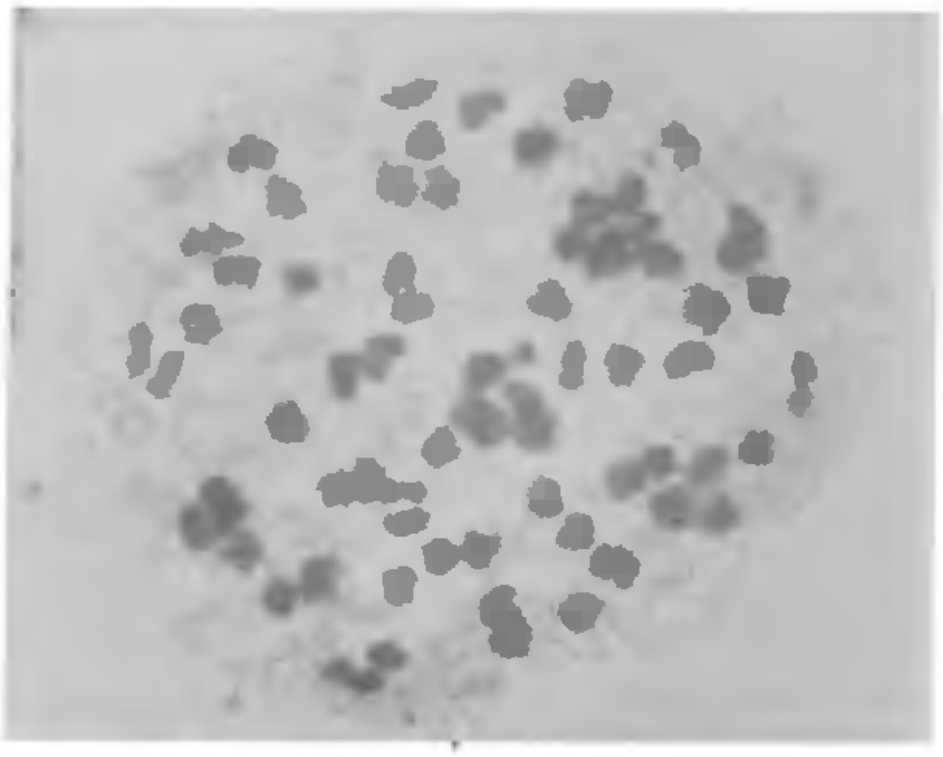

FIG. 1. Photomicrograph of a spore mother cell in *Cyathea gigantea*, $n \approx 69$, × 1100.

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

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CELLULOLYTIC ACTIVITY OF *MYCELIOPHTHORA THERMOPHILA* D-14

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QUITE a large number of fungi are known to produce extracellular cellulase. Some of these are *Trichoderma viride*[1], *Penicillium 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 cellulosic materials. As a result, a few more celluloytic fungi, such as, *Sporotrichum thermophila*[7] and *Chaetomium cellulolyticum*[8] are reported. It is also well demonstrated that decomposition of cellulosic wastes occur maximum at higher temperatures[9] 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 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° and 60° C, maximum growth however is attained at 45° C. In CD medium[10], 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 μm in diameter, bearing 1–6 or more blastoconidia on hyphal apical region (Figs. 1 and 2); conidia obovoid to pyriform, 4·2 to 7·0 μm × 3·5–4·5 μm; most of the conidia are hyaline and smooth, few with orientations and thick walled. The strain was identified as *Myceliophthora thermophyla* by the Commonwealth Mycological Institute, Kew, Surrey, England. The genus *Myceliophthora* was reported first by Costantin[11]. 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 (10×10^6/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° 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 Berghem and Pettersson[13] 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° C for 1 hr. The final volume of the reaction mixture was adjusted to

FIG. 1. Microscopic view of *Myceliophthora thermophila* D-14—young mycelial mat showing development of blastoconidia (× 400).

