Chapter 4

Biological Control of Crown Gall

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INTRODUCTION

Crown gall is caused by the soil-borne bacterium *Agrobacterium tumefaciens*. The disease manifests itself as a disorganized and uncontrolled cell division in the host plant, usually on the roots or around the crown (Figure 4.1). The disease is especially important in stone fruits, roses and grape but a wide variety of dicotyledonous plants are susceptible to the disease through natural infection, and an even larger range of plants, including a few monocotyledonous species, can be infected by artificial inoculation after wounding the plant (DeCleene and DeLey, 1976). Losses in production due to the disease in the USA were estimated by Kennedy and Alcorn (1980), who listed *A. tumefaciens* as the third most important procaryotic plant pathogen. There have been numerous reviews on crown gall, and many aspects of the biology of the disease have been covered in the book edited by Kahl and Schell (1982).

The majority of the genes responsible for crown gall induction by pathogenic agrobacteria are plasmid-borne. In the case of crown gall, a part of the tumour-inducing (Ti) plasmid, the T-DNA, is transferred to the plant chromosomal DNA where it is expressed and stably maintained. The expression of T-DNA genes coding for enzymes involved in the metabolism of plant growth substances leads to the production of a tumour on the plant. This subject has been reviewed extensively (Bevan and Chilton, 1982; Nester *et al.*, 1984).

Other T-DNA genes code for enzymes that produce compounds called opines. Opines are low molecular weight metabolites that act as a source of nutrition for the crown gall-incitant bacteria. The genes for use of opines are located on the Ti plasmid. Some opines promote bacterial conjugation and hence the spread of the Ti plasmid to non-pathogenic agrobacteria,

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Figure 4.1 Crown gall caused by *Agrobacterium tumefaciens* on a seedling peach tree.

thereby making these bacteria potential pathogens. Ti plasmids are classified according to the type of opine(s) present in the tumours they induce. Two of the Ti plasmid types are, respectively, nopaline/agrocinopine A (Ellis and Murphy, 1981) and octopine/agropine (Firmin and Fenwick, 1978).

Strains of *Agrobacterium* are classified into three biovars on the basis of carbon source utilization and other biochemical tests (Kerr and Panagopoulos, 1977). The biovars correspond to different chromosomal forms of the bacterium. In Australia, the main pathogenic forms on almond and stone fruit are nopaline/agrocinopine A strains of biovar 2. In Spain, biovar 2 strains are more prevalent on stone fruits and biovar 1 more common on roses (López *et al.*, 1987). The biovar 3 strains that infect grapevine have a worldwide distribution. The host range of biovar 3 is comparatively small and is usually determined by the Ti plasmid (Thomashow *et al.*, 1980).

BIOLOGICAL CONTROL OF CROWN GALL

A successful biological control for crown gall has been developed by Kerr and associates (New and Kerr, 1972; Htay and Kerr, 1974; Kerr and Htay, 1974). The method involves the inoculation of planting stock with the non-pathogenic *Agrobacterium radiobacter* strain K84. Kerr and coworkers have amply demonstrated the usefulness of strain K84, which has been sold commercially in Australia since 1973 and is now used in many countries in different parts of the world.

Crown gall has been controlled by commercially available antibacterial chemicals (Schroth *et al.*, 1971; Moore, 1977), but biological control using strain K84 is in many situations used instead of chemical control because it is both more effective and less expensive. Moore (1977) showed that strain K84 performed better than commercial chemical treatments in preventing crown gall on cherry seedlings. The ability to produce the antibiotic agrocin 84 is an important factor in biological control by strain K84 (Kerr and Tate, 1984).

Strain K84, like most other methods of crown gall control, can only be used as a preventative measure and cannot stop the disease after infection has taken place. Strain K84 does not control crown gall in all situations. In particular, octopine strains of biovar 1 and strains that cause crown gall in grapevine (biovar 3) are resistant to agrocin 84 and therefore not controlled. Agrocin 84-resistant strains of biovar 1 and 2 have on occasions been partially controlled by strain K84, possibly due to its ability to colonize roots effectively (Moore, 1977; López *et al.*, 1987). Searches for new biological agents to control crown gall where K84 is not successful have yielded strains that have been effective on an experimental scale but these have not been scaled up for use in the field. Most research

programmes have concentrated on organisms closely related to the pathogen.

Hendson *et al.* (1983) reported that *A. tumefaciens* strain D286 (biovar 1), which was isolated from *Eucalyptus* in South Africa, produces an antibiotic that inhibits octopine strains. Although it was originally pathogenic, strain D286 has now lost its pathogenicity and is a possible biocontrol agent for all biovars. Webster *et al.* (1986) isolated *A. tumefaciens* strain J73 (biovar 2) from a gall on *Prunus* in South Africa. Strain J73 produces an antibiotic and has now been cured of its Ti plasmid. Preliminary tests using strain J73 showed that it can decrease the severity of crown gall on grapevine. In China, *A. radiobacter* strain HLB-2 (biovar 1) which was isolated from crown gall on hops (*Humulus lupulus*) controlled crown gall (biovar 3) on grapevine shoots (Chen and Xiang, 1986). Strain HLB-2 produces an antibiotic inhibitory to biovar 3 strains. Fungal isolates also have been tested for their ability to control crown gall (Cooksey and Moore, 1980), but have not been developed further, even though some were effective.

