PRODUCTION AND PROPERTIES OF α-AMYLASE FROM THERMOPHILIC BACILLUS SP.

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ABSTRACT

α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) production by thermophilic Bacillus sp strain SMIA-2 cultivated in liquid media containing soluble starch reached a maximum at 48h, with levels of 57U/mL. Studies on the α-amylase characterization revealed that the optimum temperature for activity was 70°C. The enzyme was stable for 2h at 50°C, while at 60°C, 70°C and 90°C, 4%, 13% and 38% of the original activities were lost, respectively. The optimum pH of the enzyme was 7.5. After incubation of crude enzyme solution for 24h at pH 7.5, a decrease of about 5% of its original activity was observed. The enzyme was strongly inhibited by Co²⁺, Cu²⁺ and Ba²⁺, but less affected by Ca²⁺, Mg²⁺, Ni²⁺, Sr²⁺ and Mn²⁺. The enzyme in 1M and 5M NaCl solutions the enzyme retained 70% and 47% of the original activity after 24h of incubation at 4°C, respectively.

Key-words: α-amylase, thermophilic bacterium, Bacillus sp.

INTRODUCTION

Thermophilic and extremely thermophilic microorganisms have gained a great deal of attention recently (2,3,11,21). Enzymes from these microorganisms are of special interest since they are not usually denatured by high temperatures and are even active at elevated temperatures (1,6,10,23,24). The genus Bacillus produces a large variety of extracellular enzymes, some of which such as the amylases are of significant industrial importance (4). Among these enzymes, the thermostable varieties are more versatile with respect to industrial significance. Thermostable α-amylases have had many commercial applications for several decades. These enzymes are used in the textile and paper industries, food, adhesive, and sugar production (12,15,16,18,21,22).

In this article the production of thermostable α-amylase by thermophilic Bacillus sp strain SMIA-2, previously isolated from a soil sample collected in Campos dos Goytacazes City, Rio de Janeiro, Brazil, is reported.

MATERIALS AND METHODS

Organism

The bacterial strain used in this study was a thermophilic Bacillus sp strain SMIA-2 (19), previously isolated from a soil sample collected in Campos dos Goytacazes City, Rio de Janeiro, Brazil. Phylogenetic analysis showed that this strain is a member of the Bacillus rRNA group 5. This group includes Bacillus stearothermophilus and other thermophilic Bacillus spp. The optimum temperature and pH for growth of this organism were around 55°C and pH 7.0, respectively. The organism was found to produce α-amylase on culture medium composed of 1% Soluble starch, 0.2% Yeast extract, 0.5% Peptone, 0.05% MgSO₄, 0.05% NaCl, 0.015% CaCl₂ and 2% agar at 55°C (pH 7.0).
Enzyme production

The culture medium used in this work for α-amylase production contained (g/L): NaH₂PO₄.2H₂O-1.56, NH₄Cl-5.35, KCl-0.745, Na₂SO₄, 10H₂O-0.644, Citric acid-0.42, MgCl₂.6H₂O-0.25, CaCl₂−2.2x10⁻³, ZnO-2.5x10⁻³, FeCl₃.6H₂O-2.7x10⁻², KCl-0.745, Na₂SO₄.10H₂O-0.644, Citric acid-0.42, MgCl₂.6H₂O-

Effect of temperature on activity and stability of α-amylase

Optimum pH was found to be 7.5. The enzyme activity at pH 5.5 and 10.0 were 73% and 55% of that at pH 7.5, respectively. After incubation of crude enzyme solution for 24h at pH 5.0–10, a decrease of about 5% of its original activity at pH 7.5 was

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 4°C and in each case activity of the enzyme was measured in the same way as mentioned earlier.

RESULTS AND DISCUSSION

Enzymatic production

Fig. 1 reports the time-course of α-amylase production by Bacillus sp. strain SMIA-2 grown in basal medium supplemented with 0.5% Soluble starch. α-amylase production reached a maximum at 48h, with levels of 57U/mL. Subsequently, α-amylase levels remained more or less constant up to 96h and after 144h it dropped to 23U/mL. It was observed that maximum α-amylase production occurred when the cell population reached the peak, suggesting that this organism may be unusually sensitive to metabolite repression. Effective induction may not occur until the stationary phase has been reached and the readily available carbon source was depleted.

Effect of pH on activity and stability of α-amylase

The effect of pH on α-amylase activity is shown in Fig.2. Optimum pH was found to be 7.5. The enzyme activity at pH 5.5 and 10.0 were 73% and 55% of that at pH 7.5, respectively. After incubation of crude enzyme solution for 24h at pH 5.0–10, a decrease of about 5% of its original activity at pH 7.5 was

Effect of temperature on activity and stability of α-amylase

The activity of α-amylase was assayed by incubating 0.3 mL enzyme with 0.5 mL Soluble starch (1%, w/v) prepared in 0.05M Phosphate buffer, pH 6.5. After incubation at 90°C for 10 min the reaction was stopped and the reducing sugars released were assayed colorimetrically by the addition of 1 mL of 3-5-dinitrosalicylic acid reagent (17). An enzyme unit is defined as the amount of enzyme releasing 1 mmole of glucose from the substrate in 1 min at 90°C.

