KOR. JOUR. MICROBIOL. Vol. 18 No.4, 188-192, 1980

Studies on Mold Dextranases

1. Dextranase Production by a Strain of *Aspergillus ustus*

Kon Joo, LEE

(Dae Han Choong-Wae Pharm.)

Mold Dextranases에 關한 硏究

1. *Aspergillus ustus* 의 Dextranase生産에 관하여

李　　建　　柱

(大韓中外製藥株式會社)

ABSTRACT

In search of dextran-hydrolyzing enzymes, approximately 500 strains of molds were checked for their ability to produce extracellular dextranase. Seven strains capable of producing dextranase were screened, and among them, one strain belonging to *Aspergillus* genus showed greater activity than the other. The strain was identified to be *Aspergillus ustus* and the most suitable culture conditions for the enzyme production were determined.

INTRODUCTION

Enzymes capable of degrading dextran are potentially valuable for fundamental research as well as for practical application.

Recently, many experiments have been made on the inhibition of dental caries and plaques by hydrolysis of extracellular polysaccharide with dextranase(Guggenheim and Schroder, 1968; Caldwell *et al.*, 1971; Keys *et al.*, 1971; Murakami *et al.*, 1971; Numan *et al.*, 1972; Staat and Schachtele, 1974; Schachtele and Staat, 1975).

Several molds and bacteria have been reported to produce extracellular dextranases (EC 3, 2, 1, 11) when grown in media containing dextran as the sole source of carbon.

Reports have been made on dextranase originating from *Penicillum*(Pen.), *Spicaria*, *Aspergillus*(*Asp.*)(Tsuchiya *et al*, 1952), *Bacteroides*(Sery and Hehre, 1956; Staat and Schachtele, 1976), *Lactobacillus bifidus*(Bailey and Clarke, 1959), *Cytophaga*(Janson and Porath, 1966), *Fusobacterium*(daCosta *et al.*, 1974), *Streptococcus mutans*(Guggenheim and Burckhardt, 1974).

Tsuchila *et al.*(1952) have reported that

Pen. lilacinum, *Pen. funiculosum*, *Pen. verruculosum*, *Asp. niger*, *and Spicaria* produced considerable amounts of extracellular dextranase in submerged culture.

Miraoka *et al.*(1972) have reported that *Asp. carneus* shows good dextranase producing ability.

In the present paper, molds producing dextranase were screened and identified the strain, and the culture conditions suitable for the enzyme production determined.

MATERIALS AND METHODS

1. Experimental materials

One hundred strains of molds received from Dept. of Biology, Kon Kuk University and approximately 400 strains were isolated from decayed leaves, soils, and food. These strains were incuvated on selective medium.

2. Detecting of dextran-splitting molds

The molds were grown on selective medium at 30°C for 5 days forming a clear zone around the colony several hours after the addition of ethanol(99.5%) to the plate were considered to be the dextran-splitting molds (Matsuda, 1976). The selective medium containing 1% dextran(M.W. 70,000, Meito Sangyo Co.), 0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.1% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, and 2% agar.

3. Identification of strains

The selected 7 strains of mold were incubated on Czapek's solution agar, Malt extract agar at room temperature for 12 days to 2 weeks, and were observed colony morphology and characters under the microscope. Taxonomical studies were based upon examinations of morphological and cultural characteristics by Raper and Fennels method(1965) and Raper and Thom method(1945).

4. Enzyme activity

The mold was grown in 100ml of culture on a reciprocating shaker. The medium used for the propagation of the mold and the produciton of the enzyme contained dextran and yeast extract(Table 1). After incubation, the mycelium was removed by filteration and the culture liquors assayed.

Table 1. Chemical composition of media

Dextran	10g	$FeSO_4 \cdot 7H_2O$	0.001g
$NaNO_3$	2g	Yeast extract	5g
K_2HPO_4	1g	Malt extract	5g
$MgSO_4 \cdot 7H_2O$	0.5g	pH 7 D.W.	1L
KCl	0.5g		

To determine dextranase activity, 5ml of culture filtrates were adjusted to about pH5.1 and added to 10ml of acetate buffered (pH 5.1) dextran solution(2.5%). The mixtures were held at 40 °C for 10 minutes at which time enzyme action was stopped by acidification with sulfuric acid. Reducing power was measured by the method of Somoyi (1945).

