CHAPTER 5

EFFECT OF SOME GROWTH FACTORS AND METABOLIC INHIBITORS ON THE PRODUCTION OF PECTINASES BY *ASPERGILLUS niger* JU

Investigations on the mineral nutrition of *A. niger* JU for production of pectinases in a synthetic medium indicate the essential requirements of salts like K_2HPO_4, $MgSO_4.7H_2O$, KCl, Na_2SO_4 and $CaCl_2.2H_2O$ in amounts of 0.2%, 0.025%, 0.05%, 0.05% and 0.022% respectively and trace elements like Fe, Zn, Mo and Cu in concentrations of 1.0, 0.8, 4.0 and 1.0 µg/ml respectively. It has been well established by a number of investigators that stimulating agents like p-aminobenzoic acid, sodium phytate and yeast extracts and also metabolic inhibitors like sodium fluoride, sodium azide, methylene blue and 2:6-dichlorophenol indophenol play important role in the production of enzymes by *Aspergillus* species and during the germination of plant seeds (98-105). In the present work, a study has, therefore, been made to find out the effect of paraaminobenzoic acid, sodium phytate, yeast extract and some metabolic inhibitors like sodium fluoride, sodium azide, methylene blue and 2:6-dichlorophenol indophenol on growth of *A. niger* JU and production of pectinases.

EXPERIMENTAL AND RESULTS

(i) Effect of Some Growth Factors on the Production of Pectinases by A. niger JU

The medium employed in this experiment consisted of Pectin - 4%, Peptone - 1.5%, 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%. To this medium were added p-aminobenzoic acid, sodium phytate and yeast extract either singly or in combination. The fermentation experiments and the assay methods were the same as described before. The results are given in Tables 25, 26, 27 and 28.

TABLE 25

Effect of p-Aminobenzoic Acid on Growth of A. niger JU and Production of Pectinases

P-aminobenzoic acid concentration (ug/ml)	Cellular growth (gm/litre)	Enzyme concentration units/ml)
0.00	8.99	139.0
0.50	9.08	140.8
1.00	9.15	141.5
2.00	9.21	143.5
4.00	9.30	145.2
6.00	9.38	147.0
8.00	9.38	146.8
10.00	9.37	146.5

TABLE 26

Effect of Sodium Phytate on Growth of A. niger JU and Production of Pectinases

Sodium phytate concentration (%)	Cellular growth (gm/litre)	Enzyme concentration (units/ml)
0.00	8.98	139.3
0.05	9.01	139.8
0.10	9.06	140.7
0.50	9.15	141.0
1.00	9.20	141.9
1.50	9.19	141.5
2.00	9.16	141.0

TABLE 27

Effect of Yeast Extract on Growth of A. niger JU and Production of Pectinases

Concentration of yeast extract (%)	Cellular growth (gm/litre)	Enzyme concentration (units/ml)
0.00	8.99	139.5
0.05	9.08	140.2
0.10	9.17	141.0
0.25	9.28	144.0
0.50	9.36	151.5
0.75	9.45	158.5
1.00	9.46	157.9
1.25	9.44	158.1

TABLE 28

Effect of a Mixture of Yeast Extract and p-Aminobenzoic Acid on Growth of A. niger JU and Production of Pectinases

Concentration of yeast extract (%)	Concentration of p-aminobenzoic acid (µg/ml)	Cellular growth (gm/litre)	Enzyme concentration (units/ml)
0.0	0.0	8.98	139.0
0.75	0.0	9.46	158.8
"	0.50	9.44	153.3
"	1.00	9.47	158.0
"	2.00	9.44	157.9
"	4.00	9.45	158.1

It appears from the results in Tables 25 and 27 that both p-aminobenzoic acid and yeast extract have got stimulatory effects on the production of pectinases by A. niger JU, yeast extract being more effective. However, in the presence of the optimal concentration of yeast extract, p-aminobenzoic acid is without any effect on the production of pectolytic enzymes (Table 28). Sodium phytate has got practically no effect on the production of pectinases by A. niger JU (Table 26).

(ii) Effect of Some Metabolic Inhibitors on the Growth of A. niger JU and Production of Pectinases

The medium used for investigating the effect of metabolic inhibitors consisted of Pectin - 4%, Peptone - 1.5%, Yeast extract - 0.75%, K_2HPO_4 - 0.2%, $MgSO_4,7H_2O$ - 0.025%, KCl - 0.05%, Na_2SO_4 - 0.05%, $CaCl_2,2H_2O$ - 0.002%, $FeSO_4.7H_2O$ - 0.0005%, $ZnSO_4.7H_2O$ - 0.0004%, $Na_2MoO_4.2H_2O$ - 0.001% and $CuSO_4.5H_2O$ - 0.0004%. To this medium was added a metabolic inhibitor in different concentrations. The fermentation experiments and the assay methods were the same as described before. The results are given in Tables 29, 30, 31 and 32.

TABLE 29

Effect of NaF on the Synthesis of Biomass and Pectolytic Enzymes by A. niger JU

NaF (Millimolar concentration)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.0	158.2	9.47
2.0	160.5	9.43
5.0	162.0	9.01
10.0	168.0	8.85
15.0	164.0	8.64
20.0	153.0	8.31

TABLE 30

Effect of NaN_3 on the Synthesis of Biomass and Pectinases by A. niger JU

NaN_3 (Millimolar concentration)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.0	158.1	9.46
0.1	164.0	9.28
0.5	180.0	8.98
1.0	139.0	8.01
2.0	negligible	-

TABLE 31

Effect of Methylene Blue on the Synthesis of Biomass and Pectinases by A. niger JU

Methylene blue (Millimolar concentration)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	158.0	9.45
0.01	188.0	9.49
0.025	230.0	9.56
0.05	150.4	8.82

TABLE 32

Effect of 2:6-Dichlorophenolindophenol on the Synthesis of Biomass and Pectinases by A. niger JU

2:6-dichlorophenol indophenol (Millimolar concentration)	Enzyme activity (units/ml)	Cellular growth (gm/litre)
0.00	158.4	9.47
0.01	162.0	9.43
0.025	168.0	9.36
0.05	175.0	9.29
0.10	59.5	3.59

It is quite evident from the Tables 29, 30, 31 and 32 that all the metabolic inhibitors tested (NaF, NaN_3, Methylene blue and 2:6-dichlorophenol (indophenol) activate the synthesis of pectinases by A. niger JU to different extents. Among all these metabolic inhibitors, methylene blue has got the maximum stimulatory effect on the production of pectinases without any significant effect on the growth of the organism. But NaF, NaN_3, and 2:6-dichlorophenol indophenol have some inhibitory effect on cellular growth.