experiments indicate that affinity of peptides R and L for the DNA fragment containing the 3′-flanking region of the gene was greater than for the fragment containing the 5′-flanking region.

The results thus demonstrate specific interaction of peptides R and L with regulatory sequences of the IL 2 gene. The negative or positive character of regulation of IL 2 production by T lymphocytes may evidently be due to complex formation between specific nucleotide sequences of the IL 2 gene and the above-mentioned peptides. So far there has been only one indication in the literature of the existence of a protein nuclear factor AP-I [3], capable of regulating expression of the inducible IL 2 gene through interaction with its sequences. It is probable that peptide factors R and L may give an independent; regulatory effect, whereas peptide SKD may act indirectly through other protein factors.

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NEW HOST DNA SPECIFICITY SYSTEMS PAE 610 AND PAE 603

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The rapid development of genetic engineering has been largely due to the discovery and use of highly specific restriction endonucleases and modifying methylases, constituting the host specificity system (HSS) of DNA. Several hundreds of these enzymes are now known [13], but the broad front of research requires expansion of the arsenal of enzymes and identification of new restriction endonucleases and methylases. This task is linked, in turn, with the search for and study of new DNA restriction and modification (r-m) systems.

The aim of this investigation was to discover HSS among strains of *Pseudomonas aeruginosa* and to study them. Identification of RM systems in these microorganisms in the proposed research, by contrast with systems described previously [5, 9, 10, 11], is based on priority of biological testing, which enables a large number of strains to be tested and the distribution of HSS among microorganisms of a given species to be tested and, finally. the discovery of RM systems to be differentiated from the HSS already known.

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TABLE 1. Determination of Seeding Efficiency (SE) of Certain Phages of *Ps. aeruginosa* on Different Strains of *Ps. aeruginosa*

Phage, phenotype	SE on strains of Ps. aeruginosa**			
	573	1610	1603	PAO 303 (PMG 7)*
FP 638·573	1,0	$1,5\times10^{-5}$	$1,0\times10^{-4}$	$1,5\times10^{-3}$
FP 638·PMG 7	1,0	$1,0\times10^{-5}$	$5,0\times10^{-5}$	1,0
FP 638·1610	1,2	1,0	1,0	$1,1\times10^{-4}$
FP 638·1603	1,1	1,0	1,0	$5,0\times10^{-3}$
FP 31·1610	1,25	1,0	1,0	—
FP 31·573	1,0	$2,0\times10^{-4}$	$1,0\times10^{-3}$	$1,8\times10^{-4}$
FP 644·PMG 7	1,0	—	—	1,0
BI 16·573	1,0	$2,0\times10^{-3}$	—	$2,5\times10^{-3}$
BI 16·1610	1,0	1,0	1,0	—
BI 16·1603	1,0	1,0	1,0	—
BI 16·PMG 7	1,0	—	—	1,0

*PMG 7) Plasmid determining HSS of Pae R7.

**Degree of adsorption in all cases 78-87%; adsorption time 5-7 min.

TABLE 2. Seeding Efficiency of Some FP Phages with Different Phenotypes on Lysogenic Clones of *Ps. aeruginosa* 573 (FP 610)

Phage, phenotype	SE on strains of Ps.aeruginosa		
	573	573 (FP 610)	1610
FP 638·573	1,0	0,6	5×10^{-5}
FP 638·573 (FP 610)	1,0	1,0	1×10^{-5}
FP 638·573 (FP 610)	1,3	1,1	2×10^{-5}
FP 638·610	1,1	1,0	1,0
FP 610·573	1,0	0	0

EXPERIMENTAL METHOD

We used 102 freshly isolated strains of *Ps. aeruginosa,* bacteriophages of the BT series, and five temperate bacteriophages of the FP series from the collection of the "Bakteriofag" Research and Production Combine. LB or M9 nutrient media were used [3, 4]. Biotyping was carried out as in [2]. The strains were screened by the S test [6] and lines of bacteriophages were prepared by the agar ovrerlay method [8]. Temperate phages were isolated and studied as in [1]. Conjugation was carried out by the method in [15], transformation of plasmid DNA as in [13]. Plasmid DNA was isolated by the standard method [3, 4].

