Influence of the culture medium on the biosynthesis of the amilolytic enzymes obtained from Aspergillus strains

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M. TAPAI (STOICA)*, GH. CAMPEANU*, S. JURCOANE*, D. BALAN*
*University of Agronomic Sciences and Veterinary Medicine, Faculty of Biotechnologies,
Bd. Marasti 59, Bucharest, Romania,
mihaiela_stoica2007@yahoo.com

Abstract

The researches were performed on two Aspergillus strains: Aspergillus niger F2T from Microorganisms Collection of Centre of Microbial Biotechnologies Bucharest and Aspergillus awamori ICCF165 from Microorganisms Collection of Institute of Chemical and Pharmaceutical Researches Bucharest.

The culture media used in this experiment: medium for fungal strains (malt extract 4% and Czapek Dox medium) and different selective culture medium (starch 2%, glycogen 0,5%; maltose 0,5%, lactose 0,5%; fructose 0,5%, soy meal 1%, sun-flower meal 2%, yeast extract 1%, peptone 1%, corn flour 0,5%, wheat bran 2%, rice bran 2%). The cultivation of the fungal strains was made in Erlenmeyer flasks under permanent stirring for 72 hours. During the cultivation samples were taken and analysed in order to estimate their amilolytic activities. It was established an optimal composition of the culture medium for an enzymatic product with increased amilolytic activity.

Key-words: α-amylase, fungal strain, wheat bran, soy meal, corn flour

Introduction

Some of the most important enzymes obtained by using modern technologies are amilolytic enzymes (α-amylases, β-amylases, amyloglucosidases), which have high applicability in many industrial processes. Enzymatic products of microbial provenience are used on a large scale in the food industry because of their advantages regarding the improvement of the quality and the storage capacity of the food products.

Microorganisms represent a great source for obtaining large amounts of enzymatic products, with minimum expenses.

The most important species of microorganisms used for obtaining of the amilolytic enzymes are:

- Aspergillus (A. oryzae, A. niger, A awamori, A. flavus, A. fumigatus, A. usanii);
- Bacillus (B. subtilis, B. amyloliquefaciens, B. licheniformis).

The composition of the culture medium represents an important factor which must be considered for the obtaining of some microbial enzymatic products.

The culture medium must contain balanced amounts of carbon and nitrogen sources destined for the biosynthesis of the amilolytic enzymes, inductors which may play role of enzymes, microelements, forerunners and cofactors as well. The biosynthesis of the amylase is highly induced by the presence of the starch in the culture medium, this compound being the natural substrate of the enzyme. Maltose, lactose and galactose also favour accumulation of the amylase.

Feniksova and all. (year?) indicated that if in the composition of the Czapek medium the starch is replaced with an equal amount of corn flour, malt extract or soy meal, a stimulatory effect appears, induced by the presence of some amino acids, which are less available for the amylase biosynthesis in usual medium, because their difficult synthesis by the fungal strains.
Materials and methods

Fungal strains
The researches were performed by using two *Aspergillus* strains:

- *Aspergillus niger* F2T from Microoganism Collection of Centre of Microbial Biotechnologies Bucharest;
- *Aspergillus Aspergillus awamori* ICCF 165 from Microorganism Collection of Institute of Chemical and Pharmaceutical Researches Bucharest.

Culture mediums:
- medium for fungal strains (malt extract 4% and Czapek Dox medium);
- selective culture mediums (with starch 2%, glycogen 0,5%; disaccharide - maltose 0,5%, lactose 0,5%; monosaccharide – fructose 0,5%, soy meal 1%, sun-flower meal 2%, yeast extract 1%, peptone 1%, corn flour 0,5%, wheat bran 2%, rice bran 2%).

Cultivation conditions:
Cultivation was made in Erlenmeyer flasks (200 ml capacity) with 50 ml medium/flask, under follow circumstances:

- cultivation period 72 hours, under permanent stirring (200 rpm);
- temperature 28°C;
- pH of the medium 5.5 (at the beginning of the experiment).

During the cultivation period samples were taken and analysed from the point of view of enzymatic activity, protein content and variation of the medium pH during the fermentation.

Methods of analysis:

- **The activities of α-amylase were** determined by using the Hostettler and all. method, that is based on the hydrolysis of starch by amylases and the obtained fragments are measured through reducing groups, spectrophotometrically dosed with 3,5-dinitrosalicilic acid (DNS).

