Research Note

Isolation and Characterization of a Locus from *Azospirillum brasilense* Sp7 That Complements the Tumorigenic Defect of *Agrobacterium tumefaciens* chvB Mutant

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The chromosomal virulence gene *chvB* of *Agrobacterium tumefaciens* is required for pathogenesis. A DNA fragment from the *chvB* locus can hybridize to DNA from *Azospirillum brasilense* Sp7. This DNA fragment could restore the tumorigenic activity of the *chvB* mutant strain *A. tumefaciens* A1011 towards leaf disks of *Nicotiana tabacum*. An NH$_2$-terminal open reading frame, 480 codons long, was most likely responsible for the restoration of the tumorigenic activity. The *A. brasilense* sequence showed good homology with the NH$_2$-terminal region of the *ndvB* gene of *Rhizobium meliloti*.


The diazotroph *Azospirillum brasilense* can adsorb to root hairs of monocotyledenous plants (Elmerich 1984; Kapulnik et al. 1985; Okon 1985) and also to individual cells (Eyers et al. 1988). *Agrobacterium tumefaciens* induces tumors in dicotyledonous plants, while *Rhizobium meliloti* induces nitrogen-fixing nodules on the roots of alfalfa plants. The processes of attachment and infection by both bacteria require, in addition to genes encoded by resident plasmids, a related set of chromosomal genes designated *chvA* and *chvB* in *A. tumefaciens* (Douglas et al. 1985) and *ndvA* and *ndvB* in *R. meliloti* (Dywan et al. 1986). DNA fragments containing the *ndvA* and *ndvB* genes can complement mutations in *chvA* and *chvB*, respectively, in *A. tumefaciens* (Dywan et al. 1986).

Could there be a genetic locus in *A. brasilense* that is analogous to *chvB* and *ndvB*? From the results of Southern blot hybridization of genomic DNA of several *Azospirillum* species including *Sp7*, Altabe et al. (1990) reported that these species did not have *chvB* homologous sequences. Contrary to this, Waelkens et al. (1987) had reported that genomic DNA from *A. brasilense* Sp7 hybridized with probes containing the *chvB* locus of *A. tumefaciens*.

We report here that *A. brasilense* Sp7 does have a locus homologous to the *chvB* locus of *A. tumefaciens*, and the cloned *A. brasilense* locus can complement a *chvB* mutational defect in *A. tumefaciens* with respect to tumor formation in leaf disks of *Nicotiana tabacum*. The segment of DNA involved in complementation can encode only a 480 amino acid-long NH$_2$-terminal polypeptide fragment that has sequence homology with the NH$_2$-terminal end of NdvB protein of *R. meliloti*.

A sequence homologous to a 1.25-kb EcoRI fragment internal to the *chvB* locus (Douglas et al. 1985) was found in the *A. brasilense* Sp7 genome by Southern hybridization. These were a 6.5-kb EcoRI fragment, a 5.1-kb BamHI fragment, and a 4.6-kb HindIII fragment (data not shown). Restriction analysis revealed that these three were overlapping fragments (Fig. 1).

Complementation studies were done to determine if the *chvB*-homologous DNA from *A. brasilense* constituted a locus functionally equivalent to the *chvB* locus. The recombinant plasmids pSR12 (6.5-kb EcoRI fragment cloned in pRK405) and pSR14 (9.5-kb EcoRI–HindIII fragment cloned in pRK405) were mobilized into *A. tumefaciens* A1011 (chvB::Tn5) from *E. coli* S17.1 (Simon et al. 1986) by conjugation. The vector pRK405 is a derivative of pRK404 (Ditta et al. 1985) in which the EcoRI site outside the polylinker has been destroyed by filling. The exconjugants were used to infect the *Nicotiana tabacum* leaf disks. The *A. tumefaciens* strains A348 (wild type) and A1011 (chvB::Tn5) (Douglas et al. 1985) were used as positive and negative controls, respectively. The defect due to *chvB* mutation in the strain A1011 could be complemented by both pSR12 and pSR14 plasmids, since virulence was exhibited towards tobacco leaves (Fig. 2). We propose to designate the *A. brasilense* locus *cviB* (complements virulence). The sequence on the right side of the broken arrow in the restriction map (Fig. 1) may not be essential for virulence, since pSR12 can also complement the defects of the *chvB* mutant.

