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Published in:
Nature

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1984

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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trans-Acting Virulence Functions of the Octopine Ti Plasmid from Agrobacterium tumefaciens

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Received 13 September 1983/Accepted 7 February 1984

All Ti plasmid-encoded virulence functions that were studied act in trans. An octopine Ti plasmid-specific vir operon, called vir-O, located on an EcoRI restriction fragment has been characterized. Sequences with promoter activity in Escherichia coli were identified for a second vir operon, called vir-C, which was located close to the position of vir-O.

A large plasmid, called the Ti plasmid, which is present in oncogenic Agrobacterium tumefaciens strains, is essential for crown gall tumor induction (18, 19, 20). Part of this plasmid, the T region, is transferred to and integrated in the plant genome during tumorigenesis (2, 17). Physically distinct from the T region, a second region on the Ti plasmid which is essential for tumor induction has been identified (3, 6, 16). Several mutations in this region are complementable in trans, suggesting that this region has to function inside the bacterium for virulence (7, 8, 13). Therefore, this segment of the Ti plasmid is called Vir (virulence) region.

In a previous study, we demonstrated complementation of virulence functions for some Ti::Tn5 mutations (7). These results have now been extended by using 23 different Ti::Tn5 and 2 different Ti::Tn1831 plasmids, in which the transposons were inserted at various positions within the Vir region (Fig. 1). Agrobacterium strains, carrying these mutated Ti plasmids, were used in complementation studies with R-prime plasmids, which are compatible with Ti plasmids, and carry different segments of the Vir region of the wild-type octopine Ti plasmid (Fig. 1). In all cases, complementation was observed, demonstrating that all these functions act in trans.

This observation is in contradiction with results obtained by Klee et al. (13), who showed that two mutations in the Vir region (within Hpal fragment 15) could not be complemented in trans by cosmid clones. The same A. tumefaciens mutants were used in this study, and were shown to be complementable in trans by R-prime plasmids. The reason for this contradiction is as yet not understood, but might be due to differences in size of cloned Vir region fragments. Recently, it has been shown that Vir region and T region of an octopine Ti plasmid can physically be disconnected (10), which excludes the possibility that these virulence functions act in cis with regard to the T region.

For several reasons, we have concentrated on EcoRI fragment 4, a relatively small section of the Vir region. First, heteroduplex mapping between the octopine Ti plasmid pTiAch5 and the nopaline Ti plasmid pTiC58 revealed an area that is not homologous between the plasmids in this fragment (reference 5 and Fig. 1). Second, mutations in the homologous part cause the bacterium to be avirulent. And third, some mutations in the nonhomologous part also cause avirulence of the bacterium. These data suggest the existence of octopine Ti plasmid-specific virulence functions, which for EcoRI fragment 4 might be distinguished between general and octopine-specific virulence functions.

We used three different Ti::Tn5 plasmids for cloning EcoRI fragment 4 and for subsequent complementation studies. Two of the Tn5 insertions (in pAL1583 and pAL1586) are located in the homologous region, and one (in pAL1597) is positioned in the nonhomologous region (Fig. 1). The different EcoRI-4::Tn5 fragments were cloned on the IncP plasmid pRK290 (4), and the IncQ plasmid pKT247 (1). The broad-host-range vector plasmids pRK290 and pKT247 themselves did not have an effect on the virulence of A. tumefaciens (9). One set of mutations (in pAL1597, pAL1541, and pAL245) could only completely be restored to virulence if complementation was performed with IncP plasmids. With IncQ plasmids, however, a partial recovery of virulence (small tumors) was found for the same set of mutations. In one case, an IncQ plasmid carrying EcoRI fragment 4 even reduced the normal virulence of an A. tumefaciens strain. This might indicate that a higher copy number of plasmids, carrying vir genes, influences virulence. The virulence of none of the other avirulent mutants tested was restored completely with IncP EcoRI fragment 4 vir clones. Complementation was ineffective for LBA-1583(pAL1583), whereas only small tumors were formed with LBA1586(pAL1586). From these results, we conclude that a new vir operon, which is called vir-O, is present on EcoRI fragment 4. This operon, mutated in pAL1597, pAL1541, and pAL245, was fully restored to virulence by plasmids carrying EcoRI fragment 4, which harbors Tn5 insertions surrounding vir-O. Moreover, a second operon, referred to as vir-C, may also be present on EcoRI fragment 4, since the mutation in LBA1586(pAL1586) could be partially restored (small tumors occurred) by the other vir::Tn5 mutations cloned on pRK290.

