SOME BIOLOGICAL PROPERTIES OF CELLS OF *Escherichia coli* SK

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Cells of Escherichia coli SK are not colicinogenic but they possess multiple resistance to antibiotics (tetracycline, kanamycin, penicillin, polymixin, ampicillin). By cloning of the original culture the whole series of variants sensitive to one or more of the above-mentioned antibiotics was obtained. Resistance of the E. coli SK cells to all five antibiotics could not be completely removed with acridine orange. The partial changes in the spectrum of antibiotic resistance did not affect the system of host specificity present in the SK cells. Capacity for restriction and activity of methylating enzymes were identical in all the clones studied and in the original strain.

KEY WORDS: multiple resistance to antibiotics; cloning; modification; restriction.

As the writers showed previously [9] strain E. coli SK has a unique system of host specificity, which differs from other systems of modification and restriction in bacteria of the genus Escherichia.

The object of the present investigation was to determine the connection between the system of host specificity of the SK type and certain biological properties of the cells of this strain which may be due to the presence of plasmids in them.

EXPERIMENTAL METHOD

E. coli strains SK, C, φ, and P 678 and phages PBV-1 and PBV-3, belonging to the group of DNA-containing viruses [11], were used.

Bacteriocinogenicity was determined by the method described by Fredericq [7]. Sensitivity to antibiotics was determined qualitatively by means of indicator disks and quantitatively by serial dilutions of antibiotics in dishes [2]. The antibacterial activity of the antibiotics was expressed in activity units (a.u.) corresponding to the activity of a known weight of the chemically pure preparation on a test microorganism. For most antibiotics 1 a.u. corresponded to 1 μg of the chemically pure preparation.

Phages PBV-1 and PBV-3 were titrated on cultures of E. coli C., E. coli SK, and its variants by the agar layers method [5].

Elimination with acridine orange was carried out by Hirota's method [8]. DNA of phage S_q was isolated by the phenol method [4]. Methylating activity was determined in a crude extract of E. coli SK cells of the wild type and its variants, as described previously [3].

EXPERIMENTAL RESULTS

1. Colicinogenicity. Strain E. coli SK was tested for colicinogenicity by using the known international strains E. coli φ and E. coli P 678, sensitive to all colicins, as the indicator cultures. The results showed that the cells of E. coli SK do not synthesize colicin.

2. Resistance to Antibiotics. It was shown by the disk method that the test culture is sensitive to streptomycin (Sm), chloramphenicol (Cm), and neomycin (Ne) but resistant to penicillin (Pn), tetracycline (Tc), kanamycin (Km), polymixin (Pol), and ampicillin (Amp).

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The results obtained by determination of resistance to antibiotics indicated that the population of the strain is heterogeneous for this feature and has a low level of resistance to Tc (5-10 units/ml) and Km (5-10 units/ml). The culture was cloned and selected clones were tested for sensitivity to Pn, Tc, Km, Amp, and Pol. The results of the study of resistance of the original strain E. coli SK and its clones to antibiotics are given in Table 1.

It will be clear from Table 1 that cells of the original strain of E. coli SK possessed multiple drug resistance. They were resistant to Tc, Km, Pn, Amp, and Pol and retained their sensitivity only to Sm, Cm, and Ne. The clones of strain E. coli SK had narrower spectra of resistance to antibiotics than the original strains. For instance, clone SK_1 had lost its resistance to Km and Amp and clone SK_2 to Km; clone SK_5 remained resistant only to Pn, clone SK_4 only to Tc, and clone SK_9 only to Km, and so on. On the whole, the pattern of multiple resistance to antibiotics observed and the appearance of clones in the R^+ strain in which only one resistance was absent, the low level of resistance to certain antibiotics, and the instability of these features are characteristic of species of enterobacteria which carry transmissive plasmids of the PTF series [1]. However, this unequivocal interpretation of the results is somewhat complicated by the fact that by using acridine orange as eliminating agent it has so far proved impossible to obtain cells that are completely antibiotic-sensitive. Meanwhile these negative data cannot be taken as evidence of the chromosomal localization of the determinants of antibiotic resistance, for several plasmids are known which are not eliminated by acridine orange [1].

