Pakistan J. Sci. Ind. Res., Vol. 29, No. 1, February 1986

THE USE OF PENICILLIN WASTE MYCELIUM IN FERMENTATION MEDIUM (III) PRODUCTION OF PROTEASES BY *BACILLUS SUBTILUS*

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(Received November 14, 1984)

The protease synthesis in the starch salt medium by *Bacillus subtilus* in shake flasks was stimulated by the addition of mould mycelium, spent broth or their ash as compared with control culture. However, stimulation was found in the presence of mould mycelium and its ash.

INTRODUCTION

This is in continuation of our work [5] on the supplementation of culture medium with mould mycelium, a waste from penicillin plant at Daud-Khel, for the biosynthesis of antibiotics. The present studies describe the results of neutral as well as alkaline proteases syntheses by *Bacillus subtilus* in a synthetic starch salt medium. The influence of the addition of the mould mycelium spent broth and their ash to the fermentation medium has been studied in shake flasks.

MATERIALS AND METHODS

Organism. A strain of *Bacillus subtilus WRL 1036,* was used in the present investigation. The culture was maintained on an agar medium consisting of (g/l), glucose 10.0, beef extract 1.0, yeast extract 1.0, case in hydrolysate 2.0, and agar 20.0. The cultures were gorwn at 30° ± 2 for one to two days and then stored in a refrigerator.

Inoculum preparation. Vegetative inoculum was used in the present study. The composition of the inoculum (g/l), glucose 10.0, beef extract 2.0, yeast extract 2.0, peptone 1.0, NaCl, 1.0 and 25 ml of the inoculum medium was placed in 300 ml conical flask and inoculated by transferring small mycelium from the agar slant asceptically. It was allowed to grow at 30° for 24 hr. on a rotary shaker.

Fermentation medium. The composition of the basal medium was (g/l), starch 10.0, ammonium citrate 2.80, KH_2PO_4 1.3, $MgSO_4.7\ H_2O$ 0.5, KCl 0.1, and $CaCl_2$ 0.1, All reagents were of analytical grade and glass distilled water was used for the preparation of the solution. All media unless otherwise stated were autoclaved at 121° for 15 min. The initial pH of the medium was 6.8. For shake flask culture a 25 ml fermentation medium including 1 ml vegetative inoculum was held in 300 ml conical flask. The flasks were shaken on a rotary shaker rotated at 125 rev/min.

Penicillin waste mycelium: Wet mycelium from penicillin plant was dried at 100° overnight and then added to the culture medium to study its influence on enzyme formation. The amount of nitrogen in the dried mycelium was about 3% and thus protein was 20%.

The effect of inorganic constituents of the mycelium was also investigated by adding different levels of mycelial ash. It was prepared by ashing waste mycelium in a furnace at 600° for 6 hr.

Penicillin free spent broth: The penicillin free spent was concentrated on a water bath and then dried at 100° in the oven. The influence of the dried spent broth as was also investigated. The ashing of the dried spent broth was carried out as described above for waste mycelium.

Assay of the proteases: The proteolytic activity was determined by the method of Kokichi Oshima [1] and the protease unit was expressed as the amount of activity which hydrolysed 5 mg. of casein in 1 hr. under specific conditions. The activity of the culture liquid was measured in tris buffer solutions of pH 7.0 and 9.0 at a temperature 40° for 1 hr.

RESULTS AND DISCUSSION

Table 1 shows the effect of addition of penicillin waste mycelium (0-5.0 g/l) on the production of proteases by *Bacillus subtilus.* Enzyme formation was increased by increasing mycelial concentration. The optimum amount of mould mycelium was 3.0 g/l and the enzyme yield was 5.5 units/ml as compared with the control culture 2.5 units/ml. Further increase in the concentrations of

Table 1. Effect of penicillin waste mycellium on the production of proteases by *Bacillus subtilus* WRL-1036

Penicillin waste mycellium g/l	Protease Units/ml			
	24 hr.		48 hr	
	Neutral	Alkaline	Neutral	Alkaline
Control	1.60	0.80	2.5	1.25
1.0	1.80	0.90	2.8	1.40
2.0	2.00	1.00	3.50	1.80
3.0	2.20	1.10	5.50	2.25
4.0	1.20	0.60	2.20	1.10
5.0	1.00	0.50	2.00	1.00

Table 2. Effect of penicillin waste mycellium ash on the production of proteases by *Bacillus subtilus* WRL-1036

Penicilline waste mycellium ash g/l	Protease Units/ml			
	24 hr		48 hr	
	Neutral	Alkaline	Neutral	Alkaline
Control	1.60	0.80	2.50	1.25
0.5	1.80	0.90	2.80	1.40
1.0	2.00	1.00	3.60	1.80
1.5	2.20	1.05	5.80	2.90
2.0	1.40	0.70	2.40	1.20
2.5	1.10	0.50	1.90	1.00

mould mycelium resulted in the lowering of the proteolytic enzyme synthesis. The stimulatory effect of the addition of mould mycelium on protease formations is in accordance with the findings reported earlier in case of antibiotic synthesis [2, 3, 4].

The production of enzymes by bacteria was also increased by the addition of mycelial ash to shake flask cultures. The amount of enzyme produced was 5.8 units/ml in the presence of 1.5 g/l of mycelial ash. Further increase in its level reduced the enzyme formation.

Effect of penicillin free spent broth: Spent broth left after the recovery of penicillin was the source of amino acids and mineral. The effect of dried spent broth (0.5-2.5 g/l) was also investigated on the biosynthesis of proteases (Table 3). The proteolytic enzyme formation was increased with increase in the concentration of the dried spent broth. The optimum level of the dried spent broth was 1.5 g/l and the enzyme synthesis was (5.0 units/ml). The enzyme yield went on decreasing with increase in the dried spent broth amount. The addition of spent broth ash (0.4-2.0 g/l) to the culture medium also resulted in increasing the production protease (Table 4). The optimum level of the ash was 1.2 g/l and the amount of enzyme yield was 4.8 units/ml. The protease synthesis, however, was reduced by further increasing the concentration of spent broth ash. The enzyme formation was stimulated by the addition of mycelium spent broth, or their ash. It follows that the inorganic constituents of the mycelium or spent broth are important factors in stimulating the synthesis of protease by *Bacillus subtilus.*

Tabl 3. Effect of dried spent broth on the production of Proteases by *Bacillius subtilus* WRL-1036

Dried spent broth g/l	Protease Units/ml			
	24 hr		48 hr	
	Neutral	Alkaline	Neutral	Alkaline
Control	1.60	0.80	2.5	1.25
0.5	1.80	0.90	2.6	1.30
1.00	2.00	1.00	3.4	1.70
1.50	2.10	1.05	5.00	2.50
2.00	1.00	0.50	2.10	1.05
2.50	0.80	0.40	1.20	0.60

Table 4. Effect of spent broth ash on the production of Proteases by *Bacillius subtilus* WRL-1036

Spent broth ash g/l	Protease Units/ml			
	24 hr		48 hr	
	Neutral	Alkaline	Neutral	Alkaline
Control	1.60	0.80	2.5	1.25
.40	1.75	0.90	2.6	1.30
.80	2.00	1.00	3.00	1.30
1.20	2.30	1.15	4.80	2.40
1.60	1.50	0.75	2.00	1.00
2.00	1.00	0.50	0.80	0.40

Acknowledgement: The author acknowledge the assistance of Mr. Muhammad Hamid in the maintenance of cultures.

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