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Transduction and Genetic Homology Between *Pseudomonas* Species *putida* and *aeruginosa*

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Genetic crosses occur by transduction between the species *Pseudomonas aeruginosa* and *P. putida*. The frequency relative to intraspecific transfer is reduced and varies among markers, suggesting that these genomes contain discrete regions of homology and nonhomology.

We have demonstrated marked similarities in the chromosomes of the two *Pseudomonas* species *aeruginosa* and *putida* in the region of the genes for biosynthetic enzymes (1-3, C. F. Gunsalus and I. C. Gunsalus, *in preparation*). Kemp and Hegeman (5) showed for the central catabolic sequences of ketoadipate oxidation an indistinguishable gene organization and regulation. Very distinct differences, however, were observed for genes of the peripheral mandelate to benzoate sequence (5). F. Queener and Gunsalus (*in preparation*) studied the two-protein component, anthranilate synthase. They found equivalent activity of homologous and hybrid complexes when measured in the amid-transferase conversion of chorismate plus glutamine to anthranilate.

In contrast, broad differences between the species were suggested by earlier taxonomic studies including analyses for the deoxyribonucleic acid (DNA) base composition (6) and nutritional characters (10). Strains of *P. aeruginosa* were quite homogeneous, whereas diversity was noted among the available *P. putida* strains, even within several separate subgroups (10). Recently completed studies of DNA-DNA hybridization in pseudomonads by Palleroni and Ballard (*personal communication*) again emphasized the macrohomology among *P. aeroginosa* strains, diversity among *P. putida* strains, including PRS1 and PpG1 (3), and a low and variable degree of hybridization between *P. aeruginosa* and several *P. putida* strains. The latter is true especially under "stringent" conditions, 15 C below the DNA melting temperature. Detailed interpretation of the hybridization must await the full data.

Current data do not reveal whether a low degree of DNA hybridization reflects limited homology between all segments of the two chromosomes, i.e., low macrohomology, or whether isolated regions are nearly identical in base sequence, microhomology, whereas other regions are totally different. To illuminate this problem, we undertook interspecies transduction on the hypothesis that regions of microhomology should permit gene integration, possibly at high frequency. The data reported here suggest that distinct regions of the DNA forms of *P. putida* and *P. aeruginosa* exhibit either very high homology or virtually none at all.

The bacterial strains and mutants of *P. aeruginosa* were from the collections of Holloway (4); *P. putida*, except those strains prepared in our laboratories by nitrosoguanidine treatment or synthesized by genetic crosses were from the collection of Stanier (10).

Three phages were used, the selection depending on the cell strain employed. For *P. aeruginosa* crosses, the F116 phage of Holloway (5) was employed; for *P. putida* pf16h2 (2) and for the interspecies transduction we employed pf20, a mutant prepared from Olsen's (7) PX4 phage by ethyl methane sulfonate (EMS) treatment. For the latter, we used the transduction conditions of Olsen and Metcalf (8), for the others those previously suggested for each phage. Cells for all genetic experiments were grown overnight (15 to 16 hr) in L broth (*see* 2) at 30 C for *P. putida* and at 37 C for *P. aeruginosa* to yield about 10^{10} cell/ml.

Data for two types of interspecies gene transfer by transduction are presented. (i) Phage pf20 was propagated in the wild-type *P. aeruginosa* or

P. putida donor, and, as recipients, mutants of both the homologous and the heterologous strain were used. (ii) The phages pf16h2 and F116, respectively, were grown on *P. putida* and *P. aeruginosa*, and, as recipients, mutants of the homologous species were used. The comparison was of donor strains containing homologous genes versus those prepared by transduction with genes from the opposite species in the first set of interspecies crosses.

The inter- and intraspecies transduction frequencies for mutations in genes for tryptophan, D-mandelate, *p*-hydroxybenzoate, and benzoate enzymes are shown in Table 1. All transduction frequencies are lower with phage pf20 than with phage pf16h2 or F116, due either to transduction conditions or to phage size or properties. The relevant comparisons are between loci and between donors. The genes of two tryptophan clusters *trpABD* and *EF* are transferred in both directions between *P. putida* and *P. aeruginosa* at frequencies at 10 to 20% of the intrastrain rates. Thus, the earlier evidence of similar gross chromosomal arrangement may be extended to sufficient homology to permit genetic exchange. These transductants are stable, indicating integration of heterologous gene segments into the the chromosome. The parallel enzyme experiment with the anthranilate synthase *trpA* gene products gave further indications of homology in this genetic region. Similarly, the genes specifying benzoate and *p*-hydroxybenzoate enzymes are transferred, though at reduced frequency. These genes are, as previously stated, known to be similarly arranged and induced in the two pseudomonas species (5). In contrast, attempts to transfer from *P. putida* the *mdlA* gene, or the whole *mdlABCD* gene cluster (2) to *P. aeruginosa*, were unsuccessful. Wild-type PAT lacks *mdlA* gene activity, whether by deletion or mutation is unclear. The whole *mdlABCD* gene cluster has been transferred from PRS1 to the mandelate-deleted strain PpG1 where it replicates independently of the bacterial chromosome (2). In *P. aeruginosa*, the *mdlBCD* genes are scattered rather than clustered as in *P. putida* (9). In view of the interspecies transfer of the *trp* and aromatic pathway genes, we attribute lack of transfer of the *mdlA* gene to lack of a homologous region, possibly related to the different organization of the chromosome. An examination of the proteins of these two sets of enzymes should give further clarification as to relatedness and origin. Since most of the interspecies transductants are stable, we presumed that the heterologous genes were integrated into the recipient genomes and could be transferred to other recipients by a second transduction.

