

Supplementation of Carbohydrate to Enhance the α -amylase Production by *Bacillus licheniformis* ATCC 6346 in Presence of Seed Cakes

Arumugam Vengadaramana^{1*}, Santherasekarampillai Balakumar² and Vasanthi Arasaratnam²

¹Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka

²Department of Biochemistry, Faculty of Medicine, University of Jaffna, Sri Lanka
E.mail: vengad@jfn.ac.lk

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ABSTRACT

Aim: The effect of carbohydrate and amino acids on the production of α -amylase by *Bacillus licheniformis* ATCC 6346 was investigated.

Methodology and results: To find out the influence of carbohydrate the total carbohydrate content of the medium containing different concentration (2-18 gL⁻¹) of defatted seed cake powder of sesamum and mustard containing medium was kept constant by the addition of soluble starch separately. The highest α -amylase activity obtained in the medium containing 18gL⁻¹ mustard (59.11 \pm 1.48 UmL⁻¹) and sesamum seed cake powder (55.23 \pm 1.55 UmL⁻¹). The results indicated that under these conditions the carbohydrate content had no effect on the production of α -amylase. Effect of amino acids (0.2gL⁻¹ of Glycine, Methionine, Proline, Lysine, Leucine, Threonine, Serine, Arginine, Alanine, Glutamic acid, Tryptophan, Glutamine, Asparagine, Histidine, Valine, Phenylalanine, Isoleucine and mixture of amino acids) on the production of α -amylase in fermentation medium was investigated. Among the different amino acids supplemented, eight amino acids improved the α -amylase production but casaminoacids slightly inhibited the enzyme production. In presence of Tryptophan highest enzyme activity was obtained than control.

Conclusion, significance and impact of study: In these study amino acids especially Tryptophan takes part in a particular role rather than carbohydrate in the production of α -amylase from *B.licheniformis* ATCC 6346.

Keywords: Amino acids, α -amylase, casaminoacids, *Bacillus licheniformis*

INTRODUCTION

It is a common practice to use carbohydrates as the carbon source in microbial fermentation process. The rate of bacterial α -amylase biosynthesis is controlled by both substrate induction and catabolite repression (Laoide *et al.*, 1989 and Antrinikian, 1990) the composition and concentration of the medium play an important role in the growth and production of extracellular amylase by bacteria, yeast and *Aspergillus* sp (Zhou, 1990). It is now recognized that the rate at which the carbon sources is metabolized, can often influence the formation of biomass or production of primary or secondary metabolites. The carbon sources greatly affect the production of thermophilic α -amylases (Srivastava and Baruah, 1986 and Kumar *et al.*, 1990). Of carbohydrates used, starch is demonstrated to be a good carbon source for the synthesis of amylases in *B.sterothermophilus* (Welker *et al.*, 1963) and other thermophilic *Bacillus* sp. (Srivastava *et al.*, 1986).

Degradation of starch to maltodextrins by many bacteria is catalyzed by α -amylase and is followed by its hydrolysis to glucose by the action of either intracellular or extracellular α -glucosidase (Vihinen *et al.*, 1989). Agger *et al.*, (2002) have reported that starch was the best inducer for α -

amylase production in TA1 strain of *Aspergillus nidulans*, which was comparable with only glucose. Lower levels of nitrogen sources are inadequate for the enzyme production and excess nitrogen is equally detrimental causing enzyme inhibition (Aiyer, 2004). The regular use of peptone based fermentation medium is not commercially viable for industries. For efficient commercial production, a continuous effort is being made to find cheaper substrate sources.

The expensive products can be replaced in the fermentation medium with the economically available agricultural by products (Ghosh and Chandra, 1984). These oil cakes are fairly rich in protein and are traditionally used as feed aquaculture feeds (Singh *et al.*, 2003). Several oil seed cakes, because of their abundant, availability and low price, are used as cattle feed (Norton *et al.*, 1946), fertilizer (Salgado *et al.*, 1940) and in rare cases after proper processing as food for human (Rastogi *et al.*, 1960). The present study was planned to use defatted mustard and sesamum seed cakes which are available locally in jaffna, Sri Lanka to produce α -amylase from *Bacillus licheniformis* ATCC 6346 by supplementing starch and improve the enzyme production by the addition of amino acids and casaminoacids presence and absence of peptone.

