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Compatibility Properties of R483, a Member of the I Plasmid Complex

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R483, an I pilus-determining plasmid previously reported as belonging to a distinct incompatibility group, Iβ, proved to be an atypical Iα plasmid; in a growing culture, the degree of inhibition of replication of one Iα plasmid by the presence of another was not uniform within the Iα group.

R483 is an R factor, derived from a wild strain of *Escherichia coli*, that confers resistance to trimethoprim, streptomycin, and spectinomycin (10) and determines I pili (4; this paper). It replicates in *E. coli* K-12 to give one to two copies of the plasmid per chromosome (1). Its deoxyribonucleic acid shows substantial homology with that of typical Iα plasmids (8).

The compatibility properties of R483 are anomalous. Typical I pilus plasmids such as R64, R144, or ColIb-P9 (i.e., the Iα compatibility group) are so strongly mutually incompatible that we have never been able to observe strains carrying two such plasmids. R483, however, is capable of coexisting through many generations with typical Iα plasmids.

The difference between R483 and the typical Iα plasmids cannot be explained as a failure of the former to eliminate and be eliminated by incompatible plasmids since the I pilus-determining R factors JR66a and R805a efficiently eliminate, and are eliminated by, R483. JR66a and R805a are also incompatible with Iα plasmids (4, 6). On the basis of this evidence, Hedges and Datta (9) proposed that R483 should be regarded as the prototype of a compatibility group, Iβ, distinct from Iα. It was further suggested that JR66a might represent an ancestral compatibility group (Iω) from which both Iα and Iβ groups had diverged. Further investigation of the interactions of R483 with typical Iα plasmids are reported here and have led us to revise the classification of these plasmids.

MATERIALS AND METHODS

Media. Media were as described previously (3).

Plasmids. Plasmids were R483 (described below) and R144. The latter carries genes determining I pili and resistance to tetracycline and kanamycin and genes for production of colicin Ib. It is a prototype of group Iα (9). R144$^+$ cultures of *E. coli* K-12 carry one to two plasmid molecules per chromosome (1, 8).

Bacterial hosts. Hosts were *E. coli* K-12, J53 F$^-$ *pro met*, and J62 F$^-$ *pro his trp lac* (2).

Construction of doubles. Construction of doubles was as described under Results.

Test for resistance to trimethoprim and kanamycin. Individual colonies were streaked over double-ditch plates of Oxoid DST (direct sensitivity test) agar (CM 261) incorporating 4% lysed horse blood; the two ditches contained the same medium with added trimethroprim (10 μg/ml) and kanamycin (25 μg/ml), respectively.

Test for transmissibility of resistance. Resistant clones from the ditch plates were tested for transmissibility of resistance by patch-testing on an R$^-$ recipient. J53 resistant clones were inoculated in patches on minimal agar plates previously seeded with J62R$^-$. The plates contained either trimethoprim (10 μg/ml) or kanamycin (25 μg/ml) and the nutrient required by J62. Resistant clones of J62 were patched onto J53R$^-$, seeded on equivalent plates with appropriate nutrients. Control patches of R$^-$ bacteria were included on all plates.

RESULTS

Phenotypic characters determined by R483. The genes for resistance to trimethoprim, streptomycin, and spectinomycin are closely linked and transposable en bloc from R483 to other replicons (1).

Since R483$^+$ cultures permitted increase in titer of phage If1, it was concluded that R483 determined I pili (9). This conclusion was confirmed by the finding that an ethyl methane sulfonate-induced mutant of R483 (R483*drd2*), selected for its high efficiency of transfer between strains, permitted visible lysis by If1 (R. W. Hedges, unpublished observation).

R483 determined a colicin active against *E. coli* K-12 strains. This was colicin Ia on the following evidence. It was inactive against strain BC3, which lacks the colicin I receptor (J. Konisky, personal communication); ColIa-CA53, but not ColIb-P9, conferred partial immunity to it; and R483 conferred immunity to colicin Ia but not colicin Ib.

R483 is stably maintained through many generations in *E. coli* K-12.

Segregation of doubles carrying R483 and R144. Two doubles were constructed as follows. J62(R483) was mated in nutrient broth with J53(R144), and plasmids were transferred in each direction, selection being for the nutritional requirements of either J62 or J53 and for both plasmids. Each double was purified on plates containing trimethoprim and kanamycin. One colony each of J62(R483)(R144) and J53(R144)(R483) was suspended in drug-free broth. Viable counts on nutrient agar (with no antibiotic) were made at once and at intervals during 8 h of logarithmic aerated growth (Fig. 1). Cell densities were kept below 10^7/ml by dilution with broth at 37 C to minimize reinfection. Colonies from the viable counts were tested for resistance to trimethoprim and kanamycin and for ability to transfer resistance.

