Homology with a Repeated *Yersinia pestis* DNA Sequence IS100 Correlates with Pesticin Sensitivity in *Yersinia pseudotuberculosis*

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We have identified IS100 sequences in a specific subset of *Yersinia pseudotuberculosis* isolates that were also sensitive to the *Y. pestis*-produced bacteriocin, pesticin. In contrast, *Y. pseudotuberculosis* strains which did not contain IS100 sequences were not sensitive to pesticin. We propose that IS100 serves as a molecular marker that identifies a subset of *Y. pseudotuberculosis* isolates that have a particularly close evolutionary and/or ecological relationship with *Y. pestis*.

Yersinia pestis, the causative agent of bubonic plague (1, 21), and *Y. pseudotuberculosis* are closely related at the genetic level, having more than 90% DNA homology overall (3, 14, 18). Despite this genetic similarity, these two species differ greatly in their means of transmission and disease presentation, indicating that important genotypic differences between the agents must also exist. Unlike plague, *Y. pseudotuberculosis* infection is rarely fatal and cannot be transmitted by fleas (32). Understanding the factors that differentiate *Y. pestis* from *Y. pseudotuberculosis* at the molecular and genetic levels may facilitate plague control efforts and provide insight into the evolution of pathogenic microorganisms.

In previous studies, we identified two contiguous DNA fragments (probe fragments B and C) from the 9.5-kbp “pesticin plasmid,” pKYP1, that hybridized with all *Y. pestis* isolates and a subset of *Y. pseudotuberculosis* strains that we tested (16). We were intrigued by the possibility that these DNA probes may identify a subset of *Y. pseudotuberculosis* isolates that are more closely related to *Y. pestis* than others and undertook a study to characterize further the nature of these cross-hybridizing DNA sequences. We now report that DNA probe fragments B and C encode IS100 and that the presence of this repeated sequence correlates with pesticin sensitivity in a clonal subset of *Y. pseudotuberculosis* isolates. In addition, we suggest that the distinctive and variable restriction fragment length polymorphism (RFLP) patterns observed with *Y. pestis* and *Y. pseudotuberculosis* make IS100 useful as a molecular epidemiological tool in investigating the spread of these organisms in the environment.

Characterization of DNA probe fragment C. Initial experiments to define the boundaries and specificity of the cross-hybridizing DNA sequences within fragment C were performed by colony blot hybridization analysis as described previously (16). Except as noted, *Y. pestis* and *Y. pseudotuberculosis* were collected during plague surveillance operations and were generously provided by T. Quan, Division of Viral and Vector-Borne Disease, Centers for Disease Control and Prevention (CDC), Fort Collins, Colo. All *Y. pseudotuberculosis* isolates were phenotypically Lcr⁺ when tested on magnesium-oxalate agar for calcium dependence (12, 17). *Yersinia* species were grown at 28°C in brain heart infusion broth or on

solid agar (Difco Laboratories, Detroit, Mich.). All of 27 *Y. pestis* and 12 (36%) of 33 *Y. pseudotuberculosis* isolates hybridized with the DNA probe fragment C. Hybridization with fragment C did not correlate with serotype; however, we noted that only 20% of human and/or nonhuman primate isolates were probe positive, whereas 50% of the isolates from nonprimate sources hybridized with the probe (Table 1).

To determine the extent of cross-hybridizing sequences within this DNA probe, subfragments from either end of fragment C (C_L and C_R in Fig. 1) were used to probe an assortment of *Y. pestis* and *Y. pseudotuberculosis* isolates as described previously (16). Both DNA fragments produced hybridization patterns that were identical to what was observed with fragment C, indicating that the region of homology included most, if not all, of fragment C. Therefore, the entire DNA fragment C was chosen as a probe in all of our later experiments.

