J Biochem Tech (2015) 6(3): 1030-1033
ISSN: 0974-2328

Cold active alpha amylase from a psychrophilic bacterial isolate

Kalpana Hiteshi, Supriyanka Rana and Reena Gupta*

Received: 17 March 2015 / Received in revised form: 08 September 2015, Accepted: 03 March 2016, Published online: 25 March 2016

Abstract

In the present study an attempt was made to isolate a novel psychrophilic bacterial strain producing cold active alpha amylase. Among the 16 isolates observed on starch agar plate, six showed zone of hydrolysis on treatment with Gram's iodine. Out of these 6 isolates, isolate KS-5 gave maximum alpha amylase production. During optimization of production conditions, 6 days old inoculum at a concentration of 0.5% gave maximum production of α-amylase when the inoculated production medium was incubated for 96 h. The isolate gave maximum production of α-amylase in medium 5 with starch as carbon source at a concentration of 9%. Approximately 2-fold increase in activity was observed after the optimization of above parameters, further on complete optimization of production conditions, a great increase in amylase production can be achieved. The properties of this cold active α-amylase suggest its usefulness in commercial sectors.

Keywords: Starch, Fermentation, Optimization, Alpha amylase, Cold-active.

Introduction

α-Amylase (E.C.3.2.1.1) is the enzyme that acts upon the starch to yield glucose and maltose by catalysing the hydrolysis of internal α-1, 4-glycosidic linkages (Sundarram and Murthy 2014). There is a great demand for microbial amylase production now-a-days because of its immense importance in wide spectrum industries (Saha et al 2014). Among all the industrial enzymes, amylase is one of the most important enzymes. This enzyme has completely replaced chemical hydrolysis of starch in starch processing industries.

Kalpana Hiteshi, Supriyanka Rana and Reena Gupta*

Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla-171005

*Tel: +91-177-2831948;
*E-mail: reenagupta_2001@yahoo.com

Extremozymes are the enzymes from extremophiles having great stability at extreme temperatures. As these are active under conditions that were previously regarded as incompatible, the extremozymes are gaining a great attention now-a-days (Hiteshi and Gupta, 2014). Cold active α-amylases are active at low temperatures as they have very low activation energies. This property of enzymes is of great importance as this leads to energy saving (Kuddus et al. 2014).

Few literatures are available on production of α-amylase from psychrophilic isolate. Therefore, the main objective of this research work was to isolate a novel cold active α-amylase producing psychrophilic isolate.

Materials and Methods

Starch soluble used was from S D Fine Chemicals Ltd., dinitrosalicylic acid and nutrient agar used were from HIMEDIA, Mumbai, India. All other chemicals were of analytical grade procured from various commercial sources.

Sample collection

Samples were collected from different cold areas. Four soil samples were collected from Kufri, Distt. Shimla. One soil sample was collected from Tapan Valley, Distt. Lauhal Spiti. One soil sample and one water sample was collected from Narkanda, Distt. Shimla. Four ice-cream samples were collected from Shimla.

Preliminary screening of amylase producing microorganisms

Samples were inoculated in minimal medium containing (g/L) Disodium hydrogen phosphate 2.5; Potassium dihydrogen phosphate 2.0; Magnesium sulphate 0.5; Ferrous sulphate 0.03; Calcium chloride 0.06 and Lactose 10.0 with 2% starch. These were incubated at 4°C for 3 days. After 3 days, 1 ml from each flask was inoculated in fresh medium and again incubated at 4°C for 3 days. After 3 days incubation, samples were spread on plates containing nutrient agar with 2% starch and were incubated at 4°C for 15 days.

After incubation, plates were flooded with Gram's iodine to observe a halo formation around the amylase producing colonies.

Secondary screening of amylase producing isolates

The seed culture for each of the amylolytic bacterial strain was prepared in LB broth (Tryptone 1%, yeast extract 0.5% and NaCl 1% w/v, pH 7.0) and was incubated for 4 days at 4°C in refrigerator. After 4 days incubation, 500 µl sample was inoculated in production medium containing g/L, soluble starch, 50; yeast extract, 0.5; KH_2PO_4, 10; $(NH_4)_2SO_4$, 10.5; $MgSO_4.7H_2O$, 0.3; $CaCl_2$, 0.5; $FeSO_4.7H_2O$, 0.013; $MnSO_4.7H_2O$, 0.004; $ZnSO_4.H_2O$, 0.004 and $CoCl.6H_2O$, 0.0067 (Kathiresan and Manivannan, 2006). The autoclaved production media were inoculated with different isolates and incubated at 4°C for 4 days.

