Beyond 20th day, the process of regeneration sets in[6] which is well characterized in the present study by a progressive fall in lytic enzyme concentrations. Elevated levels of hydrolytic enzymes have also been reported in a number of muscle diseases including those of neuromuscular disorders. Alkaline phosphatase has been characteristically reported to show a proportionate elevation with the onset and progression of fibrosis during neuromuscular disorders[9,11]. Acid hydrolases also exhibit increase in their levels in diseased muscle[8,10]. The progressive morphological distintegration of muscle fibers following xylotox administration as such, results from stimulated levels of both acid and alkaline phosphatases. Phosphatases thus serve as biochemical markers of myonecrosis following local anesthetic administration. The increased levels of lytic enzymes result from accumulated macrophages and polymorphonuclear leukocytes in the affected muscle fibers. These cells contain lytic enzymes in them. It can be concluded that synthesis and subsequent accumulation of both acid and alkaline phosphatases act as a prelude to myofibrillar degeneration following local anesthetic injections. Regeneration finally ensures when the lytic enzymes have returned or are tending to return to the normal value.

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PRODUCTION OF CELLULASES FROM *CURVULARIA LUNATA**

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RECENTLY much attention has been paid to the utilization of cellulosic materials as renewable resources of energy, chemicals and food. Most of the studies are concerned with enzymatic hydrolysis of the polysaccharide to glucose[1]. Cellulolytic enzymes produced by the members of the *Trichoderma* have been used extensively in these studies, because they produce the most potent enzyme system. In our pursuit to isolate cellulytic fungi from soil, we have assayed several fungi for cellulases, of which *Curvularia lunata*, a potent cellulase producing organism, has been reported here.

C. lunata isolated from soil, was maintained on PDA slants. Production of enzymes was studied in 500 ml Erlenmeyer flasks containing 250 ml of modified basic salts medium[2] containing 50 g of substrate (cellulose, lactose and glucose) per litre at pH 5. The medium was inoculated from one-week-old PDA slants and incubated on a rotary shaker at room temperature (28–30°C). After eight days, the cultures were filtered and the filtrate analysed for enzyme activity.

Mycelial dry weight was determined by filtering the samples through preweighed filter papers, rinsing thrice with water, and drying at 80°C for 24 hr. Enzyme activities, such as filter paper activity (FPA), carboxymethylcellulase (sodium salt) activity and cellobiase activity were measured by the methods described earlier[3]. Arsenomolybdate method[4] was used to estimate the substrate level as well as liberated reducing sugars in terms of glucose. Activities are expressed in International Units (IU) as micromoles of equivalent glucose produced per ml per min. Extracellular protein was estimated by Lowry's[5] method without precipitation, using bovine serum albumin as the standard.

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Table 1 *Production of cellulases from C. lunata*

Substrate	Biomass g/l	FPase[a] IU/ml	CMcase[b] IU/ml	Cellobiase IU/ml	Protein mg/ml	Specific activity[c]	Level of substrate[d]
Cellulose	0.65	0.02	0.06	0.04	0.06	0.33	64
Lactose	3.25	0.21	1.27	0.17	0.18	1.17	800
Glucose	4.71	0.22	1.23	0.18	0.31	0.71	816

[a], filter paper activity; [b], carboxymethyl cellulase activity; [c], filter paper units/mg of protein, units/mg; [d], estimated in terms of glucose, μg/ml.

Production of three different cellulase components from the eight-day-old cultures grown in three different substrates is shown in table 1. Among the three cellulases, carboxymethyl cellulase was higher than the other two enzyme systems. The cellulase system was poor in cultures grown on cellulose. The specific activity in terms of filter paper units produced per mg of protein was greater in lactose than in glucose, though glucose supported more biomass. It is interesting to note that cellulases are induced even in the presence of substrate (glucose). Lactose and glucose have been established as good inducers of cellulase by many workers[6] and lactose as the good productive substrate in specific enzyme (protein) productivity[7]. Further improvements in cellulase production are in progress.

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OCCURRENCE OF URSOLIC ACID AND RELATED COMPOUNDS IN *EUCALYPTUS* HYBRID LEAVES

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EUCALYPTUS hybrid (Mysore gum, mainly *E. tereticornis*) is extensively grown in India under the social forestry programme due to its high biomass yield in a short span of time. The wood is mainly used for paper and pulp while the leaves give an essential oil consisting of cineole, pinenes and other monoterpenes[1]. Since there is no report on the other chemical constitutents of the leaves, the present investigation was taken up.

The leaves (400 g) were extracted with petroleum ether, acetone and alcohol respectively. Column chromatography of the acetone extract over silica gel gave three pure compounds A, B and C.

Compound A (800 mg) crystallized from chloroform-methanol as colourless needles m.p. 255–56°, acetate m.p. 260–62°. It was identified as ursolic acid lactone[2] by NMR, MS and direct comparison with an authentic sample (m.m.p., Co-TLC and superimposable IR spectra).

Compound B (4.800 g) crystallized from chloroform methanol as white needles, m.p. 291–92°, acetate m.p. 285–86°, $[\alpha]_D$ + 58.5° (chloroform), methyl ester acetate m.p. 232–33° and gave + ve L.B. test (pink → blue). It was characterized as ursolic acid[3] by NMR, MS and by direct comparison with an authentic sample (m.m.p., Co-TLC and superimposable IR spectra).

Compound C (400 mg) crystallized from methanol as white needles, m.p. 243–44°. It formed diacetate, m.p. 196–97°, methyl ester m.p. 208–209°, $[\alpha]_D$ + 48° (chloroform) and methyl ester diacetate m.p. 144–45°. Its identity was confirmed as 2-α-hydroxy ursolic acid[4] by NMR studies of the methylester diacetate and direct comparison with an