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Identification of a fungal contaminant in a culture of Dunaliella salina

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Acremonium sp. was identified as a contaminant in the culture of the halophilic algal strain *Dunaliella salina* V63. The morphological details of this fungus – algae relationship were determined by growing the association in a slide cavity culture. The interaction between *Dunaliella salina* and the contaminant is described and illustrated.

Key words: *Acremonium, Dunaliella salina*, vzájemný vztah.

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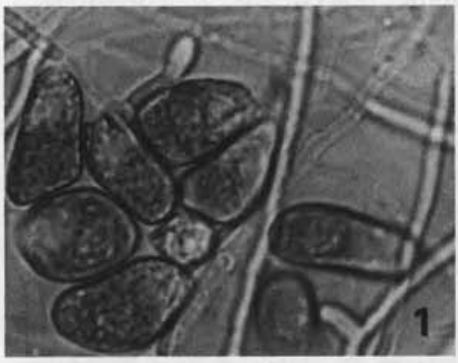
Acremonium sp. byl identifikován jako kontaminant v kultuře halofilního kmenu řasy *Dunaliella salina* V63. Morfologické detaily soužití houby a řasy byly popsány při společném výskytu ve sklíčkové kultuře. Vzájemný vztah mezi *Dunaliella salina* a kontaminantem je popsán a ilustrován.

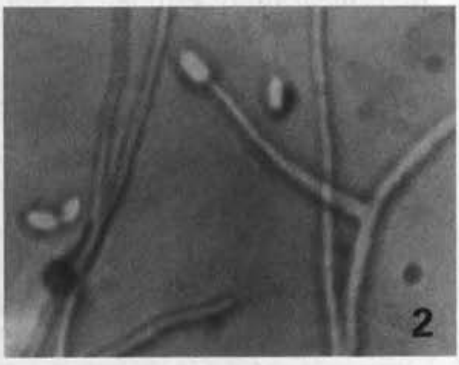
The development of unicellular algae in nature and laboratory conditions depends on different contaminated organisms – protozoa, bacteria, viruses, fungi and others (Gromov 1976). It is suggested that algae growing in extreme salinity can avoid competition and contamination with other less tolerant organisms (Brock 1975). During the cultivation of the halophilic *Dunaliella salina* in laboratory conditions we had problems with different contaminants. After treatment with antibiotics (Toncheva-Panova and Naneva 1987) bacteria-free *Dunaliella salina* was obtained but increasing occurrence of a fungal contaminant was observed. To our best knowledge there are no other data on fungal contaminants of *Dunaliella salina* besides the data of Bednářová et al. (1976) on the occurrence of *Cladosporium* in laboratory culture of *Dunaliella acidophila* (Kalina) Masjuk.

The aim of the present paper is to present the results from studies of the association of *Dunaliella salina* and the fungus including the identification of the fungus and the interaction between the two organisms.

MATERIAL AND METHODS

The investigation was carried out with *Dunaliella salina* strain V63, kindly donated by M. Tzolova, Institute of Plant Physiology, Bulgarian Academy of





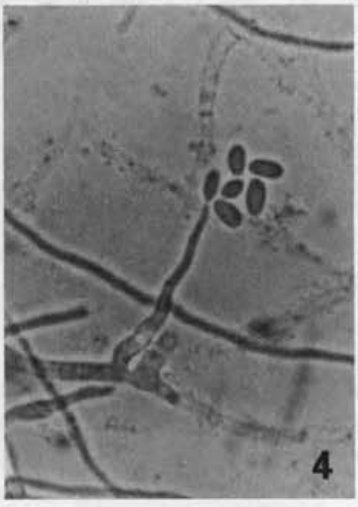


Fig. 1. *Dunaliella salina* V63 enclosed in fungal hyphae. A fungal protuberance contacted an algal cell.
Fig. 2 and 3. Variations of phialides and phialoconidia in the algal-fungus association.
Fig. 4. Aggregate droplet of phialoconidia (phialospores) at the apex of phialides of the well-developed mycelium.

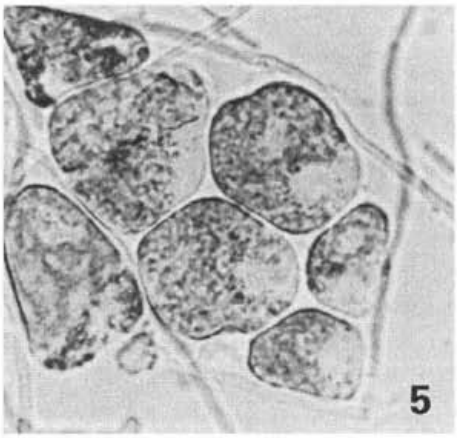
Sciences. The algae was cultivated extensively on Eddy's medium, at 26 °C, light intensity 6000 lux and treated with kanamycin (Toncheva-Panova and Naneva 1973). The morphological details of the alga and fungus community developed thereafter were determined by growing the association in cavity slide culture, placed in a moist chamber for 90 days. Microscopic observations and photographs were made.

