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SHORT NOTES

# Merodiploidy and Expression of Episomal Genes in *Pseudomonas putida*

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Abstract

A genetic locus which, as pos$^-$, results in nonformation of a cell wall polysaccharide component in Pseudomonas putida, is shown to be dominant as an episomal allele, but recessive on the chromosome. The level of enzymatic activities in strains with episomal alleles of the trpABD operon suggest their control by a regulatory locus via synthesis of repressors.

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Variations in bacterial colony morphology resulting from changes in cell wall composition are well documented. Two clonal variants of Pseudomonas putida recently employed in genetic analyses are opaque (op) as observed in strain PpG1, and translucent (tr) as formed by strains PpG2 and PpS1.[1] These strains were respectively termed C1B, C1S and A312 in earlier chemical studies.[2,3] We found[4] the phenotypic change op → tr to result from a single mutational event, occuring spontaneously at a frequency of ca. $10^{-6}$/cell division, with loss of a surface polysaccharide component. The lack of this wall component was accompanied by loss in the adsorption of phage pf15 and the exposure of receptor sites for phage pf1 and pf6 which plate readily on the translucent PpG2 strain. The genetic locus associated with this change is termed pos, with pos$^+$ denoting the opaque and pos$^-$ the translucent form. The polysaccharide component of pos$^+$ PpG1 can be removed phenotypically by growing the cells in presence of high concentration of glucose (2%)

in tryptone broth or by treating the PpGl cells with a phage pf15-induced polysaccharide depolymerizing enzyme, α-lysin.[4] Both treatments lead to translucent PpG2 clones whose cells are sensitive to phage pf6 but no longer adsorb phage pf15.[4]

We have recently obtained merodiploids in *P. putida* strain, PpGl, by the transfer of gene fragments from cells harboring a defective phage element pf*dm* as sex factor.[5] The pf*dm*, a hybrid of the phage pf16 genome with the mandelate genetic region from the bacterial strain PpSl, is formed by transductional crosses between PpSl and PpGl, or PpG2 with phage pf16h2 on mandelate selection.[5]

Table 1 presents the data from reciprocal crosses by conjugation with formation of merodiploids for the *pos* locus, and shows that *pos*$^-$, as well as *pos*$^+$, are dominant as the episomal allele. Segregation, as well as chromosomal integration, of genes from the *pos*$^-$ bearing episomal element provide further evidence for the dominance of this gene in episomal form. In the first cross between a *pos*$^+$ PpGl parent bearing pf*dm* and *pos*$^-$*trpA513* recipient, with Trp$^+$ selection the merodiploid PpG898 was isolated, see Table 1. The phenotype--opaque, pf15 sensitive, pf6 resistent is typical of PpGl wild type, and mutants, ie. Pos$^+$. Thus a *pos*$^+$ allele in the episome is dominant over the *pos*$^-$ mutation on the chromosome.

In the second cross 822 X 328, where the donor is $Str^S$ and the recipient $Str^R$, by employing streptomycin contra selection, the merodiploid formed is $pos^-$ and thus dominant to the chromosomal $pos^+$. This cross was performed by mixing equivalent volumes of overnight cultures of the parents and after 1 hour, 30°C. in stationary condition, the cells were collected by centrifugation, suspended in saline and plated on minimal plates containing 1 mg/ml streptomycin.[6] After 3 days at 30° the recombinants were purified and found also to be $Trp^+Mdl^+$. These recombinants were diploids which segregated both the trp and mdl character simultaneously at high frequency, even after purification by several single colony reisolations. The segregants were always $pos^+$trpD633, and thus it was possible to select for growth of the merodiploids on minimal media in the absence of tryptophan. Diploids differed from the segregants by the translucent → opaque change and the associated phage sensitivities as shown, Table 1. Those few cells which integrated a part of the episome--in the region of the trpABD gene cluster--were prototrophic but had regained the $pos^+$ character as did the segregants.