Y. enterocolitica is similar to *Y. pseudotuberculosis* in its transmission and disease presentation, but it has only 50% DNA homology with either *Y. pseudotuberculosis* or *Y. pestis* (4). We investigated the degree of specificity of fragment C within the genus *Yersinia* by using colony blot hybridization analysis to test 26 *Y. enterocolitica* isolates as well as five additional strains representing three nonpathogenic *Yersinia* species, including *Y. aldovae*, *Y. intermedia*, and *Y. frederiksenii* (strains were kindly provided by W. Hill, Division of Microbiology, Food and Drug Administration, Washington, D.C., and J. Farmer III, Enteric Identification Laboratory, CDC, Atlanta, Ga.). None of these *Y. enterocolitica* or nonpathogenic *Yersinia* isolates showed homology with fragment C, suggesting that fragment C is specific for *Y. pestis* and *Y. pseudotuberculosis* isolates within the genus *Yersinia* (data not shown).

Fragments B and C encode the repeated DNA sequence IS100. Southern hybridization analysis (31) of the 70-kbp plasmid of EV76 showed that the probe C homology mapped to the same location on this plasmid as a repeated sequence termed IS100 by Portnoy and Falkow (22) (not shown). To determine whether the repeated sequences that we had identified on pKYP1 contained IS100, we sequenced fragment C and a portion of flanking fragment B DNA by the Sanger dideoxy method (26). This DNA sequence analysis confirmed the results of our hybridization studies and showed that pKYP1 contains a 1,954-bp copy of IS100 (IS100kyp) that encompasses virtually all of probe C and extends part way into probe B (Fig. 1).

The DNA sequence that we derived from IS100kyp is nearly identical to other IS100 sequences recently deposited into the

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TABLE 1. Sources and phenotypes of *Y. pseudotuberculosis* strains used in this study^a

Strain ^b		Serotype	Pesticin sensitivity ^c	Probe ^d	Source	Other
Designation used in this study	CDC reference no.					
PTB1	70-365-11	ND ^e	S	+	Ground squirrel	Fresno, Calif., 1970
PTB2	713429	III	S	+	Monkey	UC Davis, ^f 1971
PTB3	730440-1	IB	S	+	Human	Fort Huachuca, Ariz.
PTB4	760635D	IA	S	+	Blesbok	National Zoo, Washington, D.C.
PTB5	760989	IA	S	+	Rat (wild)	National Zoo, Washington, D.C.
PTB6	77CO315	III	S	+	Domestic cat	Loveland, Colo.
PTB7	733700	ND	S	+	Human	Vancouver, B.C.
PTB8	79EA234A	III	S	+	Patagonian cavy	Salisbury Zoo, Salisbury, Md.
PTB9	80CO70	IA	S	+	Elk	Fort Collins, Colo.
PTB10	713425	IA	R	—	Monkey	UC Davis, 1971
PTB11	721647	ND	R	—	Human	Mountain View, Calif.
PTB12	722080	IB	R	—	Human	Portland, Oreg.
PTB13	730315	I	R	—	Toucan	California
PTB14	730316	III	R	—	Toucan	California
PTB15	730317	I	R	—	Goat	California
PTB16	731794	III	R	—	Human	West Haven, Conn.
PTB17	760133	III	R	—	Monkey	Yates Primate Center, Atlanta, Ga.
PTB18	78EA336A	III	R	—	Human	Evanston, Ill.
PTB19	FW81-2	IA	R	—	Red-winged blackbird	Wildlife refuge, New York, 1978
PTB20	79DOD44	IA	R	—	Human	Tacoma, Wash.
PTB21	79EA84	III	R	—	Human	Oklahoma
PTB22	79WA486	IA	R	—	Rat (wild)	Seattle, Wash.
PTB23	80WA109	III	R	—	Human	Seattle, Wash.
PTB24	OR81-66	III	R	—	Capuchin monkey	Wildlife Safari, Oregon
YPIII	YPIII	III	R	—	Human	Laboratory strain

^a All strains except YPIII were provided by the CDC Plague Branch, Fort Collins, Colo., along with serotype and source information. In most cases, duplicate isolates from the same source were omitted from this list.

