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PLASMID PROFILES OF *YERSINIA PESTIS* STRAINS ISOLATED IN NORTHEAST BRAZIL

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ABSTRACT

Plasmid composition in 182 *Y. pestis* strains from six natural plague foci of Northeast Brazil was analyzed by gel electrophoresis. One hundred and six strains (61.5%) displayed the classical plasmid pattern composed of the three well-characterized *Y. pestis* plasmids, fifty-six strains (33.3%) missed at least one of them and twenty (11.0%) strains displayed additional cryptic plasmids. These variations on the plasmid profile were observed among strains originated from the foci of Chapada do Araripe, Planalto da Borborema, Serra da Ibiapaba and Bahia while all the strains tested from the Triunfo and Serra de Baturité foci displayed the classical plasmide profile.

Key words: *Y. pestis*, plasmids, plague.

INTRODUCTION

Based on the study of a few laboratory strains (6, 9, 14) it was postulated that *Y. pestis*, the causative agent of plague, has a typical plasmid profile (pYV ≅ 70kb, pPst ≅ 9.5kb and pFra ≅ 90kb) 6, 9, 14, 16.

The pYV codes for calcium-dependent growth of cells incubated at 37°C and synthesis of a set of proteins called Yops (9, 15). pPst codes for pesticin as well as the production of a plasminogen activator and a coagulase (18). Finally, pFra codes for the F1 capsular antigen and the murine toxin (16).

Screening of the plasmid profiles in bacteria proved to be a rather simple and fast technique able to offer useful epidemiological data (13). Attempts to characterize the plasmid content of wild type *Y. pestis* strains have been restricted to samples isolated in Asia, mainly in the territory of the formerly called URSS (10) and in Mongolia (4). Most of the strains have shown to carry the three classical plasmids. However, it was observed that strains isolated from voles, rodents obtained in the Caucasus, lack the pPst plasmid. A few additional cryptic plasmids as well as a considerable size variation of the pFra and pYV plasmids have been observed, and a possible correlation with the geographical characteristics of these strains has been put forward (4, 10).

In Brazil, there are several independent *Y. pestis* foci in rural areas, mainly in the Northeast region (5, 20). A preliminary evaluation of the plasmid profiles of 26 strains, isolated during a plague outbreak at one of these foci (2) has revealed a homogeneous pattern composed of the three ubiquitous *Y. pestis* plasmids and an additional 22.5 kb extra chromosomal DNA band not recoverable by plasmid isolation techniques based on alkaline lysis performed in small scale extraction (11, 12).

In our present work we analyzed the plasmid content in 182 *Y. pestis* strains isolated from six plague foci in Northeast Brazil (1,2). The results of this study confirm the presence of the three classical *Y. pestis* plasmids in most of the Brazilian strains. Surprisingly,

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a fraction of the tested strains missed at least one of these plasmids whereas a few strains carried additional cryptic bands. These variations on the plasmid profile had been found among strains from the foci of Chapada do Araripe, Planalto da Borborema, Serra da Ibiapaba and Bahia. All the strains tested from the Triunfo and Serra de Baturité foci displayed the classical plasmide profile. No clear relationship could be drawn between plasmid profiles and geographical or epizootiological characteristics of the strains analysed.

MATERIALS AND METHODS

The 182 *Y. pestis* strains studied were from the bacterial strain collection of the "Centro de Pesquisas Aggeu Magalhães". These strains had been isolated from different hosts and distinct geographic foci from the Northeast Brazil during the period between 1966 to 1986 (1,2) and maintained at +4°C in stabs of Blood Agar Base (BAB, Difco Laboratories, Michigan USA). The reference strain *Escherichia coli* 39R861 from the National Collection Institute of Public Health Laboratory (Collin Dale, London) the vaccine strain *Y. pestis* EV 76 or our *Y. pestis* PPB 862 previously studied (2, 11, 12) were employed as controls. Before plasmid examination the strains were cultivated at 28°C in Brain Heart Infusion Broth (BHI = Difco) during 24 up to 72 h, plated on BAB plates to ensure purity, and grown for 24 h in BHI for plasmid extraction.

Plasmid extraction was performed by a small scale alkaline lysis technique based on the procedure described by Birnboim and Doly (7) followed by electrophoresis on 0,6% agarose gels and ethidium bromide staining.

The phenotypic plasmid markers calcium-dependent growth at 37°C coded by the pYV (9), synthesis of the bacteriocin pesticin coded by the pPst (18), and the synthesis of the F1 antigen coded by the pFra (16) were studied as described previously (3).

