Proline content and varying barometric pressure: an analysis of six genes, across three marine a*ltererythrobacter s*trains

Environmental stresses select for suitably adapted genes. These stresses will promote the propagation of certain mutations, and the loss of others, throughout the evolution of a species. For organisms living two thousand meters below the ocean's surface, one of these stresses is certainly high barometric pressure. Due to the imino ring structure of proline it was hypothesized that bacteria sampled from greater depths would show a larger proportion of content in their proteins. This hypothesis was examined by analyzing the proline content of 6 proteins common to 3 species from the *altereythrobacter* speciesl

All the selected strains are Gram negative, moderately halophilic marine bacteria with optimum temperatures around 30^{0}C. *A. marensis* strain KCTC22370 (taxonomy ID: 543877) was sampled, and genomically annotated, by Soo and Lee from the surface of the sea near Jeju island in the Republic of North Korea (2010). *A. epoxidivorans* strain JCS350 (taxonomy ID: 361183) was sampled from cold-seep sediment in the Kagoshima Bay, off the coast of Japan, at an approximate depth of 120m. The strain was isolated and genomically annotated by Kwon *et.al.* (2007). *A. atlanticus* strain 26DY36 (taxonomy ID: 1267766) was sampled from a North Atlantic mid ocean range at a depth of 2577m. The strain was isolated and annotated by Wu *et. al.* (2015).

The data showed no significant differences in the proline content of the 3 species examined, however a more expansive study would be required to fully reject the hypothesis.

Proline Content and Varying Barometric Pressure: an Analysis of Six Genes, Across Three Marine *Altererythrobacter* Strains

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Environmental stresses select for suitably adapted genes. These stresses will promote the propagation of certain mutations, and the loss of others, throughout the evolution of a species. For organisms living two thousand meters below the ocean's surface, one of these stresses is certainly high barometric pressure. The proteins of bacterial species living under barometric extremes must have sufficient strength to maintain their structural integrity. The mechanism by which enzymes can function under barometric extremes is of commercial interest, especially in terms of developing high pressure bioreactors (Horikoshi, 1998). As such, the aim of this research is to examine the content of proline in the gene products of three marine bacterial species isolated from varying depths, thus living under varying barometric pressures. The imino ring side chain of proline gives it a distinct structural and characteristic profile from the other 19 common amino acids. Proline is exceptionally rigid and it provides regional stability to proteins. As such, it is hypothesized that microorganisms living under high barometric stress will have a higher proline content in their proteins, relative to phylogenetically related organisms living under lower barometric pressure.

To examine this hypothesis three bacterial strains, from the *Altererythrobacter* genus, with annotated and open source genomes were selected. All the selected strains are Gram negative, moderately halophilic marine bacteria with optimum temperatures around 30°C. *A. marensis* strain KCTC22370 (taxonomy ID: 543877) was sampled, and genomically annotated, by Soo and Lee from the surface of the sea near Jeju island in the Republic of North Korea (2010). *A. epoxidivorans* strain JCS350 (taxonomy ID: 361183) was sampled from cold-seep sediment in the Kagoshima Bay, off the coast of Japan, at an approximate depth of 120m. The strain was isolated and genomically annotated by Kwon *et.al.* (2007). *A. atlanticus* strain 26DY36 (taxonomy ID: 1267766) was sampled from a North Atlantic mid ocean range at a depth of 2577m. The strain was isolated and annotated by Wu *et. al.* (2015). The barometric pressure experienced at the surface of the ocean is 1 atm, at a depth of 120m the pressure is roughly 13 atm and at a depth of 2577m the pressure is roughly 256 atm.

Six genes, common to each of the three *Altererythrobacter* strains, were chosen for analysis. The genes were: a) the NADH dehydrogenase gene (gene product is involved in electron transport); b) RNA polymerase sigma rpoD (codes for a subunit of RNA polymerase) (Burton *et. al*, 1983); c) the DNA primase gene (gene product is involved in DNA replication); d) the triose phosphate isomerase gene (the gene product is a central enzyme in glycolysis); e) the heat shock protein (hsp) 33 gene (normally inactive, but gets expressed during high intracellular acidity) (Graumann *et. al.*, 2001); and f) the ferrous iron transport B gene (the gene product is involved in the import and export of Fe^{2+}). The NCBI protein ID numbers, of all 18 proteins examined, can be found in table A1 of appendix 1. The genome-wide similarity between the strains is as follows: *A. atlanticus* and *A. epoxidivorans* are 92% identical; *A. atlanticus* and *A. marensis* are 85% identical; and *A. epoxidivorans* and *A. marensis* are 89% identical (all percentages were obtained using NCBI BLAST).

Using the translational outputs generated from the NCBI databases the proportion of proline content was determined. The number of prolines; the total number of amino acids; the proportion of proline content; and the mean proportion of proline content for each strain can be found below in table 1.

Table 1: Table showing the strain (depth in m), as it corresponds to the proportion of proline content in each strain's six proteins. The total number of prolines per gene, the total number of amino acids per gene and the mean proportion of proline content for each strain is also shown.

strain (depth)	proteins: (# of proline/total # of amino acids= proportion of proline)						mean proportion of proline
	NADH dehydrogenase	RNA pol sigma rpoD	DNA primase	triose P isomerase	HSP 33	ferrous iron transport B	
A. atlanticus (≈2500m)	8/133= 0.0602	20/671= 0.0298	44/639=0.0689	9/252= 0.0357	11/300= 0.0367	36/617= 0.0583	0.04826
A. epoxidivorans (≈120m)	14/231=0.0606	19/686=0.0277	39/629=0.0620	6/180=0.0333	10/296= 0.0338	31/618= 0.0502	0.04460
A. marensis (≈0m)	16/222=0.0721	19/673=0.0282	41/629=0.0652	8/254=0.0315	14/297=0.0471	34/618= 0.0550	0.04985

The assumptions for an ANOVA test were checked using a Levene's test and a Shapiro-Wilk test. The ANOVA was performed (using R 3.1.1) on the mean proline content in each strain to determine whether they differed significantly at α=0.05. The analysis found that the proline content did not differ significantly between any of the strains (ANOVA; $F = 0.1642$; $df = 2, 15$; $P = 0.8501$). A visualization of the proline content in the analyzed proteins, from all three strains, can be found in figure 1.

