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Thermal Optimization of α-amylase Production in *Brevibacillus sp*

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Thermostability is a desired characteristic of most of the industrial enzymes. The production of α-amylases has been thoroughly investigated and observed that it was affected by a variety of physiochemical factors, such as the composition of the growth medium under various temperature parameters with constant pH 7.0. The enzyme production was assayed in submerged fermentation (SmF) condition. The optimum production of α-amylase by SmF was observed at 4^0C in Clarks and Lub Medium (2551 ± 1 U/ml), Soybean Casein Digest Medium (920± 0.13 U/ml) and Tryptone Glucose Beef Extract Medium (260 U/ml). The optimum production of α-amylase by SmF was observed at 25^0C in Nutrient Broth (270 U/ml). The optimum production of α-amylase by SmF was observed at 37^0C in Pikovskaya's Medium (3371 ± 0.50 U/ml), Tendler's Non-synthetic Medium (761 ± 0.50 U/ml), Amylase Production Medium (580 U/ml) and Soluble Starch Beef Extract Medium (1930 U/ml). The optimum production of α-amylase by SmF was observed at 50^0C in Luria Bertain Broth (240 ± 0.12 U/ml) and Yeast extract peptone Glycerol Glucose Medium (265 U/ml). Thermal variability of α-amylase produced by *Brevibacillus borostelensis* R1 in various media may have a significant role in many applications like bakery industry, treatment of effluents from sago and rice industry, sewage water treatment, fodder production, laundry industry and textile industry.

Introduction

High temperatures damage bacteria by denaturing enzymes, transport carriers, and other proteins. Bacterial membranes are also disrupted by temperature extremes; the lipid bilayer simply melts, disintegrates and leaks. At very low temperatures, membranes solidify and enzymes do not work rapidly. The broad range of temperatures and the enzyme's high activity at both moderate and lower temperature make this enzyme highly attractive for both basic research studies and industrial processes. A modern trend among consumers is to use colder temperatures for laundry or dishwashing. The removal of starch from cloth and porcelain becomes more problematic at low temperatures; to overcome this problem, detergents with α-amylases at low or moderate temperatures can be used (Van der Maarel, 2002). Thermostability is a desired characteristic of

most of the industrial enzymes. Thermostable α-amylases are reported from the mesophile *Bacillus species* Teodoro & Martin, 2000; Burhan *et al.,* 2003). Therefore, a high value is placed on extreme thermostability and thermoactivity of the enzymes. Many authors reported the α-amylase production in psychrophilic bacteria (Feller *et al.,* 1999; Michael *et al.,* 2005; Siddiqui *et al.,* 2006), mesophilic bacteria (Rajagopalan *et al.,* 2008; Kuddus *et al.,* 2012) and thermophilic bacteria (Mukherjee *et al.,* 2009; Asoodeh *et al.,* 2010; Abha Singh *et al.,* 2012; Akcan *et al.,* 2012; Sailas Benjamin *et al.,* 2013).

Materials and Methods

Collection of the Marine Water Samples

Marine water samples were collected from coastal areas of Visakhapatnam across the Bay of Bengal, Rushikonda(R), Visakhapatnam, Andhra Pradesh, India. The water samples were collected from the above site in sterile BOD bottles (Borosil) and brought to the laboratory for study.

Primary Screening of α-amylase Producing Bacteria

The collected marine water samples were diluted by serial dilution technique. The diluted samples of 10^{-4} to 10^{-6} (0.1ml) were spreaded with L-shaped glass rod by spread plate technique on the starch agar plates. After incubation at 37^0C for 24hours, the plates were flooded with Lugol solution (1% iodine in 2% potassium iodide w/v) (Amoozegar *et al.,* 2003). The zone of hydrolysis measuring more than 11mm were selected for further screening of amylase activity.

