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Ministry of Environment Water & Agriculture

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Microbial Diversity Succession During *Arthrospira maxima* Adaptation and Mass Cultivation Under Desertic Outdoors Conditions

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Introduction

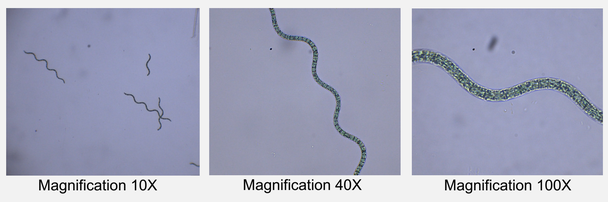
- The cultivation of microalgae in seawater-based media is fundamental for fighting worldwide freshwater shortage and food security.
- The capacity of *Arthrospira maxima* to tolerate a wide range of salinities [1] demonstrate that this species as an ideal candidate to be cultured in arid countries.
- In their natural environment, microalgae are tighly associated to planktonic bacteria in the phycosphere [2]. In open raceways in industrial setups, together with the main microalgae culture, aquatic eukaryotes and bacteria are also present.
- The microbial diversity of natural and industrial microalgal cultures is not well known in such arid environments.
- If the associated microbes contribute to the overall microalgae capacity to resist different environmental stresses it is not known.

Materials and Methods

- **Micoalgal strain:**

***Arthrospira maxima* LJGR1**

UNAM Microalgae Collection Culture
Isolated from Texcoco Lake, Mexico



Magnification 10X | Magnification 40X | Magnification 100X

- **Growth conditions: Adaptating *A. maxima* to desertic outooors mass cultivation**

Indoors: Closed System

Flask
150 $\mu E * m^2 * s^{-1}$
12h
27-30ºC

Outdoors: Closed System

Carboy
800-1000 $\mu E * m^2 * s^{-1}$
13h
30-37ºC

Outdoors: Open System

Raceway
>2800 $\mu E * m^2 * s^{-1}$
13h
30-38ºC

T_0	T_1=7d	T_3=21d	T_5=35d	T_6=42d	T_7=49d	T_8=56d
Jourdan $CO(NH)_2$ $(NH_4)_2HPO_4$ KNO_3 freshwater	Jourdan 10g/L sea salt	Jourdan 25g/L sea salt	Jourdan 50% F/2 modif. 50%	Jourdan 25% F/2 modif. 75%	F/2 modif. $(NH_4)_2SO_4$ KH_2PO_4 seawater	F/2 modif.
2 PSU	10 PSU	25 PSU	25 PSU	25 PSU	25 PSU	44 PSU

- **Genomic workflow:**

Genomic DNA Extraction → Quality Control → Metagenomic Amplicon Libray Preparation → Quality Control → Amplicon Metagenomic Sequencing → Bioinformatic Data Analysis

- **Metabarcoding markers and primers:**

Prokaryotes:

16S rRNA hypervariable region V4-V5 **515F-Y/926R**	515F-Y	[overhang]-GTGYCAGCMGCCGCGGTAA	[3]
	926R	[overhang]-CCGYCAATTYMTTTRAGTTT	

Eukaryotes:

Internal transcribed spacer 2 (ITS2) **5.8SbF/ITS4R**	5.8SbF	[overhang]-GATGAAGAACGCAGCG	[4]
	ITS4R	[overhang]-TCCTCCGCTTATTGATATGC	
18S rRNA small subunit (SSU) **NF1/18 Sr2b**	NF1	[overhang]-GGTGGTGCATGGCCGTTCTTAGTT	[5]
	18 Sr2b	[overhang]-TACAAAGGGCAGGGACGTAAT	

Results

Table 1. Number of obtained reads per sample.