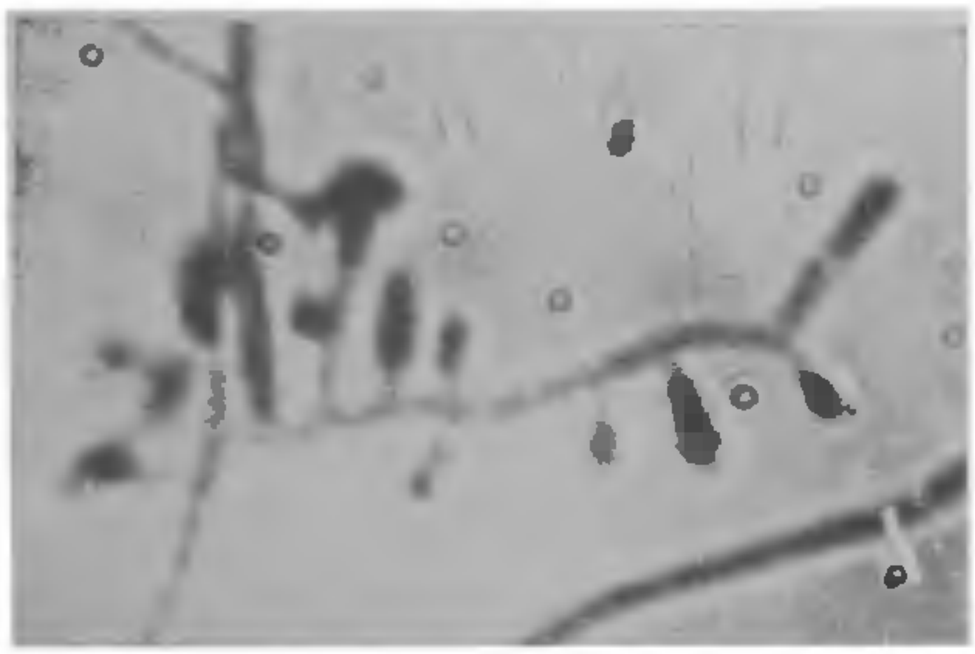

FIG. 2. *Myceliophthora thermophila* D-14—part of aerial hypha bearing blastoconidia (× 1000).

2 ml with the same buffer solution. The amount of reducing sugar produced was estimated by Nelson[14] and Somogyi[15] method taking glucose as standard.

The assay of *ß*-glucosidase was done according to the method of Eberhert *et al.*[16] 0·2 ml of culture filtrate was added to the reaction mixture containing $6{\cdot}29 \times 10^{-3}$ M of *p*-nitrophenol-*ß*-D-glucoside in 0·01 M Na-acetate buffer of pH 4·8. The final volume was adjusted to 2 ml with the same buffer solution and incubated at 60° C for 1 hr. After incubation 3 ml of 0·1 N NaOH was added. The enzyme activity was determined by calculating the *p*-nitrophenol released from the absorbance at 420 nm.

Determination of protein of the culture filtrate was done following the method of Lowry *et al.*[17], using bovine serum albumin as standard.

The results of the experiment are presented in Table I. It indicates that the enzyme production started from the 3rd day of incubation. The optimum production of CMCase was on the 11th day and then decreased gradually while that of *ß*-glucosidase increased upto 13th day and thereafter remained the same. The extracellular protein production was in continuous increase. It is evident from the above that the production of extracellular protein, though

TABLE I

Effect of incubation period on production of extracellular protein, cellulase (CMCase) and ß-glucosidase by M. thermophila D-14

Days of incubation	Extracellular protein[a]	CMCase activity[b]	Specific activity of CMCase[c]	ß-glucosidase activity[d]	Specific activity of ß-glucosidase[e]
3	44	1·25	2·63	1·08	0·40
5	85	2·10	2·29	2·90	0·56
7	105	3·48	3·06	3·30	0·52
9	178	3·56	1·85	4·00	0·37
11	210	3·80	1·67	5·00	0·39
13	248	3·46	1·29	5·07	0·34
15	274	3·40	1·15	5·07	0·31

[a] µg/ml culture filtrate.
[b] Reducing sugar produced in mg/ml culture filtrate.
[c] µ moles of reducing sugar as glucose/mg protein/min.
[d] µ moles of *p*-nitrophenol released/ml/hr.
[e] µ moles of *p*-nitrophenol/mg protein/min.

constantly increased, the CMCase and *ß*-glucosidase activity did not however increase. This might be due to the effect of long term incubation. In such a system, mycelial autolysis occurs and thus interferes with the actual enzyme production which is determined from the specific activity of the extracellular enzymes (CMCase and *ß*-glucosidase). The enzymes produced by *M. thermophila* D-14 also act on cotton and filter-paper which are the native forms of cellulose. Moreover, the organism is non-pathogenic and shows enough potency for the decomposition of native cellulosic materials. Thus the strain, reported in this investigation, can be better utilized for the conversion of huge cellulosic wastes into various useful substances of economic importance, such as, production of organic manure, glucose, ethanol, etc. Further work is however needed for increasing enzyme production by this organism in comparison to those of other high cellulase yielding cultures.

