Inducible Permease Involved in the Uptake of Octopine, Lysopine and Octopinic Acid by *Agrobacterium tumefaciens* Strains Carrying Virulence-associated Plasmids

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[³H]Octopine is transported by 'octopine' strains of *Agrobacterium tumefaciens* after induction by either octopine, lysopine or octopinic acid and by 'nopaline' strains after induction by nopaline. The analogues noroctopine, homo-octopine and desmethylhomo-octopine had no inducing effect. Lysopine and octopinic acid showed a competitive influence on octopine uptake, as would be expected if there was one permease for all three compounds. The Michaelis-Menten constant for octopine transport was about 1.5 μ M. A sharp decline in transport activity was observed at pH values below 6.0. Evidence is provided that the octopine permease gene (*uadB*) and the accessory regulator gene (*uadR*) are carried by TI plasmids.

INTRODUCTION

Octopine, lysopine, octopinic acid and nopaline are amino-acid derivatives (Fig. 1) detected in plant tumours induced by *Agrobacterium tumefaciens* (Smith and Townsend) Conn but not in healthy plant tissue. A tumour may contain either octopine, lysopine and octopinic acid or nopaline (Goldmann, Thomas & Morel, 1969; Goldmann-Ménagé, 1970), depending on the type of virulence (TI) plasmid carried by the inducing bacteria (Bomhoff, 1974; Watson *et al.*, 1975; Bomhoff *et al.*, 1976; Hooykaas *et al.*, 1977). The TI plasmids also carry genes that determine whether the bacteria can degrade octopine, lysopine and octopinic acid or nopaline: strains that evoke tumours containing nopaline are also able to degrade nopaline, while the others degrade octopine, lysopine and octopinic acid. Whether these observations can be explained by transfer of bacterial plasmid nucleic acids to plant cells during tumour induction has still to be determined (Lippincott & Lippincott, 1975; Drlica & Kado, 1975; Kado, 1976).

We are trying to compare the octopine-synthesizing enzyme isolated from tumour tissue with the degrading enzyme from the bacteria to see how they are related. In a previous report (Klapwijk *et al.*, 1976) we described the isolation of several *A. tumefaciens* mutants altered in the utilization of octopine, lysopine and octopinic acid. These so-called *Uad*⁻ mutants show an absolute coupling of the utilization of these three compounds. This phenomenon suggests that they are taken up and degraded by a common system. The mutants were most likely defective in the octopine oxidase gene(s) yet they were still able to induce tumours containing octopine. These observations were confirmed by Tempé (unpublished results) and by Montoya *et al.* (1977). Therefore if there is gene transfer from the bacteria to plant cells during tumour induction and the genes responsible for octopine synthesis in the plant are of bacterial origin, they are not identical to the octopine oxidase gene(s) in the bacterium. More information about the regulation and number of *uad* genes involved in the bacterial degradation of amino-acid derivatives is required, not only to clarify these results,

Fig. 1. Structural formulae of the amino-acid derivatives used in this study.

but also because recently it has been found that octopine stimulates the transfer of the TI plasmids between *Agrobacterium* strains (Genetello *et al.*, 1977; Kerr, Manigault & Tempé, 1977).

We assume that there are four factors involved in the utilization of the above mentioned amino-acid derivatives as bacterial nitrogen sources: (i) induction; (ii) uptake by a permease; (iii) splitting into amino acid and α -keto acid by an oxidase; and (iv) degradation of the amino acid by catabolic enzymes. We describe here the role of induction and uptake of amino-acid derivatives and provide evidence that both functions are coded for by the TI plasmid genes.

METHODS

Bacterial strains are listed in Table 1. All incubations were carried out at 29 °C. The wild-type strains were checked by phage typing (Van Larebeke *et al.*, 1975).

Media. The minimal medium (SM) contained (g l⁻¹): K₂HPO₄, 10.25; KH₂PO₄, 7.25; (NH₄)₂SO₄, 3.0; MgSO₄·7H₂O, 0.50; NaCl, 0.15; CaCl₂·6H₂O, 0.1; FeSO₄·7H₂O, 0.0025; and glucose, 4.0. When octopine or another compound was used as the sole source of nitrogen, (NH₄)₂SO₄ was omitted (SM-N medium).