^b Isolates PTB2 and PTB10 were isolated from different monkeys at the same location on the same day. Isolates PTB13 and PTB14 were derived from different birds in different locations.

^c S, pesticin sensitive; R, pesticin resistant.

^d +, strain was detected by probe fragment C by colony blot hybridization; —, strain was not detected.

^e ND, serotype is not known.

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GenBank database, including one of 1,953 bp from the pesticin plasmid of *Y. pestis* 106 Otten (20), one of 1,948 bp from the Lcr plasmid of *Y. pestis* EV76 (GenBank accession number X78302), and one with no target duplication derived from an unspecified location in *Y. pestis* EV76-51F (GenBank accession number L19030). These *IS100* sequence variations are most likely due to the differential accumulation of mutations over

time in different strains and at the various loci within each strain.

The RFLP pattern of *Y. pestis* is distinct from that of *Y. pseudotuberculosis*. The nature of *IS100* DNA homology observed in colony blots was further examined by Southern hybridization analysis in several strains each of *Y. pestis* and *Y. pseudotuberculosis*. As shown in Fig. 2, all *Y. pestis* isolates

FIG. 1. Partial restriction map of plasmid pKYP1. Striped boxes represent the regions of the plasmid used as DNA probes designated fragments B and C. Genes encoding plasminogen activator (pla) (15, 30), pesticin immunity (pim) (19, 30), and pesticin (pst) (7, 30) are defined by open boxes under the restriction map. *IS100* is marked by a solid bar that is expanded underneath to illustrate the structural relationship of the two putative open reading frames, ORFA and ORFB, with inverted repeats (triangles) and putative -10 and -35 consensus sequences. Solid boxes within *IS100* mark the locations of putative helix-turn-helix (ORFA) and ATP binding (ORFB) motifs. Abbreviations for restriction endonuclease sites: C, *Cla*I; B, *Bam*HI; BII, *Bgl*II; K, *Kpn*I; H, *Hind*III; E, *Eco*RI; EV, *Eco*RV; P, *Pst*I; BI, *Bgl*I; S, *Sma*I.

FIG. 2. Autoradiograph of *EcoRV*-digested chromosomal DNA from various *Y. pestis* isolates probed with DNA probe C. Lanes: a and b, avirulent lab strains EV76-6 and TRU, respectively; c to i, virulent isolates TQ1, TQ6, TQ7, TQ8, TQ9, TQ10, and TQ11, respectively. Arrows mark bands missing in group I isolates (the bottom arrow marks the pKYP1-derived band); arrowheads mark conserved bands in the *Y. pestis* consensus RFLP in one or more lanes. Lane d contained less DNA than others.

showed similar, but not identical, RFLP patterns when hybridized with probe C (see Table 2 for strain information).

The patterns observed could be separated into three groups: strains that have lost bands (group I), strains that have gained bands (group II), and strains that have undergone no changes in banding relative to a consensus pattern comprised of bands that are present in each of at least five isolates (group III).

Group I contains both avirulent strains, EV76-6 and TRU, each of which lack at least three consensus bands, although TRU also has gained a large unique band. Loss of some of these missing bands can be explained by the absence of the 70-kbp plasmid in both strains as well as lack of the 9.5-kbp pesticin plasmid in TRU and deletion of the Pgm locus in EV76-6. The absence of other bands may be due to additional deletions that encompass probe sequences.

Group II is comprised of six strains from a variety of sources, including a laboratory reference strain isolated from a human in India in 1948 and five isolates obtained in the United States during the last 20 years from humans, fleas, and a cat. Five of these isolates carry one additional band, and one contains two extra bands. In all cases except one (the laboratory reference strain 195/P), these additional bands represent a net increase in bands over the consensus, suggesting further duplication or