The DNA sequence of both strands of the segment between the solid vertical arrows (Fig. 1) was determined by the dideoxy chain termination method (Sanger et al. 1977). An open reading frame (ORF) was found, which was preceded by the two terminator codons TAA and TAG (Fig. 3, underlined). The first ATG in this frame (underlined) has been assumed to be the translation initiator codon. An imperfect Shine and
Dalgarno sequence (CGAGC, broken underlined) exists immediately upstream. There is a GTG (underlined) in the same frame, upstream of the putative initiator codon ATG, but there is no semblance of any Shine and Dalgarno sequence preceding it. The ORF extends beyond the sequenced region that ends in a BglII site (Fig. 3, boxed). The NH$_2$-terminal segment of the putative CviB polypeptide from *A. brasilense* capable of correcting the chvB mutant phenotype with respect to virulence, is only 480 amino acids long.

The direction of transcription was confirmed by constructing a cviB::lacZ promoter fusion. A 1.4-kb SalI fragment (hatched rectangle, Fig. 1) was cloned into the broad host range transcription fusion vector pGD499 (Ditta et al. 1985) in both orientations. The construct with the promoter in the correct orientation, in which the HindIII site within the SalI fragment was closer to the lacZ gene (pSR22.2) and the construct with the promoters in opposite orientation (pSR21.2), were mobilized into *A. brasilense* Sp7 by conjugation. The colonies of exconjugants with pSR22.2 and pSR21.2 elicited β-galactosidase activities of 2,120 and in 180 Miller units (Miller 1972), respectively. Thus, the promoter of cviB is likely to be within the 1.4-kb SalI fragment, and the direction of transcription is from left to right (horizontal arrow, Fig. 1).

The DNA sequence of the cviB locus was found to have considerable homology with the relevant portion of the ndvB gene of *R. melliloti* (Jelpe et al. 1990), the gene that could also complement the defect in the chvB locus of *A. tumefaciens*. The homology was poor, with bases upstream of the base number 32 (underlined C, Fig. 3), which corresponded to base number 377 designated by Jelpe et al. (1990) in the *R. melliloti* ndvB sequence.

The derived amino acid sequence of CviB had 60% homology with the derived amino acid sequence of NdV at the level of identical amino acids and 76% homology if similar amino acids were considered. However, the first 38 amino acids at the NH$_2$-terminal end of NdV were absent at the NH$_2$-terminal end of CviB (Fig. 4). The base sequence of the corresponding region has been rechecked by using the oligonucleotide primer, 5'-ATAGGACGCGCGGATCGAATC3' (bases complementary to base numbers 87–67, Fig. 3). The hydrophathy plot of the NH$_2$-terminal region of the putative CviB polypeptide, determined according to Kyte and Doolittle (1982), has revealed the presence of a possible transmembrane segment that corresponds to one of the transmembrane segments of the NdV polypeptide (box, Fig. 4).

Since *A. brasilense* is not known to produce tumors or nodules in plants, but adsors to the roots, we speculate one of the functions of this locus is to participate in the adsorption.

![Fig. 1](image1.png)

![Fig. 2](image2.png)
Fig. 3. Partial nucleotide sequence of the *Avena sativa* *mut* B locus. The deduced amino acid sequence is shown below the nucleotide sequence. The presumed initiator ATG is underlined as also the termination codons TAA and TAG upstream of initiator ATG. The imperfect Shine-Dalgarno sequence is represented by the broken line under the sequence. The restriction endonuclease sites, *Bgl* II (AGATCT), *Hind* III (AAGCTT), *Sst* I (GTCGAC), and *Eco* RI (GAATTC) are boxed. Significant homology with the *ndb* gene of *R. meliloti* begins at the base C at position 32 (underlined).
Fig. 4. Amino acid sequence comparison of NH2-terminal regions of CviB and NdVB proteins. Identical amino acids are represented by vertical bars and the similar amino acids are represented by plus signs. The potential transmembrane domains deduced from the hydrophathy plots of the two polypeptides are boxed.

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