

# The *virB* operon of the *Agrobacterium tumefaciens* virulence regulon has sequence similarities to B, C and D open reading frames downstream of the pertussis toxin-operon and to the DNA transfer-operons of broad-host-range conjugative plasmids

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The 28.6 kDa virulence regulon of the pTiC58 plasmid is comprised of six major operons (1), of which the *virB* operon is required for the transfer of the T-DNA from the resident Ti plasmid in *Agrobacterium tumefaciens* to plant cells. A growing body of indirect evidence suggests that this operon encodes proteins that may simulate a bacterial conjugative transfer

apparatus to facilitate T-DNA transfer to plants (2-7). Comparative sequence analyses between the *virB* operon and those present in the data bank revealed striking similarities to the Tra2 operon of RP4 (8) and to the PilW operon of R388 (9-11) that are required for conjugative transfer between bacteria. Here, we report that these DNA transfer related proteins have striking sequence similarities to the open reading frames downstream of the PT operon (12) involved in pertussis toxin biosynthesis in *Bordetella pertussis* (Figure 1). The amino acid sequence similarity (and identity) between VirB2 and PT-orfB, between VirB3 and PT-orfC, and between the amino terminal portion of VirB4 and PT-orfD are 54% (31%), 54% (26%) and 53% (25%), respectively (calculated by the BESTFIT program, GCG, Wisconsin). Noteworthy is that the genetic arrangement are also alike (Figure 2). Although the function of each of the genes of the above operons (*virB*, Tra2, PilW and PT-downstream orfs') remain to be identified, these genes (and orfs') are likely encoding accessory proteins to facilitate the secretion of either DNA or polypeptide, or both as complexes. That VirB11 protein sequence is similar to secretory proteins of Gram-negative bacteria (13) support this concept that the *virB* operon encodes the secretion machinery necessary for the transfer of T-DNA-protein complexes from *A. tumefaciens* to plant cells.

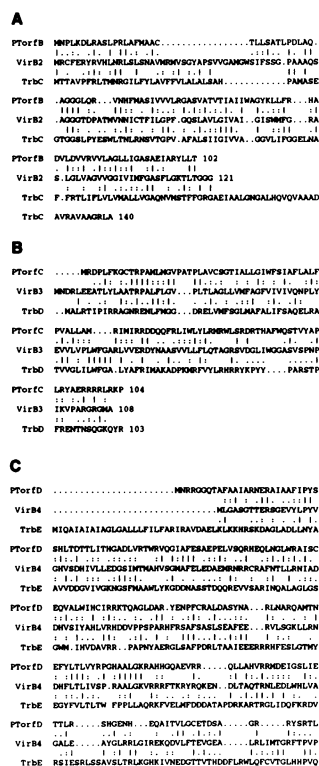


Figure 1. Amino acid sequence comparisons between VirB, PT and Trb proteins. A. VirB2 compared with PTorfB and TrbC. B. VirB3 compared with PTorfC and TrbD. C. VirB4 compared with PTorfD and TrbE.

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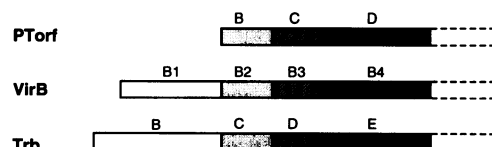


Figure 2. Identical genetic arrangement of the ORFs of P, VirB and Trb operons.

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## REFERENCES

1. Rogowsky, P.M., *et al.* (1990) *Plasmid* **23**, 85106.
2. Ream, W. (1989) *Annu. Rev. Phytopathol.* **27**, 583–618.
3. Kado, C.I. (1991) *Crit. Revs. Plant Sci.* **10**, 1–32.
4. Zambryski, P. (1992) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **43**, 465–490.
5. Winans, S.C. (1992) *Microbiol. Rev.* **56**, 12–31.
6. Beijersbergen, A., *et al.* (1992) *Science* **256**, 1324–1327.
7. Kado, C.I. (1993) In: Clewell, D.B., *Bacterial Conjugation*. Plenum Publ. NY.
8. Lessl, M., *et al.* (1992) *J. Biol. Chem.* **267**, 20471–20480.
9. Bolland, S., *et al.* (1990) *J. Bacteriol.* **172**, 5795–5802.
10. Shirasu, K., *et al.* (1993) *Proc. Natl. Acad. Sci. USA*, submitted.
11. Bolland, S., Llosa, M. and de la Cruz, F. unpublished.
12. Nicoisa, A., *et al.* (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4631–4635.
13. Dums, F., *et al.* (1991) *Mol. Gen. Genet.* **229**, 357–364.