Transcriptomic, proteomic and phenotypic analysis of the *Vibrio campbellii* stationary phase alternative sigma factors RpoS1 and RpoS2

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Introduction

The alternative σ factor RpoS is the central regulator of the bacterial stress response and stationary phase in *Escherichia coli* and other bacteria (Hengge-Aronis 2002) and it is known to be essential for the adaptation of vibrios in the marine environment (Hèulsmann *et al.* 2003). *Vibrio campbellii*, a free-living Gram-negative bacterium, is generally found in the surface waters of tropical marine environments and is an ecologically and commercially important shellfish pathogen (Macey *et al.* 2008; Sung *et al.* 2008). This microorganism's ability to survive within these varied environments, in which it routinely encounters fluctuations in temperature, salinity, nutrient levels, UV radiation and oxidative stress, is dependent upon its capacity to rapidly adapt to these changes. While almost all bacterial systems studied to date are known to utilize a single *rpoS* gene to mediate these adaptations, the genome of the bioluminescent marine bacterium *Vibrio campbellii* BAA-1116 (formerly known as *Vibrio harveyi* BB120/BAA-1116 (Lin *et al.* 2010)) harbors two alternative sigma factor *rpoS* genes: the *rpoS* ortholog (*rpoS1*) and a second, horizontally-acquired *rpoS* xenolog (*rpoS2*). The objective of this study was to determine whether *rpoS2* is expressed and functional and to elucidate the stationary phase RpoS1 and RpoS2 regulons in *V. campbellii*.

Materials and Methods

In order to begin to understand the functional roles of RpoS1 and RpoS2 in *V. campbellii* physiology, we examined stationary phase cultures of wild type *V. campbellii* and its isogenic Δ*rpoS1*, Δ*rpoS2*, and Δ*rpoS1*/Δ*rpoS2* deletion mutants using reverse transcription PCR, Western blotting, whole genome expression profiling, whole cell LC-MS/MS proteomics and traditional phenotypic analyses (growth, motility, bioluminescence, hemolysin production, protease secretion, biofilm formation, survival during prolonged starvation and oxidative stress).

Results and Discussion

Sequence similarity searches using the assembled genome of *V. campbellii* strain BAA-1116 revealed two chromosome I CDS (orf03062 and orf03598) that were identified as *rpoS* genes. Orf03062 encodes a 328 AA protein that is 97%, 79% and 70% identical to the *V. harveyi*

B392 RpoS (Lin *et al.* 2002), *V. cholerae* O1 RpoS (Yildiz *et al.* 1998) and *E. coli* RpoS, respectively and phylogenetic analyses demonstrated that it formed a distinct cluster with other known *Vibrio* RpoS orthologs. Based on its sequence identity, genetic locus and phylogeny, we identified orf03062 as the *rpoS* ortholog and designated it *rpoS1* (RpoS1). In contrast, the second identified CDS (orf03598) encodes a 287 AA protein that is 49% identical to *V. campbellii* BAA-1116 RpoS1 and 47% identical to *E. coli* RpoS. Phylogenetic analysis of orf03598 demonstrated that it distinctly resided outside the *Vibrio* RpoS ortholog cluster and in between the RpoS and RpoD clades. Its relative phylogenetic position and clear lack of a near neighbor suggested that this CDS has likely been acquired from an as yet unsequenced lineage. Taken together, these findings suggested that orf03598 is a recent horizontally-acquired *rpoS* xenolog and thus was designated *rpoS2* (RpoS2).
Reverse transcription PCR and Western blot time course experiments demonstrated that while both alternative sigma factors were expressed during the lag, mid-log, late-log and stationary phases of growth, peak expression of both RpoS1 and RpoS2 occurred during the stationary phase. This finding led us to perform comparative whole genome expression profiling experiments using stationary phase cultures of wild type *V. campbellii* BAA-1116 and its isogenic Δ*rpoS1*, Δ*rpoS2* and Δ*rpoS1*/Δ*rpoS2* deletion mutants to begin to elucidate the RpoS1 and RpoS2 regulons. When compared to the wild type strain, the expression profiles of the three mutant strains revealed that 7.5% - 10.2% of the genome was significantly differentially expressed in the absence of one or both alternative sigma factors (Table 1).

