Microalgae in Aquaponics Wastewater Systems

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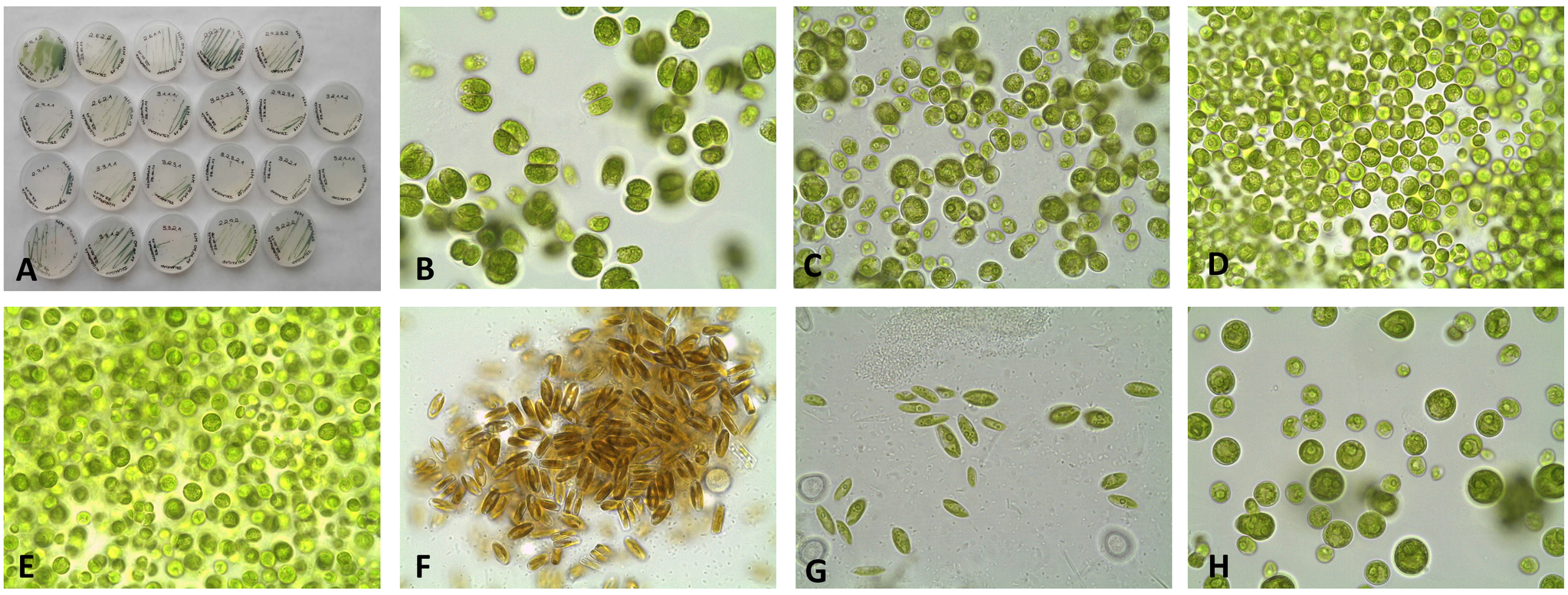
Aquaponics refers to any system that combines conventional aquaculture (fish, copepods, prawns, etc.) with hydroponics (vegetable, flower, and/or herb production) in a symbiotic environment. The nitrifying bacteria living in the gravel and in association with the plant roots play a critical role in nutrient cycling; without these microorganisms the whole system would stop functioning. Microalgae, although they are a member of the microorganism community in the aquaponic systems, they must be controlled and mitigated in order not to interfere negatively in the aquaponics system (ex: competition with oxygen and nutrients).

