GENETIC ASPECTS OF THE INDUCTION AND BIOLOGICAL CONTROL OF CROWN GALL

by

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SUMMARY

Genetic aspects of arginine catabolism in *Agrobacterium radiobacter* were studied. At least one step in the degradation of arginine to supply a carbon source is controlled by the octopine and nopaline Ti plasmids. Ti plasmid linked arginine catabolism is expressed when strains harbouring octopine or nopaline Ti plasmids are induced by the respective opine but not by arginine itself. This character is also expressed by strains harbouring Ti plasmids with regulatory mutations of the octopine or nopaline catabolic system. The ability to catabolise arginine was transferred with the Ti plasmids to recipient strains and provided a useful selective marker for the plasmids when the recipient was unable to catabolise arginine.

A second form of transmissible arginine catabolism was studied in octopine strains NCPPB 1001 and R10. Although transfer was mediated by the octopine Ti plasmid, it frequently occurred without transfer of this plasmid to the transconjugant. An unsuccessful search was made for a hypothetical plasmid controlling arginine catabolism that was mobilised from the donor by the Ti plasmid. Since the techniques used could detect plasmids of molecular weights up to 300×10^6 daltons, the possibility was considered that the Ti plasmid can mobilise chromosomally-linked arginine catabolic genes.

Further evidence is presented that biological control of crown gall by *A. radiobacter* var. *radiobacter* strain 84 is due to production of a bacteriocin, agrocin 84. The biosynthesis of agrocin 84 is

controlled by a $30x10^6$ dalton plasmid, pAt-84a. When this plasmid was transferred from the donor strain 84 to avirulent recipients, most transconjugants inherited the ability to produce agrocin 84 and the ability to act as biological control agents. This plasmid was also transferred to pathogenic strains. These strains produced agrocin 84 and were immune to its action. Such genetic transfers occurring in the field would threaten the effectiveness of this method of biological control. The bacteriocinogenic plasmid also confers immunity on its host to agrocin 84 and to a toxic degradation product of agrocin 84 described by Tate *et al.* (1979). This product lacks the bacteriocin-like specificity of agrocin 84. Immunity to it is a selectable trait of pAt-84a that is expressed even in the absence of the Ti plasmid-linked agrocin sensitivity marker. Further genetic studies of pAt-84a will therefore be facilitated.

The genetic basis of agrocin production in several other strains was investigated. Agrocin production by strains NCPPB 398 and Bo542 is also controlled by plasmids of similar size to pAt-84a.

Agrocin 84 sensitivity (Agr^S) in *Agrobacterium radiobacter* is due to a nopaline Ti plasmid-linked permease (Murphy and Roberts, 1979). A search for a non-toxic substrate for this permease revealed a new group of crown gall specific metabolites called agrocinopines. Two members of this group, agrocinopines A and B, occur in tumours induced by nopaline strains and two others, agrocinopines C and D, in tumours induced by agropine strains. Synthesis and catabolism of these compounds is controlled by Ti plasmid-linked genes.

The agrocinopines are phosphorylated sugar derivatives. Sucrose, phosphorous, arabinose and glucose were identified in hydrolysates of

agrocinopine A and sucrose, phosphorous and glucose were identified in hydrolysates of agrocinopine C. Agrocinopine A can be readily converted to agrocinopine B by loss of glucose and agrocinopine C was converted to agrocinopine D presumably by loss of fructose. Therefore agrocinopines B and D may be artifacts of purification of agrocinopines A and C respectively.

Agrocinopines A and B induce the conjugative transfer of nopaline Ti plasmids and agrocinopines C and D induce transfer of agropine Ti plasmids. Mutant plasmids constitutive for transfer (Tra^C) can be isolated in transconjugants when the donor has not been induced by agrocinopines. A study of several Tra^C mutants of pTi-C58 revealed several interesting properties. Tra^C strains are constitutive for uptake of agrocinopine A. Tra^C mutant plasmids also confer on their hosts supersenstivity to agrocin 84 due to an increased uptake of the bacteriocin. When such a Tra^C plasmid is harboured in a cell that also contains the bacteriocinogenic plasmid pAt-84a, agrocin 84 biosynthesis does not occur. The ability to "turn-off" agrocin 84 biosynthesis is controlled by genes at or near the Agr^S (agrocin 84 sensitivity) region of the Ti plasmid. When a Tra^C plasmid carries an Agr^r mutation, agrocin 84 biosynthesis occurs. Possible mechanisms for this process are discussed.

A study of 79 Agr^r mutants of a Tra^C nopaline Ti plasmid was carried out. Some of these plasmids carried small deletions that could be detected by agarose gel electrophoresis. Three phenotypic classes were conferred by these Agr^r mutant plasmids, namely $Agr^rTra^+Onc^+$, $Agr^rTra^-Onc^-$ and less frequently $Agr^rTra^-Onc^+$. These data confirm

the results of Holsters *et al.* (1980) who showed that Onc and Tra functions map in the Agr^S region of the nopaline Ti plasmid, pTi-C58.