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Interplay between Plasmid Partition and Postsegregational Killing Systems

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Active partition systems and postsegregational killing (PSK) systems are present together in naturally occurring low-copy-number plasmids. Theory suggests that PSK may act as the ultimate determinant of plasmid retention, whereas the partition system may minimize the growth penalty to the host, resulting in a near-ideal symbiosis when the systems combine. Here, we prove the validity of this principle for a specific case involving the P1*par* system and the *mvp* PSK system.

Low-copy-number bacterial plasmids cannot rely on random dispersal to ensure their propagation to daughter cells. Rather, they make use of several different types of strategies to ensure their distribution (8). The major players are active partition systems and toxin-antidote systems. Partition systems encode a centromere analog, the partition site, which allows the plasmid to be actively segregated to daughter cells (8, 9). In theory, each daughter cell should receive a plasmid copy if one is available. Toxin-antidote systems are generally believed to function by postsegregational killing (PSK) (4, 6). PSK kills cells that lose the plasmid. A toxic product and its specific antidote are produced from two contiguous genes. If the plasmid carrying them is lost, the antidote decays but the toxin persists, killing the cell (13). At least in theory, this should eliminate all plasmid-free cells from the population, irrespective of the manner by which the plasmid was lost, thus ensuring plasmid maintenance. In practice, the majority of toxin-antidote systems have not proven very efficient in this respect (4). Indeed, some systems, such as the *parD* function of plasmid R1, may act not by killing but by delaying cell division (4).

Do any PSK systems act in the simple way that theory suggests? In an ideal system, almost any event leading to plasmid loss should kill the cell. Thus, replication errors, a lack of partition to daughter cells, and the formation of unsegregatable plasmid multimers, etc., should all give rise to inviable cells. Plasmid-free cells should be undetectable in the population. A plasmid containing only a low-copy-number replicon with the PSK system should be completely stable. However, this stability should come at the cost of reduced cell growth. This is because, in the absence of active partition to daughter cells, the plasmid would be poorly distributed. Many plasmid-free cells would arise and be killed, thus slowing the growth of the population. A similar plasmid containing a partition system but no PSK system should be reasonably stable, because the products of replication are actively distributed to daughter cells. However, other problems leading to plasmid loss, such as mutations and replication errors, would not be corrected, so that a low rate of loss should remain. This type of plasmid should not cause a measurable slowing of cell growth without selection because no killing of cells occurs.

If both a PSK system and a partition system are present, the plasmid should be completely stable while minimizing the growth rate penalty. The partition system should distribute the plasmid copies to daughter cells properly. The low rate of loss due to replication errors, etc., would be dealt with by the PSK system, killing only those rare cells that still lose the plasmid. This type of plasmid should approach the ideal: a plasmid that is stably maintained without disadvantage to its host. Although this principle may be readily inferred from the proposed mode of action of these plasmid maintenance determinants, it has not been directly demonstrated. Moreover, the relatively poor performance of most PSK systems when tested in isolation casts some doubt on the theory.

Model plasmid construction. We constructed a set of plasmids to test these principles directly. Each plasmid contained the P1 replicon and a selectable *cat* marker. In addition, each plasmid had the replication origin of pBR322. This allowed genetic engineering for wild-type *Escherichia coli* strains, in which the plasmids replicate at a high copy number. Testing was done in a *polA* strain, in which the pBR322 origin is inactive and all replication proceeds via the low-copy-number P1 origin (1). The PSK system used was the *mvp* locus from the large virulence plasmid pMYSH6000 of *Shigella flexneri* (11). The toxin and antidote are small proteins, the products of the *mvpA* and *mvpT* genes. The toxin, MvpT, is remarkably efficient at killing *E. coli* host cells (12). The partition system used was the P1*par* system from the P1 plasmid prophage. It consists of an operon encoding the ParA and ParB proteins, followed by the partition site, *parS*, at which the proteins act (2). The structures of the plasmids are illustrated in Fig. 1.

