody of *Oenothera hookeri* (Sears et al., 1989), western aster yellows (AY) (Kuske and Kirkpatrick, 1990), chrysanthemum yellows, tomato big bud, periwinkle little leaf, American eastern AY (Bertaccini et al., 1990), witches' broom disease of pigeon pea (Harrison et al., 1991), walnut witches broom (Chen et al., 1992), numerous phytoplasmas from herbaceous plants, and woody dicots (Schneider et al., 1992); sugarcane white leaf (Nakashima and Hayashi, 1995); peanut witches' broom, and aster yellows (unpublished GenBank Entry Accession No. AY270152 and NC_003353); onion yellows (OY) (Kuboyama et al., 1998; Nishigawa et al., 2003; Nishigawa et al., 2002a; Nishigawa et al., 2002b; Nishigawa et al., 2001; Oshima et al., 2001a), beet leafhopper-transmitted virescence agent (BLTVA) (Liefting et al., 2004; Shaw, 1991); aster yellows witches' broom (AYWB) (Bai et al., 2006); TBB (Australian strain), and sweet potato little leaf strain V4 (Schneider et al., 1999a).

Although phytoplasma eDNAs have been detected, nothing is known about the role of these eDNAs in phytoplasma pathogenicity, if indeed they do have such a role. Only the seven phytoplasma eDNAs from OY, two from BLTVA and four from AYWB have been fully sequenced and published. This study aimed to expand our knowledge of eDNAs from Australian phytoplasmas.

2. Materials and methods

2.1. Sources of plants and DNA

Tomato big bud and *Ca.* P. australiense phytoplasma DNA were isolated from periwinkle (*Catharanthus roseus*) plants maintained at Charles Darwin University (CDU). The TBB phytoplasma strain was originally transmitted from tomato (*Lycopersicon esculentum*) collected near Adelaide, South Australia. The *Ca.* P. australiense phytoplasma strain was originally transmitted from *Gomphocarpus physocarpus*, Nambour, QLD. These TBB and *Ca.* P. australiense phytoplasma strains were transmitted to periwinkle by grafting and maintained in periwinkles by serial grafting. Field samples of the TBB phytoplasma were obtained from capsicum samples with little leaf (Childers, South QLD) and a chicory plant with little leaf and phyllody (Bundaberg, central QLD). A field sample of the *Ca.* P. australiense phytoplasma was obtained from strawberry (*Fragaria x ananassa*) with green petal symptoms (Deception Bay, southeast QLD). Other sources of TBB phytoplasma DNA were from a DNA archive maintained at CDU. Random clone TBB88 containing TBB from periwinkle was obtained as described in Schneider et al. (1999a).

2.2. DNA extractions

To identify the best method for isolating phytoplasma eDNA, several extraction methods were used. The methods included a small-scale extraction from fresh material (Doyle and Doyle, 1990) and total DNA extraction using the Dellaporta method (Dellaporta et al., 1983). Genomic DNA was extracted from 100 mg of lyophilised plant material using the DNeasy Kit (Qiagen, Australia) following the manufacturer's instructions. DNA prepared for Southern hybridisation was extracted using 2 g of lyophilised plant material according to Doyle and Doyle (1990) and resuspended in 200 μL TE buffer pH 8.0. To separate chromosomal host and phytoplasma DNA in a large scale total DNA extraction, 30 g of lyophilised strawberry plant material was extracted as described by (Porebski et al., 1997), resuspended in 5 mL of TE buffer and partially purified using a cesium chloride (CsCl) bisbenzimide gradient (Kollar and Seemüller, 1989; Kollar et al., 1990). Briefly, the DNA suspension was centrifuged for 70 h at 33,000 rpm. Hoechst stain preferentially binds the AT-rich phytoplasma DNA, thereby lowering the DNA buoyant density, and facilitating separation of phytoplasma and plant host DNA. The phytoplasma DNA band was located by comparing with a healthy plant DNA control. The phytoplasma band was extracted using a small Teflon tube attached to a micropipette tip, stained with Hoechst solution again and centrifuged at 33,000 rpm for a further 70 h, in total three spins. The final partially purified DNA solution was destained using isopropanol and precipitated