Another control method currently under investigation is the use of calcium alginate gel (Deacon *et al.*, 1988). The gel appears to protect the plant surface physically from infection by pathogenic agrobacteria. This method could allow the control of pathogenic strains not inhibited by strain K84, or could be combined with a biocontrol strain.

The biological control of crown gall has been reviewed regularly (Moore and Warren, 1979; Kerr, 1980; Kerr and Tate, 1984; Thomson, 1987; Glenister, 1987). The recent review by Thomson covers many topics not discussed in detail in this paper, which focuses on the biological control of crown gall using strain K84. The discussion centres on (a) the mechanisms involved in disease control and (b) the development of a genetically modified strain K84 that gives equal control of crown gall to the original strain, but which is much less likely to lead to breakdown in control when used commercially.

BIOLOGICAL CONTROL USING STRAIN K84

Agrobacterium radiobacter strain K84 was originally isolated from soil around a crown gall-affected peach tree near Adelaide, South Australia (New and Kerr, 1972). It is non-pathogenic and belongs to the biovar 2 subgroup of the genus.

There are numerous publications on the experimental use of strain K84 to control crown gall. Moore (1979) tabulated accounts of the use of strain K84 against crown gall on different host plants, both experimentally and in nurseries. His list included plant species from many families including Rosaceae, Juglandaceae, Compositae and Salicaceae. The degree of control achieved varied, but strain K84 was very effective in many cases,

often giving 100% control in naturally infested soil. In Australia strain K84 is effective mainly on stone fruits, almond and rose.

The control method involves dipping the planting material (either seeds or seedling roots) in a tap water suspension of strain K84 at 10^7 to 10^8 cells per millilitre immediately before sowing or planting. The widespread commercial use of the method is a testament to its practical nature and usefulness. The success of strain K84 is remarkable, considering the range of conditions in which it has been used: these include many different kinds of crop plants on at least three continents (Australia, Europe and North America).

Mechanisms in the Control of Crown Gall by Strain K84

Production of agrocin 84 is the major but not the only reason for the success of strain K84 (Kerr and Tate, 1984). Survival of the biocontrol bacteria in the soil and colonization of the root surface also play important roles.

Agrocin 84 production and sensitivity

The antibiotic agrocin 84 is specifically toxic to agrobacteria carrying a nopaline/agrocinopine A type Ti plasmid, and strain K84 is normally only effective against such strains (Kerr and Htay, 1974; Roberts and Kerr, 1974). These nopaline/agrocinopine A strains are responsible for most crown gall damage in orchards and nurseries (Kerr and Tate, 1984).

Kerr (1980) summarized the evidence that production of agrocin 84 is important in the control of crown gall. Two key lines of evidence are: (a) only agrocin-sensitive strains are controlled by strain K84 (Kerr and Htay, 1974; Kerr and Panagopoulos, 1977) and (b) the transfer of pAgK84 to other agrobacteria gives them the ability to synthesize agrocin and also to control crown gall (Ellis and Kerr, 1978).

Agrocin 84 is an adenine nucleotide with two substituent groups, and is thought to act as an inhibitor of DNA synthesis (Murphy and Roberts, 1979). It is taken up by nopaline/agrocinopine A strains via the agrocinopine permease (Ellis and Murphy, 1981). Part of the agrocin 84 molecule mimics the structure of agrocinopine A, the usual substrate recognized by the agrocinopine permease. The origin of the name "agrocinopine" lies in the discovery of this interaction (Ellis and Murphy, 1981). Bacterial mutant strains that do not take up agrocinopine are no longer sensitive to agrocin 84 (Hayman and Farrand, 1988).

The agrocin plasmid

Production of agrocin 84 is coded for by a plasmid, designated pAgK84. pAgK84 is 48 kilobases in length, i.e. about one fourth of the size of many Ti plasmids. The plasmid has been characterized by Farrand and co-workers, and regions coding for agrocin production, immunity to agrocin, and plasmid transfer have been located (Slota and Farrand, 1982; Farrand *et al.*, 1985; Ryder *et al.*, 1987). These regions are shown on a physical map of pAgK84 (Figure 4.2).

The agrocin plasmid can be mobilized to other agrobacteria at the same time as pNOC, the nopaline catabolic plasmid of strain K84, (Ellis *et al.*, 1979), but also has its own transfer functions (Farrand *et al.*, 1985). The

