

# Enhanced production of maltase ( $\alpha$ -glucosidase) from newly isolated strain of *Bacillus licheniformis* KIBGE-IB4

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**Abstract:** Maltase ( $\alpha$ -glucosidase) hydrolyzes  $\alpha$ -(1 $\rightarrow$ 4) glucosidic bond of maltose into two glucose molecules. It is widely used in various foods, beverages and also in textile and biofuel industries. During current study, various physicochemical parameters for maltase production from newly isolated strain of *Bacillus licheniformis* KIBGE-IB4 were optimized using one-factor-at-a-time methodology. It was found that *Bacillus licheniformis* KIBGE-IB4 produced maximum maltase at 37°C, pH-7.0 after 48 hours using wheat starch (2.5%) as carbon source along with peptone (1.0%), yeast extract (0.2%) and meat extract (0.4%) as nitrogen sources in fermentation medium. It is concluded that the optimization of various medium ingredients and conditions increases maltase production upto 6.74 fold from *B. licheniformis* KIBGE-IB4 as compared to previously reported media and this strain could be used for the commercial production of maltase.

**Keywords:** Maltase, maltose, wheat starch, fermentation, *Bacillus licheniformis*.

## INTRODUCTION

Maltase [EC 3.2.1.20] which is also known as  $\alpha$ -glucosidase is an amylolytic enzyme that catalyzes the degradation of maltose into two units of  $\alpha$ -D-glucose by hydrolysis of  $\alpha$ -(1 $\rightarrow$ 4) glycosidic linkage. Maltase is the second most essential enzyme, which is involved in the early phase of enzymatic degradation of unrefined starch (Emmanuel *et al.*, 2000). Maltase is mainly used for the enzymatic production of high-glucose syrup from starch beside brewing and distilling industries. Maltase ( $\alpha$ -glucosidase) could also be useful for the enzymatic manufacturing of pharmaceuticals and fine chemicals (Teague and Brumm, 1992). Clinically, this important enzyme is used for the treatment of pompe disease, caused due to the deficiency of maltase (Wagner, 2007). Maltase is classified into three types: Type I which hydrolyses sucrose and aryl glucosides more rapidly than maltose; type II prefers maltose and isomaltose as a substrate and has low activity toward aryl glucosides; Type III has specificity similar to type II but it also attacks polysaccharides such as amylose and starch (Frandsen and Svensson, 1998). Maltose is a low molecular weight disaccharide of two units of glucose, widely used as a sweetening agent and present in different fermented foods (Shirokane *et al.*, 2000).

Maltase has been produced by different live forms present on earth including plants (Marshall, 1971); animals (Lejeune *et al.*, 1963) and microorganisms (Gupta *et al.*, 2003). The microbial sources are more preferable for the industrial production of maltase due to its cost effectiveness, consistency, less time and space required

for the production and also due to the effortlessness of process modification and optimization (Gupta *et al.*, 2003). However, the cost of enzyme production is a major limitation for its commercialization and it is mainly depends on the physico-chemical fermentation conditions (Beg *et al.*, 2002; Nascimento and Martins, 2004). It is necessary to explore novel enzyme from microbial source with cost effective production for different industrial applications. The current study was designed to isolate maltase producing strain and optimization of fermentation conditions for maltase production by *Bacillus licheniformis* KIBGE-IB4.

## MATERIALS AND METHODS

### *Screening and selection of maltase producing bacterial strain*

Five newly isolated strains of *Bacillus licheniformis* were screened for maltase production. For this purpose standardized inocula of all the strains were inoculated in maltose containing medium and incubated at 37°C for 24 hours using batch fermentation technique (Suzuki *et al.*, 1976a). After 24 hours of fermentation the cells were harvested by centrifugation at 10,000 rpm for 10 minutes at 4°C and cell free filtrate (CFF) was used for further studies.

### *Determination of maltase activity*

The activity of maltase was detected by the estimation of glucose released after the enzymatic action of maltase on maltose (5.0mM in 100mM potassium phosphate buffer of pH 6.5) by glucose oxidase (GOD-PAP) method (Trinder 1969a; Trinder 1969b) using glucose as a standard. One unit of maltase was defined as! "Amount of

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enzyme required to release 1.0  $\mu$ mol of glucose per minute under the standard assay conditions”.

