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Nucleotide Sequence of the Plasminogen Activator Gene of *Yersinia pestis*: Relationship to *ompT* of *Escherichia coli* and Gene *E* of *Salmonella typhimurium*

OLA A. SODEINDE AND JON D. GOGUEN*

Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, Massachusetts 01655

Received 17 October 1988/Accepted 18 January 1989

We have determined the nucleotide sequence of the 1.4-kilobase DNA fragment containing the plasminogen activator gene (*pla*) of *Yersinia pestis*, which determines both plasminogen activator and coagulase activities of the species. The sequence revealed the presence of a 936-base-pair open reading frame that constitutes the *pla* gene. This reading frame encodes a 312-amino-acid protein of 34.6 kilodaltons and containing a putative 20-amino-acid signal sequence. The presence of a single large open reading frame is consistent with our previous conclusion that the two Pla proteins which appear in the outer membrane of *pla*$^+$ *Y. pestis* are derived from a common precursor. The deduced amino acid sequence of Pla revealed that it possesses a high degree of homology to the products of gene *E* of *Salmonella typhimurium* and *ompT* of *Escherichia coli* but does not possess significant homology to other plasminogen activators of known sequence. We also identified a transcription unit that resides on the complimentary strand and overlaps the *pla* gene.

Plasminogen activators and coagulases have long been considered to contribute to the pathogenic potential of the bacterial species that produce them. Coagulases, produced by a variety of staphylococcal species, are thought to result in the formation of fibrin barriers around the bacteria, thereby protecting them from host defenses. A mutation resulting in the loss of coagulase activity of a coagulase-positive strain of *Staphylococcus aureus* has been reported to result in a reduction in the virulence of the strain (17). The bacterial plasminogen activators, on the other hand, are thought to enhance the invasiveness of the species that produce them because of the proteolytic activity of plasmin, which may degrade fibrin barriers and/or other host proteins surrounding the foci of infection, thus facilitating dissemination of the bacteria. Thus far, there is no definitive experimental evidence to support this notion.

Yersinia pestis, the causative agent of plague, possesses both coagulase and plasminogen activator activities (2, 4). These activities are encoded by pPCP1, the 9.5-kilobase (kb) plasmid harbored by fully virulent strains of *Y. pestis* (3, 7, 28). Strains of *Y. pestis* that have lost this plasmid exhibit a marked increase in 50% lethal doses when administered to mice or guinea pigs by peripheral routes of injection, i.e., either intraperitoneally or subcutaneously. However, such strains are as virulent as wild-type strains when the bacteria are administered intravenously (4, 5). These observations demonstrate that the plasmid encodes a factor(s) that enhances the invasiveness of *Y. pestis*. The plasminogen activator is a likely candidate for this factor. Only two other genes have been identified on pPCP1 (28). One of these encodes a bacteriocin known as pesticin (3, 7), and the other provides pesticin immunity (28).

We recently reported that both coagulase and plasminogen activator activities of *Y. pestis* are encoded by the same gene, *pla*, contained on pPCP1 (28). This gene encodes two proteins, α-Pla and β-Pla, that are derived from a common precursor, pre-Pla. Both the α and β species are prominent proteins in outer membrane preparations of *Y. pestis* and *Escherichia coli* containing Pla-producing plasmids (29, 31). It is not yet known how the biochemical activities are distributed between the proteins. Expression of the Pla proteins is also responsible for the in vitro degradation of a set of outer membrane proteins, the yersinial outer membrane proteins (YOPs), that are encoded by the 75-kb plasmid of *Y. pestis* (29). It has been shown that mutations in some YOP genes result in avirulence in mice (30). The significance or mechanism of YOP degradation by a *pla*-encoded product(s) has yet to be determined.

In this paper, we present the nucleotide sequence of the *pla* gene and show that it possesses a high degree of homology to gene *E* of *Salmonella typhimurium* and *ompT* of *E. coli*. We also show that an active transcription unit resides on the complimentary strand and within the coding region of the *pla* gene.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used are described in Table 1.

Media and growth conditions. *E. coli* strains were grown in Luria broth (22) at 37°C. The medium was supplemented with ampicillin, chloramphenicol, tetracycline, or streptomycin as required at final concentrations of 50, 30, 40, and 100 μg/ml, respectively.

