Supplementary Online Material

Supplementary experimental procedures

Construction of P. aeruginosa mutants and plasmids

Construction of P. aeruginosa mutants- The non-polar chromosomal gene deletions in *P. aeruginosa* were obtained as previously described (1) by double cross over events and pKNG101 suicide vector properties (2). 500-bp upstream and downstream regions of target genes were PCR amplified, and tandemly cloned into pKNG101. One of the resulting construct, pKNGΔ*hxcQ* was transferred by conjugation in wild type *P. aeruginosa* in order to generate the *hxcQ* mutant and in an *xcpQ* mutant leading to the double *hxcQ xcpQ* mutant (Table 1). As it was shown that the residual T2SS protein secretion observed in a *xcpQ* mutant was related to the presence of XphA (P_A) and XqhA (Q_A), a functional secretin homolog to XcpQ, another construction, pKNGΔP_AQ_A (1) was used to generate a quadruple mutant deleted of *hxcQ, xcpQ, xqhA* and *xphA* (*hxcQ/xcpQ/*p_A/q_A) (Table 1).

Construction of pKNGΔ*hxcQ* was performed as followed: 500-bp regions upstream and downstream the *hxcQ* gene were PCR amplified using HiFi DNA polymerase (Roche) according to the manufacturer's protocol with the pairs of oligonucleotides (*hxcQ -500*/*hxcQ rev*) and (*hxcQ for*/*hxcQ +500*), respectively, and PAO1 genomic DNA as matrix. "rev" and "for" oligonucleotides were designed in order to create an overlapping region between the two fragments. The two fragments were ligated by overlapping PCR using the upstream *hxcQ -500* and downstream *hxcQ +500* primers and a mix of the two previously generated products as DNA matrix. The resulting PCR product was cloned into the pCR2.1 vector according to the manufacturer's protocol (TA cloning kit; Invitrogen). A 1,000-bps *Bam*HI-*Eco*RV DNA fragment, (containing the -500/ +500 *hxcQ* amplicons) was then subcloned into the suicide vector pKNG101.

Construction of pJNhxcQ$_{V5}$- pJN*hxcQ*$_{V5}$ encoding HxcQ with a C-terminal V5-hexahistidine (V5) tag ($HxcQ_{V5}$) was constructed using the Gateway PAO1 collection following the protocol described by Voulhoux *et al*; (4). Briefly, all individual PAO1 open reading frames (ORF) were amplified by PCR and cloned into an entry vector constituting the PAO1 Gateway library (3). Subsequently, any ORF can be easily moved into the desired destination vector by using phage *attR* and *attL* recombination sites flanking the cloned gene in the entry vector and in the destination vectors to allow phage recombinase-mediated cloning. We moved ORF PA0685 encoding HxcQ into the destination vector pET-DEST42 (Invitrogen) to produce a C-terminal V5-tagged HxcQ, called $HxcQ_{V5}$. The *hxcQ*$_{V5}$ gene was then subcloned into the broad-host-range vector pMMB67HE using *Xba*I/*Eco*RV and *Xba*I/*Sma*I restriction sites from pET-DEST42 and pMMB67HE, respectively. From the resulting plasmid pMMB67HE*hxcQ*$_{V5}$, a 3,139 bps fragment containing *hxcQ*$_{V5}$ was subcloned using X*ba*I/*Sac*I restriction sites into the broad-host-range vector pJN105 to yield pJN*hxcQ*$_{V5}$ in which *hxcQ*$_{V5}$ is placed under the tight control of the arabinose-inducible *bad* promoter.

