Impact of carbon source for the synthesis of α-amylase in rice callus culture

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Abstract. Various carbon sources such as fructose, glucose, sucrose and maltose were tested at different concentrations to control enzyme synthesis in callus culture. Among them, 3% maltose was found to be the best carbon source for effective synthesis and secretion in the callus induction medium. The effect of various carbon sources on the synthesis of α-amylase in rice callus culture was studied. The features of the production of α-amylase in rice callus culture were studied on media containing as a carbon source a natural polysaccharide – starch and the end products of its enzymatic hydrolysis – maltose and glucose. A study of the dynamics of growth of callus culture of rice embryos on media with various carbon sources showed that the greatest growth of calli during the cultivation cycle was observed in the variant with glucose and corresponded to an almost 7-fold increase in cell mass. Using different concentrations of sucrose, it was found that the onset of induction of synthesis and secretion of cathodic isoforms of AMY1 α-amylase in rice suspension cells coincided exactly with the moment of depletion of the carbon source in the nutrient medium. The synthesis of the anodic forms of the AMY2 enzyme was constitutive.

1 Introduction

Callus tissue obtained from rice seeds has a unique ability to synthesize and secrete α-amylase in large quantities [1]. α-Amylase can account for up to 45% of the total protein secreted by cells. It was noted that the level of enzyme synthesis does not increase with the addition of exogenous gibberellic acid [2].

Later it was found that the induction of α-amylase synthesis in calli and suspension cells is regulated by the concentration of sucrose in the nutrient medium [3]. In the presence of an adequate amount of sucrose, the synthesis of the enzyme is repressed, while repression is removed (derepression) occurs under conditions of sucrose starvation. Further studies showed that, in addition to sucrose, other sugars, glucose and fructose, can act as effectors of α-amylase gene activity [4, 5].

In the presented work, the features of α-amylase production in rice callus culture were studied on media containing a natural polysaccharide, starch and the end products of its enzymatic hydrolysis, maltose and glucose, as a carbon source.

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2 Materials and Methods

Callus tissue obtained on standard MS medium (3% sucrose, 2 mg/ml 2,4-D) was passaged in equal portions onto MS medium with various carbon sources: glucose, maltose and starch [6]. A nutrient medium with sucrose was used as a control. Carbohydrates were added to the medium before autoclaving at a final concentration of 3%. Calli were cultured for a 4-week cycle. Every four days, the fresh weight of the callus mass was determined, and tissue extracts were used to determine the activity and electrophoretic spectrum of α-amylase produced by the cells.

α-Amylase activity was determined by the starch-iodine method [7]. Electrophoretic separation of α-amylase was carried out in 10%polyacrylamide gel plates in the Davis system [8], followed by staining of enzyme activity zones according to the method starch-iodine.

To prepare nutrient media (standard MS), we used sucrose (3%), glucose (3%), maltose (3%) and starch (3%) from potato starch (Serva, Germany).

The experimental and analytical data were evaluated in triplicate, graphs and electropherograms reflect the average data.

3 Results and Discussion

Our preliminary experimental work on the cultivation of callus culture of rice germs [9] showed that sucrose in the nutrient medium can be replaced by other carbon sources, such as reducing sugars – glucose, fructose and maltose, as well as oligomeric and soluble starch. In this case, the cells remain completely viable and retain, although to varying degrees, their growth and proliferative abilities. Preliminary results indicated that these carbon sources were metabolizable by cultured rice cells.

A study of the dynamics of growth of callus culture of rice embryos on media with various carbon sources showed that the greatest growth of calli during the cultivation cycle was observed in the variant with glucose and corresponded to an almost 7-fold increase in cell mass (Fig. 1).

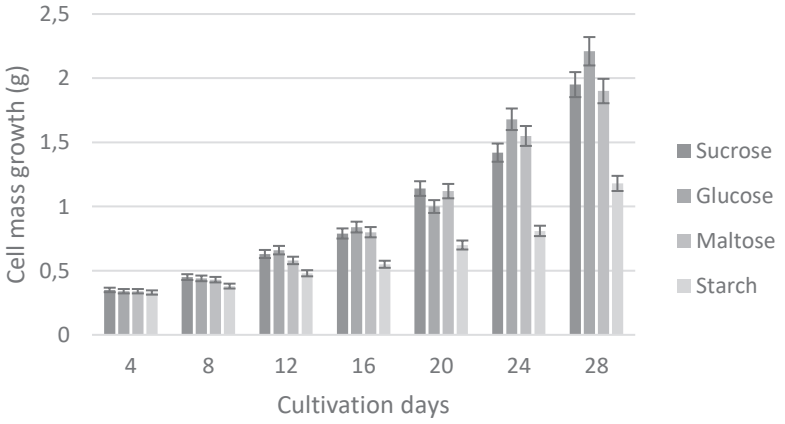

Fig. 1. Dynamics of growth of callus culture of rice embryos on media with various carbon sources

In the variant with starch in the nutrient medium, the increase in biomass was the smallest, however, it was 3.5-fold. The variant with maltose was characterized by an average increase in cell mass. It should be noted that the variant with glucose is characterized by uneven, spasmodic growth dynamics (from the 20th to the 28th day), while the variant with starch

was characterized by a gradual, smooth increase in callus mass throughout the entire cultivation period.

