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THE EFFECT OF R FACTOR TRANSFER ON BACTERIAL GROWTH RATES

AN IN VITRO STUDY

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SØGAARD, HENRY: *The effect of R factor transfer on bacterial growth rates. An in vitro study.* Acta vet. scand. 1975, *16*, 388—395. — It has been studied whether R factor transfer had an inhibitory effect on the growth rate of Escherichia coli in mixed cultivation. A total of 39 antibiotic resistant E. coli strains were grown together with E. coli K12 W 3132 as prospective recipient. Thirteen crosses turned out successfully. When comparing generation times for these crosses with those of negative crosses no difference could be demonstrated. This, however, does not exclude that R factor transfer impairs bacterial growth capacity at a level which can not be detected in a short-term experiment.

R factor transfer; growth rate.

Resistance to antibiotics in gram-negative bacteria is to a large extent mediated by R factors, a class of plasmids transferred by direct cell-to-cell contacts. This mechanism of genetic recombination is known as conjugation.

In the transfer of R factors, three stages can be distinguished: The first stage is the formation of sex pili which form intercellular bridges. During the second stage, transfer of genetic material takes place through the sex pili, and finally a third stage is the phenotypic expression of the genes taking place in the recipient cell (*Mitsuhashi* 1971).

R factor transfer is an energy requiring process. Substances with an inhibitory effect on oxidative phosphorylation reduce transfer frequency in vitro (*Egawa et al.* 1961, *Mitsuhashi* 1965). It is not known at which stage this inhibition is effective. The present study was designed to investigate the possibility that the process of R factor transfer in mating cultures could have a measurable impact on the growth rate of donor and recipient strains.

MATERIAL AND METHODS

Bacterial strains

A total of 39 Escherichia coli strains were used as potential donors of R factors. The strains were all resistant to one or more of the following antibiotics: sulphonamides, tetracyclines, streptomycin, chloramphenicol, and ampicillin. They were isolated from faeces of healthy pigs. The techniques applied in isolation and sensitivity testing have been described previously (*Søgaard* 1973). A nalidixic acid resistant mutant of E. coli K12 W 3132 was used as prospective recipient in crosses with the resistant strains. W 3132 is an auxothroph strain requiring methionine for growth, and it is sensitive to the five antibiotics mentioned above. Its competence as recipient of R factors has been demonstrated in previous experiments.

Genetic transfer experiments

Donor strains and the recipient strain were grown separately overnight at 37°C in nutrient broth. Donor cultures, 0.02 ml, were then transferred to 10 ml of fresh nutrient broth together with 0.1 ml of W 3132. The mixed cultures were incubated at 37°C for 24 hrs. After incubation it was examined whether or not R factor transfer had occurred by plating onto Conradi-Drigalski agar plates supplemented with nalidixic acid (25 µg/ml) and one of the antibiotics to which the donor strain was resistant. This selective medium should allow only recombinant cells of the recipient strain to multiply. Ten-fold serial dilutions were prepared in saline, and 0.1 ml of the following dilutions were plated on the selective medium: 10^0, 10^{-2}, and 10^{-4}. The number of recombinant colonies were counted after incubation overnight at 37°C. One colony from each plate with growth was picked and identified as W 3132 by requirement for methionine. The transfer frequency was calculated and expressed as the ratio between the number of R+ recipient cells and the total number of recipient cells present after 24 hrs.' mixed cultivation.

Determination of generation times

The generation time (g) was determined for donor and recipient strains separately during mixed cultivation. During incubation, 0.1-ml aliquots of the cultures were removed at intervals of 1 hr. From these samples, ten-fold dilutions were made in