Enzyme assay

Amylase assay was performed by spectrophotometeric method described by Sengupta *et al.* (2000), using starch as substrate and DNS as coupling reagent.

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of reducing sugars as glucose per minute under standard assay conditions.

Morphology and Gram's staining of α-amylase producing bacterium

The colony morphology was noted with respect to colour, shape, size, nature of colony and pigmentation. The bacterial isolate was Gram stained and observed under a high power magnifying lens in light microscope.

Studies on production of cold active alpha amylase from psychrophilic bacterial isolate

Inoculum age was optimized by incubating the seed culture at 4°C for 8 days. Sample was taken from the seed culture after every 24 h and checked for its absorbance at 600 nm and also for the amylase activity. The inoculum size was optimized by incubating the production medium with a range of inoculum sizes (0.2%, 0.5%, 1%, 1.5%, 2% and 2.5%) and incubated at 4°C for optimized inoculum age. The amylase activity was observed after incubation.

To study the optimal incubation time for the maximum production of enzyme, the production medium was incubated in the shaker for the time intervals of 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h and amylase activity was then determined. Various reported media (Medium 1, (Ozean et al. 2010); Medium 2, (Srivastava and Baruah 1986); Medium 3, (Bhaskara et al. 2011); Medium 4, (Dalvi and Anthappan 2007); Medium 5, (Kathiresan and Manivannan 2006); Medium 6, (Hamilton et al. 1999); Medium 7, (Makky 2009) were used to optimize nutrient medium for maximum enzyme production. Various carbon sources such as sodium-acetate, sodium-citrate, sucrose, glycerol, starch and wheat flour were used in the production medium at a concentration of 5% (w/v) to check the effect of carbon source on α-amylase production. The culture supernatants were assayed for α-amylase activity. To optimize the concentration of carbon source for maximal enzyme production the selected carbon source was used at different concentrations [0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10% (w/v)] in the production medium and the culture supernatant was assayed for the enzyme activity.

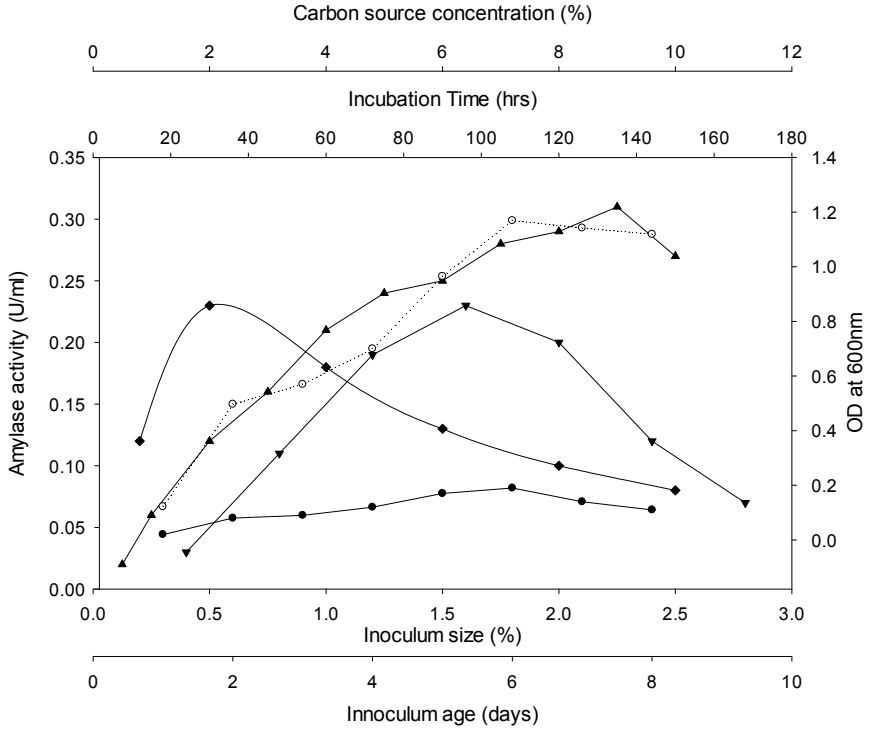

Figure 1a. Effect of inoculum age on production of α-amylase from the psychrophilic bacterial isolate KS-5 (●) and OD at 600nm (o). b. Effect of inoculum size on production of α-amylase from the psychrophilic bacterial isolate KS-5 (♦). c. Effect of incubation time on production of α-amylase from the psychrophilic bacterial isolate KS-5 (▼). and d. Effect of concentration of carbon source on production of α-amylase from the psychrophilic bacterial isolate KS-5 (▲).