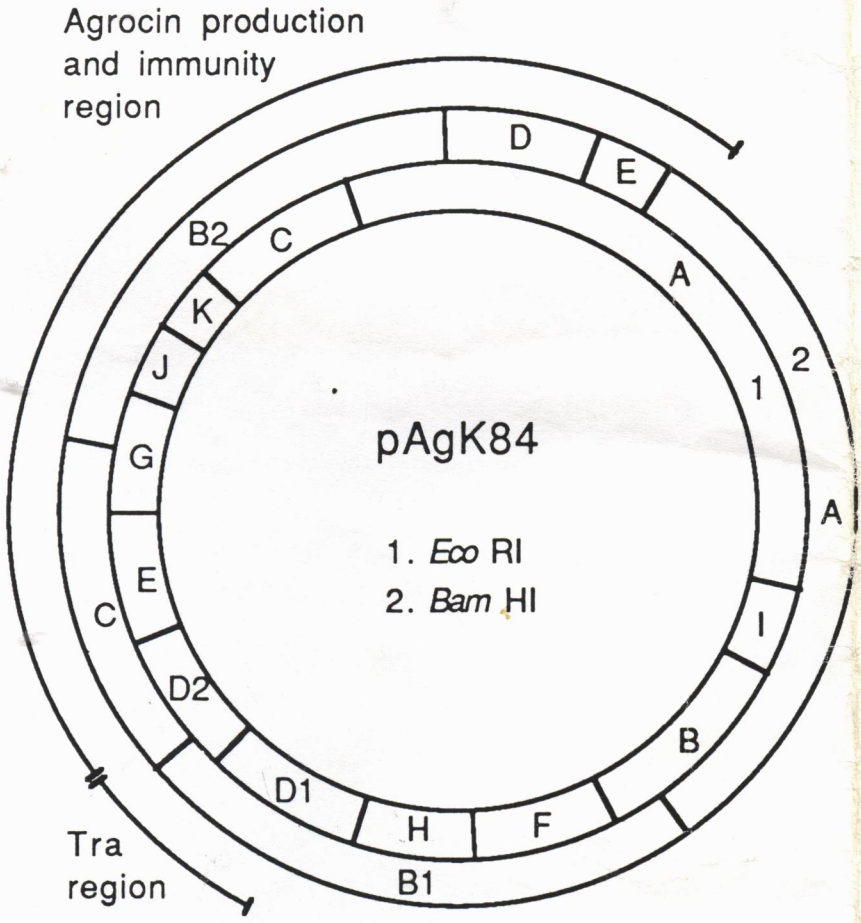

Figure 4.2 *Bam*HI and *Eco*RI restriction map of pAgK84 showing the regions coding for plasmid transfer, agrocin 84 synthesis, and immunity to agrocin.

frequency of transfer of pAgK84 is highest (a) in the presence of pNOC, and (b) in the presence of nopaline which induces the conjugal transfer of pNOC.

The transfer (Tra) region of pAgK84 was mapped by mutagenesis using the transposon Tn*5* (Farrand *et al.*, 1985) and is 3.5 kb long (Figure 4.2). Derivatives of pAgK84 carrying Tn*5* insertions in the Tra region were not transferred at a measurable frequency to other agrobacteria in mating experiments.

Root colonization

It has been shown that the chromosomal background (biovar) in which pAgK84 resides influences the ability of the strain to control disease. Thus, not all strains carrying pAgK84 are effective control agents. When agrocin production was transferred from strain K84 (biovar 2) to strains of biovar 1, the new strains produced agrocin 84 but did not control disease as effectively as K84 (Ellis *et al.*, 1979; Shim *et al.*, 1987). Biovar 2 strains containing pAgK84 were much more efficient than biovar 1 strains in colonizing the roots of almond seedlings (Shim *et al.*, 1987); initially colonization was similar, but 3 or more months after inoculation the populations of biovar 2 strains on the root surface were consistently three to four times higher than those of biovar 1 strains.

Macrae *et al.* (1988) compared the colonization of strain K84 with that of strain J73, also a biovar 2 strain. J73 produces an antibiotic that is toxic to a range of agrobacteria irrespective of Ti plasmid type and has been used experimentally for control of crown gall on grapevine. Strain K84 was superior to strain J73 in its ability to colonize the roots of tomato plants grown in sterile and in non-sterile soil. The reasons for these differences are not known.

There is ample evidence from these studies, that, in addition to any other attributes it may have, an effective biocontrol strain must be able to survive in the soil and rhizosphere of the plant to be protected from crown gall. The ability of the control organism to maintain a sufficient population density for a sufficient length of time is critical to the success of this method of biological control.

POSSIBLE REASONS FOR BREAKDOWN OF BIOLOGICAL CONTROL OF CROWN GALL

Strain K84 normally controls crown gall caused by the nopaline/agrocinopine A type pathogenic agrobacteria. However, biological control could break down and there are several ways that this might happen. The reasons listed here are all related to the production of agrocin 84. Other characteristics important in biological control, such as root colonization, appear to be stable in strain K84.

(a) Panagopoulos *et al.* (1979) reported that in a field experiment where strain K84 and a pathogenic biovar 1 strain were co-inoculated on to almond seedlings in a 1:1 ratio, pathogens resistant to agrocin 84 were subsequently isolated. The pathogenic strains had acquired resistance to agrocin 84 and they also produced agrocin 84. The occurrence of these events was undoubtedly due partly to the high population densities of both the pathogen and of the control agent, which were each introduced artificially. Nevertheless, the report caused concern that such a breakdown could also occur during the normal practice of biological control using strain K84.

This type of breakdown in control was caused by the transfer of the agrocin plasmid (pAgK84) from strain K84 to the pathogenic agrobacteria. Such a transfer, in which the pathogenic bacteria become resistant to agrocin 84 and also produce the antibiotic, was demonstrated *in vitro* by Ellis *et al.* (1979). The frequency of transfer of pAgK84 *in vitro* is markedly increased in the presence of nopaline. The transfer frequency presumably would also be higher in the vicinity of a gall containing nopaline than in the soil or on the root at some distance from a gall.