#### **Estimation of total protein**

The total protein was estimated by Lowry’s method using BSA (bovine serum albumin) as a standard (Lowry *et al.*, 1951).

#### **Selection of medium for maltase production**

The influence of various nutritional components on maltase production from *Bacillus licheniformis* KIBGE-IB4 was investigated in different previously reported media; Medium-1 (Suzuki *et al.*, 1976a), Medium-2 (Mcwethy and Hartman, 1979), Medium-3 (Gupta and Gautam, 1993), Medium-4 (Jansen *et al.*, 2006) Medium-5 (Suzuki *et al.*, 1976b) and Medium-6 (Thirunavukkarasu and priest 1984) at 37°C for 24 hours. After 24 hours of incubation the cells were harvested and CFF was used for the enzyme activity.

#### **Influence of various carbon sources on maltase production**

For the optimization of maltase production different available pure carbon sources including wheat starch, potato starch, commercial starch (Arrowroot), general soluble starch (Corn starch) were incorporated in separate media as carbon source to investigate the role of different available carbon sources on the production of maltase.

#### **Influence of different nitrogen sources on maltase production**

For the selection of appropriate nitrogen source for the maltase production, *B. licheniformis* KIBGE-IB4 was inoculated in the media containing different nitrogen sources such as yeast extract, tryptone, ammonium sulphate, ammonium acetate, ammonium chloride, sodium nitrate, potassium nitrate and urea and incubated at 37°C for 24 hours.

#### **Modification of medium components**

Various concentrations of peptone (0.0-2.5%), yeast extract (0.0-0.5%) and meat extract (0.0-1.0%) were incorporated in the production medium for the selection of appropriate concentration of these ingredients in medium for the maximum maltase production.

#### **Influence of substrate concentration on maltase production**

Concentration of wheat starch in the production medium was varied from 0.0-3.0% to optimize the substrate concentration for the maximum maltase production.

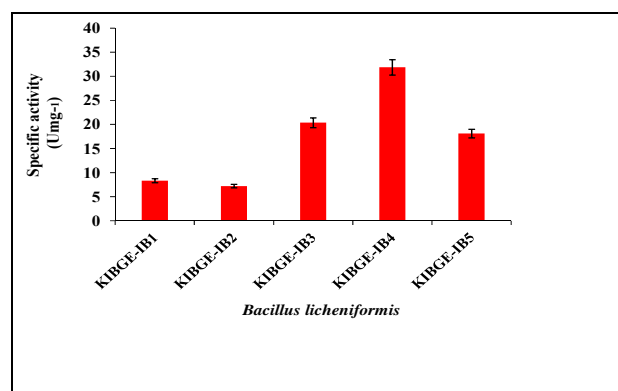
#### **Effect of temperature, pH and incubation time on maltase production**

For the determination of optimum temperature for maximum maltase production from *B. licheniformis*

KIBGE-IB4, the fermentation was carried out at different temperatures (30°C-60°C) for 24 hours. For the selection of optimum pH for maltase production, pH of the production medium was varied from pH4-9 and incubated at 37°C for 24 hours. In order to optimize the fermentation time for maximum maltase production from *B. licheniformis* KIBGE-IB4, the strain was incubated for different time intervals (6.0-96.0 hours) at constant pH (7.0), temperature (37°C) and substrate concentration (2.5 %).

## **RESULTS**

The genomic 16S rDNA sequence analysis data for *Bacillus licheniformis* KIBGE-IB4 used in the current study was deposited in the Genbank with an accession number: GU216161 (Ghani *et al.*, 2013). Quantitative screening for five available strains of *B. licheniformis* through batch fermentation technique revealed that *B. licheniformis* KIBGE-IB4 is capable of producing maximum extracellular maltase among all the strains tested (fig. 1). After 24 hours of incubation *B. licheniformis* KIBGE-IB4 produced 31.88 U mg<sup>-1</sup> of maltase. Initially, several reported media were used to test for extracellular maltase production and among the tested media, the nutritional components of medium-5 were found more supportive for maltase production (fig. 2). After medium selection, various pure carbon sources were incorporated and it was detected that in the presence of wheat starch maximum maltase production was achieved (table 1). Afterwards the major components of the selected medium were varied in terms of their concentrations in order to enhance the production of the enzyme and it was observed that maltase production can be increased by varying the concentration of the different components of the medium.



