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Cytochemical localization of peroxidase activity in early developmental stages of the moss *Ceratodon purpureus*. Light-microscopic observations

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Abstract

Peroxidase activity was localized in the cell walls and minute cytoplasm granules of swollen and germinating spores and in the several-celled protonema of the moss *Ceratodon purpureus* kept in darkness. Kinetin in a concentration of 100 μM inhibited the protonema development and also depressed the activity of this enzyme.

INTRODUCTION

It results from numerous papers that peroxidases are enzymes occurring commonly in plant material.

Changes in peroxidase activity accompanying the induction of gametophore buds in the moss *Ceratodon purpureus* under the influence of kintein were demonstrated cytochemically (Sobkowiak et al., 1976) and biochemically (Schneider and Szweykowska, 1974).

The present study aims at localization of the peroxidase activity in early developmental stages of the moss *Ceratodon purpureus* and establishment of the influnce of kinetin on this activity.

MATERIAL AND METHODS

The object of the studies were early developmental stages (i.e. unswollen, swollen and germinating spores and several-celled protonema) of the moss *Ceratodon purpureus* Brid. Spores were sown on modified

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(Szweykowska et al., 1971) mineral Kofler medium (1959) with addition of Heller microelements (1953) and 0.25% glucose in combination with kinetin (5,10 and 100 μM). Protonema developed in culture room conditions, i.e. at about 25°C and air moisture about 70%, in darkness. After 4 days of culture the peroxidase activity was determined by the 3,3′-diaminobenzidine method of Graham and Karnovsky (1966) in material previously fixed with 2.5% glutaraldehyd (in 0.05 M phosphate buffer at pH 7.2). For control purposes material incubated in KCN (Goff, 1975) was used.

The photographs were take with a Zeiss microscope and original nipple with outfit for automatic determination of the exposition time.

RESULTS AND DISCUSSION

In the 4-day culture the following developmental stages of the moss were observed: unswollen, swollen and germinating spores and several-celled protonema (Fig. 1).

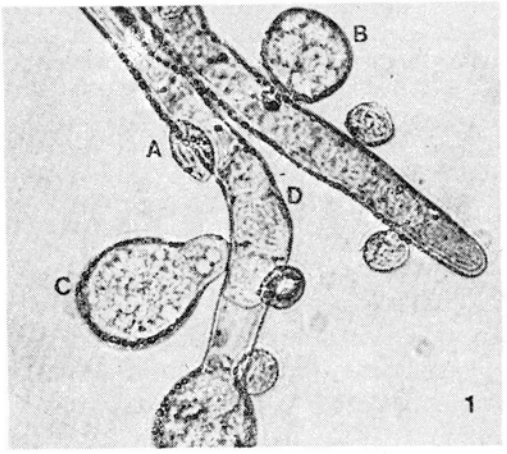
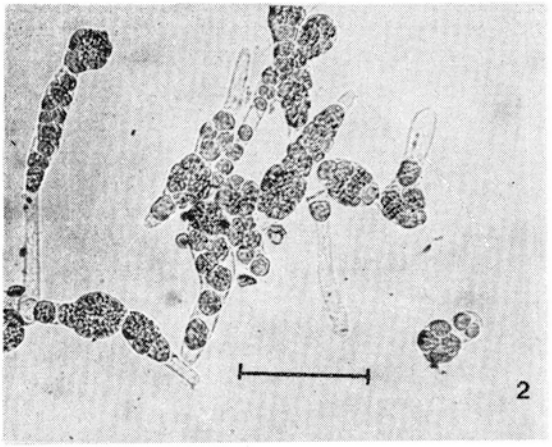
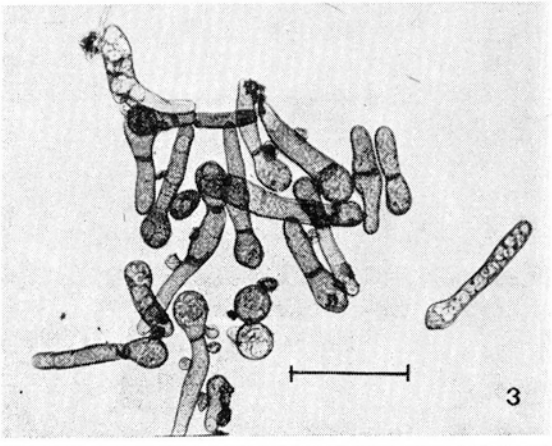


Fig. 1. Early developmental stages of *Ceratodon purpureus*.