General procedures. Screening of plasmid sizes, isolation of plasmid DNA, restriction enzyme digestions, ligations, transformations, and agarose gel electrophoresis were performed as previously described (28).

Construction of pJH502E::kan. pJH502 was digested with *Nde*I, which cuts at a unique site within gene *E*, and then treated with the Klenow fragment of DNA polymerase I to produce blunt ends. The resulting linear DNA was ligated to the *Hin*dIII-*Nru*I fragment of Tn*5*, also treated with the Klenow fragment to produce blunt ends. The latter fragment contains the Tn*5* kanamycin resistance determinant. Km^r Cm^r transformants were selected, and the structures of the

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or description	Source or reference
Strains		
E. coli		
JM101	K-12 *supE thi* Δ(*lac-proAB*) F′ (*traD36 proAB lacI*^{q}*ZM15*)	34
CSH50	K-12 F$^-$ *ara* Δ(*lac-pro*) *strA thi*	CGSC[a]
LE392	K-12 F$^-$ *hsdR514 supE44 supF58* Δ(*lacIZY*)*6 galK2 galT22 metB1 trpR55*	33
S. typhimurium		
DB7000	LT2 *leuA414*	A. Poteete
WB600	Virulent strain of *S. typhimurium*	W. Benjamin
Y. pestis KIM5	*pgm-1* (spontaneous nonpigmented mutant of *Y. pestis* KIM)	R. Brubaker
Plasmids		
pPCP1	9.5-kb plasmid of *Y. pestis* KIM	
pRS415	*lacZ* operon fusion vector	27
pOS31	0.5-kb *Sma*I-*Bam*HI fragment of pPCP1 inserted upstream of promoterless *lacZ* of pRS415	This study
pOS32	0.4-kb *Dra*I-*Bam*HI fragment of pPCP1 inserted upstream of promoterless *lacZ* of pRS415	This study
pJH502	Contains cloned copy of gene *E* of *S. typhimurium*	35
pJH502E::kan	Contains Kan^r fragment inserted in gene *E* of pJH502	This study
Bluescript M13 plasmids	Cloning and sequencing vectors	Stratagene

[a] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

resulting plasmids were confirmed by additional restriction mapping.

Enzyme assays. A fibrin film lysis assay for plasminogen activator activity and a rabbit plasma clotting assay for coagulase activity were performed as described by Beesley et al. (2). These assays were modified for semiquantitative comparison of activities between strains as follows. Cells were harvested from fresh overnight cultures of each strain, washed once in 1 volume of fibrinolysis buffer (2), and suspended in a 0.1 volume of fibrinolysis buffer. Seven serial 10-fold dilutions were prepared from each suspension. For the plasminogen activator assay, 10 μl of the original suspension and every dilution was spotted onto the fibrin film. For the coagulase assay, 100 μl of the original suspension and every dilution was added to 0.4 ml of rabbit plasma. The greatest dilution showing obvious lysis of the fibrin film or clotting of the plasma was noted. The fibrin films used in the plasminogen activator assays contained chloramphenicol or tetracycline to prevent further growth of the bacteria. To assess the effect of bacteria or bacterial extracts on the fibrin films in the absence of plasminogen, we heated the preformed films at 70°C for 1 h (2). This treatment irreversibly denatures plasminogen.

β-Galactosidase assays were performed as described by Miller (22).

DNA sequencing. The DNA fragment containing the *pla* gene was cloned into Bluescript M13 plasmids (Stratagene Cloning Systems, San Diego, Calif.). Unidirectional deletions from both ends of the fragment were obtained by exonuclease III-mung bean nuclease digestion as described by Henikoff (15). Single-stranded DNA templates were obtained by using an M13 helper phage as described in the Stratagene manual. A portion of the sequence was determined by using double-stranded DNA templates as described by Chen and Seeburg (6). The DNA was sequenced by the chain termination method of Sanger et al. (25) by using either the Klenow fragment of DNA polymerase I (Amersham Corp., Arlington Heights, Ill.) or Sequenase (U.S. Biochemical Corp.). Structural analysis of the deduced primary amino acid sequence was performed as described by Hopp and Woods (16) and implemented in the DNASTAR PROTEIN program (DNASTAR, Inc., Madison, Wis.) by using a window size of 9. Amino acid sequence alignments were determined as described by Lipman and Pearson (21) and implemented in the DNASTAR AALIGN program by using a gap penalty of 4 and a deletion penalty of 12.