Construction of pJNhxcQnl$_{V5}$- Firstly, the 5' region of *xcpQ*$_{V5}$ gene encoding the XcpQ signal peptide was PCR-amplified with oligos *petDEST42 for* and *xcpQ114 rev* using pET-DEST42*xcpQ*$_{V5}$ as matrix, yielding the "XcpQ SP" amplicon. Secondly, the *hxcQ*$_{V5}$ DNA sequence encoding the mature $HxcQ_{V5}$ was amplified with oligos *hxcQ85 for petDEST42 rev* using pET-DEST42*hxcQ*$_{V5}$ as matrix, yielding the "HxcQ M" amplicon. "rev" and "for" oligonucleotides were designed in order to create an overlapping region between the two amplicons. Both fragments were ligated by overlapping PCR using the most upstream *petDEST42 for* and downstream *petDEST42 rev* primers. The resulting PCR product was cloned into the pCR2.1 vector and then subcloned into the broad-host range vector pJN105 using *Eco*RI restriction site to give pJN*hxcQnl*$_{V5}$.

Construction of pJNhxcQnl- The 3' end region of *hxcQnl*$_{V5}$ gene was PCR amplified with oligo *hQnl_1 for* and *hQnl_4* using *pCR2.1hxcQnl*$_{V5}$ as the template and cloned into pCR2.1, yielding pCR2.1*hQnlend*. The *Xba*I/*Cla*I fragment from pCR2.1*hQnlend* was substituted to the *Spe*I/*Cla*I fragment of pCR2.1*hxcQnl*$_{V5}$ and renamed pCR2.1*hxcQnl*. The recombinant *hxcQnl* was then subcloned into the broad-host range vector pJN105 using *Eco*RI restriction site to give pJN*hxcQnl*.

References
1. Michel, G. P., Durand, E., and Filloux, A. (2007) *J. Bacteriol.* **189**, 3776-3783
2. Kaniga, K., Delor, I., and Cornelis, G. R. (1991) *Gene* **109**, 137-141
3. Labaer, J., Qiu, Q., Anumanthan, A., Mar, W., Zuo, D., Murthy, T. V., Taycher, H., Halleck, A., Hainsworth, E., Lory, S., and Brizuela, L. (2004) *Genome Res.* **14**, 2190-2200
4. Voulhoux, R., Filloux, A., and Schalk, I. J. (2006) *J. Bacteriol.* **188**, 3317-3323

Supplementary figures

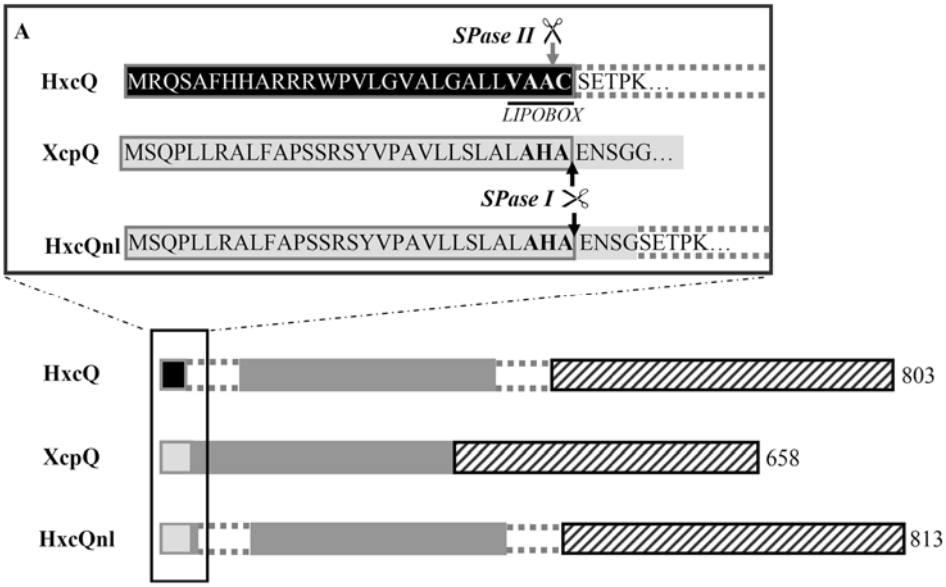

Fig. S1. Schematic representation of HxcQ, XcpQ and HxcQnl signal sequences. HxcQ, HxcQnl and XcpQ secretins are represented following the color code used in figure 2. The sizes (in amino acids) of the different secretins are indicated on the right. The upper panel highlights signal sequences. The typical lipoprotein motif (lipobox) found in HxcQ is underlined. Recognition sites for signal peptidases I and II (SPase I and SPase II) are also indicated.

