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Mutation Rate to Nonpigmentation in *Pasteurella pestis*[1]

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The ability of *Pasteurella pestis* to absorb exogenous hemin on a solid synthetic medium (7) and thus form dark pigmented (P^+) colonies is essential for the expression of virulence (8). Of equal importance is the ability to produce a bacteriocin-like substance termed pesticin I (PI^+); mutation to PI^- results in concomitant loss of the plague coagulase and fibrinolysin (3; E.D. Beesley et al., Bacteriol. Proc., p. 80, 1963). However, the virulence of P^- and PI^- isolates is enhanced in white mice receiving Fe^{+2} by injection (2, 8). Hemin and Fe^{+3} also inhibit the antibacterial activity of pesticin I (5). The purpose of this note is to describe an additional relationship between these determinants of virulence.

Of 13 PI^- strains of *P. pestis*, only cells of the P^+ isolates A12, OX/G32, and TSX were inhibited by pesticin I; all but one of the remaining nonsensitive strains (Dodson) were P^-. Cells of 50 P^- mutants of strain A12, obtained from light sectors of separate colonies on hemin agar (7), were resistant to pesticin I, whereas this substance was active against P^+ cells obtained from the same colonies. Conversely, all of 50 pesticin I-resistant clones of strain A12, isolated on pesticin-agar (4) containing pesticin I, proved to be P^-. Identical results were obtained in more limited experiments with strains OX/G32 and TSX.

This relationship (Fig. 1) was exploited to determine the mutation rate to P^-. Crude pesticin I was prepared as previously described (4), dialyzed for 6 hr against cold distilled water, and sterilized by ultrafiltration; final preparations contained about 10^5 units per ml as defined by Beesley et al. (1). As judged by the results of reconstruction experiments, the addition of this preparation to pesticin agar resulted in a medium which was selective for one P^- cell per 10^7 P^+ cells of strain A12.

A liquid culture of strain A12 was diluted in Heart Infusion Broth (Difco) containing 0.2% D-xylose and 0.001 M $MgCl_2$ (6) to yield a concentration of 5×10^2 cells per ml. An 0.5-ml amount of this suspension, which was also assayed to assure the absence of P^- mutants, was added to sterile tubes (10 by 100 mm). The

TABLE 1. *Determination of the mutation rate to nonpigmentation in P. pestis strain A12*[a]

Expt no. 1[b]		Expt no. 2[c]	
Culture no.	Colonies[d]	Culture no.	Colonies
1	7	1	113
2	138	2	3
3	4	3	37
4	1	4	15
5	14	5	60
6	0	6	312
7	6	7	6
8	40	8	6
9	35	9	35
10	8	10	40
11	12	11	31
12	5	12	4
13	12	13	44
14	11	14	12
15	2	15	105
16	17	16	24
17	5	17	18
18	1	18	28
19	23	19	36
20	10	20	7

[a] Determined by the equation $M = aN \ln(aCN)$, where M is the average number of mutants, a is the mutation rate, N is the total number of cells per culture, and C is the number of cultures.

[b] Average: 17.5; total cells per culture observed on pesticin-agar alone: 3.45×10^5; mutation rate: 1.16×10^{-5} mutations per bacterium per generation.

[c] Average: 46.8; total cells per culture observed on pesticin-agar alone: 9.4×10^5; mutation rate: 9.6×10^{-6} mutations per bacterium per generation.

[d] Observed on pesticin-agar containing crude pesticin I.

[1] Presented in part at the 67th Annual Meeting of the American Society for Microbiology, 30 April-4 May 1967, New York, New York.

