GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

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GÖTTINGEN CAMPUS PLUS

Pflanzliche Mikroorganismen und Cyanobakterien zur Produktion von Wertstoffen und Biomasse mittels CO2-reicher Industrieabgase in Israel: Isolate, DNA-barcodes und Wachstumsexperimente

Biomass produced by microalgae provides a resource of a broad range of valuable compounds, such as essential amino acids, unsaturated fatty acids, carbohydrates, pigments, various vitamins and others. They are very useful in pharmacy, cosmetics, as well as food and feed supplement. Microalgal biomass can also be used to produce high-value biofuels, e.g. derived from algal oil as well as biohydrogen and bioethanol. Also biogas, i.e. methane, can be produced by anaerobic fermentation of algal biomass.
However, currently there are probably no more than about 20 species and genera from no more than six taxonomic classes of autotrophic microalgae of microalgae in use.

The objective of this project is to isolate novel strains from extreme habitats as well as a screening of available strains of microalgae (from the SAG culture collection, Göttingen, and research projects associated with this collection) which can grow well in simple liquid growth media and are capable to produce useful biomass.

Fig. 1

Fig. 1. Sampling at Haifa, Israel

Sampling from extreme habitats

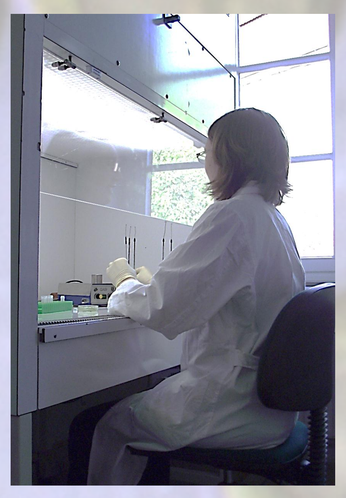
The samples were collected from:
- soil habitats near power plant (Haifa, Israel) (Fig. 1);
- fumarole with high CO2 content in Dunsthöhle (Bad Pyrmont, Germany) (Fig. 2);
- soil habitats near a open algal mass production plant (Bad Hersfeld, Germany);
- various soil habitats in forests of Solling mountains (Schlarpe, Germany).

Fig. 2

Fig. 2. Sampling at Dunsthöhle

Isolation of the strains

A total of 47 strains were isolated: 28 green algae, 9 cyanobacteria, 9 diatoms and 1 from eustigmatophytes.
The isolates from CO2 fumarole showed the slowest growth rate. Some of the algae were not isolated due to slow growth rate (were overgrown by faster competitors) or due to medium selectivity. Isolates from Haifa (Israel) were the most contaminated with fungi and bacteria.



Morphological characterization of the strains.

According to the morphological features strains can be divided in several distinct morphotypes.

Cyanobacteria: filamentous without heterocytes (*Pseudoanabaena*, Fig. 4, K, *Oscillatoria*, Fig. 4, N, *Leptolyngbya* Fig. 4, L,) and filamentous with heterocytes (*Nostoc*, Fig. I, M, *Cylindrospermum* Fig.4, J)

Green algae: "*Chlamydomonas*" green, motile with two flagella and one chloroplast (Fig. 3, E,F), "*Chlorella*" green small coccoid, with one chloroplast with or without pyrenoid (Fig. 3, A,B, C), "*Parietochloris*" green coccoid, one chloroplast with visible vacuoles, with "granulated" pyrenoid (Fig. 3, G), "*Scenedesmus*" green coccoid sometimes in coenobia, cells ellipsoidal (Fig. 3, D), "*Klebsormidium*" green, filamentous, one chloroplast with pyrenoid (Fig. 3, H).

