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SHORT PAPERS

Evidence for transductional shortening of the plasmid obtained by recombination between the TOL catabolic plasmid and the R91 R plasmid

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SUMMARY

The previously isolated plasmid pND3, arising from recombination between the TOL catabolic plasmid and the R plasmid R91, was transduced by pf16 in *Pseudomonas putida*. Apparent transductional shortening was evident in 25 % of the transduced pND3 plasmids. Transductants were isolated which had segregated the antibiotic resistance marker, transfer ability and some of the catabolic functions of the parent plasmid.

1. INTRODUCTION

We are conducting a study aimed at increasing the transfer range of the TOL catabolic plasmid. One method being employed is to attempt *in vivo* fusion with several R plasmids. Ideally it is desirable to obtain a recombinant plasmid which carries the catabolic information of the TOL plasmid, the transfer information of the R plasmid and which has had resistance to the antibiotics deleted.

We recently reported the isolation of a plasmid (pND3) which arose from apparent recombination between the TOL catabolic plasmid and the R91 R plasmid (White & Dunn, 1977). This recombinant plasmid encodes for the degradation of *m*-toluate, *p*-toluate, *m*-xylene, *p*-xylene and toluene, as does the parent catabolic plasmid, and encodes carbenicillin resistance from the R plasmid. Furthermore, pND3 is incompatible with the NAH catabolic plasmid, is able to mediate its own transfer and promote transfer of the bacterial host chromosome and carries at least the plasmid transfer system of R91. pf16 mediated transductants could be readily obtained in which all encoded properties could be cotransduced. However a significant proportion had lost at least one phenotypic property, probably as a result of transductional shortening.

This paper describes the transductional shortening data.

2. MATERIALS AND METHODS

Bacterial strains: PP1-2, wild type strain of *P. putida* derived from ATCC 17453 (Wong & Dunn, 1974); PP1-8, methionine requiring mutant of PP1-2 (Wong & Dunn, 1974) and PP2-4 (NAH), leucine requiring mutant of the *P. putida* strain ATCC 17485 or PpG379 as referred to by Dunn & Gunsalus (1973).

Bacteriophage: pf16, virulent transducing phage of *P. putida* specific for PP1-2 and mutant derivatives (Gunsalus *et al.* 1968).

Plasmids: TOL, catabolic plasmid which encodes degradation of the toluates

(Williams & Murray, 1974; Wong & Dunn, 1974) and toluene and xylene (Worsey & Williams, 1975); NAH, catabolic plasmid which encodes the degradation of naphthalene and salicylate (Dunn & Gunsalus, 1973); R91, R plasmid which encodes resistance to carbenicillin (Chandler & Krishnapillai, 1974*a*) and pND3, recombinant between TOL and R91 (White & Dunn, 1977). The usage of the M plasmid designation in a previous publication (Wong & Dunn, 1976) has been discontinued in view of the recent plasmid nomenclature recommendations (Novick *et al.* 1976).

The basal salts medium used in all carbon source tests was PAS (Chakrabarty, 1972). Transduction with pf16 was carried out using a modification of the technique of Gunsalus *et al.* (1968) in that the transducing preparation was irradiated for only 60 seconds. Plasmid transfer from the transductants was tested using PP1-2 as recipient in a standard plate mating experiment using auxotrophic contraselection (White & Dunn, 1977).

To test growth on the aromatic compounds, *m*-toluate and *p*-toluate were incorporated into the growth medium at 5 mM and *m*-xylene and *p*-xylene were used in vapour phase in a basal solid PAS medium supplemented with methionine. Resistance to carbenicillin was tested on nutrient agar plates supplemented with 1000 μg/ml carbenicillin.

3. RESULTS AND DISCUSSION

A transducing preparation of pf16.PP1-2(pND3) was used for the pf16 mediated transduction of pND3. Using PP1-8 as the recipient and selecting for the ability to utilize *m*-toluate (PAS + methionine + 5 mM *m*-toluate), transductants were obtained at a frequency of 10^{-9} per phage particle. Following purification by single colony isolation, it was found that all clones required methionine and a number of the transductants had lost plasmid encoded properties. Phenotypic properties of the transductants are listed in Table 1.

Table 1. *Phenotypic properties of pf16 mediated transductants of pND3*

Group no.	No. of transductants	*m*-Tol	*p*-Tol	*m*-Xyl	*p*-Xyl	CB	Tra
1	41	+	+	+	+	R	+
2	14	+	+	+	+	S	−
3	12	+	−(L)	+	+	S	−
4	1	+	+	−	−	S	−
5	1	+	−(L)	−	−	S	−

Abbreviations: CB, carbenicillin; *m*-Tol, *m*-toluate; *p*-Tol, *p*-toluate; *m*-Xyl, *m*-xylene; *p*-Xyl, *p*-xylene; R, resistant to carbenicillin; S, sensitive to carbenicillin; Tra, plasmid transfer by conjugation; +, normal growth response or plasmid transfer; −, no growth or no plasmid transfer; −(L), no growth but large colonies appear at a low frequency.

Transductants in group 1 retained all characteristics known to be associated with the parent plasmid pND3. Group 2 transductants were unable to transfer and had lost resistance to carbenicillin. Group 3 transductants were similar to those of group 2 in that they had lost resistance to carbenicillin and the ability to transfer. Furthermore it was thought that these transductants had lost the ability to grow on *p*-toluate, however it was noted that colonies appeared at a frequency of approximately 10^{-7} per cell plated. These colonies could be seen growing up after 4 days incubation compared to the 2 days normally required for growth of strains harbouring pND3. This suggests that the genetic information encoding the enzymes responsible for the utilization of *p*-toluate

was always present, but that they had become phenotypically suppressed as a result of the transduction. Experimental work is currently under way to characterize this phenomenon. The other unusual property of transductants from groups 4 and 5 was that they had lost the ability to utilize *m*- and *p*-xylene.

