Lab Report 5

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Metagenomic analysis was conducted on coastal seawater incubated for 15h with various different nutrients added. Prior to incubation, 450L of seawater was passed through a $1.5\mu m$ filter to remove most Eukaryotes and large bacteria. One of the following treatments were applied to subsamples of the filtered seawater: Amino acids, protein, acetate, lipids, glucose, starch. After 15 hours of incubation, the water was passed through a $0.2\mu m$ filter to remove cells and the filtrate was subjected to a variety of genomic and proteomic techniques, including shotgun DNA metagenomics with 300bp paired-end MiSeq sequencing.

Metagenomic reads were assembled and annotated with COG functions a categories. The number of sequences supporting each category in each sample was compiled into a contingency table of counts. This contingency table was then uploaded into STAMP for statistical analysis. For all analyses, I used a two-sided Fisher's exact test and a Bonferroni correction for multiple comparisons. Unclassified sequences were used only to calculate frequency profiles.

Using STAMP, I first checked whether there was a difference between the polymer nutrients and their constitutive monomers: amino acids vs proteins and starch vs glucose. In both these comparisons there were no significant differences between any COG categories or annotations, as might be expected. Since the monomers and polymers are likely interchangeable, for further comparisons I will only use protein and starch. Acetate and lipids also had no significant differences in COG frequencies. When comparing protein and acetate, a methyl-accepting chemotaxis protein was more frequent in the protein treatment. This was also the case when protein was compared with lipids and starch. In all significant differences between protein COG frequencies and those of other treatments the protein COGs annotations were more frequent. Acyl-CoA synthetases and Arylsulfatases A COG frequencies were also found to be significantly different in multiple comparisons.

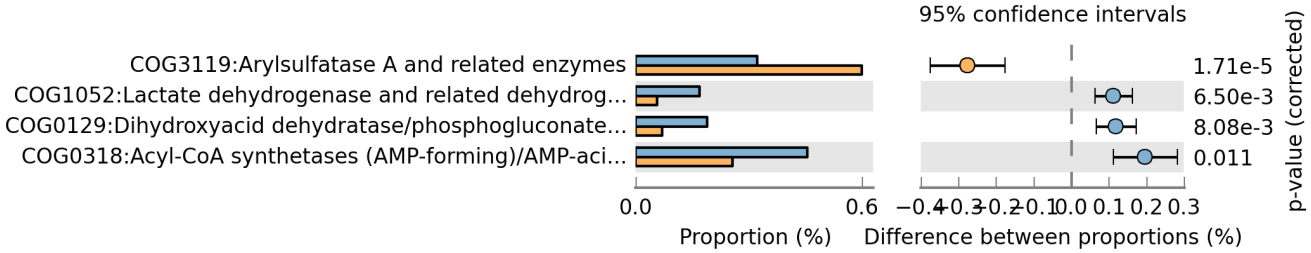

Figure 1: acetate vs lipids

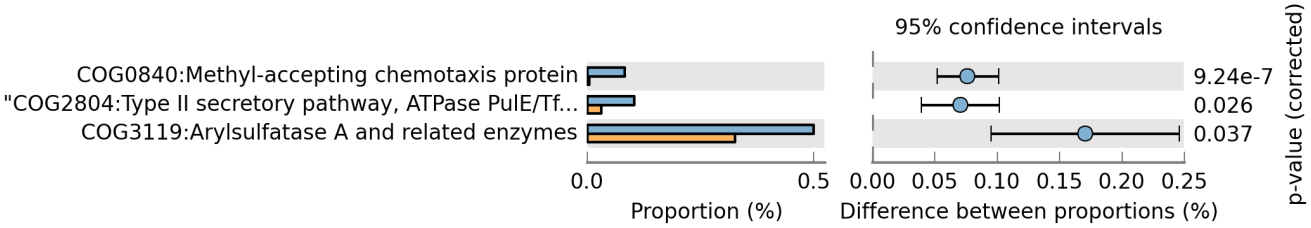

Figure 2: protein vs acetate

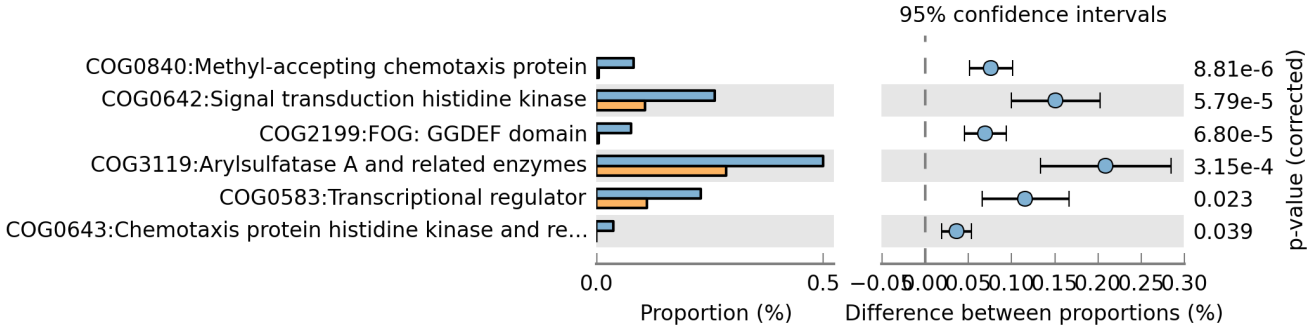

Figure 3: protein vs lipids

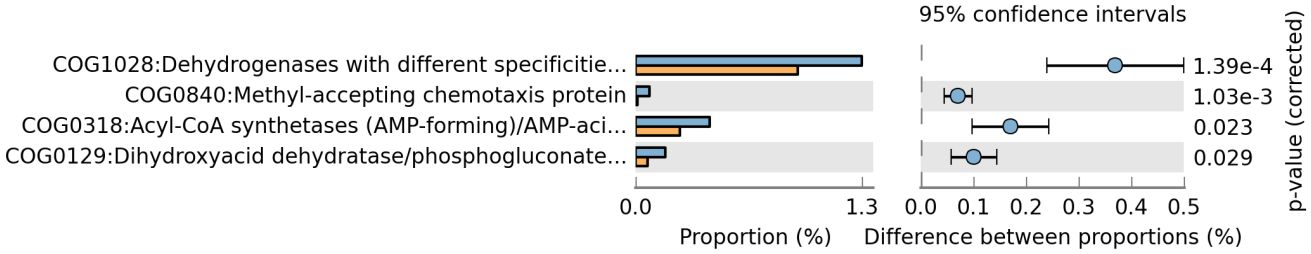

Figure 4: protein vs starch

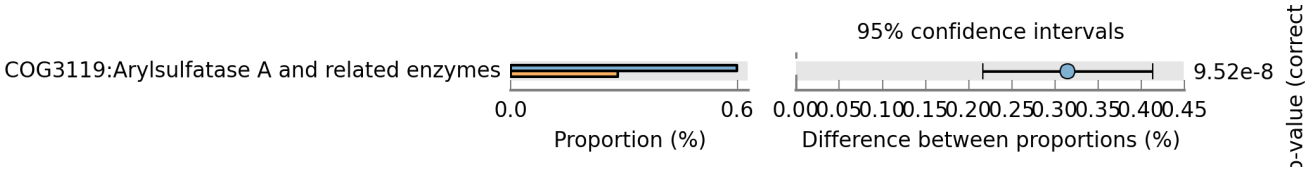

Figure 5: starch vs lipids