

stability have been described by Daniels *et al.* (1973). It is an aminoglycoside akin to gentamycin and kanamycin. Unlike other aminoglycosides, G418 has an inhibitory effect on eukaryotes (Jimenez and Davies 1980). We hope to develop a fern transformation system and intend to employ G418 as an aid to selection of transformed individuals of *P. aquilinum*.

G418 effects on germination were assessed by sowing spores on plates of Moore's medium containing 0 to 2,000 µg/ml G418. The effects on growth were monitored using germinated spores. Three-day-old gametophytes were plated on medium containing 0 to 100 µg/ml G418.

The results showed that G418 has a strong inhibitory effect on both germination and growth in *Pteridium*. It is effective at very low concentrations: 50 µg/ml is sufficient to prevent both germination and growth.

The mode of action has not yet been elucidated, but G418 is clearly an excellent candidate for use in eukaryotic selection systems, as transformed individuals will be easily identified by their ability to grow on media containing 50 µg/ml G418 or above.

Daniels, P. J. L., Yehaskel, A. S. and Morton, J. 1973. The structure of antibiotic G418. *13th Interscience Conf. Antimicrobial Agents and Chemotherapy*, Washington. Abstract 137.

Jimenez, A. and Davies, J. 1980. Expression of a transposable antibiotic resistance element in *Saccharomyces*. *Nature* **287**, 869–871.



E. EXPERIMENTAL STUDIES ON FERN-ALLIES

Spore germination in *Psilotum*

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Previous attempts at germinating the spores of *Psilotum* resulted in less than 0.1% germination after 6 months in the dark (light prevents germination). These experiments were carried out on a nutrient medium containing Knudson's solution of mineral salts which contains both nitrate and ammonium ions, minor elements, FeEDTA, 0.5% sucrose, and 0.6% agar. The form of the nitrogen in the medium greatly affects germination. Media which contain nitrate (50 mg l⁻¹) with or without ammonium ions inhibit germination. Spore germination on media with 50–0.05 mg l⁻¹ nitrate increases as the nitrate concentration decreases. Germination is excellent for spores cultured on media containing ammonium ions as the nitrogen source or a medium without nitrogen. Tests with several ammonium salts, supplying 50 mg l⁻¹ ammonium ions, give 85–90% germination. In addition to

increasing germination, spores germinate more rapidly, after 4 months, on media with ammonium ions or without nitrogen. Early gametophyte growth is faster with ammonium–nitrogen.

Effects other than those of the culture conditions on spore germination or viability can be investigated by using a nutrient medium with ammonium–nitrogen which allows almost total spore germination. The ability of fresh spores to germinate varies from plant to plant with it being as high as 92% and as low as 5%. Storage of spores at room temperature for 3 years eliminates their viability. Spores stored at 4°C for 6 months give 50% better germination than spores from the same batch stored at room temperature; however, fresh spores from this batch gave 20% better germination than the spores stored at 4°C. Spore storage at –20°C is under investigation; hopefully the below freezing temperature will extend the viability of *Psilotum* spores for many years.

Electrophoretic studies of proteins in *Selaginella kraussiana* stems, leaves, roots and ‘rhizophores’

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The enigmatic nature of ‘rhizophores’ in *Selaginella* has stimulated much work on the structure, development and physiology of organs in this genus. One of the goals of such work has been to understand the morphological homology of rhizophores, while another has been to understand the developmental relationships between rhizophores and subterranean roots. This work represents the application of biochemical techniques to the analysis of the similarities and distinctions in soluble proteins between the various organs, and provides new information for application to classical problems in the genus.

Soluble proteins were precipitated from homogenates of fresh stems, ventral leaves, aerial roots (rhizophores) and subterranean roots of greenhouse-grown clones of *Selaginella kraussiana* A. Br. Proteins were separated by one- and two-dimensional polyacrylamide gel electrophoresis. Some 44–51 protein bands were resolved on 1-D gels stained with Coomassie brilliant blue, with approximately 75% of these bands common to stems, leaves, roots and rhizophores. Two-dimensional separation followed by silver staining permitted detection of 200–250 protein spots per organ, again with approximately 75% of the spots common to all organs. However, qualitative and quantitative differences existed between organs. Based on 1-D analysis, stems and rhizophores had the largest number of bands in common (46 out of a possible 52) and the largest number of bands (6) common only to them. Subterranean roots and rhizophores had 41 out of 53 bands in common but no bands common only to them. Two-dimensional gels, while more complicated to analyse, showed similar trends, with the greatest quantitative and qualitative similarities between stems and rhizophores and lesser similarities between rhizophores and roots.