

Supplementary material

Detection and level evaluation of antibodies specific to environmental bacteriophage I11mO19 and related coliphages in non-immunized human sera

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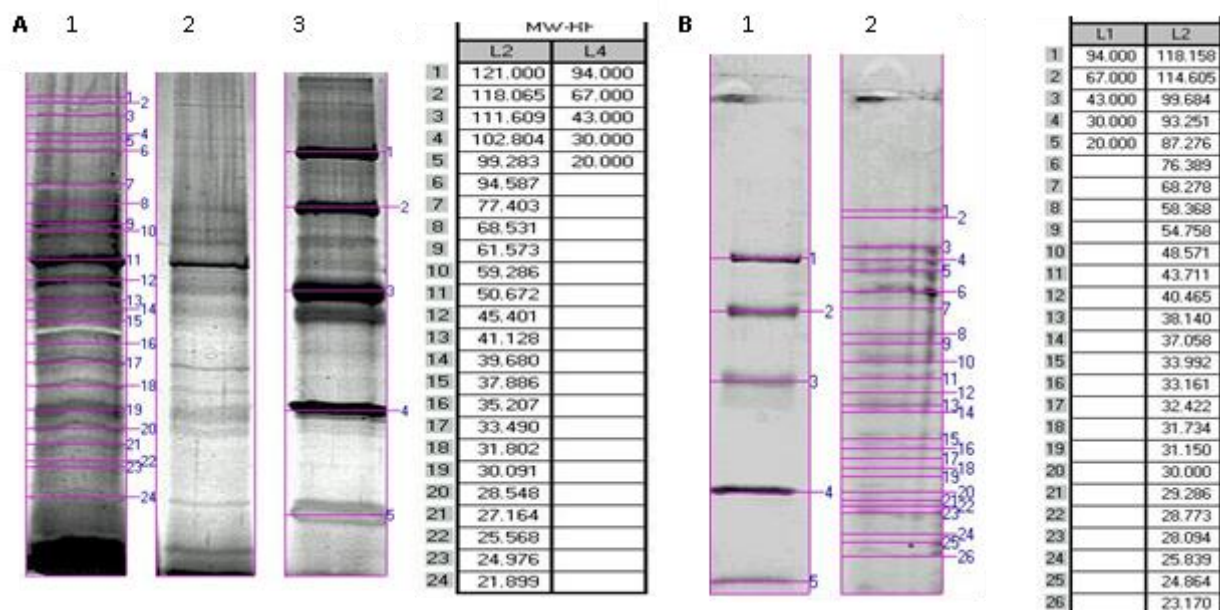


Figure S1. Molecular weights of bacteriophage I11mO19 proteins (A) – lanes 1 and 2), 3) molecular weight standard. (B) Proteins of *E. coli* D- the host of I11m19 phage – lane 2, 1) molecular weight standard. Proteins were separated in a 12.5% gel by electrophoresis under denaturing conditions. Bacterial lysate proteins were stained with Coomassie Brilliant Blue R-250 and phage proteins with silver. Molecular weights were analyzed using Bio-Profile Bio-1D++ Windows Application V99.04.

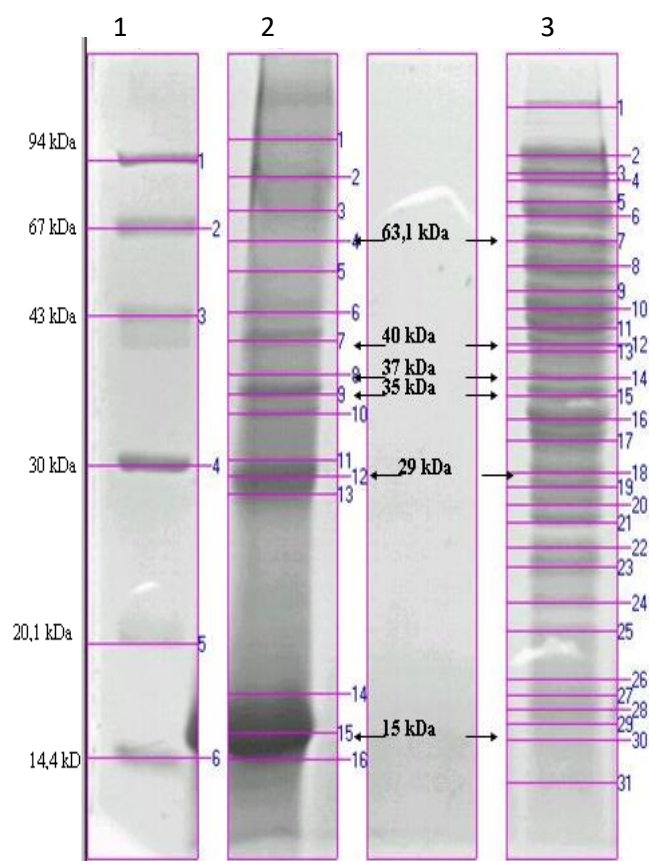


Figure S2. Comparison of Φ K1E bacteriophage (2) and *E. coli* K1 proteins (3). (1) Molecular weight standard. Proteins were separated in a 12.5% gel by electrophoresis under denaturing conditions. Proteins were stained with Coomassie Brilliant Blue R-250. Molecular weights were analyzed using Bio-Profile Bio-1D++ Windows Application V99.04