Optimization of Temperature

Hundred ml of the ten media: Nutrient Broth (NB), Luria Bertain Broth (LB), Clarks and Lub Medium (CL), Pikovskaya's Medium (PK), Tendler's Non-synthetic Medium (TNS), Amylase Production Medium (APM), Soluble Starch Beef Extract Medium (SB), Soybean Casein Digest Medium (SCD), Yeast Extract peptone Dextrose Glucose Medium (YPDG) and Tryptone Glucose Beef Extract (TGB) Medium are procured from Himedia, India were taken in Erlenmeyer flasks. Two percent of pure culture of *Brevibacillus borostelensis* R1 isolated from coastal waters of Bay of Bengal, Visakhapatnam from pre-incubated pure strain was inoculated to each of Erlenmeyer flask. All the ten media were shaken gently and incubated at different (4^0C, 25^0C, 37^0C, 50^0C and 60^0C) temperatures for 24 hours. After incubation, the sample was subjected for centrifugation (Remi) at 5,000 rpm for 15 minutes at room temperature. The supernatant was collected in sterile test tubes (Borosil) and the pellet was discarded.

Assay of α-amylase

The starch substrate [0.5ml of 0.5% in 0.1M phosphate buffer (pH 6.8)] was mixed with 1% (0.2ml) NaCl in a test tube and pre-incubated at 37^0C for 10 minutes. The supernatant collected from the centrifugation of the production media was used as enzyme source, 0.5ml of this was added to the reaction mixture. The reaction was terminated by the addition of 1.0 ml of 3, 5-dinitrosalicylic acid reagent [1.0 gm DNS in 0.8% NaOH, 60% Na K tartrate] after incubation at 37^0C for 15 minutes. The contents were mixed well and kept in boiling water bath for 10 minutes. Then they were cooled and diluted with 10 ml of distilled water. The color developed was read at 520nm in colorimeter. One unit of enzyme activity is defined as the amount of enzyme that that releases one micromole of maltose per minute under the standard assay conditions. Estimation of α-amylase activity

was carried out according to the dinitro salicylic acid (DNS) method (Miller, 1959). All chemicals are procured from MERCK, Mumbai, India. All the above experiments were carried out in quadrant sets and standard deviation was calculated.

Results and Discussion

The potent α-amylase producing *Brevibacillus borostelensis* R1 was isolated by using primary screening. The *B.borostelensis* R1 was identified by colony, morphological, biochemical and 16S gene sequencing. The scanning electron micrograph of *B. borostelensis* R1 is shown in figure1.

The highest α-amylase activity was observed in various media at different temperatures. At 4°C the optimum amylase observed in CL (2551 ± 1 U/ml), SCD (920± 0.13 U/ml) and TGB medium (260 U/ml); At 25°C the optimum amylase observed in NB (270 U/ml), at 37°C the optimum amylase observed in PK (3371 ± 0.50 U/ml), TNS medium (761 ± 0.50 U/ml), APM (580 U/ml) and SB medium (1930 U/ml). At 50°C the optimum amylase observed in LB medium (240 ± 0.12 U/ml) and YPDG medium (265 U/ml) the results are shown in figures 2 a-f and 2 g-j.

The lowest amylase activity was observed in different media at different temperatures. At 4°C the lowest amylase activity was observed in NB (80U/ml), LB medium (160U/ml), TNS medium (310U/ml), APM (200U/ml), SB medium (315U/ml). At 25°C the lowest amylase activity was observed in CL medium (940 U/ml) and TGB medium (120U/ml), at 50°C the lowest amylase activity was observed in SCD medium (110U/ml), at 60°C the lowest amylase activity was observed in PK medium (1280±0.25U/ml), at 4°C and 25°C the lowest amylase activity was observed in YPDG medium (170U/ml). The range of amylase activity observed in different media at different temperatures is shown in table 1.

The amylase produced by *Brevibacillus borostelensis* R1 at various temperature conditions (4°C, 25°C, 37°C, 50°C and 60°C) in ten different media was estimated. The outcome displayed the peak production of amylase occurred in psychrophilic condition when grown in media - CL, SCD and TGB; in mesophilic condition the peak production of amylase observed in NB, PK, TNS, APM and SB media and in thermophilic condition the highest production was observed in LB and YPDG media.