RESULTS

Structure of the *pla* gene. The *pla* gene had previously been localized to the 1.4-kb *Sma*I-*Hin*dIII fragment of pPCP1 (Fig. 1) (28). The sequence of both DNA strands of this 1,453-base-pair fragment was determined (Fig. 2). The sequence revealed the presence of a single long open reading frame (ORF) extending from positions 277 to 1212. A polypurine tract, AAGGG (positions 266 to 270), that possesses strong homology to consensus *E. coli* ribosome-binding sites (12, 26) was located 6 base pairs upstream of the putative initiation codon. Two pairs of sequence elements that bear close resemblance to the consensus *E. coli* promoter sequence (24) were located upstream of the ribosome-binding site. The −35 and −10 sites were located between positions 212 to 217 and 237 to 242 for the first pair of sequence elements and between positions 197 to 202 and 221 to 226 for the second.

Coding region of the *pla* gene. The *pla* gene has been shown to encode two proteins, α-Pla and β-Pla, β-Pla being derived from α-Pla by a posttranslational processing event and α-Pla being derived from pre-Pla, probably through processing by signal peptidase (28). The ORF described above must encode pre-Pla, since it is the only frame of sufficient length present in the sequenced fragment. This ORF encodes a protein containing 312 amino acids and having a deduced M_r of 34.6 kilodaltons. The observation that deletions extending from either the 5′ end (position 357 and downstream) or the 3′ end (position 1017 and upstream) resulted in the loss of both plasminogen activator and coagulase activities confirms that this ORF determines both activities.

An examination of the deduced amino acid sequence of the *pla* gene revealed the presence of an N-terminal 20-amino-acid sequence that possesses structural similarity to a typical procaryotic signal sequence (23), i.e., a positively charged N-terminal region (Lys-Lys) followed by a stretch of uncharged and mostly hydrophobic residues that terminate in a signal peptidase recognition sequence (Ala-Asn-Ala). A structural analysis of the deduced primary sequence of Pla predicted the presence of at least four hydrophobic

