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A new method in collection and cultivation of aerophytic and endolithic algae

by

Georg GÄRTNER, Maya P. STOYNEVA, Antonia D. MANCHEVA & Blagoy A. UZUNOV*)

Summary: The paper presents a new method for collecting aerophytic and endolithic algae (incl. cyanoprokaryotes), in which algal material is scraped directly in sterile test tubes, filled with agarized medium. The further steps necessary to obtain clonal algal cultures, follow standard isolation methods. The main advantages of the innovation proposed are in the significant reduction of the time between sampling and incubation on agar plates for enrichment cultures, as well as in the keeping of collected algae in fresh conditions, without passing through desiccation or freezing before cultivation.

1. Introduction:

Aerophytic algae (incl. cyanoprokaryotes) represent a significant ecological group, which includes the highly specialized lithobionts (epi-, endo- and hypolithes), as well as the bark- and soil-crust inhabitants among the others. These organisms are of widespread occurrence in nature but yet the information on their diversity, biology and ecology is quite sparse and scattered. All algae, represented in the aerophyton, are also taxonomically difficult, and identification requires their isolation with further cultivation on defined media. Most of the applied methods are species-selective, and a variety of media and culture techniques have to be utilized. During the last century the main sampling and cultivation methods have been developed, refined and generally standardized (BOLD 1942, PRINGSHEIM 1946, LEWIN 1959, STEIN 1973, ANDERSEN 2005, ANDERSEN & KAWACHI 2005). However, still the efforts are focused more on the cultivation techniques than on the sampling details, and, additionally, all procedures are time-consuming. This "time-lag" problem has two important aspects. On one hand, it concerns researcher's time-table, but more important is the fact that usually during the time elapse, desiccation of algal material, ingestion by animals or contamination by fungal organisms happens. In order to avoid these problems, the period between collecting and primary cultivation has to be shortened as much as possible.

*) Addresses of the authors: tit. Univ.-Prof. Dr. Georg Gärtner, Institut für Botanik der Leopold-Franzens-Universität Innsbruck, Sternwartestrasse 15, 6020 Innsbruck, Austria; Assoc. Prof. Dr. Maya P. Stoyneva, Mag. Antonia D. Mancheva and Assist. Dr. Blagoy A. Uzunov, Department of Botany, Faculty of Biology, Sofia University 'St Kliment Ohridski', 8 bld Dragan Zankov, 1164-Sofia, Bulgaria; E-Mails: georg.gaertner@uibk.ac.at; mstoyneva@abv.bg; antoniamancheva@abv.bg; blagoy_uzunov@abv.bg

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Therefore our target was to reduce the time between the field sampling and first enrichment cultures. This task was achieved by using a "direct collecting method" (DC-method), which is presented below.

2. Material and methods:

In April and September 2009, after receiving a special permission from the Bulgarian Ministry of Environment and Waters, 158 samples of aerophytic epilithic and endolithic algae (including Cyanoprokaryota) were collected from 12 localities of the nature rock phenomenon and protected area "Belogradchishki Skali", situated in the vicinity of the town Belogradchik (Northwestern Bulgaria, figs. 1,2). The material from the visible with naked eye spots and layers on rock surface and fissures, tree-barks and soil crusts was scraped off from the substratum with sterile knife, or needle, and directly transferred into sterile glass

Figs.1, 2: Rock formations of the Nature Monument "Belogradchishki Skali" (Northwestern Bulgaria).

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Fig. 3: Sampling of algal material directly into agar tubes by scraping off the rock surface with a sterile needle.

Fig. 4: Sampling of algal material directly into agar tubes by scraping off the rock surface with a sterile knife.

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tubes containing BBM-agar (Bischoff & Bold 1963) (Figs. 3, 4). The tubes were immediately closed with aluminium caps and sealed up with Parafilm to avoid further contamination. In the laboratory, the tubes were incubated at room temperature (21 °C) with an irradiance of ~ 60 µmol photons m^{-2} s^{-1} and a light dark regime of 14:10 h. After 6 months incubation, algal material was transferred from the tubes onto agar plates and single cells of different colonies were isolated by streaking across the agar surface (Pringsheim 1946, Ettl & Gärtner 1995, Andersen & Kawachi 2005, Uzunov 2009). After repeated procedure of cell streaking and continuous incubation, colonies from single cells were transferred into new agar tubes and maintained as a part of the Algal Collection of the University of Sofia (ACUS).

