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SPOROPOLLENIN IN THE SPORE WALL OF SPIROGYRA (ZYGNEMATACEAE, CHLOROPHYCEAE)

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SUMMARY

The acetolysis resistant mesospore walls of *Spirogyra* were investigated by infrared spectrometry and chromic acid degradation. It was demonstrated that the mesospore wall contains chromic acid and hydrofluoric acid resistant sporopollenin. The biological significance could be a protection of the spores against desiccation and fungal attack.

1. INTRODUCTION

Features of the spore wall are of considerable importance for the recognition of species in the genus *Spirogyra*. In a previous study the technique of acetolysis has been applied to reveal the finer structures of the spore wall (SIMONS et al. 1982).

The spore wall of *Spirogyra* consists of four layers: one exo- and endospore, containing cellulose and/or pectine and two acetolysis resistant mesospore layers, of which the inner one is often sculptured (SIMONS et al. 1982).

The resistance against acetolysis suggests the presence of sporopollenin in the mesospore wall (ASHRAF & GODWARD 1980). Sporopollenin is considered one of the most extra-ordinary resistant materials in the organic world. According to HESLOP-HARRISON (1971) it is the only known organic constituent of plant cell walls that withstands acetolysis.

This study intends to determine the presence of sporopollenin in the *Spirogyra* mesospore wall by infrared spectrometry and by chromic acid degradation.

2. MATERIALS AND METHODS

Field samples, fixed in F.A.A., containing ripened spores of *Spirogyra gracilis* (Hassall) Kützing, *S. longata* (Vaucher) Kützing, *S. weberi* Kützing, *S. acanthophora* (Skuja) Czurda and *Mougeotia laevis* (Kützing) Archer, as well as spores of an unialgal culture of *Spirogyra hassallii* (Jenn) Petit., which was induced to sexuality in Woods Hole medium (STEIN 1973) at 16 °C; 4500 lx and a 12:12 light-dark regime were used. As a reference pollen grains of *Taxus baccata* L. and *Pinus sylvestris* L. were collected from the field.

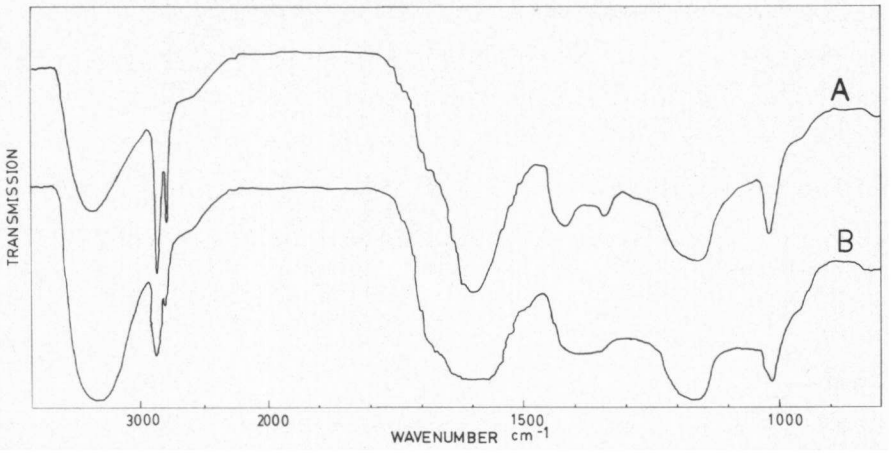

Fig. 1. Infra-red transmission spectra of acetolysed residues from (A) mesospore of *Spirogyra hassallii*, and (B) pollen grains of *Taxus baccata*.

Previous to acetolysis algal material containing ripened spores or pollen grains was washed twice in glacial acid, 5 min, 300 g. Acetolysis was carried out in a waterbath, 90 °C for 15 min, using 9 parts acetic anhydride plus 1 part concentrated sulphuric acid (ERDTMAN 1969). The resistant material was centrifugated 5 min, 300 g, followed by washing the pellet twice in ethanol 100%, 5 min, 300 g.

Acetolysed spores of *Spirogyra hassallii* and pollen grains of *Taxus baccata* were separately prepared for infrared spectrometry as follows: the acetolysed material was rinsed with 0.02 M potassium phosphate buffer, pH 6.8 and boiled for 10 min. in 1 N sodium hydroxide. After rinsing in buffer, distilled water and absolute methanol (GOOD & CHAPMAN 1978), the material was placed in ether and dried at 40 °C. 1.2 mg material was mixed with potassium bromide at a ratio of 1:25 and grinded. The pellet was pressed into a tablet, placed in a Jasco-IRA 2 Grating infrared spectrophotometer and emission was measured from 4000 cm^{-1} till 800 cm^{-1}.