(b) The second way in which biological control could break down is via the transfer of a Ti plasmid from a pathogenic *Agrobacterium* to strain K84. The control agent would then become pathogenic while retaining agrocin 84 production and immunity. There are no data available on the frequency of Ti plasmid transfer into strain K84. Such an event is probably less common than the frequency of transfer of pAgK84 into a pathogen described in (a) above. The reason for this is that strain K84 also carries a Ti-like pasmid, pNOC, which codes for the catabolism of nopaline but does not confer virulence on strain K84. In most cases where strain K84 is successfully used, pNOC and the Ti plasmid of the pathogenic strains present in the soil are incompatible, i.e. the two plasmids cannot coexist in the same cell. Strain K84 would first (or simultaneously) need to lose pNOC in order to accept an incoming Ti plasmid. Because pNOC is stably maintained in strain K84, the occurrence of this event appears unlikely. It has not been observed experimentally.

(c) Laboratory studies (Süle and Kado, 1980; Cooksey and Moore, 1982) have shown that strains of *Agrobacterium* sensitive to agrocin 84 can mutate at a relatively high frequency to become resistant to the antibiotic. These resistant mutants arise in the zone of inhibition around a colony of K84 on solidified agar medium that has been overlaid with a sensitive strain. Cooksey and Moore (1982) showed that some of the resistant mutants retained their pathogenicity while others appeared to have lost their Ti plasmid. The genetic locus for sensitivity to agrocin 84 is located on the Ti plasmid (Engler *et al.*, 1975; Hayman and Farrand, 1988), and this is presumably where the mutation to resistance occurs, either through loss of part of the plasmid, or through a point mutation.

strains of *Agrobacterium* not controlled by strain K84. At present we do not know whether such resistant mutant strains occur in the soil, and if so, with what frequency they arise. Such agrocin-resistant mutants would be difficult to combat. Mixed inoculation using strain K84 and a strain producing a different agrocin might give some protection against spontaneous agrocin 84-resistant mutants of the pathogen.

Given that a type (c) breakdown in control would be difficult to overcome and that a type (a) breakdown had already been observed in a field experiment, it was reasoned (Ellis and Kerr, 1979; Kerr, 1980) that the best and most practical way to extend the effectiveness of this approach to biological control of crown gall would be to produce a derivative of strain K84 in which the agrocin plasmid could no longer be transferred to pathogenic strains.

CONSTRUCTION OF STRAIN K1026, A TRANSFER-DEFICIENT (Tra$^-$) DELETION MUTANT OF STRAIN K84

Shim *et al.* (1987) demonstrated that two strains of biovar 2, containing pAgK84 which had been made transfer-deficient (Tra$^-$) by insertion of Tn*5*, controlled crown gall just as effectively as did the unmodified strain K84. However the Tn*5*-induced Tra$^-$ derivatives were not considered suitable for the commercial biological control of crown gall for two reasons. Firstly, the insertion of Tn*5* confers on strain K84 the genes for resistance to three antibiotics (Genilloud *et al.*, 1984) that strain K84 does not have, and secondly there is the possibility that Tn*5* can be lost, thereby resulting in the reappearance of the Tra$^+$ parent strain.

It was decided that the best strategy for guarding against the breakdown in biocontrol of crown gall was to use a Tra$^-$ variant of strain K84 containing pAgK84 from which a part of the transfer region had been deleted. The construction of a deletion derivative of pAgK84 was described by Jones *et al.* (1988). Using the restriction endonuclease map (Figure 4.2), it was decided that at least *Eco*RI fragment D1 should be removed, since this would delete 2.8 kb (i.e. 80%) of the 3.9 kb transfer region. To make the deletion, *Bam*HI fragment B1 of pAgK84 (Figure 4.2) was first cloned into a cloning vector, namely plasmid pBR325 of *Escherichia coli*. Both *Eco*RI fragments D1 and H were then removed using recombinant DNA techniques. A total of 5.9 kb of pAgK84 DNA had been deleted. The removal of *Eco*RI fragment H as well as fragment D1 did not affect the replication or stability of pAgK84 in *Agrobacterium*.

After the two *Eco*RI fragments had been deleted, the cloned *Bam*HI B1 fragment was extended at one end by adding *Bam*HI fragment C, which normally adjoins fragment B1 on pAgK84 (Figure 4.2). The deletion

site was now flanked at each end by enough pAgK84 DNA to allow a reasonable frequency of homologous recombination between this plasmid and pAgK84.

The cloned segment of pAgK84 containing the deletion was transferred to pAgK84 by a deletion-marker exchange. Firstly, a mating was performed between the *E. coli* strain with pBR325, which carried the segment of pAgK84 with the deletion, and an *Agrobacterium* strain containing pAgK84 marked with the transposon Tn*5*. The Tn*5* insertion in pAgK84 was just outside the transfer region but still inside the area covered by the deletion. The selection in this mating was for an *Agrobacterium* strain that had both the chloramphenicol resistance of pBR325, and the kanamycin resistance of Tn*5*. Because the *E. coli* plasmid pBR325 does not survive by itself in *Agrobacterium* strains, it could only be recovered as a combination of both plasmids (a cointegrate plasmid) following homologous recombination with pAgK84.

The cointegrate plasmid was then transferred to a derivative of strain K84, which had spontaneously lost pAgK84. It was important to have the Tra^- version of pAgK84 in the same chromosomal background as the original pAgK84 because (a) strain K84 possesses the ability to colonize the rhizosphere and root surface, and (b) when pAgK84 was transferred to other strains of *Agrobacterium*, the resulting strain was usually not as effective as strain K84 in controlling crown gall (Shim *et al.*, 1987).