The *mvp* locus confers complete stability on an isolated replicon. When the plasmid vector pALA136 was present in the *polA* strain BR825, the plasmid was unstable in the absence of selection (Table 1). This is because it is replicated from the low-copy-number P1 origin and has no plasmid stability determinants. When the *mvp* locus was added to the plasmid (pALA2529), a remarkable increase in stability was seen. We were unable to reproducibly detect any loss of the plasmid in

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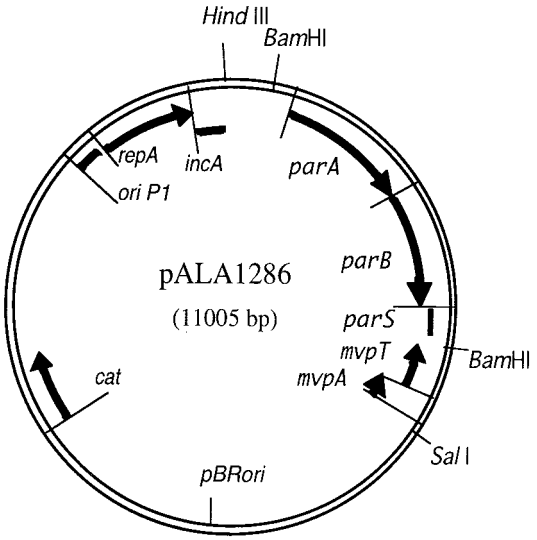

FIG. 1. Plasmid pALA1286 and related plasmids. A series of plasmids was derived from plasmid pALA136 (7). Plasmid pALA2529 has the BamHI-SalI *tet* gene fragment of pALA136 replaced by a BamHI-SalI fragment from pMYSH6000 containing the *mvp* locus (11). Plasmid pALA1557 (11) has a BamHI fragment containing the P1*par* locus inserted into the BamHI site of pALA136. Plasmid pALA1286, shown above, was derived by substituting the BamHI fragment of pALA1557 into the BamHI site of pALA2529.

100 generations of unselected growth in rich medium (Table 1).

Plasmids pALA136 and pALA2529 were equally unstable in strain CC5220 (Table 1). CC5220 is derived from BR825 and blocks the action of the plasmid-borne *mvp* system because it produces the MvpA antidote from a gene integrated into the chromosome (12). We conclude that the complete stability of pALA2529 in strain BR825 is due to the PSK effect of the *mvp* system.

TABLE 1. Plasmid loss rates[a]

Host strain	Plasmid	Stability determinant	Loss per generation (%)
BR825	pALA136	None	10
	pALA2529	*mvpA-mvpT*	<0.02[b]
	pALA1557	P1*par*	0.9
	pALA1286	*mvpA-mvpT* P1*par*	<0.02[b]
CC5220	pALA136	None	15
	pALA2529	*mvpA-mvpT*	13
	pALA1557	P1*par*	1.1
	pALA1286	*mvpA-mvpT* P1*par*	1.1
CC4118	pALA136	None	10
	pALA2529	*mvpA-mvpT*	<0.1
	pALA1557	P1*par*	6
	pALA1286	*mvpA-mvpT* P1*par*	<0.1

[a] Plasmid loss was determined by scoring the loss of chloramphenicol resistance during unselected growth at 30°C. CC5220 differs from BR825 in having an *mvpA-mvpT* kanamycin resistance cassette integrated into the chromosome at *araD* by λ*red*-promoted recombination (14). The MvpA expressed from this cassette blocks the PSK effect when a plasmid carrying the *mvp* locus is lost from the cell (12). CC4118 is a derivative of BR825 that contains plasmid pALA1838. Plasmid pALA1838 carries the P1*parS* site as an EcoRI and BamHI fragment from plasmid pALA1952 (10) inserted between the same sites of plasmid pGB2 (5). The *parS* site blocks the action of plasmid-borne P1*par* partition systems by competition (3).

[b] Values were determined during 100 generations of unselected growth. All other values were determined after 10 generations of unselected growth in Luria-Bertani broth at 30°C. Average values from duplicate experiments were used.

