Indian J. mycol. Res. 19 *(1), 21—26 (1981)*

PRODUCTION OF DEXTRANASE BY *ASPERGILLUS NIGER*

BY

PONTY ROY AND ARATI DAS

Department of Microbiology, Bose Institute, Calcutta-700 009

A survey of seventy fungal isolates revealed maximum dextranase activity in a strain of *Aspergillus niger*. Its cultural characters were studied for optimal dextranase production. Incubation for 11 days in a culture medium at initial pH 8.0 was recorded optimal for the strain. The dextranase was judged to be an inducible enzyme system.

INTRODUCTION

Dextranase is an inducible enzyme capable of hydrolysing ∝-(1-6) glucosidic linkages of dextran. Its importance in various industries has led to intensive studies of microorganism for commercial production of the enzyme. Microbial dextranases are produced mostly by fungi belonging to *Aspergillus, Penicillium, Verticillum, Spicaria* and *Fusarium,* (Nordstrom and Hultin, 1948 ; Tsuchiya *et. al.,* 1952 ; Whiteside-Carlson and Carlson, 1952 ; Fukumoto *et. al.,* 1971 ; Tsuru *et. al.,* 1971 ; Dasmohapatra and Thangamoni, 1974 ; Simonson and Liberta, 1975). However, it must be admitted that in the field of industrial microbiology there is a considerable scope for developing more useful strains showing higher dextranase activity. An investigation was therefore undertaken whereby 70 fungal isolates were surveyed to select a good dextranase producing strain. The best strain was found to be *Aspergillus niger* which had not yet been reported. Cultural factors affecting dextranase production by the strain had been studied and some interesting results were observed as presented in this paper.

MATERIALS AND METHODS

Microorganism and Media

Screening tests were performed with 70 fungal isolates belonging to Ascomycetes, Basidiomycetes and Deuteromycetes obtained from the culture stock of this laboratory. Dextran-Pepton-Yeast extract medium (DPY) was used for screening and dextranase production. Its composition was (g/1) : Dextran (Rallis India Ltd.) 10, peptone 10 and Yeast extract 20, pH 7.0. The medium was solidified with 2% Agar.

Screening

The cultures were grown in DPY medium in petridishes for 5 days at 30°C, and then flooded with 95% ethanol (Simonson *et. al.* 1972). A clear zone appeared around the cultures which hydrolysed dextran while a white precipitate was formed in the nondegraded dextran portion. The enzyme activity was studied qualitatively by dividing the diameter of the clear zone by that of the colony.

Culture Methods

Experiments were conducted under stationary culture condition in 250 ml. Erlenemeyer flasks containing 50 ml DPY medium which was inoculated with 2 ml spore suspension (5-10 x 10^6/ml.). All experiments were set up in triplicates and the average of the readings were recorded. Incubations were always carried out at 30°C unless mentioned otherwise.

In studying the effects of carbon sources on dextranase production, DPY broth lacking dextran was used as the basal medium. The different carbohydrates were incorporated into this broth.

Following incubation, contents of each flask were filtered through weighed filter papers and mycelial dry weights were taken by drying overnight at 80°C. The filtrates were assayed for enzyme production.

Enzyme Assay

Dextranase activity of the culture filtrate was assayed in a reaction mixture containing 1 ml of the appropriately diluted filtrate and 4 ml of a 2% (W/V) dextran (Sigma, St. Louis, U. S. A. Mol. Wt. 80700) solution in 0.1M phosphate buffer (pH 6.0). This was incubated at 37°C for 2 hours and total reducing sugars (R. S.) released therein were determined by Clarks' method (1964). The original concentration of reducing sugars present in the filtrate before enzyme assay was also determined similarly by using a heat inactivated (15 min at 80°C) enzyme solution (filtrate). The difference in R. S. values between the two readings represents the actual R. S. concentration produced in the reaction mixture by dextranase activity. One unit of dextranase was defined as that amount of enzyme required to release from the dextran substrate 1 mg of reducing sugars (calculated as isomaltose, measured as maltose).

RESULTS AND DISCUSSION

Selection of the strain

All the isolates were grown in petridishes at 30°C for 5 days and then the plates were flooded with ethanol. On the basis of zone diameter 10 most active strains were selected and these were grown in DPY medium for 14 days for quantitative estimation of dextranase production. The best strain was

found to be a strain of *Aspergillus niger* with dextranase yield of 20.5 units/ml. So this strain was selected for further studies.

Table 1. *Effects of time course on Dextranase production*

Days of incubation	Dextranase production (Units/ml.)	Biomass (mg/flask)
2	2.0	150
4	8.0	750
6	12.5	850
8	13.9	890
9	21.0	950
10	28.3	1560
11	28.5	1665
12	30.1	1656
13	30.0	1600
14	29.0	1610

Time course of dextranase production

The strain was next grown in DPY medium at 30°C and dextranase production was studied following incubation for different periods. It is apparent from the Table 1 that production was maximum (30.1 units/ml) on 12th day of incubation, followed by a decline in subsequent days. Mycelial growth also increased gradually up to 12 days of incubation. So the amount of dextranase production by the strains seems to be related to its mycelial growth. Similar observations were reported in *Aspergillus luchuensis* (Joshi & Tamhane, 1975) where enzyme production was maximum on the day of maximum mycelial growth. On the other hand in *Penicillium janthinellum* (Dasmohapatra & Thangamani, 1974) dextranase production continued to increase in spite of a decline in mycelial growth. However in view of optimal enzyme production in 12 days, in all subsequent experiments fermentation was carried out for this period.

