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Plasmids R577 and R785 decrease the resistance of *Escherichia coli* K12 strain W1485 to bactericidal action of normal serum

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Abstract. The role of plasmids of drug resistance R577 and R785 in the development of susceptibility of *Escherichia coli* K12 strain W1485 to the bactericidal action of serum was studied. Plasmids R577 and R785 were transfered to cells of strain W1485 by means of conjugation. The susceptibility of cells containing the plasmid to serum was compared to that of cells lacking the plasmid. It was found that plasmids R577 and R758 sensitize bacteria to the action of serum.

Key words: *Escherichia coli*, plasmid, serum.

Introduction

The complement plays a decisive role in the bactericidal activity of serum, which constitutes one of the mechanisms protecting higher organisms against infection with Gram-negative bacteria (JANKOWSKI, GRZYBEK-HRYNCEWICZ 1995). The bactericidal activity of newborn sera against various Gram-negative bacteria is lower than that of normal adult sera (JANKOWSKI 1995a). Drug resistance plasmids (R plasmids), particularly of the FII incompatibility group, are known to make Gram-negative strains more resistant to the complement-mediated bactericidal action of sera (REYNARD, BECK 1976, ABUL-MILH et al. 1987, SUKUPOLVI, O'CONNOR 1990, SIEGFRIED, PUZOVA 1991, PRAMOONJAGO et al. 1992, JANKOWSKI 1993, TAYLOR 1995). The resistance of bacteria to serum due to R plasmids is connected with a protective action of surface

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protein TraT which is encoded by the gene localized in the plasmid (MOLL et al. 1980, SUKUPOLVI, O'CONNOR 1990, PRAMOONJAGO et al. 1992).

It is interesting that some R plasmids can increase the susceptibility of bacteria to the action of sera. WRETLIND et al. (1985) have shown that cells of *Pseudomonas aeruginosa* strains bearing plasmids RP1 and RP8 are more susceptible to NHS than analogous plasmid-free cells. Concordant results have been obtained by ONAOLAPO et al. (1987) for *Proteus mirabilis* strains containing plasmid RP1.

Results indicating that other R plasmids increase the susceptibility of *E. coli* K12 W1485 cells to the action of NHS and NCS are described in the present work.

Material and methods

Strains and plasmids. Plasmid R577 bearing markers of resistance to tetracycline (Tc), chloramphenicol (Cm), vibramycin (Vb), sulphonamide (Su) and streptomycin (Sm) as well as plasmid aggregate R785 (Tc, Cm, Vb, Su) were obtained from two enteropathogenic *E. coli* strains (No. 577 and 785) isolated from children suffering from diarrhoea (ABUL-MILH et al. 1987). Both these strains were susceptible to nalidixic acid (Nal^S). *E. coli* K12 strain W1485 met $Nal^R Tc^S$ was used as a recipient of R plasmids. This strain was obtained from the collection of the Institute of Microbiology, Wrocław University.

Sera. Normal human serum (NHS) was derived from a regular donor. Pooled normal cord serum (NCS) was a mixture of sera obtained from three children. These sera, in samples of 0.1 mL, were kept frozen (–20°C). In some experiments NHS and NCS was inactivated for 30 min at 56°C (HS56°C, CS56°C). The donors of blood were not recently treated with antibiotics and did not suffer from *E. coli* infections.

Bactericidal assay of NHS and NCS. Bactericidal activity of serum was determined as described previously (JANKOWSKI et al. 1996). To 0.4% solution of NCS and 2.3% solution of NCS (diluted with 0.1M NaCl), bacteria were added. Their initial density of about 10^5 cells/mL derived from the early phase of logarithmic growth. Bacteria were incubated in serum at 37°C. After 0, 30, 60, 90 and 120 min samples of the suspensions were collected, diluted with 0.85% NaCl and plated on agar. After 18 h of incubation, the number of colonies was calculated taking the number of bacteria at 0 time as 100%. The results are means of three separate experiments.

Mating procedure. Conjugational assay was performed as previously described (ABUL-MILH et al. 1987): Conjugational plasmid transmission was

carried out by mixing exponentially growing broth cultures of donor and recipient followed by incubation of the mixture at 37°C for 2 h. The transconjugants were selected on minimal agar (Difco USA) plates supplemented with L-methionine (50 mg mL^{-1}), nalidixic acid (65 mg mL^{-1}) and tetracycline (20 mg mL^{-1}).