EXPERIMENTAL RESULTS

I. Detection of HSS. Detection of RM systems by cross titration of phages assumes, as we know, the existence of a "null" strain, known not to contain an HSS, and also a number of bacteriophages which have a relatively broad spectrum of lytic action and are active against the "null" strain. As the latter we used strain *Ps. aeruginosa* 573. Cross titration was carried out with 14 bacteriophages, including nine virulent phages of the BT series and five temperate phages of *Ps. aeruginosa,* series P, isolated in the laboratory. Thus a collection of 102 strains of *Ps. aeruginosa* was tested.

The ability of the bacteriophages to undergo restriction was tested in a preliminary series of experiments, and phages reproducing in *Ps. aeruginosa* PAO 303 cells, possessing the Pae R7 HSS with an efficiency $\leq 1 \cdot 10^{-2}$ were selected for future work. Table 1 shows as an example that temperate phages FP 638, FP 647, and FP 644 underwent restriction in cells of *Ps. aeruginosa* PAO 303 (PMG 7). Besides those already mentioned, other test phages for cross titration included

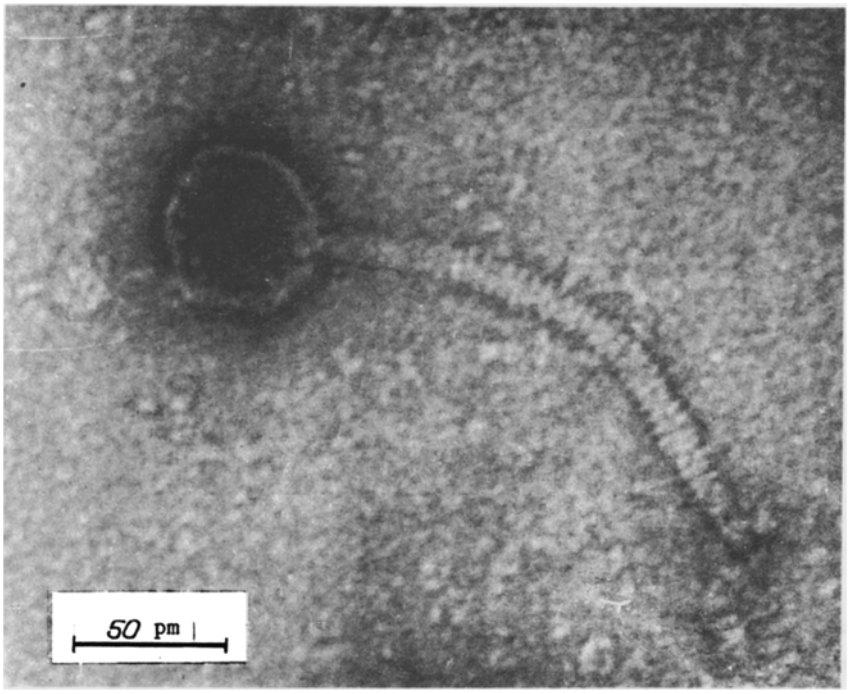

Fig. 1. Electron micrograph of bacteriophage FP 610 of *Ps. aeruginosa.* JEM-CX-1200 electron microscope. Stained with 1% uranyl acetate.

