NEW BIOMEDICAL TECHNOLOGIES

Construction of a *Yersinia pestis* Strain with High Protective Activity

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An experimental genetically engineered plague vaccine strain LAG1 has been constructed, ensuring 27 times better protection (due to capsular antigen) than the maternal culture of commercial live antiplague vaccine (EV strain of the Research Institute of Epidemiology and Hygiene).

Key Words: *Yersinia pestis; genetically engineered vaccine*

Capsular antigen fraction 1 (F1) is the main component of the *Yersinia pestis* capsule [6]. In animals infected with plague the highest titers of antibodies are detected to this antigen [11]. Correlation of the degree of humoral response to F1 with the protective properties of antiplague vaccines containing it as a component indicates that it is the principal immunogen of *Y. pestis* [7]. However, the live and killed vaccines used today may cause side effects due to their high content of "ballast" (from the viewpoint of protective properties) antigens of *Y. pestis* [4,9,10]. In Russia commercial live antiplague vaccine (CAV) prepared on the basis of the attenuated strain of *Y. pestis* EV is traditionally used for immunoprophylaxis [4].

The purpose of this study was to construct an experimental vaccine strain of *Y. pestis* with a reduced content of ballast antigens by superproduction of the main protective antigen of plague agent F1.

MATERIALS AND METHODS

The strains of *Y. pestis* used in this study are presented in Table 1, and plasmids in Table 2.

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Cultures of *Y. pestis* were grown for 48 h and of *Escherichia coli* for 24 h in solid nutrient media based on enzymatic meat hydrolysate. Bacterial cells of *Y. pestis* for serological tests and *E. coli* cells were cultured at 37°C; *Y. pestis* bacteria for immunization and infection of outbred white mice weighing 20±2 g were cultured at 28°C.

Genetic engineering manipulations were carried out as described elsewhere [3] using *E. coli* strain HB101. The SalGI fragment with the *kan* gene from plasmid pUC4K was inserted at the SalGI site of the EcoRI fragment of the pFra plasmid cloned as a component of the pFS1 plasmid [2]. The EcoRI fragment with a locus of the *fra* operon labeled with the *kan* gene from the thus obtained pFSK1 plasmid was inserted in the EcoRI fragment of plasmid pPst including the plasmid replication domain (*ori*) and the genes coding for plasminogen activator (*pla*), pesticine production (*pst*), and immunity to pesticine (*imm*). The resultant construction was named pFSK3 (Fig. 1).

Plasmid pFSK3 was transferred to *Y. pestis* cells by electrostimulated transformation.

The level of capsular antigen production was determined in the passive hemagglutination test with commercial immunoglobulin erythrocytic diagnosticum [5].

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TABLE 1. Characteristics of *Y. pestis* and *E. coli* Strains Used in the Study

Strain	Virulence determinants	Level of production of F1 antigen, mg/ml medium	Source
Y. pestis 231 (708)	Fra⁺Tox⁺Lcr⁺V⁺Pst⁺Pla⁺Pgm⁺	Not measured	Museum of Live Cultures, Russian Research Antiplague Institute *Mikrob*
Y. pestis EV (maternal culture)	Fra⁺Tox⁺Lcr⁺V⁺Pst⁺Pla⁺Pgm⁻	0.078	L. A. Tarasevich State Research Institute for Standardization and Control of Biomedical Preparations
Y. pestis Km217 (EVpFra⁻pCad⁺pPst⁻)	Fra⁻Tox⁻Lcr⁺V⁺Pst⁻Pla⁻Pgm⁻	-	Provided by O. A. Protsenko
Y. pestis LAG1 (Km217pFSK3)	Fra⁺Tox⁻Lcr⁺V⁺Pst⁺Pla⁺Pgm⁻	>9.6	Obtained by the authors
E. coli HB101	-	-	[3]

Note. *Fra:* production of capsular antigen; *Tox:* synthesis of "murine" toxin; *Lcr:* relationship between *Y. pestis* growth and synthesis of external membrane proteins by its cells, on the one hand, and presence of Ca^{2+} ions in the nutrient medium at 37°C, on the other; *Pst:* pesticine production; *Pla:* fibrinolytic and plasma–coagulase activity (plasminogen activator); *Pgm:* pigment sorption.

