

Table S1. Real time qRT-PCR analysis^a

Parameter and gene expressed	<i>V. anguillarum</i> strain ^b	Average (\pm SD ^c)	
		Log phase	Stationary phase
<i>rtxA</i>			
RNA Copy number	M93sm	$2.66 \times 10^4 (\pm 3.76 \times 10^3)$	$8.57 \times 10^3 (\pm 3.60 \times 10^2)$
	<i>hns</i> -	$7.75 \times 10^4 (\pm 1.11 \times 10^4)$	$1.83 \times 10^4 (\pm 2.13 \times 10^3)$
	<i>hns</i> +	$9.72 \times 10^2 (\pm 10)$	$2.94 \times 10^2 (\pm 19)$
	<i>hlyU</i> -	$2.38 \times 10^2 (\pm 1.38 \times 10^2)$	$6.02 \times 10^2 (\pm 90)$
	<i>hns</i> -/ <i>hlyU</i> -	$6.47 \times 10^4 (\pm 9.92 \times 10^3)$	$1.23 \times 10^4 (\pm 1.47 \times 10^3)$
	<i>hns</i> -/ <i>hlyU</i> +	$1.43 \times 10^5 (\pm 2.00 \times 10^4)$	$2.13 \times 10^4 (\pm 4.91 \times 10^2)$
	<i>hns</i> +/ <i>hlyU</i> -	$1.24 \times 10^4 (\pm 1.75 \times 10^2)$	$3.74 \times 10^3 (\pm 4.65 \times 10^2)$
Relative change (fold) to M93Sm ^d	M93Sm	1.00 (± 0.14)	1.00 (± 0.04)
	<i>hns</i> -	2.91 (± 0.42)	2.14 (± 0.25)
	<i>hns</i> +	-27.37 (± 0.00)	-29.15 (± 0.00)
	<i>hlyU</i> -	-11.18 (± 0.01)	-14.23 (± 0.01)
Relative change (fold) to <i>hlyU</i> - ^d	<i>hlyU</i> -	1.00 (± 0.06)	1.00 (± 0.15)
	<i>hns</i> -/ <i>hlyU</i> -	27.21 (± 4.17)	20.50 (± 2.43)
	<i>hns</i> +/ <i>hlyU</i> -	5.22 (± 0.07)	6.22 (± 0.77)
Relative change (fold) to <i>hns</i> - ^d	<i>hns</i> -	1.00 (± 0.14)	1.00 (± 0.12)
	<i>hns</i> -/ <i>hlyU</i> -	-1.20 (± 0.13)	-1.48 (± 0.08)
	<i>hns</i> -/ <i>hlyU</i> +	1.84 (± 0.26)	1.16 (± 0.03)
<i>rtxH</i>			
RNA Copy number	M93Sm	$6.80 \times 10^4 (\pm 1.80 \times 10^4)$	$2.45 \times 10^4 (\pm 3.19 \times 10^3)$

	<i>hns</i> -	$3.10 \times 10^5 (\pm 8.58 \times 10^4)$	$5.85 \times 10^4 (\pm 3.56 \times 10^3)$
	<i>hns</i> +	$9.43 \times 10^3 (\pm 1.31 \times 10^3)$	$8.59 \times 10^3 (\pm 6.12 \times 10^2)$
Relative change (fold) to M93Sm ^d	M93Sm	1.00 (± 0.26)	1.00 (± 0.13)
	<i>hns</i> -	4.56 (± 1.26)	2.39 (± 0.15)
	<i>hns</i> +	-7.21 (± 0.02)	-2.85 (± 0.02)