Cultures (10 ml) were grown in 100 ml Erlenmeyer flasks in a linear shaker. Turbidity (as *E*₆₆₆) was used as a measure of the number of organisms ml⁻¹; the relation between them was determined by plating serial dilutions of known *E*₆₆₆. The dry weight of 10⁸ organisms of strain B6S3 grown in SM was 30 µg. The protein content of lyophilized bacteria was 52 % when determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Induction. Bacteria were grown overnight in SM containing 0.5 g (NH₄)₂SO₄ l⁻¹, with 0.1 g octopine l⁻¹ or an equal amount of one of the other compounds. Uninduced cultures were grown in SM without further additions.

Determination of octopine uptake. Cultures were grown overnight, diluted with an equal volume of fresh medium and incubated for another 150 min. The bacteria were harvested by centrifuging and resuspended

Table 1. *Relevant properties of bacterial strains*

Strain*	TI plasmid	Virulence	[³ H]Octopine uptake after growth with:			Amino-acid derivatives utilized	Amino-acid derivatives in tumours	Origin
			Octopine	Nopaline	None			
B6s3	+	+	+	—	—	octopine	octopine	J. Schell, Gent
C58	+	+	—	+	—	nopaline	nopaline	E. Nester, Seattle
LBA270	+	+	+	+	+	nopaline and octopine	nopaline	Our laboratory
SI005	—	—	—	—	—	none	—	—
SI005(TI-Kerr14)	+	+	—	+	—	nopaline	nopaline	J. Schell, Gent
SI005(TI-B6s3)	+	+	+	—	—	octopine	ND	Our laboratory
Kerr14	+	+	—	+	—	nopaline	nopaline	A. Kerr, Adelaide, Australia
Kerr14R1†	+	—	—	+	—	none	—	J. Schell, Gent
ACH5	+	+	+	—	—	octopine	octopine	J. Schell, Gent
ACH5-C3	—	—	—	—	—	none	—	J. Schell, Gent
C58-C9	—	—	—	—	—	none	—	J. Schell, Gent
C58-C9(TI-B6s3, RP4)	+	+	+	—	—	octopine	octopine	Our laboratory

ND, Not determined.

* The presence of a virulence-associated plasmid (TI) is shown in parentheses with the origin of the plasmid: for example, SI005(TI-B6s3) denotes strain SI005 with a TI plasmid which was transferred from wild-type strain B6s3. This system is not used for wild-type strains in order to avoid confusion with the existing literature. Whether or not a wild-type strain carries a TI plasmid is shown in this Table.

† Strain Kerr14R1 carries a TI plasmid with a 10 µm deletion affecting AP1 exclusion, virulence, sensitivity to the agrocin of Kerr84 and nopaline utilization (Schell *et al.*, 1977).

in SM-N at 5×10^8 organisms ml^{-1} . To this bacterial suspension [^3H]octopine was added to a final concentration of $8.5 \mu\text{M}$ ($6.75 \mu\text{Ci } \mu\text{mol}^{-1}$). Samples (0.25 ml) were taken at 0, 1, 2, 4 and 6 min, immediately diluted with 5 ml 0.9 % (w/v) NaCl, filtered on a Millipore filter ($0.45 \mu\text{m}$ pore size) and washed with 20 ml 0.9 % NaCl. The filters were dried and counted with 8 ml 0.4 % (w/v) 2,5-diphenyloxazole (PPO; Merck) in toluene. Arginine uptake was determined in the same way, but using 1×10^8 organisms ml^{-1} and [^3H]arginine at $58 \mu\text{M}$ ($35 \mu\text{Ci } \mu\text{mol}^{-1}$).

To determine the quantity of [^3H]octopine taken up by the bacteria, the dried filters with the bacteria were solubilized with 1 ml Soluene (Packard) per filter for 15 min. Then 0.1 ml 30 % (w/v) H_2O_2 was added, followed after 3 h by 13 ml toluene/Triton X-100 (3:1, v/v) containing 0.4 % PPO. After vigorous shaking the samples were counted and the d.p.m. were calculated using the counting efficiency determined with an external standard.

Variation of the pH value of the medium. For determination of the effect of pH on [^3H]octopine uptake, the usual phosphate addition to the incubation medium was omitted and different ratios of KH_2PO_4 (9.08 g l^{-1}) and Na_2HPO_4 (11.88 g l^{-1}) were added (Sørensen's phosphate buffer; Sober, 1970). The total concentration of phosphate was 0.067 M. In other experiments McIlvaine citrate/phosphate buffer (Sober, 1970) was used at one-tenth of its original strength.