Results and discussion

Tables 1 and 2 illustrates the bactericidal action of NHS and NCS against *E. coli* K12 strain W1485 $R577^-$ as well as against cells of the same strain which acquired plasmid R577 ($R577^+$). The tables show that transconjugate cells were more susceptible to NHS (Table 1) and NCS (Table 2) than cells free of the plasmid. The sensitivity of strain W1485, whose cells contain

Table 1. Bactericidal action of normal human serum (NHS) against *Escherichia coli* K12 strains W1485 $R577^+$ and W1485 $R577^-$

Time of incubation (min)	Percentage of colony-forming units			
	Bactericidal action of NHS		Control*	
	K12 W1485R577$^-$	K12 W1485R577$^+$	K12 W1485R577$^-$	K12 W1485R577$^+$
30	80.2 ± 5.3	68.4 ± 37.2	135.4 ± 11.6	141.0 ± 7.4
60	52.4 ± 7.8	35.3 ± 4.5	172.5 ± 19.7	198.5 ± 11.1
90	35.3 ± 11.5	10.0 ± 0.8	222.3 ± 18.6	224.6 ± 31.5
120	7.2 ± 1.03	2.1 ± 0.7	244.6 ± 0.23	249.3 ± 12.8

*Growth of *E. coli* strains $R577^+$ and $R577^-$ in HS56°C.

plasmid R785, to the bactericidal action of NCS as well as of an isogenic strain without this plasmid is presented in Table 3. Cells of both forms grown after introduction to NCS, continued to divide after 30 minutes. This increase was greater in the case of $R785^-$ cells as compared to $R785^+$ cells. In the following period a more significant decrease of colony-forming units was observed for cells containing plasmid R785. After 90 min of incubation about 70% of $R785^-$ cells and only 10% of $R785^+$ cells survived. Tables 1 and 3 demonstrate that growth of *E. coli* cells with or without plasmid, in HS56°C and CS56°C, was comparable.

The results described in the present work indicate that plasmid R577 and plasmid aggregate R785 enhance the susceptibility of *E. coli* K12 strain W1485

Table 2. Bactericidal action of normal cord serum (NCS) against *E. coli* K12 strains W1485 R577$^+$ and W1485 R577$^-$

Time of incubation (min)	Percentage of colony-forming units	
	K12 W1485R577$^-$	K12 W1485R577$^+$
30	80.4 ± 3.7	72.3 ± 7.06
60	75.0 ± 5.2	52.5 ± 10.2
90	51.0 ± 7.9	35.2 ± 4.3
120	4.2 ± 1.8	2.2 ± 0.8

to the action of NHS and NCS. This effect is most probably caused by structural changes in the bacterial cell wall concerning proteins and/or lipopolysaccharides (LPS) which are the most important protective barriers against bactericidal action of serum (JANKOWSKI 1995b). This conclusion is supported

Table 3. Bactericidal action of normal cord serum (NCS) against *Escherichia coli* K12 strains W1485 R785$^+$ and W1485 R785$^-$

Time of incubation (min)	Percentage of colony-forming units			
	Bactericidal action of NCS		Control*	
	K12 W1485R785$^-$	K12 W1485R785$^+$	K12 W1485R785$^-$	K12 W1485R785$^+$
30	170.8 ± 7.5	142.2 ± 7.03	172.1 ± 8.6	157.4 ± 12
60	145.0 ± 8.6	50.0 ± 7.7	214.1 ± 25.6	208.2 ± 6.6
90	65.1 ± 9.5	15.7 ± 4.4	254.3 ± 22.7	257.0 ± 11.2

*Growth of *E. coli* strains R785$^+$ and R785$^-$ in CS56°C.

by an observation of SUKUPOLVI et al. (1987). These researchers noticed that a mutation of the insertion type in plasmid R6-5 is responsible for a variation in amino acid sequence of TraT protein consisting in an introduction of negatively charged asparagine or glutamine residues into this region, which resulted in increased outer membrane permeability of *E. coli* K12 LE390 cells to hydrophobic antibiotics and detergents. Additionally, the ability of the protein to increase serum resistance was diminished in these mutants.

Another factor sensitizing bacterial cells may be structural changes in LPS taking place in the presence of plasmids. DERYŁO et al. (1975) have shown

that some plasmids occurring in *Salmonella typhimurium* rods cause definite changes in the structure of LPS. Importance of LPS for resistance of bacteria against bactericidal action of serum has been verified by experements performed on Ra-Re mutants with various degrees of LPS damage. The mutants Ra characterized by a low degree of LPS damage are in most cases more resistant to the action of serum than the Re forms in which the LPS defect is serious (JANKOWSKI 1995b). Small changes in LPS consisting in replacement of some sugar moiety by another or its removal, change considerably the susceptibility of a strain to the action of serum. This has been observed, e.g., in the case of *Shigella flexneri* rods (DOROSZKIEWICZ et al. 1994).

A damage to the structure of LPS is frequently accompanied by a loss of some surface proteins, e.g., OmpA. It is therefore quite probable that sensitization of the cells of *E. coli* K12 strain W1485 which aquired plasmids R577 or R785 is conditioned by a mechanism which compares variation both in the structure of proteins and of LPS.

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