rtxB

RNA Copy number	M93sm	$4.17 \times 10^4 (\pm 4.01 \times 10^3)$	$1.71 \times 10^4 (\pm 1.23 \times 10^3)$
	<i>hns</i> -	$5.35 \times 10^4 (\pm 9.58 \times 10^3)$	$2.44 \times 10^4 (\pm 3.10 \times 10^3)$
	<i>hns</i> +	$3.89 \times 10^3 (\pm 5.19 \times 10^2)$	$1.59 \times 10^3 (\pm 18)$
	<i>hlyU</i> -	$9.54 \times 10^3 (\pm 5.96 \times 10^2)$	$3.62 \times 10^3 (\pm 1.41 \times 10^2)$
	<i>hns</i> -/ <i>hlyU</i> -	$7.31 \times 10^4 (\pm 7.23 \times 10^3)$	$3.56 \times 10^4 (\pm 7.66 \times 10^2)$
	<i>hns</i> -/ <i>hlyU</i> +	$2.92 \times 10^4 (\pm 8.00 \times 10^3)$	$1.85 \times 10^4 (\pm 2.48 \times 10^3)$
	<i>hns</i> +/ <i>hlyU</i> -	$1.14 \times 10^4 (\pm 1.42 \times 10^3)$	$1.47 \times 10^4 (\pm 5.74 \times 10^2)$
Relative change (fold) to M93Sm ^d	M93Sm	1.00 (± 0.10)	1.00 (± 0.07)
	<i>hns</i> -	1.28 (± 0.23)	1.43 (± 0.18)
	<i>hns</i> +	-10.72 (± 0.01)	-10.77 (± 0.00)
	<i>hlyU</i> -	-4.37 (± 0.01)	-4.73 (± 0.01)
Relative change (fold) to <i>hlyU</i> - ^d	<i>hlyU</i> -	1.00 (± 0.06)	1.00 (± 0.04)
	<i>hns</i> -/ <i>hlyU</i> -	7.67 (± 0.76)	9.84 (± 0.21)
	<i>hns</i> +/ <i>hlyU</i> -	1.20 (± 0.15)	4.06 (± 0.16)
Relative change (fold) to <i>hns</i> - ^d	<i>hns</i> -	1.00 (± 0.18)	1.00 (± 0.13)
	<i>hns</i> -/ <i>hlyU</i> -	1.37 (± 0.14)	1.46 (± 0.03)
	<i>hns</i> -/ <i>hlyU</i> +	-1.83 (± 0.15)	-1.32 (± 0.10)

vah1

RNA Copy number	M93sm	$1.40 \times 10^3 (\pm 1.76 \times 10^2)$	$6.19 \times 10^2 (\pm 94)$
	<i>hns</i> -	$2.27 \times 10^4 (\pm 6.40 \times 10^3)$	$1.24 \times 10^4 (\pm 2.69 \times 10^3)$
	<i>hns</i> +	$1.06 \times 10^3 (\pm 3.62 \times 10^2)$	$1.88 \times 10^2 (\pm 17)$
	<i>hlyU</i> -	$3.24 \times 10^3 (\pm 1.94 \times 10^2)$	$4.74 \times 10^2 (\pm 88)$
	<i>hns</i> -/ <i>hlyU</i> -	$1.68 \times 10^4 (\pm 3.21 \times 10^3)$	$1.95 \times 10^4 (\pm 2.90 \times 10^3)$
	<i>hns</i> -/ <i>hlyU</i> +	$1.30 \times 10^4 (\pm 1.48 \times 10^2)$	$3.04 \times 10^4 (\pm 1.93 \times 10^3)$
	<i>hns</i> +/ <i>hlyU</i> -	$5.34 \times 10^3 (\pm 5.30 \times 10^2)$	$6.45 \times 10^3 (\pm 4.17 \times 10^2)$
Relative change (fold) to M93Sm ^d	M93Sm	1.00 (± 0.13)	1.00 (± 0.15)
	<i>hns</i> -	16.21 (± 4.57)	20.01 (± 4.35)
	<i>hns</i> +	-1.32 (± 0.26)	-3.30 (± 0.03)
	<i>hlyU</i> -	2.31 (± 0.14)	-1.30 (± 0.14)
Relative change (fold) to <i>hlyU</i> - ^d	<i>hlyU</i> -	1.00 (± 0.06)	1.00 (± 0.19)
	<i>hns</i> -/ <i>hlyU</i> -	5.20 (± 0.99)	41.02 (± 6.11)
	<i>hns</i> +/ <i>hlyU</i> -	1.65 (± 0.16)	13.60 (± 0.88)
Relative change (fold) to <i>hns</i> - ^d	<i>hns</i> -	1.00 (± 0.28)	1.00 (± 0.22)
	<i>hns</i> -/ <i>hlyU</i> -	-1.35 (± 0.14)	1.57 (± 0.23)
	<i>hns</i> -/ <i>hlyU</i> +	-1.75 (± 0.01)	2.46 (± 0.16)

plp

RNA Copy number	M93sm	$8.50 \times 10^2 (\pm 2.07 \times 10^2)$	$3.02 \times 10^2 (\pm 8)$
	<i>hns</i> -	$2.66 \times 10^4 (\pm 4.08 \times 10^3)$	$1.11 \times 10^4 (\pm 2.43 \times 10^2)$
	<i>hns</i> +	$4.21 \times 10^2 (\pm 1.08 \times 10^2)$	$3.18 \times 10^2 (\pm 73)$
	<i>hlyU</i> -	$1.14 \times 10^3 (\pm 3.38 \times 10^2)$	$3.46 \times 10^2 (\pm 50)$