TABLE 1. *Intra- versus interspecies transduction by phage pf20*

Recipient parent genotype[b]	Selected phenotype	Donor[a]	
		PRS1	PAT
PRS1			
trpD1032	Trp+	~100	10
F1061	Trp+	~100	20
mdlA1001	D-Mdl+	~100	0
pobA1001	Pob+	~80	10
ben-1002	Ben+	~100	4
PAT			
trpB2011	Trp+	20	50
F2011	Trp+	30	80
mdlA	D-Mdl+	0	Wt-

[a] Values indicate transductants per 10^{10} recipients.

[b] PRS, *P. putida* A312 from Stanier (3); PAT, *P. aeruginosa* strain (two) of Holloway-Gunsalus collection PaG 12. The *P. aeruginosa trpB* and *F* mutants are 408 and 464 of Holloway, respectively, PaG 176 and 179.

TABLE 2. *Transfer frequencies for homologous and heterologous genes between P. putida and P. aeruginosa*

Recipient parent genotype	Selected phenotype	Phage	Donor gene[a]	
			Homologous	Heterologous[b]
PRS1			PRS1	PRS-*ben-a*+
pobA1001	Pob+	pf16h2	100	100
ben-1002	Ben+	pf16h2	100	4
PAT			PAT	PAT *trpF-p*+
trpF2053	Trp+	F116	1000	300

[a] Values indicate transductants per 10^{9} recipients.

[b] Designations: *ben-a*+ and *trpF-p*+ from crosses shown in Table 1 with heterologous donor; *a*, aeruginosa; *p*, putida in opposite recipient.

Table 2 contains the results of several transductions with the interspecies hybrid regions for intrastrain repair as compared to transduction by the homologous wild-type donor. Both the *ben* and *trpF* genes are transferred in the homologous and heterologous state by phages pf16h2 and F116. For example, a wild-type *P. putida* PRS1 with homologous *pobA*, unlinked to a *ben-a*+ locus from *P. aeruginosa* shows a frequency of 10^{-7} for the *pobA* gene and a 25-

to 50-fold reduction in transfer of the *ben-a*$^+$ locus. In this case, an equivalent number of minute colonies appear on benzoate plates after 48 hr, suggesting that the integration of the *P. aeruginosa ben* gene fragment is delayed. In the crosses in Table 2, the minute colony formation occurred only when the selection was for the *ben*$^+$ gene of heterologous origin. In contrast the transduction frequencies among *P. aeruginosa* PAT mutants using the *trpF* genetic region of *P. putida* (*trpF-p*$^+$) was reduced only three to fivefold from those receiving the *P. aeruginosa trpF*, suggesting enough microhomology between the two species in this chromosomal region to allow efficient pairing and integration. The delayed or small colony phenomenon was not observed. This type of analysis is now being extended to determine the genetic homology at a number of other sites on the chromosomes of *P. aeruginosa* and *P. putida*.

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LITERATURE CITED

1. Chakrabarty, A. M., C. F. Gunsalus, and I. C. Gunsalus. 1968. Transduction and the clustering of genes in fluorescent pseudomonads. Proc. Nat. Acad. Sci. U.S.A. **60**:168–175.
2. Chakrabarty, A. M., and I. C. Gunsalus. 1969. Autonomous replication of a defective transducing phage in *Pseudomonas putida*. Virology **38**:92–104.
3. Gunsalus, I. C., C. F. Gunsalus, A. M. Chakrabarty, S. Sikes, and I. P. Crawford. 1968. Fine structure mapping of the tryptophan genes in *Pseudomonas putida*. Genetics **60**:419–435.
4. Holloway, B. W. 1965. Variation in restriction and modification of bacteriophage following increase of growth temperature of *Pseudomonas aeruginosa*. Virology **25**:634–642.
5. Kemp, M. B., and G. D. Hegeman. 1968. Genetic control of the β-ketoadipate pathway in *Pseudomonas aeruginosa*. J. Bacteriol. **96**:1488–1499.
6. Mandel, M. 1966. Deoxyribonucleic acid base composition in the genus *Pseudomonas*. J. Gen. Microbiol. **43**:273–292.
7. Olsen, R. H., E. S. Metcalf, and J. K. Todd. 1968. Characteristic of bacteriophages attacking psychrophilic and mesophilic pseudomonads. J. Virol. **2**:357–364.
8. Olsen, R. H., and E. S. Metcalf. 1968. Conversion of mesophilic to psychrophilic bacteria. Science **162**:1288–1289.
9. Rosenberg, S. L., and G. D. Hegeman. 1969. Clustering of functionally related genes in *Pseudomonas aeruginosa*. J. Bacteriol. **99**:353–355.
10. Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonad: a taxonomic study. J. Gen. Microbiol. **43**:159–271.