Figure.1 Scanning Electron Micrograph of *Brevibacillus borostelensis* R1

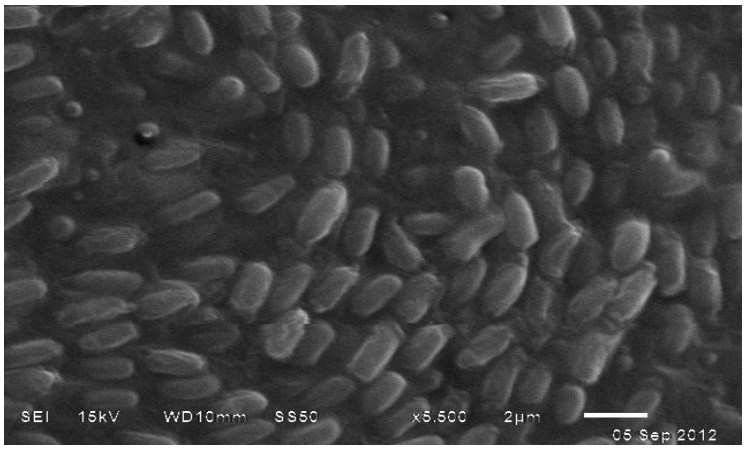

Figure.2 a-f Effect of Temperature on the Production of α-amylase by *Brevibacillus borstelensis* R1 in Various Media: a, NB; b, LB Medium; c, CL Medium; d, PK Medium;e, TNS Medium and f, APM