Time point	Amplicon	Raw reads	Trimmed reads	Kept reads (%)	Counts	Filtered counts	Classified Domain counts (%)
T0	16S V4-V5	82188	81341	98.97	78085	78015	95.91
T1	16S V4-V5	76927	76184	99.03	74604	74450	97.72
T3	16S V4-V5	56218	55366	98.48	54759	54704	98.80
T5	16S V4-V5	91668	90569	98.80	82857	81991	90.53
T6	16S V4-V5	106556	105275	98.80	104550	104404	99.17
T7	16S V4-V5	78800	77978	98.96	77057	76878	98.59
T8	16S V4-V5	104943	103626	98.75	101487	99219	95.75
T0	ITS rDNA	43232	42402	98.08	38594	38354	90.45
T1	ITS rDNA	56531	56082	99.21	55092	49289	87.89
T3	ITS rDNA	34315	33914	98.83	33647	33647	99.21
T5	ITS rDNA	52149	51729	99.19	50508	50483	97.59
T8	ITS rDNA	69578	69005	99.18	68643	60321	87.42
T0	18S SSU	10667	10323	96.78	5480	5480	53.09
T1	18S SSU	17840	17275	96.83	12306	12204	70.65
T3	18S SSU	7885	7569	95.99	5292	5274	69.68
T5	18S SSU	30948	30328	98.00	26370	26226	86.47
T6	18S SSU	12894	12380	96.01	8359	8359	67.52
T7	18S SSU	5314	5171	97.31	3465	3297	63.76
T8	18S SSU	31537	31025	98.38	27837	27663	89.16

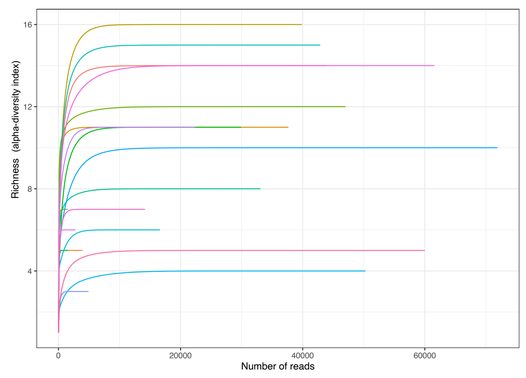
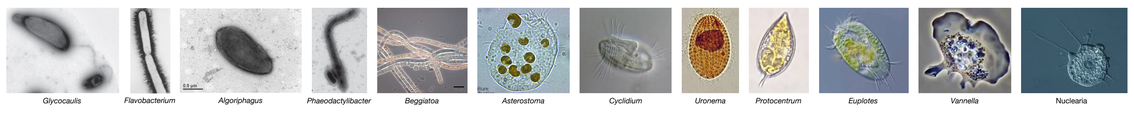


Figure 1. Rarefraction curve. Evaluation of the increase in the species richness in each sample with the increase of sequencing depth. All rarefraction curves converge reaching an horizontal asymptote, what means that the number of sequenced reads is enough and that all sample libraries have been successfully sequenced to represent its identity.

(Images obtained from Google search)