3. The Restricting Capacity of Clones of E. coli SK. The next step was to study to what extent differences in antiobiotic resistance of the isolated clones correlate with changes in the properties of the system of host specificity.

Experiments accordingly were carried out on the cross titration of phages PBV-1 and PBV-3, capable of replication in cells of both E. coli SK and E. coli C, which do not possess host specificity systems [10]. The results, given in Table 2, show that within the limits of accuracy of the titration experiments, the host specficity system in the original strain SK did not differ from that in its clones. The effectiveness of titration of PBV-1 (SK_{orig}) and of PBV-3 (SK_{orig}) phages on wild-type cells and on the various clones was the same. The effectiveness of titration of these phages on cells of E. coli SK_{orig} also was the same after their propagation in cells of the different clones – SK_5, SK_7, etc. (see Table 2). Acquistion of sensitivity to seven antibiotics (SK_5 and SK_7), just as to six antibiotics (SK_{30}, SK_{36}), is thus unaccompanied by any changes in the ability of the cells of these clones to modify and restrict phages with the corresponding phenotypes.

It must, however, be emphasized that one or other form of resistance to antibiotics was present in all the clones of E. coli SK studied. Unfortunately we were unable (see above) to obtain a variant completely sensitive to the antibiotics tested. It cannot therefore be categorically asserted as yet that the host specificity system is determined by the same genetic determinants as control antibiotic resistance.

4. Methylating Activity of Variants of E. coli SK. Modification of DNA is known to be based on a methylation reaction, which is carried out by specific DNA-methylases [6]. To study the possible relationship between changes in antibiotic resistance and methylase activity, experiments were carried out to determine the comparative activity of these enzymes in extracts from cells of the wild type of E. coli SK and three of its variants: SK_5, SK_7, and SK_9.

TABLE 1. Sensitivity of Original Strain E. coli SK and Its Clones to Antibiotics

Strain, clone	Resistance to antibiotics	Sensitivity to antibiotics	
		initial	acquired
E. coli SE_{orig}	Tc, Km, Pn, Amp, Pol	Sm, Cm, Ne	
Clone SK_1	Tc, Pn, Pol	The same	Km, Amp
" SK_2	T, Pn, Pol, Amp	"	Km
" SK_4	Tc, Km, Pn, Pol	"	Amp
" SK_5	Pn	"	Tc, Km, Amp, Pol
" SK_6	Km, Pn, Pol	"	Tc, Amp
" SK_7	Tc	"	Km, Pn, Amp, Pol
" SK_9	Km	"	Tc, Pn, Amp, Pol
" SK_{23}	Tc, Km	"	Pn, Amp, Pol
" SK_{30}	Pn, Pol	"	Tc, Km, Amp
" SK_{36}	Pol, Amp	"	Tc, Km, Pn

TABLE 2. Effectiveness of Infection of Different Clones of E. coli SK by Phages PBV-1 and PBV-3*

Strain† of bacteria	Phenotype of phage‡							
	PBV-1				PBV-3			
	C	SK_{orig}	SK_5	SK_7	S	SK_{orig}	SK_5	SK_7
E. coli C	100	100	—	—	100	100	—	—
E. coli SK_{orig}	0,005	100	—	60	0,001	133	—	60
E. coli SK_5	—	71	100	60	0,001	90	100	120
E. coli SK_7	0,005	140	120	100	0,001	130	110	100
E. coli SK_9	0,003	85	—	—	0,001	115	—	—
E. coli SK_{20}	0,004	100	—	—	0,001	95	—	—
E. coli SK_{26}	0,004	110	—	—	0,001	120	—	—

*Effectiveness of infection given as a percentage of infectious titer in a homologous system, taken as 100%. For example, the titer of phage PBV-1, expressed in cells of E. coli C and titrated on cells of the same strain, is taken as 100%, and the effectiveness of titration in the heterogeneous system, i.e., on strains SK_{orig} and its variants, is calculated relative to this value.

