SIGNIFICANCE OF THE PRESENCE OF STREPTOMYCIN RESISTANT PLANT PATHOGENIC AND EPIPHYTIC BACTERIA IN NEW ZEALAND

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SUMMARY

Streptomycin resistant strains of epiphytic bacteria (*Erwinia herbicola* and *Pseudomonas fluorescens*) and plant pathogenic bacteria (*Erwinia amylovora*) have been isolated from apple orchards in New Zealand since 1991. The risk that streptomycin resistance becomes widespread and is transmitted to other bacterial species, including other pathogens, is dependent on the genetic basis of the resistance. In this study, we analysed the genetic determinants of streptomycin resistance in strains of *E. amylovora* and *Pseudomonas*. Results suggest that resistance in *E. amylovora* was most probably due to chromosomal mutation, while strains of *Pseudomonas* might carry genes that degrade the antibiotic.

Keywords: streptomycin resistance, genetics, *Erwinia amylovora*, *Pseudomonas syringae*, epiphytic bacteria

INTRODUCTION

Fire blight of apple and pear is an economically important disease which is effectively controlled by spraying streptomycin during flowering. However, the use of streptomycin carries the risk of selecting strains of the pathogenic bacterium resistant to this antibiotic. Streptomycin resistant strains of several plant pathogenic bacteria have already been isolated (Minsavage *et al.* 1990; Norelli *et al.* 1991; Sundin and Bender 1993) including, since 1972 in the USA, strains of *Erwinia amylovora*, the causal agent of fire blight (Miller and Schroth 1972). In New Zealand streptomycin resistant strains were isolated for the first time during the 1991-1992 season (Thomson *et al.* in press).

The possibility that streptomycin resistance may become widespread in New Zealand and be transmitted to other bacterial species, including animal and human pathogens, depends essentially on the genetic determinants of the resistance. Two mechanisms for streptomycin resistance have been described in plant pathogenic bacteria: i) chromosomal mutation which alter the binding affinity of ribosomal proteins for streptomycin ii) enzymatic inactivation of the antibiotic, which is often plasmid encoded. Chromosomal mutations are transmitted, almost exclusively, by cell division, thus they are not transmitted to bacteria from other species and their geographical spread is usually relatively slow. In contrast, transposons are able to move from one bacterium to another even if they belong to a different species or different genus. Thus streptomycin resistance carried by a transposon may spread rapidly and be transmitted to different bacterial species.

To assess the risk associated with the presence of streptomycin resistance in New Zealand, we analysed the genetic basis of this resistance in plant pathogenic and epiphytic strains isolated in New Zealand. This paper reports the results obtained with strains of *E. amylovora* and *Pseudomonas* isolated during the 1992 - 1993 season.

MATERIAL AND METHODS

We analysed the 18 strains of *E. amylovora* resistant to streptomycin that were isolated in New Zealand (Hawkes Bay area) during the 1992 - 1993 season. Other strains of *E. amylovora* were also used: Ea273 a wild type streptomycin sensitive

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strain, and CA11 (Chiou and Jones 1991) a streptomycin resistant wild type strain, which harbours a 34 Kb conjugative plasmid (pEA34) coding for streptomycin resistance. Both strains were used in all the experiments as a positive and as a negative control respectively. Among the plant pathogenic (eg. *Pseudomonas syringae*) or epiphytic (eg. *Pseudomonas fluorescens*) streptomycin resistant strains of *Pseudomonas* isolated last season in New Zealand, 17 strains were analysed. The strain of *Pseudomonas syringae* pv. *papulans* Psp36 (Norelli *et al.* 1991) was also used as a positive control in DNA/DNA hybridization experiments.

Matings between strains of *E. amylovora* or *Pseudomonas*, and *Escherichia coli* DH5a used as a recipient, were carried out at 28°C. Exponential cultures of both donor and recipient strains were washed with MgSO₄ 10 mM, mixed and spread on a fresh plate of complete medium. After 18 to 36 h of incubation, cells were resuspended in MgSO₄ 10 mM and spread on medium with streptomycin to counter select the recipient cells and incubated at 37 °C to counter select the donor.

