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EFFECTS OF NUTRITION ON AMYLASE PRODUCTION BY *MONASCUS PURPUREUS*

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Summary

*The extracellular amylase of mycelial fungus, **Monascus purpureus**, is an inducible enzyme. The amylase activity was induced by various concentrations of starch, in the presence of yeast extract and ammonium nitrate. **M. purpureus** grew better at 37°C than 25°C on 15 g/l of starch, 2 g/l of yeast extract and 4 g/l of ammonium nitrate. There was a higher concentration of reducing sugar when the medium contained an increasing amount of starch (5-15 g/l) at 37°C. The repression phase occurred after two days of growth and could be detected both in starch containing medium and starch yeast extract medium, except in NH_4NO_3 containing medium. The highest overall activity was induced by yeast extract (2 g/l) at 37°C. The activity pattern was slightly affected by temperature variations from 37°C to 25°C, with NH_4NO_3 added to the medium. However, the pattern of enzyme production was not related to the mycelial growth phase of the fungus.*

Introduction

"Ang-kak" is the name of red rice which has the fungus, *Monascus purpureus*, grown on rice grains. The method for producing good quality Ang-kak has been kept secret in certain localities. Recently the mold has been adopted for fermentative pigment production for coloring foodstuffs. The pigments have been studied by Yoshimura *et al.*[1] Different environmental conditions and various carbon and nitrogen sources have been tried[2-4]. McHan[5] reported his comprehensive nutritional study of *M. purpureus* Went, which grew better in glucose-peptone-yeast extract broth than in any other natural medium. Starch or bread used as substrates apparently promotes growth of the fungus. Certainly, the high amylase activity indicated the ability of the fungus to grow on rice. However, no preliminary study of the relationship between starch and amylase production has been made. The present paper investigates the effects of the use of various concentrations of starch, inorganic nitrogen (ammonium nitrate) and yeast extract on amylase production.

Materials and Methods

Isolation and Cultivation

Monascus purpureus was isolated from red rice from two different sources, the United Kingdom and the Republic of China. After soaking the red grains of each strain in sterilized water overnight, they were cultured on Sabouraud agar medium. Small red colonies were isolated after 3 days of incubation at 25°C. Because of its consistent production of deep red color, the China strain was selected for the purpose of enzymatic detection.

The use of solid medium for detection of enzyme production developed by Henkin[6] and the technique established by Pichyangkura *et al.*[7] were adopted. The basic nutrient in starch-containing medium which was used in this experiment followed Barnett[8]. The methods used for the detection of amylase activity followed Bernfeld[9]. The growth rates were measured in terms of the diameter of the colonies before detecting the amylolytic enzyme.

The media used for cultivation contained various concentrations of starch, yeast extract (Difco) and NH_4NO_3. However, each of the various media contained basic components of KH_2PO_3 1 g and $MgSO_4.7H_2O$ 0.5 g and the pH was adjusted to 6.5. The concentration of NH_4NO_3 and yeast extract were varied, but 10 g of starch was constant. Seven-day-old stock cultures which were grown on Sabouraud agar were used as inoculum on 37 ml of solidified agar medium. Duplicate sets of each of the various media were inoculated with 0.5 mm of inoculum size at the center of the agar plates, then one set was incubated at 25°C. Enzyme detection was performed at two day intervals for a period of twelve days.

Enzyme activity determination

The agar block, 4 mm in diameter was cut with cork borer at the edge of the fungal colony, One block was taken into a test tube and used as an enzyme source. A solution (0.5 ml) of 1% non-reducing sugar test starch (Pfanstiebl Lab, Waukegan, Illinois) was added, then incubated at 25°C after shaking for 5 min. The enzyme reaction was stopped by adding 1 ml solution that contained 1.0 g of 3,5-dinitrosalicylic acid, 20 ml of 2N sodium hydroxide, and 30 g of sodium potassium tartrate in 100 ml. The mixture was boiled in a water bath for 5 min; then 20 ml of water was added. Absorbance was determined in a Bausch-Lomb spectronic-20 at 470 nm. The blank was prepared by using agar plug with substrate at time zero. Units of amylase activity were expressed as micromoles of reducing sugar liberated per minute[2]. A standard curve was obtained by determining absorbance of known standard aqueous maltose solutions of 0.1, 0.2, 0.5, 1.0 and 2.0 mg per ml to calculate the amount of reducing sugars.

