-Amylase Activity Of Wild And Mutant Strains Of Bacillus Sp

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Citation

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Abstract

-Amylase is an enzyme capable of hydrolyzing starch. Alternate names of this enzymes are 1,4--D-glucan glucanohydrolase and glycogenase. Amylase enzymes are used extensively in bread making to break down complex sugars such as starch (found in flour) into simple sugars. Recently, various strategies in the pharmaceutical and chemical industries have used amylases in the synthesis of optically pure drugs and agrochemicals. The production of amylase by bacteria and fungi has been studied in terms of enzyme production, protein properties and purification. The aim of this work was to describe the production of an extracellular amylase by wild and UV mutated Bacillus sp. UV mutagenesis made the organism to produce more quantity of amylase.

INTRODUCTION

Enzymes are the proteins capable of catalyzing biochemical processes. Some of these are capable of catalyzing hydrolytic cleavage (digestion) of such biological polymers as proteins, carbohydrates and fats. These are known as hydrolases. Amylase is the name given to glycoside hydrolase enzymes that break down starch into glucose molecules. Although the amylases are designated by different Greek letters, they all act on -1,4-glycosidic bonds. Under the original name of diastase, amylase was the first enzyme to be found and isolated (by Anselme Payen in 1833). The -amylases are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, -amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and “limit dextrin” from amylopectin. Starch-degrading, amylolytic enzymes are widely distributed among microbes. These enzymes include alpha-amylase, beta-amylase, glucoamylase, alpha-glucosidase, pullulan-degrading enzymes, exoacting enzymes yielding alpha-type endproducts, and cyclodextrin glycosyltransferase. Properties of these enzymes vary and are somewhat linked to the environmental circumstances of the producing organisms (Cotta, 1988).

Among all the amylolytic enzymes, the genetics of alpha-amylase in Bacillus subtilis are best known. Alpha-Amylase production in B. subtilis is regulated by several genetic elements, many of which have synergistic effects. Genes encoding enzymes from all the amylolytic enzyme groups dealt with here have been cloned and the sequences have been found to contain some highly conserved regions thought to be essential for their action and/or structure. Glucoamylase appears usually in several forms, which seem to be the results of a variety of mechanisms, including heterogeneous glycosylation, limited proteolysis, multiple modes of mRNA splicing, and the presence of several structural genes (Vihinen and Mäntsälä, 1989).

Bacterial amylases have been extensively researched and are prolifically described in the patent literature. Unfortunately, only two Bacillus enzymes are readily available to the conservation community in acceptable purity. Both B. subtillis and B. licheniformis require sodium ion and calcium ion in order to preserve the enzyme’s full activity. That of B. licheniformis, however, requires only 5 ppm Ca2+, while that of B. subtillis requires 150 ppm Ca2+. The heat stability of B. subtillis amylase extends as high as 80-85°C and that of B. licheniformis extends to 110°C; both species’ amylases nevertheless show comparable activities at room temperature (Thippeswamy et al., 2006). By introducing defined deletions in recA and an essential sporulation gene (spoIV), stable mutant strains of Bacillus licheniformis were obtained which are totally asporogenous and severely affected in DNA repair and thus being UV-hypersensitive. Studies on growth in various liquid media as

well as on amylase production revealed no differences of the mutants when compared to the wild type. Hence, such genes appear to be suitable disruption targets for achieving passive biological containment in this industrially exploited species (Nahrstedt et al., 2005). A novel recombinant Bacillus bacterial strain is constructed by genetic engineering which has high productivity of alpha-amylase. The Bacillus strain comprising an alpha-amylase gene inserted in the Bacillus chromosome under transcriptional control of a phage promoter. An efficient process for the rapid production of large amounts of alpha-amylase is also disclosed (www.freepatentsonline.com/20020187541.html).

Having known the importance of amylase enzyme this work was aimed to isolate amylase from soil bacteria and to mutate the bacteria to overproduce the same.

MATERIALS AND METHODS

COLLECTION OF SAMPLE

Soil samples from various places were collected in sterile container. Samples were serially diluted and subjected for total colony count. Colonies isolated were subjected for the production of amylase.

