Envelope Alterations Produced by R Factors in *Proteus mirabilis*

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(Received 3 June 1974)

SUMMARY

R-1818 increases swarming and abolishes proteolytic enzyme excretion by *P. mirabilis* F67. R-TEM reduces swarming, increases proteolytic enzyme excretion by this organism and renders it susceptible to sodium deoxycholate. The properties of strains harbouring both R-1818 and R-TEM simultaneously are dependent on the order of introduction of the R factors, the R factor first present being dominant. Recombination between the R factors in this organism does not seem to be responsible for these observations.

INTRODUCTION

Studies on the behaviour of R factors in several bacterial hosts have been well documented in recent years, but reports on how R factors affect bacterial physiology are fewer in number. R factor Rts 1, thermosensitive for DNA replication at 42 °C, has been shown to interfere with the normal growth of the host at this temperature and also caused changes in the appearance of the membrane (Di Joseph, Bayer & Kaji, 1973). A change in the permeability of the host was suggested as an explanation of the increased initial sensitivity of *Escherichia coli* to nalidixic acid and acriflavine caused by R₁₀₀ (Yoshikawa, 1971).

Certain R factors have also been shown to render their *E. coli* host more susceptible to rifampicin than the R⁻ parent strain (Romero, Riva, Fietta & Silvestri, 1971). This increase in susceptibility was not due to the synthesis of an R factor-mediated, rifampicin-sensitive, RNA polymerase or to a modification of the host enzyme. It was again suggested that an increase in permeability to the drug was responsible for the observations.

During a study of several R⁺ strains of *Proteus mirabilis* we found that the ability of the host to swarm on minimal agar could be increased by some R factors but abolished by others. In addition, these R factors affected the level of host-mediated proteolytic enzyme and in some cases rendered the host bacteria more susceptible to sodium deoxycholate.

METHODS

Bacteria. Organisms used were *P. mirabilis* F67 (Smith, 1969); *E. coli* K12 *met* (Meynell & Datta, 1966); *E. coli* J6-2 *lac*⁻ *pro his trp* (Clowes & Rowley, 1954) and its rifampicin resistant derivative *E. coli* J6-2-2 (Datta & Hedges, 1972).

R factors. These are listed in Table 1; R-TEM is also known as R6K and R-1818 is also known as R46.

Media. Minimal medium used was that of Davis & Mingioli (1950). Solid media were

Table 1. *R* factors

R factor	Compatibility group	Resistances*
1818	N	Am, Sm, Sp, Tc, Su
TEM	X	Am ^H , Sm ^H

* Am, ampicillin; Sm, streptomycin; Sp, spectinomycin; Tc, tetracycline; Su, sulphonamide; Am^H, Sm^H, resistance to high levels of ampicillin and streptomycin sufficient to differentiate R-TEM from R-1818.

prepared and supplemented as described by Smith (1969) using 1.5% Davis agar. Plates were overdried for 1 h at 44 °C before use. Nutrient broth No. 2 and MacConkey agar were obtained from Oxoid.

Transfer of R factors. The general methods of Smith (1969) were used. Strains of *P. mirabilis* harbouring simultaneously R-TEM and R-1818 were constructed using *E. coli* K12(TEM) or K12(1818) as donors. The presence of R-TEM in *P. mirabilis* was scored by its ability to grow on 200 µg ampicillin/ml and R-1818 by growth on 10 µg sulphadiazine/ml. Transfer of R-TEM from *P. mirabilis* into *E. coli* J6-2-2 was detected on media with 300 µg ampicillin/ml and that of R-1818 on media with 10 µg tetracycline/ml. *Proteus mirabilis* was contraselected by 25 µg rifampicin/ml. Control experiments had shown that the presence of R-TEM or R-1818 did not render *E. coli* J6-2-2 susceptible to rifampicin, as had been observed for certain R⁺ strains of *E. coli* (Romero *et al.* 1971).

Proteolytic enzyme determinations. Stationary phase broth cultures of *P. mirabilis* strains grown at 37 °C with vigorous aeration were centrifuged and the supernatant passed through a membrane filter (0.45 µm, Millipore). The proteolytic activity of the supernatant at pH 8.0 was measured by the casein digestion method of Kunitz (1947) using a digestion time of 120 min. One unit of proteolytic enzyme was defined as that activity which caused an increase in extinction of 0.1 at 280 nm. Activities were calculated with amounts of enzyme causing an increase in extinction of 0.3 or less.

