CHAPTER 4

EFFECTS OF MINERALS ON THE PRODUCTION OF PECTINASES BY *ASPERGILLUS niger* JU

The medium employed in the previous experiment for determining the effect of different carbon and nitrogen sources on the production of pectolytic enzymes by *A. niger* JU contained the basal mineral salts. It is well known that a microorganism must be supplied a proper balance of minerals along with carbon and nitrogen compounds for good growth and efficient metabolism. However, the requirement of a particular mineral and its amount vary for different strains, species and nutrilities. The specific function of any one of the metals can only be determined in a chemically defined medium which provides a scope for correct assessment of the role played by individual minerals with respect to growth and enzyme production. In the present work, a study has, therefore, been made to assess the individual role played by minerals on growth of *A. niger* JU and production of pectinases.

EXPERIMENTAL AND RESULTS

Although it has been observed during studies on the production of pectinases by A. niger JU that pectin and peptone are superior carbon and nitrogen sources respectively for the production of pectolytic enzymes it is difficult to obtain pectin and peptone free from trace elements. So in the present study, maltose was selected as the carbon source and NH_4NO_3 as the nitrogen source in the preparation of the medium, the C/N ratio being kept at 10. The following synthetic medium was used for investigating the effects of minerals on the production of pectinases by A. niger JU :

Maltose - 4.0%, NH_4NO_3 - 0.48%, Na_2SO_4 - 0.05%, $MgSO_4,7H_2O$ - 0.05%, KCl - 0.05%, K_2HPO_4 - 0.1% and Fe - 1 p.p.m. (pH 4.5). In a typical experiment the inorganic salt under test was first omitted from and then added to the basal medium in graded doses in separate flasks to determine optimal concentration. In each subsequent experiment, the composition of the basal medium was so altered as to include an optimal amount of a mineral. Normally, rigorous purification of medium is necessary for studies on trace element nutrition. In the present experiment, a rigorously purified basal medium was used in studying the requirement of minerals (including trace elements) of A. niger JU for the production of pectinases. The chief

source of trace elements as impurities lies in the carbon source, nitrogen source and K_2HPO_4 of the medium. Only analytical grade reagents were, therefore, used after a special purification process which was as follows :

The necessary amounts of ammonium nitrate, K_2HPO_4 and maltose were each dissolved separately in 200 ml of triple glass-distilled water, and the resulting solution was shaken twice with a mixture of 0.1 gm of 8 hydroxyquinoline and 5 ml of chloroform in a separating funnel, first at pH 7.2 and then at pH 5.2. After each extraction, the solution was washed three times with 5 ml and then once with 10 ml of chloroform to free the medium from traces of 8-hydroxyquinoline. Another source of trace element contamination is glassware for which clean pyrex was used throughout the study. Ammonium nitrate solution was sterilized separately. The medium was dispensed in 30 ml volumes in 100 ml Erlenmeyer flasks (pyrex) and inoculated with 0.5 ml of spore suspension containing 5×10^3 spores. After inoculation the flasks were incubated at $30^{\circ}C$ on a rotary shaker (150 r.p.m.) for 7 days. For the preparation of the inoculum, the culture was previously grown on agar slants of the synthetic medium at $30^{\circ}C$ for 5 days and then spore suspension was prepared with water. After the fermentation was over, the activity of pectinases in the broth and cellular growth were determined by usual method as described before. The results on the effect of K_2HPO_4, $MgSO_4$, KCl,

Na_2SO_4, $CaCl_2$ and of trace elements Fe, Zn, Mo, Cu, Co and Mn on the production of pectinases by A. niger JU and cellular growth are shown in Tables 14 to 24.