ASSAY FOR AMYLASE PRODUCTION

MEDIA COMPOSITION

Soluble starch-1g, Yeast extract - 0.2g, Peptone - 0.5g, $MgSO_4$ - 0.05g, NaCl -0.05g, $CaCl_2$ - 015g, Agar - 2g,, Distilled water - 100mL, pH 7.0

METHOD

Starch agar plates were prepared. Isolated colonies were inoculated unto the medium. Plates were incubated at 370C for 24 hours. Iodine solution was added and checked for the production of amylase enzyme in the starch agar plate. Formation of zone after iodine solution confirms the presence of amylase. All the starch hydrolyzing organisms were identified using bergey's manual (Claus and Berkeley, 1986).

IDENTIFICATION OF BACTERIA

The isolated bacteria were identified according to Gram characteristics, spore morphology and motility. In addition, the following identified tests were carried out: utilization of citrate, VP test, casein hydrolysis, gelatin liquefaction starch hydrolysis and production of acid from D-glucose, L-arabinose and D-xylose. The production of gas from glucose, catalase production and nitrate reduction were also tested (Claus and Berkeley, 1986).

ENZYME PRODUCTION

MEDIA COMPOSITION(IN GMS)

$NaH_2PO_4.2H_2O$ - 1.56, NH_4Cl - 5.35, KCl - 0.745, $Na_2SO_4.10H_2O$ -0.644, Citric acid -0.42, $MgCl_2.6H_2O$ -0.25, $CaCl_2$ - 2.2x10-3, $FeCl_3.6H_2O$ -2.7x10-2 , $MnCl_2.4H_2O$-1.0x10-2, $CuCl_2.2H_2O$ -8.5x10-4, H_3BO_3 - 3.0x10-4, Na_2MoO_4 -1.0x10-3, Bactotryptone - 10.0, Yeast extract - 2.5, Soluble starch –5, pH - 6.9-7.0, pH was adjusted with 1.0 M NaOH and this basal medium was sterilised by autoclaving at 121ºC for 15min used in this work for -amylase production contained (g/L): Yeast extract, Bacto-tryptone and Soluble starch were sterilised separately and aseptically added to the flasks containing the liquid medium, after cooling. The above medium (50 mL in 250 mL Erlenmeyer flasks) was inoculated with 1 mL of an overnight culture and incubated at 37ºC with vigorous aeration in a rotary shaker at 150 rpm for 144h. At time intervals, the turbidity of the cultures was determined by measuring the optical density at 470 nm in a Systronics spectrophotometer (India). Before assay, the cells were separated by centrifugation at 13.000 rpm for 15 min and the clear supernatant was used as crude enzyme preparation (Cordeiro et al.,2002).

AMYLASE ASSAY (MILLER, 1959)

The activity of -amylase was assayed by incubating 0.3 mL enzyme (crude enzyme preparation) with 0.5 mL Soluble starch (1%, w/v) prepared in 0.05M Phosphate buffer, pH 6.5. After incubation at 37ºC for 10 min the reaction was stopped The reducing sugars released were assayed colorimetrically by the addition of 1 mL of 3-5-dinitrosalicylic acid reagent. An enzyme unit is defined as the amount of enzyme releasing 1 mM of glucose from the substrate in 1 min at 90ºC.

EFFECT OF PH ON ACTIVITY AND STABILITY OF -AMYLASE

Effect of pH on the activity of -amylase was measured by incubating 0.3 mL of enzyme and 0.5 mL of buffers, adjusted to pH of 5.5 to 8.5, containing Soluble starch (0.5%). The buffers used were: sodium acetate pH 5.5; phosphate pH 6.0 – 8.0; Tris-HCl pH 8.5. Stability of the enzyme at different pH values was also studied by incubating the enzyme at various pH values ranging from 5.5 – 8.5 for 24h and then estimating the residual activity.

EFFECT OF TEMPERATURE ON ACTIVITY AND STABILITY OF -AMYLASE

The effect of temperature on the enzyme activity was determined by performing the standard assay procedure as mentioned earlier for 10 min at pH 6.5 within a temperature range of 40 –100ºC. Thermostability was determined by incubation of crude enzyme at temperatures ranging from 40-100ºC for 2hr in a constant-temperature water bath. After treatment the residual enzyme activities were assayed.