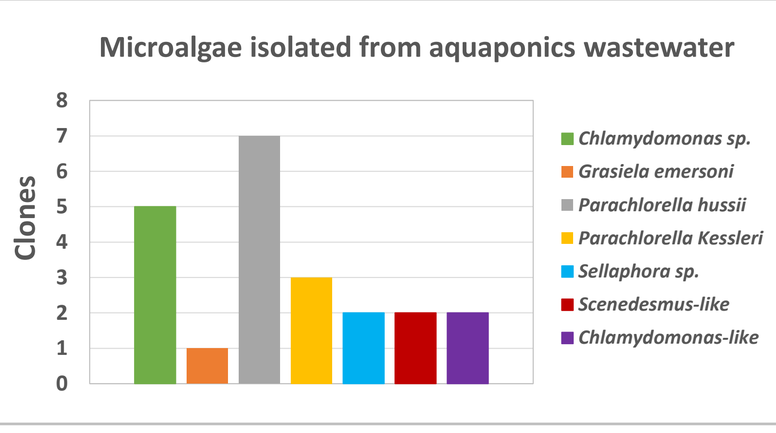
The aim of this work was to acquire knowledge of the community of microalgae that are present in our aquaponic system (Tilapia, *Oreochromis* sp. was the culturing fish, and lettuce, *Lactuca sativa* was the culturing vegetable). For this purpose, we isolated a total of 22 clones and identified them with traditional and molecular techniques. Of these 22 clones, 20 corresponded to chlorophytes and two to diatoms. Cyanobacteria was not detected in our aquaponic system. From the resulted study, we obtained monoalgal strains of *Chlamydomonas* sp., *Grasiella emersonii*, *Parachlorella kessleri*, *Parachlorella hussi* and *Sellaphora* sp.

METHODOLOGY: **After isolation and verification that the 22 clones obtained were monoalgal, DNA extraction was performed with a protocol based on chelex-100 (Biorad, CA, USA; Giraffa et. al. Journal of Microbiological Methods 42 (2000) 175–184) and purified with the Real Clean Spin Kit (Durviz SL., Valencia, Spain). PCR was performed with the primers described in the table on the right in a C1000 thermocycler (Bio-Rad) and purified with the Illustra ExoStar 1-step (GE Healthcare Life Sciences) following manufacturer's instructions. Sequencing was performed by Macrogen. The sequences obtained were compared with the ones available at GenBank (http://www.ncbi.nlm.nih.gov/genbank/) through the Blast tool.**

Primers ID	Sequences (5'-3')	Gene	References
Diatoms_C Diatoms_D	CTGCCCTATCAGCTTTGG CGGCCATGCACCACC	18S rDNA	Armbrust et al. 2001. Appl. Environmental Microbiol., 67:8, 3501–3513
18Seuk-F 18Seuk-R	GTCAGAGGTGAAATTCTTGGATTTA AGGGCAGGGACGTAATCAACG	18S rDNA	Ferris et al. 2005. App. Environ. Microbiol. 71(11), 7164-7171.
Euk1A Euk516r	CTGGTTGATCCTGCCAG ACCAGACTTGCCCTCC	18S rDNA	Díez et al., 2011
ITS5 ITS4	GGAAGTAAAAGTCGTAACAAGG TCCTC GCTTATTGATATGC	ITS rDNA	Sato et al. 2011. Plos One, Volume 6 (12) e27983



Microalgae isolated from the aquaponic wastewater system (A-H). The 22 clones obtained (A), microscope observations of monoalgal cultures, *Chlamydomonas* sp. (B), *Grasiela emersoni* (C), *Parachlorella hussii* (D), *Parachlorella kessleri* (E), *Sellaphora* sp. (F), *Scenedesmus*-like (G), *Chlamydomonas*-like (H); (magnification: 1000x).



Frequency of microalgae isolates of aquaponic wastewater. Total clones were 22.

REMARKS

- **Chlorophytes were the predominant group of microalgae detected in aquaponic wastewater (microscopic observations), even before isolation procedures.**
- **In general, 18S rDNA only allowed an identification until the genus level. ITS rDNA allowed a more precise identification, but the problem encounter in some cases was the lack of similar sequences to compare to ours, which prevented an identification at species level.**
- **On the other hand, in public genetic databases like GenBank, there is a huge amount of microalgae sequences with an identification only at family or genus level, which do not contribute at all to molecular taxonomy.**
- **The identification of *Scenedesmus*-like and *Chlamydomonas*-like clones are pendent due to interference of protozoa DNA in the PCR step, which claims for more specific primers for microalgae DNA amplification.**

ACKNOWLEDGEMENT

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