The dominance of an episomal $pos^-$ allele to the chromosomal $pos^+$ can be explained on the hypothesis that this cistron controls an enzyme responsible for the biosynthesis of the polysaccharide component and the $pos^-$ results in the formation of an enzymically inactive protein still capable of competing

with the active enzyme in substrate binding and other related properties. This might be the reason that wild type ($pos^+$) cells are opaque and phenotypically Pos$^+$ in a growth medium with 0.2% glucose, while with 2% glucose, the cells are phenotypically Pos$^-$ (tr). The excess glucose may interfere with formation of the polysaccharide component.

A parallel hypothesis would be a regulator gene mutation of the *pos* locus specifying the synthesis of a repressor protein which is multimeric and the *pos*$^-$ mutation coding for functional repressors of the synthesis of a polysaccharide, while the *pos*$^+$ allele produces non-functional monomers of the multimeric repressor molecule, and allows synthesis of the polysaccharide component. The episomal *pos*$^-$ allele would be dominant over chromosomal *pos*$^+$ because of the synthesis of an excess of functional repressor monomers. The episomal *pos*$^+$ allele would be dominant if a large excess of altered monomers were formed and could combine with chromosomally coded functional monomers to form a non-functional multimer. A regulator gene of this type is in fact known in *E. coli* K12, and has been shown to control the synthesis of a capsular polysaccharide and to be dominant on an episome, but recessive on the chromosome.[7]

Both of these alternate explanations are based on the supposition that enzyme levels directed by the genes on an episome are higher than those specified by the chromosomal genes.

In P. putida this condition is met in the case of the mandelate pathway enzymes which are always present in 2 to 4-fold higher levels when the genes are present on the episome.[5] Similarly the tryptophan biosynthetic enzymes occur at higher levels in cells which carry their genes on an episome as is shown in Table 2. In the case of anthranilate synthase, the regulator negative strains, (trpR$^-$), produce 4 to 5-fold the wild type enzyme level; the merodiploid (PpG899) more then 7-fold; and the merodiploid of the trpR$^-$ regulator negative strain (PpG900) 40 to 50-fold wild type AS activity, Table 2. The PRT and InGPS levels are parallel but less responsive...as was shown by Queener and Gunsalus[8] for regulation of trpA vs. BD genes in numerous mutants. For comparison is shown the levels of the trpC gene product, phosphoribosyl anthranilic isomerase (PRAI), which is on the chromosome. This gene is unlinked to the trpABD gene cluster and, in the strains employed, the enzyme level is low as is characteristic of the wild type. The regulator negative strains, trpR$^-$, are from the collection of Maurer and Crawford[9] who observed that spontaneous mutations to 5-fluoroinal resistance produce strains forming elevated levels of the enzymes specified by the trpABD gene cluster, but not by other loci. Mutations in this regulatory gene, R, are unlinked to the trpABD structural genes and so far remain unmapped. The higher enzyme levels with the trpABD$^+$ cistrons in the episomal

position suggests either a more rapid translation, a higher level of transcription, or more likely an occurrance of several copies of the episome per cell. One can attribute to the R locus the coding of a repressor for the *trpABD* operator. The $R^-$ mutation can be interpreted as specifying the formation of a non-functional repressor permitting non-repressed levels of expression of the *trpABD* operon, when such operon copies are present as chromosomal or episomal alleles. Mapping and cis-trans dominance tests for the R locus are presently being attempted in our laboratory and will be the subject of a future communication.