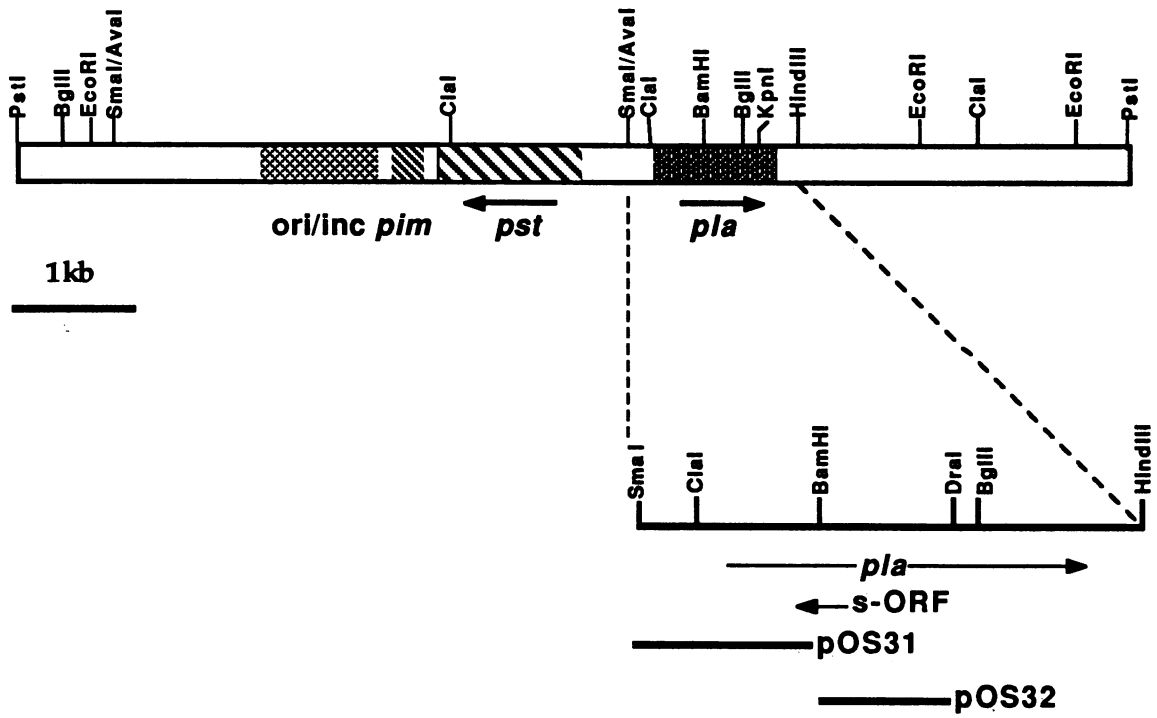

FIG. 1. Physical and genetic maps of pPCP1. The positions of the origin of replication-incompatibility control region (ori/inc) and the structural genes encoding plasminogen activator-coagulase (*pla*), pesticin (*pst*), and pesticin immunity (*pim*) are shown. s-ORF, Short ORF on the complimentary strand of *pla* (see the text). The arrows indicate the direction of transcription. The bars below the expanded portion of the map show the restriction fragments cloned into pRS415 to yield pOS31 and pOS32. A more detailed description of pPCP1 is given in reference 28.

domains: residues 78 to 99, 159 to 179, 185 to 204, and 242 to 261 (Fig. 3). These may be the regions of the protein associated with the outer membrane.

Relationship of Pla to protein E of *S. typhimurium* and OmpT of *E. coli*. The *pla* gene does not possess significant homology to known plasminogen activators of bacterial (streptokinase and staphylokinase) or eucaryotic (urokinase and tissue plasminogen activator) origin at either the DNA or the deduced primary amino acid sequence levels. However, it possesses a significant degree of homology to gene *E* of *S. typhimurium* (35) and *ompT* of *E. coli* (14). The degrees of homology based on the alignment of DNA sequences within the coding regions were as follows: 69% between *pla* and gene *E*; 61% between gene *E* and *ompT*; and 59% between *pla* and *ompT*. The homology between the genes did not extend beyond the limits of the coding regions. The degrees of homology between the proteins based on the alignment of the deduced amino acid sequences were as follows: 71% between Pla and protein E; 49% between protein E and OmpT; and 47.5% between Pla and OmpT (Fig. 3). A total of 41% of the residues were conserved in all three proteins.

This observation prompted us to investigate whether protein E and OmpT also possessed plasminogen activator and coagulase activities (Table 2). OmpT has recently been reported to activate plasminogen (13), but no enzymatic activity has been associated with protein E. We were unable to detect either plasminogen activator or coagulase activity in the OmpT$^+$ *E. coli* K-12 strains LE392 and CSH50. The discrepancy in these results is presumably due to the much higher sensitivity of the fluorescence assays used in the studies of OmpT (20). Nonetheless, it indicates that plasminogen activator activity is much higher in *Y. pestis* than in *E. coli*.

S. typhimurium DB7000 and WB600 also failed to produce detectable levels of either activity. However, *E. coli* K-12 strain LE392 harboring pJH502, a multicopy plasmid containing a copy of cloned gene *E*, expressed both activities but at lower levels than *Y. pestis*. In particular, plasminogen activator activity was 1,000-fold lower (Table 2). When a DNA fragment encoding kanamycin resistance was inserted into the *Nde*I site within the coding region of gene *E* in pJH502, both plasminogen activator and coagulase activities were lost. This result shows that these activities are gene *E* dependent.

Identification of a transcription unit on the complimentary strand and within the coding sequence of the *pla* gene. The DNA sequence of the *pla* gene also revealed the presence of a short ORF on the complimentary strand and within the coding sequence of *pla*. This frame could encode a 48-amino-acid polypeptide. Immediately upstream were two sequences with significant homology to the consensus *E. coli* promoter sequence (24). The first sequence, TATAAT, located between positions 715 and 710, was identical to the consensus *E. coli* −10 site, and the second, CTGATA, located between positions 741 and 736, possessed a four out of six nucleotide match to the consensus *E. coli* −35 site, TTGACA (Fig. 4). Preceding the initiation codon by 5 nucleotides was a potential ribosome-binding site located between positions 642 and 639.

