Short Communication

Production of Extracellular Polyamine Oxidase by *Penicillium* sp. No. PO-1

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Recently, many investigators have reported on the relationship between the amount of polyamine in body fluids and cancer.^{1~5)} Therefore, enzymatic assay of polyamine has become important in diagnostic analysis. Yamada *et al.* reported that polyamine oxidase was widely distributed among fungi.⁶⁾ They purified polyamine oxidase of *Penicillium chrysogenum* IFO 4626 and *Aspergillus terreus* IFO 6346, and investigated the properties of these enzymes in detail.^{7~8)} They have also reported the differential determination method of putrescine, spermidine and spermine using polyamine oxidase of these fungi and putrescine oxidase of *Micrococcus rubens*.⁹⁾ Enzymes of *P. chrysogenum* and *A. terreus* were induced by spermidine or spermine as intracellular enzymes.

We tried to isolate microorganism which could produce extracellular polyamine oxidase and found a fungus which produced the enzyme in the medium containing polyamine as a sole source of nitrogen. A restrict monoverticillate penicillus was observed on the top of the conidiophore of the isolated fungus. The strain was designated as *Penicillium* sp. No. PO-1.

Polyamine oxidase activity was assayed spectrophotometrically by measuring the amount of quinoneimine dye based on the formation of H₂O₂. Assay system contained

0.2 ml of 500 mM sodium acetate buffer (pH 4.0), 0.2 ml of 0.2% (v/v) *N,N*-dimethylaniline solution, 0.2 ml of 0.2% (w/v) 4-aminoantipyrine solution, 0.2 ml of peroxidase solution (90 U/ml), 0.1 ml of 10 mM spermine tetrahydrochloride solution and 0.3 ml of the enzyme solution of appropriate dilution in a cuvette with a light path of 1 cm. The increase in the absorbance at 565 nm was followed with a Hitachi Spectrophotometer Model 200-10 at 30°C. One unit of the enzyme activity was defined as the amount of the enzyme which formed 1 nmol of H₂O₂ per min under the conditions described above.

Penicillium sp. No. PO-1 produced an extracellular polyamine oxidase in the medium containing spermidine or spermine as an inducer. Since spermidine and spermine were to

TABLE I. EFFECT OF AMINES ON THE PRODUCTION OF POLYAMINE OXIDASE

One ml of the spore suspension of *Penicillium* sp. No. PO-1 was inoculated to 50 ml of the medium in 300-ml Erlenmeyer flask and cultured for 72 hr at 30°C on a rotary shaker. The medium contained 0.1% amine, 0.1% glucose, 0.1% K₂HPO₄, 0.05% MgSO₄·7H₂O (pH of the medium was adjusted to 5.0 with HCl). Polyamine oxidase activity was assayed by the standard assay method.

Amine	Activity (u/ml)
Ethylamine	0
Methylamine	0
<i>n</i> -Propylamine	0
<i>n</i> -Butylamine	10.1
<i>n</i> -Heptylamine	0
Benzylamine	0
<i>n</i> -Amylamine	8.1
Histamine	0
Tyramine	0
Tryptamine	0
Ethylendiamine	0
3,3'-Diaminodipropylamine	0
1,3-Propanediamine	36.7
1,4-Butanediamine (Putrescine)	10.6
1,5-Pentanediamine (Cadaverin)	15.6
1,6-Hexanediamine	7.5
1,7-Heptanediamine	0
1,8-Octanediamine	0
1,9-Nonanediamine	5.6
1,10-Decanediamine	0
Spermidine	11.2
Spermine	10.7

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TABLE II. EFFECT OF THE CONCENTRATION OF 1,3-PROPANEDIAMINE ON THE PRODUCTION OF POLYAMINE OXIDASE

Penicillium sp. No. PO-1 was cultured by the similar method described in Table I except adjusting pH of the medium to 6.0 every 24 hr with NaOH solution.

Concentration (%)	Activity (u/ml)	
	48 hr culture	72 hr culture
0.02	0	19.2
0.05	6.5	21.1
0.07	11.1	54.6
0.10	8.4	61.4
0.15	19.2	82.3
0.20	31.4	119
0.30	24.9	78.6

TABLE III. EFFECT OF THE CONCENTRATION OF GLUCOSE ON THE PRODUCTION OF POLYAMINE OXIDASE

Penicillium sp. No. PO-1 was cultured by the same method described in Table II using 0.2% 1,3-propanediamine as an inducer.

Concentration (%)	Activity (u/ml)	
	48 hr culture	72 hr culture
0	5.8	18.4
0.05	57.6	99.9
0.1	29.9	95.4
0.2	33.3	104
0.3	30.9	52.7
0.5	30.9	64.3
1.0	29.9	53.2
2.0	20.7	48.3

expensive for a large scale production, the effect of several kinds of amines on polyamine oxidase production was investigated. As shown in Table I, nine kinds of amines were effective. From the view point of the price of chemicals and the productivity of the enzyme, 1,3-propanediamine was the most suitable inducer. From Table II, the optimal concentration of 1,3-propanediamine for the enzyme production was 0.2%. Table III shows the effect of the concentration of glucose on the production of polyamine oxidase. It was found that glucose was essential for the fungal growth, but a high concentration of glucose

FIG. 1. Time Course of Fungal Growth and Polyamine Oxidase Production in Jar Fermentor.

Penicillium sp. No. Po-1 was cultured in the medium described in the text. Cultural conditions were as follows; volume of the medium was 7 liters in a 10-liter jar fermentor, temperature was 30°C, seed was 5%, aeration was 1/2 vvm., agitation was 300 rpm and pH of the medium was controlled between 4 and 6 with NaOH solution. Fungal growth (●), polyamine oxidase activity (○).

FIG. 2. Optimal pH of Polyamine Oxidase.

Buffer systems used are as follows; pH 2~4, 100 mM glycine-HCl buffer (○), pH 4~6, 100 mM sodium acetate buffer (△), pH 6~8, 100 mM phosphate buffer (□) and pH 7.5~9, 100 mM Tris-HCl buffer (●).

inhibited the production of the enzyme. From the above results, the composition of the medium was determined as follows; 0.2% 1,3-propanediamine, 0.1% glucose, 0.1% K_2HPO_4 , 0.05% $MgSO_4 \cdot 7H_2O$ (pH was adjusted to 5.5 with HCl). Time course of the fungal growth and the enzyme production is shown in Fig. 1. It was found that pH of the medium had to be kept between 4 and 6,

because the lowering of pH of the medium below 3 with the assimilation of 1,3-propanediamine by the fungus inactivated the enzyme.

The preliminary experiments showed that the optimal pH of the enzyme was 4.0 (Fig. 2) and the optimal temperature was 45°C.

Further works on the purification and characterization of the enzyme are in progress.

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