Fig. 3

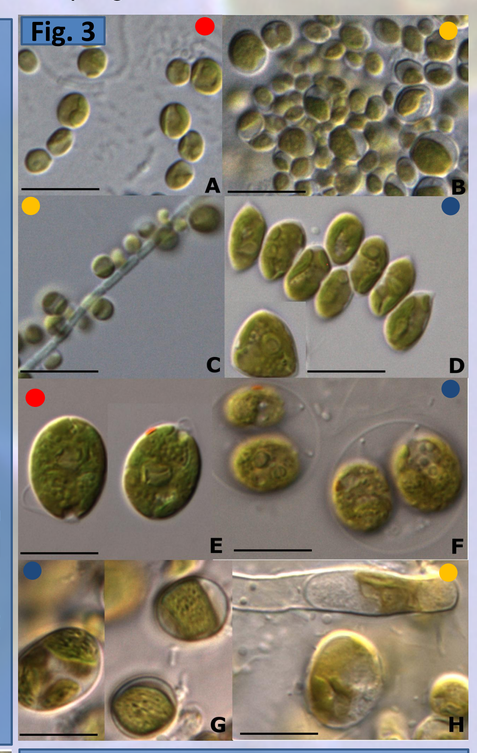


Fig. 4

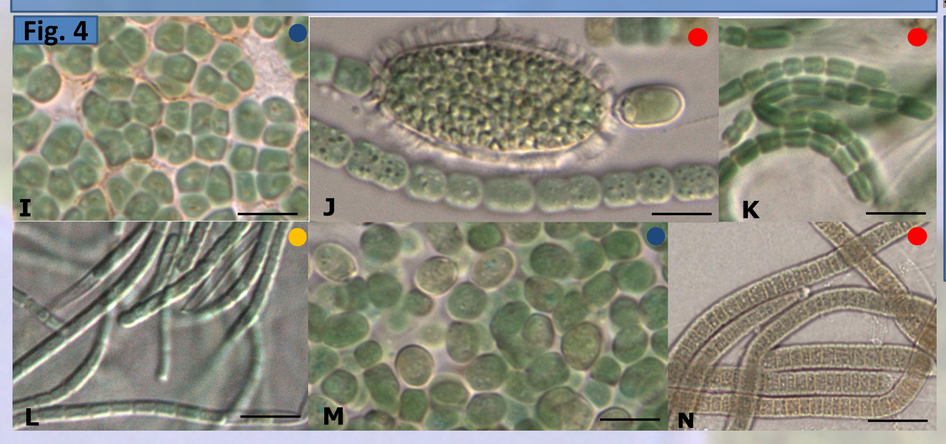


Fig. 3. Images of green algae cultures isolated in the frame of FoLL project
Fig. 4. Images of cyanobacteria cultures isolated in the frame of FoLL project
Scale – 10 µm

- Isolates from Haifa (Israel)
- Isolates from Dunsthöhle
- Isolates from Schlarpe

DNA barcoding for characterization of the new isolates

Genomic DNA was extracted from 16 strains of green algae.

ITS2 rDNA sequences were used as a DNA barcode to unambiguously characterize green algal strains. Sequence stretches extending from the 3'-end of 18S rDNA until the 5'-end of LSU rDNA (which covers the ITS2 region) were amplified using PCR primers which preferentially bind to green algal rDNA. Within the given time of the project, from 16 strains for which DNA was extracted 11 strains were successfully sequenced for their entire 18S-LSU rDNA sequence stretches (Fig 5.).

Queries at public DNA sequence databases using the BLASTn were used to obtain a preliminary identification of the sequenced strains. Three strains were assigned to *Chlorella vulgaris*, one to a still unidentified *Chlorella* sp. Representatives of the *Scenedesmus* morphotype were found to be close relatives to *Scenedesmus obliquus* (3 strains), *S. dissociatus* (1 strain), *Desmodesmus multivariabilis* (1 strain) and *Graesiella emersonii* (1 strain). One strain of the *Chlamydomonas* morphotype belonged to *Chlamydomonas reinhardtii*.

Fig. 5

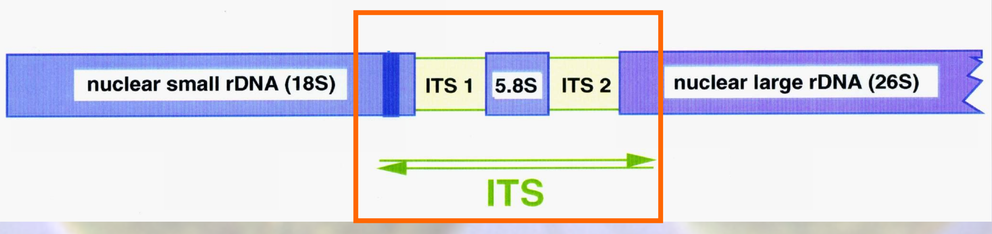


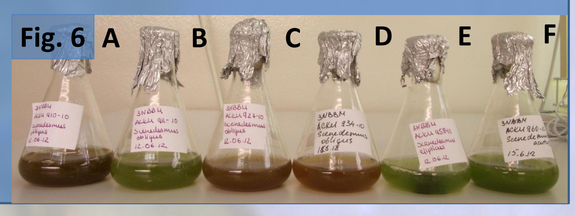
Fig. 5. Schema of 18S-LSU rDNA region

Growth experiments

For 19 strains from the SAG and ACKU culture collections absorption at 688 nm was measured every 3 days in order to establish a growth curve. Also for representatives of *Scenedesmus* pigments were extracted using 100% of acetone and absorption at 470, 645 and 662 nm was measured for chlorophyll *a, b* and total carotenoid content estimation.
The *Scenedesmus* representatives grew faster and accumulated larger amounts of biomass than representatives of *Stichococcus* and *Chlorella*.
The *Scenedesmus* strains can be divided in two groups "green" and "orange". In "orange " the total carotenoids content was rising in absolute values and in comparison with chlorophyll a content . The total carotenoid content can be even bigger than chlorophyll *a*.

Fig. 6. *Scenedesmus* strains from "green" (B, E, F) and "orange" groups (A, C, D) in liquid culture

Fig. 6



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