Genetic data indicated that a vir-O operon could be present on a 3-kilobase segment within PstI-O, which is part of EcoRI fragment 4 (between the positions of the transposon insertions in pAL1636 and pAL1586 [Fig. 1]). This fragment contains the intact vir-O operon indeed, since it restores virulence of A. tumefaciens vir-O mutants, but not of a vir-C mutant. Because of the positions of different Tn5 insertions, the right-hand end of the vir-O operon could be placed in a 300-base-pair segment, and the left-hand end could be placed in an 1,100-base-pair HindIII-PstI segment. The size of the vir-O operon is therefore estimated to be in the range of 800 to 2500 base pairs (Fig. 2).

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FIG. 1. Location of vir mutations. The position of the different transposon insertions is indicated on a physical map of the Vir region for the restriction enzymes EcoRI and HpaI. Only in EcoRI fragment 4, also PstI sites are shown. The numbers above the insertion positions correspond to pAL numbered Ti plasmids. All insertions cause Agrobacterium to be avirulent on tomato, except for LBA1636(pAL1636) which is normally tumorigenic. Homology between octopine and nopaline Ti plasmids has been indicated by double-hatched boxes. In dashed lines the R-prime plasmids pAL1813 and pAL1818, used in the complementation studies, are indicated. Blocks refer to different Vir transcription units (12, 14).

From the present study, an indication has been found for both existence and position of an octopine-specific vir operon, the function of which is still unknown. Mutations in this operon cause a host-range effect on Agrobacterium spp. (on tomato, no tumor formation; on petunia, normal tumor formation); i.e., the vir-O product is not an absolute requirement for virulence in all plant species. Moreover, it has been shown that in the presence of a nopaline Ti plasmid, the octopine T region is transferred to plant cells (11). Thus, vir products, and in this case the vir-O product, obviously can be replaced by a product encoded by the nopaline Ti plasmid.

The vir-C mutation, present in pAL1586 (causing avirulence on all plant species tested), is located in PstI-O; however, it was located in a homologous region between octopine and nopaline Ti plasmids. This PstI fragment, cloned on an IncP plasmid, did not complement the mutation in vir-C, indicating that the vir-C operon cannot be present on this fragment completely. We cloned PstI-O in the EcoRI site of the galK promoter probe plasmid pKO-1 (15), and in

FIG. 2. Localization of vir-O and a putative promoter of vir-C. The 3.5-kilobase PstI fragment O, cloned in the EcoRI site of IncP plasmid pRK252, was used for complementation of Agrobacterium vir-O mutants. The numbers 1 to 6 refer to the positions of different Tn5 insertions into this fragment. Indications + or – above the numbers show whether complementation of vir-O mutations occurs. Thus genetically vir-O must be positioned between Tn5 insertions 1 and 5. The numbers 1, 2, 3, and 6 were recloned as EcoRI fragments in both orientations on the galK promoter probe plasmid pKO-1, and tested for promoter activity in E. coli. –, No blocking; +, blocking of promoter activity in orientation I by Tn5. H, HindIII; X, XhoI; B, BamHI; S, Smal.
E. coli significant promoter activity was detected only for the fragment in one orientation (pKO-1; 1 unit of galK activity; pKO-1 harboring PstI-O in orientation I, 30 units, and in orientation II, 1 unit). To map the position of the promoter, we recloned a number of mutated PstI-O::Tn5 fragments on plasmid pKO-1. In this way, the promoter was localized to a 500-base-pair segment, somewhere at the right-hand end of the vir-O operon, which is at the position of divergence of homology between the octopine and the nopaline Ti plasmid (Fig. 2). The direction of this promoter activity is towards the homologous region. This promoter has been identified by its activity in E. coli, but no evidence is available yet that this promoter is also functional in A. tumefaciens.

We thank Dave Garfinkel for sending us the Ti::Tn5 mutant strains and Marie-Louise Koop for skillful technical assistance in part of this work. We gratefully acknowledge the help of all our colleagues of the MOLBAS research group, and we acknowledge Ron van Veen and Paul Hooykaas for critical reading of the manuscript.

LITERATURE CITED