Results and discussion

Isolation of amylase producing bacteria

A total of 16 different bacterial strains were isolated on starch agar plates (Nutrient agar containing 2% starch).

All isolates were primarily screened for amylase production by starch plate method. Among 16 isolates, 6 isolates hydrolysed the starch on starch agar plate showing zone on treatment with Gram's iodine.

Secondary screening of bacterial isolates

Among 16 isolates, 6 isolates were screened on the basis of their amylase activity at 4°C. Out of these isolates, isolate KS-5 (Table 1) showed maximum activity and was selected for further studies.

Table 1 Amylase activity shown by 6 different isolates

Isolate Number	Enzyme activity (U/ml)
KS-1	0.023
KS-2	0.021
KS-3	0.020
KS-4	0.021
KS-5	0.19
KS-6	0.086

Morphology and Gram's staining of α-amylase producing bacterium

The isolated bacterial culture showed round configuration of colony with smooth margins, raised and smooth surface, opaque density and off-white in colour. The bacterial isolate KS-5 was observed to be Gram –ve.

Studies on production of cold active alpha amylase from psychrophilic bacterial isolate

Six days old inoculum gave maximum production of amylase from bacterial isolate KS-5 (Figure 1a). Biomass yield was found to increase with an increase in the inoculum age. Cell mass also increased upto six days, after which it attained stationary phase. These results suggested that enzyme production has direct relationship with cell growth. Inoculum size affects substrate utilization rate. It also affects the growth and primary metabolic production (Shah et al. 2014).

Maximum amylase production was observed with 0.5% of inoculum size (Figure 1b). In earlier studies, 4% inoculum was used for α-amylase production from source (Jogezai et al. 2011). Metabolic activity and growth are dependent on time of incubation. The incubation time varied between different enzymes produced from one substrate (Shah et al. 2014). During optimization of incubation time, 96 h of incubation gave maximum production of enzyme (Figure 1c). After further incubation, a decrease in amylase production was observed. This might be due to the accumulation of other by-products after certain period of incubation (Riaz et al. 2014).

In previous studies, incubation time for the production of α-amylase from *Microbacterium foliorum* GA2 from Gangotri Glacier was found to be about 120 h (Roohi et al. 2011). Out of various media used for the best production of amylase from bacterial isolate KS-5, medium 5 containing (g/L) Soluble starch 50.0, Yeast extract 0.5, Potassium dihydrogen phosphate 10.0, Ammonium sulphate 10.5, Magnesium sulphate 0.3, Calcium chloride 0.5, Ferrous sulphate 0.013, Manganese sulphate 0.004, Zinc sulphate 0.004 and Cobaltous chloride 0.0067 showed maximum activity (Figure 2). Similar medium was used for α-amylase production by *Penicillium fellutanum* (Kathiresan and Manivannan 2006).

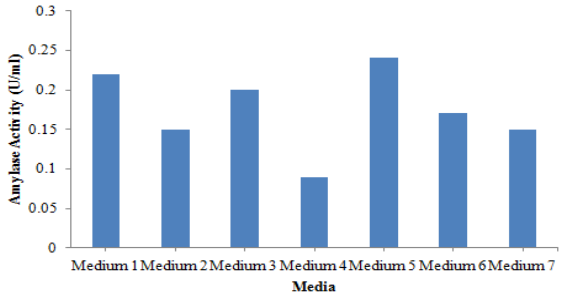

Figure 2: Optimization of medium for production of α-amylase from the psychrophilic bacterial isolate KS-5.

Carbon is a major component of the cell and the rate at which a carbon source is metabolized can often influence the production of metabolites. Among the various carbon sources used for optimization, starch was found to be the best carbon source for maximum α-amylase production (Figure 3). Similar results were observed by Stergiou *et al*. for amylase production from *Kluyveromyces marxianus* IF0 0288 (Stergiou et al. 2014).

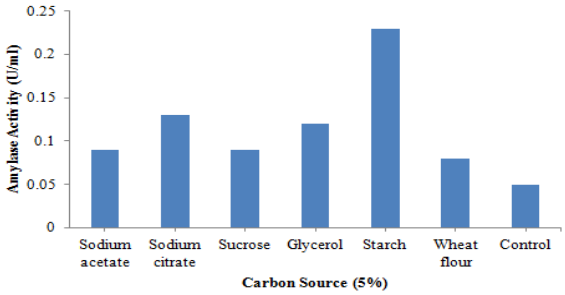

Figure 3: Effect of carbon source on production of α-amylase from the psychrophilic bacterial isolate KS-5.