Results

For growth of *M. purpureus* in various starch media at concentrations 5, 10 and 15 g/l at 25°C, the results are presented in Figs. 1,3 and 5. The growth was faster

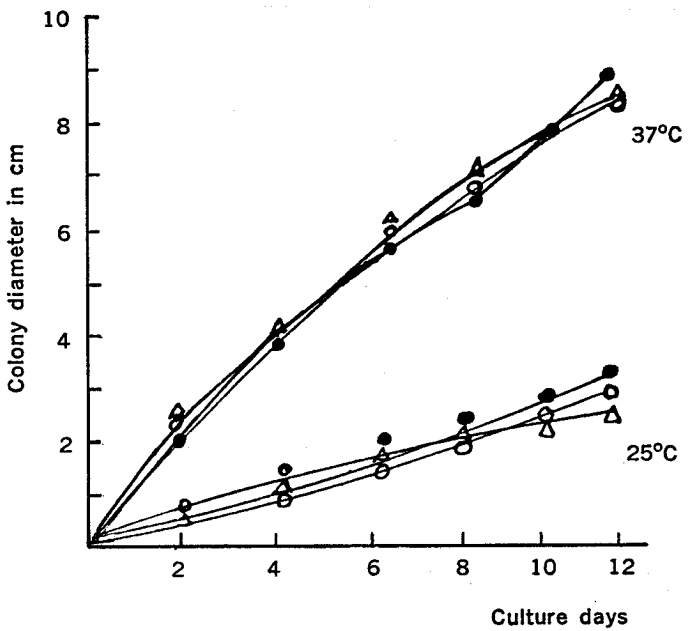

Fig. 1. Growth curve of *M. purpureus* grown on the solid medium containing 5 (○), 10 (●) and 15 (△) g/l of starch and mineral salts at 25°C and 37°C.

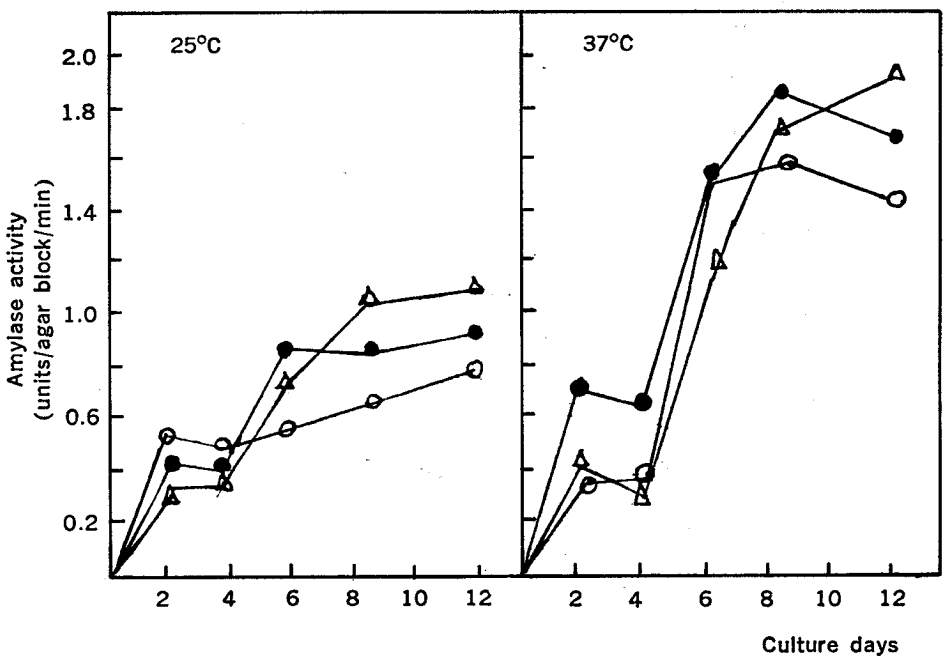

Fig. 2. Amylase activity of *M. purpureus* grown on 5 (○), 10 (●) and 15 (△) g/l of starch at 25°C and 37°C. Experimental concentration of mineral salts were same as in Fig. 1.