FIG. 3. Autoradiograph of *EcoRV*-digested chromosomal DNA from an assortment of *Y. pseudotuberculosis* isolates probed with DNA probe C. Lanes: a and b, *Y. pestis* isolates TQ7 and TQ11, respectively; c to j, *Y. pseudotuberculosis* isolates PTB11, PTB19, PTB1, PTB2, PTB3, PTB5, PTB6, and PTB7, respectively. The arrow denotes the pKYP1-derived band; arrowheads mark conserved bands in *Y. pseudotuberculosis*, the lower of which appears to comigrate with a *Y. pestis* band. Some lanes are lighter because they contain less DNA.

acquisition of the repeated sequence. However, strain TQ8 (195/P) in lane f of Fig. 2 has also lost one of the consensus bands, making it difficult to evaluate the source of the new band. DNA mutation, plasmid integration into the chromosome, or separate *IS100* deletion and addition events could explain this change in banding. All of the additional bands observed in the group II strains are unique except for one band that occurs in each of two isolates from different regions of Colorado in the same year. However, a second unique band in TQ11 prevents these strains from having identical RFLP patterns.

Group III contains one strain, designated TQ6, which matches the consensus banding pattern. That is, it has all bands that are present in at least five other isolates and no bands that are not present in at least five other isolates.

The low degree of variation among isolates from diverse sources in this study is notable and most likely reflects the clonal nature of *Y. pestis* bacteria, a characteristic feature of many pathogens (27, 29) and bacteria under a continuously high degree of selective pressure. However, the subtle differences in RFLPs from *Y. pestis* strains isolated at similar times in the same geographic region suggest that RFLP analysis may be useful for epidemiologic studies.

In contrast, we found more diversity in RFLPs among the *Y.*

TABLE 2. Sources of *Y. pestis* strains used in this study^a

Strain		Hyb ^b	Source	Reference or year	Location	Other
Designation used in this study	CDC reference no.					
	EV76-6	I		22		Avirulent laboratory strain
	TRU	I		2		Avirulent laboratory strain
TQ1	CO87-1239	II	Cat	1987	Loveland, Colo.	Throat, blood, bubo, nonfatal
TQ6	NM87-3406	III	Human	1987	New Mexico	Source not available
TQ7	AZ87-3329-636	II	Fleas	1987	Coconino City, Ariz.	From prairie dog burrows
TQ8	195/P	II	Human	1948	Poona, India	Virulent laboratory strain
TQ9	OR87-4607	II	Human	1987	Douglas City, Oreg.	Lymph node, fatal
TQ10	77NM538	II	Human	1977	Sante Fe, N. Mex.	Lymph node, nonfatal
TQ11	CO87-3724-838	II	Fleas	1987	Montezuma City, Colo.	From prairie dog burrows

^a Except as noted, all strains and source information were provided by the CDC Plague Branch, Fort Collins, Colo.

^b Designation of hybridization group as determined by RFLP analysis.

pseudotuberculosis isolates that we tested (Fig. 3). *Y. pseudotuberculosis* RFLP patterns were significantly different from those of *Y. pestis*, but similarities within the *Y. pseudotuberculosis* group itself that also suggest a clonal origin were observed. Two bands were present in all probe-positive *Y. pseudotuberculosis* strains, and one of these two conserved bands comigrated with one of the *Y. pestis* consensus bands. Southern hybridization and sequence analyses indicate that this DNA band is homologous in *Y. pestis* and IS100-positive *Y. pseudotuberculosis* isolates (11a). However, further work is needed to determine whether this conserved comigrating DNA band represents a primordial copy of IS100 in both of these strains or evidence of horizontal transmission of IS100-containing DNA sequences between these strains. As with *Y. pestis*, the variability in the *Y. pseudotuberculosis* RFLPs suggests that IS100 DNA sequences may provide useful epidemiological markers for distinguishing among *Y. pseudotuberculosis* isolates, as well as between *Y. pseudotuberculosis* and *Y. pestis* strains, during an epizootic.