General DNA manipulations, such as plasmid preparation, genomic DNA isolation, DNA/DNA hybridizations, were as described earlier (Vanneste *et al.* 1992). The probe used for the hybridisation was SMP3, a 500 bp *Ava*I-*Bam*HI DNA fragment that corresponds to DNA sequences isolated from a transposon which confer streptomycin resistance to different plant pathogenic bacteria including *P. syringae* and *E. amylovora* (Chiou and Jones 1991; Norelli *et al.* 1991; Sundin and Bender 1993). This probe, isolated from the plasmid pCPP505 (Norelli *et al.* 1991), has been used in different studies to detect bacteria that acquired a plasmid coding for streptomycin resistance (Chiou and Jones 1991; Norelli *et al.* 1991).

RESULTS

Strains of *E. amylovora*

No transfer of streptomycin resistance between any of the strains of *E. amylovora* isolated from New Zealand and *E. coli* could be detected even after 36 h matings. Under the same conditions, streptomycin resistance was readily transferred to *E. coli* from CA11.

The only plasmid that could be detected in the streptomycin resistant strains isolated from New Zealand was the pEA28, a plasmid of ca. 30 Kb common to all strains of *E. amylovora* (Laurent *et al.* 1989).

No hybridization between the total DNA of the *E. amylovora* strains from New Zealand and the probe SMP3 could be detected; while the total DNA of CA11 showed strong hybridization.

Strains of *Pseudomonas*

The plasmid content of 17 strains has been analysed by agarose gel electrophoresis. All but one strain carried at least one plasmid and some strains carried up to six plasmids. However, no plasmid was common to all the strains. Five strains chosen because they carry a total of 21 different plasmids representative of the plasmids found in the 17 strains were conjugated with *E. coli* DH5a. No streptomycin resistant transconjugants were detected, after 18 h matings.

Of the 15 strains analysed by DNA/DNA hybridization, 12 showed homology with the probe SMP3, suggesting that genes coding for streptomycin resistance could be present in these strains.

DISCUSSION

To assess the risk that streptomycin resistance could spread throughout New Zealand and could be transferred to numerous bacterial species, we analysed the genetic basis of streptomycin resistance in strains isolated from New Zealand. Our effort has been focused on *E. amylovora*, the fire blight pathogen, and different *Pseudomonas* strains isolated from diseased plants during the 1992 -1993 season.

No transfer of streptomycin resistance was detected between *E. amylovora* and *E. coli*, suggesting that either streptomycin resistance is carried by a non conjugative plasmid, or that resistance is not plasmid borne. This second hypothesis is in agreement

with the fact that the only plasmid detected is the common plasmid of 28 Kb. To check that streptomycin resistance was not due to a transposon inserted in the chromosome, we looked for possible homology between the total DNA of these *E. amylovora* strains and the probe SMP3. Since no hybridization was detected, streptomycin resistance is, for these strains, most probably due to a chromosomal mutation that affects the binding of streptomycin to the ribosome.

Results with *Pseudomonas* are ambiguous. Total DNA of 12 strains out of the 15 analysed showed homology with the probe SMP3, suggesting that these strains might harbour genes whose product inactivate streptomycin. Such genes are usually on transposons or on plasmids (Minsavage *et al.* 1990; Chiou and Jones 1991; Norelli *et al.* 1991; Sundin and Bender 1993); however, no plasmid common to these strains of *Pseudomonas* was found. Furthermore, while four of the five strains used for the matings which total DNA hybridize with the probe, transfer of streptomycin resistance to *E. coli* has not been detected. These strains could carry genes for streptomycin resistance on non conjugative plasmids, or on the chromosome. Alternatively, since SMP3 also contains DNA sequences that are not related to streptomycin resistance (Chiou and Jones 1993), the hybridization that has been detected might not be linked to antibiotic resistance. Experiments to lift this ambiguity are in progress.

Results reported in this paper are similar to those obtained with strains isolated from New Zealand during the 1991-1992 season. *E. amylovora* acquired resistance to streptomycin most probably by mutation of a chromosomal gene (Thomson *et al.* in press), while epiphytic bacteria might harbour genes whose product inactivate the antibiotic (Vanneste and Yu unpublished results).

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