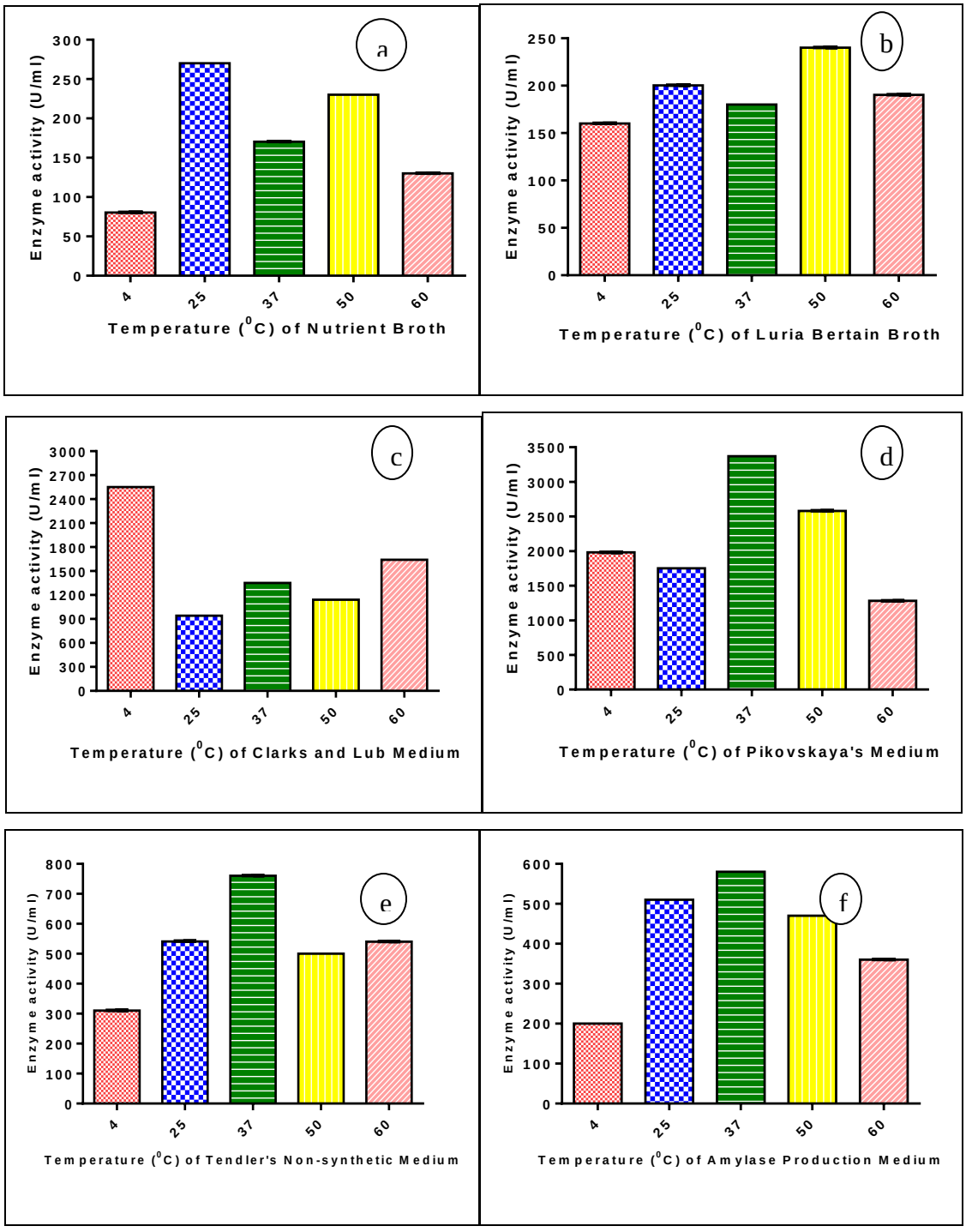

Figure.2 g-j Effect of Temperature on the Production of α-amylase by *Brevibacillus borstelensis* R1 in Various Media: g, SB Medium; h, SCD Medium; i, YPDG Medium and j, TGB Medium