Probe-positive *Y. pseudotuberculosis* isolates are pesticin sensitive (Pst^s). The shared presence of a unique insertion sequence suggested an evolutionary link between *Y. pestis* and the probe-positive population of *Y. pseudotuberculosis*. In addition, a lower incidence of probe-positive isolates associated with human disease in this study led us to hypothesize that the probe-positive isolates may be more zoonotic in nature, and more like *Y. pestis* in the niches that they occupy, than those isolates which fail to hybridize with IS100. Bacteriocins are often active against very closely related strains of bacteria because they compete for limited resources in similar niches (25). Therefore, we reasoned that pesticin sensitivity in *Y. pseudotuberculosis* might be used as an additional indicator of genetic relatedness to *Y. pestis*.

Pesticin is a lysozyme-like bacteriocin that is produced by *Y. pestis* and is active against *Y. pestis*, as well as some strains of *Y. pseudotuberculosis*, *Y. enterocolitica*, and *Escherichia coli* (2, 5, 6, 24). Susceptibility to pesticin requires expression of a receptor (*fyuA/psn*) for pesticin uptake (8, 10, 23, 28). Most nonrelated bacterial species are resistant to pesticin, presumably because they lack the appropriate receptor. In contrast, most *Y. pestis* isolates are Pst^s but avoid its bactericidal activity by expressing an immunity factor, which is encoded adjacent to the pesticin structural gene (19, 30).

We tested our *Y. pseudotuberculosis* strains for sensitivity to pesticin by using EV76-6 as the pesticin-producing strain as described by Hu et al. (13), with modifications. Sterile toothpicks were used to stab EV76-6 bacteria into the center of an L-agar plate. After allowing for 2 days growth of *Y. pestis* at 28°C, we transferred plates to 37°C for 2 h to stimulate pesticin activity. *Y. pestis* bacteria were then killed by treatment with chloroform, and the plates were seeded with the appropriate *Y. pseudotuberculosis* test strain by application of a sheet of Whatman filter paper that had been soaked in a dilute suspension of *Y. pseudotuberculosis* bacteria. Plates were incubated overnight at 37°C and then examined for zones of clearing around the original EV76-6 stab site in an otherwise confluent lawn of bacteria. Zones of clearing with Pst^s isolates typically had diameters of approximately 1.75 cm.

All *Y. pseudotuberculosis* strains which had hybridized with probe C were found to be Pst^s, whereas no strain which failed to hybridize showed sensitivity to pesticin (Table 1). These findings indicate a strong correlation between IS100 and pesticin sensitivity in *Y. pseudotuberculosis* that is consistent with a genetic linkage between IS100 and the gene encoding the pesticin receptor *fyuA/psn*, as exists in *Y. pestis*.

psn, the gene encoding pesticin sensitivity in *Y. pestis*, is

located proximal to a copy of IS100 in the Pgm locus of this bacterium (10), and it is possible that a similar relationship exists in *Y. pseudotuberculosis*. Others have suggested that *psn/fyuA* and *irp2*, a second virulence-associated gene within the Pgm locus in *Y. pestis*, are part of a pathogenicity island that is shared by both *Y. pestis* and highly pathogenic strains of *Y. pseudotuberculosis* and *Y. enterocolitica* (24). Our findings are consistent with this possibility but suggest that IS100 is linked to this pathogenicity island in *Y. pestis* and *Y. pseudotuberculosis* but not in *Y. enterocolitica*.

In this study, which included both serogroup I and serogroup III isolates, neither pesticin sensitivity nor IS100 carriage in *Y. pseudotuberculosis* was restricted to the serogroup I isolates, as has been observed by others (7, 9, 20, 24). However, geographic heterogeneity has been reported for the distribution of the *ypm* gene within serotypes in *Y. pseudotuberculosis* isolates from Europe and the Far East (33), and it is possible that the probe-positive *Y. pseudotuberculosis* isolates that we have identified represent a geographic population of clonal variants.

In addition, pesticin sensitivity was recently correlated with the presence of *psn/fyuA* and *irp2* genes in serogroup I *Y. pseudotuberculosis* isolates (24). In the same study, some serogroup III strains were found to carry *irp2* but not *fyuA*, and the authors suggested that *fyuA* was deleted in the serogroup III isolates. It is possible that the Pst^s, IS100-positive serogroup III isolates that we identified in this study represent wild-type progenitors of the *irp2*-positive, *fyuA*-negative isolates described by others (24).

