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PRELIMINARY OBSERVATIONS ON THE PRODUCTION OF AMYLOGLUCOSIDASE BY THERMOPHILIC FUNGI

KERR *et al* (1941) and Underkofler (1953) among others recognised that enzymes of amyloglucosidase type were present in preparations which produced fermentable sugars from starch. Philips and Caldwell (1951), Arima (1964), Nehira and Nomi (1956), Aschengreen (1969), Underkofler (1969), Mangallam (1973) and others studied the production of amyloglucosidase by species of *Aspergilli* and *Rhizopus*. Thus, in literature several reports of fungi producing this enzyme are available but none deals with thermophilic fungi. Therefore, a preliminary investigation on the production of amyloglucosidase by thermophilic fungi was carried out, with the hope to isolate a thermostable enzyme.

Four thermophilic fungi *viz.*, *Torula thermophila* Apinis; *Mucor miehe* Cooney and Emerson; *Humicola lanuginosa* (Griffon and Maublanc) Bunce; *Sporotrichum thermophile* Cooney and Emerson, which are unable to grow below 25° C and grow well above 50° C, thus satisfying the definition of Cooney and Emerson (1964) were screened for amyloglucosidase activity in the following manner.

100 ml. of the seed medium of the following composition (Tapioca starch 1%, CSL 1%, NH_4NO_3 0·25%, KH_2PO_4 0·2%, $MgSO_4$ 0·1%, pH 6) was distributed in 500 ml. Erlenmeyer flasks, plugged with non-absorbent cotton and sterilised for 45 mts., at 120° C and 15 lb./sq. in. pressure. After cooling they were inoculated with 2·5 ml. fungal spore suspension prepared from five-day old, heavily sporulating cultures grown on potato dextrose agar slants. They were incubated at 50° C for 48 hrs. on a rotary shaker with 280 r.p.m. and 2" throw.

Production of enzyme: Sterilized 500 ml. Erlenmeyer flasks containing 100 ml. production medium (Composition: Tapioca starch 2·5%, Peanut meal 2%, ammonium nitrate 0·5%, KH_2PO_4 0·25%, K_2HPO_4 0·2%, $MgSO_4$ 0·1%, pH 6·5) were inoculated with 2·5 ml. seed. The mold was grown for 96 hrs. At the end of the fermentation cycle the mycelium of each species was separately harvested and filtered. The culture filtrate was assayed and quantitative estimation of the enzyme in terms of glucose was carried out by Hane's method (1947). The conversion of starch to glucose by the enzyme was first identified by preparing glucosazone with phenyl hydrazine and later confirmed by paper chromatography. The enzyme is found to be stable for 60 mts. at 60° C. Mean of triplicate results are given in Table I.

TABLE I

Amyloglucosidase production by thermophilic fungi

Sl. No.	Organism	Temp. (° C)	Age (Hrs)	pH	Filtrate activity* μ/ml.
1.	*Terula thermophila*	50	96	7·0	195·14
2.	*Mucor miehe*	50	96	6·7	102·7
3.	*Humicola lanuginosa*	50	96	7·0	75·6
4.	*Sporotrichum thermophile*	50	96	6·7	149·4

* One amyloglucosidase unit is defined as the amount of enzyme releasing 1 mg. of glucose in one hour from a 4% starch solution at pH 4·5 at 60° C.

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