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PHOMA LUCKNOWENSIS SP. NOV. FROM INDIAN ALKALINE SOILS

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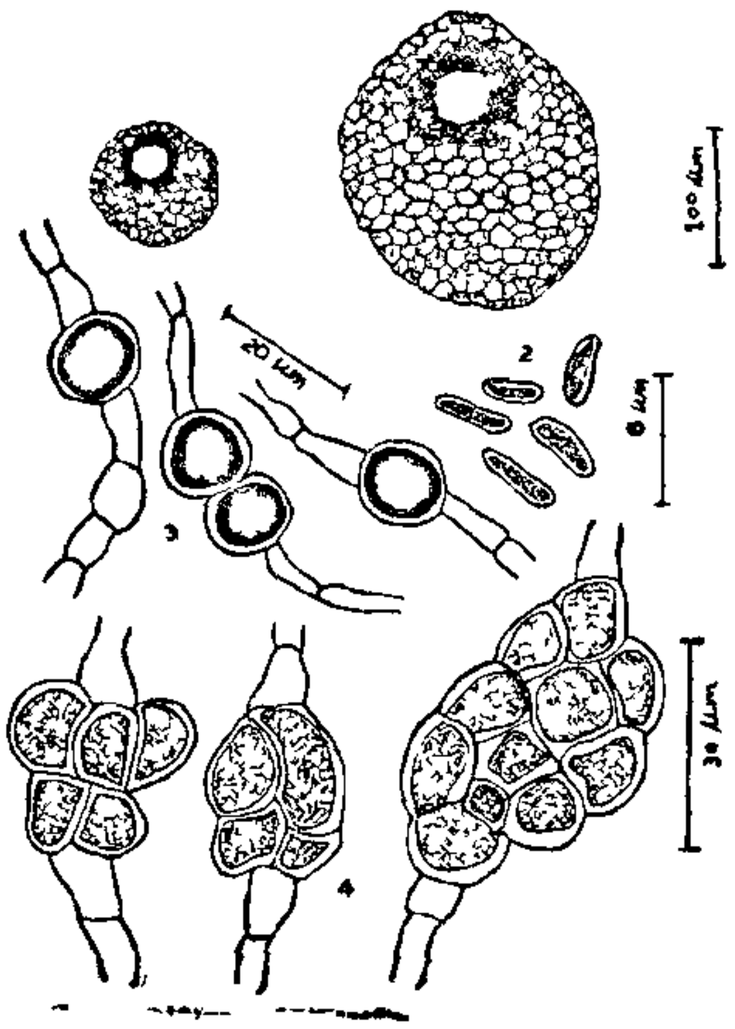
Phoma lucknowensis sp. nov.

HYPHAE hyalines vel leviter fuscescense, septatae bis 6·4 μm in diam. Pycnides brunneis vel nigris, globosa vel irregularia, non numerosa, 72·0–216·0 μm × 72·0–158·0 μm. Pycnidiosporae minutae, unicellularae, hyalinae, ellipsoideae, 3·1–4·6 μm × 1·5–2·0 μm. Dictyochlamydosporae fusco-nigrescens, muriformibus, intercalaribus, 24·0–64·0 μm × 16·0–48·0 μm. Chlamydosporae fusco-nigrescense, singularis, vulgo, 8·0–12·0 μm in diam.

Lectus mense julio ex solo usar (pH 8·0) and Hindnagar, Lucknow.

Colonies on potato-dextrose agar growing rather very slowy, forming a tough mycelial felt over the substratum, gradually deepening and in age becoming almost black, reverse and reverse agar dark greyish, exudates abundant in the form of colourless droplets. Hyphae hyaline to light brownish, septate upto 6·4 μm in width. Pycnidia produced in limited numbers after a few weeks, dark brown to black, globose to irregular, 72·0–216·0 μm × 72·0–158·0 μm. Pycnidiospores extruded in a mucilage droplet, small, unicellular, hyaline, ellipsoidal 3·1–4·6 μm × 1·5–2·0 μm. Dictyochlamydospores dark brown, muriform, produced in chains, measuring 24·0–64·0 μm × 16·0–48·0 μm. Chlamydospores dark brown, single, frequently produced in long chains and measuring 8·0–12·0 μm in diameter.



FIGS. 1–4. *Phoma lucknowensis* sp. nov. Fig. 1. Pycnidia. Fig. 2. Pycnidiospores. Fig. 3. Chlamydospores. Fig. 4. Dictyochlamydospores.

Isolated in July from Usar soils (pH 8·0) collected from Hindnagar, Lucknow. Type, in the form of dried culture, deposited in the Department of Botany, Lucknow University, Lucknow, India. A subculture has also been deposited in the Commonwealth Mycological Institute, Kew, England, as IMI 132164.

Of all the described species of the genus *Phoma*[1,2] *P. lucknowensis* sp. nov. comes closer to *P. eupyrena* but clearly differs in the size of pycnidia and pycnidiospores and the presence of dictyo-chlamydospores which is characteristic of this form.

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