Table 2. Chemical composition of media and factors (per l)

	$NaNO_3$	2g		K_2HPO_4	1g
Basal Medium	KCl	0.5g		$FeSO_4 \cdot 7H_2O$	0.001g
	$MgSO_4 \cdot 7H_2O$	0.5g		Malt extract	5g
Dextran concentration	0.25%	0.5%		1.0%	2.0%
Yeast extract concentration	0.5%	1.0%		2.0%	5.0%
Effect of temperature	20°C	24°C		28°C	32°C
Effect of initial pH	5	6	7	8	9

Table 3. Morphology of *Penicillium sp.*

		A	B	C	D	E
Conidial stage	*Penicili* Color	Biv.-As. Dull green	Monov. Blue green	Biv.-S Pinkish brown	As.-D. Brownish white	As.-Ve. Green
Condiophore	Origin Marking	Basal Smooth	Basal Smooth	Basal Smooth	Basal Smooth	Basal Rough

Biv. : Biverticillata As. : Asymmetric D. : Divaricata Monov. : Monoverticillata
S. : Symmetrica Ve. : Velutina

One unit of dextranase activity was defined as the enzyme quantity which liberated reducing sugar equivalent to one μ mole of glucose per minute under the condition.

5. Cultural factors affecting dextranase production

Optimum temperature, dextran concentration, yeast extract concentration, and pH were examined(Table 2).

RESULTS AND DISCUSSION

1. Comparison of dextran-splitting ability among surveyed strains

Of 500 strains checked, only 7 strains were found to secrete dextranase. One of them belonged to genus *Aspergillus*, five of them to genus *Penicillium*(Table 3), and one was not identified.

A strain belonging to *Aspergillus* was most active in producing dextranase.

2. Identification of strain

Colonies on Czapek's solution agar at 12 days at room temperature(24~26°C) spreaded broadly, 5.0 to 6.0cm in diameter.

Sporuating abundantly from the basal mycelium and from aerial hyphae, exudate lacking, reverse in yellow shades. Conidial heads radiate, persistently gray from the yellow brownish conidiophores, mostly 100 to 120 μm in diameter.

Conidiophores mostly 600 to 800 μm long by 5 to 6 μm but commonly up to 2 to 3 mm in length when produced at margins of older colonies. Vesicles hemispherical to subglobose, 10 to 20 μm in diameter. Sterignata in two series, primaries 4.3 to 6.5 by 2.5 to 3.5 μm, secondaries 4.3 to 6.0 by 2.5 to 3.0 μm.

Conidia globose, 3.0 to 4.5 μm, roughened. Hülle cells are elongated and much twisted. Colonies on malt extract agar growing more rapidly attaining up to 7 cm in diameter in one week to 10 days, sporulating abundantly in slightly dark shade near gray.

Exudate lacking, reverse in dull olive-brown shades.

Hülle cells produced more abundantly than on Czapek's agar.

The strain was identified as *Aspergillus ustus*.

3. Cultural factors affecting dextranase production

The effect of varying the concentraion of dextrans and yeast extract is shown in Fig. 1 with *Asp. ustus*. 1% dextran and 2% yeast extract were the optimal nutrients levels(Fig. 1).

The effect of temperature of culture on production of the enzyme was investigated. As shown in Fig. 2, enzyme yields was highest at 28°C.

The enzyme production in media of various initial pH values is illustrated in Fig. 3. The optimum pH was 8.