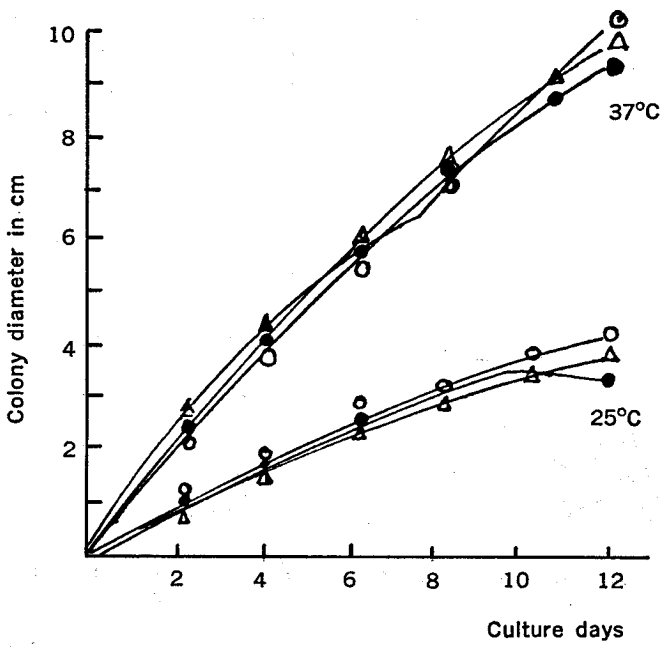

Fig. 3. Growth curve of *M. purpureus* grown on the solid medium containing 1 (○), 2 (●) and 3 (△) g/l of yeast extract, and 10 g/l of starch and mineral salts at 25°C and 37°C

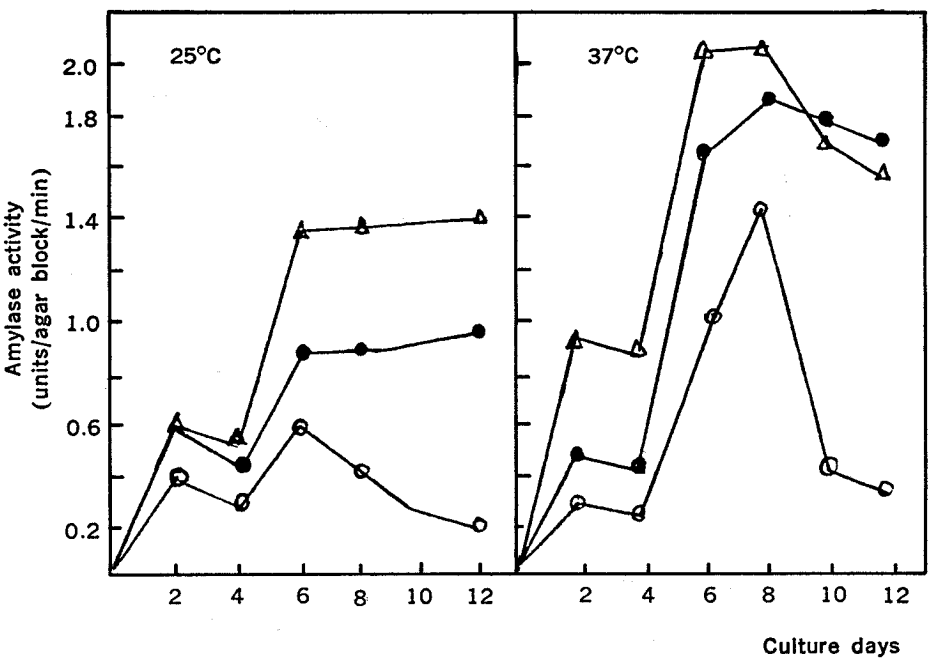

Fig. 4. Amylase activity of *M. purpureus* grown on 1 (○), 2 (●) and 3 (△) g/l of yeast extract at 25°C and 37°C. The experimental conditions were the same as in Fig. 3.