Table 1: Summary of modulated genes and proteins.

Strain	No. of DE[1] genes[2]	Median coefficient (-0.5>x>+0.5) Down-regulated	Up-regulated	No. of DE proteins[3]
Δ*rpoS1*	362	97	13	41
Δ*rpoS2*	486	117	13	ND[1]
Δ*rpoS1*/Δ*rpoS2*	493	95	70	ND

[1] DE: differentially expressed; ND: not determined.
[2] Based on the expression profiling of 4,831 CDS (adj. p value = 0.001).
[3] 725 unique proteins were identified and quantified.

Median coefficient values of < -0.5 or > +0.5 were used as the threshold for down selecting the most significantly modulated genes. Analysis of the down-regulated CDS from Δ*rpoS1* (n=97) revealed that RpoS1 coordinates the expression of genes involved in oxidative stress response, polyhydroxybutyrate synthesis, membrane transport, bioluminescence, the TCA cycle, electron transport, amino acid metabolism and nucleotide metabolism. A similar analysis of Δ*rpoS2* (n=117) revealed that RpoS2 coordinates the expression of genes involved in membrane transport, bioluminescence, flagellar biosynthesis, branched amino acid metabolism and ribosome synthesis. Interestingly, the RpoS1 and RpoS2 regulons only appear to overlap by ~20% whereas the Δ*rpoS1* and Δ*rpoS1*/Δ*rpoS2* strains share 90% of their down regulated genes. This high percentage of RpoS1 controlled genes found in the double mutant suggests that RpoS1 is the primary transcriptional regulator of the stationary phase (when compared to RpoS2). The expression profiles of the Δ*rpoS1*/Δ*rpoS2* mutant also revealed an up-regulation of 70 genes. This subset of up-regulated genes may be the result of a potential synergistic action of RpoS1 and RpoS2 to function as negative regulators of gene expression or σ factor competition where the absence of these stationary phase σ factors enables another to better compete for finite numbers of core RNAP and shared promoters thus

resulting in the perceived outcome (Farewell *et al*. 1998).
To verify a subset of these findings, a quantitative two-dimensional LC-MS/MS proteome analysis was performed comparing stationary phase cultures of the wild type and Δ*rpoS1* strains. This comparison revealed that 41 of the 725 identified proteins were differentially expressed in the Δ*rpoS1* mutant. Importantly, each of the 41 proteins identified either confirmed its cognate transcript modulation data (e.g. catalase/peroxidase KatG, serine protein kinase PrkA, outer membrane protein OmpN, luciferase LuxAB, enolase Eno, acetyl-CoA dehydrogenase CaiA, pyruvate dehydrogenase PdhAB) or belonged to the same pathway or process (e.g. oxidative stress response, central metabolism, membrane transport, bioluminescence, motility) as the identified differentially expressed genes. Where possible, the transcriptomic and proteomic findings were further supported by accompanying phenotypic assays (motility, bioluminescence, stationary phase survival and oxidative stress assays).

Conclusions

Our results confirm that the horizontally-acquired *rpoS* xenolog *rpoS2* is expressed and functional in *V. campbellii* BAA-1116. Thus, in addition to *rpoS1*, this strain appears to uniquely utilize a second σ^S factor to aid in adaptive genetic regulation. These findings, which were confirmed at the transcriptomic, proteomic and phenotypic levels, begin to outline the size and composition of the RpoS1 and RpoS2 regulons and provide the first global view of sigma factor-mediated stress response and its impact on *V. campbellii* physiology.

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