INFLUENCE OF THE NATURE AND CONCENTRATION OF CALCIUM IONS ON PRODUCTION AND ACTIVITY OF PROTEASE OF *STREPTOMYCES* SPP.

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Calcium chloride at a concentration of 3 g/litre accelerated the production of protease from 11.59 to 142.60 units/ml in case of *S. noursei* and from 9.06 to 86.97 units/ml in case of *S. echinatus*. It was followed by calcium acetate, calcium carbonate and calcium sulphate. It was the presence of Ca^{++} and not Cl^- which increased protease production. Calcium chloride also increased the protease activity when the filtrate was obtained from the medium free from $CaCl_2$.

INTRODUCTION

Chahal and Nanda (1975) reported that *Streptomyces noursei* and *S. echinatus* were the best protease producers amongst the fourteen different actinomycetes tried. They further reported that elimination of calcium carbonate from the medium reduced the protease production drastically which indicated that calcium might be playing a great role in the protease production or its activity. Nomoto *et al.* (1960) observed a protective effect of calcium ions on protease produced by *Streptomyces griseus*. Davies (1963) reviewed that calcium was the most essential metal for the activity and stability of many microbial extracellular enzymes. In the present study the role of calcium in the production and activity of protease by *Streptomyces noursei* and *S. echinatus* has been reported.

MATERIALS AND METHODS

The materials and methods for the growth of test organisms and estimation of protease activity were the same as described earlier by Chahal and Nanda (1975).

RESULTS AND DISCUSSIONS

Effect of different calcium compounds

Presence of calcium chloride ($CaCl_2$) in the medium had zoomed up the production to about three times from that of $CaCO_3$ in case of *S. noursei*. It was followed by calcium acetate. Production remained exactly the same when $CaCO_3$ or $CaSO_4$ were supplied for *S. noursei*. Presence of $CaCl_2$ had also increased protease production by *S. echinatus* in which case, the production was almost doubled. *p*H decreased to 6.5 when $CaCl_2$ was supplied for both the organisms (Table I).

Good effect of $CaCl_2$ as compared to other calcium compounds can be attributed to its high solubility in the medium which is dependent on dissociation constant. $CaCO_3$ on the other hand is slightly soluble in the medium.

TABLE I

Effect of different calcium compounds

Calcium compound	*S. noursei*			*S. echinatus*		
	Final *p*H	Protease activity (units/ml)	Dry wt. of mycelium and undigested soybean (g)	Final *p*H	Protease activity (units/ml)	Dry wt. of mycelium and undigested soybean (g)
No calcium compound	6.9	11.59	2.192	6.8	9.06	2.120
Calcium carbonate	6.8	54.36	2.374	6.8	40.77	2.100
Calcium chloride	6.5	141.33	2.104	6.5	74.29	2.493
Calcium acetate	6.7	117.78	2.216	6.6	67.04	2.317
Calcium sulphate	6.8	54.36	2.312	6.7	38.05	2.093

Medium used : The composition of modified medium used in this study is as follows : Whole soybean flour, 30.0 g; Glucose, 30.0 g; Dried beer yeast, 3.0 g; $(NH_4)_2SO_4$, 2.0 g; $CaCO_3$, 2.0 g; NaCl, 2.0 g; KH_2PO_4, 1.2 g for *S. noursei*, 1.0 g for *S. echinatus*; $MgSO_4.7H_2O$, 0.5 g; $MnCl_2$, 0.01 g; $FeCl_3$, 0.01 g; Distilled water, to make one litre; *p*H, 7.5 for *S. noursei*, 7.0 for *S. echinatus*.

Effect of calcium and chloride ions

Previous experiment indicated that calcium chloride increased the production of protease in *S. noursei* and *S. echinatus*. But it was not clear whether the effect was due to calcium or chloride ions. Hence sodium chloride to give chloride ions and calcium acetate to give calcium ions were supplemented in the medium in place of calcium chloride.

Chloride ions alone had doubled the protease production in both the test organisms (Table II). *S. noursei* produced protease about four times more when calcium ions were present as compared to that of chloride ions. Calcium ions also increased protease production three times to that of chloride ions in case of *S. echinatus*. Effect of calcium ions was still more when it was present in combination with chloride ions in the form of calcium chloride.