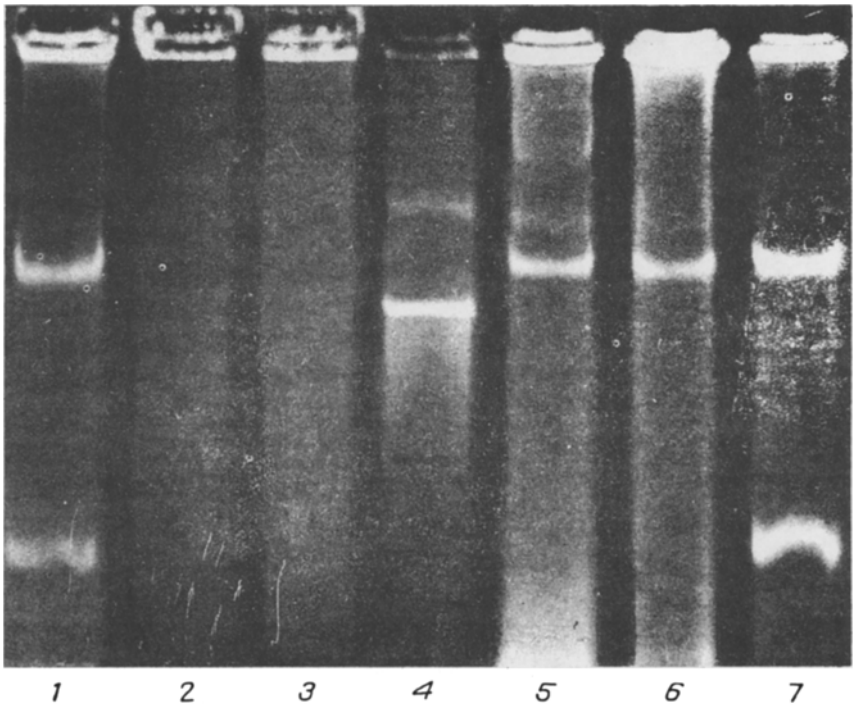

Fig. 2. Plasmid composition of different strains. 1) K-PBR 322 (4.4 kbp); 5) *Ps. aeruginosa* 1647; 6) *Ps. aeruginosa* 1721; 7) *Ps. aeruginosa* 1610 plasmid DNA was isolat;ed by alkaline extraction and electrophoresis in 1% agarose gel.

TABLE 3. Seeding Efficiency of Phage FP 638 with Different Phenotypes on Some Strains of *Ps. aeruginosa* and Their Derivatives

Bacterial strain		Markers of antibiotic resistance	SE for infection by phage			
			FP 638·573	FP 638·M 4262	FP 638.EL 1*	FP 638.TR 4**
Ps. aeruginosa	573	—	1,0	1,4	1,2	1,2
»	610	Km Cb Tc Em	5×10^{-5}	4×10^{-4}	2×10^{-5}	1,0
»	ML4262	—	0,5	1,0	—	1,0
»	610 EL 1	Em	0,22	—	1,0	—
»	610 EL 2	Km Em	0,4	—	1,0	—
»	610 EL 3	Em	0,5	—	1,0	—
»	ML 4262 TR 1	Km Tc	1×10^{-5}	1×10^{-4}	—	1,0
»	ML 4262 TR 2	Km	1×10^{-4}	5×10^{-4}	—	1,0

Legend. EL) eliminated clones; TR) clones of transformants; *) similar results were obtained in all cases on elimination of the plasmids; **) the results were similar for all transformants studied.

FP 631, BT 16, BT 35, BT 34, BT 10, and BT 8. According to data in Table 1, the seeding efficiency (SE) of phages FP 638.573, FP 31.573, and BT 16.573 on strains *Ps. aeruginosa* 1610 and 1603 was 3-5 orders of magnitude less than the titer on the "null" strain. Given a high degree of adsorption, the result justifies the conclusion that HSS is present in strains *Ps. aeruginosa* 1610 and *Ps. aeruginosa* 1603. According to the accepted nomenclature [17] these RM systems can be designated Pae 610 and Pae 603 respectively. Table 1 also shows that HSS Pae 610 and Pae 603 are identical, for phages with the "610" and "603" phenotype are not mutually limiting on cross titration, and a higher degree of restriction relative to heterologous phages is observed in cells of *Ps. aeruginosa* 1610.

It is important to note that according to data in Table 1, strains of phage FP 638 with the PMG 7 phenotype, on the one hand, and with the 610 and 603 phenotype, on the other hand, on cross titration are mutually restricting, indicating that the HSS discovered differ from the known Pae R7 system.