	<i>hns-/hlyU-</i>	$8.46 \times 10^3 (\pm 1.64 \times 10^3)$	$1.18 \times 10^4 (\pm 2.15 \times 10^3)$
	<i>hns-/hlyU+</i>	$8.58 \times 10^3 (\pm 8.21 \times 10^2)$	$2.39 \times 10^4 (\pm 8.47 \times 10^2)$
	<i>hns+/hlyU-</i>	$2.02 \times 10^3 (\pm 5.75 \times 10^2)$	$3.58 \times 10^3 (\pm 1.54 \times 10^2)$
Relative change (fold) to M93Sm ^d	M93Sm	1.00 (± 0.24)	1.00 (± 0.03)
	<i>hns-</i>	31.27 (± 4.80)	36.88 (± 0.81)
	<i>hns+</i>	-2.02 (± 0.13)	1.05 (± 0.24)
	<i>hlyU-</i>	1.34 (± 0.40)	1.15 (± 0.17)
Relative change (fold) to <i>hlyU-</i> ^d	<i>hlyU-</i>	1.00 (± 0.30)	1.00 (± 0.15)
	<i>hns-/hlyU-</i>	7.46 (± 1.44)	34.02 (± 6.21)
	<i>hns+/hlyU-</i>	1.78 (± 0.51)	10.35 (± 0.44)
Relative change (fold) to <i>hns-</i> ^d	<i>hns-</i>	1.00 (± 0.15)	1.00 (± 0.02)
	<i>hns-/hlyU-</i>	-3.14 (± 0.06)	1.06 (± 0.19)
	<i>hns-/hlyU+</i>	-3.10 (± 0.03)	2.15 (± 0.08)

^a The data presented is a representative experiment of two independent experiments. Each sample is the average of three replicates.

^b *V. anguillarum* strains: M93Sm (wild type), *hns-* (*hns* mutant, M114), *hns+* (*hns* complemente, M116), *hlyU-* (*hlyU* mutant, S305), *hns-/hlyU-* (*hns/hlyU* double mutant, ES114), *hns-/hlyU+* (*hns/hlyU* double mutant with *hlyU* complemented, ES115), *hns+/hlyU-* (*hns/hlyU* double mutant with *hns* complemented, ES116)

^c Standard Deviation

^d Gene expression is up-regulated (positive number) or down-regulated (negative number) compared to expression in M93Sm.