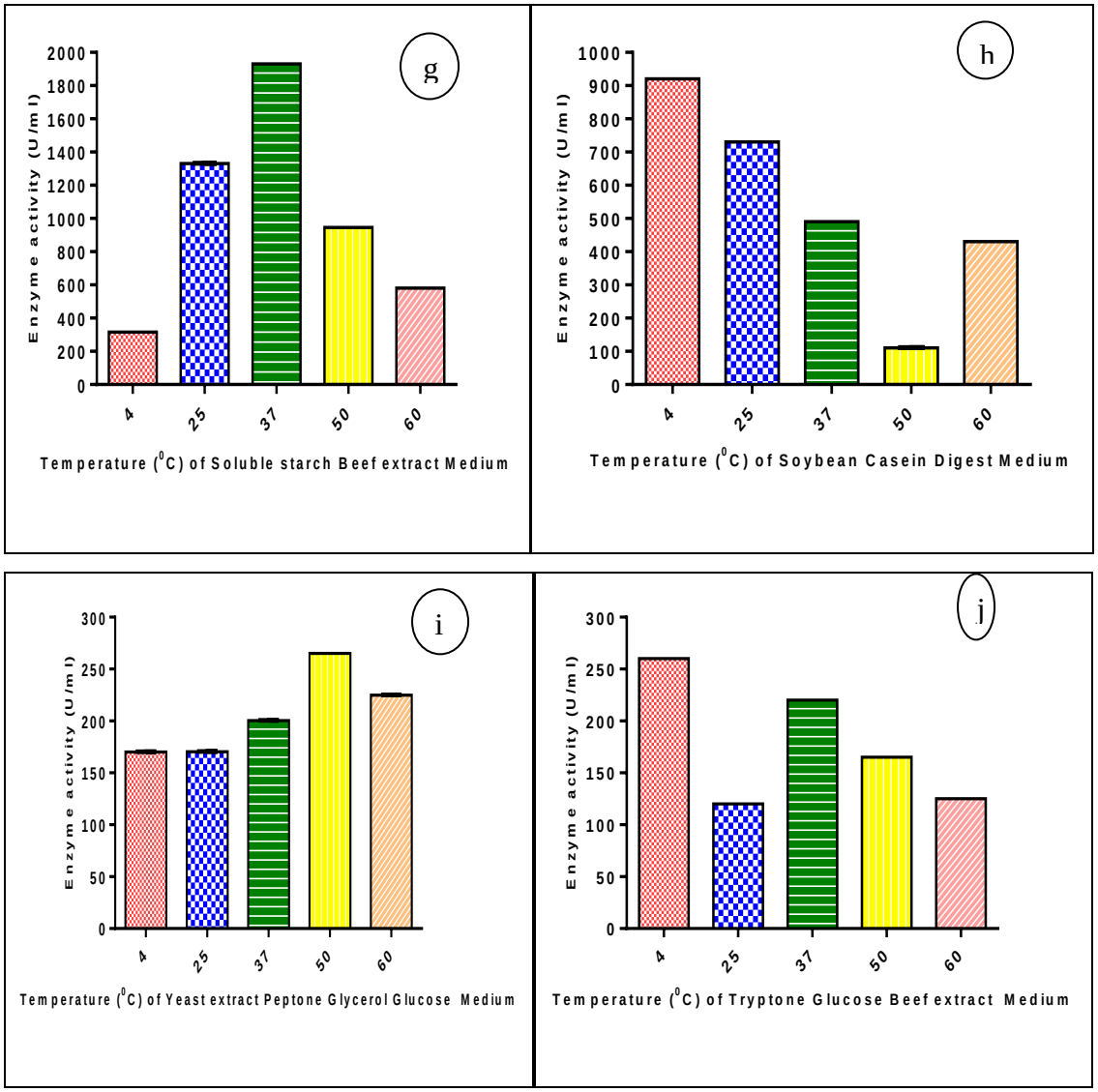

Table.1 The Range of Amylase Activity at Various Temperatures with Different Media

Media	Amylase activity Range in U/ml
Nutrient Broth (NB)	80-280
Luria Bertain Broth (LB)	160-250
Clarks and Lub Medium (CL)	940-2560
Pikovskaya's Medium (PK)	1280-3380
Tendler's Non-synthetic Medium (TNS)	310-770
Amylase Production Medium (APM)	200-590
Soluble Starch Beef Extract Medium (SB)	315-1940
Soybean Casein Digest Medium (SCD)	10-930
Yeast Extract peptone Dextrose Glucose Medium (YPDG)	170-270
Tryptone Glucose Beef Extract (TGB) Medium	120-270

Nevertheless, the production was highest (3371 ± 0.50 U/ml) in Pikovskaya's medium at 37^0C. The literature reviewed the amylase production in different media in psychrophilic condition (4°C) in *Alteromonas haloplanktis*[16], *Nocardiopsis sp.* 7326 (Groudieva *et al.,* 2004) and *Arthrobacter psychrolactophilus* (Declerck *et al.,* 2003). The zenith amylase production of mesophilic *Bacillus species* (30^0C-45^0C) was communicated (Nagarajan *et al.,* 2010; Pushpendra Singh *et al.,* 2012; Francisco Fábio Cavalcante Barros *et al.,* 2013; Hanumanthu Prasanna Lakshmi *et al.,* 2013). Peak production of amylase in *Bacillus species* in thermophilic condition (50°C -100°C) was documented (Sodhi *et al.,* 2005; Schwab *et al.,* 2009). Isolation of α-amylase producing bacteria from the coastal waters of Bay of Bengal, Visakhapatnam (Suribabu et al., 2015). The optimization of physical parameters, carbon, nitrogen, mineral sources parameters of alpha amylase producing *Brevibacillus borostelensis* R1 (Suribabu *et al.,* 2014).

In conclusion, the present studies were carried out to optimize the α-amylase production of *Brevibacillus borstelensis* R1 using ten different media at various temperatures (4^0C, 25^0C, 37^0C, 50^0C and 60^0C) with constant pH 7.0. Among ten media taken, Pikovskays's medium proved to be optimal α-amylase production medium for *Brevibacillus borostelensis* R1 at physical parameters of pH 7.0, and at 37^0C. The optimum production of α-amylase by submerged fermentation (SmF) was observed at 37^0C in Pikovskaya's Medium (3371 ± 0.50 U/ml) and at 4^0C in Clarks and Lub Medium (2551 ± 1 U/ml).

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