RESULTS

Swarming ability

The ability of *P. mirabilis* to swarm over the surface of nutrient agar is notorious and to reduce swarming, minimal agar was used. Even on this latter medium, some colonies of *P. mirabilis* F67 swarmed whereas others did not (Fig. 1*a*). However, *P. mirabilis* F67(TEM) never gave rise to swarming colonies (Fig. 1*b*) whereas all colonies of *P. mirabilis* F67(1818) were found to swarm (Fig. 1*c*). Consequently, it was of interest to see how a strain harbouring both R factors behaved. Hence R-TEM was introduced into F67(1818) and R-1818 was introduced into F67(TEM). These organisms are referred to as F67(1818/TEM) and F67-(TEM/1818), respectively. Control tests on the antibiotic resistances of these strains showed that each R factor was stably maintained in both strains.

It can be seen (Fig. 1*d,e*) that the effect on swarming was determined by the R factor first present in the cell because F67(TEM/1818) did not produce any swarming colonies the same as F67(TEM), whereas F67(1818/TEM) gave rise to exclusively swarming colonies, like F67(1818).

Proteolytic enzyme production

The R factors also altered the level of extracellular proteolytic enzyme produced by *P. mirabilis* F67. The level was higher in supernatants from cultures of F67(TEM) and F67(TEM/1818) than in the supernatant from the parent R⁻ strain (Table 2). This increase

Fig. 1. Swarming of *Proteus mirabilis* on minimal agar. (a) F67; (b) F67(TEM), (c) F67(1818); (d) F67(TEM/1818); (e) F67(1818/TEM).

Table 2. *Proteolytic enzyme activities of supernatants from R⁺ cultures of P. mirabilis F67*

Organism	Proteolytic activity (units/10 ⁹ organisms)
F67	0.25
F67(TEM)	0.67
F67(TEM/1818)	0.76
F67(1818)	0
F67(1818/TEM)	0

Fig. 2. Sensitivity of R⁺ *P. mirabilis* F67 cultures to sodium deoxycholate. ○, R⁻; □, R-1818; △, R-TEM; ■, 1818/TEM; ▲, TEM/1818.

was not due to an R factor-mediated proteolytic enzyme, since no proteolytic activity was detected in cultures of *E. coli* J6-2(TEM). On the other hand, *P. mirabilis* F67(1818) or F67(1818/TEM) were found to have no detectable level of extracellular proteolytic activity.

Susceptibility to sodium deoxycholate

The previous effects may have been due to changes in the cell envelope and if this was the case, such changes could alter the response of the host cell to surface active agents. Hence the survival of R⁻ and R⁺ strains of *P. mirabilis* F67 on minimal agar containing increasing concentrations of sodium deoxycholate was determined by plating suitable dilutions of 24 h minimal medium cultures.

Whereas F67(1818) and F67(1818/TEM) behaved similarly to the R⁻ parent, F67(TEM) and F67(TEM/1818) were strikingly more sensitive to sodium deoxycholate (Fig. 2).

Transfer of R factors from *P. mirabilis* F67(TEM/1818) and F67(1818/TEM)

In strains harbouring both R-TEM and R-1818, it has been found that the R factor first resident in the cell is dominant in terms of its effect on swarming, proteolytic enzyme activity and susceptibility to sodium deoxycholate. One explanation for dominance could be that the incoming R factor had integrated into the genome of the resident plasmid to give a complex under the control of the R factor first present. To test this hypothesis, *P. mirabilis* F67(TEM/1818) and F67(1818/TEM) were mated with *E. coli* J6-2-2 and colonies selected for R-1818 or R-TEM were tested for co-transfer of the unselected R factor. The results (Table 3) show that both donors transferred R-1818 to *E. coli* approximately 100 times more frequently than they transferred R-TEM. Furthermore, colonies selected for R-1818 did

Table 3. *Transfer of R-TEM and R-1818 from P. mirabilis* F67(1818/TEM) and F67(TEM/1818) to *E. coli* J6-2-2

Donor	Transfer rate of R-TEM/donor	Co-transfer of R-1818*	Transfer rate of R-1818/donor	Co-transfer of R-TEM†
F67(1818/TEM)	9.7×10^{-5}	19/20	1.2×10^{-2}	0/20
F67(TEM/1818)	1.8×10^{-7}	5/9	1.2×10^{-5}	0/20

* Co-transfer of R-1818 was assessed by streaking colonies selected for R-TEM on to minimal agar containing 10 µg/Tc ml, 10 µg/Su ml and 100 µg/Sp ml.

