

# Characterization of bacterial community profiles associated with *Chlorella vulgaris* cultivations

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## 1 Introduction

A growing world population demands the development of sustainable food resources providing nutrients for a balanced diet. Microalgae, such as *Chlorella vulgaris*, could contribute solving the challenge. To provide microalgal biomass for human consumption, downstream processes must be developed ensuring safe products. This requires an understanding of the microbial community profile within microalgae cultivations to effectively remediate harmful bacterial species.

## 2 Method overview

### Sampling

Non-axenic *Chlorella vulgaris* SAG 211-12 cultivations from a  $\mu$ -gravity adapted photobioreactor ( $\mu$ -PBR; Fig. 1) and a shaking incubator (SI).

Sampling of SI samples was done over a 4 week period.

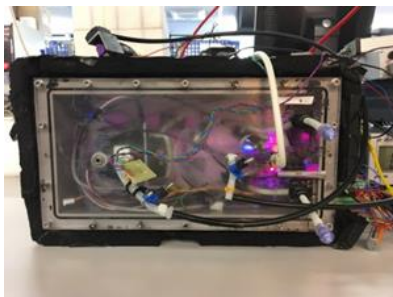


Fig. 1. Image of a  $\mu$ -PBR.



Fig. 2. Erlenmeyer flask with *Chlorella vulgaris*.

### Analysis

The microbial community profile was investigated using two approaches:

1. Metagenomics based characterization of bacterial sequences and *Chlorella vulgaris*.
2. Characterization of bacterial isolates and *Chlorella vulgaris* to complement the data obtained from metagenomics analysis.

## 3 Materials

### 1. Metagenomics:

- Tag-encoded 16S rRNA gene MiSeq based (Illumina) high throughput sequencing of V4 region.

### 2. Isolates:

- Characterization of bacterial isolates using morphological and biochemical analysis including microscopy, oxidase, peroxidase, and katalase tests.
- Verification of *Chlorella vulgaris* based on 16S and 18S rRNA sequencing.

## 4 Results and discussion

A dynamic and diverse microbiota was identified in all samples.

### 1. Metagenomics:

- 9 bacterial families (Fig. 3) and *Chlorella vulgaris*

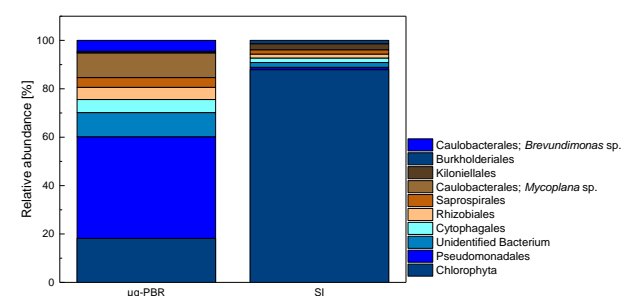


Fig. 3. Relative abundance (%) microorganisms identified in  $\mu$ -PBR and shaking incubator (SI) samples.

### 2. Isolates:

- Identification of 5 bacterial families: *Pseudomonadaceae*, *Phyllobacteriaceae*, *Sphingomonadaceae*, *Rhodospirillaceae*, *Microbacteriaceae* and *Chlorella vulgaris*.
- *Chlorella vulgaris* growth associated increase in bacterial counts (Fig. 4).

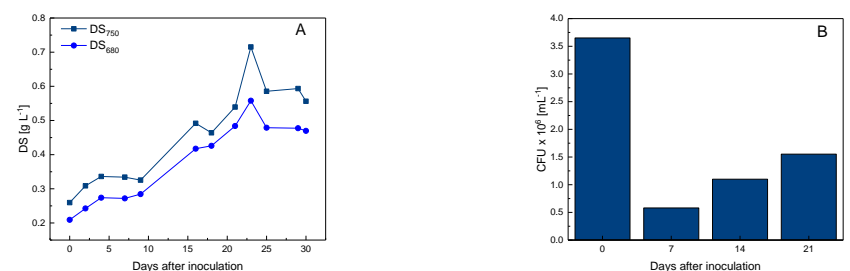


Fig. 4. *Chlorella vulgaris* growth and bacterial counts over a 4-week experiment in a shaking incubator: Growth was monitored using ODs at 680 nm and 750 nm and converted to dry substance (DS) (A). According colony forming units (CFU) in *Chlorella vulgaris* cultivations, based on duplicate measurements (B).

## 5 Conclusion

The research project established a base for the development of gentle microalgae downstream processes.

Divergent cultivation conditions impact microbial community structures.

The adaptability of the microbial community profile bears potential. Engineering a bacterial consortium could facilitate downstream processing and increase biomass yields.

## 6 References

1. Helisch, H., Keppler, J., Bretschneider, J. & Zentrum, D. Preparatory ground-based experiments on cultivation of *Chlorella vulgaris* for the ISS experiment PBR @ LSR. 1–16 (2016).
2. Melrose, J., Perroy, R. & Careas, S. World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241. United Nations, Dep. Econ. Soc. Aff. Popul. Div. 1, 1–59 (2015).