Effect of pH on activity and stability of α-amylase

Effect of pH on the activity of α-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 metal ions

The effect of different metal ions on α-amylase activity was determined by the addition of the corresponding ion at a final concentration of 1mM to the reaction mixture, and assayed under standard conditions. The enzyme assay was carried out in the presence of Ca²⁺, Mg²⁺, Fe²⁺, Fe³⁺, Co²⁺, Zn²⁺, Mn²⁺, Hg²⁺, Cu²⁺, Cs²⁺, Ni²⁺, Sr²⁺, Ba²⁺, Ag¹⁺ chlorides, Pb²⁺acetate, and Cu²⁺sulphate.

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 4°C and in each case activity of the enzyme was measured in the same way as mentioned earlier.

Effect of pH on activity and stability of α-amylase

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α-amylase from Bacillus sp

observed. At pH 10.0, the decrease was of 44%. Thus, α-amylase of Bacillus sp. strain SMIA-2 strain seems to be active in very broad pH range.

**Effect of temperature on activity and stability of α-amylase**

The supernatant amylolytic activity were assayed at different temperatures ranging from 40ºC-100ºC at a constant pH of 7.5 and a substrate concentration of 0.5% as shown in Fig. 3. Enzyme activity increased with temperature within the range of 40ºC to 70ºC. A reduction in enzyme activity was observed at values above 70ºC. The optimum temperature of this α-amylase was 70ºC, which was higher or similar to that described for other Bacillus α-amylases (4,7,8,13). The residual activity of crude α-amylase incubated at different temperatures for a period of 2h and 24h was estimated at optimum temperature. The enzyme was stable for 2h at temperatures ranging from 40-50ºC while at 60ºC, 70ºC and 90ºC, 4%, 13% and 38% of the original activities were lost respectively.

**Effect of metal ions**

Because metal ions could be generated from equipment corrosion, specially when subject to acid hydrolysis, the effect of some metal ions at the concentration of 1 mM in the activity of α-amylase was investigated. As can be observed in Table 1, the α-amylase did not require any specific ion for catalytic activity. A slight activity inhibition was produced in the activity by Cu²⁺, Mg²⁺, Ni²⁺, Sr²⁺ and Mn²⁺; and a stronger inhibitory effect was observed in the presence of Co²⁺, Cu²⁺ and Ba²⁺. Some amylases are metalloenzymes, containing a metal ion for catalytic activity. The inhibition of Bacillus sp. strain SMIA-2 α-amylase by Co²⁺, Cu²⁺ and Ba²⁺ ions could be due to competition between the exogenous cations and the protein-associated cation, resulting in decreased metalloenzyme activity (12).

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**Figure 2.** Optimum pH and stability pH of α-amylase produced by Bacillus sp. strain SMIA-2 grown at 55ºC for 48h. Relative activity is expressed as a percentage of the maximum (100% of enzyme activity = 92U/mL).

**Figure 3.** Optimum temperature and stability temperature of α-amylase produced by Bacillus sp. strain SMIA-2 grown at 55ºC for 48h. Relative activity is expressed as a percentage of the maximum (100% of enzyme activity = 78U/mL).

**Figure 4.** Effect of NaCl concentration on α-amylase produced by Bacillus sp. strain SMIA-2 grown at 55ºC for 48h. Relative activity is expressed as a percentage of the maximum (100% of enzyme activity = 79 U/mL).
Table 1. Effect of different ions on α-amylase activity produced by Bacillus sp. strain SMIA-2 grown at 55°C during 48h.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Percentage of activity (%)</th>
<th>I mM</th>
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<tbody>
<tr>
<td>Control (no addition)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>97</td>
<td>97</td>
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<td>Fe²⁺</td>
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<td>75</td>
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<td>Co²⁺</td>
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<td>26</td>
</tr>
<tr>
<td>Zn²⁺</td>
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<td>73</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Hg²⁺</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>Cs⁺</td>
<td>101</td>
<td>101</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Sr²⁺</td>
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<td>91</td>
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<td>Pb²⁺</td>
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<td>101</td>
</tr>
<tr>
<td>Ba²⁺</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Ag¹⁺</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

The percentage of activity is expressed as a percentage of the control (100% of enzyme activity = 42U/mL).

The effects of metal ions on the activity of α-amylase in Bacillus sp. strain KSM-1378, a relative of Bacillus firmus, was investigated by Igarashi et al. (7). Ni²⁺, Cd²⁺, Zn²⁺, and Hg²⁺ ions strongly inhibited the enzymatic activity by 82, 91, 100, and 100% respectively. On the other hand, in Bacillus sp. TS-23, Ni²⁺ and Cd²⁺ slightly inhibited amylase activity.

Salt tolerance test

This test is important in treatment of effluent with high salinity containing starch or cellulosic residues in pollution control mechanism. The enzyme in 1.0 M and 5 M NaCl solution retained 70% and 47% of the original activity after 24h at 4°C, respectively. The α-amylase produced by Bacillus sp. MD 124 (9) was stable in 5 M NaCl solution and retained 75% of its original activity after 24h.

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REFERENCES