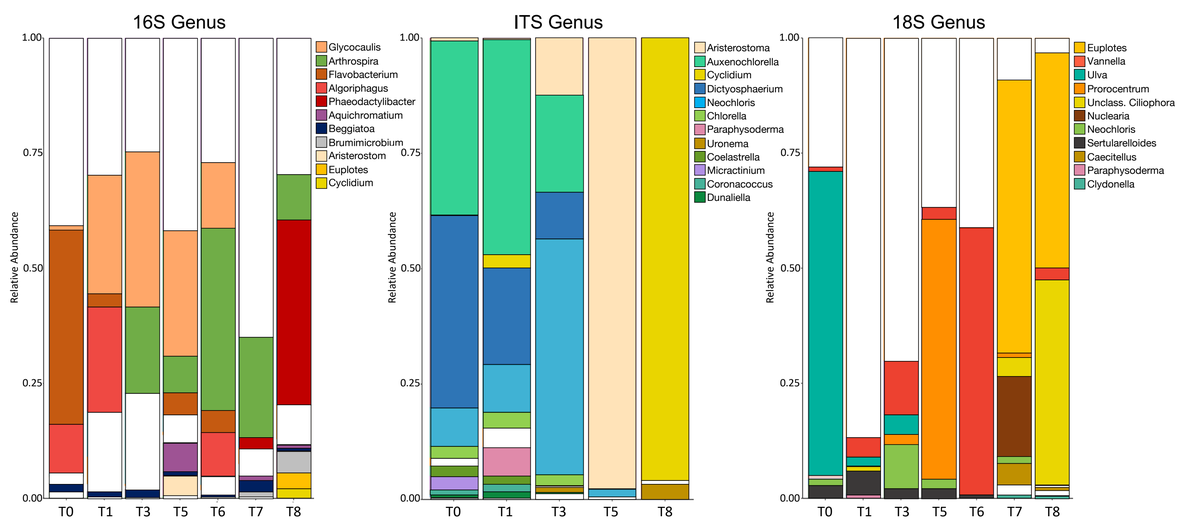


Figure 2. Taxonomoy barplots. Representation of the compositioin based on the relative abundance of different taxa at different taxonomic levels. The y-axis shows the relative abundance of each taxon. The x-axis represents the different samples being compared, ordered based on the culture adaptation stage. Thhe taxa with the highest mean relative abundace across all samples for each taxonomic rank are shown. Other taxa and those identified as "unclassified" are shown in white (□).

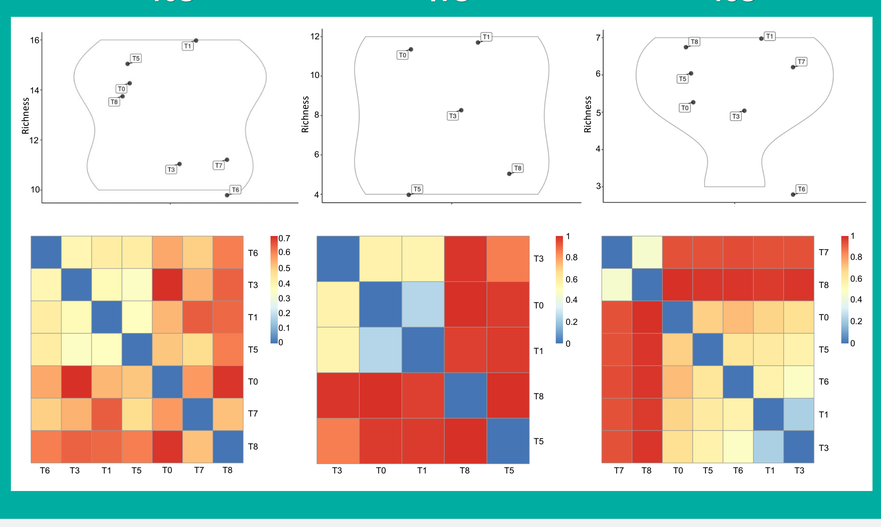


Figure 3. Alpha diversity (Richness). Refers to the species biodiversity in a local community. This richness metric represents the number of species observed in each sample, computed at the Genus level.

Figure 4. Beta diversity (Bray-Curtis dissilimarity). Heatmaps showing the beta diversity index at the Genus level. This diversity metric provides a way to asses the similarity or dissimilarity between two samples. A ß index equal to 0 indicates the samples are identical, whereas a ß index equal to 1 indicates the samples are completely different.

Conclusions

- The sequencing depth reached during the sequencing process was enough to reveal the totallity of the taxa present in the samples. This is consequence of a global taxonomical richness rather low compared to samples from other natural environments.
- The microbial diversity changes along the culture adaptation process. Competitors (algae) and predators (e.g. ciliates) appear at the latest stages of the cultivation process (open system). This is in accordance with our previous work (*Alamari* et al., 2023 [6]). MiSeq sequencing is a tool that can be used to complement the routine Contamination Monitoring Program in industrial setups.
- ITS libraries revealed green algae and ciliates, while 18S libraries allowed the identification of more diverse members of zooplankton, including ciliates, flagelates and amoebas. A broader range of primer pairs for both prokaryotes and eukaryotes will allow the identification of a more diverse species.

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