MATERIALS AND METHODS

Materials

Sesamum seed cake was obtained from local market in Jaffna and powdered with a domestic grinder. Mustard seed cake powder was prepared in the laboratory at Department of Biochemistry, Faculty of Medicine, University of Jaffna, Sri Lanka by crushing the seeds purchased in the local market with motor and pestle, air drying and powdering with a domestic grinder.

Strain of α -amylase producer and enzyme production

Bacillus licheniformis ATCC 6346 from Heriot-Watt University U.K was used in this study. The nutrient agar medium contained (g L^{-1}) nutrient agar, 25.0 and soluble starch, 3.0 and the activation medium contained (g L^{-1}) Nutrient broth, 25.0 and soluble starch 3.0 at pH 7.0. The fermentation medium contained (g L^{-1}) soluble starch, 4.0; $(\text{NH}_4)_2\text{SO}_4$, 5.0; peptone, 6.0; FeCl_3 , 0.01; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.01; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01; KH_2PO_4 , 4.0 and K_2HPO_4 , 7.5 at pH 7.0. A loopful of *Bacillus licheniformis* ATCC 6346 from nutrient agar slants with 0.3 % soluble starch (grown at 37 °C for 24 h) was transferred to 10 mL activation medium which was incubated at 42 °C in a rotary shaker (100 rpm) for 12 h and used as inoculum. The fermentation medium was inoculated with 20 % (v/v) inoculum and the inoculated flasks were incubated for 48 h at 42 °C with shaking at 100 rpm. The culture filtrate was used as source of α -amylase.

Measurement of α -amylase activity

Enzyme was diluted with 0.01M phosphate buffer (pH 7.0). The diluted enzyme and soluble starch (2g L^{-1}) in 0.01M phosphate buffer (pH 7.0) were pre incubated for 3 min at 85 °C. Then 0.5mL of the enzyme was mixed with 0.5mL substrate and incubated for 5min at 85 °C. Reducing sugar was measured by the DNS method (Miller, 1959). One unit of α -amylase activity is defined as the amount of enzyme that produces one μmole of reducing sugar in one minute at 85 °C, and pH 7.0 from soluble starch (20g L^{-1}) as substrate.

Estimation of fat content from seed cakes

Fat in mustard seed cake powder (3 g) taken in a thimble was extracted with petroleum ether (250 mL) in a continuous extraction apparatus of soxhelt type for 8 hours. After fat extraction the Mustard seed cake powder was dried at 40 °C for overnight to evaporate the ether. This procedure was repeated with sesamum seed cake powder.

Estimation of total Carbohydrate content of defatted seed cakes

The alpha-glycosidic bonds are acid labile. Hence, the starch was acid hydrolyzed with 1N HCl and the reducing sugar was estimated by DNS method (Miller, 1959).

Estimation of total Nitrogen content of defatted seed cakes

The micro Kjeldhal method was used (Bremner and Keeney, 1966)

Effect of maintaining the total carbohydrate content of the medium on the production of α -amylase in fat removed mustard powder and sesamum seed cake powder

Peptone of medium was replaced with different (2.0 to 18 g) amounts of defatted mustard and sesamum seed cake powder separately. Another set of medium were prepared with different (2.0 to 18 g) amounts of defatted mustard and sesamum seed cake powder separately while the amount of total carbohydrate content kept constant (equalized to that present in 18g L^{-1} of either mustard or sesamum) by the addition of soluble starch. Peptone (6g L^{-1}) containing medium was used as control.

Effect of amino acids on the production of α -amylase

Effect of amino acids on the production of α -amylase in presence of peptone: Amino acids such as 0.2g L^{-1} of Glycine or Methionine or Proline or Lysine or Leucine or Threonine or Serine or Arginine or Alanine or Glutamic acid or Tryptophan or Glutamine or Asparagine or Histidine or Valine or Phenylalanine or Isoleucine and their mixture were supplemented to the peptone (6g L^{-1}) containing fermentation medium separately. Peptone (6g L^{-1}) containing medium without amino acid supplementation was used as the control. Amino acids were sterilized by filtering the solution through membrane filter.

Effect of amino acids and Casamino acids on the production of α -amylase: Mixture of amino acids such as 0.2g L^{-1} of Glycine, Methionine, Proline, Lysine, Leucine, Threonine, Serine, Arginine, Alanine, Glutamic acid, Tryptophan, Glutamine, Asparagine, Histidine, Valine, Phenylalanine and Isoleucine were supplemented to medium while avoiding peptone (6g L^{-1}). Mixture of the eight amino acids (0.2g L^{-1} of Lysine, threonine, Serine, Arginine, Tryptophane, Glutamine, Histidine and Isoleucine) were added to medium in presence of 6g L^{-1} peptone. Casamino acids (6g L^{-1}) were supplemented to medium which contained 6g L^{-1} peptone. Peptone (6g L^{-1}) containing medium without amino acid supplementation was used as the control. Amino acids were sterilized by filtering the solution through membrane filter.