Effects of different carbon sources

Effects of carbon sources were studied by using starch, sucrose, maltose, dextran, lactose and glucose. These were incorporated into the basal DPY medium lacking dextran and the carbohydrates were each added in 2% concentration. The flasks were incubated for 12 days at 30°C when good mycelial growth occured in all cases (Table 2). But dextranase produced only in

Table 2. *Effects of different carbon sources on Dextranase production*

Substrate 2%	Dextranase activity (units/ml)	Biomass (mg/flask)
Sucrose	0	—
Maltose	0	—
Glucose	0	—
Lactose	0	—
Glucose & Dextran	5.5	850
Dextran	30.0	1445
Starch	0	—

presence of dextran and this indicates inducible nature of the enzyme system. In medium containing glucose and dextran enzyme production was appreciably reduced. It appears that glucose was able to counteract dextranase induction by dextran, showing examples of catabolic repression.

Effects of temperature on dextranase production

Fermentation was set up at different temperatures and after 12 days of incubation culture filtrates were assayed. The enzyme production was maximum (Table 3) during incubation at 30°C. This is in contrast to the report of Tsuru *et. al.* (1971), who observed 25°C optimal temperature for dextranase production by *Aspergillus carneus*.

Table 3. *Effects of temperature on Dextranase production by A. niger*

Incubation temp. (°C)	Dextranase production (units/ml)	Biomass (mg/flask)
25	20.8	890
30	30.5	1550
37	12.1	1260
40	10.1	720

Effects of pH on production and activity of dextranase

In order to study the effects of initial cultural pH on enzyme production the strain was grown for 12 days at 30°C in DPY medium with initial pH adjusted to different levels (6.0-8.0) by adding 0.1M phosphate buffer. The enzyme production was maximum in medium adjusted to initial pH 7.0 (Table 4)

Table 4. *Effects of pH on production of Dextranase*

Initial pH	Final pH	Dextranase activity (Units/ml)	Biomass (mg/flask)
5.0	2.4	0.4	370
5.5	2.5	0.6	505
6.0	2.7	2.9	890
6.5	2.7	30.2	1020
7.0	4.0	40.1	1550
7.5	4.0	26.1	885
8 0	6 0	16.0	500

Table 5. *Effects of pH on activity of Dextranase*

pH of the reaction mixture	Dextranase activity (units/ml)
5.0	10.9
5.5	20.0
6.0	40.5
6.5	75.5
7.0	130.2
7.5	70.6
8.0	10.5

In order to study the pH optima for dextranase activity in this culture filtrate, assay was again carried out by varying the pH of phosphate buffer in the reaction mixture. Activity was satisfactory in the pH range of 6.0-7.5 with maximum at 7.0 (Table 5). Beyond the level, the activity reduced sharply. The observation agrees with the report of Davies (1963) that most exoenzymes are produced in high amounts at growth pH near the pH level of its optimal activity. Regarding initial cultural pH for dextranase production, the same optimal pH 7.0 was also recorded for *Fusarium moniliformis* (Simonson & Liberta, 1975). But there are several other reports of varying pH optima for maximum production of dextranase. These are, pH 8.0-8.5 for *A. carneus* (Tsuru *et. al.*, 1971) ; 7.0-8.0 for *Penicillium liliacinum* (Tsuchiya *et. al.* 1952), 6.0 for *Penicillium funiculosum* (Fukumoto *et. al.*, 1971) ; 5.0 for *Penicillum verruculosum* (Tsuchiya *et. al*, 1952) ; 4.0 for *P. liliacinum, P. funiculosum* and *Verticillium coccorum* (Hultin & Nordstrom, 1949) ; and 2.5-6.5 for *A. luchuensis* (Joshi & Tamhane, 1975). All these contrasting reports may result from differences (a) in experimental conditions or (b) in molecular properties of dextranase. A striking example of experimental conditions affecting dextranase

production has been provided by Tsuchiya *et. al.* (1952). They observed strong exodextranase activity of the same strains of Penicillia which were earlier reported to be weak producers of enzyme (Hultin & Nordstrom, 1948,). Again, differences in strain specificity may also account for different observations on dextranase activity, as reported by Tsuchiya *et. al.* in *P. funiculosum*. The strain *Penicillim funiculosum* NRRL 1132 produced maximum dextranase at pH 8.0 (Tsuchiya *et. al.* 1952) while for another strain of *P. funiculosum*, dextranase production was maximum at pH 6.0 (Fukumoto *et. al.* 1971).

In view of all these observations it is worth while to isolate and study the properties of dextranase produced by the strain under study. Further work is in progress in this direction.

ACKNOWLEDGEMENT

We are grateful to Dr. S. K. Bhattacharyya and Prof. A. K. Mishra for their encouraging support to this work. Mr. B. B. Sarkar, Managing Director, Rallis India Ltd., for the gift of dextran used in this investigation. Sincere thanks are due to C. S. I. R., New Delhi, for providing financial help to one of us (P. R.).

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