3. Results and discussion:

The standard processing of aerophytic algae encompasses series of activities, which begin with the field sampling, pass through primary enrichment cultures and end in clonal cultures with relevant microscopic work for algal determination. All these well-established procedures need time, patience and sufficient skills. Beside this, a minimum of laboratory equipment is necessary to make isolation and cultivation successful (Andersen 2005).
Following the standard protocols for studying the aerophytic algae, the samples usually are collected dry in paper bags, glass or plastic tubes and stored in air-dry conditions, or are frozen after the collection and stored before they are prepared for cultivation (Friedmann & Ocampo-Friedmann 1984, Flechtner et al. 1998, Büdel et al. 2009). In spite of the fact that aerophytic and lithobiontic algae show great tolerance against desiccation and a considerable diversity in soil samples kept in air-dry conditions for years ("museum samples")

Fig. 5: Layer of endolithic algae in a stone-piece from the nature rock phenomenon "Belogradchishki Skali".

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Fig. 6: Direct collected field samples after 6 months cultivation in the Algal Collection of University of Sofia (ACUS).

is known (TRAINOR 1985, STOYNEVA 2000), the almost immediate establishment of enrichment cultures could enlarge the diversity of enlisted organisms from the relevant sample, as it is well-documented for soil algae (TRAINOR 1985). Therefore it is always strongly recommended collected material to be taken in culture as soon as possible after sampling.

The method used in this study was applied for the first time during collection of aerophytic algae (incl. endolithic rock-inhabitants) in the region of the rock-phenomenon "Belogradchishki Skali". In the field it was conducted in the following manner: aerophytic algal material from a layer, spot or crust, visible with naked eye or with a hand lens, was scraped directly into an agar tube, which was hold below the scraping area (Figs. 3, 4). The endolithic algae (Fig. 5) were sampled in the same manner from the rock fissures which were opened with hammer and chisel. The scraped algae (together with stone particles and some detritus, etc.) fell directly into the tube, which was closed immediately. In this state, closed in the agar tubes, and without any other treatment, the samples were put in bags and transported to the laboratory.

The moisture of the agarized medium kept the samples fresh and algae were in normal, unchanged living conditions, ready for further treatment in the laboratory. After 6 months incubation, considerable green and blue-green layers were visible on the agar surface in the tubes (Figs. 6-10). They consist of different algae and cyanoprokaryotes, but also of moss protonemas. Further isolation procedures were continued by transfer of well grown algal colonies onto new agar plates from the tubes.

In our opinion, in spite of the fact that during this study, the "direct collecting method" (DC-method) was tested mainly on epilithic and endolithic algae, it could be applied to all

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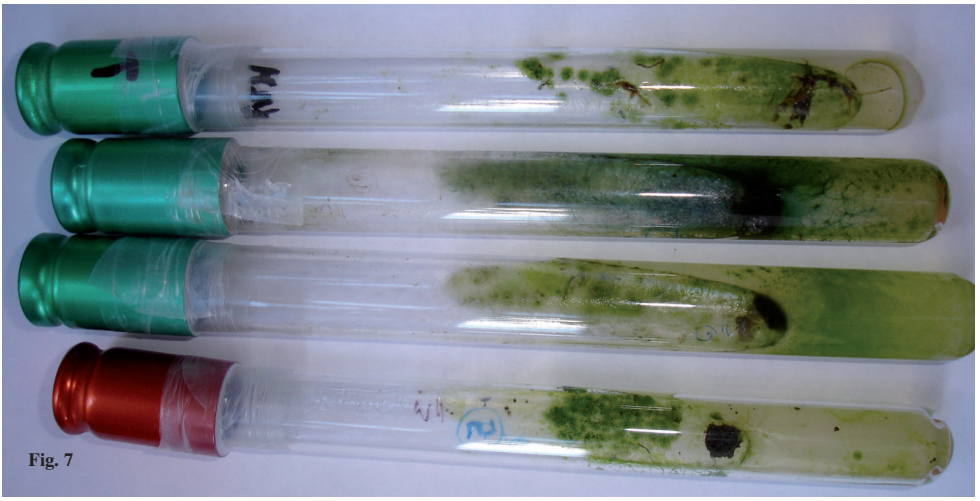
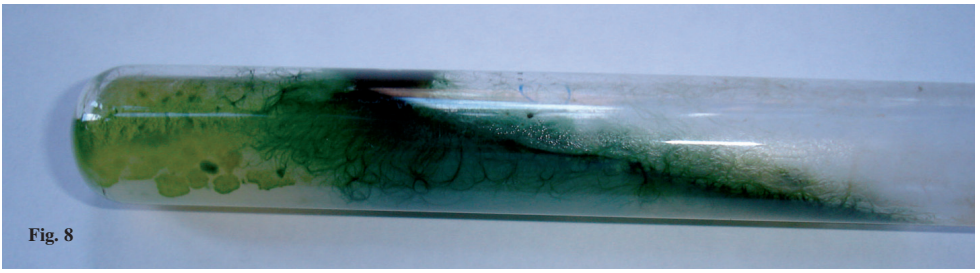