The data do not support the hypothesized trend, but further research could elicit a relationship between the barometric environment and the composition of certain amino acids. For instance, thermophilic proteins contain a high proportion of stabilizing disulfide bonds (created from cysteine residues) (Barton, 2004). This may be an avenue worth exploring in barophiles. Under the parameters of this research, the hypothesis cannot be supported. However, the hypothesis cannot be fully dismissed without a more comprehensive study. This study would entail larger sample sizes and many taxa. Nonetheless, this research shed an interesting light on the protein composition of marine microorganisms. The mechanisms of conformational rigidity, displayed by barophilic proteins, is an area that elicits further investigation.

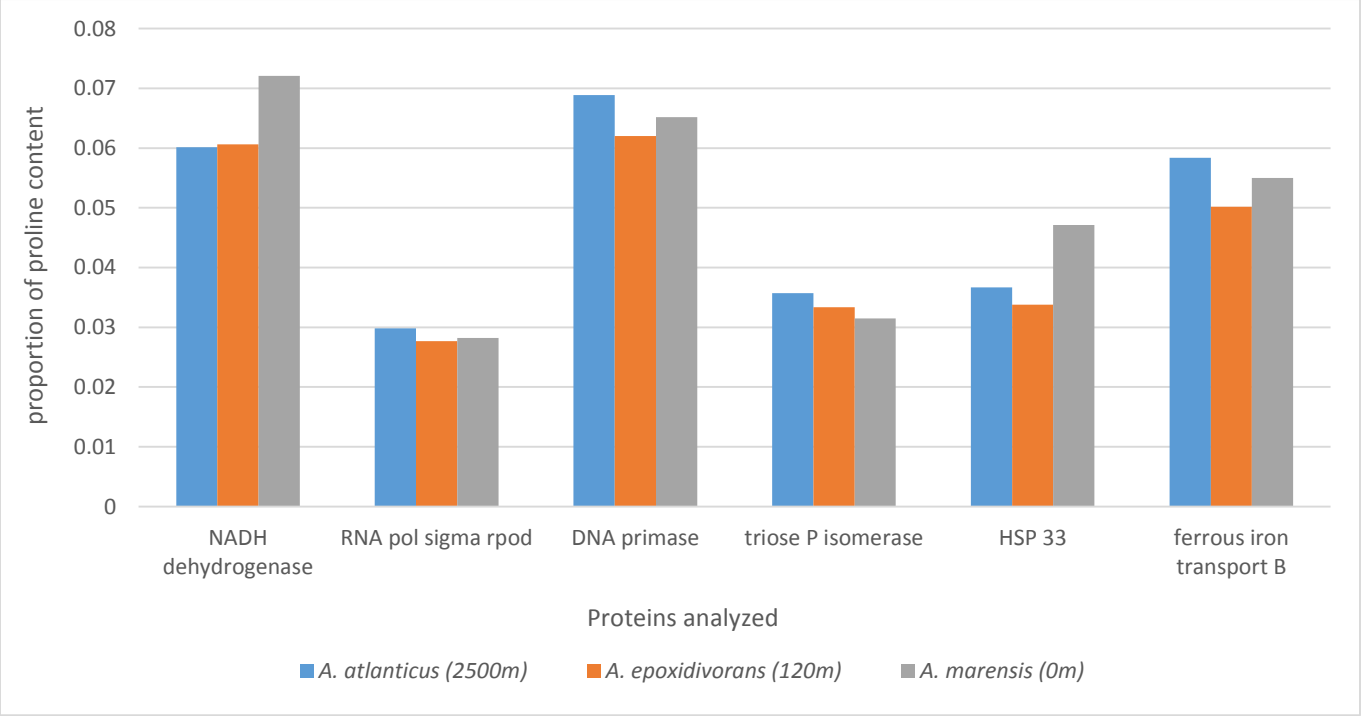

Figure 1: Grouped-bar graph showing the proportion of proline content, of the three strains of *Altererythrobacter,* for all six of the examined gene products. *A. atlanticus* was isolated from 2577m and is shown in blue, *A. epoxidivorans* was isolated from 120 m and is shown in orange, and *A. marensis* was isolated from the sea surface and is shown in grey.

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Appendix 1

Table A1: Contains the NCBI protein ID number for all 18 of the analyzed proteins. The Links will lead directly to the open source data that was used for this experiment.

	Protein name and NCBI ID number					
species (depth)	NADH dehydrogenase	RNA pol sigma rpoD	DNA primase	triose P isomerase	HSP 33	ferrous iron transport B
***A. atlanticus* (≈2500m)**	AKH43871.1	AKH41478.1	AKH41479.1	AKH41653.1	AKH41653.1	AKH43976.1
***A. epoxidivorans* (≈115m)**	ALE16090.1	ALE16372.1	ALE16373.1	ALE16516.1	ALE17990.1	ALE17414.1
***A. marensis* (≈0m)**	WP_047806393.1	WP_047807685.1	WP_047806004.1	WP_047807739.1	WP_047807012.1	WP_047806822.1