SALT TOLERANCE TEST

Enzyme was incubated in 10 mM Phosphate buffer (pH 7.0) containing various NaCl concentrations (0.05 to 5M) for 24h at 4ºC and in each case activity of the enzyme was measured in the same way as mentioned earlier.

INDUCTION OF MUTATION

Wild species was exposed to UV radiation by following method. Wild Bacillus sp was taken into LB broth medium and incubated at 370C for 24 hrs. the culture was serially diluted upto 106 dilution and 0.1 ml was plated into the LB medium to find out the CFU (Control). 5ml of LB broth culture was expressed to UV rays for various time intervals. 40 cm distance was maintained between lamp and the culture. The different broth cultures exposed to different time exposure i.e. 30, 60, 90, 120 and 150 seconds. The exposed culture was mixed well separately and immediately diluted to 10-5. the diluted culture samples were plated on the LB agar plates and incubated 370C for 24 hours. After incubation, the plates were observed and the colonies were counted. The -amylase activities were initially analysed in mutant species as did for wild strains.

RESULTS AND DISCUSSION

Totally 180 samples were isolated from the soil samples. About 28 samples showed -amylase production (Table 1). All the 28 organisms were subjected for the identification of Bacillus sp. Morphological characteristics and biochemical characteristics were done to find out the Bacillus sp. About 14 samples were found to be Bacillus sp. Among these, 14 strains B10 strain was able to produce higher quantity of –amylase than the any other wild strains (Table 2).

B10 was further explored for the UV mutagenesis study. Efficient –amylase producing B10 was exposed to UV irradiation for various time intervals viz. 30, 60, 90, 120 and 150 sec. 7, 6, 4, 3 and 1 colonies were observed in the 30, 60, 90, 120 and 150 sec UV exposed plates respectively. Reduction in colony count was due to effect of UV. All these 21 colonies were subjected for the –amylase activity. One from colony each 90, 120 and 150 sec exposed groups found to produce more –amylase than the wild strain B10 (Table 3). Among these three M3 (i.e. 150 sec UV exposed strain) produced 35U/mL of -amylase.

When studying the time course production of -amylase, B10 Bacillus sp was able to produce more –amylase in 48 hours i.e 29 U/mL, whereas, the mutant M3 produced 34u/mL of –amylase (Fig. 1). Maximal –amylase activity of B10 culture was at pH of 7.5 in 48 hours i.e 32 U/mL. Mutant M3 showed higher activity of –amylase at pH 7 and showed the higher activity as 36U/mL (Fig. 2). B10 and M3 were subjected to grow at various temperature range having constant pH and incubation time. B10 was able to produce more –amylase at 300C at pH 7.0 in 48 hours i.e 30 U/mL. whereas, the mutant showed more –amylase activity at the 32.50C at pH 7.0 in 48 hours i.e 36U/mL (Fig. 3).

B10 and M3 were also subjected to produce -amylase in various concentration of NaCl. At the concentration 0.75%, B10 (wild strain) showed –amylase activity of 29U/mL and M3 showed –amylase activity as 37U/mL (Fig.4). The overall results clearly said us that mutant strains are more capable of producing more –amylase than wild strain.

Figure 1

Table1. Colonies isolated from soil samples

s.no	Total number of colonies isolated from soil samples	Number of colonies showed amylase activity
1.	180 x 10^4	28

Figure 2

Table 2. –amylase activity in wild sp isolated from soil samples

S.No	Wild strain	Amylase activity (U/mL)
1.	B1	15
2.	B2	23
3.	B3	25
4.	B4	27
5.	B5	23
6.	B6	12
7.	B7	16
8.	B8	27
9.	B9	23
10.	B10	28
11.	B11	23
12.	B12	21
13.	B13	15
14.	B14	16