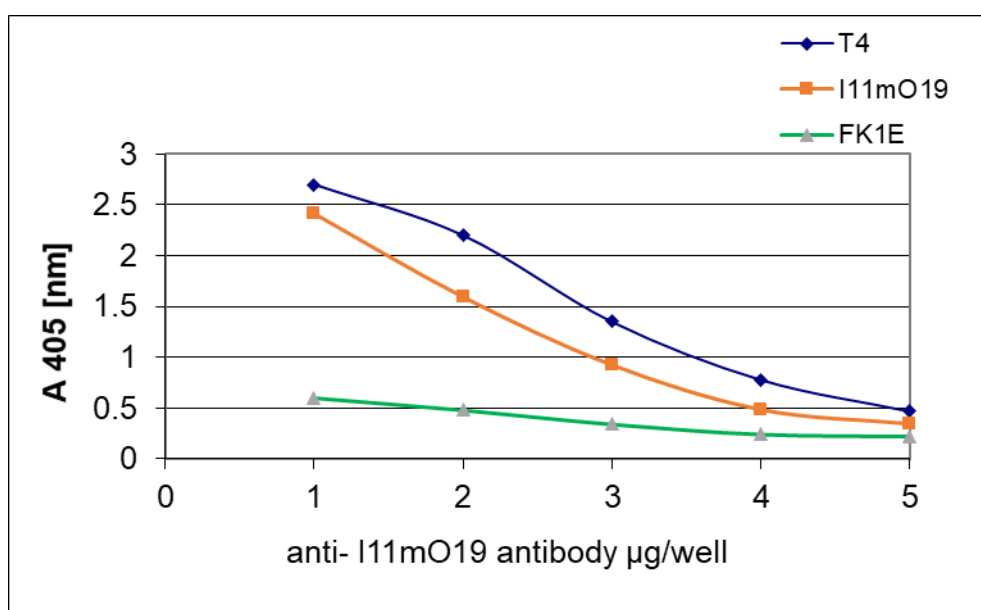


Figure S3. Immunoreactivity of isolated anti-I11mO19 antibodies with phage proteins; ϕ K1E (green), I11mO19 (orange), and T4 (dark blue). The amount of phage proteins was 1.0 μ g per well.