Fig. 2 shows data on the dynamics of growth of amylase activity in calli when cultivated on different versions of the nutrient medium.

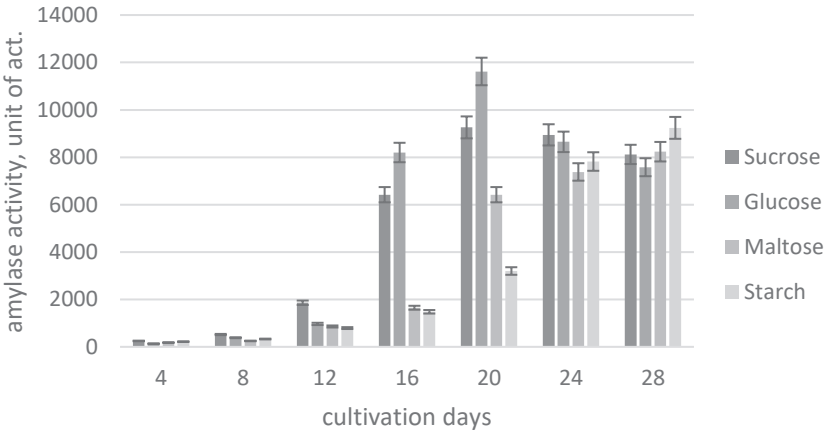

Fig. 2. Dynamics of growth of amylase activity in calli

From the above graphs it is clearly seen that as the complexity (increasing degree of polymerization) of the carbon source in the glucose-maltose-starch series occurs, there is a certain delay in the time of α-amylase induction. Thus, for the variant with glucose, a sharp jump in enzyme activity was observed already from the 12th day of cultivation, while for the variants with sucrose and maltose – from the 16th day, and for the variant with amylopectin this increase was observed only from the 20th day cell culturing.

The maximum activity of α-amylase for variants with glucose and sucrose was observed on the 20th day of cultivation, after which the level of enzyme activity decreased. In the variants with maltose and starch, the increase in α-amylase activity from the moment of its rise continued until the very end of the cell culture cycle.

Data on changes in α-amylase activity were in good agreement with the electrophoretic spectra of the enzyme (Fig. 3).

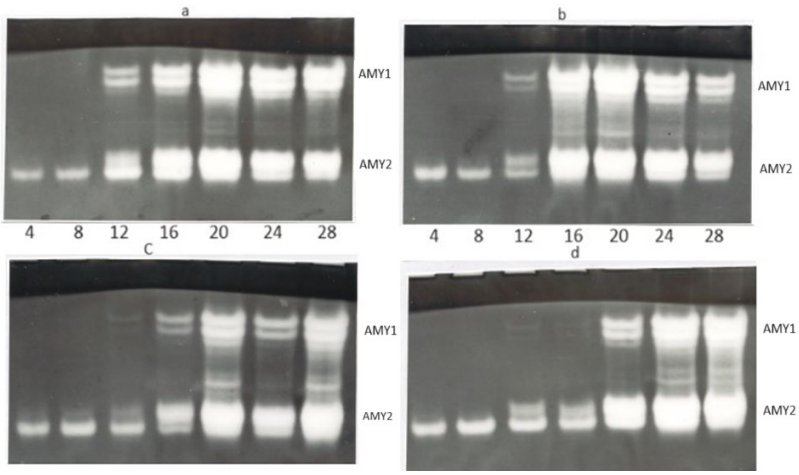

Fig. 3. Electrophoretic spectra of α-amylase activity in callus cells of all cultivation variants

The presented electropherograms show that a sharp increase in enzyme activity in callus cells of all cultivation variants is due to a sharp activation of the cathodic isoforms of group AMY2 and, especially, isoenzymes of group AMY1. The formation of the anodic components of group AMY2 of the enzyme was clearly constitutive in nature, as evidenced by the presence of activity bands in the electrophoretic spectrum from the moment the callus cells began to be cultivated.

The data obtained allow us to conclude that rice callus culture is capable of growing on a medium not only with mono- and disaccharides, but also with polymeric carbon sources. The ability of rice cells in in vitro culture to absorb the polysaccharide starch from the external environment is apparently ensured by a high level of production of the amylolytic enzyme. α-Amylase, synthesized and secreted by cells, hydrolyzes amylopectin in the culture medium to smaller fragments – dextrins and maltose. Subsequently, the sugar maltose under the action of α-glucosidase (maltase) can be converted into glucose, which is easily utilized by cells.

The results of the study indicate that there is a certain relationship between the rate of induction and accumulation of α-amylase in cultured rice germ cells and the type of carbon source of the nutrient medium.

In our previous studies using different concentrations of sucrose, it was established that the onset of induction of synthesis and secretion of cathodic isoforms of α-amylase (components of group AMY1) in rice suspension cells exactly coincided in time with the moment of depletion of the carbon source in the nutrient medium. The synthesis of the anodic forms of the enzyme (group AMY2) was constitutive in nature [10, 11].

4 Conclusion

Summarizing the data obtained, we can conclude that there are significant differences between α-amylase isoenzymes in the mechanisms of their induction, regulation and physiological role in the hydrolysis of the native substrate.

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