Epimycotic Algae on the Medicinal Fungus Trametes versicolor (L.) Lloyd

Pavel V. Videv\textsuperscript{1}, Georg Gärtner\textsuperscript{2}, Blagoy A. Uzunov\textsuperscript{1}, Petya H. Dimitrova\textsuperscript{1}\& Maya P. Stoyneva-Gärtner\textsuperscript{1}

\textsuperscript{1} Sofia University “St Kl. Ohridski”, Faculty of Biology, Department of Botany, 8 bld. Dragan Zankov, Sofia, Bulgaria

\textsuperscript{2} University of Innsbruck, Institute of Botany, Sternwartestrasse 15, Innsbruck, Austria

Corresponding Author: Maya P. Stoyneva-Gärtner: mstoyneva@uni-sofia.bg

Abstract: The paper presents the species composition of algae, which develop on the surfaces of the medicinal fungus Trametes versicolor, collected in seven different sites of Bulgaria and investigated in cultures. All ten recorded epimycotic algae belong to the green evolutionary line (Chlorophyta – 9 species and Streptophyta – 1 species). The species composition on basidiomata collected from different sites was different with number of species varying from one to five. The same is valid for the species composition on different fungi from the same site. Therefore, we claim the lack of symbiotic relations between one specific alga and Trametes versicolor. Taking into account the ecological preferences of the recorded algae, it is possible to suppose with much higher probability that there is a relation between the epimycotic algae and nearest aerophytic phycflora.

Keywords: aerophytic algae, aero-terrestrial algae, green algae, medicinal fungi, symbiosis

1. INTRODUCTION

The aero-terrestrial algae are important producers in land ecosystems but their taxonomy and biogeographic distribution have been scarcely studied at globe scale and in Bulgaria particularly [e.g. 1-3]. This is especially valid for the aerophytic algae which develop on the surface of the fungal fruiting bodies. Long they were commonly named as epiphytic and only recently the use of term epimycotic was advocated [4]. The only actual study on these algae in Bulgaria concerns their development on the surface of the basidiomycete Fomes fomentarius (L. ex Fr.). Kickx, on which four green aerophytic algae have been identified [4].

The development of algae on F. fomentarius is rare, while the presence of clearly visible algal growth on the surface of the fruiting bodies (basidiocarps, basidiomata) is typical for another basidiomycetous species with a tube-like hymenophore - Trametes versicolor (L.) Lloyd (Syn. Coriolus versicolor (L.) Quél.; Polyporus versicolor (L.) Fr.), most popular as Turkey tail. Four species from the green algal genera Chlorococcum Meneghini, Hormidium Kützing, Stichococcus Nägeli and Trebouxia Puymaly were documented from its surface in North America with the suggestion that the basidiocarps have the potential to be lichenized [5, 6].

The Turkey tail acquires high popularity due to its medical use in treatment of cancer (in particular the breast-cancer) and different infections [7, 8]. The study of the biodiversity of epimycotic species on its surface could help to clarify the nature of algal-fungal relations and to provide ideas on the potential relation between them and the medical importance of the fungus. Therefore, the present paper is aimed on the species composition of the epimycotic algae on T. versicolor based on cultural studies of field material collected from seven different sites of Bulgaria (Eastern Europe) and its comparison with the results of [5, 6] from North America.

2. MATERIAL AND METHODS

The samples were collected by picking off the basidiocarps from their typical substrata – tree trunks or stems (Fig. 1) from seven sites: 1 – South Park of Sofia (42.6691N;23.3075E); 2 – Stara planina Mts, Villa zone nearby of Trudovets village (42.9333N; 23.8449E); 3 – Stara planina Mts, locality
Dzhendema (42.6584N; 24.9265E); 4 – Stara planina Mts, locality Dzhendema (42.6943N; 24.9513E); 5 – Vitosha Mt, vicinity of Boyana village (42.6386N; 23.2640E); 6 - Vitosha Mt, vicinity of Zheleznitsa village (42.5348N; 23.3385E); 7 - Vitosha Mt, vicinity of Simeonovo village (42.6081N; 23.3308E). Sites 1-2 were situated in a large central park of the Bulgarian capital and in a small village garden, respectively, while sites 3-7 were from the natural deciduous forests. Six different fruiting bodies closely attached on two broken stems of cherry trees in the same house garden of site 2 (Fig.1e) were sampled especially for comparison of the species composition of the epimycotic algae.

**Fig 1a-f.** Basidiocarps of *Trametes versicolor*: a, b – from the vicinity of Simeonovo village on Vitosha Mt; c, d – from the region Dzhendema in Stara planina Mts; e, f – from the vicinity of village Trudovets on Stara planina Mts.
After taking off from the substratum, the basidiocarps were kept in a paper envelope. In less than 24 hours they were transported to the laboratory in the Faculty of Biology of Sofia University “St. Kliment Ohridski” for further processing.

The cultivation and isolation of monocultures was done in the Algal collection of Sofia University - ACUS [9] in Petri dishes with agar with Bold’s Basal Medium (BBM) according to standard methods and techniques [10-14]. The material from all basiomata was scrapped out from the visible green layers of their upper surface on the agar (Fig. 4), and, in addition, pieces from the fruiting bodies were dragged on the agar surface with the green layers turned to the medium (Fig. 5).
Figure 4a-b. Scratching of the material from the basidiocarp (a) and spread out of the material on the surface of the solid medium (b).