It has been reported by Nomoto *et al.* (1960) that when calcium ions were not supplemented in the purification procedures of protease of *S. griseus*, the enzyme was inactivated considerably. Kaverzneva (1966) also reported that the absence of calcium ions inactivated the protease of *S. griseus*. Effect of calcium ions in the medium is most probably due to the protective effect of these ions on protease.

Effect of different concentrations of calcium chloride

Results from previous experiment revealed that calcium chloride supplemented in the medium had a good effect on protease production. It was considered worthwhile to find out the optimum concentration of calcium chloride in the medium.

TABLE II

Effect of calcium and chloride ions

Treatments	*S. noursei*			*S. echinatus*		
	Final *p*H	Protease activity (units/ml)	Dry wt. of mycelium and undigested soybean (g)	Final *p*H	Protease activity (units/ml)	Dry wt. of mycelium and undigested soybean (g)
Without Ca^{++} or Cl^{-}	6.8	11.77	2.117	6.8	9.15	2.013
Chloride ions in the form of sodium chloride	6.8	24.46	2.293	6.7	20.18	2.352
Calcium ions in the form of calcium acetate	6.7	106.89	2.213	6.7	65.23	2.285
Calcium ions & chloride ions in the form of calcium chloride	6.5	141.33	2.117	6.5	74.29	2.493

Protease production increased with the addition of calcium chloride up to 3.0 g/litre for *S. noursei* as well as for *S. echinatus* (Table III). Decrease in *p*H was observed with an increase in addition of calcium chloride. There was increase in the dry weight of mycelium up to 4.0 g $CaCl_2$/litre for *S. noursei* and up to 3.0 g $CaCl_2$/litre for *S. echinatus.*

TABLE III

Effect of different concentrations of calcium chloride

Concentration of $CaCl_2$ (g/litre)	*S. noursei*			*S. echinatus*		
	Final *p*H	Protease activity (units/ml)	Dry weight of mycelium and undigested soybean (g)	Final *p*H	Protease activity (units/ml)	Dry weight of mycelium and undigested soybean (g)
Zero	6.8	15.22	2.017	6.8	23.55	2.256
1	6.6	77.91	1.872	6.6	67.04	2.320
2	6.4	138.25	2.022	6.5	79.72	2.243
3	6.3	142.60	2.123	6.4	86.97	2.239
4	6.4	138.49	2.181	6.4	74.29	1.956
5	6.4	113.25	1.948	6.3	70.66	1.967

Effect of different calcium compounds on protease activity

To confirm whether calcium chloride had a stimulatory effect, a protective effect during the production or its presence in the filtrate increased the protease activity,

the test organisms were grown in the absence of calcium ions and calcium ions in the form of various compounds were added at a constant M/50 concentration at the time of testing of activity in the filtrate (Table IV).

TABLE IV

*Effect of different calcium compounds on protease activity**

Calcium compound	Protease activity (units/ml)	
	S. noursei	*S. echinatus*
No calcium compound	16.30	21.93
M/50 $CaCl_2$	38.05	32.61
M/50 $Ca(CH_3COO)_2$	36.54	34.42
M/50 $CaSO_4$	28.99	30.80

*Organisms were grown in the absence of $CaCl_2$; and calcium ions in the form of various compounds were added in the filtrate to give the final concentration of M/50 Ca^{++}.

Calcium ions in the form of calcium chloride gave the maximum activity of proteases after incubating for 15 minutes in both the test organisms, but calcium acetate gave a little more activity in *S. echinatus*. It was followed by calcium sulphate and least activity was observed in the absence of calcium compounds in the filtrate.

These results confirmed that $CaCl_2$ increased the activity to a great extent. It could be attributed either to its protective effect during production or to high secretion of protease from the mycelium in the presence of $CaCl_2$. The former reason is supported by the work of Nomoto *et al.* (1960). Mizusawa (1964) also reported that activity of protease from thermophilic *Streptomyces* increased considerably by the addition of Ca^{++}.

Thus keeping in view the results given in Tables III and IV, it could be attributed that $CaCl_2$ not only protects the proteases as reported by Nomoto *et al.* (1960) but also stimulates the production and the activity of proteases in both the test organisms.

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