The cointegrate plasmid was still transfer-proficient (Tra^+), because the Tn*5* insertion in the pAgK84 part of the plasmid was outside the Tra region. It was therefore relatively easy to transfer the cointegrate by conjugation to the derivative of K84 which had lost pAgK84.

The deletion-marker exchange to form the Tra^- derivative of pAgK84 was now completed by growing the strain containing the cointegrate for many generations in a medium lacking antibiotics and then screening for a colony that had lost the kanamycin resistance coded for by Tn*5*. A kanamycin-sensitive colony would arise as a result of a homologous recombination on the side of the deletion opposite that on which the earlier recombination had occurred to form the cointegrate. Cells of this strain would contain pAgK84 lacking *Eco*RI fragments D1 and H. A colony of this type was recovered as a single occurrence amongst approximately 7,000 replica-plated colonies. This spontaneous kanamycin-sensitive derivative was designated K1026.

PROPERTIES OF STRAIN K1026

Strain K1026 was tested in laboratory experiments to ensure that it had the desired properties (Jones *et al.*, 1988). There was no difference between strains K1026 and K84 in their abilities to produce agrocin 84 *in vitro* (Figure 4.3).

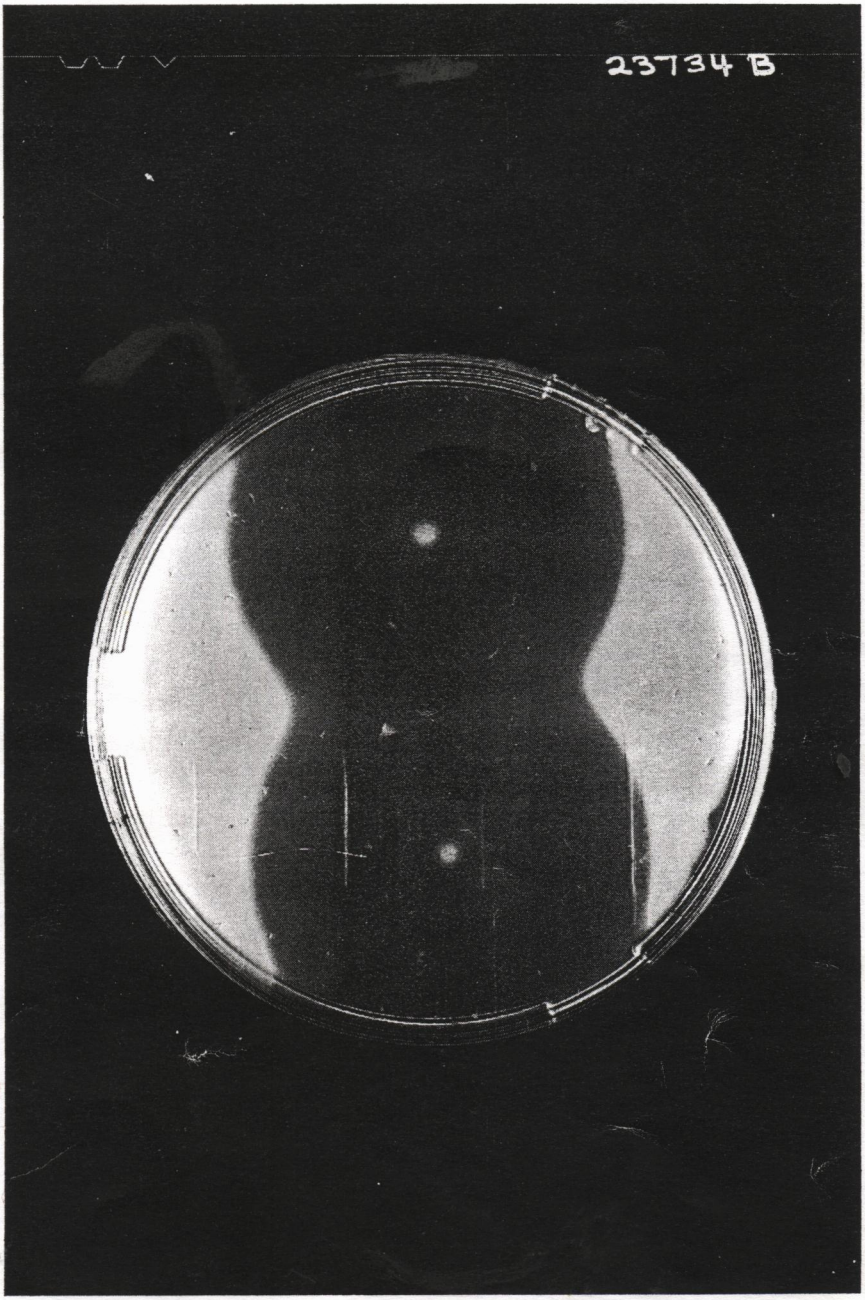

Figure 4.3 Bioassay for the production of agrocin 84 by *Agrobacterium* strains K84 (above) and K1026 (below). Agrocin production is indicated by the zones of inhibition in the growth of the agrocin-sensitive strain.