RESULTS AND DISCUSSION

Effect of maintaining the total carbohydrate content of the medium on the production of α -amylase in defatted mustard and sesamum seed cake powder

When the amount of defatted mustard seed cake powder in the medium was varied from 2.0 to 30.0g L^{-1} while the other components of the medium were kept constant, α -

Table 1: Production of α -amylase by *B.licheniformis* ATCC 6346 in medium containing defatted mustard seed cake powder supplemented with soluble starch to maintain the carbohydrate content. Fermentation was carried out at 42 °C, 100 rpm and at 48 h.

| Mustard powder (gL ⁻¹) | Without starch | | With starch | |
|---------------------------------------|----------------|---|-------------|---|
| | C/N ratio | α -Amylase (U mL ⁻¹) | C/N ratio | α -Amylase (U mL ⁻¹) |
| Control ^A | 2.2:1 | 42.16 ± 1.53 | 2.2:1 | 42.16 ± 1.53 |
| 2.0 | 4.0:1 | 39.46 ± 1.31 | 2.8:1 | 40.26 ± 1.63 |
| 6.0 | 2.8:1 | 45.72 ± 1.43 | 3.3:1 | 44.26 ± 1.47 |
| 10 | 2.5:1 | 50.31 ± 1.41 | 2.7:1 | 51.26 ± 1.01 |
| 14 | 2.4:1 | 54.21 ± 1.29 | 2.5:1 | 53.72 ± 1.12 |
| 18 | 2.5:1 | 59.11 ± 1.48 | - | - |

^A Control medium containing 6g L⁻¹ peptone

Note: Values are given as Mean ± SD of triplicate experiments

Table 2: Production of α -amylase by *B.licheniformis* ATCC 6346 in medium containing defatted sesamum seed cake powder supplemented with soluble starch to maintain the carbohydrate content. Fermentation was carried out at 42 °C, 100 rpm and at 48 h

| Sesamum powder (gL ⁻¹) | Without starch | | With starch | |
|---------------------------------------|----------------|---|-------------|---|
| | C/N ratio | α -Amylase (U mL ⁻¹) | C/N ratio | α -Amylase (U mL ⁻¹) |
| Control ^A | 2.2:1 | 43.62 ± 1.45 | 2.2:1 | 43.62 ± 1.45 |
| 2.0 | 4.0:1 | 35.95 ± 1.33 | 4.0:1 | 37.26 ± 1.24 |
| 6.0 | 3.0:1 | 46.22 ± 1.51 | 3.2:1 | 44.15 ± 1.01 |
| 10 | 2.4:1 | 47.42 ± 1.12 | 2.8:1 | 46.11 ± 0.79 |
| 14 | 2.3:1 | 53.23 ± 1.65 | 2.4:1 | 54.12 ± 1.51 |
| 18 | 2.1:1 | 55.23 ± 1.55 | - | - |

^A Control medium containing 6g L⁻¹ peptone

Note: Values are given as Mean ± SD of triplicate experiments

amylase produced in presence of 18.0 g L⁻¹ defatted mustard seed cake powder was the highest (58.14 U mL⁻¹) and 1.5 times higher than that produced in the control medium which containing 6 g L⁻¹ peptone (Vengadaramana *et al.*, 2011). Thus increase in total nitrogen and sugar contents do not simply influence the α -amylase production. When the concentration of defatted sesamum seed cake powder in the medium was changed from 2.0 to 30.0 g L⁻¹, highest α -amylase activity (56.64 U mL⁻¹) was obtained in the medium containing 18 g L⁻¹ sesamum seed cake powder, and was 1.4 fold higher than that in the control medium with 6 g L⁻¹ peptone (Vengadaramana *et al.*, 2011).

When the total carbohydrate content of the medium was kept constant, the α -amylase production in presence of 2 to 18g L⁻¹ defatted mustard seed cake powder was increased from 39.46 ± 1.31 to 59.11 ± 1.48 U mL⁻¹ (**Table 1**). Maximum α -amylase production was obtained in the medium containing 18 g L⁻¹ mustard seed cake powder. Highest α -amylase production was obtained in the medium with Carbon (Total) to Nitrogen (Total), ratio of 5:2. After equalizing the carbohydrate content in the medium, production of α -amylase at different mustard seed cake powder containing medium were almost same to that in the medium where carbohydrate was not supplemented (**Table 1**).