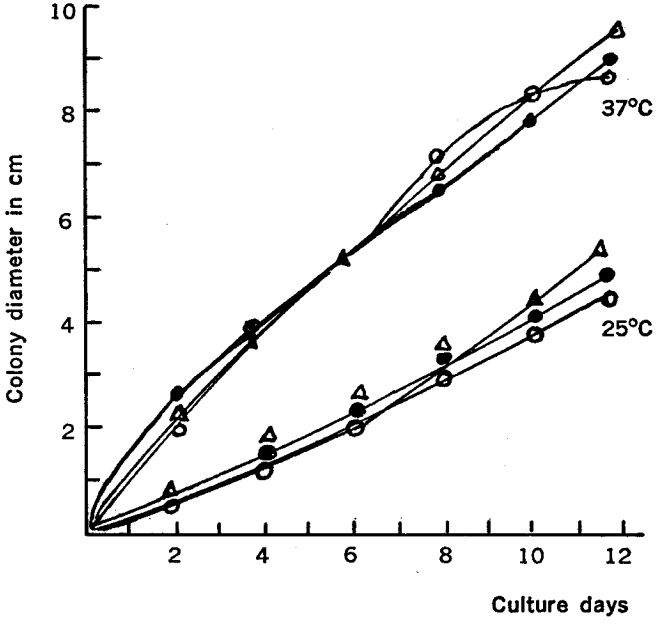

Fig. 5. Growth curve of *M. purpureus* on the medium containing 1 (○), 2(●) and 4(△) g/l of NH_4NO_3, 2 g/l yeast extract, 10 gm/l of starch and mineral salts.

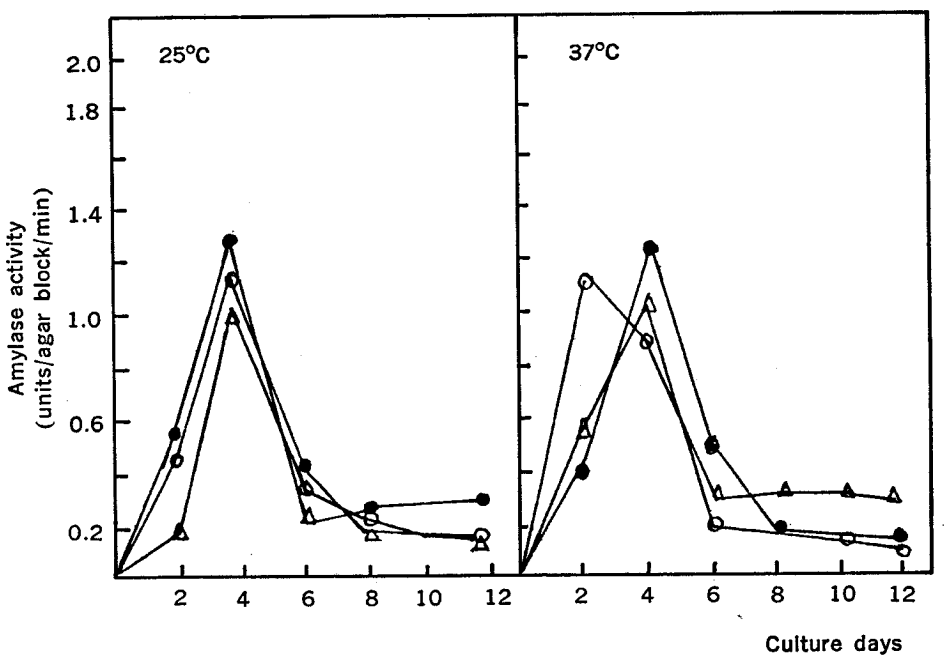

Fig. 6. Amylase activity of *M. purpureus* grown on 1 (○), 2 (●) and 4 (△) g/l of NH_4NO_3 at 25°C and 37°C. The experimental conditions were the same as in Fig. 5.

at 37°C than at 25°C, but the effects of starch concentration on the growth of mold was small.

Amylase activity determined by measuring the quantities of total reducing sugar in the medium culture at 25°C and 37°C, is shown in Fig. 2. Higher enzyme activity was obtained when an increasing amount of starch 5–15 g/l was used at 25°C and 37°C. Maximum enzyme activity was obtained after 6 days. The effect of varying the concentration of yeast extract in the medium is measured at 25°C and 37°C (Fig. 3). Increasing yeast extract concentration did not significantly promote growth but increased the enzyme activity (Fig. 4).