RESULTS

Plasmid analyses were carried out in 182 *Y. pestis* strains recovered from patients (76 strains), rodents (68 strains) and flea vectors (38 strains) from the plague foci of Chapada do Araripe (126 strains), Planalto da Borborema (3 strains), Triunfo (15 strains), Serra de Baturité (6 strains), Serra da Ibiapaba (29 strains) and Bahia (3 strains). Out of these, 113 strains (62.08%) displayed the classical plasmid profile composed by the pYV, pPst and pFra plasmids (Table 1).

Fifty-six strains (33.3%) missed at least one of these plasmids (incomplete classical plasmid profile) (Table 1). Among them, 46 strains missed 1 plasmid, 9 strains missed 2 and 1 strain missed all the three. Between these 56 strains, 22 (12,08%) lacked pPst (pPst$^-$ strains), 4 strains (2.19%) lacked pYV (pYV$^-$ strains) and 40 (21.97%) strains lacked pFra (pFra$^-$ strains). The pFra was the most frequently missing

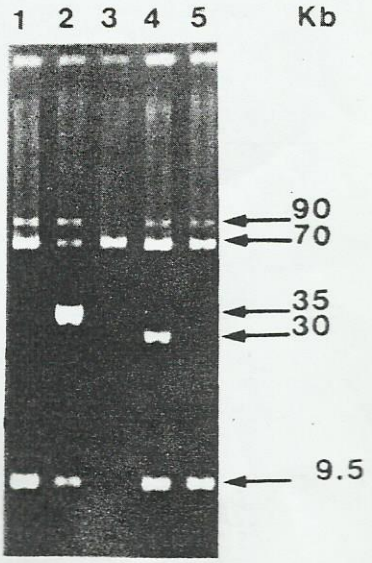

Figure 1: Plasmid profiles of some representative *Y. pestis* strains from different plague foci in Northeast Brazil. Lines 1, P.EXU 315; 2, P.EXU 216; 3, P.EXU 189; 4, P.EXU 115; 5, EV76 (vaccinal *Y. pestis* strain).

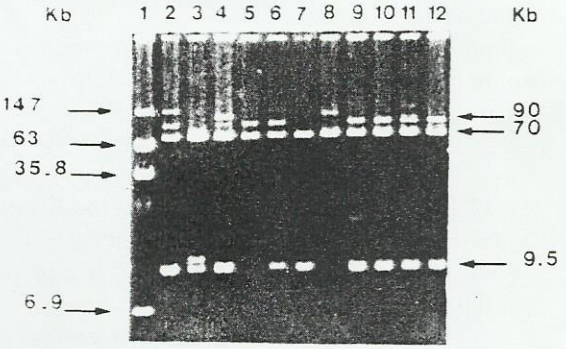

Figure 2: Plasmid profiles of some representative *Y. pestis* strains from different plague foci in Northeast Brazil. Lines 1, *E.coli* 39R861 (reference strain); 2, P.EXU 260; 3, P.EXU 264; 4, P.EXU 266; 5, P.EXU 267; 6, P.EXU 270; 7, P.EXU 271; 8, P.EXU 289; 9, P. EXU 275; 10, P.EXU 277; 11, P.EXU 420; 12, EV76 (vaccinal *Y. pestis* strain).

Table 1: Plasmid profile of the brazilian *Yersinia pestis* strains.

Origin of the strains			Plasmid Profile			
Foci	Year	N° strains	A	B	C	D
Chapada do Araripe	1966-1975	126	73	08	40	05
Ibiapaba	1971-1982	29	16	04	07	02
Planalto da Borborema	1979-1986	03	01	-	02	-
Triunfo	1967-1979	15	15	-	-	-
Bahia	1978	03	02	01	-	-
Baturité	1978	06	06	-	-	-
Total		182	113	13	49	07

A = classical plasmid profile
B = classical plasmid profile with additional cryptic bands
C = incomplete classical plasmid profile
D = incomplete classical plasmid profile with additional cryptic bands

Table 2: *Yersinia pestis* strains cured of at least one of the virulence-associated plasmids

Plague foci	N° Strains	Cured plasmid[a]			Phenotipic expression[b]		
		pFra	pYV	pPst	$F1^+$	Cad^-	Pst^-
Chapada do Araripe	45	31	2	17	29	2	17
Serra da Ibiapaba	9	7	2	4	7	2	4
Planalto da Borborema	2	2	-	1	2	-	1

a = number of strains in which each plasmid was missing
b = +, expression of the plasmid-encoded phenotype; -, no detectable expression of plasmid-encoded phenotype
$F1^+$ = F1 production.
Cad^- = No detectable expression of calcium dependence at 37°C.
Pst^- = No detectable expression of Pesticin production.

plasmid (40/56), followed by the pPst (22/56) and pYV (4/56). These results could suggest that plasmid pYV is more stable than the other two plasmids. Plasmid-lacking strains were isolated from the foci of Chapada do Araripe (45/126), Serra da Ibiapaba (9/29) and Planalto da Borborema (2/3) while all the strains from Triunfo, Serra de Baturité and Bahia had all the three classical plasmids (Table 2).

