# BIOLOGICAL AND CHEMICAL ASPECTS OF AGROCIN 434 AS A SUPPLEMENTARY BIOCONTROL AGENT FOR CROWN GALL

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# SUMMARY

The involvement of factors other than agrocin 84 in the biological control of crown gall by *Agrobacterium* strains K84 and K1026 has been suggested in a number of studies. This thesis examines the possible role in the biological control process of an antibiotic compound, agrocin 434, produced by the biocontrol strains in addition to the well-characterized adenine nucleotide agrocin 84.

A total of 66 *Agrobacterium* isolates from Australia, Europe and North America were tested for sensitivity to agrocin 434 using standard agrocin bioassay methods. All strains of biovar 2 tested were sensitive to agrocin 434 with the exception of 6 strains which produced agrocin 434 themselves. These included K84 and two agrocin 434-producing derivatives plus three isolates from New South Wales which were subsequently shown to be pathogenic. In contrast, all strains of biovars 1, 3 and *A. rubi* tested were resistant to agrocin 434. Selected isolates representing these three groups were also tested for agrocin 434 production to check if the resistance could be due to the production of this agrocin. None of those strains produced agrocin 434. It appears that sensitivity to agrocin 434 is a general characteristic of biovar 2 agrobacteria and may, at least in part, be chromosomally encoded.

It was also shown that media had an influence on the expression of sensitivity to agrocin 434. Whether this is due to inactivation of the agrocin, or to changes in the test strains brought about by growing on a particular medium remains to be clarified.

The genes involved in the biosynthesis of agrocin 434 are encoded on a large plasmid (300-400 kb) carried by K84 and some of its derivatives (Donner *et al.*, 1993). A derivative of K84 (strain K1143) which carries only this large plasmid, designated

pAgK434, has been isolated (Donner *et al.*, 1993). A buffered glucose-glutamate medium was found to support both the growth of K1143 and agrocin 434 production. Agrocin 434 was produced throughout the growth cycle even though its antibiotic activity is characteristic of a secondary metabolite. Agrocin production did not appear to be regulated by glucose.

Another derivative of K84, strain K1318, carries a deleted version of pAgK434 (the designation pAgK1318 is recommended). This strain produces a biologically inactive fragment of agrocin 434, designated nucleoside 4176, suggesting that part of the agrocin 434 biosynthetic pathway is encoded on pAgK1318 and that genes for this would most probably be located in the vicinity of the deletion. The smaller size of pAgK1318 (80-90 kb) facilitates the localization of genes involved in agrocin 434 synthesis as compared to the much larger pAgK434. On the assumption that the relevant genes are clustered in a manner similar to the agrocin 84 synthesis genes, mapping of pAgK1318 was undertaken. Conditions were optimized for the preparation and purification of plasmid DNA from strain K1318. A protocol for the minipreparation of plasmid DNA by the alkaline lysis method was scaled up and DNA was recovered from low melting point agarose gels using the enzyme agarase. The procedure is simple, fast and very reliable, yielding high quality DNA useful for most recombinant DNA applications.

*Xba*I digests of plasmids pAgK1318 and pAgK434 were compared and the results supported the assumption that a single large deletion event had given rise to plasmid pAgK1318 (McClure NC, unpublished). A novel 10.5 kb *Xba*I fragment of pAgK1318 encompasses the site of deletion and could be expected to contain some of the genes that encode the production of the biologically inactive agrocin 434 fragment. A physical map of the region inclusive of, and flanking, the novel 10.5 kb *Xba*I fragment was constructed with respect to the restriction endonucleases *Bam*HI, *Hin*dIII and *Sma*I

using the techniques of molecular cloning, DNA-DNA hybridization, and multiple and partial endonuclease digestions.

A provisional structure had been proposed for agrocin 434 as a disubstituted cytidine nucleoside (Donner SC, unpublished). The substituent at the $N^4$-position of cytidine was described as a β-1,4-hexuronosyl-glucopyranosyl moiety. The nature of the uronic acid component and its linkage remained to be established. Strong acid hydrolysis of agrocin 434 yielded glucuronic acid and glucose by electrophoretic comparisons with authentic samples. Sodium borohydride reduction of the acid hydrolysate yielded products whose electrophoretic properties in alkaline, as well as in borate and zinc complexing buffers, were identical with the reduction products of glucuronic acid and glucose. NMR studies have confirmed the nature of the linkages. The available evidence is consistent with a β-1,4-glucuronopyranosyl-α-1,$N^4$-glucopyranosyl substituent on the cytidine moiety of the nucleoside.