In both doubles the Iα plasmid, R144, was retained throughout the period of growth in the great majority of clones. In J53(R144)(R483), where R144 had been resident and R483 introduced, the latter was unstable and was lost from the majority of clones in 8 h (Fig. 2). In J62(R483)(R144), where R483 had been resident and R144 introduced, the resistance markers of both plasmids were retained, but the transmissibility of R483 was lost at a rate similar to the rate of loss of R483 in the J53 culture (Fig. 3). Loss of transmissibility reflected the loss of the plasmid, R483, with its colicin Ia and I pilus genes; its resistance genes had, however, been transposed to the host chromosome (1, 4). No loss of transmissibility of kanamycin resistance was ever observed.

The kinetics of loss of R483 from both doubles is shown in Fig. 2 and 3. The rate of loss indicated that the plasmid was replicating, although less frequently than its host. Assuming that R483 segregates as symmetrically as the number of plasmid copies allows, then the exponential rate of loss (time for 50% loss [$t_{1/2}$] = 156 min; Fig. 2) would be given by a plasmid doubling on average every 26.3 min in its host, J53(R144)(R483), which had a mean generation time of 22.5 min (Fig. 1). The comparable figures for the J62 double were: 28.3 min for doubling of transmissible R483 ($t_{1/2}$ for loss of transmissibility = 120 min) and 22.9 min for the host

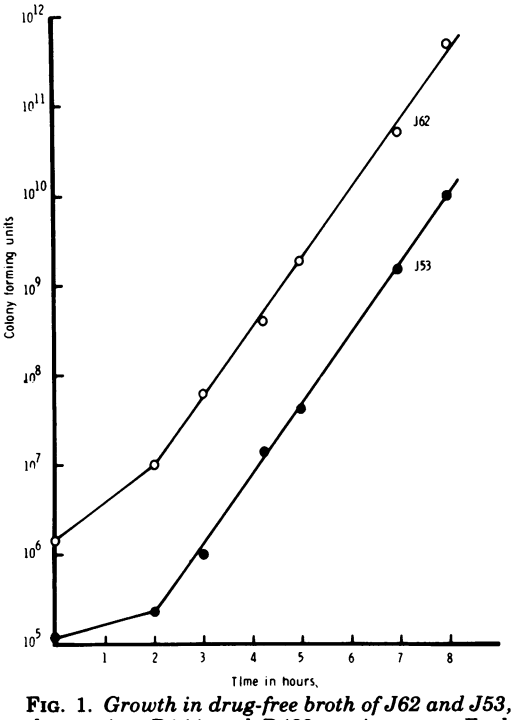

FIG. 1. *Growth in drug-free broth of J62 and J53, each carrying R144 and R483 at time zero. Each culture was inoculated at time zero with a colony taken from plates incorporating drugs selective for both plasmids. Broth cultures were grown at 37 C with shaking and diluted at intervals to maintain cell titers <10^7/ml. The ordinate is calculated from the viable counts, multiplied by these dilutions.*

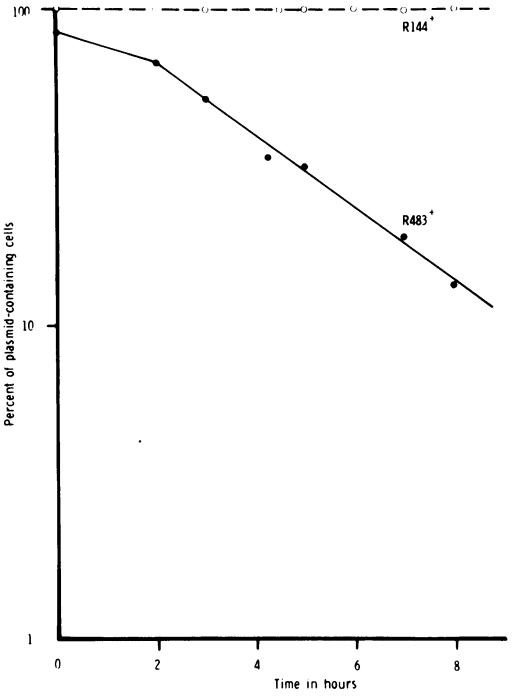

FIG. 2. *Loss of plasmids from J53(R144)(R483) in drug-free broth. About 100 colonies from each of the viable counts shown in Fig. 1 were tested for the presence of each plasmid as described in the text. Loss of R483 was indicated by loss of trimethoprim resistance. The drug resistance was transmissible in all clones that retained it.*

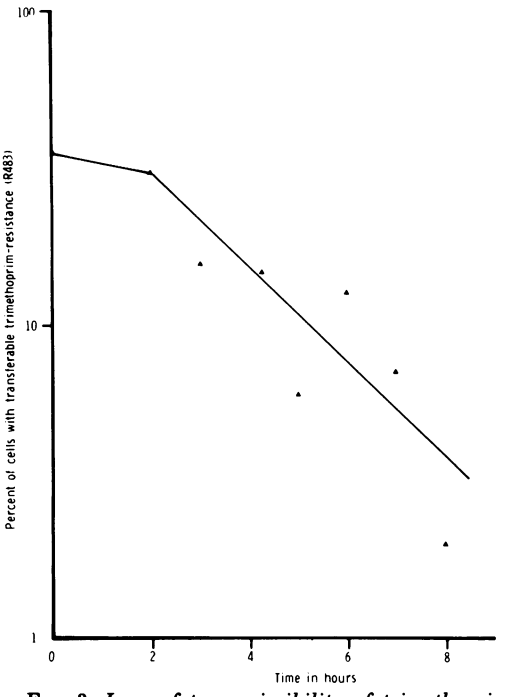

FIG. 3. *Loss of transmissibility of trimethoprim resistance (i.e., loss of R483 plasmid) from J62(R483)(R144) in drug-free broth. About 100 colonies from each of the viable counts shown in Fig. 1 were tested for the presence of each plasmid as described in the text. No loss of trimethoprim resistance was detected, but its transmissibility declined exponentially, indicating loss of the plasmid R483. R144 was retained by all clones tested.*