**Fig. 1:** Selection of bacterial strain for maximum maltase production.

When the nitrogen source was varied, maximum maltase production was achieved in the presence of peptone (1.0%), yeast extract (0.2%) and meat extract (0.4%). Upon further variation in the concentration of these

nitrogen sources, a decline in enzyme production was noticed (table 2). However in the presence of other organic nitrogen sources such as urea, the enzyme production reduced. In case of inorganic nitrogen sources, maltase production declined and a profound lost in the enzyme production was witnessed under the influence of ammonium sulphate, ammonium chloride, potassium nitrate, sodium nitrate and ammonium acetate (table 3). The use of suitable substrate concentration in the production medium is also very important for optimum enzyme production. The effect of substrate concentration on maltase production by *B. licheniformis* KIBGE-IB4 was monitored at different concentrations of wheat starch in the fermentation medium and it was found that maximum maltase production was achieved at 2.5% concentration (fig. 3). It was also found that low concentrations of substrate cause lower induction of maltase and no enzyme production was found in the absence of a suitable substrate.

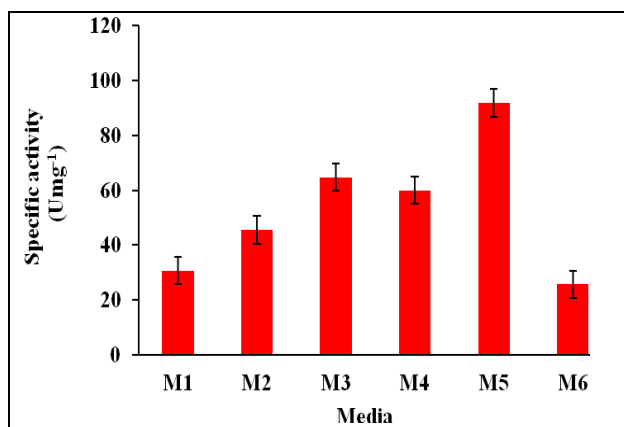


Fig. 2: Effect of different reported media on maltase production by *Bacillus licheniformis* KIBGE-IB4.

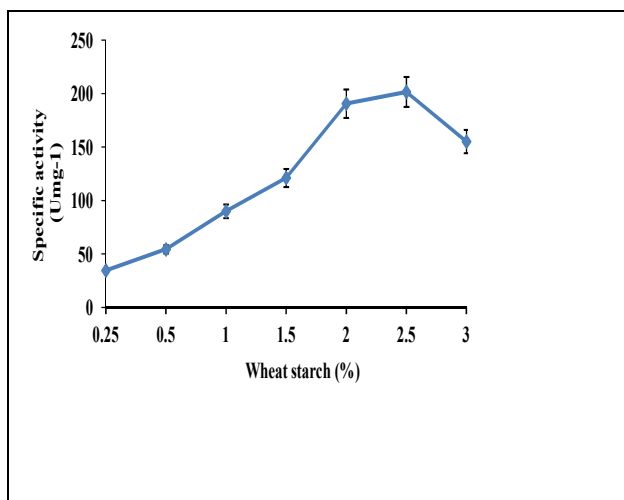


Fig. 3: Influence of various concentrations of substrate on maltase production by *B. licheniformis* KIBGE-IB4.

Table 1: Influence of various pure carbon sources on maltase production by *B. licheniformis* KIBGE-IB4.