Competition with octopine uptake. The usual concentration of [^3H]octopine was added to the incubation mixture and uptake was determined over 6 min in a series of cultures to which increasing amounts of the compound being tested for competition were added. A reference line was determined by adding unlabelled octopine.

Chemicals. [^3H]Octopine was synthesized from L-[β - ^3H]arginine and 2-bromopropionic acid following the procedure of Bomhoff (1974). The product was purified by column chromatography using BioRad anionic exchanger Ag-2-X8 and subsequent preparative paper electrophoresis as previously described (Bomhoff, 1974; Schilperoort & Bomhoff, 1975). The final product was more than 99 % pure.

Homo-octopine was synthesized from L-homoarginine (Koch-Light) and 2-bromopropionic acid (Merck, zur Synthese) following the procedure of Bomhoff (1974). Desmethylhomo-octopine was synthesized by mixing equimolar amounts of L-homoarginine and bromoacetic acid (Merck, zur Synthese) in water in the presence of excess $\text{Ba}(\text{OH})_2$. The reaction at 37°C was allowed to proceed for 72 h. Desmethylhomo-octopine and homoarginine were separated by column chromatography with Dowex anionic exchanger 50-W-X8, 100 to 200 mesh (H. C. M. Kester, unpublished results).

Lysopine and noroctopine were generous gifts from Dr B. Lejeune, Orsay, France and Dr J. Tempé, Versailles, France, respectively. D-Nopaline was a gift from Dr M. van Montagu, Brussels, Belgium. L-Arginine, L-serine, L-lysine and D-alanine were from Merck. L-[β - ^3H]Arginine was obtained from New England Nuclear. D(+)-Octopine and D(+)-octopinic acid were from Sigma. Oxytetracycline was from Mycofarm, Delft, The Netherlands.

Symbols. Uad^- is used to designate strains that are not able to utilize the amino-acid derivatives octopine, lysopine and octopinic acid, but are unaffected in the utilization of the corresponding amino acids (Klapwijk *et al.*, 1976).

RESULTS

General properties of the octopine uptake by strain B6S3

When an exponentially growing culture of *A. tumefaciens* B6S3 was harvested and inoculated into fresh medium containing octopine, there was no lag phase in the disappearance of octopine from the medium if the bacteria had previously been grown in medium containing octopine as a nitrogen source, but there was a lag phase of 30 min if the bacteria had been grown with arginine (Fig. 2). Therefore the enzymes responsible for the conversion of octopine into arginine and pyruvate may need to be induced. Octopine uptake was shown to depend on induction (Fig. 3), and was not observed until 40 min after the addition of octopine (Fig. 4). No induction of uptake was observed when an inhibitor of protein synthesis, such as tetracycline, was added simultaneously (Fig. 4).

The uptake of octopine was dependent on the external concentration of this compound and showed saturation as would be expected for an enzyme-catalysed process. The Michaelis-Menten constant (K_m) was $1.5 \pm 0.2 \mu\text{M}$. A Lineweaver-Burk plot showed a linear relationship between v^{-1} and s^{-1} ; only at low s^{-1} values was there some divergence. The V_{max} of the uptake system was about $10 \text{ nmol mg}^{-1} \text{ min}^{-1}$. This value is higher than that for lysopine transport by the closely related *A. tumefaciens* strain B6 ($1.4 \text{ nmol mg}^{-1} \text{ min}^{-1}$; Jubier, 1975).

Fig. 2

Fig. 3

Fig. 2. Disappearance of octopine from the medium of cultures of strain B6S3. Exponential-phase bacteria were washed and resuspended in SM-N plus octopine (0.1 g l^{-1}). ○, Bacteria grown previously in SM-N plus arginine; ●, bacteria grown previously in SM-N plus octopine. The octopine concentration was measured as described by Bomhoff (1974) using α -naphthol-diacetyl reagent.

Fig. 3. Uptake of $[^3\text{H}]$ octopine by strain B6S3, measured as described in Methods. ○, Uninduced culture; ●, culture induced by growth with octopine (0.1 g l^{-1}).