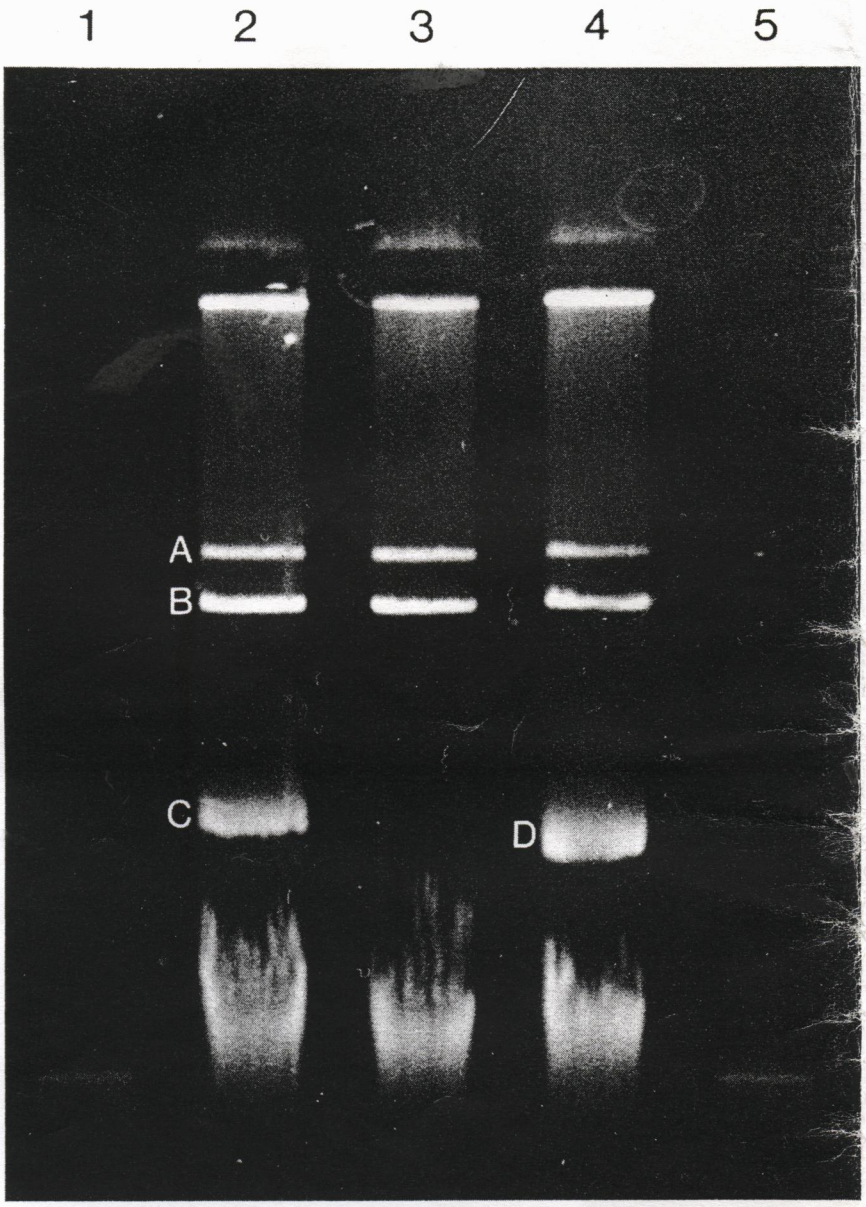

Figure 4.4 Plasmids contained in *Agrobacterium* strain K84 (lane 2), a derivative of strain K84 lacking pAgK84 (lane 3), and strain K1026 (lane 4). Lanes 1 and 5 contain undigested lambda phage DNA. Plasmids were separated by electrophoresis in a 0.7% agarose gel. Band A contains the large cryptic plasmid; band B, the plasmid pNOC; band C, the plasmid pAgK84; band D, the plasmid pAgK1026.

The agrocinogenic plasmids of strain K1026 and strain K84 were compared for size and restriction fragment patterns. Plasmids were isolated from strains K1026, K84 and the K84 derivative lacking pAgK84. The plasmid sizes were compared by electrophoretic separation in an agarose gel (Figure 4.4). The smallest plasmid in strain K1026, the agrocinogenic pAgK1026, is clearly smaller than the corresponding agrocinogenic plasmid (pAgK84) from strain K84 because of the deletion. When the plasmids were digested with the restriction endonuclease *Eco*RI, pAgK1026 did not contain fragments D1 or H. In addition, no DNA fragments from the *E. coli* vector were detected, confirming that no foreign DNA remained in strain K1026. All three strains contain a large cryptic plasmid and the slightly smaller pNOC, which codes for nopaline catabolism.

To determine plasmid stability, strains containing pAgK1026 and pAgK84 marked with resistance to chloramphenicol were grown without antibiotic selection for ten subcultures. There was no loss of pAgK1026 or pAgK84, indicating that both are stable plasmids.

Plasmid transfer frequency was determined from experiments in which strains harbouring antibiotic resistance-marked derivatives of pAgK1026 and pAgK84 were mated with a strain of *Agrobacterium* biovar 1. There were no transconjugants in the cross with the pAgK1026 derivative, whereas many transconjugants were observed from the mating with the pAgK84 derivative (Figure 4.5). Clearly pAgK1026 is a Tra^- mutant of pAgK84.