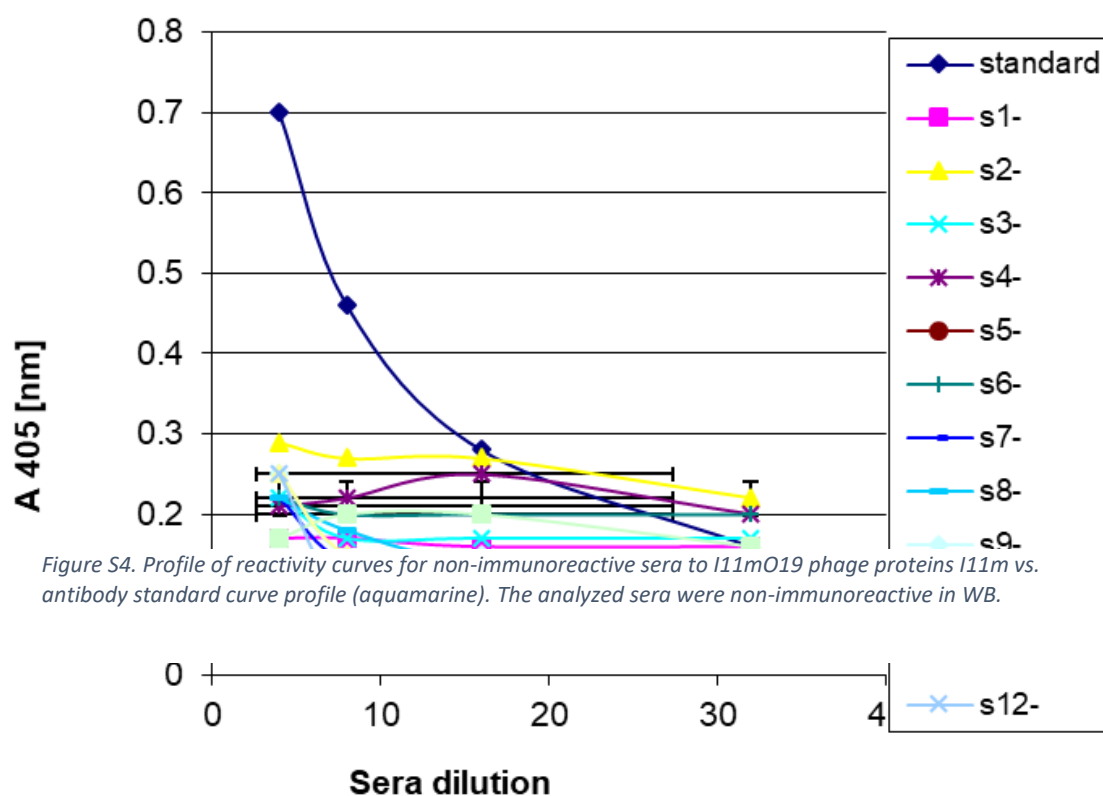


Figure S4. Evaluation of immunoreactivity of human sera determined as negative to I11mO19 phage in ELISA. Standard (dark blue) stands for anti-I11mO19 antibody isolated in affinity chromatography. 1.0 μ g of I11mO19 phage proteins coated the plate well, and human sera were diluted 4; 8; 16; and 32 times. The amount of 'standard' was in the range of 0.06 to 1.2 μ g. The conjugate - goat anti-human IgG with alkaline phosphatase was diluted 1/10000.

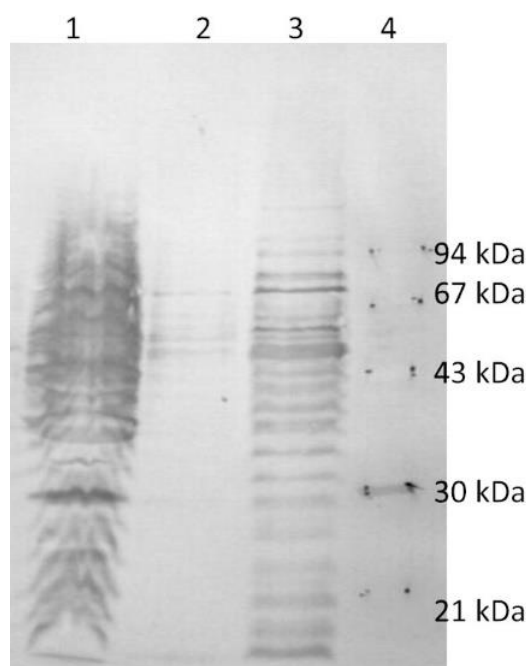


Figure S5. Immunoreactivity analysis of human serum to proteins of *E. coli* D – the host for I11mO19 phage (1) and phage I11mO19 (2) and the host's LPS (3). The human serum 250 times diluted.

Table S1. Analysis of molecular weights of bacteriophage T4 and *E. coli* B proteins separated by electrophoresis in a 12.5% polyacrylamide gel under denaturing conditions. Molecular weights were analyzed using Bio-Profil Bio-1D++ Windows Application V99.04. The same MW in phage and in bacteria protein's panel are marked in blue

Molecular weights of bacteriophage T4 proteins [kDa]	Molecular weights of <i>E. coli</i> B proteins [kDa]
133.7	96.8
123.8	90.7
115.3	74.0
101.1	57.9
79.6	51.1
74.0	45.4
68.2	40.5
56.2	35.6
46.3	32.9
44.3	31.6
38.3	27.2
35.6	23.3
33.9	19.8
30.9	16.5
22.5	12.6
14.8	

Table S2. Analysis of molecular weights of bacteriophage F8 and *P.aeruginosa* proteins separated by electrophoresis in a 12.5% polyacrylamide gel under denaturing conditions. Molecular weights were analyzed using Bio-Profile Bio-1D++ Windows Application V99.04

Molecular weights of bacteriophage F8 proteins [kDa]	Molecular weights of outer membrane proteins of <i>Pseudomonas aeruginosa</i> [kDa]
92.83	96.54
57.64	85.48
47.98	75.81
35.7	72.38
27.82	65.35
15.86	58.07
	55.73
	45.28
	41.64
	40.28
	37.83
	32.12
	28.03
	27.15
	24.26
	21.54
	20.74
	19.11
	17.63
	16.35
	15.79
	15.05

Table S3 Table showing the average A405 values obtained in the ELISA test in the reaction of the standard (i.e., anti-I11m antibodies)diluted from ¼ to 1/128, with I11m phage proteins.

Standard dilution	Arithmetic mean of A ₄₀₅	Standard deviation (sD)
¼	0.765	0.086
1/8	0.527	0.053
1/16	0.315	0.026
1/32	0.157	0.029
1/64	0.082	0.010
1/128	0.034	0.004