II. Biological characteristics of strains carrying HSS, namely *Ps. aeruginosa* 1610 and *Ps. aeruginosa* 1603, were determined by comparison with the "null" strain *Ps. aeruginosa* 573. All the microorganisms mentioned exhibit similar staining and cultural-biological properties: they are Gram-negative, are identical in spectrum of carbohydrate fermentation, and in mobility. The spectrum of antibiotic resistance (Km_{100}, Cb_{200}, Tc_{50}, Em_{100}) and phage sensitivity of *Ps. aeruginosa* 1610 and 1603 is identical and differs from that for the control strain (Km_{100}, Em_{100}). Both species studied possess bactericidin-producing activity relative to the control variant. Cells of strains *Ps. aeruginosa* 1610 and 1603 are lysogenic and spontaneously produce temperate phages, designated FP 610 and FP 603 respectively, and are closely related to one another in their lytic spectrum and virion morphology. Phages FP 610 and FP 603 have a hexagonal head measuring 50.0 × 56.0 nm and a long tail measuring 185.0 × 10.0 nm. According to Slopek's classification [16] the phages studied belong to the Styloviridae class, type II. Analysis of the plasmid content in strains *Ps. aeruginosa* 1610 and 1603 (Fig. 1) leads to the conclusion that the plasmid profile of these two strains is identical (Fig. 2, tracks 1 and 7).

We chose for further investigation strain *Ps. aeruginosa* 1610, as providing a higher degree of restriction of the infecting phage.

III. Genetic Determinant of the RM System of Pae 610. Genes of the restriction-modification system can be coded by chromosomal, phage, or plasmid DNA [7, 9].

To identify the role of prophage in the formation of the RM system of Pae 610 experiments were carried out with artificial lysogenization of *Ps. aeruginosa* 573 cells by temperate phage Pae 610. After necessary subculture on solid nutrient media the clones of *Ps. aeruginosa* 573 thus obtained were tested for immunity to lysogenizing phage and sensitivity to other phages. The presence of lysogenizing phage FP 610 in the cells of the test clones was revealed by seeding on cells of the nonlysogenic parent *Ps. aeruginosa* 573. Of the 22 clones 12 were found to be immune to superinfecting homologous phage and they produced phage FP 610, which appeared on a lawn of *Ps. aeruginosa* 573. Thus these 12 clones of *Ps. aeruginosa* 573 were lysogenic for phage FP 610 and were called *Ps. aeruginosa* 573 (FP 610).

To study the problem of transmission of the r^+m^+ system during artificial lysogenization, cross titration of phage FP 638 with the phenotype "573" and "573" · (FP 610) was carried out on *Ps. aeruginosa* 610, *Ps. aeruginosa* 573, and the test lysogenic clones *Ps. aeruginosa* 573 · (FP 610) cells.

As Table 2 shows, lysogenization of cells of *Ps. aeruginosa* strain 573 by phage FP 610 makes these cells immune to phage FP 610, but does not endow them with the ability to restrict proliferation of bacteriophage FP 638.573. Under these circumstances, after repeated passage on "573 · (FP 610)" cells phage 638 is again restricted on the r^+ host *Ps. aeruginosa* 1610. On the basis of the above description it can be concluded that the Pae 610 HSS discovered is not phage-specific.

During elimination of determinants of resistance to antibiotics by the use of mitomycin C, loss of resistance to Tc, Cb, Em, and Km by 94.5, 89.3, 75.3, and 32% respectively took place in *Ps. aeruginosa* 1610 cells. In clones undergoing elimination, the presence of restricting properties characteristic of Pae 610 was tested (Table 3). Loss of plasmid was monitored electrophoretically. In another series of experiments recipient cells of plasmid DNA were transformed. Transformation of *Ps. aeruginosa* ML 4262 cells by plasmid DNA from *Ps. aeruginosa* 610 took place with a high frequency, of the order of $1 \cdot 10^2$. Transformants were screened in order to detect r — m properties in relation to the test phages (Table 3). The transformants were selected relative to several markers: Km, Em, Cb, and Tc.

It will be clear from Table 3 that during multiplication of phage FP 638 · 573 in *Ps. aeruginosa* 1610 cells, where the plasmids were eliminated, no evidence of restriction was observed. Meanwhile, during infection of transformants, phage FP 638 · 573 was restricted by 4 or 5 orders of magnitude, just as in the case of the original r^+ strain *Ps. aeruginosa* 1610. The results indicate that genes of the Pae 610 HSS are located on plasmids, and during their transformation, the RM properties are transmitted to the recipient cells. On elimination of the plasmid, together with loss of the resistance markers, the r^+m^+ phenotype also is lost.

The plasmid location of r^+m^+ genes in strain *Ps. aeruginosa* 1610 can thus be regarded as proved.

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