We have previously reported that the NAH catabolic plasmid is incompatible with pND3 (White & Dunn, 1977). Two transductants, where possible, were chosen from each group listed in Table 1 and the NAH plasmid was transferred by conjugation into these strains using PP2-4(NAH) as the donor strain and selecting for growth on PAS + methionine + naphthalene. In all crosses transconjugants were purified by streaking twice for single colonies on the selection medium. When back tested at least 90% of each group of transconjugants had lost all phenotypic properties derived from pND3. This incompatibility suggests that the phenotypic properties listed in Table 1 are still plasmid associated.

The phenomenon of tranductional shortening has been reported in other experimental systems (Watanabe, 1963; Novick, 1969; Shipley & Olsen, 1975; Falkow, 1975). It is possible that this technique could be used specifically to delete the resistance determinants, yet retain the transfer system of pND3. Such a deletion has not yet been achieved; however preliminary deletion mapping data of pND3 suggests that resistance to carbenicillin and transfer information are very closely linked.

Another aspect of transductional shortening which may prove very useful is marker rescue by other plasmids (Novick, 1967; Fredericq, 1969; Shipley & Olsen, 1975; Stanisich, Bennett & Ortiz, 1976). In our system, marker rescue may permit recombination of the catabolic information with the R plasmids RP1 and R68, which have a much wider transfer range (Olsen & Shipley, 1973; Chandler & Krishnapillai, 1974*b*).

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REFERENCES

Chakrabarty, A. M. (1972). Genetic basis of the biodegradation of salicylate in *Pseudomonas*. *Journal of Bacteriology* **112**, 815–823.

Chandler, P. M. & Krishnapillai, V. (1974*a*). Phenotypic properties of R factors of *Pseudomonas aeruginosa:* R factors transmissible only in *Pseudomonas aeruginosa*. *Genetical Research* **23**, 251–258.

Chandler, P. M. & Krishnapillai, V. (1974*b*). Phenotypic properties of R factors of *Pseudomonas aeruginosa:* R factors readily transferable between *Pseudomonas* and the *Enterobacteriaceae*. *Genetical Research* **23**, 239–250.

Dunn, N. W. & Gunsalus, I. C. (1973). Transmissible plasmid coding early enzymes of naphthalene oxidation in *Pseudomonas putida*. *Journal of Bacteriology* **114**, 974–979.

Falkow, S. (1975). *Infectious Multiple Drug Resistance*, pp. 83–87. London: Pion.

Fredericq, P. (1969). The recombination of colicinogenic factors with other episomes and plasmids. *Ciba Foundation Symposium*: *Bacterial Episomes and Plasmids*, pp. 163–178. London: J. and A. Churchill.

Gunsalus, I. C., Gunsalus, C. F., Chakrabarty, A. M., Sikes, S. & Crawford, I. P. (1968). Fine structure mapping of the tryptophan genes in *Pseudomonas putida*. *Genetics* **60**, 419–435.

Novick, R. P. (1967). Penicillinase plasmids of *Staphylococcus aureus*. *Federation Proceedings* **26**, 29–38.

Novick, R. P. (1969). Extrachromosomal inheritance in bacteria. *Bacteriological Reviews* **33**, 210–263.

Novick, R. P., Clowes, R. C., Cohen, S. N., Curtiss, R., Datta, N. & Falkow, S. (1976). Uniform nomenclature for bacterial plasmids: a proposal. *Bacteriological Reviews* **40**, 168–189.

Olsen, R. H. & Shipley, P. (1973). Host range and properties of the *Pseudomonas aeruginosa* R factor R1822. *Journal of Bacteriology* **113**, 772–780.

Shipley, P. L. & Olsen, R. H. (1975). Isolation of a nontransmissible antibiotic resistance plasmid by transductional shortening of R factor RP1. *Journal of Bacteriology* **123**, 20–27.

Stanisich, V. A., Bennett, P. M. & Ortiz, J. M. (1976). A molecular analysis of transductional marker rescue involving P-group plasmids in *Pseudomonas aeruginosa*. *Molecular and General Genetics* **143**, 333–337.

Watanabe, T. (1963). Infective heredity of multiple drug resistance in bacteria. *Bacteriological Reviews* **27**, 87–115.

White, G. P. & Dunn, N. W. (1977). The apparent fusion of the TOL plasmid with the R91 drug resistance plasmid in *Pseudomonas aeruginosa*. *Australian Journal of Biological Science*. (In the Press.)

Williams, P. A. & Murray, K. (1974). Metabolism of benzoate and the methyl benzoates by *Pseudomonas putida* (*arvilla*) mt-2: evidence for the existence of a TOL plasmid. *Journal of Bacteriology* **120**, 417–423.

Wong, C. L. & Dunn, N. W. (1974). Transmissible plasmid coding for the degradation of benzoate and *m*-toluate in *Pseudomonas arvilla* mt-2. *Genetical Research* **23**, 227–232.

Wong, C. L. & Dunn, N. W. (1976). Combined chromosomal and plasmid encoded control for the degradation of phenol in *Pseudomonas putida*. *Genetical Research* **27**, 405–412.

Worsey, M. J. & Williams, P. A. (1975). Metabolism of toluene and xylenes by *Pseudomonas putida* (*arvilla*) mt-2: evidence for a new function of the TOL plasmid. *Journal of Bacteriology* **124**, 7–13.