JOURNAL OF BACTERIOLOGY, May 1977, p. 943-945

Vol. 130, No. 2
Printed in U.S.A.

Bacterial Mutation Affecting Plasmid Maintenance in *Pseudomonas aeruginosa*

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Received for publication 29 December 1976

A bacterial mutation, *risA*, in *Pseudomonas aeruginosa* caused growth inhibition at 43°C of *risA* strains containing P2 plasmids. Incubation at 43°C resulted in selection for clones that had lost P2 plasmids.

The precise mechanisms by which plasmids are stably maintained in bacterial cells are not yet fully understood. It has been shown that bacterial, as well as plasmid, genes may be involved in plasmid stability (1, 3, 12). Many of the host mutants found also appear to have cell envelope alterations (7, 9, 13). We have unsuccessfully screened many aeruginocin-tolerant (*tol*) mutants of *Pseudomonas aeruginosa* for any mutations that also affect plasmid stability. In the course of these experiments, a mutant strain with altered plasmid stability was fortuitously isolated.

Aeruginocin-insensitive mutants were isolated from strain PAO1670 (*pur-136 leu-38 rif-1*) carrying plasmid R38 (specifying resistance to streptomycin [Sm], tetracycline [Tc], and mercuric chloride [Hg]) by treatment with ethyl methane sulfonate and selection on nutrient agar (NA) plates spread with aeruginocin AR41 (5). The growth of these mutants at 37 and 43°C on NA containing 40 μg of $HgCl_2$ per ml was compared (for general culture methods used see reference 8). The procedure used was designed for the isolation of bacterial mutants that were temperature sensitive for plasmid stability, retaining plasmid R38 during growth at 37°C but losing it with growth at 43°C. Such mutants should not grow on NA + $HgCl_2$ at 43°C.

One mutant, PAO1713, had the desired phenotype. After 15 generations of growth at 43°C, the survival on NA containing either Sm, Tc, or Hg was reduced by a factor of 10^{-4}. Clones that failed to grow on the selective media had apparently lost the entire R38 plasmid. Such clones can be reinfected with unmutated R38 and again exhibit instability of the R-plasmid at 43°C; the mutation is thus bacterial in nature and is not on the plasmid genome. By contrast, after growth at 37°C there was 5% loss of plasmid R38 from strain PAO1713, whereas no loss (<0.04%) could be detected from PAO1670 with growth at 37 or 43°C.

Strain PAO1713 was shown to have a mutation at the *tolA* locus (8) situated at about 9 min on the *P. aeruginosa* linkage map. This is 80% cotransducible with *car-9* (carbamoylphosphate synthase). Revertants to *tolA*$^+$, shown to have reverted at the *tolA* locus by transduction studies, showed the same pattern of instability as strain PAO1713; thus, *tolA* and the locus affecting stability are not corevertable. Likewise, transductants for *tolA*$^+$ obtained from strain PAO1713 showed instability for plasmid R38 at 43°C. Hence, the locus causing this instability is a mutation different from *tolA* and is not closely linked to it. It has been denoted *risA* (R-plasmid instability).

Attempts to map *risA* by conjugation with plasmid FP2 were frustrated by the difficulty of scoring for *risA* in large numbers of recombinants. No linkage of *risA* was found with a range of auxotrophic markers situated between 0 and 50 min (4). Strains PAO1719 (*tolA*$^+$ *risA*) and PAO1720 [*tolA*$^+$ *risA*(R38)] were constructed from strain PAO1713 to enable characterization of the mutation in a genetic background not containing *tolA*. Revertants to *risA*$^+$ were isolated by plating strain PAO1720 onto NA supplemented with either Sm, Tc, or Hg and incubating at 43°C, indicating that *risA* behaves as a single-step mutation.

No other phenotypic effects of *risA* were detected. Strains PAO1719 and PAO1720 showed the same responses to antibiotics, detergents, surfactants, aeruginocins, and bacteriophages as isogenic *risA*$^+$ strains. Derivatives of PAO1719 were made which contained a range of R- and FP-plasmids (6), and the stability of each plasmid was examined (Table 1). Six of 10 group P2 plasmids were unstable in the presence of the *risA* allele. Plasmids of other incompatibility groups were unaffected. No reason can be offered at this time for this apparent specificity of action.

When the growth characteristics of strains PAO1669 [*risA*$^+$(R38)] and PAO1720

TABLE 1. *Plasmid stability in the risA host at 43°C*[a]

Plasmid	Compatibility group[b]	Cells that retained the plasmid (%)
R38	P2	0.01
R931	P2	1
p181	P2	3
pK181	P2	1
p170a	P2	3
pK170-5	P2	2
R130	P2	100
pMG2	P2	100
Rms159	P2	100
FP39[c]	P2	100
R68	P1	100
R18	P1	100
Rms163	P5	100
Rms148	P7	100
FP2	P8	100
R18-1[d]	Unclassified	100
R91[d]	Unclassified	100

[a] Cultures of strains containing the *risA* allele and each plasmid were grown at 43°C in nutrient broth for 15 generations. Cultures were not grown beyond 10^7 cells per ml to avoid reinfection by the plasmids of any cured cells. The cultures were plated onto NA and grown for 16 h at 37°C. Colonies were then scored for retention of the plasmid-coded drug resistances; at least 60 colonies were tested in each case.