Three concentrations of NH_4NO_3 were used in the media: 6, 8, 10 g/l, with constant 10 g/l of starch and 2 g/l of yeast extract. The growth was shown in Fig. 5. The fungus grew faster at 37°C than 25°C. The results show no effect on growth when high concentration of NH_4NO_3 were used. The amylase activity at 25°C and 35°C are presented in Fig. 6. Increasing the concentration of NH_4NO_3 diminished the prolonged phase of enzyme production. The results showed similarity of the enzyme activity pattern at 25°C and 37°C which reached the peak in 3 days.

Discussion

The fungal growth, in starch-containing medium, starch-yeast extract medium and starch-yeast extract NH_4NO_3 medium, presented in Figs. 1, 3 and 5 respectively, was promoted more readily at 37°C than 25°C. The results are different from those of Yoshimura[7], who reported that 25°C was optimum temperature.

Amylase, in *M. purpureus*, was an inducible enzyme. Glucose-peptone-yeast extract medium by McHan[2] had been tried and no effect was observed. Some effect of starch on amylase production was observed. Reducing sugars were detected at higher concentration when more starch was used. A comparison of enzyme activity at two different temperatures in Fig. 2 showed that more amylase activity was liberated at 37°C than 25°C. Increasing amount of glucose and polysaccharide in the medium affected the rate of enzyme reaction[10] after two days. The addition of 0.5% of glucose into the medium completely repressed α-amylase synthesis in *B. licheniformis*[4]. However, the catabolite repression phase showed a slower reaction than normal. This suggested that the effect of pH has been involved[8]. Since solid media were used the pH variation was difficult to detect in our study.

When various concentrations of yeast extract were used there was little effect on the growth rate of the fungus (Fig. 3). However, higher concentrations of yeast extract promoted production of amylase and appeared to modify catabolite repression (Fig. 4).

Various concentrations of NH_4NO_3 (inorganic) nitrogen added to the medium reduced amylase activity both at 25°C and 37°C (Fig. 6). This might indicate that 1 g/l of NH_4NO_3 used was too high to have an effect on amylase liberation and also acted as a good buffer. Seemingly, the temperature did not affect the enzyme activity pattern but did affect the intensity of enzyme and the rate of fungal growth.

In some strains of the fungi, pigment production is inhibited by inorganic nitrogen. However ammonium nitrate could be used to supplement pigment promotion in *M. purpureus.*

Acknowledgement

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บทคัดย่อ

เอมิเลสที่สร้างจากรา *Monascus purpureus* เป็นเอนไซม์ประเภทที่ถูกขับออกมาภายนอกด้วยการชักนำของแป้ง ได้ใช้ความเข้มข้นของแป้งต่าง ๆ กัน เพื่อเพิ่มการสร้างเอมิเลส *M. purpureus* โตที่ 37°C ได้ดีกว่าที่ 25°C เมื่อปริมาณของแป้ง 15 g/l ยีสต์เอกซแตรคท์ 2 g/l และแอมโมเนียมไนเตรต 4 g/l ค่าของน้ำตาลรีดิวซิงที่ตรวจพบ เมื่อใช้แป้ง 15 g สูงกว่า 10 และ 5 g/l ที่ 37°C เมื่อเติมยีสต์เอกซแตรคท์ ให้ความเข้มข้นต่างกัน โดยปริมาณของแป้งคงที่ 10 g/l การกดดันเกิดขึ้นหลังวันที่สองของการเจริญในอาหารแข็ง การกดดันเกิดได้ทั้งในอาหารเลี้ยงที่มีแป้งอย่างเดียวและแป้งกับยีสต์เอกซแตรคท์ แต่ไม่เกิดเมื่อเติมแอมโมเนียมไนเตรต อย่างไรก็ตามปฏิบัติการสูงสุดของเอมิเลสถูกชักนำได้ด้วย 15 g/l ของแป้งและ 2 g/l ของยีสต์เอกซแตรคท์ที่ 37°C สูงกว่า 25°C การแปรของเอนไซม์ที่สร้างเป็นแบบเดียวกัน แต่มีความเข้มที่ 25°C ต่ำกว่า อย่างไรก็ตามแบบการสร้างเอนไซม์ชนิดนี้ไม่แปรตามอัตราการเจริญเติบโตของเรา