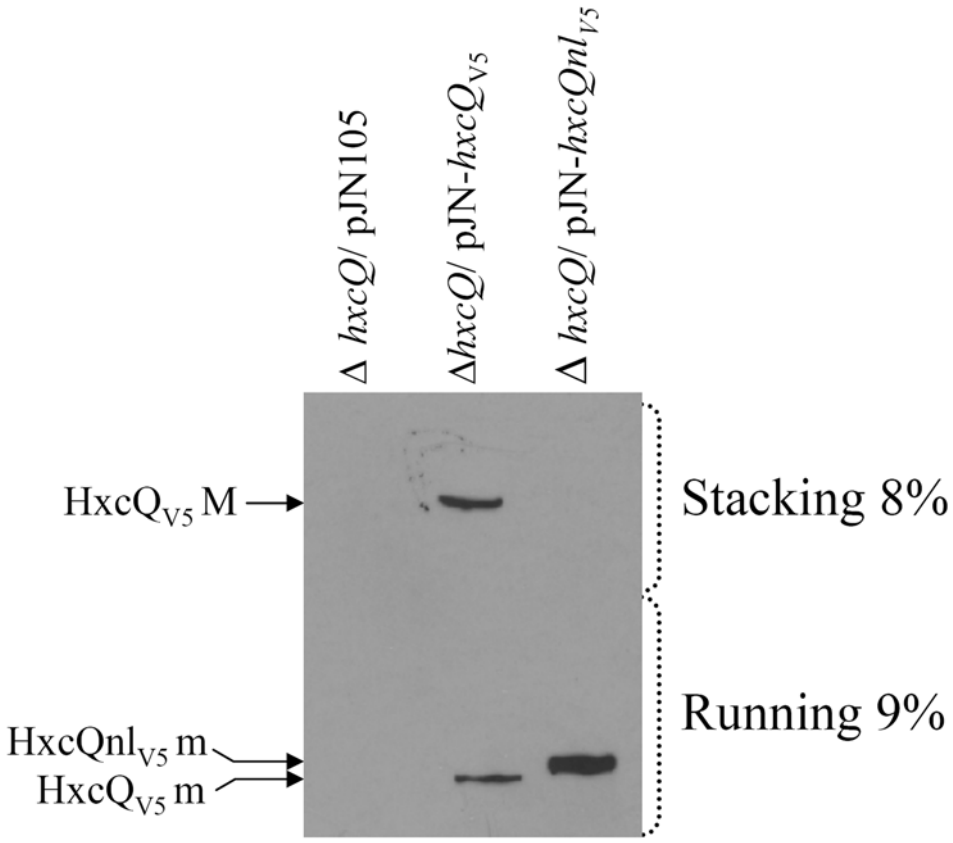

Fig. S2. Heat resistance of HxcQ and HxcQnl. Immunoblot analysis with anti-V5 serum of whole cell extracts of the *hxcQ* mutant carrying the plasmid pJN105 as control and complemented with pJN*hxcQ*$_{V5}$ or pJN*hxcQnl*$_{V5}$. Strains were grown under standard conditions to induced HxcQ$_{V5}$ or HxcQnl$_{V5}$ production. The bacteria were collected by centrifugation and equal amounts of cell (in OD equivalent units) were resuspended in SDS-PAGE sample containing 2% SDS heated at 95°C during 10 min. The proteins were separated on a 8% polyacrylamide stacking gel / 9% polyacrylamide running gel. In contrast to HxcQ$_{V5}$ which mainly migrates as an HMW complex in the stacking gel in these standard SDS- PAGE conditions, HxcQnl$_{V5}$ migrates exclusively as a monomer. The positions of the HxcQ$_{V5}$ multimers (M) and HxcQ$_{V5}$ and HxcQnl$_{V5}$ monomers (m) are indicated on the left.