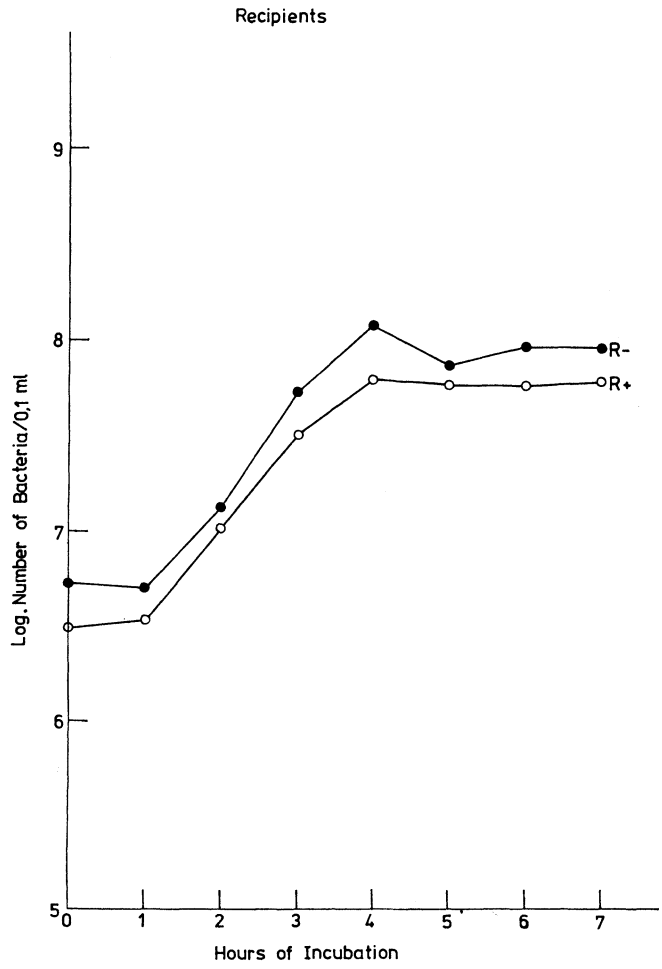

Figure 1. Growth curves for the recipient strain, E. coli K12 W 3132. Log. number of bacteria represents mean values from 13 R+ and 26 R— crosses, respectively.

saline and 0.1 ml of the dilutions plated on two different media: For selection of recipient cells was used Conradi-Drigalski agar with nalidixic acid incorporated (25 μg/ml). Donor cells were selected for on Conradi-Drigalski agar containing one of the antibiotics to which they were resistant. With this method, selection of donor cells could not be attained completely, since recipient cells which had acquired R factors at the time of sampling could

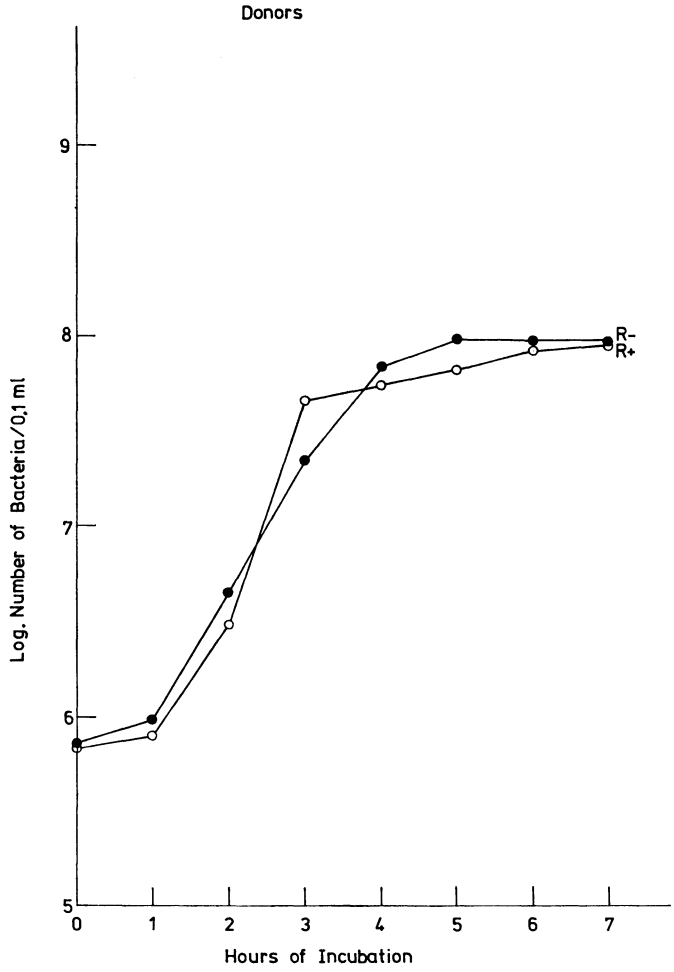

Figure 2. Growth curves for E. coli donor strains. Log. number of bacteria represents mean values from 13 R+ and 26 R— crosses, respectively.

also grow. Because of the dilution effect this error, however, was considered insignificant for the purpose.

Generation times were calculated during the exponential part of the growth curve (Figs. 1 and 2) from the equation

$$\frac{\log. N_4 - \log. N_1}{t_4 - t_1} = \frac{\log. 2}{g}$$

where N_1 and N_4 are the bacterial counts per 0.1 ml after incubation for 1 and 4 hrs., respectively (t_1 and t_4).

RESULTS

Of the 39 crosses, 13 were successful (R+ crosses), whereas in the remaining 26, no transfer of R factors could be demonstrated (R— crosses). Transfer occurred at frequencies ranging from 2.3×10^{-2} to 2.1×10^{-6} (Table 1).