generation time (Fig. 3 and 1, respectively). The equation relating the rate of loss of a plasmid to its replication rate is given in the Appendix.

After the 8 h, during which logarithmic growth at low cell density was maintained, the cultures were allowed to grow overnight to saturation and plated on nutrient agar. Examination of these colonies showed extensive reinfection, as evidenced by re-establishment of doubles.

DISCUSSION

Incompatibility between a pair of plasmids is an inability on the part of the host to support replication of both. In the case of an F'*lac* factor introduced into an Hfr strain, the kinetics of loss of F'*lac* were those expected by dilution, in the growing culture, of a nonreplicating plasmid (7).

When R483 and R144 were present together in *E. coli* K-12 the resulting doubles were unstable. In the kinetic experiments reported here, R483 was lost much more frequently than R144. However, in some earlier experiments (9) we observed loss of one or other of the two plasmids from doubles carrying R483 and R64 or R483 and R144. In some experiments R144 or R64 was lost more frequently than R483, but the results of 10 experiments showed that R483 had a greater likelihood than R144 of being lost. Our conclusion is that R144 is higher than R483 in the "hierarchy" of Iα plasmids, but its position can be overturned by relatively trivial and uncontrolled changes in the conditions of the experiment.

The rate of loss of R483 was less than one would find with a nonreplicating plasmid. We conclude that R144 and R483 both replicated in the doubles, the former almost as frequently as the host and the latter less frequently.

The doubles, constructed in parallel, both retained R144 in nearly all clones and lost R483. But J62(R483)(R144), where R483 was resident and R144 introduced, retained the R483 resistance genes, integrated in the chromosome, whereas in the J53 clone, where R144 had been resident and R483 introduced, there was no evidence of chromosomal integration of resistance genes. We interpret this difference between the two doubles as meaning that at the outset of the experiment (time zero) the J62 clone carried two copies of the R483 resistance genes, one in the chromosome and the other in the plasmid (1).

R483 and R144 do not exclude one another (9). When the segregating cultures were grown up to saturation, doubles were re-established by reinfection. This effect, together with the ability of R483 to coexist with R144 through many cell generations, meant that a test for stability of doubles after overnight growth in broth (3) gave misleading results. On the basis of that test, R483 was recorded as compatible with plasmids of group Iα and allocated to a distinct compatibility group (9, 10). Its replication is, however, inhibited by an Iα plasmid in the same host, and so it must be considered as an atypical member of group Iα. Plasmids such as JR66a, which are evidently incompatible with typical Iα plasmids as well as with R483, must now, therefore, also be included in the Iα compatibility group.

Differences in compatibility properties between plasmids within a compatibility group have been noted previously, e.g., the "hierarchy" relationship between the F factor and Col V factors, both of group FI (11), and between R factors JR72 and R1, both of group FII (5). The Iα group is also subdivisible. The "metastability" of strains carrying both R483 and a typical

Iα plasmid suggests that the Iα group has undergone evolutionary divergence that will perhaps lead to the separation of a new group.

APPENDIX

Relationship between the rate of curing of a plasmid and its rate of replication. Let τ_b and τ_p be the mean generation times, and let B_0 and P_0 be, at time zero, the number of bacteria and plasmids, respectively. Let $t_{1/2}$ be the half time of the (exponential) rate of decrease of the proportion of plasmid-containing clones; i.e., assuming balanced segregation of the plasmids, after $t_{1/2}$ twice as many bacterial cells as plasmids will have been produced.

At time t, the number of bacterial cells will be $B_0 2^{t/\tau_b}$ and the number of plasmids will be $P_0 2^{t/\tau_p}$. For two times, t_1 and t_2, where $t_2 - t_1 = t_{1/2}$,

$$B_0 2^{t_2/\tau_b} / B_0 2^{t_1/\tau_b} = (P_0 2^{t_2/\tau_p}) \times 2 / P_0 2^{t_1/\tau_p},$$

i.e., $$2^{t_2 - t_1/\tau_b} = 2^{t_2 - t_1/\tau_p} \times 2$$

or $$2^{t_{1/2}/\tau_b} = 2^{t_{1/2}/\tau_p} \times 2$$

and $$t_{1/2}/\tau_b = (t_{1/2}/\tau_p) + 1$$

thus $$\tau_p = t_{1/2}\tau_b / (t_{1/2} - \tau_b).$$

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