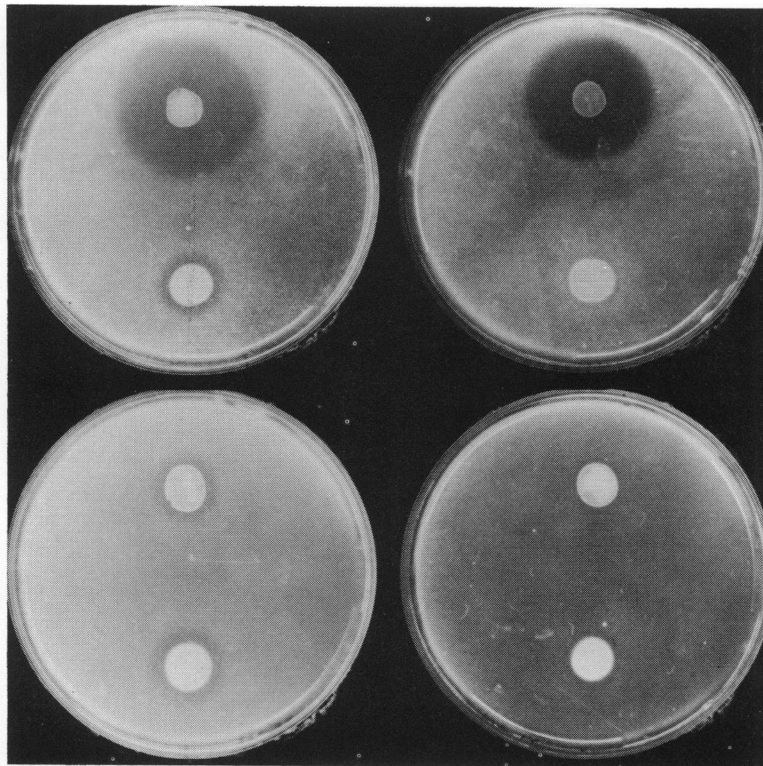

FIG. 1. *Plates containing pesticin-agar spotted with colonies of P. pestis strains (top) EV76(PI+) and (bottom) EV76X(PI−). After incubation for 48 hr at 26 C and treatment with chloroform vapor, the colonies were overlayered with 4 ml of agar containing cells of the following strains of P. pestis: (upper left) OX/G32(P+), (lower left) OX/G32(P−), (upper right) A12(P+), (lower left) A12(P−).*

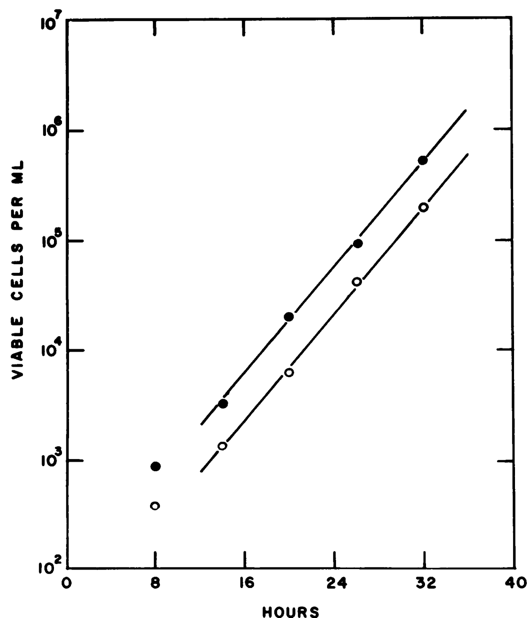

FIG. 2. *Growth of P+ (●) and P− (○) of P. pestis strain A12 in Heart Infusion Broth plus 0.2% D.-xylose and 0.001 M. $MgCl_2$.*

tubes were fitted with metal closures and slanted; the cultures were incubated for 30 hr at 26 C. Some of the cultures then received 0.5 ml of pesticin I solution and, after 10 min at room temperature, an additional 3 ml of warm pesticin-agar. After mixture, the contents of the tubes were layered onto the surface of plates of pesticin-agar. Remaining cultures were appropriately diluted and plated on pesticin-agar (without pesticin I) to obtain an estimation of total viable cells. Colonies were counted after incubation at 37 C for 4 days. Evidence that P^+ and P^- cells grew at equal rates in the liquid medium is shown in Fig. 2 (generation time ~ 2.5 hr).

The equation of Luria and Delbrück (9) was used to calculate a rate of 10^{-5} mutations from P^+ to P^- per bacterium per generation (Table 1). This rate is 10 times lower than that determined for loss of Ca^{++}-dependence, an unrelated determinant of virulence (6). The correlation between sensitivity to pesticin I and expression of the PI^-, P^+ phenotype suggests that hemin and pesticin I are absorbed at the same site. However, this correlation is not absolute as evidenced by the existence of strain Dodson (PI^-, P^+), which is not sensitive to pesticin I.

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