In conclusion, we have demonstrated a genetic linkage between pesticin sensitivity and IS100 carriage in *Y. pseudotuberculosis* and suggested that IS100 is a useful epidemiologic tool for investigating *Y. pestis* and some *Y. pseudotuberculosis* outbreaks. We have also proposed that IS100 identifies a clonal subset of *Y. pseudotuberculosis* isolates that may be closely related to *Y. pestis* with respect to their biological niche and/or evolutionary pathways. Determination of the source of IS100 and its possible evolutionary relationship to both *Y. pestis* and the pesticin receptor will require further study.

Nucleotide sequence accession number. The sequence data reported have been assigned GenBank accession number U59875.

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REFERENCES

1. Barnes, A. M. 1982. Surveillance and control of bubonic plague in the United States. Symp. Zool. Soc. London **50**:237-270.
2. Ben-Gurion, R., and I. Hertman. 1958. Bacteriocin-like material produced by *Pasteurella pestis*. J. Gen. Microbiol. **19**:289-297.
3. Bercovier, H., H. H. Mollaret, J. A. Alonso, J. Brault, G. R. Fanning, A. G. Steigerwalt, and D. J. Brenner. 1980. Intra- and interspecies relatedness of *Yersinia pestis* by DNA hybridization and its relationship to *Yersinia pseudotuberculosis*. Curr. Microbiol. **4**:225-229.
4. Brenner, D. J., A. G. Steigerwalt, D. P. Falcao, R. E. Weaver, and G. R. Fanning. 1976. Characterization of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by deoxyribonucleic acid hybridization and by biochemical reactions. Int. J. Syst. Bacteriol. **26**:180-194.