Fig. 4

Fig. 5

Fig. 4. Induction of octopine uptake activity in strain B6S3. At time 0, octopine (10 mg l^{-1} , final concentration) or octopine and oxytetracycline (5 mg l^{-1} , final concentration) were added to an exponentially growing culture in SM. Samples were withdrawn immediately and at 10 min intervals, and the bacteria were separated by centrifuging (5 min, 4000 g) and resuspended in SM-N. $[^3\text{H}]$ Octopine uptake was determined over 5 min as described in Methods. ○, Octopine added alone; ●, octopine and oxytetracycline added.

Fig. 5. Influence of the pH of the medium on octopine uptake activity. $[^3\text{H}]$ Octopine uptake was determined over 6 min in a series of cultures at different pH. ○, 0.067 M Sørensen phosphate buffer; ●, McIlvaine citrate/phosphate buffer.

Bomhoff (1974) reported that octopine utilization can be inhibited if the medium is not adequately buffered. Glucose-grown bacteria regularly produce acid which lowers the pH of the medium. We found that when the pH of the medium was varied by the addition of different phosphate buffers, there was a sharp decline in uptake activity at pH values below 6.0 (Fig. 5). The fact that in McIlvaine buffer (citrate/phosphate) there was less octopine uptake over the whole pH range may be explained by chelation of Mg^{2+} by citrate; the decrease could be compensated by adding more MgSO_4 .

Table 2. Induction of [^3H]octopine uptake by different substrates

Bacteria were grown in the presence of the test compound (0.1 g l^{-1}), washed and their uptake of [^3H]octopine over 5 min was measured as described in Methods.

Compound added to medium	[^3H]Octopine uptake	
	Strain B6S3	Strain C58
Octopine	+	—
Lysopine	+	—
Octopinic acid	+	—
Nopaline	—	+
Arginine	—	—
Lysine	—	—
Norocoptine	—	—
Desmethylhomo-octopine	—	—
Homo-octopine	—	—
None	—	—

Specificity of induction

Our hypothesis that octopine, lysopine and octopinic acid are degraded by a single system in *A. tumefaciens* B6S3 requires that the uptake system, which is essential for utilization, must be inducible by each of these three compounds. We observed uptake of [^3H]octopine after growth with either octopine, lysopine or octopinic acid, but not after growth with nopaline (Table 2). In strain C58, octopine uptake was only induced by nopaline (Table 2, Fig. 9). The synthetic analogues and amino acids tested had no inducing effect (Table 2). Therefore octopine uptake is only induced when the natural substrates that are found in the tumour are added to the culture – nopaline for a ‘nopaline’ strain, and octopine, lysopine or octopinic acid for an ‘octopine’ strain.

Competition for sites of the uptake system

If there were only one system for octopine, lysopine and octopinic acid utilization in ‘octopine’ strains, the three compounds would be taken up by a single permease and the presence of one of the compounds would influence the uptake rate of another. Because the supply of [^3H]octopine was limited we could not test this hypothesis by performing classical competition experiments. We therefore developed a probe method in which we used a fixed concentration of [^3H]octopine in the uptake medium and added increasing amounts of the competitor, using the effect of unlabelled octopine as a reference. If the addition of a given amount of a competitor had the same influence on the rate of [^3H]octopine uptake as an equimolar amount of unlabelled octopine, it seemed reasonable to assume that the octopine permease had the same affinity for the competitor as for octopine.

The octopine-transporting permease appeared to have the same degree of affinity for lysopine, octopinic acid and octopine (Fig. 6*a*). The presence of L-arginine, L-lysine, L-serine or D-alanine in the incubation medium had little effect on the uptake of octopine (Fig. 6*b*), indicating that the octopine permease has no affinity for these compounds. Conversely, the presence of an excess of unlabelled octopine had no effect on the rate of [^3H]arginine uptake (Fig. 7).

The Uad⁻ mutant LBA102 (Klapwijk *et al.*, 1976) showed no uptake of octopine, whereas arginine uptake was normal. This suggests that the uptake of octopine, lysopine and octopinic acid in ‘octopine’ strains (like B6S3) is carried out by a single permease, which is not that utilized by the bacteria for the transport of arginine or the other amino acids tested.

