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Short Communication

Effect of C:N ratio on alpha amylase production by *Bacillus licheniformis* SPT 27

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Bacillus licheniformis **SPT 27 is an isolate which produces extra cellular alpha (∝-) amylase exhibiting activity at a wide pH range and was relatively stable. The *B. lichenformis* isolate, however, produces low yields of the amylase. Our results show that the amylase production is higher in the presence of starch, with *Amarantus peniculatus* starch producing the highest amount of amylase. Amongst sugars, fructose supported maximum amylase production. Of the nitrogen sources tested, peptone and ammonium hydrogen phosphate where the best organic and inorganic sources respectively. The C:N ratio found to be the optimum was 1:1.**

Key words: *Amarantus manipulates,* alpha (∝-) amylase, *Bacillus licheniformis* SPT 27, C:N ratio.

INTRODUCTION

Bacillus licheniformis SPT 27 is a natural isolate obtained from the alkaline soil of Cambay, in the western region of Gujarat, India. The isolate produced extracellular ∝-amylase which was confirmed by percentage blue value (Moseley and Keay, 1970) and by the identification of the products obtained by starch hydrolysis (Trevelyan et al, 1950).

The amylase exhibited activity at a wide pH range, with the optimum being pH 9, desirable characteristics which can lead to its application in detergents as additive and in textile desizing. The enzyme was also relatively thermostable.

Since this natural isolate produced very low concentration of amylase, attempts were made to increase the productivity by optimizing the nature and relative concentration of carbon and nitrogen source.

MATERIALS AND METHODS

Culture of *Bacillus licheniformis* SPT 27

The stock culture of *B. licheniformis* SPT 27 was maintained on nutrient agar slant at 5^{o}C.

Preparation of inoculum

B. licheniformis were streaked on nutrient agar plate supplemented with 1% soluble starch and were incubated for 16 h at 37^{o}C. The cells from this plate were inoculated into nutrient broth and incubated at 37^{o}C for 8 h.

Production of amylase

The inoculum size taken was 1% of the production media . 50ml of production media containing test carbon source , test nitrogen source, sodium chloride(Nacl), was taken in 250ml of Erlenmeyer flask. The pH of the production media was 9. The flasks were kept on rotary shaker for 24 h at 37^{o}C. The amylase saccharolytic activity of amylase was determined by dinitrosalicylic acid method (Bernfield,1955)

Amylase assay

Amylase was assayed by adding 0.2 ml of enzyme (crude extract/fermented broth supernatant) to 0.5 ml of 1% soluble starch and incubated for 30 min at 37^{o} C. The reaction was stopped by adding 1 ml of 3, 5 dinitrosalicylic acid, followed by boiling for 10 min and to develop brown color. The final volume was made to 5 ml with distilled water and the absorbancy measured at 540 nm with a spectrophotometer (Systronics-UV-vis 108).

A calibration curve of absorbancy and concentration of maltose was established with known amount of maltose.

One unit (U) of amylase was defined as the amount of enzyme that liberates one Mole of reducing sugars, measured as maltose per min under the conditions of assay.

RESULTS

Effect of starch on enzyme production

High enzyme titers were obtained in starch supplemented production media though some amount were detected in production media without starch. (Table 1).

Table 1. Effect of starch on amylase production with or without soluble potato starch.

Hours of incubation	Amylase activity IU/ml	
	without starch	with starch (1%)
4	46	98
8	66	125
12	119	144
24	168	266
28	158	252

Standard deviation ± 0.5

Amongst the different types of starchy grains and tubers tested, highest yield were obtained with *Amarantus paniculatus* followed by *Zea mays*, potato and *Metroxylan remphii* (Table 2).

Table 2. Effect of starchy grains and tubers on amylase yields.

Starch source (1g %)	Amylase activity (IU/ml)
Potato starch	350
Hordeum vulgare	276
Amarantus paniculatus	382
Metroxylan remphii	346
Manihot utilisma	290
Triticum sativum	258
Zea mays (refined flour)	364
Pennisetium trophoides	168
Zea mays	262
Trapa bipinosa	298

Standard deviation ± 0.5

The effect of concentration of starch substrate on enzyme production was studied at 37°C using potato soluble starch and *Amarantus paniculatus* in the range of 0.5 to 5 %. Potato starch at 1% and *Amarantus paniculatus* at 2% concentration was found to be optimum (Table 3).

Table 3. Effect of starch substrate concentration on amylase production (peptone 0.5%w/v).

Substrate	Concentration (%w/v)	Amylase activity (IU/ml)
Potato soluble starch	0.5	396
	1	416
	1.5	368
	2	168
	3	74
	4	46
	5	14
A. peniculatus	0.5	378
	1	420
	1.5	441
	2	514
	3	374
	4	108
	5	94

Standard deviation ± 0.2

Effect of pure soluble sugars on ∝-amylase production

Using 1% of different soluble sugars, amylase production was highest in sucrose medium. Nonmetabolizable sugars like arabinose, raffinose, mesoinositol, sucrose, and galactose did not support amylase production. (Table 4).