The results indicated that 1% dextran and 2% yeast extract are good nutrients level

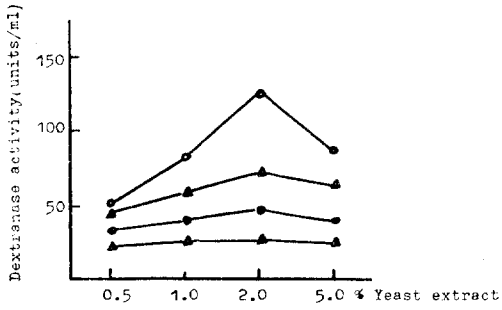

Fig. 1 Effect of yeast extract and dextran concentration on dextranase production
Concentration of dextran;
○ : 1.0% △ : 2.0% ● : 0.5% ▲ : 0.025%
(pH 7, temperature 28°C after 5 days)

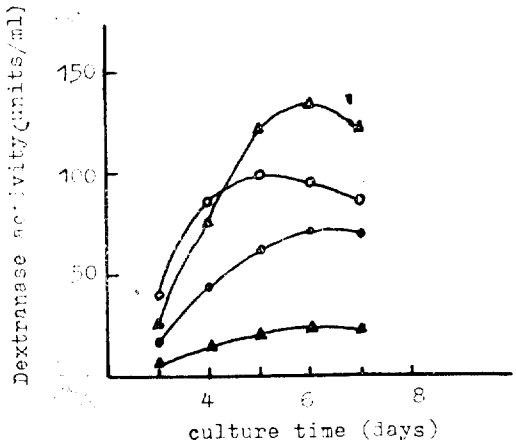

Fig. 2 Effect of temperature on dextranase production
Temperature; ▲ : 20°C ○ : 24°C △ : 28°C
● : 32°C
(pH 8, 1% dextran, 2% yeast extract)

and optimal temperature is 28°C and initial pH is 8. The time course of the enzyme production is illustrated in Fig. 4.

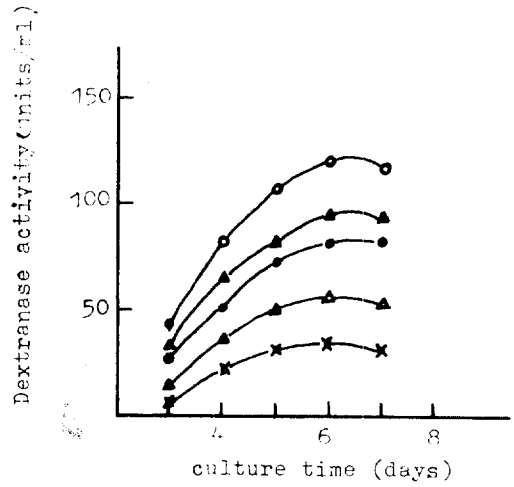

Fig. 3 Effect of initial pH of culture on dextranase production
pH × : 5 △ : 6 ▲ : 7 ○ : 8 ● : 9
(1% dextran, 2% yeast extract, 28 C)

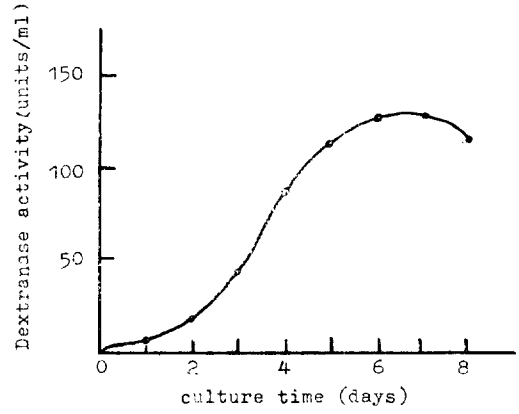

Fig. 4 Time course at optimal conditions on dextranse production

The author could obtained the culture broth showing 130 units dextranase per ml by cultivating the mold under the optimal condition(Fig. 4).

적 요

Dextran을 분해할 수 있는 molds를 선발할 목적으로 건국대학교 생물학과에 보관중인 100여 strain과 토양등에서 분리한 400여 strain에 대한 dextran분해능을 조사한 결과 7 strains가 dextran분해능을 나타냈다. 이들 균

주 중 5 strains는 *Penicillium* genus에 속하고, 1 strain은 *Aspergillus* genus에 속하며, 1 strain은 동정할 수 없었다. 7 strain중 분해능이 가장 양호한 *Aspergillus* genus에 속하는 균주를 동정한 결과 *Aspergillus ustus*였으며 dextranase를 생성하는 조건은 basal medium에 1% dextran과 2% yeast extract를 추가한 경우 양호하였고 배양온도는 28°C가 최적이였으며, initial pH는 8.0근처가 양호하였다. 최적조건하에서 6일후 *ml*당 약 130 units의 dextranase가 생성되었다.