Chemical degradation of acetolysed material of all species was attempted in an aqueous solution of 30% (wt/vol) chromic acid at room temperature. The residue was exposed to hydrofluoric acid 40% (wt/vol) to detect possible presence of silica.

3. RESULTS

The infrared transmission diagram of acetolysis resistant spore material of *Spirogyra hassallii* and *Taxus baccata* pollen is presented in *fig. 1*. It appears that the spectrum of the *Taxus* pollen grains agrees closely with the spectrum of the *Spirogyra* mesospore material. Hence it is stated that the mesospore wall of *Spirogyra* contains sporopollenin.

In general the peaks either lie at the same wavelength or else a peak in one spectrum is matched by the shoulder of the other. The diagram shows for the two objects an absorption band at 3500–3400 cm^{-1} typical for OH (O-H stretch). At about 2900 cm^{-1} a peak characteristic for aliphatic compounds is situated. At 1600 cm^{-1} a broad signal due to carbonyl compounds can be detected while the sharp peak at 1050–1030 cm^{-1} indicates aliphatic C-O stretch. The interpretation of the spectra is based on SCHEINMANN (1970).

Silica is also known to withstand acetolysis. If present, it can be demonstrated by its resistance to chromic acid (ATKINSON et al. 1972) and its degradation by hydrofluoric acid (MILLINGTON & GAWIK 1967). *Taxus baccata* and *Pinus sylvestris* pollen grains dissolved in chromic acid within one hour, while mesospore walls of *S. gracilis, S. longata, S. weberi, S. acanthophora, S. hassallii* and *Mougeotia laevis* were resistant to the treatment. The chromic acid resistant residue, however, remained insoluble when exposed to hydrofluoric acid. This indicates that no silica is present in the mesospore wall of *Spirogyra.*

On the basis of these results we conclude that the mesospore wall of *Spirogyra* contains a hydrofluoric acid and chromic acid resistant sporopollenin.

4. DISCUSSION

That resistance of mesospore walls to acetolysis is a strong indication for the presence of sporopollenin is confirmed by the infrared spectrum for *S. hassallii* which appeared to be characteristic for sporopollenin. The chemical structure of sporopollenin is still not fully elucidated, but SHAW (1971) indicates that it is composed of oxidative polymers of carotenoids and/or carotenoid esters. The presence of sporopollenin has been demonstrated in the vegetative algal cells of *Chlamydomonas* (ATKINSON et al. 1972), *Phycopeltis* (GOOD & CHAPMAN 1978) and *Trebouxia* (KÖNIG & PEVELING 1980) as well as in the spore walls of *Chara, Pithophora, Lycopodium, Selaginella, Aspergillus* and *Mucor* (SHAW 1971). The infrared transmission spectra of acetolysed *Taxus baccata* pollen and *Spirogyra hassallii* mesospore walls appear to be similar to infrared spectra of sporopollenin from green algae, spores of lower plants, pollen grains, as well as to synthetically produced sporopollenin (BROOKS 1971; BROOKS & SHAW 1971; ATKINSON et al. 1972).

An extra argument for the presence of sporopollenin could be the digestion by chromic acid. Sporopollenin is susceptible to oxidation by this acid (ATKINSON et al. 1972). However, mesospore walls of *Spirogyra* spp. and *Mougeotia laevis* withstand the treatment. These findings agree with those of SOUTHWORTH (1974) who demonstrated that *Ambrosia trifida* pollen contain sporopollenin, yet withstand chemical degradation by chromic acid.

The chromic acid and hydrofluoric acid resistant sporopollenin in the mesospore wall of *Spirogyra* could have some biological significance to protect spores against desiccation and fungal parasitism. *Spirogyra* and *Mougeotia* are often growing in temporary waters. Spores are mostly formed during late spring and

summer. After hibernation in organic mud, they generally germinate the next spring.

Hence the spore wall should be resistant to winter conditions. Pollen grains lacking the chromic acid resistance do not hibernate. The biological difference between certain spores and pollen grains may be reflected in the different resistance to chemical degradation.

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