Transcription of the region immediately downstream of this potential promoter was confirmed by subcloning a *Dra*I-*Bam*HI restriction fragment that contains it into the *lacZ* operon fusion vector pRS415 (27) to form pOS32 (Fig. 1). For comparison, a similar plasmid, pOS31, containing a *Sma*I-*Bam*HI fragment with the *pla* promoter was constructed. β-Galactosidase activity dictated by pRS415 (no

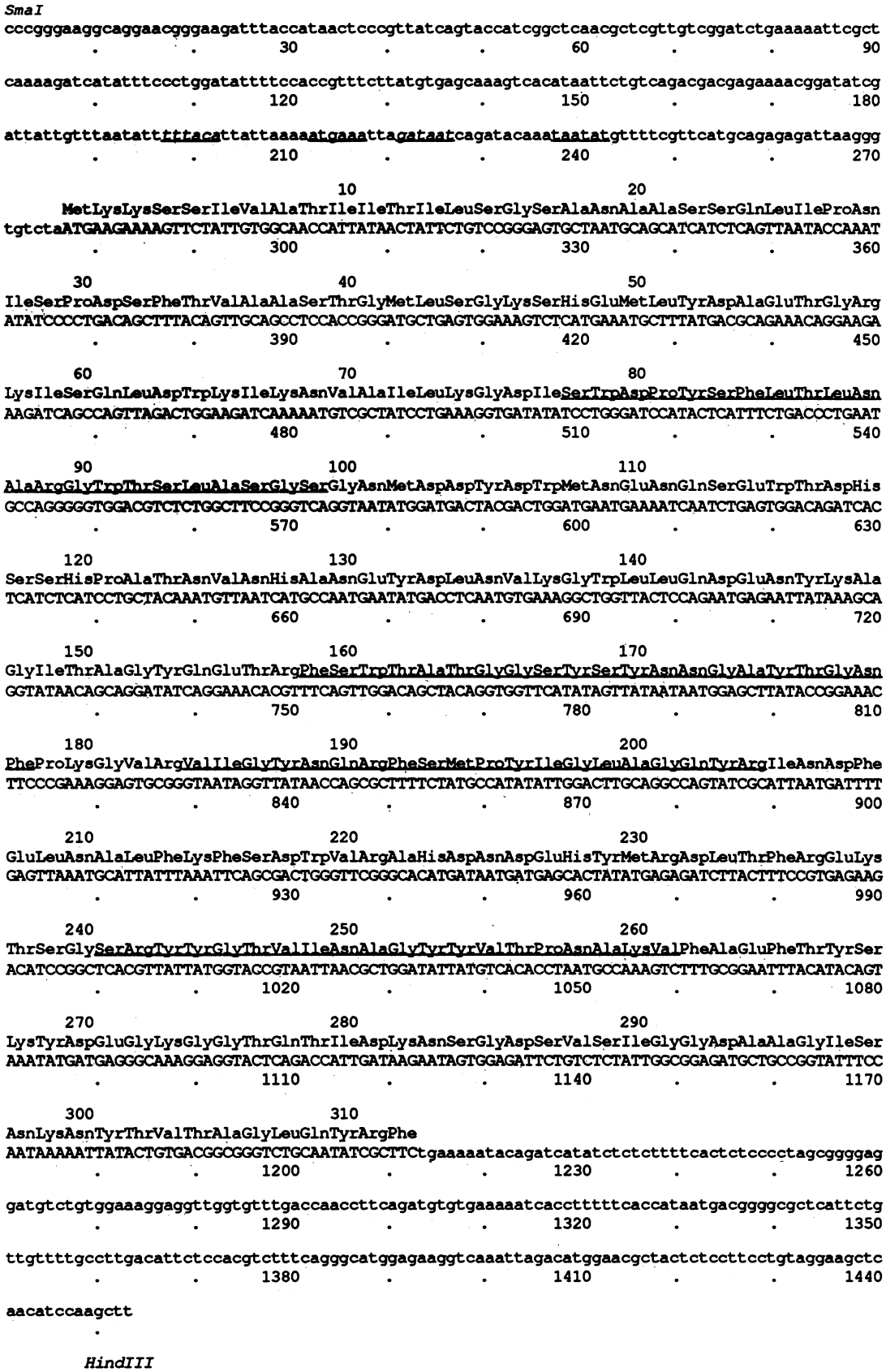

FIG. 2. Nucleotide sequence of the 1.4-kb *Sma*I-*Hin*dIII fragment of pPCP1. Underlined nucleotides (roman and italic types) at the 5′ end of the *pla* gene show its two potential promoter elements. The deduced amino acid sequence is given above the nucleotide sequence. Underlined amino acid residues indicate the predicted hydrophobic domains.

```
                                 10        20        30        40        50
Pla                     MKKSSIVATIITILSGSANAASSQLIPNISPDSFTVAASTGMLSGKSHEMLY-
E     MKTSSPLQITSEYEDKRNE•TCYCVMM•AVF•ESVY•E•ALF••DV••••V•TSL•V•V•N•••R•LV•-
OmpT                     MRAKLLGIVL•TPIAISSF••TETL-SFT••NINADI•L•T••••TK•RV•L

          60        70        80        90       100       110       120
Pla   DAETGRKISQLDWKIKNVAILKGDISWDPYSFLTLNARGWTSLASGSGNMDDYDWMNENQSE-WTDHSSH
E     •TD••••L••••••••••••T•Q••L••E••••M••D•••••••••••••H•V•H•••SSE•PG-•••R•I•
OmpT  AE•G•••V•••••••FN•A••I••A•N••LMPQISIG•A•••T•G•RG•••V•Q•••DSSNPGT•••E•R•

          130       140       150       160       170           180
Pla   PATNVNHANEYDLNVKGWLLQDENYKAGITAGYQETRFSWTATGGSYSYNNG-AY---TGNFPKGVRVIG
E     •D•S••Y••••••••••••••GD•••••V••••••••••••••R••••I•D••-R•---I••••H•••G••
OmpT  •D•QL•Y•••F•••I•••••NEP••RL•LM•••••S•Y•F••R••••I•SSEEGFRDDI•S••N•E•A••

      190       200       210       220            230       240       250
Pla   YNQRFSMPYIGLAGQYRINDFELNALFKFSDWVRAHDNDEHY--MRDLTFREKTSASRYYGTVINAGYYV
E     •S•••E••••••••D••••••••C•V•••Y••••N•••••••••--••K••••••••EN•••••AS•D••••I
OmpT  •K•••K•••••••T•S••YE••••GGT••Y•G••ESS••••••DPGKRI•Y•S•VKDQN••SVAV••••••