Fig. 7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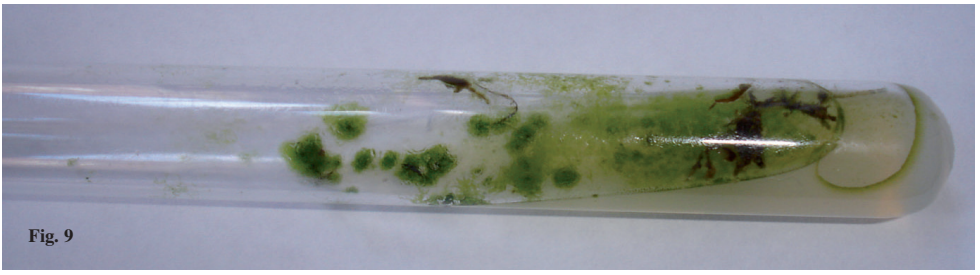
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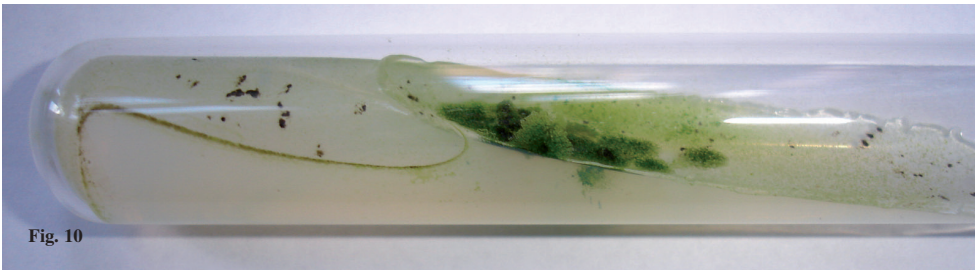
Fig. 10

Figs. 7-10: Enrichment cultures from direct collected field samples on the agar surface after 6 months cultivation in the Algal Collection of University of Sofia (ACUS); in fig. 8 mainly cyanoprocaryota are visible; in fig. 10 the greenish line on the lower agar surface is caused by zoospores from green algae.

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kinds of aerophytic algae. There are at least two advantages of the DC-method proposed here: they lie both in the saving of time (days and sometimes even weeks!) between transport and first enrichment cultures in the laboratory and work efforts for the first inclusion of the dry field material in enrichment cultures and their second labelling, as well as in economising of the first glass or plastic equipment for field collection and its labelling. Doubtless, the most important advantage is in the fact that the field samples keep fresh in the closed tubes with agarized medium and therefore the desiccation of small pieces of material is prevented. It is necessary to underline also that the transfer on agar plates needed for the isolation into clonal cultures can be done more easily from the fresh growing and easily visible original samples. In cases, when the algae are very small and delicate, such method obviously prevents possibilities to loose material during its transfer to the lab and into cultures. Finally, all the field samples, collected in this way, can be stored as "originals" for a long time.

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