† Co-transfer of R-TEM was assessed by streaking colonies selected for R-1818 on to minimal agar containing 300 µg/Am ml and 200 µg Sm/ml.

Table 4. *Transfer of R-TEM and R-1818 from E. coli* J6-2-2 to *E. coli* K12

Donor	Transfer rate of R-TEM/donor	Co-transfer of R-1818*	Transfer rate of R-1818/donor	Co-transfer of R-TEM*
J6-2-2(1818/TEM)	4.5×10^{-3}	5/20	4.0×10^{-4}	20/20
J6-2-2(TEM/1818)	1.2×10^{-3}	0/17	4.1×10^{-5}	11/14

* See Table 3.

not receive R-TEM, suggesting that R-1818 exists independently in both *P. mirabilis* F67(TEM/1818) and F67(1818/TEM). However, many colonies selected for R-TEM had also acquired R-1818. To determine whether this was due to recombination between the two R factors, *E. coli* J6-2-2 clones which had acquired both R factors were mated with *E. coli* K12. Recipients were selected for the presence of R-TEM or R-1818 and then tested for co-transfer of the unselected R factor. In all such crosses, the transfer rate of R-TEM was higher than that of R-1818 (Table 4) and thus opposite to the transfer rates observed in F67 to J6-2-2 crosses. Furthermore, most colonies selected for R-TEM had not acquired R-1818, suggesting that R-TEM is being transferred independently. However, colonies selected for R-1818 had usually also received R-TEM.

Since it has been possible to demonstrate independent transfer of R-TEM and of R-1818, albeit from different donor organisms, it would seem unlikely that the formation of a stable recombinant could have occurred in all cells of *P. mirabilis* F67(TEM/1818) or in F67(1818/TEM). Yoshikawa & Sakai (1972) have shown that recombinants between R factors exhibit a marked degree of instability. However, both strains of *P. mirabilis* which harboured R-TEM and R-1818 retained all their resistances stably.

DISCUSSION

Burman, Nordstrom & Bloom (1972) found that the susceptibility of *P. mirabilis* to cholate was increased by being grown in sub-inhibitory concentrations of ampicillin. This suggested that the mucopeptide acts as a penetration barrier, either directly or indirectly by virtue of its intimate association with the lipoprotein of the outer membrane (Braun, Rehn & Wolff, 1970). The opposing effects of R-TEM and R-1818 on swarming ability, proteolytic activity and deoxycholate sensitivity of *P. mirabilis* reported here could also be explained by these R factors affecting the structure of mucopeptide. If R-1818 increased mucopeptide bonding, the enhanced strength of the macromolecule would be commensurate with the increased swarming ability and reduced proteolytic enzyme excretion by strains harbouring R-1818; if R-TEM decreased mucopeptide bonding, this could account for the

abolition of swarming, increased proteolytic activity and susceptibility to deoxycholate observed. If these views are correct, it may be pertinent that these R factors mediate distinct β -lactamases (Dale & Smith, 1971*a,b*) since these enzymes are probably related to a wall biosynthetic enzyme (Pollock, 1967; Boman, Eriksson-Grennberg, Foldes & Lindstrom, 1967). Another possibility is that the R factors have a direct action on the cytoplasmic membrane. Brinton (1971) suggested that pilin mediated by sex factors is present in the membrane and that the stability of the membrane could be decreased by the presence of a large amount of this protein. However, this view is difficult to reconcile with the mating behaviour of *P. mirabilis* F67(TEM/1818) and F67(1818/TEM), since both organisms transferred R-1818 to *E. coli* 100 times more frequently than they transferred R-TEM.

The mating experiments also showed that both doubly R⁺ *Proteus* strains donate R-1818 independently of R-TEM. However, when transfer of R-TEM occurred, it was often accompanied by R-1818. *Escherichia coli* recipients which had received both R factors transferred R-TEM independently, while R-1818 was often transferred together with R-TEM. Therefore, in each cross, the R factor being transferred at the higher rate was also that exhibiting independent transfer. It would seem that the other R factor was rarely capable of conjugation unless in co-operation with the more active mating apparatus. Taken as a whole, these findings suggest that stable recombination in the doubly R⁺ *Proteus* strains is unlikely. Hence the dominance effects seen may result from the first R factor received by *P. mirabilis* occupying a preferred site, leaving an incoming R factor to occupy some other site from which it is unable to dictate alterations to the physiology of the host bacterium.

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