TABLE 14

Effect of K_2HPO_4 on Growth of A. niger JU and Production of Pectinases

Concentration of K_2HPO_4 (gm/100 ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.01	9	5.8
0.05	32	10.8
0.10	47	12.0
0.20	56	12.8
0.30	52	13.5
0.40	48	13.6

TABLE 15

Effect of $MgSO_4,7H_2O$ on Growth of A. niger JU and Production of Pectinases

Concentration of $MgSO_4,7H_2O$ (gm/100 ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00125	39.0	8.60
0.0025	45.5	10.20
0.0075	51.0	11.20
0.025	60.0	11.90
0.05	56.1	12.90
0.10	49.0	13.50

TABLE 16

Effect of KCl on Growth of A. niger JU and Production of Pectinases

Concentration of KCl (gm/100 ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.01	49.5	10.6
0.025	55.0	11.2
0.05	60.0	12.0
0.10	57.0	12.5
0.20	51.0	12.6

TABLE 17

Effect of Na_2SO_4 on Growth of A. niger JU and Production of Pectinases

Concentration of Na_2SO_4 (gm/100 ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.01	52.4	10.1
0.025	57.0	11.1
0.05	60.2	11.9
0.10	58.0	12.4
0.20	55.0	12.3

TABLE 18

Effect of $CaCl_2,2H_2O$ on Growth of A. niger JU and Production of Pectinases

Concentration of $CaCl_2,2H_2O$ (gm/100 ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	62.8	12.3
0.004	63.0	12.4
0.008	63.5	12.6
0.015	64.0	12.8
0.022	65.0	13.0
0.030	64.0	13.1
0.045	63.2	12.9

TABLE 19

Effect of Fe on Growth of A. niger JU and Production of Pectinases (Fe added as $FeSO_4,7H_2O$)

Concentration of Fe (µg/ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	29.0	7.8
0.10	45.0	9.2
0.50	52.5	10.1
1.00	60.5	12.0
2.00	60.0	12.9
4.00	58.0	13.0

TABLE 20

Effect of Zn on Growth of *A. niger* JU and Production of Pectinases
(Zn added as $ZnSO_4,7H_2O$)

Concentration of Zn (µg/ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	59.9	11.8
0.10	61.0	12.0
0.40	63.0	12.4
0.80	62.6	12.9
1.20	62.0	12.2
2.50	61.0	12.9

TABLE 21

Effect of Mo on Growth of *A. niger* JU and Production of Pectinases
(Mo added as $Na_2MoO_4,2H_2O$

Concentration of Mo (µg/ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	65.1	12.9
0.50	66.4	13.1
1.00	66.8	13.3
2.00	67.5	13.5
4.00	68.0	13.7
8.00	67.5	13.8
10.00	66.6	13.6

TABLE 22

Effect of Cu on Growth of *A. niger* JU and Production of Pectinases (Cu added as $CuSO_4, 5H_2O$)

Concentration of Cu (µg/ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	67.8	13.7
0.25	68.1	14.0
0.50	68.9	14.0
1.00	71.0	13.9
2.00	68.2	12.9
4.00	66.5	12.0

TABLE 23

Effect of Co on Growth of *A. niger* JU and Production of Pectinases (Co added as $CoCl_2$)

Concentration of Co (µg/ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	70.9	13.9
0.10	70.8	13.8
0.25	70.2	13
0.50	69.2	
1.0	68.1	

TABLE 24

Effect of Mn on Growth of A. niger JU and Production of Pectinases (Mn added as $MnSO_4,4H_2O$)

Concentration of Mn (µg/ml)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	71.0	13.9
0.10	69.0	13.5
0.25	68.0	13.0
0.50	66.0	12.9
0.80	60.1	12.7

It appears from the results (Tables 14 to 24) that salts like K_2HPO_4, $MgSO_4,7H_2O$, KCl, Na_2SO_4 and $CaCl_2,2H_2O$ are required at concentrations of 0.20%, 0.025%, 0.05%, 0.05% and 0.022% respectively whereas the trace elements like Fe, Zn, Mo and Cu are required at concentrations of 1.0, 0.8, 4.0 and 1.0 µg/ml respectively for the maximal production of pectinases. However, the elements like Co and Mn have no effect on the production of pectinases.

The suitable synthetic medium selected as a result of the present study consists of maltose - 4%, NH_4NO_3 - 0.48%, K_2HPO_4 - 0.2%, $MgSO_4,7H_2O$ - 0.025%, KCl - 0.05%, Na_2SO_4 - 0.05%, $CaCl_2,2H_2O$ - 0.022%, $FeSO_4.7H_2O$ - 0.0005%, $ZnSO_4.7H_2O$ - 0.0004%, $Na_2MoO_4.2H_2O$ - 0.001% and $CuSO_4.5H_2O$ - 0.0004%.