The nopaline-degrading strain C58 took up [^3H]octopine after induction by growth with nopaline (Table 2) and the uptake was influenced by the presence of nopaline in the medium. However, uptake of octopine by strain B6S3 was not sensitive to the addition of excess

Fig. 6. Competition for sites of the uptake system. Strain B6s3, grown in SM plus octopine (0.1 g l^{-1}), was incubated with a fixed concentration of $[^3\text{H}]$ octopine ($8.5 \mu\text{M}$) and increasing amounts of the compound being tested for competition. Unlabelled octopine was added in one series of experiments to obtain a reference line. (a) ○, lysopine; ●, octopine; □, octopinic acid. (b) ○, L-arginine; ●, L-lysine; □, L-serine; ■, D-alanine.

Fig. 7

Fig. 7. Influence of unlabelled octopine on the uptake of $[^3\text{H}]$ arginine by strain B6s3. The bacteria were incubated with a fixed concentration of $[^3\text{H}]$ arginine and increasing amounts of octopine (○) or arginine (●).

Fig. 8

Fig. 8. Effect of nopaline in the medium on the uptake of $[^3\text{H}]$ octopine. The bacteria were incubated with a fixed concentration of $[^3\text{H}]$ octopine and increasing amounts of nopaline. ○, Strain C58, induced by growth with nopaline; ●, strain B6s3, induced by growth with octopine.

unlabelled nopaline (Fig. 8). It seems that the permease of strain C58 has affinity for both octopine and nopaline, whereas that in B6s3 accepts octopine but not nopaline. Nopaline-degrading strains such as C58 and Kerr14 can only utilize nopaline, but mutants that can also utilize octopine have been isolated (Petit & Tempé, 1975; Hooykaas *et al.*, 1977). Petit & Tempé postulated that these mutants produced the nopaline enzymes constitutively. An octopine-utilizing variant of C58 (strain LBA270) showed a spontaneous uptake of $[^3\text{H}]$ -octopine comparable to that of C58 induced by growth with nopaline (Fig. 9). This uptake was equally sensitive to the addition of unlabelled nopaline.

Fig. 9

Fig. 9. Uptake of [^3H]octopine by strain c58 and its octopine-utilizing variant LBA270. ○, c58 grown with octopine; ●, c58 grown without nopaline or octopine; □, c58 grown with nopaline; ■, LBA270 grown without nopaline or octopine.

Fig. 10

Fig. 10. Effect of nopaline in the medium on the uptake of [^3H]octopine by strains with TI plasmids of different origins. Conditions were comparable to those in Fig. 8. ○, s1005(TI-Kerr14), induced by growth with nopaline; ●, s1005(TI-B6s3) induced by growth with octopine; □, Kerr14, induced by growth with nopaline.

Therefore it seems that the specificity of utilization of amino-acid derivatives by strain c58 is determined by the regulatory process and not by the permease or the oxidase. This is consistent with the observation that LBA270 not only used octopine and nopaline, but also lysopine, octopinic acid and noroctopine.

Evidence that the genes for octopine permease and the accessory regulatory protein are located on the TI plasmids

A previous report (Hooykaas *et al.*, 1977) showed that the ability to utilize octopine could be transferred with the TI plasmid of *A. tumefaciens* strain B6s3 to *Rhizobium trifolii*. This indicates that all the genes essential for octopine degradation are located on the TI plasmid, unless we assume that some *uad* genes were already present in *R. trifolii*. The latter seems unlikely because, to the best of our knowledge, there are no reports of octopine or nopaline utilization in *Rhizobium* strains.

We have shown that octopine uptake can be induced by octopine, lysopine and octopinic acid or by nopaline, depending on the strain under study, and we wanted to confirm that this regulation was under the control of plasmid genes. We therefore studied several isogenic strains carrying TI plasmids of different origins, and found that the response of a strain to induction by either octopine or nopaline correlated with the origin of the plasmid and was independent of the chromosomal background (Table 1). Strain s1005(TI-Kerr14), for example, reacted like Kerr14 to nopaline, while s1005(TI-B6s3) reacted to octopine like B6s3. Thus the regulation of the synthesis of octopine permease must be determined by a gene (*uadR*) on the TI plasmids.