        260       270       280       290       300       310
Pla   TPNAKVFAEFTYSKYDEGKGGTQTIDKNSGDSVSIGGDAAGISNKNYTVTAGLQYRF
E     •S•••I••••A••••E•••••••KS
OmpT  ••••••YV•GAWNRVTNK••N•SLYD-HNNNTSDYSKNG•••E•Y•FIT••••K•T•
```

FIG. 3. Comparison of the deduced amino acid sequences of *pla*, gene *E* of *S. typhimurium*, and *ompT* of *E. coli*. Numbers indicate the amino acid residues of Pla. Residues of protein E and OmpT identical to those of Pla are indicated by dots. Gaps were introduced where necessary to optimize the alignment.

promoter), pOS31 (*pla* promoter), pOS32 (short ORF promoter) in CSH50 was 7, 4,659, and 946 U, respectively. Additional work will be required to determine if the short ORF is translated, but our initial observations with *lacZ* gene fusions suggest that it is translated either at an extremely low level or not at all (data not shown).

DISCUSSION

In addition to the striking degree of homology exhibited between protein E, Pla, and OmpT, Pla and OmpT also share the following characteristics: they are outer membrane proteins (13, 29, 31); they are plasminogen activators (13, 20, 28); they cleave particular outer membrane proteins of the strains which produce them, at least in vitro (Pla cleaves the YOPs [29] and OmpT cleaves the ferric enterobactin receptor [Fep] protein [10]); their molecular masses deduced from denaturing gels exceed those predicted from their nucleotide sequences (11, 14, 28); and they are posttranslationally processed following the export of their precursors across the cytoplasmic membrane to lower-molecular-mass species (11, 28). Although protein E apparently has both plasminogen activator and coagulase activities, we do not know which, if any, of the other properties mentioned above it shares with Pla and/or OmpT. Since a name consistent with standard genetic nomenclature has not been assigned to gene *E* of *S. typhimurium*, we propose the name *prtA* (protease A).