When the total carbohydrate content of the medium containing sesamum seed cake powder was kept constant, α -amylase production was highest (55.23 ± 1.55

U mL⁻¹, **Table 2**) in the medium containing 18 g L⁻¹ sesamum seed cake powder. Carbon (Total) to Nitrogen (Total), ratio of 2 and 18 g sesamum seed cake powder containing medium were 4:1 and 2.18:1 respectively. Here also after equalization of carbohydrate, production of α -amylase in different sesamum seed cake powder containing medium were almost same to those produced in the medium where the carbohydrate content were not equalized (**Table 2**). Mustard contained starch and glucose and sesamum contained galactose, sucrose and glucose as major carbohydrates but in this experiment soluble starch was used as carbon source to adjust the carbohydrate content. Under the experimental conditions maintaining the total carbohydrate content gave no improvement on the production of α -amylase in mustard and sesamum seed cake powder containing medium. Thus the production of α -amylase was not influenced by total carbohydrate content but influenced by different sources or other nutrients. Among the other nutrients amino acids might have quantitatively and qualitatively varied in the protein and might have influence the enzyme production. Hence the effect of amino acids on the production of α -amylase was investigated in the following experiment.

Effect of amino acids on the production of α -amylase in the presence of peptone

α -Amylase production in the medium containing peptone was less than that obtained in the medium with 10.0-18.0 g L⁻¹ defatted mustard seed cake powders

(Vengadaramana *et al.*, 2011). Sesamum seed cake powder (18 gL⁻¹) and coconut seed cake powder (24 gL⁻¹) containing medium were supplemented with different amino acids or their mixture equivalent to the present in mustard seed cake (18 gL⁻¹) powder (Vengadaramana *et al.*, 2011). Supplementation of 0.0147 and 0.10801 gL⁻¹ tryptophane respectively to defatted sesamum (18 gL⁻¹) and coconut seed cake powder (24 gL⁻¹) containing medium increased the production of α -amylase to 57.42 and 58.26 U mL⁻¹, which were almost equal to that produced in defatted mustard seed cake powder (Vengadaramana *et al.*, 2011).

Reduction in α -amylase production in peptone containing medium could be due to amino acids deficiencies. Therefore different amino acids and their mixture were supplemented to peptone containing medium and their effect on α -amylase production by *Bacillus licheniformis* ATCC 6346 was studied. Medium containing 6g L⁻¹ peptone was used as control. When the peptone containing medium was supplemented with different amino acids, eight amino acids (lysine, threonine, serine, arginine, tryptophan, glutamine, histidine, and isoleucine) and their mixture improved α -amylase production by *B.licheniformis* ATCC 6346 at 42°C, 100rpm and at 48h (**Table 3**). Among the amino acids, in presence of tryptophan highest α -amylase activity was obtained (55.71 \pm 1.34 U mL⁻¹) followed by serine (53.39 \pm 0.85 U mL⁻¹), lysine (52.97 \pm 1.21 U mL⁻¹), arginine (52.47 \pm 1.61 U mL⁻¹), and isoleucine (51.39 \pm 1.48 U mL⁻¹) supplementation. Production of α -amylase in glycine (43.38 \pm 1.38 U mL⁻¹), methionine (44.17 \pm 1.43 U mL⁻¹), proline (44.13 \pm 1.51 U mL⁻¹), glutamic acid (45.63 \pm 1.31 U mL⁻¹) and phenylalanine (42.71 \pm 1.66 U mL⁻¹) containing medium were almost same as that in the control (44.45 \pm 1.46 U mL⁻¹) medium. Therefore these amino acids did not influence the production of α -amylase at 42 °C, 100rpm and at 48 h (**Table 3**).