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Table 1. Dominance of $pos^{-}$ and $pos^{+}$ as episomal alleles

| Strain | PpG# | genotypes | PHENOTYPES | | | |
|---|---|---|---|---|---|---|
| | | | clone[1] | Phage pf6 | Phage pf15 | Trp |
| Donor[2] | 892 | $mdl^{+*} pos^{+*}$ | op | − | + | + |
| Recipient | 791 | $mdl^{-} pos^{-} trpA513$ | tr | + | − | − |
| Merodiploid[3] | 898 | $pos^{-} trpA513/mdl^{+*} pos^{+*} trpABD^{+*}$ | op | − | + | + |
| Donor | 822 | $mdl^{+*} pos^{-*}$ | tr | + | − | + |
| Recipient | 328 | $pos^{+} trpD633$ | op | − | + | − |
| Merodiploid | 899 | $pos^{+} trpD633/mdl^{+*} pos^{-*} trpABD^{+*}$ | tr | + | − | + |
| Segregant[4] | (328) | $pos^{+} trpD633$ | op | − | + | − |
| Recombinant[5] | 749 | $pos^{+} trp^{+}$ | op | − | + | + |

* Denotes episomal genes

1 Op is opaque clone, genotype $pos^{+}$; Tr translucent clone, genotype $pos^{-}$, $Trp^{-}$ is tryptophan auxotroph, trpABD is a linked cluster coding early enzymes, see ref. 1. $Mdl^{+}$ phenotype coded by 4 linked genes mdlABCD required for conversion of mandelate to benzoate, see ref. 1.

2 A merodiploid formed by transduction, PpS1 donor, PpG1 ($mdl^{-}$) recipient with phage pf16h2, see ref. 5.

3 $Pos^{+}$ merodiploids are obtained at ca 3% frequency, and segregate to $Pos^{-} Trp^{-}$ at frequency ca $10^{-2}$/bacterial division.

4 Spontaneous segregant from merodiploid.

5 Recombinant selected for $Trp^{+}$ after UV treatment of merodiploid #899.

Table 2. Genetic Regulation of trpABD gene cluster in P. putida

| PpG# | Strain Genotype | Media[1] Trypt lim/xs µg/ml | AS | | PRT | | InGPS | | PRAI[3] |
|---|---|---|---|---|---|---|---|---|---|
| | | | Specific Activity in m units/mg protein[2] | | | | | | |
| 1 | wt(trpR$^{+}$ABD$^{+}$) | 0/20 | 40/40 | 1/1 | 70/60 | 1/0.9 | 150/190 | 1/1.3 | |
| 328 | trpR$^{+}$D633 | 5/50 | 100/20 | 2.5/0.5 | 50/10 | 0.7/.1 | 0/0 | 0 | 220/160 |
| 896 | trpR$^{-}$ABD$^{+}$ [4] | 0/50 | 200/190 | 5/4.8 | 220/230 | 3/3.3 | 400/300 | 2.7/2 | 160/160 |
| 899 | trpR$^{+}$D633/mdl$^{+*}$trpABD$^{+*}$ [4] | 0/50 | 290/300 | 7.3/7.5 | 190/190 | 2.7/2.7 | 370/330 | 2.5/2.2 | 220/220 |
| 900 | trpR$^{-}$D633/mdl$^{+*}$trpABD$^{+*}$ | 0/50 | 1610/1930 | 40/48 | 610/610 | 9/9 | 1300[5]/1120 | 8.7/7.5 | 130/210 |

1 Cells grown in minimal VB medium 16 hrs. 30° with tryptophan additions as indicated, lim = limiting level (derepressed), xs = excess (repressed).

Tryptophan gene and enzyme designations: trpA, AS (anthranilate synthase); trpB, PRT (phosphoribosyl transferase); trpD, InGPS (indoleglycerol phosphate synthase).

2 m unit = 1 nmole product formed/min., (unit = 1 µmole/min).

3 Gene trpC specifying PRAI (phosphoribosyl anthranilate isomerase) is unlinked to the trpABD gene cluster and in these strains is chromosomal only.

4 From crosses 892 x 328; and 892 x 897.

5 The parent mutant trpR$^{-}$D633 (PpG 897) is very low in InGPS specific activity < 20.