Maximum amylase production was achieved with 9% of substrate (starch) used. Further increase in concentration led to a decrease in activity (Figure 1d).
In a previous study, media supplemented with 1% (w/v) starch supported maximum amylase enzyme yield from *Bacillus* sp. (Zohra and Ahmad 2012).

Conclusion

Though some preliminary enzymatic parameters like inoculum age and size, incubation time, media, carbon source and its concentration have been determined for production of alpha amylase from a bacterial isolate which led to approximately 2-fold increase in activity, further on complete optimization of production conditions, a great increase in amylase production can be achieved.

References

Bhaskara KV, Kumar A, Kumar G, Karthik L (2011) Optimization, production and partial purification of

extracellular α-amylase from *Bacillus* sp. Marini. Arch Appl Sci Res 3: 33-42.

Dalvi P, Anthappan P (2007) Amylase and pectinase from single source for simultaneous desizing and scouring. Indian J Fibre Text Res 32: 459-465.

Hamilton LM, Kelly CT, Fogarty WM (1999) Production and properties of the raw starch-digesting α-amylase of *Bacillus* sp. IMD 435. Process Biochem 35: 27-31.

Hiteshi K, Gupta R (2014) Thermal adaptation of α-amylases: a review. Extremophiles 18: 937-944.

Jogezai N, Raza A, Abbas F, Bajwa M, Mohammad D, Kakar W, Saeed M, Awan A (2011) Optimization of cultural conditions for microbial α-amylase production. J Microbiol Antimicrob 3: 221-227.

Kathiresan K, Manivannan S (2006) α-Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. Afr J Biotechnol 5: 829-832.

Kuddus M, Roohi, Arif JM, Ramteke PW (2014) An overview of cold-active microbial a-amylase: adaptation strategies and biotechnological potentials. Biotechnol 10: 246–258.

Makky EA (2009) Comparison of osmotic stress on growth and pectinase production by *Aspergllus flavus* in liquid and solid state cultures. Asian J Exp Sci 23: 19-26.

Ozean BD, Baylan M, Ozean N, Tekdal D (2010) Characterization of thermostable α-amylase: Characterization of thermostacle α-amylase from thermophillic and alkaliphillc *Bacillus* sp. isolate DM-15. Res J Biol Sci 5: 118-124.

Riaz N, Haq IU, Qadeer MA (2014) Characterization of α-amylase bi *Bacillus subtilis*. Inter J Agric Biol 5: 249-252.

Roohi, Kuddus M, Ahmad IZ, Arif JM (2011) Production of Cold-active Extracellular α-Amylase by Newly Isolated *Microbacterium foliorum GA2* from Gangotri glacier, Western Himalaya, India. Asian J Biotech 3: 449-459.

Saha K, Maity S, Roy S, Pahan K, Pathak R, Majumdar S, Gupta S (2014) Optimization of amylase production from *Bacillus amyloliquefaciens* (MTCC 1270) using solid state fermentation. Int J Microbiol 2014: 1-7.

Sengupta S, Jana ML, Sengupta D, Naskar AK (2000) A note on the estimation of microbial glycosidase activities by dinitrosalicylic acid reagent. Appl Microbiol Biotechnol 53: 732-735.

Shah IJ, Gami PN, Shukla RM, Acharya DK (2014) Optimization for α-amylase production by *Aspergillus oryzae* using submerged fermentation technology. Basic Res J Microbiol 1: 1-10.

Srivastava RAK, Baruah JN (1986) Culture conditions for the production of thermostable amylase by *Bacillus stearothermophilus*. Appl Env Microbiol 52: 179-184.

Stergiou PY, Foukis A, Theodorou L, Papagianni M, Papamichael E (2014) Optimization of the Production of Extracellular α-Amylase by *Kluyveromyces marxianus* IF0 0288 by Response Surface Methodology. Braz Arch Biol Technol 57: 421-426.

Sundarram A, Murthy TPK (2014) α-Amylase production and application: A review. J Appl Env Microbiol 2: 166-175.

Zohra RR, Ahmad M (2012) Optimization of cultural conditions for production of amylase from thermophilic *Bacillus* sp. Pak J Biochem Mol Biol 45: 99-103.