Table 4. Effect of pure soluble sugars on enzyme production (peptone 0.5% w/v).

Sugars (1% w/v)	Amylase activity (IU/ml)
Mannitol	162
Fructose	424
Maltose	420
Ribitol	116
Raffinose	102
Mesoinositol	42
Sorbitol	92
Dulcitol	98
Glucose	242
Sucrose	52
Arabinose	42
Galactose	32
Glycerol	242
Trehalose	206
Xylose	234
Lactose	192
Dextrin	372

Standard deviation ± 0.5

Table 5. Effect of organic nitrogen on amylase yields (potato soluble starch 1% w/v).

Nitrogen source (1%,w/v)	Amylase activity (IU/ml)
Peptone	350
Yeast extract	224
Meat extract	304
Proteose peptone	256
Casein (vit free)	224
Gelatin	178
Tryptone	266
Peptone and Yeast extract (1:1)	280

Standard deviation ± 0.5

Table 6. Effect of inorganic nitrogen source on amylase yields (potato soluble starch 1%w/v).

Inorganic nitrogen source (1%,w/v)	Amylase activity (IU/ml)
Sodium nitrate	204
Potassium nitrate	186
Potassium nitrite	94
Ammonium chloride	164
Ammonium sulphate	94
Ammonium hydrogen phosphate	239
Ammonium oxalate	164

Standard deviation ± 0.5

Table 7. Effect of concentration of peptone/ammonium hydrogen phosphate on amylase yields (potato soluble starch 1%w/v).

Nitrogen source	Concentration (%w/v)	Amylase activity (IU/ml)
Peptone	0.2	406
	0.5	420
	1	484
	2	294
	3	280
	4	280
Ammonium hydrogen phosphate	0.2	428
	0.5	416
	1	416
	2	298
	3	98
	4	0

Standard deviation ± 0.5

Effect of nitrogen sources on ∝-amylase production

Peptone was replaced with 1% of different organic and inorganic compounds as nitrogen source keeping the rest of the media composition the same. Peptone was the best organic nitrogen source followed by meat extract (Table 5). While ammonium hydrogen phosphate was the best inorganic nitrogen source with 29% less yield than peptone (Table 6). The effects of different concentrations of peptone and ammonium hydrogen phosphate on amylase production was also studied. We observed that 1% peptone and 0.2 % of ammonium hydrogen phosphate gave highest amylase yields (Table 7).

Combination of peptone and ammonium hydrogen phosphate with different concentrations were also studied. 1% peptone and 0.5% ammonium hydrogen phosphate was the best combination (Table 8).

Table 8. Effect of peptone &ammonium hydrogen phosphate combination on amylase production (potato soluble starch 1%w/v).

Peptone	Ammonium hydrogen phosphate	Amylase activity (IU/ml)
(%)	(%)	
0.2	0.5	458
0.5	0.5	504
1	0.5	458
2	0.5	458
3	0.5	448
4	0.5	437
1	0.2	462
1	1	385
1	2	340
1	3	140
1	4	0

Standard deviation ± 0.5

Effect of C/N ratio on enzyme production

For C/N ratio study, potato soluble starch and peptone was used as carbon and nitrogen sources, respectively. 1% of potato starch was used while the amount of peptone was varied to attain desired C/N ratio. The activity of amylase in fermented broth was recorded after 24 h. The maximum amylase yields were obtained when C/N ratio was 1.0 (Table 9).

Table 9. Effect of c/n ratio on amylase production.

C/N Ratio	Amylase activity (IU/ml)
5.0	406
2.O	420
1.O	498
0.5	308
0.3	280
0.25	266

Standard deviation +/-0.5

DISCUSSION

The nature and amount of carbon source in culture media is important for the growth and production of extracellular amylase in bacteria. It is empherically known that higher yields of amylase can be obtained in media with complex rawmaterial containing starch from maize, barley, wheat and malt (Burbridge and Collier, 1968). *B. licheniformis* SPT 27 gave higher yields of amylase in presence of starch. The bacteria can produce the enzyme even in the absence of starch.

Amylase yield was high in media containing fructose as sole carbon source. Dextran and maltose also served as good substrate for enzyme synthesis. Some yields were detected in media with glucose as carbon source, and the bacteria can produce the enzyme even in the absence of carbon source. This indicates that amylase production in this organism is paraconstitutive (Chandra et al, 1980).

The nature and relative concentration of carbon and nitrogen sources are important in formation of amylase. Lower levels of nitrogen are inadequate for the enzyme production and excess nitrogen is equally detrimental causing enzyme inhibition. Tigue et al. (1994), reported C/N ration of 1:1 with starch and yeatex as carbon and nitrogen sources, respectively. With *B. licheniformis* SPT 27 the C/N ratio is also 1:1 where potato soluble starch and peptone area carbon and nitrogen sources, respectively.

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