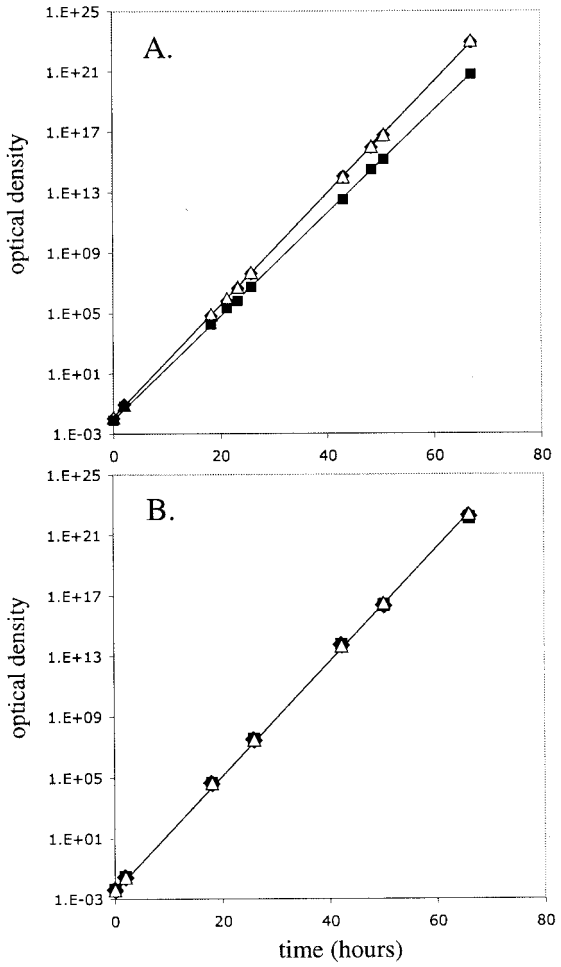

FIG. 2. Growth rate curves for plasmid-containing cells. The plasmid-containing cells were grown overnight with chloramphenicol to select for retention of the plasmid and then transferred to Luria-Bertani broth without chloramphenicol at 30°C. The cultures were periodically diluted to continuously maintain them in logarithmic growth. The optical density at 600 nanometers of the cells was recorded at the times shown. The values were corrected for the periodic dilutions. A duplicate experiment gave virtually identical results. Diamonds, no plasmid; open triangles, pALA136 (vector); squares, pALA2529 (*mvp*$^+$). Panel A, determinations for strain BR825; panel B, determinations for strain CC5220, which expresses *mvp* genes from the chromosome and is immune to killing by the plasmid *mvp* system. Virtually identical growth curves were obtained when viable counts were assayed (see Fig. 3; data not shown).

Stabilization by *mvp* incurs a growth deficit. Cultures of BR825 plasmid-containing cells were grown without selection for 80 generations, and the growth of the cultures was determined by optical density. Figure 2 shows that the pALA2529-containing cells grew more slowly than those with the pALA136 vector or with no plasmid. No measurable growth deficit was seen with the same plasmid in strain CC5220, in which *mvp* activity is suppressed. From the differences in the slopes of the growth curves, we conclude that the stabilization

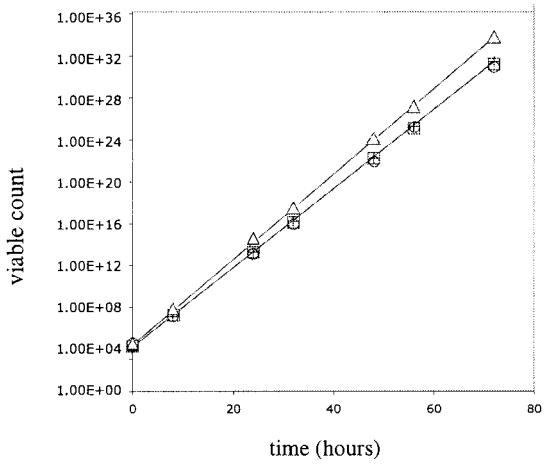

FIG. 3. Viable count growth curves. BR825 cells containing the respective plasmids were grown as described for Fig. 2. Viable counts were determined by plating suitable dilutions on solid medium, and the values were corrected for the periodic dilutions made to maintain the cultures in exponential phase. A duplicate experiment gave virtually identical results. Triangles, pALA136 (vector) viable count on Luria-Bertani agar; circles, pALA136 viable counts on Luria-Bertani chloramphenicol agar; squares, pALA2529 (*mvp*$^+$) viable counts on Luria-Bertani agar; crosses, pALA2529 viable counts on Luria-Bertani chloramphenicol agar.