Figure 3

Table 3. –amylase activity in mutant sp exposed to UV irradiation

S.No	Wild strain	Amylase activity (U/mL)
1.	M1	31
2.	M2	30
3.	M3	35

Figure 4

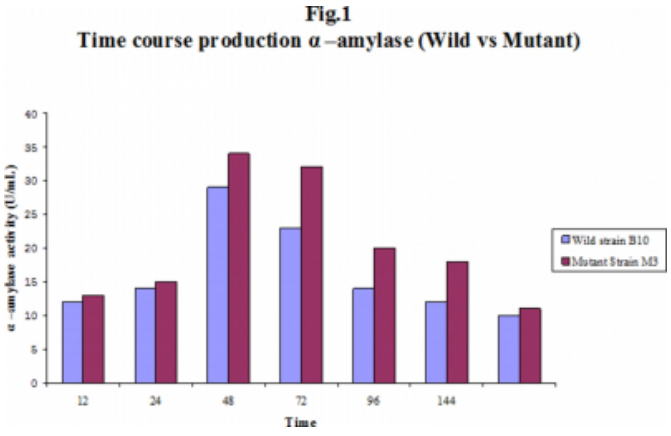


Figure 5

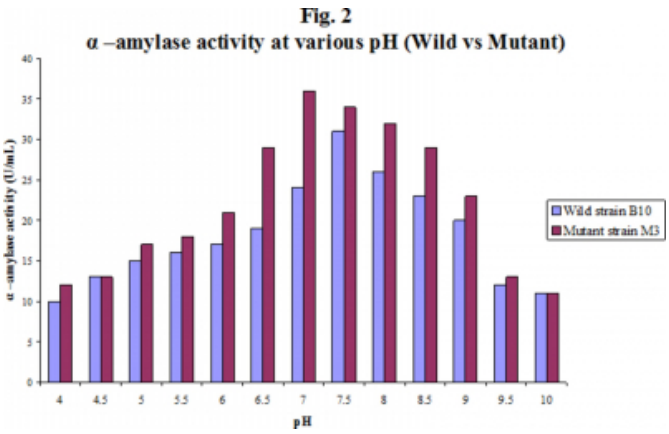


Figure 6

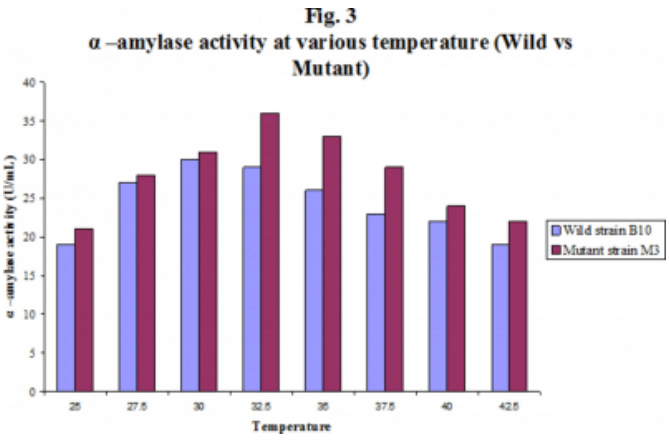
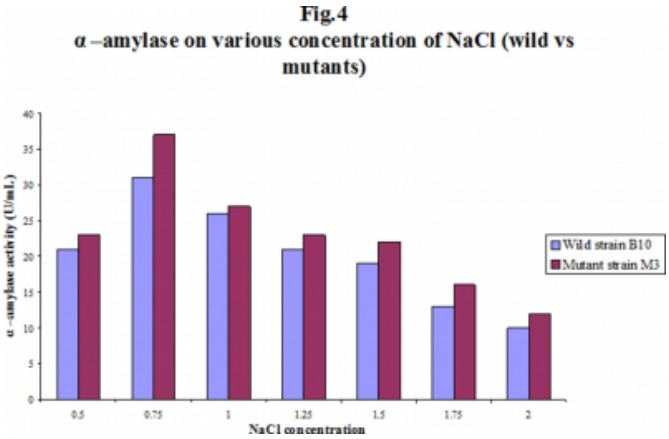


Figure 7



CONCLUSION

Mutants form of Bacillus sp are found to be more potent the wild strains, which can be further characterized used for further study.

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