TABLE 2. Characteristics of Plasmids Used in the Study

Plasmid	Marker of antibiotic resistance	Source
pFS1	Ap^R, Tc^R	[2]
pFSK1	Ap^R, Tc^R, Km^R	Obtained by the authors
pFSK3	Km^R	Obtained by the authors
pUC4K	Ap^R, Km^R	[14]

In parallel with this, the protective properties of the experimental vaccine strain and commercial antiplague vaccine were assessed. Mice were subcutaneously immunized with suspensions of 2-day cultures of the above preparations in 0.89% NaCl in doses of 10^3, 5×10^3, 2.5×10^4, and 1.25×10^5 colony-forming units (CFU) per animal, 30 mice being infected with each dose. Twenty-one days after immunization the animals were subcutaneously inoculated with a similarly prepared suspension of *Y. pestis* strain 231 in a dose of 10^5 CFU (2.9×10^3 LD_{50}) per animal. The mice were followed up for a month after inoculation. The ImD_{50} value (the dose preventing death in 50% of infected animals) and the confidence interval were calculated after a modified [1] Koerber's method. The confidence interval was calculated for 95% probability.

RESULTS

A variant of the EV strain possessing the only plasmid of its own, calcium-dependence, was used as the recipient strain during the construction of the experimental vaccine strain. Insertion of plasmid pFSK3 constructed by us restored the ability of this strain to produce plasminogen activator coded for by the pesticinogenicity plasmid and needed for providing effective invasion of the cells of the experimental vaccine strain [13]. The level of capsular antigen secretion in the cells of the genetically engineered strain surpassed that in the EV strain of the Research Institute of Epidemiology and Hygiene

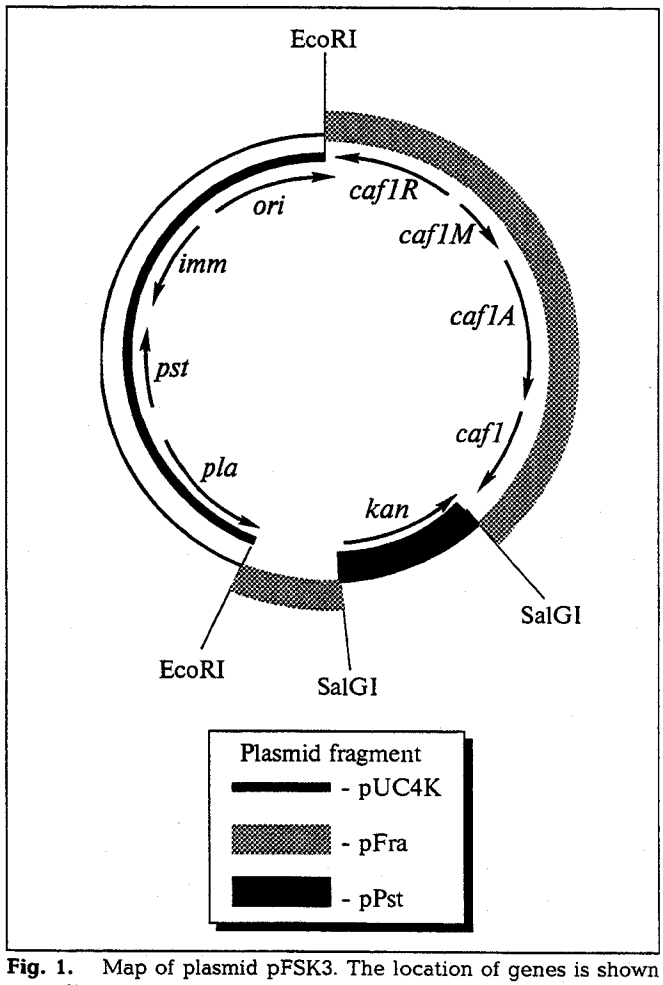

Fig. 1. Map of plasmid pFSK3. The location of genes is shown according to published data [8,12,14].

more than 100 times. This seems to be due to the increase of the gene dose.

Comparative assessment of the protective properties of the experimental vaccine strain and commercial antiplague vaccine showed that the ImD_{50} for the former was 86 (33-505) CFU and 2363 (906-16,299) CFU for the latter strain.

Hence, the LAG1 strain of *Y. pestis* which we designed may serve as the basis for the development of a new live antiplague vaccine containing fewer ballast antigens thanks to the use of a lower immunizing dose.

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