Carbon source (2.0%)	Specific activity (Umg <sup>-1</sup> )
Wheat starch	190.00±9.50
Soluble starch (Potato starch)	160.37±8.01
Commercial starch (Arrow root)	77.83±3.89
Soluble starch (Corn)	42.64±2.13

Table 2: Influence of various concentrations of yeast extract, peptone and meat extract on the production of maltase from *Bacillus licheniformis* KIBGE-IB4.

Yeast extract (%)	Specific activity (Umg <sup>-1</sup> )
0.1	98.09±4.90
0.2	163.76±8.18
0.3	113.85±5.69
0.4	99.36±4.96
0.5	47.12±2.35
Peptone (%)	
0.25	90.37±4.51
0.5	120.78±6.03
0.75	143.01±7.15
1.0	195.88±9.79
1.5	130.07±6.50
2.0	74.71±3.73
2.5	50.60±3.08
Meat extract (%)	
0.1	90.45±4.52
0.2	119.92±5.99
0.3	150.62±7.53
0.4	196.33±9.81
0.5	160.96±8.04
0.6	130.64±6.53
0.7	80.11±4.00
0.8	45.50±2.27

Table 3: Influence of various nitrogen sources on the production of maltase from *Bacillus licheniformis* KIBGE-IB4.

Nitrogen Source (0.2 %)	Specific activity (U mg <sup>-1</sup> )
Yeast extract	180.23±9.01
Tryptone	64.40±3.22
Ammonium sulphate	40.98±2.04
Ammonium acetate	37.01±1.85
Ammonium chloride	31.19±1.55
Sodium nitrate	30.19±1.50
Potassium nitrate	27.75±1.38
Urea	130.50±6.52

The effect of temperature on maltase production was also evaluated by cultivating *B. licheniformis* KIBGE-IB4 at different temperatures and the maximum production was achieved at 37°C. Any temperature change, either

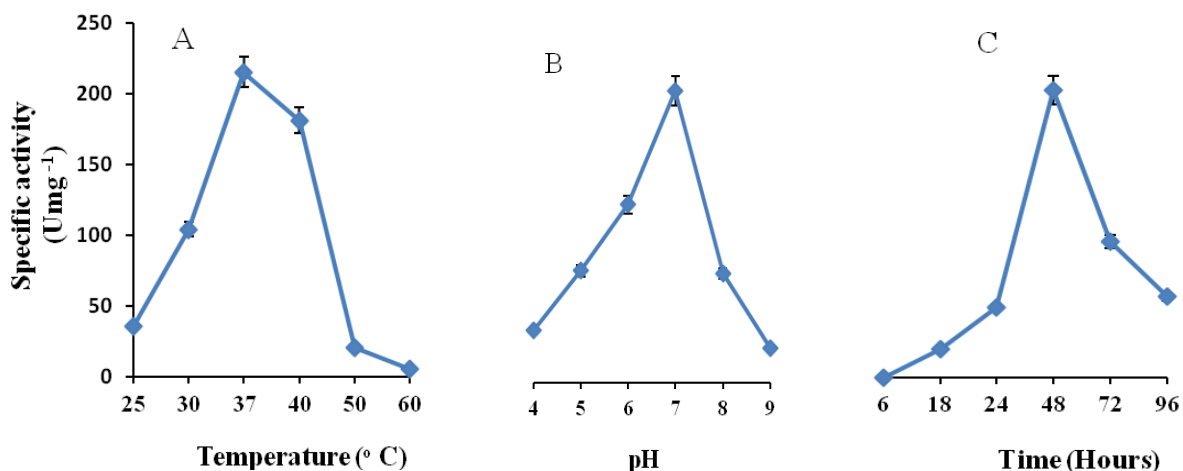


Fig. 4: Effect of Temperature, pH and time course on Maltase production by *B. licheniformis* KIBGE-IB4

decrease or increase from the optimum temperature, resulted in the reduction of maltase production (fig. 4A). When the effect of pH (4.0 - 9.0) was studied on maltase production, it was observed that the production of enzyme gradually increases from pH-4.0 to pH-6.0 with a maximum at pH-7.0 (fig. 4B). Further increase in pH of the medium beyond optimum pH leads towards decline and only 11.76 % maltase was produced at pH-9.0. The time course for maltase production was also studied and the bacterium was grown in the selected production medium at 37°C up to 96 hours and enzyme production was monitored after regular time intervals. It was detected that the enzyme production started after 18 hours of incubation and reached to its maxima at 48 hours (fig. 4C) and after this point the production started to decline.

