De novo transcriptome characterization of *Ulva lacinulata* under *in situ* emersion/immersion cyclic conditions

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1 Introduction:

Intertidal macroalgae are permanently exposed to environmental conditions that change cyclically (Hurd & Dring,1991; Parages et al., 2014), developing strategies of acclimation in response to desiccation, high irradiance, ultraviolet radiation (UVR) etc, and occasionally modifying its internal composition (pigment content, chemical composition osmolytes, and nutrients) (Karsten et al. 1999; Sampath-Wiley et al. 2008; Sánchez de Pedro 2017). The situation of **emersion** exposes intertidal organisms to a very different temperature, relative water content (RWC), carbon source, irradiance, salinity, etc. than is found when they are submerged and isolates them from the source of essential nutrients such as nitrogen and phosphorus (Thomas et al., 2008).

The green algal genus **Ulva** Linnaeus (Ulvaceae, Ulvales, Chlorophyta) displays a worldwide distribution in marine, freshwater and brackish ecosystems, and are really well adapted to fluctuating natural environments. To disentangle the genetic networks that might regulate the adaptation mechanisms of intertidal organisms in a changing environment, the characterization of the ***de novo* transcriptome** from ***Ulva lacinulata*** derived from a coastal ecosystems of southern Spain under ***in situ*** cyclic conditions of **emersion/immersion** by using Next Generation Sequencing technologies was carried out. Transcriptome sequencing and transcript-level expression analysis were performed by ***Illumina®NextSeq® 550 system platform.***

2 Methodology:

Sampling ➡ The whole thallus were collected (removing epiphytes carefully on site) under 2 different *in situ* edxperimental situations:

At **Low Tide** (Emersion): **LT** *(3 replicates)*
At **High Tide** (Immersion): **HT** *(3 replicates)*

RNA isolation ➡ Frozen thalli were ground by hand using mortar and pestle in liquid N2. Total RNA was extracted using a *RNeasy Plant Kit (QIAGEN).*
RNA yield and quality were assessed with the *NanoDropTM 1000 Spectrophotometer* (ThermoFisher Scientific, Wilmington, DE, USA).

Transcriptomic sequencing and transcrip-level expression analysis

Illumina Truseq library construction, and subsequent sequencing, were performed using the **Illumina®NextSeq® 550 system platform.**

The quality trimming of raw reads was carried out by **Trimmomatic software v. 0.36** (Bolger et al. 2014).

De novo transcriptome assembly was conducted by **Trinity** (Grabherr et al., 2011) software

3 Results:

Data Quality Control Summary

Sample	Raw Reads	Clean Reads	Raw Bases	Clean Bases	Error Rate	Q20 (%)	Q30 (%)	GC (%)
LT_1	26,638.538	26,366.348	8	7.9	0.03	97.78	93.98	55.79
LT_2	30,427.046	30,138.016	9.1	9	0.02	97.94	94.36	56.85
LT_3	31,346.684	31,044.015	9.4	9.3	0.03	97.86	94.18	56.46
HT_3	28,558.979	27,845.322	8.6	8.4	0.03	96.48	91.32	57.15
HT_4	33,439.632	32,961.158	10	9.9	0.02	98.09	94.62	57.01
HT_5	33,905.419	33,449.635	10.2	10	0.03	97.76	93.98	55.42

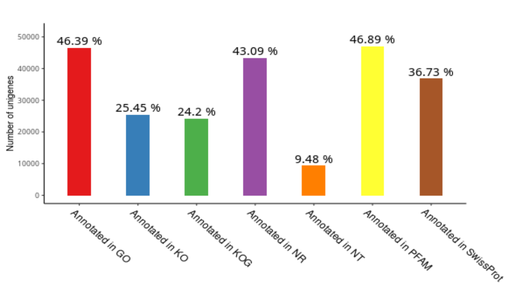
Table 1. Summary of Illumina RNA-seq data

