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Sustained Ethylene Production in *Agrobacterium*-Transformed Carrot Disks Caused by Expression of the T-DNA *tms* Gene Products

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***Agrobacterium*-infected carrot disks continually produced elevated levels of ethylene. Ethylene production was mediated by the elevated levels of auxin synthesized in transformed tissues.**

Canfield and Moore (2) demonstrated that a Ti or Ri plasmid is required for the long-term production of high levels of ethylene by carrot tissue (*Daucus carota*) inoculated with *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. The measurements of Canfield and Moore were done on primary tumors containing living bacteria. The present study addresses the question of whether a transformation event is required or whether plant-bacteria interaction is sufficient for the production of elevated levels of ethylene. Since ethylene production in normal plants is regulated by auxin (1), it might be expected that the elevated levels of ethylene produced in transformed tissues are a result of the elevated levels of indoleacetic acid synthesis in these tissues, as has been suggested by Miller and Pengelly (6). To determine whether transformation was required, we tested strain LBA4404 which carries *vir* (virulence) genes but no T-DNA. The results (see below) showed that T-DNA genes were essential to the production of high levels of ethylene. To establish which T-DNA genetic loci are involved, we measured ethylene levels in tumors induced by a series of *A. tumefaciens* strains with mutations in each of the left T-DNA transcripts of an octopine-type Ti plasmid.

The initial observations of Canfield and Moore (2) indicate that tumors induced by *A. rhizogenes* produce higher levels of ethylene than tumors induced by *A. tumefaciens* do. To determine whether such differences were due to differences in the growth rates of either the bacteria or the plant tissues, we carried out a time course study comparing ethylene production in tumors induced by wild-type *A. rhizogenes* and *A. tumefaciens* strains.

Strains of *A. rhizogenes* and *A. tumefaciens* (Table 1) were grown and maintained as described previously (11, 12). Ethylene production by carrot disks (five slices per flask) was measured essentially as described by Canfield and Moore (2), with the exception that the gas was allowed to accumulate in the flask headspace for 18 h before sampling. Initial experiments indicated that about 0.15 nl of ethylene per ml of headspace was produced by the carrot slices (including uninoculated controls and controls inoculated with sterile media) 2 days after cutting (which can be attributed to wounding). After that time, the levels of ethylene fell to less than 0.09 nl/ml of headspace in the control flasks. Strains that contained either the T-DNA alone (A136 Mini-Ti [3]) or the virulence loci and no T-DNA region (ACH5 LBA4404 [7]) when inoculated onto carrot disks did not produce significant ethylene at day 7 or day 18 (Table 2). Thus these experiments indicate that a transformation event was absolutely required for the sustained production of ethylene. The levels, of ethylene observed after inoculation of carrot disks with *A. rhizogenes* strains reached maximums by 7 to 9 days; *A. tumefaciens* strains took longer to reach maximum levels of gas production (data not shown). However, contrary to previous observations (2), these studies indicated that at 11 to 18 days after inoculation, tumors induced by some *A. tumefaciens* strains produced levels of ethylene equal to or greater than those produced by roots induced by *A. rhizogenes* strains.

We measured ethylene production in tumors induced by bacteria mutated (4) in each of the transcriptional units (5, 15) that have been defined for the left T-DNA region of the octopine-type Ti plasmid. Measurements were made 7 and 18 days after inoculation of carrot tissue with the mutant *A. tumefaciens* strains. At day 18 no ethylene was produced by tumors induced by mutations of the *tms* (tumor morphology shoot), loci, while mutations in all other loci produced normal or elevated levels of ethylene (Table 2). Schröder et al. (8) have demonstrated that the *tms* loci encode two

TABLE 1. Agrobacterium strains

Strain	Plasmid	Chromosomal background	Reference
A. rhizogenes			
A4	pRiA4	A4	13,14
TR105	pRiTR105	TR105	12,13
A. tumefaciens			
Transformant[a] R1000	pRiA4	C58	13,14
Transformant[a] R1149	pRiA4*tms1*	C58	14
Transformant[a] R1133	pRiA4*tms2*	C58	14
A136	None	C58	11
A208	pTiT37	C58	9
A277	pTiB6806	C58	9
A281	pTiBo542	C58	9
A136 Mini-Ti	Mini-Ti	C58	3
ACH5 LBA4404	pTiACH5del	ACH5	7
A136 pTiA66	pTiA66	C58	9,10
149 to 373[b]	pTiB6806	C58	4

[a] Ri plasmid transformed into strain C58 which has been cured of its Ti plasmid.

[b] Strains 149 to 373 are mutants of *A. tumefaciens* octopine-type plasmid pTiB6806 produced by site-directed transposon mutagenesis.

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TABLE 2. Ethylene production

Strain	Mutated locus[a]	Ethylene (nl/ml of headspace)[b]	
		Day 7	Day 18
325	Transcript 5	0.71	1.69
326	Transcript 5	0.35	0.99
316	Transcript 7	0.54	1.07
328	*tms-1*	0.12	<0.09
369	*tms-1*	0.12	<0.09
344	*tms-1*	0.10	<0.09
A136(pTiA66)	*tms-2*	0.12	<0.09
357	*tmr*	0.30	0.29
149	*tmr*	0.56	0.77
339	Transcript 6a	0.33	0.58
343	*tml*	0.57	1.96
351	*ocs*	0.32	0.36
373	*ocs*	0.27	0.69
R1149	*tms-1*	<0.09	<0.09
R1133	*tms-2*	0.11	<0.09
A136	No plasmid	<0.09	<0.09
ACH5(LBA4404)	No T-DNA	<0.09	<0.09
A277	None (wild type)	0.29	0.83
R1000	None (wild type)	0.45	0.14

[a] The mutated loci are as indicated in references 4, 5, and 15.
[b] Uninoculated controls produced <0.09 nl/ml at both 7 and 18 days.

proteins which enzymatically synthesize the auxin indoleacetic acid. To determine whether the *tms* loci alone were sufficient to induce the production of ethylene, we inoculated carrot disks with *A. tumefaciens* and *A. rhizogenes* containing the helper plasmid LBA4404 and a binary vector carrying T-DNA borders and the *tms* loci (16). From these strains we obtained a very weak tumor response with concomitant production of low levels of ethylene after 16 to 20 days (data not shown). There was no ethylene produced by a parallel construction carrying the *tmr* (tumor morphology root) locus, which encodes an enzyme involved in cytokinin biosynthesis. In a study of ethylene production by cloned crown-gall cell lines of *Nicotiana tabacum*, *Nicotiana glutinosa*, and *Lycopersicon esculentum*, Miller and Pengelly (6) showed that the *tms* genes stimulate 1-aminocyclopropane-1-carboxylic acid synthesis. Their results indicate that host factors in these cell lines are important in the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. Results presented here are consistent with their findings. We conclude that the *tms* loci are necessary and sufficient for the sustained production of ethylene and that this production is a secondary result of auxin synthesis in the transformed carrot root disk system examined here.

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