Genetic analysis of *Klebsiella pneumoniae* alginate lyase by transposon Tn10 mutagenesis

P. Gacesa1, G. S. Leaves2 & A. J. Weightman2

1Biochemistry Department, University College, P.O. Box 78, Cardiff CF1 1XL, Wales, UK
2Department of Applied Biology, University of Wales Institute of Science and Technology, P.O. Box 13, Cardiff CF1 3XF, Wales, UK

Key words: *Klebsiella pneumoniae*, alginate lyase, genetic analysis, transposon mutagenesis, plasmids

Introduction

Alginites are degraded by a group of enzymes that catalyze the β-elimination of the 4-O-linked glycosidic bond with formation of oligosaccharides containing unsaturated uronic acids (Boyd & Turvey, 1977). Alginate lyases have been reported to occur in a wide range of organisms including brown algae (Madgwick et al., 1978), molluscs (Favorov et al., 1979), marine fungi (Wainwright & Sherbrock-Cox, 1981) and many bacterial genera (see for example Boyd & Turvey, 1977; Dunne & Buckmire, 1985). The alginate lyase from *Klebsiella pneumoniae* strain PGI has an absolute specificity for L-guluronate at the non-reducing side of the bond that is cleaved (Boyd & Turvey, 1977), and this enzyme catalyzes the first reaction of the pathway for alginate catabolism. Extrachromosomal plasmid DNA is known to encode proteins and enzymes associated with many ancilliary reactions in microbial cells, and the involvement of catabolic plasmids in the utilization of a wide range of hydrocarbons is well documented (see Gunsalus, 1985). Plasmids, because of their ability to replicate independently of the chromosome and to transfer from one species to another, are of considerable importance in the generation of genotypic and phenotypic variation in microorganisms (Hardy, 1986).

The aim of this study was to determine whether genes associated with alginate catabolism in *K. pneumoniae* strain PGI are carried by extrachromosomal DNA. Specifically, experiments were designed to determine the location of gene(s) encoding alginate lyase. Transposon Tn10, a mobile genetic element encoding tetracycline resistance (Kleckner, 1981), was used to generate mutations by random insertion of the transposon into the genome of strain PGI. The procedure, called transposon mutagenesis (Foster, 1985), gives rise to selectable, non-leaky mutations which can be mapped, according to the location of the transposon, using restriction endonucleases.

Materials and methods

**Bacterial strains, plasmids and media**

*Escherichia coli* strains LE392 and SK1590 (Maniatis et al., 1982) and *Klebsiella pneumoniae* (*aerogenes*) strain PGI were used as hosts for different plasmids, and were maintained on antibiotic medium number 3 (AM3, Oxoid) agar at 37 ºC unless otherwise stated. Plasmid R64дрd-11 (Meynell & Datta, 1967) was used to mobilize the transposon donor plasmid, pHSG415::Tn10, a derivative of pHSG415 (Hashimoto-Gotoh et al., 1981) constructed by Dr Matthew Binns. In outline, the transposon donor comprised a Tn10-containing PstI fragment from the colicin plasmid derivative ColIb::Tn10, ligated into the PstI site of pHSG415. Plasmid pPG1 is described in this paper. Plasmid-
isolation procedures included small- and large-scale methods (Holmes & Quigley, 1981; Kado & Liu, 1981; Maniatis et al., 1982). Antibiotics (Sigma Chemical Co.) were added to media, where required, at the following concentrations: ampicillin, 50 μg·mL⁻¹; chloramphenicol, 40 μg·mL⁻¹; kanamycin, 50 μg·mL⁻¹; streptomycin, 100 μg·mL⁻¹; tetracycline, 12.5 μg·mL⁻¹. L-broth and glucose-M9 salts media were used for growing liquid cultures (Maniatis et al., 1982).

Detection of alginate lyase activity in bacterial colonies

A plate assay was used to identify whether mutants of K. pneumoniae strain PG1 were producing alginate lyase. Colonies to be tested were inoculated onto alginate-agarose plates (tryptone, 10 g·L⁻¹; yeast extract, 5 g·L⁻¹; NaCl, 5 g·L⁻¹; alginate (Manucol, DMB), 1 g·L⁻¹; agarose 10 g·L⁻¹) and incubated overnight at 37°C. Clearing zones in the alginate around colonies, indicative of lyase activity, were visualized with 10% (w/v) cetylpyridinium chloride (Sigma).

Transposon Tn10 mutagenesis

An outline of the protocol used for transposon Tn10 mutagenesis of strain PG1 is given in Fig. 1. Strain PG1 could not be transformed directly with plasmid pHSG415::Tn10 (isolated from E. coli), presumably because of an efficient restriction barrier in this strain. The transposon Tn10 donor was, therefore, mobilized into strain PG1 from E. coli LE392, with plasmid R64drd-11. Plasmid pHSG415::Tn10 was then reisolated from strain PG1(pHSG415::Tn10, R64drd-11) and was used to transform strain PG1 according to the rapid method described by Hanahan (1985). Use of the method of Holmes & Quigley (1981) for plasmid isolation and transformation of PG1 with this preparation effectively separated transposon donor pHSG415::Tn10 from residual R64drd-11 and its interfering tetracycline resistance marker. Transformants were selected at 30°C on plates containing tetracycline and chloramphenicol, and were checked for sensitivity to streptomycin, a marker for R64drd-11. The temperature-sensitivity of pHSG415::Tn10 was checked in strain PG1 and the kinetics of segregation of the plasmid in strain PG1.

Fig. 1. Transposon Tn10 mutagenesis of K. pneumoniae strain PG1 using the temperature-sensitive plasmid pHSG415::Tn10. The transposon is represented by a closed square on pHSG415::Tn10, and transposition to both chromosomal and plasmid DNA is indicated.