Substitution of GPR Grade Salts in the Production of α-Amylase by *Bacillus licheniformis* 6346 in Solid State Fermentation

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Abstract

Production of α-Amylase by *Bacillus licheniformis* 6346 may be performed in a solid fermentation medium, prepared using locally available raw materials and GPR grade salts. The solid medium contained paddy husk, rice flour, $(NH_4)_2HPO_4$, $NaH_2PO_4.2H_2O$, $NaH_2PO_4.2H_2O$, KC1, $MgSO_4.7H_2O$, sesame oil, coconut oil, and tap water. When $(NH_4)_2HPO_4$ was replaced with $(NH_4)_2SO_4$ fertilizer in the present study, the α-amylase production was reduced by 0.6 fold and pH of the medium was decreased from 6.9 to 5.0. Cuttlefish shell powder was then used to correct the pH of the medium and this also improved the α-amylase production to 777 unit g^{-1} dry bacterial material (Ug^{-1} DBM) (6^{th} day). When KCl was replaced with murated potash (K_2O) and table salt (NaCl), the α-amylase activity produced was 766 Ug^{-1} DBM (6^{th} day). Substitution of $Na_2HPO_4.2H_2O$ and $NaH_2PO_4.2H_2O$ with triple super phosphate increased α-amylase activity to 1075 Ug^{-1} DBM (5^{th} day). Hence, locally available fertilizers and table salt could be used instead of the GPR grade salts for the production of α-amylase in solid medium.

Keywords: α-Amylase, *Bacillus licheniformis,* paddy husk, solid state fermentation, fertilizers

Introduction

Economic starch hydrolysis is a recognised priority in biotechnology research. One of the ways to reduce expenses is by cutting the cost of enzymes which are used in the process operations. α-Amylase is produced by submerged [1] and solid state fermentation (SSF) [2] processes. The SSF technique is a traditional fermentation method in South-East Asia [3-7], and there are many reports on the production of α-amylase [8], glucoamylase [9], protease [10,11], gibberellic acid [12], fibrinolytic enzyme [13] and pectinase [14] by SSF. SSF is known to reduce the overall production cost [8,15], which can be further reduced by using locally available materials. The medium widely used in SSF for α-amylase production is wheat bran [16]. However, in Sri Lanka, wheat bran is not available as a waste material. Alternatively, paddy husk with nutrient supplementation has been reported as a substitution material [17].

Production of α-amylase is dependent on the strain and composition of media, and the method of cultivation [18]. α-Amylase production is also influenced by the nutrients supplementation [19]. To avoid import and transport barriers and to reduce the cost of the raw materials, a medium was formulated using locally available materials with the use of paddy husk and GPR grade salts as support for α-Amylase production by *Bacillus licheniformis* [20, 21]. To further reduce the cost of enzyme production, the possibility of introducing commercially available fertilizers in place of GPR grade salts was tried in the present study.

Materials and Methods

Materials

Paddy husk, raw unpolished rice, ammonium sulphate fertilizer, murate potash and triple super phosphate, table salt (NaCl), sesame oil (*Sesamum indicum,* L), and coconut oil (*Cocous nucifera,* L) were purchased from a local market. Cuttlefish (*Sepia sp.*) shells were collected from the fish market, washed and powdered using a domestic grinder. Raw unpolished rice was ground to powder using a domestic grinder. Nutrient Broth was from Oxoid Limited, UK. Other chemicals were from standard sources.

Microorganism

Bacillus licheniformis 6346, from the Heriot-Watt University, UK, was used.

Measurement of α-amylase activity

Enzyme was extracted by mixing 1 g of bacterial medium (wet) with 4 ml of tap water for 20 min and centrifuged (3000 rpm, 10 min). The supernatant was pre-incubated at 85°C for 3 min, mixed with 0.5 ml of 20 gl^{-1} starch in 0.1 M phosphate buffer (pH 7.0) and incubated for 5 min. Reducing sugar produced was measured by dinitrosalicylic acid method [22]. One unit (U) of α-amylase activity is the amount of enzyme that

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