Figs. 2-5. Fig. 2. Gametophytes of *Pteris vittata* grown on Knudson's medium containing 2% sucrose 2 mg/l 2, 4-D and 10% coconut milk, showing callus initiation. Incubation: 4 weeks in light at 25 ± 2°C. Fig. 3. Formation of many well developed apogamous shoots from callus grown on Knudsons' medium with 4% sucrose. Incubation: 4 weeks in light at 25 ± 2°C. Fig. 4. Formation of few apogamous shoots and then roots from callus grown on Knudson's medium containing 1% sucrose. Incubation: 4 weeks in light at 25 ± 2°C. Fig. 5. Intermediate structures developed from callus grown on Knudson's medium containing 0-5% sucrose. Incubation: 4 weeks in light at 25 ± 2°C.

these structures were found to be rich in starch when squashed and stained with iodine. Regeneration of gametophytes occurred from callus grown on sucrose-free medium after 8 weeks incubation.

Discussion

In the present studies with *Pteris vittata* gametophytes, vigorous growth occurred in the presence of standard dose of macroelements as present in the Knudson's medium along with 4% sucrose. Similar results were reported by Schwabe who showed that mineral deficiencies reduced the growth of the prothallii and apogamy was not promoted by elimination or decrease of these macroelements from the culture medium. In *Pteris vittata* the apogamous structures formed were leaves which ultimately produced sporangia while Sulkiyan and Mehra reported in *Nephrolepis cordifolia* only apogamous roots formation. They thought this might be due to high level of endogenous native auxin (IAA) present in this species.

Gametophytic callus differentiated into sporophytic structures in the presence of sucrose. This might be due to the complex sporophytic forms required more of energy source for their formation. Thorpe reported that for shoot formation and its further development from tobacco callus a continuous supply of carbohydrates was essential. In fenns such as *Pteris vittata* autotrophic gametophytic forms can be produced from the gametophytic callus in the absence of sucrose.

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**CHROMOSOME NUMBER OF THE TREE FERN—CYATHEA GIGANTEA**

P. I. KURIAKHAN

Department of Botany, Kerala University
Kariyavattom, Trivandrum, India

Cyathea gigantea (Wall. ex Hook.) Holtt. (= Alsyphiola gigantea Wall. ex Hook; Alsyphiola globula sensu Bedd.) is one of the very few tree ferns, indigenous to South India. It is usually found on banks of streams in the mountains (1000-1500 m).

Sporangial materials for the present study were collected from plants growing in the reserve forest at Bonnaceadie in Trivandrum District. The sporangia were fixed in acetic alcohol (1:3) and smeared in 1% aceticarmine. The spore mother cells showed 69 bivalents at first metaphase of meiosis (Fig. 1). This is the first report of chromosome number in the species from South India. Plants of this species from Ceylon are also known to have n = 69 bivalents in spore mother cells.

Chromosome numbers in 10 species of Cyathea are so far known. A cytogeographic analysis of the data shows that the same gametic chromosome number n = 69 occurs in specimens of both C. luciflua and C. gigantea from different geographic regions. The remaining eight species also show n = 69 bivalents. The absence of any variation in chromosome number
Trichoderma koningii\(^4\). Recently, search for new cellulase-producing organisms has received much attention for the production of glucose from cellulose materials. As a result, a few more cellulolytic fungi, such as, *Sporotrichum thermophilum*\(^7\) and *Chaetomium cellulolyticum*\(^8\) are reported. It is also well demonstrated that decomposition of cellulosic wastes occurs maximum at higher temperatures\(^6\) and hence thermophilic strains play an important role in cellulose decomposition in nature. Based on this idea, the present investigation deals with the characterization and cellulolytic activity of a thermophilic strain, *Myceliophthora thermophila* D-14, reported for the first time as a cellulose decomposer.

The strain was isolated in the course of screening thermophilic organisms from the city wastes of Calcutta and suburbs. It possesses appreciable cellulose decomposing activity. It grows well between 40\(^0\) and 60\(^0\) C, maximum growth however is attained at 45\(^0\) C. In CD medium\(^9\), supplemented with 1% carboxymethyl cellulose (CMC) as carbon source, the mycelium consists of much branched hyphae, initially white cottony growth, but later turns to pale brown; old cultures show greenish appearance. Aerial hyphae branched, septate, hyaline, 0·75 to 3·5 \(\mu\)m in diameter, bearing 1–6 or more biiastronidia on hyphal apical region (Figs. 1 and 2); conidia obovoid to pyriform, 4·2 to 7·0 \(\mu\)m \(\times\) 3·5–4·5 \(\mu\)m; most of the conidia are hyaline and smooth, few with orientations and thick walled. The strain was identified as *Myceliophthora thermophila* by the Commonwealth Mycological Institute, Kew, Surrey, England. The genus *Myceliophthora* was reported first by Costantini\(^3\). It was described by Van Oorschot\(^12\) as *M. thermophila* having keratinolytic activity. But no report regarding cellulolytic activity has yet appeared. It is for the first time that we are reporting that this organism possesses cellulose decomposing activity. To give the separate identity, the organism was named as *M. thermophila* D-14.

One ml of conidial suspension (10 \(\times\) 10\(^6\)/ml) of *M. thermophila* D-14 was inoculated with 25 ml CD medium, supplemented with 1% CMC in 100 ml Erlenmeyer flask and incubated for 15 days at 50\(^0\) C (as this temperature showed maximum enzyme production). The pH of the medium was kept constant at 5·5 by adding 4N HCl or 4N NaOH as needed. The readings were taken on every alternate day of incubation from 3rd to 15th day. The culture was centrifuged at 1000 g for 10 min and the supernatant (culture filtrate) thus obtained was used as the source of extracellular enzymes.

The CMCase assay was done following the method of Berggren and Petersson\(^9\) with little modifications. Half a millilitre of culture filtrate was incubated with 30 mg of CMC (Sigma Chemical Co., USA) in 0·01 M Na-acetate buffer of pH 4·8 at 70\(^0\) C for 1 hr. The final volume of the reaction mixture was adjusted to

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CELLULOLYTIC ACTIVITY OF *MYCELIOPHTHORA THERMOPIHLA* D-14

**Sridip Sen, T. K. Abraham and S. L. Chakrabarty**

Department of Microbiology, Bose Institute, Pashupata Chandra Road, Calcutta 700 009, India

Quite a large number of fungi are known to produce extracellular cellulase. Some of these are *Trichoderma viride*\(^1\), *Pencillium funiculosum*\(^2\), *Sporotrichum pulverulentum*\(^3\), *Fusarium solani*\(^4\), *Myrothecium verrucaria*\(^5\), *Trichoderma koningii*\(^6\). Recently, search for new cellulase-producing organisms has received much attention for the production of glucose from cellulose materials. As a result, a few more cellulolytic fungi, such as, *Sporotrichum thermophilum*\(^7\) and *Chaetomium cellulolyticum*\(^8\) are reported. It is also well demonstrated that decomposition of cellulosic wastes occur maximum at higher temperatures\(^6\) and hence thermophilic strains play an important role in cellulose decomposition in nature. Based on this idea, the present investigation deals with the characterization and cellulolytic activity of a thermophilic strain, *Myceliophthora thermophila* D-14, reported for the first time as a cellulose decomposer.

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Fig. 1. Photomicrograph of a spore mother cell in *Cyathaea gigantea*, n = 69, \(\times 1100\).

in species of *Cyathaea* from different geographic regions would suggest that the present-day forms of this primitive genus are evolutionally static.

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