CONTROL OF CROWN GALL BY STRAIN K1026

After appropriate permission had been granted by Australian authorities, strain K1026 was tested for its ability to control crown gall on almond seedlings in an open air pot experiment (Jones and Kerr, 1989). Younger (2 month) and older (10 month) seedlings were dipped in a suspension of either strain K1026 or strain K84 and planted in soil infested with a pathogenic *Agrobacterium* biovar 2, and damage due to disease was assessed after 7 months. There was no significant difference in the incidence of galls between plants treated with strains K1026 or K84 (Table 4.1). Strain K1026 is as effective as strain K84 in controlling crown gall.

REGISTRATION AND COMMERCIAL USE OF STRAIN K1026

Because strain K1026 is as effective as strain K84 in limiting damage due to crown gall, and because its agrocin plasmid cannot be transferred to other agrobacteria, it should be used in preference to strain K84. In order

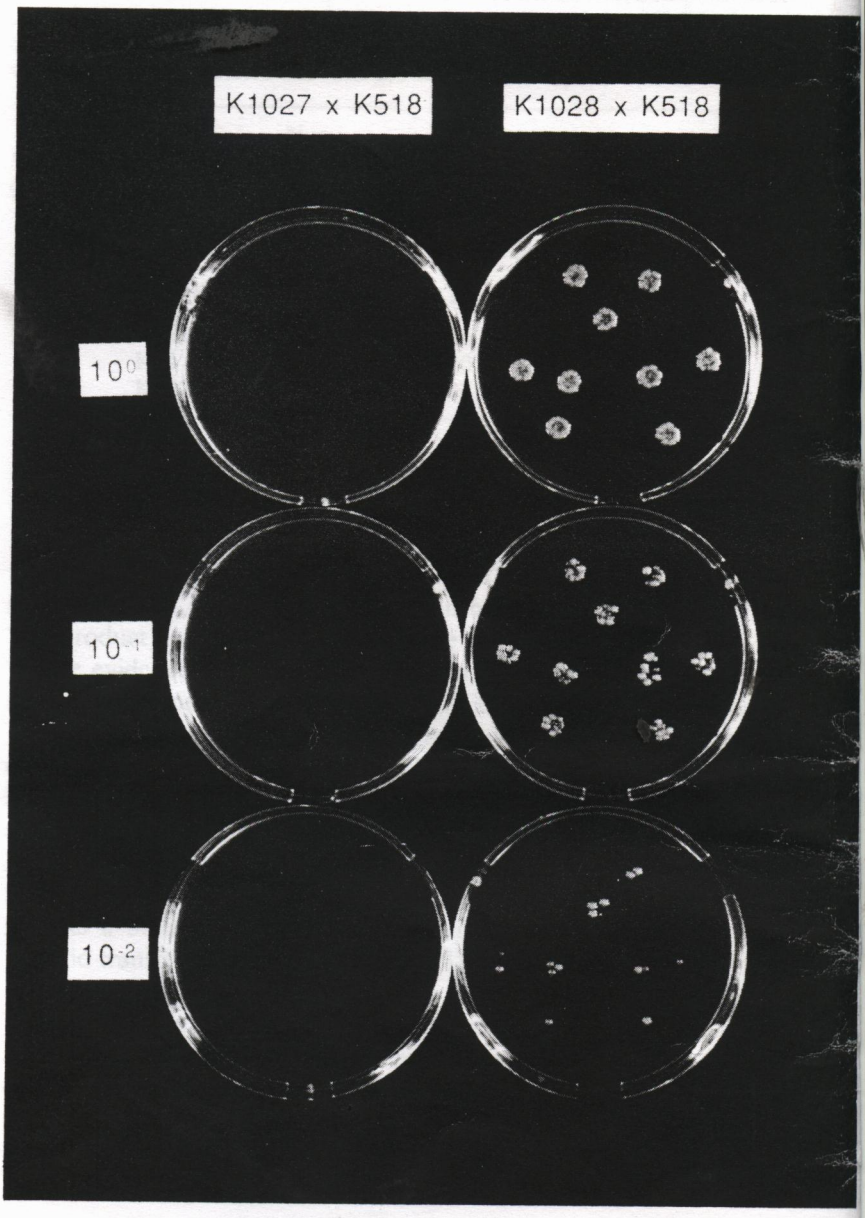

Figure 4.5 The results of matings between strains containing plasmid pAgK84 and its engineered derivative plasmid pAgK1026, showing the absence of transfer ability in the engineered plasmid. Strain K1027, containing pAgK1026, and strain K1028, containing pAgK84, were mated with strain K518 and 10-microlitre droplets of a tenfold dilution series of the mating mixture were spotted onto a medium selecting for transconjugants.