TABLE 2. Minimum number of bacteria needed to elicit observable activities[a]

Species	Minimum no. of bacteria needed for indicated activity	
	Plasminogen activator[b]	Coagulase
S. typhimurium WB600	NDt[c]	NDt[d]
S. typhimurium DB7000	NDt[c]	NDt[d]
E. coli CSH50	NDt[c]	NDt[d]
E. coli LE392	NDt[c]	NDt[d]
E. coli LE392(pJH502)	10^5	3×10^8
E. coli LE392(pJH502E::kan)	NDt[c]	NDt[d]
Y. pestis	10^2	10^8

[a] Assays were performed on sonicated and whole cells.
[b] None of the strains tested could lyse a fibrin film in which plasminogen had been denatured by heating.
[c] Not detectable (NDt) when 3×10^8 cells were used in the assays.
[d] Not detectable (NDt) when 3×10^9 cells were used in the assays.

Given the high degree of homology, it is clear that all three of these proteins are derived from a common ancestor. However, the relationships among the genes do not parallel the homology of the species that contain them (Fig. 5). The tree labeled A shows the relationship among the three genes, and that labeled B shows the relationship among the bacterial species based on hybridization of genomic DNA (19). Note that while *S. typhimurium* and *E. coli* are much more closely related than are *S. typhimurium* and *Y. pestis*, a comparison of the sequences of the three *pla*-related genes would indicate the opposite, suggesting that at least one of the genes has moved between two of these lineages.

pla would appear to be the most likely of the three to have originated in a lineage different from its current one. In both *S. typhimurium* and *E. coli* the *pla*-related genes are chromosomal, in contrast to the location of *pla* on a plasmid in *Y. pestis*. Moreover, this plasmid is stably maintained in *Y. pestis* as well as in *E. coli* and would almost certainly function well in other members of the family *Enterobacteriaceae*, including the salmonellae. The recent movement of *pla* is also supported by the observation that *Y. pseudotuberculosis*, which is very closely related to *Y. pestis*, lacks the *pla*-containing plasmid, an indication that the plasmid was acquired by *Y. pestis* subsequent to the divergence of these two species. Since the homology between *pla* and gene *E* is much less than that between the two yersiniae, it is unlikely that the divergence between the sequences of the two genes has accumulated since the arrival of *pla* in *Y. pestis*. One would therefore predict that a much closer relative of *pla* exists in a *Salmonella* lineage other than *S. typhimurium*. Note that gene exchange between such spe-

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gtgtttcctgatatcctgctgttatacctgcttttataattctcatcctggagtaaccagcctttcacattgaggtcatattcattggcat
        .     .    720      .     .    690      .     .    660

                                   10                  20
                   MetSerAspLeuSerThrGlnIleAspPheHisSerSerSerArgSerHisProTyrTyrLeuThr
gattaacatttgtagcaggatgagATGAGTGATCTGTCCACTCAGATTGATTTTCATTCATCCAGTCGTAGTCATCCATATTACCTGACC
        .     .    630      .     .    600      .     .    570