The analyses of the phenotypic expression of some prominent plasmid-encoded properties have shown that accordingly all the 22 $pPst^-$ strains were negative for the expression of pesticin (18), the 4 pYV^- strains were calcium independent as they were able to grow at 37°C in the MOX medium (14). Curiously enough, 38 $pFra^-$ strains remained proficient for the F1 antigen synthesis whereas only 2 were unable to do so (Table 2).

Twenty strains (11.0%) carrying additional cryptic bands with molecular weights (147 to 11.5 kb), distinct from those of the classical plasmids, were found (Table 3; Figs. 1, 2). Among these strains, 13 had all the typical plasmids (classical plasmid profile with additional cryptic bands) while 7 have also shown the lack of at least one of the classical *Y. pestis* plasmids. (incomplete classical plasmid profile with plasmid additional cryptic bands) (Table 3 and Fig. 1). *Y. pestis* strains carrying cryptic bands were found in only three plague foci: 13 out of 126 strains (10.3%) from the Chapada do Araripe focus, 6 out of 29 (26.7%) from the Serra da Ibiapaba focus and 1 out of 3 (33.3%) from the Bahia focus. Additional bands could be detected in strains isolated from men, rodents and fleas regardless of the isolation period (Table 3).

Based on the molecular weight, the additional cryptic bands could be grouped into three classes: one composed of bands greater than the pFra, another group with sizes ranging between those corresponding to pFra and pYV and one group smaller than the pYV but greater than the pPst (Figs. 1, 2; Table 3).

In conclusion, four major plasmid patterns can be distinguished for the brazilian *Y. pestis* strains (Table 1). Most of them (62.0%) displayed the classical plasmid profile. The others (38%), can be distributed into three different patterns: incomplete classical plasmid profile, caracterized by the absence of at least one of the typical plasmids (26.9%); classical plasmid

Table 3: The 20 *Y.pestis* strains carrying additional cryptic plasmids, their phenotypes, hosts, geographical origin and year of isolation.

Strains	Plasmids (kb)								F1	Cad	Pst	Hosts	Foci	Year
P.EXU 260	>90	90		70				9.5	+	+	+	Man	C. Araripe	1968
P.EXU 861	>90	90		70				9.5	+	+	+	Rodent	S. Ibiapaba	1982
P.EXU 266	>90	90		70				9.5	+	+	+	Man	C. Araripe	1968
P.EXU 420	>90	90		70				9.5	+	+	+	Flea	C. Araripe	1970
P.EXU 274	90			70					+	+	-	Man	C. Araripe	1968
P.EXU 289	>90			70				9.5	+	+	-	Man	C. Araripe	1968
P.EXU 114	>90	90		70				9.5	+	+	+	Rodent	C. Araripe	1967
P.EXU 273		90	82	70				9.5	+	+	+	Rodent	C. Araripe	1968
P.EXU 263		90	82	70				9.5	+	+	+	Rodent	C. Araripe	1968
P.EXU 799		90	82	70				9.5	+	+	+	Rodent	Bahia	1978
P.EXU 554		90	82	70				9.5	+	+	+	Man	S. Ibiapaba	1972
P.EXU 803		90	82	70					+	+	+	Man	S. Ibiapaba	1978
P.EXU 556		90	82	70				9.5	+	+	-	Man	S. Ibiapaba	1972
P.EXU 264			82	70			11.5	9.5	+	+	+	Man	C. Araripe	1968
P.EXU 429	>90			70				9.5	+	+	+	Flea	C. Araripe	1971
P.EXU 509			82	70				9.5	+	+	+	Man	S. Ibiapaba	1971
P.EXU 115		90		70		30		9.5	+	+	+	Flea	C. Araripe	1967
P.EXU 789		90		70	32			9.5	+	+	+	Man	S. Ibiapaba	1978
P.EXU 216		90		70	35				+	+	+	Rodent	C. Araripe	1968
P.EXU 228		90				29			+	-	-	Rodent	C. Araripe	1968

F1 - F1 production;Cad - Calcium dependence at 37°C;Pst - Pesticin production

profile with additional cryptic bands (7.1%) and incomplete classical plasmid profile with additional cryptic bands (3.8%).

