DSSG11 : A bacterial dipeptide as potential high pressure osmolyte

Adaptation in Deep Sea Bacteria The deep sea still presents many mysteries, with details of how microorganisms are capable of growth under such extreme pressures being very scarce. With regard to growth under elevated pressures, organisms can be divided into 3 classes: 1) those that grow optimally under pressures between >0.1 MPa and <60 MPa are piezophiles, 2) Organisms that are capable of growth under high pressure, but not optimally, are piezotolerant, 3) and those which have reduced growth at highpressures are piezosensitive[1].

Bacterial adaptation to high pressure that has been studied to date shows either change in membrane and/or change in enzyme composition. The outer membrane prevents the diffusion of molecules, such as detergents (SDS and DOC), antibiotics (rifampicin and actinomycin), and dyes (eosine and methylene blue). This change in membrane composition affects sensitivity of the bacteria to certain stressors[2]. It is likely that in order to maintain normal growth that primary and secondary metabolism is affected, but very little has been published in this regard. We propose that small molecule compatible solutes (osmolytes) are produced in response to elevated hydrostatic pressures and act as high pressure osmolytes (piezolytes).

Secondary metabolites from Deep Sea Microorganism *Micromonospora* is a genus of bacteria belonging to the Phylum Actinobacteria and is known as a prolific producer of novel bioactive natural products with interesting chemical structures[3]. Many secondary metabolites have been found since then such as thiocoraline from *Micromonospora* sp. L-13-ACM2-092[4], micromonospolides[5], micromonomycin[6], indolocarbazole derivatives staurosporines[7], lomaiviticin A from *Micromonospora lomaivitiensis*[8], and diazepinomicin from *Micromonospora* DPJ12[9].

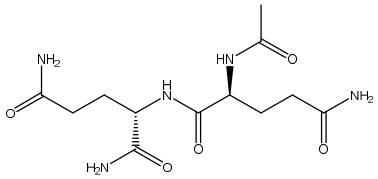

Figure 1. N-acetylglutaminyl glutamine amide from Mariana Trench *Micromonospora* strain MT25

Preliminary data obtained on the piezotolerant *Micromonospora* strain MT25[10] isolated from sediment obtained from Challenger deep in the Mariana trench (ca 11,000 m) shows that it produces a dipeptide N-acetylglutaminyl glutamine amide (NAGGN) which is a known osmolyte[11] (Wael Abdel-Mageed PhD Thesis 2010, University of Aberdeen).

The aim of this project is to investigate the correlation between concentrations of NAGGN in *Micromonospora* MT25 at different high pressures.

Table 1. Procurement of NAGGN in different media composition.

Media	Culture Growth	NAGGN Product
ISP2	+++	-
ISP2 + $CaCO_3$	+++	-
ISP2 + Monosodium Glutamic Acid	++	+
ISP2 + Monosodium Glutamic Acid + $(NH_4)_2SO_4$ in pH 9	+	-
ISP2 + L-Glutamine	-	-
GYE	+++	-
Marine Broth	+++	-

Experimental Results *Micromonospora* strain MT25 was grown at atmospheric pressure in different medium and we used standard isolation and characterisation procedures available in the Marine Biodiscovery Centre to obtain NAGGN. The result was shown in table 1.

Using HPLC-MS based quantification procedure to allow us to identify and quantify the amount of NAGGN produced under elevated pressures. The LC-MS chromatogram showed that NAGGN occurred at early retention time (Figure 2). Due to the small amount of NAGGN that being produce by *Micromonospora* MT25, two other methods to obtain NAGGN have been conducted. First method is producing NAGGN synthetically using precursor compounds and the second method is biosynthesis of NAGGN with *Sinorhizobhium meliloti*.

