Genotype/Features	Reference or Source
<u>Strains</u>	
0395	Laboratory strain
O395- <i>pspA</i> -His <sub>6</sub>	This study
$O395\Delta lacZ$	Laboratory strain
$O395\Delta pspFABC$	This study
$O395\Delta pspA$	This study
$O395\Delta pspB$	This study
$O395\Delta pspBC$	This study
$O395\Delta pspF$	This study
O395lacZΩPpspA	This study
O395lacZΩPpspA, pspA-His <sub>6</sub>	This study
DH5a	Laboratory strain
JM101	Laboratory strain
DH5alpir	Laboratory strain
SM10λpir	Laboratory strain
Plasmids	
pTTQ18	(1)
pKAS32	(2)
pBAD18-kan	(3)
pBAD33	(3)
pBAD30	(3)
pKAS32- <i>ApspA</i>	This study
pKAS32- <i>ApspB</i>	This study
pKAS32- <i>ApspC</i>	This study
рKAS32- <i>ДрspBC</i>	This study
pKAS32- <i>ApspF</i>	This study

## **Table S1. Strains and Plasmids**

pKAS32- <i>ApspFABC</i>	This study
pKAS32-PpspA::lacZ	This study
pKAS32-pspA-6xHis	This study
pTTQ18-gspD-6xHis	This study
pTTQ18- <i>tcpC</i> -6xHis	This study
pTTQ18- <i>pilQ</i> -6xHis	This study
pTTQ18-mshL-6xHis	This study
pBAD18-kan-gspD-6xHis	This study
pBAD18-kan- <i>tcpC</i> -6xHis	This study
pBAD18-kan- <i>pilQ</i> -6xHis	This study
pBAD18-kan-mshL-6xHis	This study
pBAD33- <i>pspA</i> -6xHis	This study
pBAD33-ompT-FLAG	This study
pBAD30- <i>pspA</i> -6xHis	This study
pBAD30- <i>pspBC</i> -6xHis	This study
pBAD30-6xHis- <i>pspF</i>	This study
pBAD30-crp-FLAG	This study

- 1. Stark MJR. 1987. Multicopy expression vectors carrying the lac represser gene for regulated high-level expression of genes in Escherichia coli. Gene 51:255-267.
- 2. Skorupski K, Taylor RK. 1996. Positive selection vectors for allelic exchange. Gene 169:47-52.
- 3. Guzman LM, Belin D, Carson MJ, Beckwith J. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol 177:4121-30.



**Figure S1.** *pspABC* are cotranscribed. Cultures were grown to mid-log phase when GspD expression was induced for one hour. RNA was harvested for RT-PCR analysis. Primers spanning from *pspA* (P1) to *pspC* (P2) were used for amplification from cDNA made by reverse transcription (+RT), reverse transcription negative (-RT) samples, or from genomic DNA (gDNA).



## Figure S2. PspA is primarily localized in the soluble fraction under non-

**inducing conditions.** Cultures were grown to mid-log phase before fractionation into soluble (S) and membrane (M) fractions. Crp-FLAG and ToxR were used as cytoplasmic and membrane controls, respectively. WC = whole cell lysate, S = soluble, M = membrane fractions.



Figure S3. Complementation with *pspBC* and *pspF* restore *psp* activity to their respective deletion strains. Cultures were grown to mid-log phase when GspD and *psp* expression were induced for one hour.  $\beta$ -galactosidase activity was detected from the chromosomal reporter *lacZ* $\Omega$ *PpspA*.