Figure 5a-b. Separation of small pieces of the basidiocarps (a) and dragging of the material on the surface of the solid medium (b).

Another cultivation procedure was conducted in order to obtain algal cells free from fungal hyphae. Then the green layers were scratched out in the eprouvettes with liquid media. Afterwards the liquor was shaken and drops of the material were spread on the agar with BBM (Fig. 6).

Figure 6a-d. Scrapping of the material in a liquid medium (a-b) and application of the drops with the algal material on Petri dishes with a solid medium (c-d).

For obtaining of single colonies the material from Petri dishes was inoculated in new dishes with agar medium (Fig. 7).
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The microscopic observations on the colonies were made on Motic binocular stereoscope with magnification 10x and 30x. Species identification was done on non-permanent microscopic slides on light microscope Motic BA 400 with an immersion objective. Standard staining of cell elements follows [15]. Microphotographs were taken by Moticam 2000 digital camera and were processed by the Motic Images Plus 2.0 program.

The identification of algae was done on the basis of standard taxonomic literature [16-36] and the taxonomy was consulted with AlgaeBase [37], following the International Code for Nomenclature of Algae, Fungi and Plants [38].

3. RESULTS AND DISCUSSION

In total 10 algal species were identified. All of them were green algae from the divisions Chlorophyta (9) and Streptophyta (1):

**CHLOROPHYTA**

*Apatococcus lobatus* (Chodat) J. B. Petersen: Distribution: Site 2.

*Chlamydomonas moewusii* Gerloff (Fig. 8): Distribution: Site 6.

*Chlorella vulgaris* Beijerinck (Fig. 8): Distribution: Sites 4, 5, 6.

*Chlorella minutissima* Fott et Nováková (Fig. 8): Distribution: Sites 1, 3, 4, 6.

*Coccomyxa confluens* (Kützing) Fott (Fig. 8): Distribution: Sites 3, 4, 6, 7.

*Desmococcus olivaceus* (Persoon ex Acharius) J. R. Laundon: Distribution: Site 2.

*Sphaerocystis* sp. (Fig. 8): Cells are spherical to elongated (before division), 9-10 µm long and 5-6 µm wide, covered with a mucilage sheath. The chloroplast is singular, parietal and slightly lobed and bears a pyrenoid with attached starch grains. The species identification was impossible because of lack of sporangia and zoospores in the materials. Distribution: Site 7.

*Trebouxia* cf. *aggregata* (Archibald) Gärtner (Fig.8): Distribution: Sites 2, 4.

*Stichococcus bacillaris* Nägeli (Fig. 8): Distribution: Site 7.

**STREPTOPHYTA**

*Klebsormidium dissectum* (F. Gay) H. Ettl et Gärtner: Distribution: Site 2.

The species found to develop epimycotically on *T. versicolor* are well-known representatives of the aerophytic algae [12, 13], most of which have been recorded on different substrata in Bulgaria [2-4, 39-41]. The algal distribution on basidiomata was different – in site 1 only *Chlorella minutissima* was detected, in site 5 - only *Chlorella vulgaris*, whereas in the other localities two to four species developed together on the fungal surface. Some of them were common for both mountain regions of Vitosha and Stara planina (*Chlorella minutissima*, *Chlorella vulgaris* and *Coccomyxa confluens*). By contrast, *Apatococcus lobatus*, *Desmococcus olivaceus*, *Trebouxia* cf. *aggregata* and *Klebsormidium dissectum* were recorded only in the samples from Stara planina Mts, and *Chlamydomonas moewusii*, *Stichococcus bacillaris* and *Sphaerocystis* sp. were found only in the samples from Vitosha Mt.
Fig. 8. Epimycotic species on Trametes versicolor: a - Coccomyxa confluens (Kützing) Fott, 2 - Chlorella vulgaris Beijerinck; c - Chlamydomonas moewusii Gerloff; d – Desmococcus olivaceus(Persoon ex Acharius) J. R. Laundon; e – Trebouxia cf. aggregata (Archibald) Gärtnér; f - Stichococcus bacillaris Nägeli; g - Sphaerocystis sp.; h - Chlorella minutissima Fott et Nováková. Scale bar – 10 µm.

The comparison between the species composition on six different basidiomata from the region of Trudovets on Stara planina Mts (site 2) shows that Desmococcus olivaceus developed on all of them, but in various combinations with Apatococcus lobatus, Coccomyxa confluens, Klebsormidium dissectum and Trebouxia cf. aggregata.

4. CONCLUSION

Ten green algal species were recorded on the surface of T. versicolor, most of which have been documented also from the surfaces of other basidiomycetes (for details see [4]). This species composition is almost twice richer when compared with the four species published earlier for the
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surface of T. versicolor from North America [5, 6]. The common result from both studies is that all algae which develop epimycotically belong to the green evolutionary line and two genera coincide: Stichococcus and Trebouxia.

The detected difference in the species composition at the sites with different location and at different basidiomata from the same site clearly shows the lack of symbiotic relations between one specific alga and T. versicolor. Taking into account the statement of [6] that the occurrence of algae on this fungus is common, but not universal and the ecological preferences of the recorded algae, it is possible to suppose with much higher probability a relation between the epimycotic algae and the nearest aerophytic phycoflora. Doubtless, further studies with more data on epimycotes of T. versicolor from different sites with comparisons with their nearest aerophytes (on the tree barks, rocks or stone surfaces, soils, etc.) will provide more knowledge on the algal distribution on fungal surfaces.

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