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A Recombinant, Prototrophic *Yersinia pestis* Strain Over-produces FI Antigen with Enhanced Serological Activity

SVETLANA V. DENTOVSKAYA, RIMA Z. SHAIKHUTDINOVA, and ANDREI P. ANISIMOV
State Research Center for Applied Microbiology, Obolensk, Moscow Region, Russia

1. INTRODUCTION

Immunization with the capsular FI antigen induces protection against lethal plague challenge (Meyer *et al*., 1974; Simpson *et al*., 1990). Our recent studies have shown that *Y. pestis* cells expressing the recombinant pFSK3 plasmid produce FI antigen with 10^2-10^4-fold enhanced ability to react with antibody to FI when compared to the amount of FI obtained from the wild type *Y. pestis*. Moreover, the recombinant strain possessed 30-fold improved protection when compared with the Russian commercial live vaccine (EV line NIIEG) (Anisimov *et al*., 1995). Over-production of FI along with its enhanced potency is of obvious for formulation of a subunit plague vaccine.

2. ENGINEERING AND STUDYING *YERSINIA PESTIS* STRAIN OVER-PRODUCING FI ANTIGEN WITH ENHANCED SEROLOGICAL ACTIVITY

In this study plasmid pFSK3 was transferred into the prototrophic, plasmid-less derivative of *Y. pestis* vaccine strain EV, EV11M. Bacteria were grown for 24 h at 28 ºC or 37 ºC in LB broth. The results of

determinations of the total-protein concentrations (Lowry *et al*., 1951) in the culture supernatants are shown in the table 1.

Table 1. Concentration of total protein in cell-free supernatant

Strain	Total protein in culture liquid under different growth temperatures, mg/ml	
	28 ºC	37 ºC
EV line NIIEG	0.013	0.011
EV11MpFSK3	0.461	0.001

Indirect (passive) hemagglutination test was performed with the broth cultures, supernatants, and bacterial pellets to determine comparative serologic activity (table 2).

Table 2. FI antigen serological activity

Strain	Indirect hemagglutination test (reciprocal titers)					
	28 ºC			37 ºC		
	Broth culture	Supernatant	Pellet	Broth culture	Supernatant	Pellet
EV line NIIEG	4	-	-	64	64	64
EV11MpFSK3	8192	8192	65536	32	64	512

Cell-free FI antigen was precipitated directly from culture supernatants by adjusting the pH to its isoelectric point, 4.1. The insoluble pellets were suspended in 0.9% NaCl solution, pH 7.2, (one fiftieth of the initial supernatant volume). This FI antigen preparation was evaluated in denaturing PAGE (data not shown) and an indirect hemagglutination test (table 3), which confirmed the advantage of producing the antigen from the recombinant strain in comparison with wild type *Y. pestis*.

Table 3. Total yields of FI from supernatants of broth cultures of *Y. pestis* strains

Source of FI	FI yield, mg/liter		Relative value of serologic activity [a]	
	28 ºC	37 ºC	28 ºC	37 ºC
EV line NIIEG	13.4	11	9.3×10^{-3}	9.0×10^{-2}
EV11MpFSK3	230.6	1.4	9.0×10^{4}	2.8

[a] Expressed as FI units per milligram of protein. One FI unit is serological activity of 1 mg/ml solution of commercial preparation of FI (Stavropol' Research Anti-Plague Institute, Russia) in indirect hemagglutination assay.

Strain EV11MpFSK3 was able to produce significant amounts of FI antigen even in minimal nutritional media supplemented with glucose 0.2% (data not shown).

3. CONCLUSION

Growth of *Y. pestis* EV11MpFSK3 at 28 °C yields high levels of highly serologically active FI antigen, which is a promising component of subunit plague vaccines.

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