Functional Annotation of the Transcriptome

A

Data Base	Number of Unigenes	Percentage (%)
NR	43,202	43.09
NT	9,507	9.48
PFAM	47,015	46.89
SwwissProt	36,825	36.73
GO	46,508	46.39
KO	25,517	25.45
KOG	24,265	24.2
Total Unigenes	100,251	100

B



C

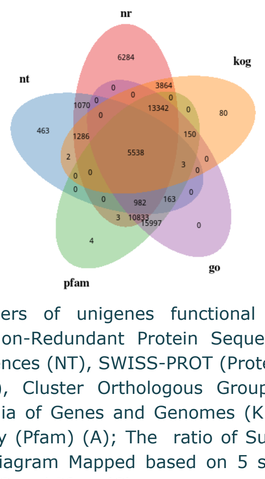
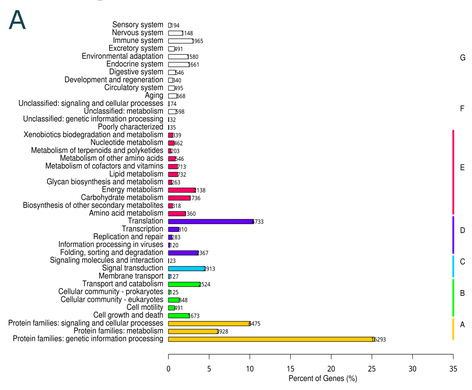


Figure 1. Numbers of unigenes functional annotation in 7 different database:NCBI Non-Redundant Protein Sequences Database (NR), NCBI nucleotides sequences (NT), SWISS-PROT (Protein sequence annotation and review database), Cluster Orthologous Groups of Proteins (COG/KOG), Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), and Protein family (Pfam) (A); The ratio of Successfully Annotaded Genes (B); The Venn Diagram Mapped based on 5 selected databases (NR, NT, SWISS-PROT, KOG and Pfam (C).

KEGG/KOG Gene Functional Annotation

A



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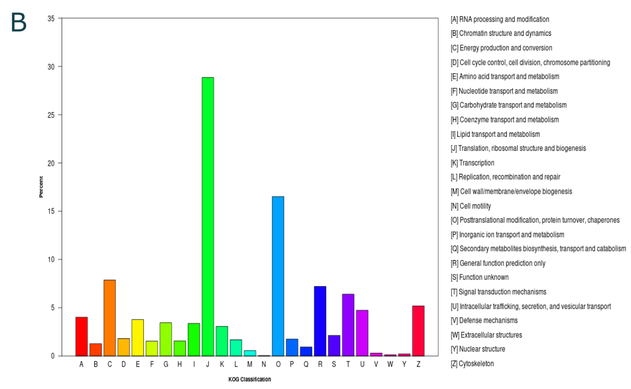
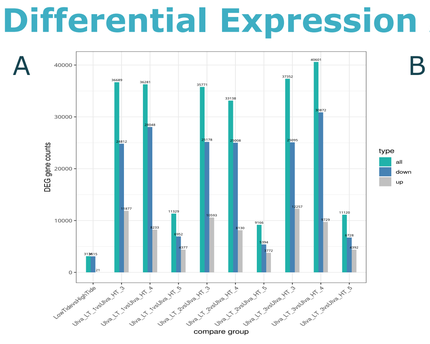


Figure 2. KEGG Function Classification (A); The KEGG metabolic pathways gene involved in are divides into 5 branches: A: Cellular Processes; B:Environmental Information Processing, C: Genetic Information Processing, D:Metabolism, E: Organismal Suystems. KOG Function Classification (B); The X-axis is the names of KOG groups, and the Y-axis is the percentage of genes annotated under this group in the total annotated genes.