5. Brubaker, R. R. 1972. The genus *Yersinia*: biochemistry and genetics of virulence. *Curr. Top. Microbiol.* **57**:111–158.
6. Brubaker, R. R., E. D. Beesley, and M. J. Surgalla. 1965. *Pasteurella pestis*: role of pesticin I and iron in experimental plague. *Science* **149**:422–424.
7. Brubaker, R. R., and M. J. Surgalla. 1961. Pesticins. I. Pesticin-bacterium interrelationships, and environmental factors influencing activity. *J. Bacteriol.* **82**:940–949.
8. Brubaker, R. R., and M. J. Surgalla. 1962. Pesticins II. Production of pesticin I and II. *J. Bacteriol.* **84**:539–545.
9. Burrows, T. W., and L. D. Bacon. 1960. V and W antigens in strains of *P. pseudotuberculosis*. *Br. J. Exp. Pathol.* **41**:38–44.
10. Fetherston, J. D., J. Lillard, Jr., and R. D. Perry. 1995. Analysis of the pesticin receptor from *Yersinia pestis*: role in iron-deficient growth and possible regulation by its siderophore. *J. Bacteriol.* **177**:1824–1833.
11. Fetherston, J. D., P. Schuetze, and R. D. Perry. 1992. Loss of the pigmentation phenotype in *Yersinia pestis* is due to the spontaneous deletion of 102 kb of chromosomal DNA which is flanked by a repetitive element. *Mol. Microbiol.* **6**:2693–2704.
- 11a. Hare, J. M., and K. A. McDonough. Unpublished results.
12. Higuchi, K., L. L. Kupferberg, and J. L. Smith. 1959. Studies on the nutrition and physiology of *P. pestis*. III. Effects of calcium ions on the growth of virulent and avirulent strains of *P. pestis*. *J. Bacteriol.* **77**:317–321.
13. Hu, P. C., G. C. Yang, and R. R. Brubaker. 1972. Specificity, induction, and absorption of pesticin. *J. Bacteriol.* **112**:212–219.
14. Ibrahim, A., B. M. Goebel, W. Liesack, M. Griffiths, and E. Stackebrandt. 1993. The phylogeny of the genus *Yersinia* based on 16S rDNA sequences. *FEMS Microbiol. Lett.* **114**:173–178.
15. McDonough, K. A., and S. Falkow. 1989. A *Yersinia pestis*-specific DNA fragment encodes temperature-dependent coagulase and fibrinolysin-associated phenotypes. *Mol. Microbiol.* **3**:767–775.
16. McDonough, K. A., T. G. Schwan, R. E. Thomas, and S. Falkow. 1988. Identification of a *Yersinia pestis*-specific DNA probe with potential for use in plague surveillance. *J. Clin. Microbiol.* **26**:2515–2519.
17. Mehigh, R. J., A. K. Sample, and R. R. Brubaker. 1989. Expression of the low calcium response in *Yersinia pestis*. *Microb. Pathog.* **6**:203–217.
18. Mollaret, H. H. 1965. Sur la nomenclature et la taxonomie du bacille de malassez et vignal. *Int. Bull. Bacteriol. Nomencl. Taxon.* **15**:97–106.
19. Pils, H., H. Killmann, K. Hantke, and V. Braun. 1996. Periplasmic location of the pesticin immunity protein suggests inactivation of pesticin in the periplasm. *J. Bacteriol.* **178**:2431–2435.
20. Podladchikova, O. N., G. G. Dikhanov, A. V. Rakin, and J. Heesemann. 1994. Nucleotide sequence and structural organization of *Yersinia pestis* insertion sequence IS100. *FEMS Microbiol. Lett.* **121**:269–274.
21. Poland, J. D., and A. M. Barnes. 1979. Plague, p. 515–516. In J. F. Steele (ed.), *CRC Handbook series in zoonoses*, vol. 1, section A. Bacterial, rickettsial and mycotic diseases—1979. CRC Press, Inc., Boca Raton, Fla.
22. Portnoy, D. A., and S. Falkow. 1981. Virulence-associated plasmids from *Yersinia enterocolitica* and *Yersinia pestis*. *J. Bacteriol.* **148**:877–883.
23. Rakin, A., E. Saken, D. Harmsen, and J. Heesemann. 1994. The pesticin receptor of *Yersinia enterocolitica*: a novel virulence factor with dual function. *Mol. Microbiol.* **13**:253–263.
24. Rakin, A., P. Urbitsch, and J. Heesemann. 1995. Evidence for two evolutionary lineages of highly pathogenic *Yersinia* species. *J. Bacteriol.* **177**:2292–2298.
25. Reeves, P. 1972. *The bacteriocins*. Springer, New York, N.Y.
26. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing and chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
27. Selander, R. K., J. M. Musser, D. A. Caugant, M. N. Gilmour, and T. S. Whittam. 1986. Population genetics of pathogenic bacteria. *Microb. Pathog.* **3**:1–7.
28. Sikkema, D. J., and R. R. Brubaker. 1987. Resistance to pesticin, storage of iron, and invasion of HeLa cells by yersiniae. *Infect. Immun.* **55**:572–578.
29. Smith, J. M., C. G. Dowson, and B. G. Spratt. 1991. Localized sex in bacteria. *Nature* **349**:29–31.
30. Sodeinde, O. A., and J. D. Goguen. 1988. Genetic analysis of the 9.5-kilobase virulence plasmid of *Yersinia pestis*. *Infect. Immun.* **56**:2743–2748.
31. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503–517.
32. Une, T. 1977. Studies on the pathogenicity of *Yersinia enterocolitica*. III. Comparative studies between *Y. enterocolitica* and *Y. pseudotuberculosis*. *Microbiol. Immunol.* **21**:505–516.
33. Yoshino, K., T. Ramamurthy, G. B. Nair, H. Fukushima, Y. Ohtomo, N. Takeda, S. Kaneko, and T. Takeda. 1995. Geographical heterogeneity between Far East and Europe in prevalence of *ypm* gene encoding the novel superantigen among *Yersinia pseudotuberculosis* strains. *J. Clin. Microbiol.* **33**:3356–3358.