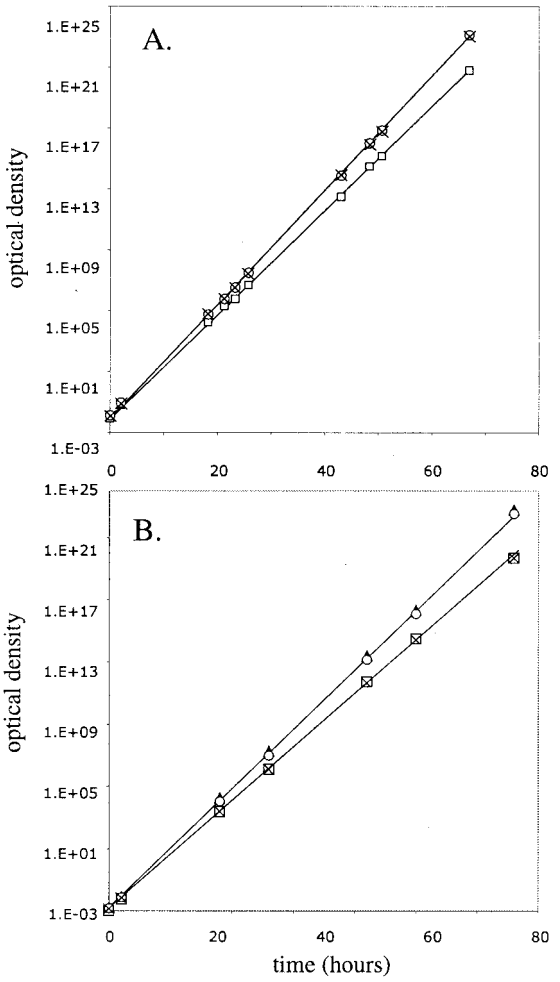

FIG. 4. Growth curves of cells by optical density. Panel A, BR825 cells carrying pALA2529 (*mvp*$^+$) (squares; shown also in Fig. 2 and repeated here for comparison), pALA1557 (*par*$^+$) (circles), or pALA1286 (*mvp*$^+$ *par*$^+$) (crosses); panel B, growth of strain CC4118 carrying pALA136 (triangles), pALA2529 (squares), pALA1557 (circles), or pALA1286 (crosses). Cells were grown as described in the legend to Fig. 2, except that 25 μg of spectinomycin/ml was added to the CC4118 cultures to ensure the retention of the element that blocks P1*par* activity (see footnote to Table 1). A duplicate experiment gave virtually identical results.

of the plasmid by *mvp* imposes a growth deficit on the host cells of about 9% per generation.

The growth deficit is due to the killing of plasmid-free cells by *mvp*. Figure 3 shows the viable counts of the total cells and the plasmid-containing (chloramphenicol-resistant) cells from a growth experiment parallel to that illustrated in Fig. 2. Again, cells containing pALA2529 grew more slowly than those containing the pALA136 vector, as judged by the total viable count. However, the plasmid-containing (chloramphenicol-resistant) cells of the two populations increased at the same rate (Fig. 3). In addition, the growth rates of the total population and the chloramphenicol-resistant population of pALA2529 cells are identical (Fig. 3), reflecting the fact that within the limits of detection, there are no viable plasmid-free cells (Table 1). We conclude that the two plasmids are lost from the cells at the same rate (approximately 9% per generation, as determined by a comparison of the slopes of the growth curves [Fig. 3]). However, when pALA136 is lost, the plasmid-free cells contribute normally to the total population, so that their proportion increases with time. When pALA2529 is lost, the plasmid-free cells are killed. Although cell killing may never be complete, any remaining viable cells are below the limits of detection. Only plasmid-containing cells contribute to the viable population, so that growth curves for the resistant and total cells are identical.