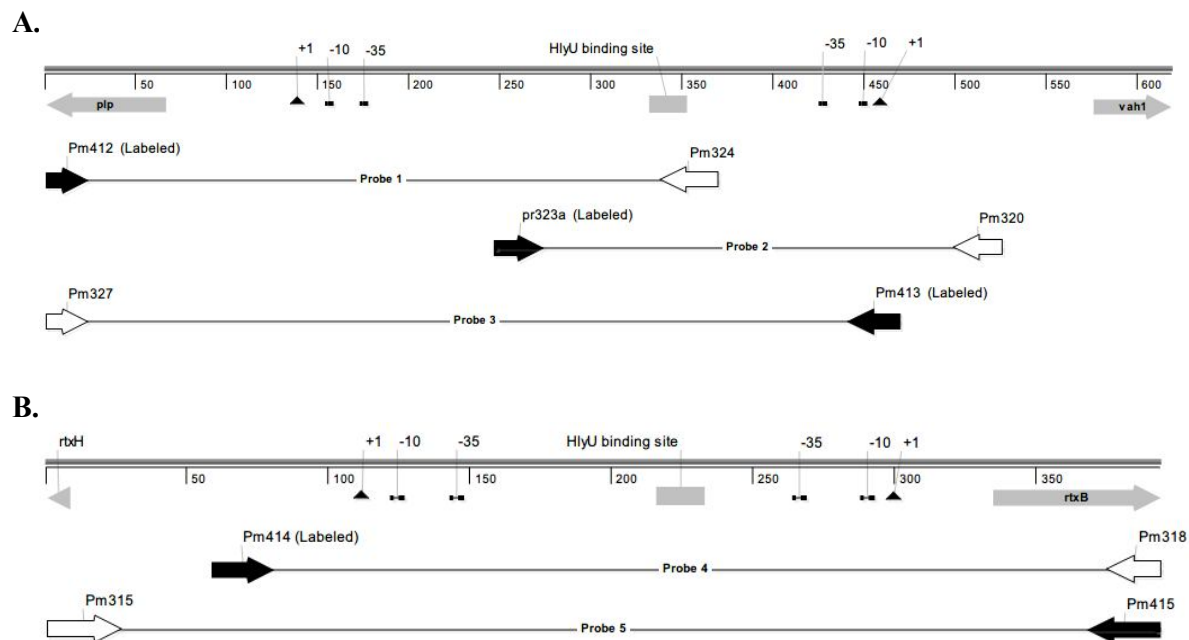


Figure S1. 6-FAM labeled DNA probes (probes 1-5) used for DNaseI protection studies of the intergenic regions between **A)** *plp* and *vah1* and **B)** *rtxH* and *rtxB* were obtained by PCR amplification using the primers in Table 2.

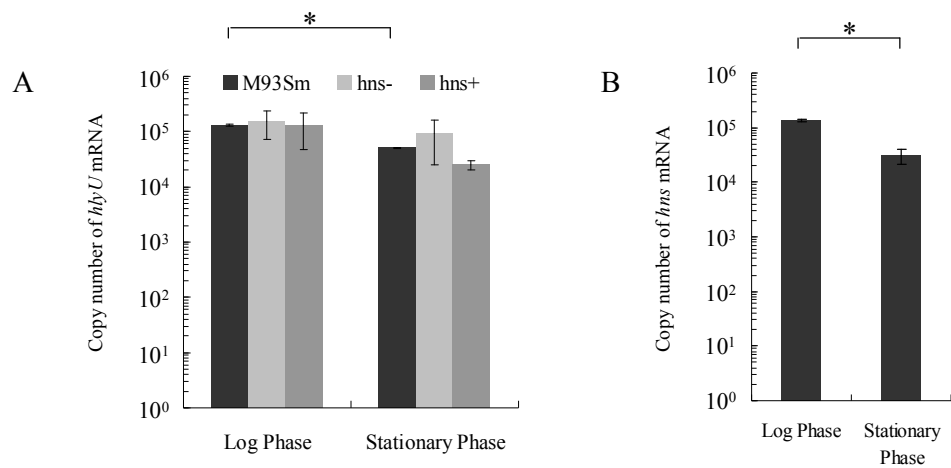
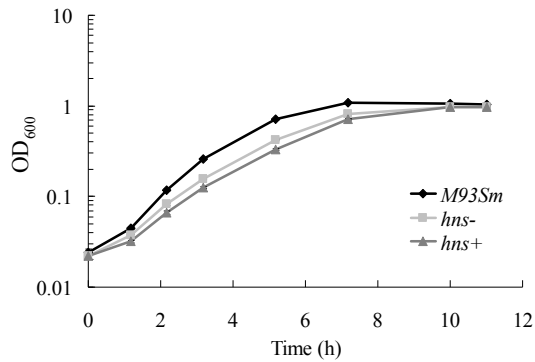


Figure S2. Real-time qRT-PCR was performed to determine **A)** the expression level of *hlyU* in the wild-type strain (M93Sm), *hns* mutant (*hns*⁻), and the *hns* complement (*hns*⁺) during exponential and stationary growth phases. Each sample is the average of three replicates. The data presented are the averages of two independent experiments. **B)** Expression level of *hns* in the wild-type strain (M93Sm) during exponential and stationary growth phases. Each sample is the average of three replicates. Statistically significant differences between samples are marked with a bracket and an * symbol. Error bars represent 1 standard deviation.

A.



B.

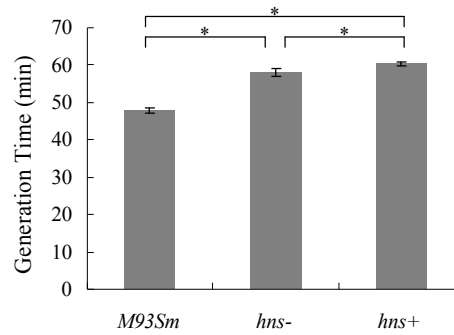


Figure S3. A) Growth curve of *V. anguillarum* wild type (M93Sm), *hns* mutant (*hns*-) and *hns* complemented (*hns*+) strains in LB20. **B)** Generation time of *V. anguillarum* wild type (M93Sm), *hns* mutant (*hns*-) and *hns* complemented (*hns*+) strains grown in LB20. Statistically significant differences between samples are marked with a bracket and an * symbol. Error bars represent 1 standard deviation.