Differential Expression Analysis

A



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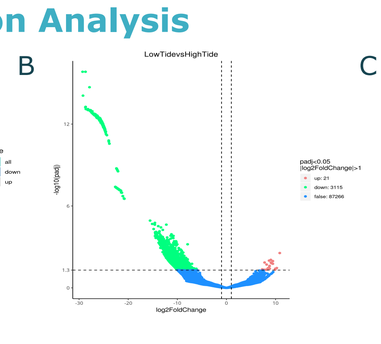

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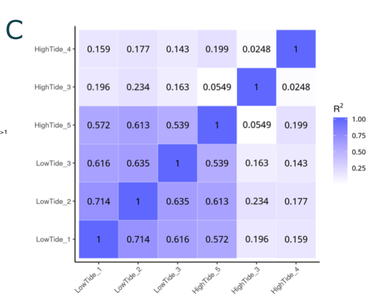

Figure 3. The number of differentially expressed genes (DEGs), including up-regulated and down regulated genes, in each comparison combination. A total number of 3136 differentially expressed genes (including 21 up-regulated and 3115 down regulated genes) showed under LowTide (LT) versus HighTide (HT) condition (A); Gene expreswsion levels shown in volcano plot (B); Pearson correlation coefficients for comparision among all samples (B)

Diffenrentially Expressed Genes (DEGs)

Two of the top5 DEGs are:

RPL31 Gene (P45841_ Large ribosomal subunit protein eL31): involved in **ribosome biogenesis.** With a 8631,0277 read counts under HighTide condition with a log2FoldChange of -28.694, a pvalue of 3.238e-21 and a padj of 1,4636e-16.

PRDX2 Gene (Q2PFZ3_Peroxiredoxin-2): involved in **oxidoreductase activity/ peroxiredoxin activity/antioxidant activity.** With a 4842,3393 read counts under HighTide condition with a log2FoldChange of -27.878, a pvalue of 6.5966e-20 and a padj of 1,9878e-15.

Enrichment Analysis of DEGs

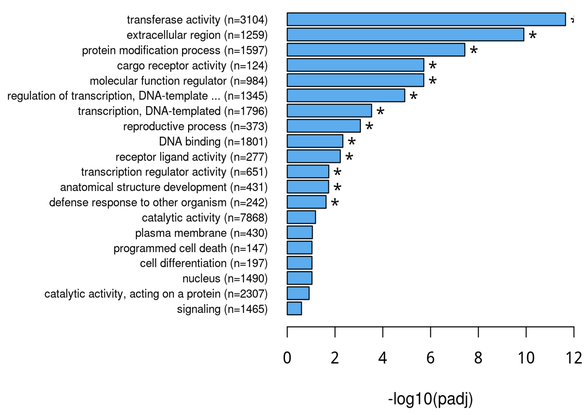

Figure 4. Gene Ontology (GO) Enrichment Analysis of DEGs

4 Concluding Remarks:

- In *Ulva lacinulata* a total of **100,251 unigenes** were expressed during emersion/immersion process
- The total number of **3136 differentially expressed genes** (including **21 up-regulated** and **3115 down regulated** genes) were showed under LowTide *versus* HighTide condition
- Based on the differentially expressed genes (DEGs), genes like ***RPL31 Gene*** and ***PRDX2 Gene*** associated with **ribosome biogenesis** and **antioxidant activity** respectively were annotated according to Gene Ontology and KyotoEncyclopedia of Genes and Genomes Orthology (KEGG)
- After KEGG and GO functional annotation different molecular pathways like **starch and sucrose metabolism** (ko00500), **fructose and mannose metabolism** (ko00051), **glycolysis/gluconeogenesis** (ko00010) **spliceosome** (ko03040) and **transferase activity** (GO:0016740) were matched.
- Based on the results, the rapid response to intertidal dehydration/rehydration cycling within *U. lacinulata* might include the activation of signal transduction mechanisms, a readily capacity to utilize ribosomal stores,an increased carbohydrate metabolism activity, a protein turnover and chaperones activity and a strong anti-oxidation system to dissipate excess redox energy upon exposure to air
- These findings shed light on the molecular mechanisms underlying rapid and successful ecophysiological response of marine macroalgae in cyclic tidal conditions

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