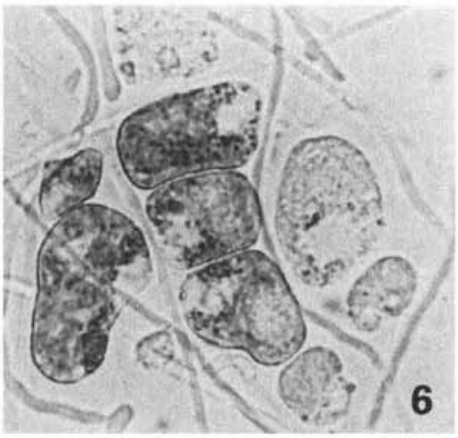
RESULTS AND DISCUSSION

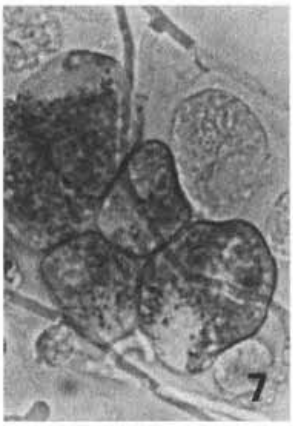
The fungus developed together with D. salina and produced slowly a hyaline mycelium consisting of white or pale yellow hyphae, 0.8–1.5 μm wide, with thin cell walls, branched and septate (Fig. 1). Vegetative growth of the fungus was observed together as well as sporulation. Two types of fungal conidia associated with different stages of mycelial maturation were present: 1) abundant, globose, solitary aleurioconidia – 4.0–7.3 μm diam., borne usually on the top of conidiophores or on lateral short conidiophores, and 2) oval phialoconidia formed at the tips of long and slender phialides of the hyphae of the well-developed mycelium (Fig. 2, 3 and 4). The phialides erected from the substratum, have a maximum width of 1.0–2.0 μm at the base and taper to 0.2–0.5 μm at the tip. Phialides produced oval phialoconidia, measuring 1.0–1.5 μm to 0.4 μm (Fig. 3). The phialoconidia are hyaline – separate or in clusters of two to six, parallel to each other or to the phialides producing them. They are deposited in slimy heads at the apex of the phialide (Fig. 3 and 4). According to the features of fine, slow growing mycelium, simple one-celled in slimy heads conidia, the pigmentation and the presence of phialoconidia, the contaminant of *Dunaliella salina* V63 was identified as *Acremonium* sp. (Domsch et al. 1980; Gams 1971).

When the mycelium of *Acremonium* grew together with *D. salina* V63 and the hyphae became intermixed with the algal cells, the first observable reaction was the formation of short protuberances from the fungus towards the algal cells (Fig. 1). First at a distance from the *Dunaliella* cells, the protuberances later elongated and developed into a single hook which appeared to clamp onto algal cells (Fig. 5–7). The algal cell reaction to the fungal invasion was invagination of the cell wall and redrawment of the chloroplast. It was evident from the photographs that in the 40 days old association the algal division was inhibited and the fungus grew over the enormous bigger non-divided *Dunaliella* cells. Only in some cases fungal filaments in the intracellular space of the algae were observed (Fig. 8). We suggest that exhaustion of nutrients in the association caused by the development of algae induces the formation of a hook from the fungus toward the algae. The interaction between the fungus and *Dunaliella* have some similarity to the way some mycoparasites make contact with their hosts (Rakvidhyasastra and Butler 1973). The mycoparasites are deficient in the growth factor mycotropheine and, like the fungal contaminant of *Dunaliella*, produce a special branche of absorptive hyphae for their hosts so as to assure nutrients and the growth factor.

The *Acremonium* sp. occurring in the *Dunaliella salina* culture appeared to be an algal antagonist. Although there is no information about *Acremonium* species – algal parasites, our observations showed that under defined conditions and a proper algal strain some *Acremonium* species act as undesirable contaminants. Our suggestions that *Acremonium* possesses such negative potentials, are supported







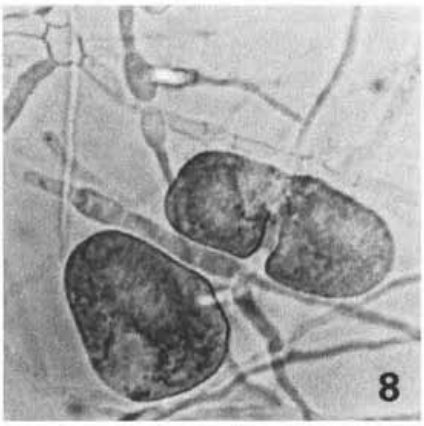


Fig. 5. A single hook formation from the fungal hyphae towards the algae cause invagination of the algal cell.
Fig. 6. The next step of the fungal attack – fungal hook in the closest contact with the Dunaliella cell.
Fig. 7. The hook developed into a clamp directly on the algal cell.
Fig. 8. Some fungal hyphae seemed to enter the algal cells.

All photographs × 200

by the data on proteolytic activity of some *Acremonium* representatives, parasites on sporangia of Myxomycetes, different insects, some higher plants, causal pathogens of gummatous ulcers, and antagonists against *Fusarium oxysporum* f.sp. *lycopersici* (Domsch et al. 1980; Gams 1971).

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