A — unswollen spore, B — swollen spore, C — germinatin gspore, D — several-celled protonema.

The germination power of spores and further development of protonema growing on medium without or with kinetin in a concentration of 5 μM did not differ. Neither did peroxidase activity in the same concentration of cytokinin differ from material growing on medium without kinetin. Kinetin in a 10 μM concentration exerted a similar influence as 100 μM kinetin but, considerably weaker. Therefore the results regarding peroxidase activity were obtained from variants grown on medium without kinetin and with kinetin in 100 μM concentration.





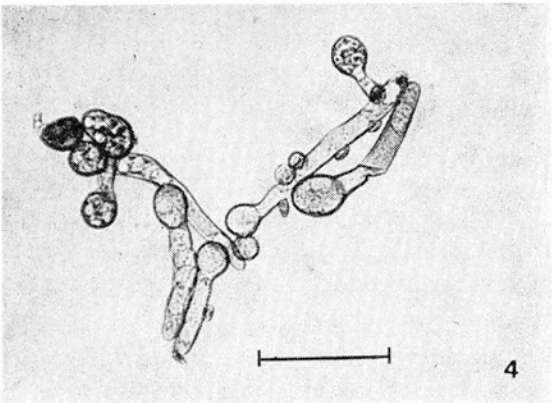


Fig. 2—4. *Ceratodon purpureus* — 4-day culture. Segment of scale = 100 μm.

Fig. 2. Kofler medium with 0.25% glucose (without kinetin). Basic control. Incubation in KCN — lack of reaction for peroxidase

Fig. 3. Kofler medium with 0.25% glucose (without kinetin).Reaction with diaminobenzidine high peroxidase activity

Fig. 4. Kofler medium with 0.25% glucose (kinetin in concentration of 100 μM). Reaction with diaminobenzidine — low peroxidase activity.

The presence of kinetin in the medium in a concentration of 100 μM inhibited spore development so that unswollen and swollen spores were most numerous, while on the medium without kinetin germinating spores and several-celled protonema prevailed. The inhibitory influence of kinetin on germination of moss spores had been shown earlier (Zajchert — unpubl.).

In this case the peroxidase activity differed not in dependence on the presence, or lack, of kinetin in the medium, but rather in dependence on the developmental stage of the moss.

Kinetin in the analysed concentration inhibited the growth of protonema and considerably decreased the activity of the examined enzyme in all developmental stages (Fig. 3, 4).

In the unswollen spores the reaction characteristic of peroxidase was not observed. The highest activity was observed in swollen and germinating spores. On the other hand, in the several-celled protonema the highest activity was found in the oldest and apical cells.

Analysis in the light microscope revealed that peroxidases were localized mainly in the cell walls and in the minute spherical granules of the cytoplasm. Especially high peroxidase activity was observed in the cross walls (Fig. 3).

In earlier cytochemical (Sobkowiak et al., 1976) and biochemical (Schneider and Szweykowska 1974) studies of *Ceratodon purpureus* an increase of the peroxidase activity under the influence of kinetin was observed. It was connected with initiation and acceleration of the gametophore buds which showed a high activity of the enzymes. An increase of peroxidase activity was noted also in the degenerated intercalary cells which produced the buds.

As shown in the present study, the effect of kinetin on the peroxidase activity in early stages described earlier (Sobkowiak et al., 1976), agrees with the results achieved by de Boer and Feierabend (1974). Studying rye seedlings they found sensibility to cytokinins manifested by changes in activity of some enzymes dependent on the developmental stage. It is probable that the endogenous cytokinin level is higher in earlier developmental stages of plants than in the later ones.

Particularly high activity of peroxidases in the newly forming walls points to some function they perform in formation of the cell wall. However, it is not connected with lignification in this case. The granules in the cytoplasm are not microbodies (as described at the electron microscope level by Młodzianowski, 1970) with properties of peroxisomes, because the material was grown in darkness. They can be Golgi bodies what may be confirmed by the occurrence of an intensive reaction in the apical cell of the several-celled proto-