                30                  40
ArgLysProGluThrSerThrProTrpHisSerGlySerGluMetSerMetAspProArgIleTyrHisLeuSerGly
CGGAAGCCAGAGACGTCCACCCCCTGGCATTCAGGGTCAGAAATGAGTATGGATCCCAGGATATATCACCTTTCAGGAtagcgacatttt
        .     .    540      .     .    510      .     .    480
```

FIG. 4. Nucleotide sequence of the transcriptional unit on the complimentary strand of *pla*. Potential promoter and Shine-Dalgarno sequences are underlined, and the deduced amino acid sequence is given above the nucleotide sequence. The numbering of the nucleotide sequence is consistent with that of the *pla* sequence presented in Fig. 2.

cies and *Y. pestis* is not necessarily an unlikely event given their similar ecology: both are natural pathogens of rodents, and both grow within macrophages (8, 32).

An alternative source for the *pla* gene of pPCP1 is an inactive or poorly expressed *pla* homolog on the chromosome of *Y. pestis*. An explanation based on this source requires strong selection to maintain the homology of the chromosomal gene subsequent to the divergence of the *S. typhimurium* and *Y. pestis* lineages to account for the high degree of homology between *pla* and gene *E* of *S. typhimurium*. Selection for increased plasminogen activator activity, satisfied by an increased copy number of *pla*, would then account for the plasmid location of the gene. This hypothesis can be readily tested by hybridization experiments with *pla*-derived probes.

Despite the similarities among these proteins, *Y. pestis* has much greater plasminogen activator activity than have the

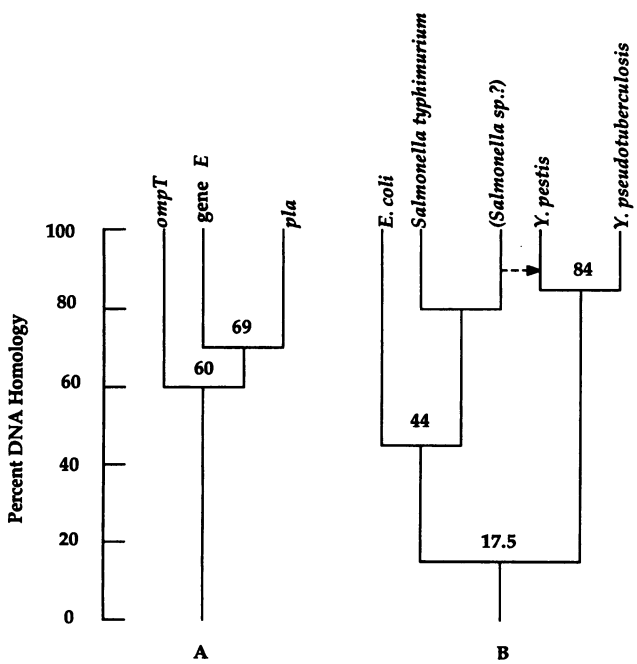

FIG. 5. Evolutionary trees based on the assumption of a common ancestor showing the degree of DNA homology among *pla*, *ompT*, and gene *E* (A) and the relatedness among the species based on hybridization of genomic DNA (B) (19). The numbers above the horizontal lines indicate the percentages of DNA homology at the points of divergence. The broken arrow indicates the possible origin of *pla*.

other two species. In our crude fibrin film lysis assay, plasminogen activator activity could be detected in *Y. pestis* but not in wild-type strains of the other species. This was true even when the number of bacteria applied to the fibrin film was a million times greater than necessary to detect the activity in *Y. pestis* (Table 2), suggesting that Pla may have a much higher specific activity against plasminogen than do OmpT and protein E. Measurement of the relative amounts of the proteins produced by the respective species will be necessary to confirm this interpretation.

Coagulase activity, even in *Y. pestis*, is very weak, suggesting that plasminogen activation is the major *pla* activity (Table 2); at least 10^8 cells must be added to 400 μl of plasma to observe the reaction, even with overnight incubation. In addition, the resulting clots are soluble in 5 M urea, an indication of incomplete cross-linking of the fibrin. This incomplete cross-linking may be the result of the unavoidable plasminogen activator activity present during the clotting reactions or of the induction of coagulation without the activation of thrombin. The latter is required to activate factor XIII, which catalyzes the cross-linking reaction.

The presence of *pla* may well explain important differences in the pathogenesis of the diseases caused by *Y. pestis* and *Y. pseudotuberculosis*. Unlike *Y. pseudotuberculosis*, which is transmitted by the fecal-oral route, *Y. pestis* relies on fleas for transmission between mammalian hosts. Consequently, it must produce bacteremia from an intradermal site of infection. *Y. pestis* strains lacking the *pla*-encoding plasmid are unable to do so (4, 5), suggesting that activation of plasminogen to the trypsinlike plasmin at foci of infection promotes invasiveness. Another major feature of plague possibly resulting from plasminogen activator activity is hemorrhaging, particularly in the lungs following primary pneumonic infections (9). If *pla* is responsible for these properties of *Y. pestis*, a seemingly innocuous plasmid transfer between two rodent pathogens may have had drastic consequences for humans.

The significance of the transcription unit on the complimentary strand of *pla* remains to be determined. It is plausible that a product derived from this transcription unit has a physiological function. For example, a transcript derived from this ORF may act as an antisense RNA that regulates the expression of *pla*, a situation similar to the regulation of *ompF* by *micF* antisense RNA in *E. coli* (1).

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

The homology of *ompT* and gene *E* was recently reported by K. Sugimura and T. Nishihara (J. Bacteriol. **170**:5625–5632, 1988). These investigators also showed that OmpT cleaves peptide substrates between consecutive basic (Arg or Lys) residues.

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