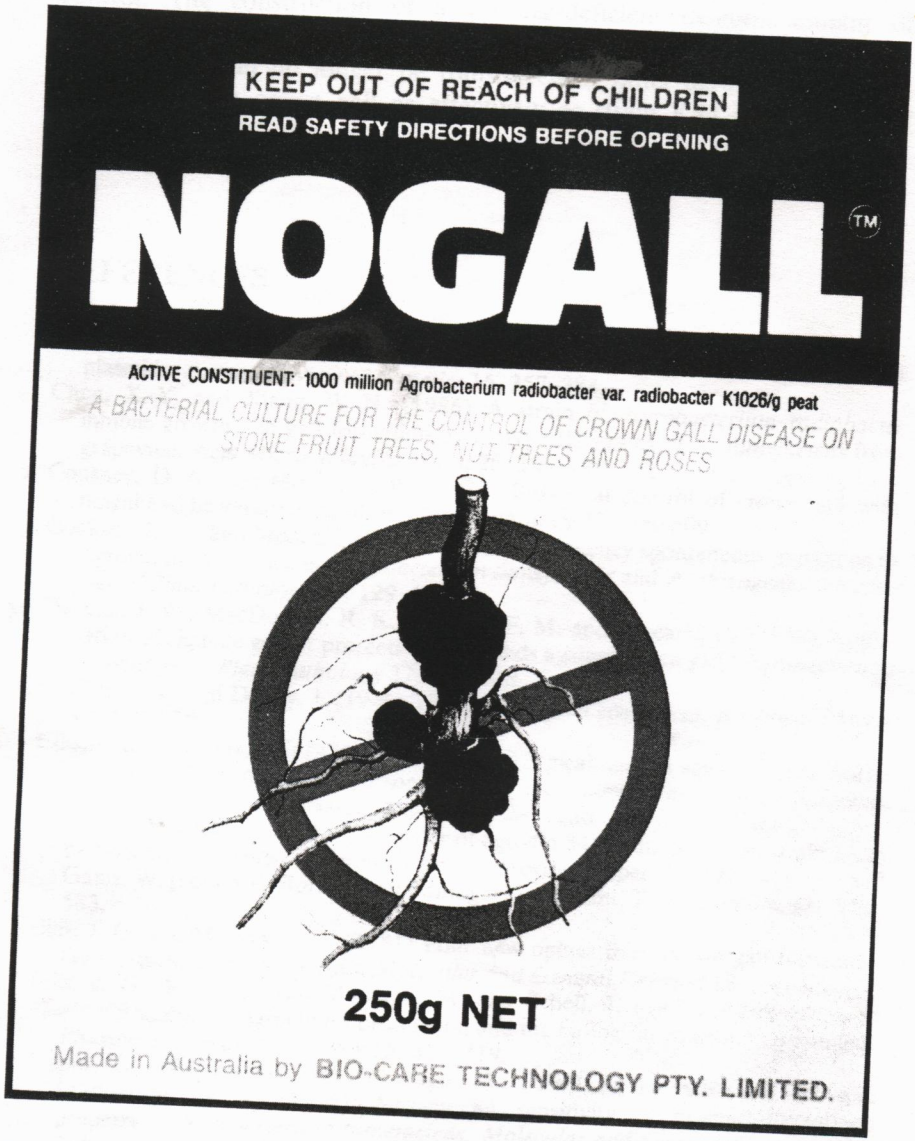

Figure 4.6 Label for Nogall™, a peat-based formulation of strain K1026 produced by Bio-Care Technology Pty. Ltd.

Table 4.1 The effect of treating almond seedlings with water, a suspension of *Agrobacterium radiobacter* strain K84 or a suspension of *A. radiobacter* strain K1026 on crown gall induced by *A. tumefaciens* strain K27. Fifteen plants per treatment were inoculated and then grown outdoors in pots for seven months.

Plant age (months)	Treatment	Number of plants surviving	% of plants with galls	No. of galls/plant		
				Mean	Median	Range
2	water	12	100	9.33	7.5	3–23
	K84	14	14	0.21	0	0–2
	K1026	12	25	0.33	0	0–2
10	water	15	100	46.33	41	13–103
	K84	15	20	0.20	0	0–1
	K1026	15	27	0.67	0	0–5

to make strain K1026 commercially available, it has been registered as a pesticide. The strain has now been registered and is being sold in the Australian state of New South Wales and can be sold to growers in any state. It has been available since late 1988. The strain is sold as Nogall™ (Figure 4.6), a peat-based formulation containing 10^9 bacteria per gram. The product has an advertised shelf life of over 6 months.

The evidence that strain K1026 is harmless to humans, animals, plants and the environment was listed by Jones and Kerr (1989). As this bacterial strain is the first genetically engineered microbe to become available as a commercial product, it will be worthwhile to repeat this information here. Strain K1026 is regarded as safe because: (1) strain K84, the progenitor of strain K1026, has been registered as a pesticide and used commercially in many countries, including Australia and the USA, for over 15 years with no reports of harm; (2) strain K1026 is identical to strain K84 except that it lacks a 5.9 kb portion of the agrocin-84 plasmid, thereby preventing plasmid transfer (Jones *et al.*, 1988); (3) no foreign DNA remains in strain K1026; (4) strain K1026 contains no Ti-plasmid-encoded genes involved in crown gall induction (B. G. Clare, unpublished data); (5) strain K1026 is a biovar 2 strain of *Agrobacterium* and cannot grow at 37°C (human body temperature; Kerr and Panagopoulos, 1977); (6) agrocin 84 is specifically toxic to agrocinopine-catabolizing agrobacteria, most of which are crown gall pathogens (Engler *et al.*, 1975).

CONCLUSIONS

Crown gall has been successfully controlled on a commercial scale for over 15 years by the use of *A. radiobacter* strain K84. The continued success of strain K84 has been jeopardized by the possibility of transfer of the agrocin plasmid, pAgK84, to pathogenic agrobacteria, making them resistant to

control. The construction of a transfer-deficient deletion mutant of pAgK84 has resulted in a strain (K1026) from which the agrocin plasmid (pAgK1026) can no longer be transferred to other agrobacteria. Strain K1026 controls crown gall as effectively as strain K84 and the use of K1026 in place of strain K84 should ensure that the danger of breakdown of biological control is minimized.

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