Table 1. Frequency of R factor transfer.

Donor strain	Frequency of transfer*	% R+ recipient cells
S 105 II	2.3×10^{-2}	2.3
S 103 III	1.9×10^{-2}	1.9
S 104 I	1.1×10^{-2}	1.1
S 106 I	0.8×10^{-2}	0.8
S 104 III	0.5×10^{-2}	0.5
S 99 I	0.4×10^{-2}	0.4
S 90 II	0.4×10^{-2}	0.4
S 102 II	0.6×10^{-3}	0.06
S 89 I	1.2×10^{-4}	0.01
S 108 II	0.1×10^{-4}	0.001
S 103 I	0.6×10^{-5}	0.0006
S 96 I	2.1×10^{-6}	0.0002

* Frequency of transfer indicates the ratio between number of R+ recipient cells and the total number of recipient cells after 24 hrs.' mixed incubation.

Growth curves were traced for recipient and donor strains separately from both positive and negative crosses. In Figs. 1 and 2, the curves for donors and recipients from both types of crosses are shown together. It appears that logarithmic growth occurred between 1 and 4 hrs. after inoculation. The courses of the curves two and two appear to be practically identical. The ratio between donor and recipient cells at the beginning of mixed cultivation was 0.22 in R+ crosses and 0.14 in R— crosses.

Generation times were calculated from the logarithmic part of the growth curves (Table 2). For donor strains it was 0.49 hr. in both cases. The recipient strain was growing at a lower rate,

Table 2. Mean generation times ($\bar{g}$) and standard deviations (s) for donor and recipient strains in 13 R+ and 26 R— crosses.

Outcome of crosses	Recipients		Donors	
	$\bar{g}$	s	$\bar{g}$	s
R—	0.61	0.09	0.49	0.05
R+	0.64	0.17	0.49	0.05

the generation times being 0.61 hr. and 0.64 hr., respectively. This discrepancy, however, was statistically insignificant ($t = 0.60$, $P > 0.1$).

DISCUSSION

Since it was recognized that antibiotic resistance in pathogenic and commensal enteric bacteria could be mediated by extrachromosomal elements, the ecological and epidemiological aspects of this phenomenon has been intensively discussed. It is evident that an increase in R factor mediated resistance to commonly used antibiotics has occurred in pathogenic species of Enterobacteriaceae like Salmonella and Shigella (*Anderson* 1968, *Guinée* 1971, *Mitsuhashi* 1971). Evidence regarding E. coli is much more conflicting. *Smith & Halls* (1966) reported on increased resistance among strains isolated from pigs suffering from neonatal diarrhoea. *Slocombe & Sutherland* (1973) did not find any appreciable increase in the overall incidence of antibiotic resistance among human enteropathogenic strains of E. coli between 1957 and 1968. Several investigators on the other hand have reported on trends towards decreasing incidence both in humans (*Price & Sleigh* 1970, *Søgaard* 1974) and in animals (*Smith* 1973).

The latter reports indicate that cutting down of antibiotic consumption within an environment leads to ecological changes favouring sensitive bacteria. These changes suggest that bacteria carrying R factors are at an ecological disadvantage in vivo in the absence of selection pressure from antibiotics (*Anderson* 1974). This author demonstrated that strains of E. coli bearing R factors did not survive in the intestinal tract as well as strains without R factors when both were ingested simultaneously by human volunteers.

Other explanations can be suggested as to the disappearance of R+ bacteria in nature. Loss or segregation of R factors is

known to occur spontaneously (*Smith & Halls*). In this study it was attempted to study the possibility that the transfer process had an inhibitory effect on the growth capacity of bacteria. If this was the case it might contribute to levelling off of antibiotic resistant bacteria competing in nature with sensitive organisms. The study does not show any measurable effect during a single growth cycle of five-six generations. The results, however, do not exclude that R factor transfer impairs the growth rate at a level which can not be measured in a short term experiment, but which could be effective by evolutionary standards.

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SAMMENDRAG

Indvirkning af R faktor overførsel på bakteriers væksthastighed in vitro.

Det er undersøgt, hvorvidt overførsel af R faktorer har en hæmmende virkning på E. coli's væksthastighed i blandingskulturer. Ialt 39 antibiotikaresistente E. coli stammer isoleret fra svin er dyrket sammen med E. coli K 12 W 3132 som potentiel recipient. I 13 af disse krydsningsforsøg skete overførsel af R faktorer. En sammenligning af generationstider for positive og negative krydsninger viste ingen forskel. Det kan imidlertid ikke udelukkes, at R faktor overførsel hæmmer bakteriers væksthastighed på et niveau, som ikke lader sig påvise i et kortvarigt forsøg.

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