Synthesis of α -amylase by *Bacillus subtilis* was stimulated by the growth medium which was separately supplemented with alanine and arginine (Sema *et al.*, 2000). These two amino acids also improved the α -amylase production by *B. licheniformis* ATCC 6346 under the experimental conditions (**Table 3**). Cystine, arginine and leucine increased the transport of α -amylase through the membrane, while alanine and serine inhibited it (Sema *et al.*, 2000). We have not studied the effect of cystine under the experimental conditions. Arginine, alanine, leucine and serine have increased α -amylase activity in the spent medium. Secreted α -amylase activity was appreciably activated by alanine, arginine, glutamine, glycine, leucine, phenylalanine, proline and especially cystine while it was repressed by asparagin, glutamic acid, lysine, methionine, serine, threonine and tryptophan (Sema *et al.*, 2000). In our experiment glycine, phenylalanine and proline have not increased the enzyme activity and asparagine, glutamic acid, lysine, serine, threonine and tryptophan have not reduced the α -amylase

Table 3: Effect of amino acids (0.2 gL⁻¹) on the production of α -amylase and growth (600 nm) of *B.licheniformis* ATCC 6346 in medium (which contained 6 gL⁻¹ peptone) at 42 °C and at pH 7.0 while shaking (100 rpm) at 48 h.

| Amino acids | α -Amylase (U mL ⁻¹) |
|-----------------------------|---|
| Control ^A | 44.45 \pm 1.46 |
| Glycine | 43.38 \pm 1.38 |
| Methionine | 44.17 \pm 1.43 |
| Proline | 44.13 \pm 1.51 |
| Lysine | 52.97 \pm 1.21 |
| Leucine | 48.30 \pm 1.36 |
| Threonine | 49.30 \pm 1.43 |
| Serine | 53.39 \pm 0.85 |
| Arginine | 52.47 \pm 1.61 |
| Alanine | 48.88 \pm 1.12 |
| Glutamic acid | 45.63 \pm 1.31 |
| Tryptophan | 55.71 \pm 1.34 |
| Glutamine | 50.05 \pm 1.56 |
| Asparagine | 48.38 \pm 1.45 |
| Histidine | 49.13 \pm 1.29 |
| Valine | 47.22 \pm 1.65 |
| Phenylalanine | 42.71 \pm 1.66 |
| Isoleucine | 51.39 \pm 1.48 |
| Mixture of A.A ^B | 52.39 \pm 1.72 |

^A Control medium containing 6 gL⁻¹ peptone

^B Mixture of seventeen amino acids such as glycine, methionine, proline, lysine, leucine, threonine, serine, arginine, alanine, glutamic acid, tryptophan, glutamine, asparagine, histidine, valine, phenylalanine and isoleucine.

Note: Values are given as Mean \pm SD of triplicate experiments.

activity (**Table 3**). When the medium (contained 6 gL⁻¹ peptone) was supplemented with different amino acids separately, highest growth was obtained in the medium supplemented with threonine at 48 h (Results are not shown). Following thereonine higher growth of *B.licheniformis* ATCC 6346 was seen in the medium supplemented with lysine, methionine, arginine and valine. Other amino acids supplementation has not influenced the growth of *B.licheniformis* ATCC 6346.

Highest α -amylase activity (55.71 \pm 1.34 U mL⁻¹) was obtained in the medium supplemented with tryptophan at 48h while highest growth was obtained in the medium supplemented with threonine at 48h (Results are not shown). This suggests that the enzyme production is not growth associated and threonine might be influencing the growth while tryptophan is influencing the α -amylase production by *B. licheniformis* ATCC 6346. Srivastava and Baruah (1986), showed that addition of cystine and glycine have inhibited the growth of *B. stearothermophilus*, and the highest reproductive values were obtained with phenylalanine and aspartic acid. α -Amylase production by *B. stearothermophilus* was studied, by adding aspartic acid, cystine, glutamic acid,

glycine, and methionine separately to the medium. Glutamic acid, glycine and methionine partly decreased α -amylase production, while other amino acids, increased α -amylase production (Srivastava and Baruah, 1986). In our experiment glycine and methionine have slightly decreased the production of α -amylase.

In lysine, threonine, serine, arginine, tryptophan, leucine, alanine, asparagine, valine, glutamine, histidine, and isoleucine supplemented medium higher α -amylase activity was obtained than in control medium (contained 6 gL⁻¹ peptone) (Table 3). Therefore following experiment was carried to find the effects of the amino acids mixture which gave positive effect on α -amylase production in the absence of 6 gL⁻¹ peptone. Further the effect of casamino acids in presence of 6 gL⁻¹ peptone was also studied.