An indication that the gene for octopine permease is also plasmid-borne is the absence of

octopine uptake activity in plasmid-less strains such as C58-C9 and ACH5-C3. The possibility that transcription of a chromosomal permease is activated by a plasmid-borne regulatory gene is unlikely on the following grounds. As already mentioned, we found an important difference between B6s3 and C58 in the effect of nopaline on octopine uptake: uptake of octopine in B6s3 was insensitive to the presence of nopaline, while uptake by C58 was very sensitive to nopaline (Fig. 8). We therefore examined strains to see if the permease of a given strain showed the same degree of sensitivity to nopaline as that of another with the same TI plasmid. We found this was so: strain S1005(TI-Kerr14), which like Kerr14 and C58 had the capacity to utilize nopaline, had a permease that took up octopine and that was sensitive to nopaline, whereas strain S1005(TI-B6s3), like B6s3, had a permease insensitive to nopaline (Fig. 10). These observations suggest that the octopine permease is coded for by a TI plasmid-borne gene (*uadB*) and was transferred with the TI plasmid gene from one strain to the other.

Strain Kerr14 with a deleted TI plasmid

By selection for clones resistant to agrocin K84, Schell *et al.* (1977) isolated several *Agrobacterium* mutants with deletions in the TI plasmid. One of these strains, mutant Kerr14R1, was unable to grow on nopaline as sole source of nitrogen, was avirulent and was no longer able to exclude bacteriophage AP1; it had a deletion of up to 10 μ m in the TI plasmid. It seemed likely that one or more components of the nopaline degradation system were missing in this strain. However, after induction by growth with nopaline, it took up [3 H]-octopine. Thus the regulator gene and the permease gene were still present and were located outside the deleted region, whereas one or more genes responsible for the synthesis of an active nopaline oxidase had disappeared with the deletion.

DISCUSSION

Our results are consistent with the hypothesis that genes located on the TI plasmids in *A. tumefaciens* are responsible for the synthesis and the regulation of a permease with high affinity for both nopaline and octopine or for octopine alone. They support our opinion that all specific *uad* genes are localized on the TI plasmid. This opinion is based not only on TI transfer between agrobacteria (Bomhoff *et al.*, 1976) and into *Rhizobium* (Hooykaas *et al.*, 1977), but also on the fact that with a cointegrate consisting of the TI plasmid and the R factor RP4 it could be proven that *Uad*⁻ mutations are located on the TI plasmid (Klapwijk, unpublished results). The results are also in agreement with our experiments with *Uad*⁻ mutants (Klapwijk *et al.*, 1976) which indicated that octopine, lysopine and octopinic acid share one common degradation system.

We have shown that octopine-utilizing variants of 'nopaline' strains produce the permease constitutively. This confirmed the work of Petit & Tempé (1975) who postulated that these octopine-utilizing strains were constitutive. It seems that whether a compound will be degraded depends on the specificity of the inducing regulatory system. Once the system is induced or constitutively present it may accept related compounds. Thus the mutant of C58 (LBA270) was able to grow equally well on octopine, lysopine, octopinic acid and noroctopine. The low K_m value of the octopine permease coded for by the TI plasmid present in B6s3 means that this is a high affinity permease compared with other amino-acid permeases (Oxender, 1972). We found that the activity of the octopine permease is influenced by the pH of the medium and that it is not detectable in rich media. In addition we never observed degradation of octopine in media with high concentrations of amino acids. These considerations must, therefore, be taken into account when determining whether a strain can utilize octopine, nopaline or related compounds.

Although octopine stimulates both the expression of octopine enzymes and the transfer of TI plasmids (Genetello *et al.*, 1977), the relation between these phenomena remains an open question. That there is a common factor can be deduced from the enhanced TI

transfer frequency in constitutive mutants (Kerr *et al.*, 1977). If the common factor is one repressor for both transfer and *uad* genes, there may be two types of constitutive mutants – one with normal TI transfer and increased levels of octopine enzymes (operator mutants) and another type with high TI transfer and also high levels of octopine enzymes (repressor mutants). Constitutive derivatives of both ‘nopaline’ and ‘octopine’ strains have been isolated. This indicates that there is only one repressor for all *uad* genes and that mutation of the repressor relieves both the permease and the oxidase genes. If these *uad* genes were in an operon and thus closely linked, a very big deletion (as in Kerr14R1) should cause the loss of all *uad* genes. Since this did not occur in Kerr14R1, it is unlikely that the *uad* genes are in one operon.

The method for measuring octopine permease activity described here permits an easy survey of the factors that influence expression of the plasmid-borne octopine genes.

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