DISCUSSION

The growing information on the plasmid content of wild strains of *Y. pestis*, originated from different natural plague foci in Asia, has allowed to relate distinct plasmid patterns to specific hosts or geographic origins of the strains (4, 10).

Our present work conducted on 182 *Y. pestis* strains recovered from diverse hosts and plague foci from Northeast Brazil has revealed that most of the strains displayed a classical plasmid profile composed of the three well-characterized plasmids: pYV, pPst and pFra. This might reflect the origin of the plague in Brazil which was introduced through a single entry site, the port of Santos in the Southeast region of the country, during the last great pandemic of plague at the end of the 19th century (20). The fact that a single strain has colonized the region and the relatively short period since then could explain this prominent plasmid pattern.

Strains lacking one, two or all the three classical *Y. pestis* plasmids have been observed. However, no correlation could be established between these strains and their origins. We do not believe that these strains could represent true wild type spontaneous variants since most of them were collected from diseased mammalian hosts, and essential virulence-associated factors in *Y. pestis* are plasmid encoded (8). Therefore, it is quite probable that some of these variants have been selected during the prolonged storage (up to 25 years). On the other hand, insertion sequences promoting the integration of the plasmids pYV and pFra into the chromosome of *Y. pestis* have been previously detected (17, 21). Therefore, it is also reasonable to speculate that the absence of plasmids in some strains represents their integration into the chromosome of the bacteria. Furthermore, the occurrence of 38 pFra$^-$ strains synthesizing F1 strengthens this hypothesis and is in agreement with previous observations that the pFra can integrate into

the *Y. pestis* chromosome without losing plasmid-encoded functions (17). A better evaluation of the integration of plasmids into the chromosome of the bacteria would demand the use of specific probes in Southern-blot experiments.

Another observation was the presence of additional cryptic extra chromosomal DNA bands of varied molecular mass on 20 strains originated from three foci. These findings confirm previous observation that the presence of additional plasmids in *Y. pestis* is a rather frequent phenomena (10, 19). Five different plasmids with molecular mass ranging from 35 to 11.5 kb were found respectively in each one of the five strains originated from two plague foci. Even though we could not establish any relevant epidemiological feature associated with these additional plasmids, they were remarkable for their stability on the respective strains, high copy number and their similarity with previously observed plasmids in wild (10) and laboratory *Y. pestis* strains (17). By contrast, the additional high molecular weight bands (a group higher than the pFra, and the other between the pFra and pYV) found in 16 strains are quite unstable since they can disappear by handling of the strains. Furthermore, the bacteria population in the strains carrying these high molecular weight bands is heterogeneous as such bands could not be recovered from all colonies obtained from a single plate (to be published elsewhere). These bands could represent an increase on the molecular mass of the pFra or the pYV, but as the pYV was nearly always present in the strains carrying extra bands and the pFra was present in 5 out of 8 strains harbouring higher bands this hypothesis is unlikely. On the other hand, it may be possible that these bands could represent an artifact generated by handling the samples during the plasmid extraction, open circular or linearised forms of one of the classical plasmids present in the same strain. Clear demonstration of any relationship among the high molecular weight bands with any one of the classical *Y. pestis* plasmid should await the use of specific molecular probes. Studies to disclose specific phenotypic traits associated with strains harbouring the additional cryptic bands are in progress in our laboratory.

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RESUMO

Perfil plasmidial de cepas de *Yersinia pestis* isoladas no Nordeste do Brasil

Foi analisada a composição plasmidial de 182 cepas de *Yersinia pestis* isoladas em seis focos naturais de peste do Nordeste do Brasil. Cento e seis cepas (61,5 %) apresentaram o perfil plasmidial clássico composto de três plasmídeos bem caracterizados. Cinquenta e seis cepas (33,3 %) perderam pelo menos um deles e 20 cepas (11,0 %) apresentaram plasmídeos crípticos adicionais. Estas variações no perfil plasmidial foram observadas entre cepas originadas dos focos da Chapada do Araripe, Planalto da Borborema, Serra da Ibiapaba e Bahia enquanto todas as cepas analisadas provenientes dos focos de Triunfo e Serra de Baturité apresentaram o perfil plasmidial clássico.

Palavras-chave: *Y. pestis*, plasmídios, peste.

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