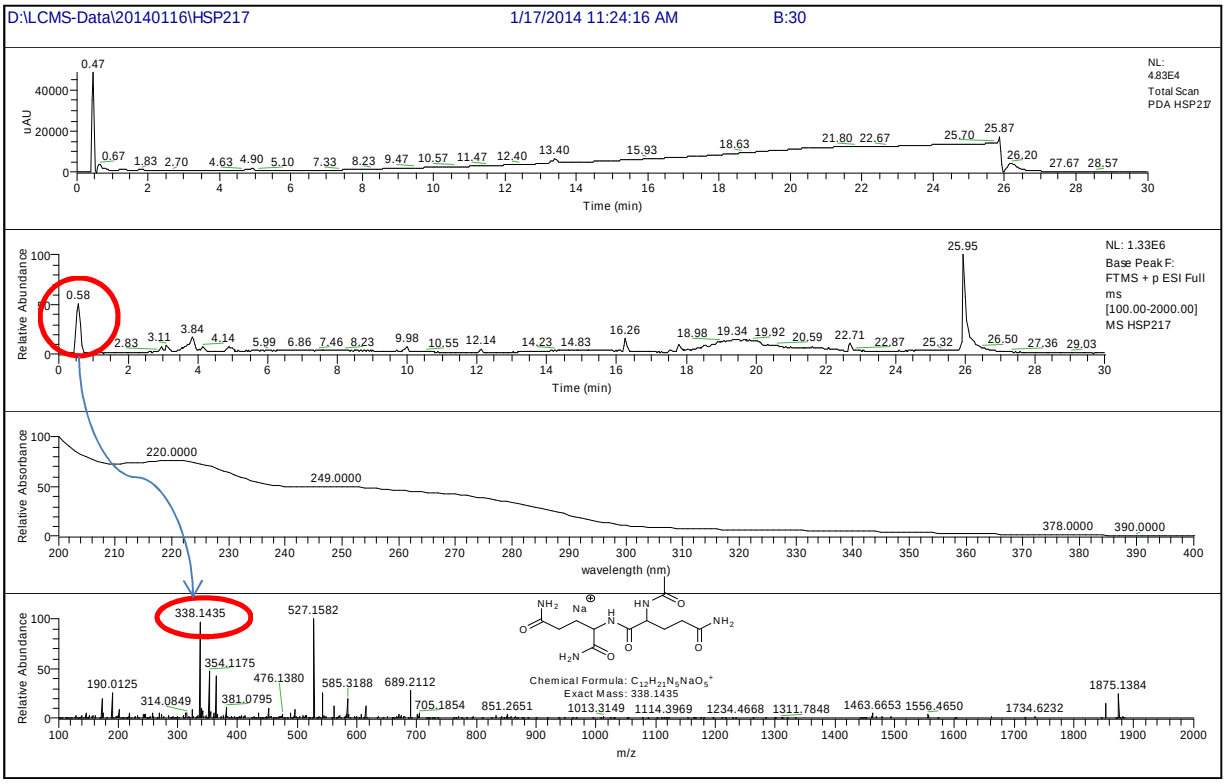

Figure 2. Presence of NAGGN $[M+Na]^+$ in media ISP2+MSG

Synthesis of NAGGN using Rapid Repetitive Solution Phase Peptide Synthesis (RRSPS)[12] is undergoing and showed positive result in its half steps. In the same time, the production of NAGGN using biosynthesis from *Sinorhizobium meliloti*[13] is being carried out at early step of cultivation and upscaling the culture.

The *Micromonospora* MT25 culture in the high-pressure vessel is being carried out at constant temperature (28^{o}C) in ISP2+MSG in sealed bags containing a small amount of Fluorinert FC72 saturated with oxygen to provide oxygen during cultivation.(Wael Abdel-Mageed PhD Thesis 2010, University of Aberdeen).

At the moment, the experiment results have not shown that NAGGN works as an piezolytes just yet, since obtaining pure NAGGN as calibration standard was challenging. But the results had shown that for Micromonospora MT25, NAGGN did not show any activity as an osmolytes. Hence, the possibilities of the use of NAGGN in *Micromonospora MT25* still remain undiscovered.

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