†Strains of bacteria on which titration was carried out. Significance of SK_{orig}, SK_5, and SK_7 the same as in Table 1.

‡The phenotype of a phage, according to the generally accepted nomenclature, is named after the strain of the host on which it grew. For instance, PBV-1 (SK_5) signifies phage PBV-1 grown in cells of E. coli variant SK_5.

TABLE 3. Methylating Activity of Crude Extract of Cells of Original Strain E. coli SK and Its Clones*

Strain, clone	Resistance	Methylating activity	
		cpm/0.5 ml incubation mixture	units of enzyme activity†
SK_{orig}	Tc, Pn, Km, Pol, Amp	5,800±500	24±0.5
SK_5	Pn	5,300±700	22±0,7
SK_7	Tc	6,100±400	25±0,4
SK_9	Km	5,900±500	24±0,5

*Methylating activity was determined in 0.5 ml of incubation mixture containing 0.1 M potassium phosphate buffer, pH 7.0, 100 μg acceptor DNA of phage Sd, 2 ml protein of crude extract, and 2.5 μCi [^{3}H]SAM. Mixture incubated for 2 h at 37°C.

†The unit of enzyme activity is expressed in cpm calculated per 100 μg acceptor DNA and per milligram protein per minute.

The results are summarized in Table 3 and they show that activity of DNA methylation enzymes is identical in the cells of the original strain E. coli SK and the various clones obtained from it.

It can thus be concluded on the basis of existing data that a change in sensitivity to individual antibiotics is not accompanied by corresponding changes in the modification-restriction. Further identification of the genetic elements responsible for the host specificity system of SK type will require the performance of transfection and conjugation experiments, and these have now been begun.

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SPECIFIC BLOCKAGE OF THE IMMUNE RESPONSE BY AN EXCESS OF Salmonella typhi Vi-ANTIGEN

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Intravenous injection of 200 μg of Vi-antigen into adult mice induces a state of short-term (10-12 days) areactivity in the animals. The observed depression of the immune response is due to blockage of the immunocompetent cells and not to masking of antibody production through binding with the excess of free antigen. Washing a suspension of spleen cells twice before using them in the local passive hemolysis in gel test did not reduce the blockage of the immune response; moreover, no free antigen capable of binding antibodies produced by cells of the immune animal could be found in the spleen of the experimental animals. Blockage of the immune response could be abolished by injecting heterologous antiserum against Vi-antigen into the animals 18-24 h before Jerne's test. Injection of 6-thioguanine after 200 μg of Vi-antigen prevented restoration of the immune response by means of the antiserum. It is concluded from the results that injection of a massive dose of Vi-antigen does not block proliferation and differentiation of antigen-recognizing cells but inhibits the synthesis or secretion of antibodies.

KEY WORDS: Vi-antigen; tolerance; immunologic paralysis.

Injection of a large dose of polysaccharide antigens induces immunologic paralysis in mice [6-8]. The mechanism of this phenomenon has been adequately studied for paralysis induced by the polysaccharide of type III pneumococcus and by other antigens of similar structure [7, 8]. The form of areactivity observed has been shown to be due to blockade of lymphocytes by antigen and not to binding of the antibodies by the excess of antigen (peripheral neutralization). With respect to the Vi-antigen of Salmonella typhi it is known that injection of a massive dose of this polysaccharide into mice sharply reduces the blood antibody titers [1, 3].

The object of this investigation was to study whether this effect is due to peripheral neutralization of antibodies or to blockade of lymphocytes by the antigen.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male mice weighing 18-20 g. A commercial preparation of S. typhi Vi-antigen produced by the Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR, was used as the antigen [4].

The experimental mice were given an intravenous injection of 200 μg Vi-antigen and the controls an injection of 10 μg of the same antigen (the optimal immunogenic dose). The number of antibody-forming cells (AFC) in the spleen was determined 4 days later by the passive hemolysis in gel method (Jerne's test) using sheep's red cells loaded with Vi-antigen [2]. Hyperimmune rabbit sera against Vi-antigen were obtained by injecting 100-200 μg Vi-antigen at monthly intervals several times into rabbits. Blood was taken on the seventh

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