Effect of amino acids and casamino acids on the production of α -amylase

When compared to defatted mustard seed cake powder the amount of amino acids content in peptone was more. However the enzyme production in the former medium was more than that in the later. To find whether the supplementation of the amino acids which gave positive effect on α -amylase production could show a positive effect this experiment was performed. Another commonly available product which is rich in most of the amino acids is casamino acid. Therefore this was also supplemented to peptone containing medium. In this experiment medium, which contained peptone 6gL⁻¹ was used as the control. To study the effects of amino acids, the medium (presence of peptone) was supplemented with a mixture of 17 amino acids. To find the effect of the amino acids which enhanced the α -amylase production to medium, eight amino acids were supplemented. In another set, to find whether the peptone is essential and the amino acids present in peptone are inhibiting the enzyme production, only the mixture of 17 amino acids was added to medium while avoiding the peptone.

Peptone in the medium was replaced with a mixture of all 17 amino acids showed better production of α -amylase (47.42 \pm 0.89 U mL⁻¹) than control medium (43.63 \pm 1.68 U mL⁻¹) which contained 6 gL⁻¹ peptone (Table 4). However the growth of *B. licheniformis* ATCC 6346 was reduced when peptone was replaced with amino acids mixture at 48 hours (Results are not shown). This could be because peptone contains not only amino acids but also other important nutrients such as vitamins and minerals essential for the growth of bacteria. The enzyme production in the medium with peptone and mixture of amino acids was 52.39 \pm 1.23 U mL⁻¹ and this was 12.5 % higher than that in the medium without peptone. Therefore the addition of peptone is essential and the amino acid mixture alone cannot completely replace peptone for the production of α -amylase by *B. licheniformis* ATCC 6346. Based on the experimental results, eight amino acids which influenced the α -amylase production above 49

Table 4: Effect of amino acids (0.2 gL⁻¹) and casamino acids (6 gL⁻¹) on the production of α -amylase by *B. licheniformis* ATCC 6346 in Medium (in presence and absence of 6 gL⁻¹ peptone) while shaking (100 rpm) at 48 h and at 42 °C

| Nitrogen sources | α -Amylase (U mL ⁻¹) |
|--|---|
| Control ^A | 43.63 \pm 1.68 |
| All amino acids ^B (with peptone) | 52.39 \pm 1.23 |
| All amino acids ^B (without peptone) | 47.42 \pm 0.89 |
| Eight amino acids ^C + Peptone | 51.03 \pm 1.34 |
| Peptone + Casamino acids | 41.75 \pm 1.28 |

^A Control medium containing 6gL⁻¹ peptone

^B Mixture of seventeen amino acids such as glycine, methionine, proline, lysine, leucine, threonine, serine, arginine, alanine, glutamic acid, tryptophan, glutamine, asparagine, histidine, valine, phenylalanine and isoleucine.

^C Mixture of eight amino acids such as lysine, threonine, serine, arginine, tryptophane, glutamine, histidine and isoleucine

Note: Values are given as Mean \pm SD of triplicate experiments.

U mL⁻¹ were selected. This was 10 % above that produced in control medium. When the mixture of eight amino acids (0.2gL⁻¹ of lysine, threonine, serine, arginine, tryptophan, glutamine, histidine and isoleucine) were supplemented to medium which contained 6 gL⁻¹ peptone (Table 4), α -amylase production was increased by 16.96 % (51.03 \pm 1.34 U mL⁻¹) at 42 °C, 100 rpm and at 48 h (Table 4) than in the control medium (43.63 \pm 1.68 U mL⁻¹). The mixture of all 17 amino acids with peptone in medium gave better results than that with mixture of eight amino acids with peptone (Table 4).

When 6 gL⁻¹ peptone in the medium was replaced with 6 gL⁻¹ casamino acids, production of α -amylase in casamino acids containing medium (39.83 U mL⁻¹) was less than that in the control medium (44.43 U mL⁻¹) which contained 6 gL⁻¹ peptone (Vengadaramana *et al.*, 2011). Further the production of α -amylase in medium containing 6 gL⁻¹ of casamino acids and peptone was also less (41.75 \pm 1.28 U mL⁻¹) than that in the control (43.63 \pm 1.68 U mL⁻¹) medium (Table 4). Therefore casamino acids have inhibited the production of α -amylase. Salt content present in casamino acids was higher than that of salt present in peptone. Therefore the high salt content could be inhibiting α -amylase production in casamino acids containing medium.

CONCLUSION

Amino acids are essential for growth and enzyme production of Bacteria. Some amino acids have inhibited the production of α -amylase while some have increased the enzyme production. Therefore amino acid needs are

specific for different bacteria. From these experiments it can be concluded that tryptophan and lysine are essential for production of α -amylase by *B.licheniformis* ATCC 6346.

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