## DISCUSSION

In the current study various fermentation conditions including physicochemical parameters were optimized to enhance the production of maltase from *B. licheniformis* KIBGE-IB4. After analyzing the results, it is suggested that maltase production is exclusively dependent on the carbon sources used and any variation in the utilization of carbon source can affect the enzyme production. The selective inducer of maltase for this strain was wheat starch. The physicochemical characteristics as well as the amylose and amylopectin content of starch plays an important role for its utilization as a carbon source by the microbes for enzyme production (Lehmann and Robin, 2007; Cummings and Roberfroid, 2004; Kaur *et al.*, 2007; Singh *et al.*, 2010). Among various carbon sources, wheat starch was found to be the suitable substrate in term of its composition and ionic state for the production of maltase by *B. licheniformis* KIBGE-IB4. Any increase in the concentration of wheat starch resulted in the reduction of enzyme production, which could be due to the substrate inhibitory effect on maltase but sometime the depletion of

the nutritional components of the medium and oxygen supply can also ultimately effect the microbial growth which might leads towards low enzyme production. Along with this various organic and inorganic nitrogen source also have a crucial role on enzyme productivity and *B. licheniformis* KIBGE-IB4 is capable of producing maximum maltase by utilizing only more complex organic nitrogen sources as compared to inorganic sources.

Incubation temperature always plays a key role on the production and stability of the enzymes. The optimum temperature for the growth of microorganisms usually varies from one microorganism to another (Banargee and Bhattacharya, 1992; Kumar and Takagi, 1999) Therefore, any decline in the enzyme production at high temperature might be due to the reason that the temperature enhanced the metabolic activity of microbial cells by increasing the kinetic energy of biomolecule and after reaching a certain level it started to denature these biomolecules which finally ceased the growth as well as the enzyme production. The results reveal that *B. licheniformis* KIBGE-IB4 is mesophilic in nature and can therefore produce maximum maltase at 37°C. Similar findings were also reported by Li and Chan (1983) during the production of  $\alpha$ -Glucosidase from *Lactobacillus acidophilus*. pH is also a very significant physical factor affecting microbial growth and metabolites production (Banargee and Bhattacharya, 1992). Most of bacterial strains can cultivate at pH in the range of 6.0 to 7.0 for the growth and enzyme production (Gupta *et al.*, 2003; Kundu *et al.*, 1973; Castro *et al.*, 1992) and any change in the pH of the medium may influence the ionization state of various nutritionally important components and can also reduce their availability for microbial cells. Slight decrease in the enzyme production may also be due to the two important factors first, the accumulation of toxic byproducts and secondly the depletion of important

nutrients in the medium, which can halt further multiplication of the bacterial cells responsible for the secretion of extracellular enzyme. Similar results were observed when thermophilic *Bacillus sp.* KP 1035 was used for maltase production (Suzuki *et al.*, 1976). The most important factor about this study is the less fermentation time as *Bacillus licheniformis* KIBGE-IB4 can produce higher quantity of maltase in less fermentation time (48 hours) as compared to the previously reported fungal strains, which showed 120 hours of incubation time (Gianesi *et al.*, 2006).

## CONCLUSION

Various strains of *B. licheniformis* were screened for maltase production and among them *B. licheniformis* KIBGE-IB4 showed maximum maltase production. The physicochemical parameters of fermentation were optimized using one-factor-at-a-time methodology to enhance the production of maltase. *Bacillus licheniformis* KIBGE-IB4 produced maximum maltase by utilizing wheat starch (2.5%) as carbon source and peptone (1.0%), yeast extract (0.2%) and meat extract (0.4%) as nitrogen sources at 37°C, pH-7.0 after 48 hours of incubation time. In a nutshell, the hyper production of maltase by optimizing physicochemical parameters of fermentation could be beneficial for the commercial production.

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