[b] The P-incompatibility groups are those summarized by Jacoby (6).

[c] FP39 was reported to be in group P2 (11), but Jacoby (6) was unable to confirm this.

[d] R18-1 and R91 have not been assigned to compatibility groups, but they appear to be compatible with each other and the P1 group (V. Krishnapillai, personal communication).

[*risA* (R38)] at 43°C in nutrient broth were studied, the *risA*$^+$ strain was found to have a lag phase of 2 to 3 h (Fig. 1a), compared with the 4- to 6-h lag phase of strain PAO1720 (Fig. 1b). Viable counts of the PAO1720 culture showed that R38 was absent from the cells of PAO1720 that eventually grew, suggesting that R$^-$ *risA* cells grow much faster than R$^+$ *risA* cells.

The generation times of strains PAO1670 (*risA*$^+$) and PAO1719 (*risA*), both without plasmid R38, were each found to be 40 min at 43°C; thus, the *risA* mutation per se has no overt effect on the rate of bacterial growth in the absence of the plasmid. Since all cells of PAO1720 that grow at 43°C lost the plasmid, it can be concluded that *risA* (R38$^+$) cells are unable to divide at 43°C. The eventual growth of the *risA* (R38$^+$) strain at 43°C is believed to be due to the small proportion of cells in the original inoculum that have lost R38 during growth at 37°C as described above.

Thus, *risA* strains cannot grow at 43°C if

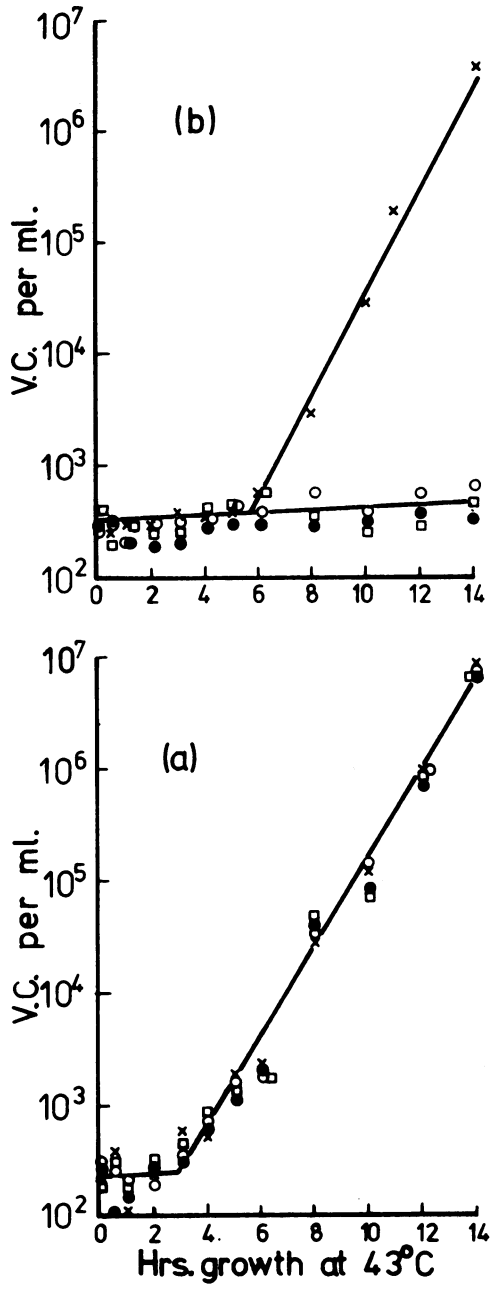

FIG. 1. *Stability of plasmid R38 at 43°C in strains (a) PAO1669(risA$^+$) and (b) PAO1720(risA). Nutrient broth was inoculated with 10^2 cells per ml from an overnight culture grown at 37°C. This was grown, with aeration at 43°C, up to a cell density of about 10^7 cells per ml. Viable counts were made at intervals on the following media, which were incubated at 37°C overnight: ×, NA; ○, NA supplemented with Sm (500 μg/ml); ●, NA supplemented with Tc (250 μg/ml); □, NA supplemented with $HgCl_2$ (40 μg/ml).*

they possess certain group P2 plasmids. Cells surviving and growing at 43°C have lost these P2 plasmids, thus giving the impression of gross plasmid instability in such strains at this temperature.

To our knowledge, a bacterial mutation affecting host cell growth in the presence of R-plasmids has not been described previously. The *risA* mutation may render the bacterium sensitive to a gene product of certain P2 plasmids, a product, for example, that prevents bacterial deoxyribonucleic acid replication. Alternatively, there may be competition between such plasmids and the host for an essential enzyme (2, 10), the level of which is reduced by the *risA* mutation. Strains possessing *risA* may be of value in studies where curing of P2 plasmids is needed, since this cannot be accomplished by other means in *P. aeruginosa*. The identification of the lesion involved will undoubtedly be of value in the study of a variety of plasmid-bacterium interactions.

This work was supported by the Australian Research Grants Committee.

We are grateful to G. A. Jacoby, S. Mitsuhashi, H. Matsumoto, and P. Kontomichalou for the generous gifts of R-plasmids.

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