MICROBIAL ATTACK ON CELLULOSIC MATERIALS FROM GROUNDNUT CAKE

Of late, there has been a considerable amount of interest in the microbial cellulolytic activities as seen from the recent symposium.[1] The cellulolytic activity is sought to be exploited for the production of single cell protein. The feasibility of this was shown by Srinivasan and Han[2] with a *Cellulomonas* species with bagasse as the cellulosic material and Han, Dunlap and Callihan[3] designed a pilot plant for the purpose. Besides, cellulases of fungal origin have been employed to develop a continuous saccharification process[4] and have been tried on cellulosic materials of paddy hulls[5]. Oil-seed cakes, particularly groundnut cake, contains from 4–7% cellulosic material in the form of crude fibre. In the present studies, an attempt was made to screen microbes having preference for hydrolysis of this material with the objectives of facilitating extraction of proteins, enrichment of the cake with microbial protein at the expense of cellulose, production of single cell-protein and destruction of aflatoxin.

A number of soil samples were screened for microbial cultures by the enrichment culture technique in Czapek's medium using groundnut cake cellulose obtained by acid and alkali digestion of the cake as a sole carbon source at a level of 1%. A culture indicating cellulolysis of the substrate from the dinitro salicylic acid (DNS) positive reaction[6] was identified as a *Streptomyces albus* species according to the procedures described by Waksman.[7] Growth studies were carried out in 50 ml aliquots of the medium containing 1% cellulose in 250 ml Erlenmeyer flasks both under stationary and shake flask cultures on a rotary shaker rotating at 200 r.p.m. In view of the insoluble nature of the substrate, growth was followed by measurement of cell-protein.[8] Growth under aeration was better as seen from the protein values of 39·8 mg/50 ml culture as compared to 6·30 mg/50 ml culture under stationary condition for a 7-day fermentation cycle. Results on the utilization of different carbon sources (Table I) show a clear preference for the groundnut cake cellulose amongst the cellulosic materials tried though the organism could utilize glucose as well. Comparative studies on the utilization of various nitrogen sources at a level of 200 mg % (Table II) show preference for organic nitrogen, peptone being the most effective one. This is seen from values of cell-protein as well as reducing sugars. The results obtained demonstrate the cellulolytic potential in the organism. Patni[9,10] has isolated a number of *Streptomyces* species having cellulolytic activities and has reported extensive studies on *Streptomyces caespitosus*. His collection does not mention *Streptomyces albus* nor were his organisms tried on cellulosic materials from seed cakes. Fermentation of groundnut cake slurry containing 70% water with *S. albus* species gave an increase in protein upto 5% in a period of 3 days. This compares favourably with 3·25% enrichment with protein of cassava cakes obtained by Stanton and Wallbridge[11] using *Rhizopus* species.

TABLE I

Utilization of carbon sources by S. albus *species*

Carbon source		Cell protein (mg/50 ml culture)
Glucose	..	35·0
Sucrose	..	30·0
Groundnut cake cellulose	..	33·3
Carboxy methyl cellulose	..	25·3
Insoluble cellulose powder	..	22·2

72-hour shake flask cultures at room temperature.

TABLE II

Growth of S. albus *species with different nitrogen sources*

Nitrogen source		Reducing sugar (mg/50 ml culture)	Growth (mg protein/ 50 ml culture)
Peptone	..	4·72	44·7
Yeast extract	..	1·63	41·1
$NaNO_3$	..	1·63	35·2
$(NH_4)_2 HPO_4$	..	1·30	32·3

72-hour shake flask cultures at room temperature.

The studies reveal the possibility of preparing cellulolytic enzymes having a degree of specificity for cellulosic materials from seed proteins. In this connection Ghose and Haldar[12] have reported better extraction of protein from Khesari and Gram plants on treatment with fungal cellulase. Preliminary studies on hand also reveal that *Streptomyces albus* could attack the cellulosic materials from mustard and linseed cakes as well. The possibility of enriching the cakes with mycelial proteins is being worked out in great detail. This could have importance in feed if supported heavily with careful nutritional evaluation and solutions of bioengineering problems associated with the fermentation.

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DERIVATIVES OF BENZOXAZOL-2-YL-ACRYLIC ACID

MUSCLE relaxant and antiinflammatory properties have been reported for derivatives of cinnamic acid amides[1] and benzoxazole[2]. This prompted us to study the pharmacological properties of the derivatives of benzoxazol-2-yl-acrylic acid reported in this communication.

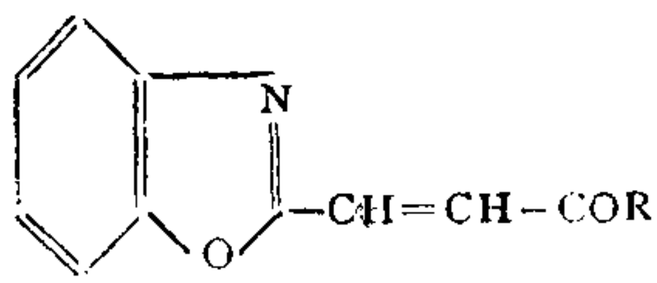
β-(Benzoxazol-2-yl)-acrylic acid was synthesised by reported methods[3]. It was then condensed with various amines in pyridine in presence of phosphorus trichloride to obtain the compounds listed in Table I. They were screened for hypnotic, anticonvulsant, analgesic and antiinflammatory properties by reported methods[4], using methaqualone, phenobarbital and dilantin sodium, aspirin and butazolidine respectively as standards.

Compound 1 showed analgetic potency equivalent to that of aspirin in mice at 200 mg/kg dose level; compound 12 showed antiinflammatory properties at 400 mg/kg dose level equivalent to those of butazolidine (at 100 mg/kg dose level) in rats. The rest of the compounds showed no significant pharmacological properties.

β-(Benzoxazol-2-yl)-acrylic acid anilide.—β-(Benzoxazol-2-yl)-acrylic acid (1·9 g; 0·01 mole) and aniline (0·93 g; 0·01 mole) were taken in dry pyridine (10 ml). Phosphorus trichloride (0·5 ml) was added to the above reaction mixture at 5° C. It was then stirred for 1 hour at 80° C. The reaction mixture was cooled and acidified with dilute hydrochloric acid (40 ml; 4 N). The solid obtained was filtered and triturated with sodium bicarbonate solution. The insoluble solid residue was crystallised from benzene-hexane to obtain the title product in 55% yield.

The rest of the compounds in Table I were similarly prepared.

TABLE I



No.	R	m.p. °C*	Nitrogen % Found	Nitrogen % Calc.
1	Anilino	.. 198–200	10·56	10·60
2	*o*-Toluidino	.. 183–84	9·82	10·07
3	*m*-Toluidino	.. 172–74	9·76	10·07
4	*p*-Toluidino	.. 174–75	9·89	10·07
5	*o*-Anisidino	.. 122–23	9·47	9·53
6	*m*-Anisidino	.. 86–88	9·35	9·53
7	*p*-Anisidino	.. 177–78	9·61	9·53
8	*o*-Chloroanilino	.. 156–57	9·29	9·38
9	*m*-Chloroanilino	.. 161–62	9·22	9·38
10	*p*-Chloroanilino	.. 248–49	9·31	9·38
11	*m*-Trifluoromethylanilino	128–29	8·26	8·44
12	3-Pyridylamino	.. 246–47	15·96	15·85
13	*p*-Acetamidophenoxy	.. 225–26	8·43	8·68

* All melting points are uncorrected.

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