Stabilization by P1*par*. Plasmid pALA1557 was derived from the pALA136 vector by an insertion of the P1*par* partition locus. Table 1 shows that P1*par* reduces the loss rate of the plasmid some 10-fold. Some of the residual instability is likely to be due to plasmid multimer formation: unlike naturally occurring plasmids, the test system lacks a site-specific recombination system that can resolve any multimers that form by generalized recombination (8). The rest of the instability is likely to be due to replication errors or fluctuations that reduce the plasmid copy number in a given cell to less than the two copies needed for partition to work.

The partition activity of the P1*par* locus can be eliminated by the introduction of a competing high-copy-number plasmid carrying the P1*parS* partition site (3). Table 1 shows that the stability of pALA1557 is reduced to that of the vector when assayed in strain CC4118, which carries such a plasmid. Stabilization by P1*par* does not impose a growth deficit on the host cell (Fig. 4A).

P1*par* and *mvp* combine to achieve complete plasmid stability without a growth penalty to the host. Plasmid pALA1286 carries both the *mvp* and P1*par* loci (Fig. 1). It showed no measurable loss from strain BR825 after 100 generations of unselected growth (Table 1). The growth curves in Fig. 2A and

Fig. 4A show that this plasmid imposed no measurable growth penalty on the host. Thus, the deleterious effect of *mvp* is nullified by the presence of the P1*par* locus. This effect was lost in strain CC4118, in which the partition activity of P1*par* is blocked; in CC4118, the plasmid imposed the same growth rate deficit as its counterpart pALA2529, which contains *mvp* but not P1*par* (Fig. 4). We conclude that active partition of the plasmid reduces plasmid loss, so that very little cell killing by the *mvp* system is needed. As expected, when pALA1286 was introduced into strain CC5220, in which the activity of *mvp* is blocked, the plasmid behaved as if only P1*par* were active; the plasmid was partially stabilized without a growth penalty (Table 1; data not shown).

Concluding remarks. The *mvp* PSK system from the *S. flexneri* virulence plasmid pMYSH6000 acts as the perfect killer in our experimental system. Every cell that loses the plasmid is killed. This results in complete stability of the plasmid that is otherwise lost from the host at a very high rate. The efficient killing of the cells that lose the plasmid results in the expected reduction of the growth rate of the unselected population. This growth rate deficit was alleviated if the P1*par* partition system was also present. Thus, the theoretical principle holds, at least in this particular case.

The almost absolute plasmid stability conferred by the *mvp* system acting alone mimics the stability of many low-copy-number plasmids found in nature. However, stability due to *mvp* action comes at a cost. The growth rate of the population is decreased, and energy is expended in producing cells that are destined to die. This puts plasmid-bearing cells at a disadvantage over plasmid-free competitors. However, naturally occurring plasmids have both a PSK system and an active partition system. Active partition greatly reduces the number of cured cells by distributing the available plasmid copies equitably to daughter cells. Very few plasmid-free cells are now produced. These constitute the daughters of those rare cells that, due to mutation, replication errors, or multimer formation, have too few plasmids to allow a distribution to their progeny. Only the rare cured cells are killed by the PSK system. Complete stability is therefore achieved without a measurable effect on the growth of the population.

How widespread is this efficient dual strategy in nature? Although the large low-copy-number plasmids that are broadly distributed in nature always appear to have both types of stability determinants, their PSK systems often appear to be inefficient at killing their hosts and at promoting plasmid stability (4). However, such systems are generally assessed in test vectors, away from their natural contexts. This may compromise their efficiency. We suggest that these systems might function efficiently in the context of the plasmids in which they occur naturally. Thus, they may kill their hosts efficiently and play a key role in plasmid survival. Alternatively, some plasmid PSK systems may be inherently inefficient but are backed up by the presence of other PSK systems present in the same plasmid. Natural plasmids often encode more than one PSK system. Thus, the dual PSK-*par* strategy is likely to be a widespread, if not universal, method employed by low-copy-number plasmids to ensure their long-term survival.

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