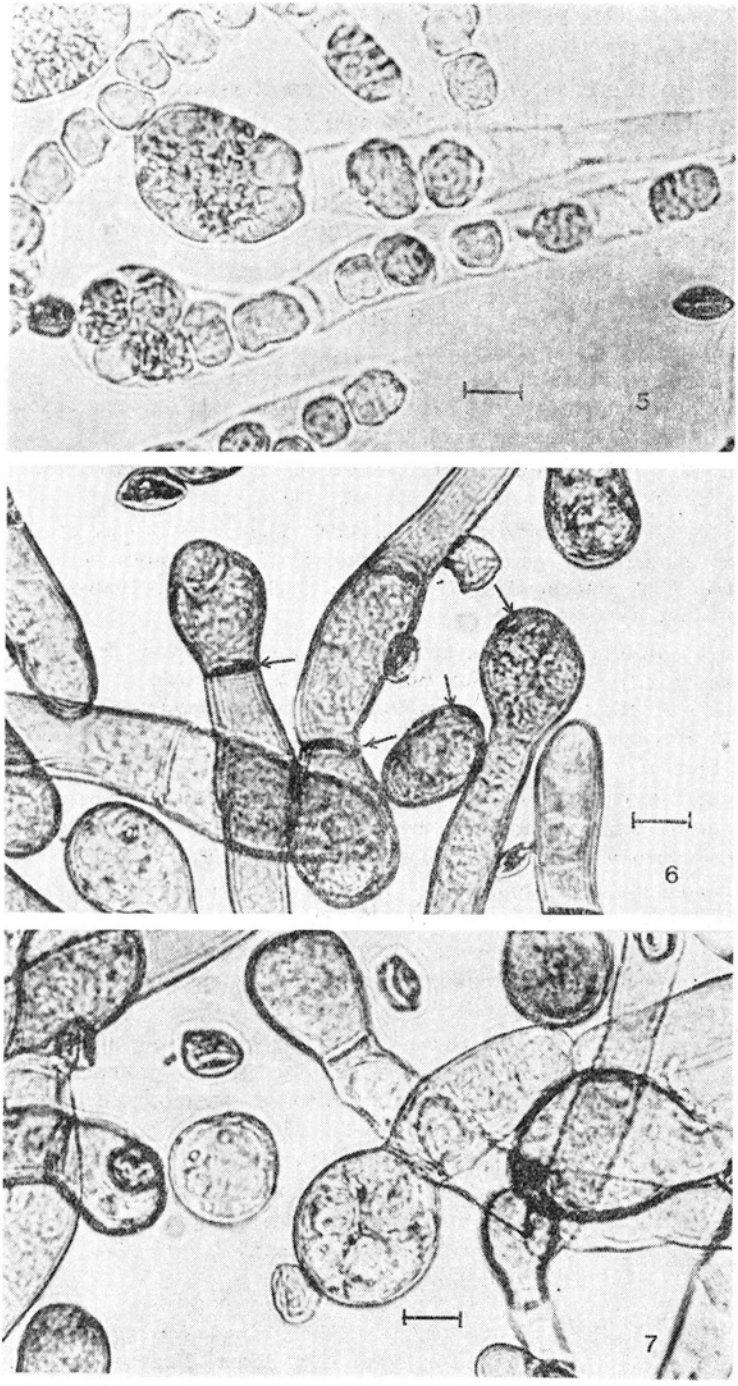


Fig. 5—7. *Ceratodon purpureus* — the 4 days culture. Segment of scale = 100 μM.

Fig. 5. Kofler medium with 0.25% glucose (without kinetin). Basic control. Incubation in KCN — lack of reaction on peroxidase.

Fig. 6. Kofler medium with 0.25% glucose (without kinetin). Reaction with diaminobenzidine — high peroxidase activity (see arrows).

Fig. 7. Kofler medium with 0.25% glucose (kinetin in concentration of 100 μM). Reaction with diaminobenzidine — low peroxidase activity.

nema, since in the case of moss protonema it is known that the greatest number of these structures is observed in the apical cells (Idzikowski, 1974).

At present the studies are continued at the electron microscope level in order to localize these enzymes more accurately.

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Cytochemiczna lokalizacja aktywności peroksydazy we wczesnych stadiach rozwojowych mchu Ceratodon purpureus

Streszczenie

Aktywność peroksydazy w ścianach komórkowych i drobnych ziarnistościach cytoplazmatycznych spęczniałych i kiełkujących zarodników oraz kilkukomórkowego splątka mchu *Ceratodon purpureus* hodowanego w ciemności. Kinetyna w stężeniu 100 μM hamowała rozwój splątka oraz obniżała aktywność tego enzymu.