REFERENCES

1. Bailey R.W. and Clarke T.I., 1959. A bacterial dextranase. *Biochem. J.* **72**, 49—54.
2. Caldwell, R.C., Sandham, I.H., Mann, Jr., W. V., Finn, S.B., and Formicola, A.J., 1971. The effect of a dextranase mouthwash on dental plaque in young adults and children. *J.A.D.A.* **82**, 132—135.
3. daCosta, T., Bire, L.C., and Gaida, F., 1974. Dextran hydrolysis by a *Fusobacterium* strain isolated from human dental plaque. *Archs. oral Biol.*, **19**, 341—342.
4. Guggenheim, B., and Burckhardt, J.J., 1974. Isolation and properties of a dextranase from *Streptococcus mutans* OMZ 176. *Helv. odont. Acta.* **18**, 101—113.
5. Guggenheim, B., and Schroeder, H.E., 1968. Biochemical and morphological aspects of extracellular polysaccharides produced by cariogenic *streptococci. Helv. odont. Acta.* 11, 131—152.
6. Hiraoka, N., Fukumoto, J., and D. Tsuru., 1972. Purification and some enzymatic properties of *Aspergillus carneus* dextranase. *J. Biochem* (Tokyo) **71**, 57—64.
7. Janson, J.C., and Porach, J., 1966. A bacterial dextranase. *Methods in enzymology.* **8**, 615—621.
8. Keys, P.H., Hicks, M.A., Goldman, B.M., Mc Cabe, R.M., and Fitzgerald, R.J., 1971. Dispersion of dextranous bacterial plaque on human teeth with dextranase. *J.A.D.A.* **82**, 136—141.
9. Matsuda, Y., 1976. Dextran degrading bacteria in human oral cavity and their activity against insoluble glucan from *Streptococcus mutans. Bull. Tokyo. Med. Med. Dent. Univ.* **23**, 27—40.
10. Murakami, Y., Tsuchimoto, M., Hayashi, H., Unhida, T., and Yokomizo, I., 1971. Experimental studies of the effect of dextranase on human dental plaque. *Japan J. Periodont.*, **13**, 68—73.
11. Numan, S., Lindhe, J., and Janson, J., 1972. The effect of a bacterial dextranase on dental plaque formation and gingivitis development. *Odont. Revy.*, **23**, 243—252.
12. Sery, T.W., and Hehre, D.G., 1956. Degradation of dextrans by enzymes of intestinal bacteria. *J. Bact.*, **71**, 373—380.
13. Somogyi, M., 1945. A new reagent for the determination of sugars. *J. biol. Chem.* **160**, 61—73.
14. Staat, R.H., Gawronski, T.H., and Schachtele, C.F., 1973. Detection and preliminary studies on dextranase-producting micorrganisms from human dental plaque. *Infect. Immunity*, **8**, 1009—1016.
15. Staat, R.H., and Schachtele, C.F. 1974. Evaluation of dextranase production by the cariogenic bacterium *Streptococcus mu tans. Infect Immun.* **9**, 467—469.
16. Staat, R.H., and Schachtele, C.F., 1975. Characterization of a dextranase produced by an oral strain of *Actinomyces israelii Infect. Immun.* **12**, 556—563.
17. Schachtele, C.F., Staat, R.H., and Harlander, S. K., 1975. Dextranase from oral bacteria inhibition of water-insoluble glucan production and adherence to smooth surfaces by *Streptococcus mutans. Infect. Immun.* **12**, 309—317.
18. Tsuchiya, H.M., Heans, A., Bricker, H.M., and Wilhelm, C.A., 1952. Dextran-degrading enzymes from molds. *J. Bact.* **64**, 513—519.
19. Fennell, D.I., Raper, K.B. andThe genus *Aspergillus*, 1973.