  A unit of amylolytic activity is defined as the amount of reducing sugars (expressed in μmolls) produced by the hydrolysis of the substrate per minute at 30 0°C.

  The specific activity (U/mg protein) is defined as the ratio between enzymatic activity and the amount of protein.

- **The content in proteins** was determined spectrophotometrically by Lowry method, which is based on the producing of a copper complex as a result of the reaction of protein with an alkaline copper reagent (the biuret reaction), followed by reducing of the phosphomolibdates and of the phosphowolframates contained by the Folin-Ciocâlteu reagent with the phenolic compounds from the protein. The results were expressed in mg protein/ml product.

Results and discussions

1. **Influence of the carbon source on the biosynthesis of the fungal amylases**

1.1. **Selection of the optimum carbon source by testing of some products.**

In order to study the influence of the carbon source, the fungal strains *Aspergillus awamori* ICCF 165 and *Aspergillus niger* F2T were cultivated on different experimental variants of M2a culture medium (starch 2%, soy meal 0,5%), which was modified by replacing the starch with other product as source of carbon.

The main sources of carbon used in this experiment were: polysaccharides – starch (2%), glycogen (0,5%); disaccharides – maltose (0,5%), lactose (0,5%); monosaccharides – fructose (0,5%).

The activities of the amilolytic enzymes (A.E.) and the specific amylolytic activities (A.E.S.) of the fungal strains were analysed after 60 hours of growth on the mentioned culture
Influence of the culture medium on the biosynthesis of the amilolytic enzymes obtained from *Aspergillus* strains

media, when the cell concentration is high and the maximum accumulation of the enzymes in the culture medium occurred (table 1 and table 2).

**Table 1. Effect of carbon source on the activity and specific activity by amylases of *Aspergillus awamori* ICCF 165 strain**

<table>
<thead>
<tr>
<th>Source of carbon</th>
<th>AE (U/ml/min)</th>
<th>AES (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>4,3</td>
<td>2,7</td>
</tr>
<tr>
<td>Glycogen</td>
<td>3,5</td>
<td>2,1</td>
</tr>
<tr>
<td>Maltose</td>
<td>3,9</td>
<td>2,6</td>
</tr>
<tr>
<td>Lactose</td>
<td>2,7</td>
<td>1,6</td>
</tr>
<tr>
<td>Fructose</td>
<td>2,2</td>
<td>1,3</td>
</tr>
</tbody>
</table>

**Tableul 2. Effect of source of carbon on the activity and specific activity by amylases of *Aspergillus niger* F2T strain**

<table>
<thead>
<tr>
<th>Source of carbon</th>
<th>AE (U/ml/min)</th>
<th>AES (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>4,5</td>
<td>3,1</td>
</tr>
<tr>
<td>Glycogen</td>
<td>3,5</td>
<td>2,2</td>
</tr>
<tr>
<td>Maltose</td>
<td>3,9</td>
<td>2,9</td>
</tr>
<tr>
<td>Lactose</td>
<td>2,1</td>
<td>1,4</td>
</tr>
<tr>
<td>Fructose</td>
<td>1,5</td>
<td>1,0</td>
</tr>
</tbody>
</table>

The obtained results show that the highest values of the enzymatic activity (4,3 U/ml/min for *Aspergillus awamori* ICCF 165 and 4,5 U/ml/min for *Aspergillus niger* F2T) were registered on starch medium, so this was the most appropriate source of carbon. Similar results were obtained also on the maltose medium (3,9 U/ml/min for *Aspergillus awamori* ICCF 165 and 3,9 U/ml/min for *Aspergillus niger* F2T).

Significant values of the enzymatic activities were noticed also for the strain cultivated on the medium containing glycogen, but only moderate activities were determined in the case of using lactose and fructose as sources of carbon.

Considering these results, starch and maltose were selected for further experiments.

**1.2. Selection of the optimum concentration of saccharides**

In order to establish the optimum concentration of the selected saccharides used for the preparation of the culture medium, different variants were tested: medium M2a (starch 2%, soy meal 0,5%) containing different concentrations (0,5%; 2%; 3,5% and 5%) of starch, respectively maltose.

The data obtained in the experiments with *Aspergillus awamori* ICCF 165 strain (fig. 1) and *Aspergillus niger* F2T strain showed that the initial value of the starch concentration (2%) established according to other authors for the composition of M2a medium was the most appropriate for the obtaining of maximum amylolytic activities for both the tested fungal strains.

![Figure 1. Influence of the concentration of starch (a) and maltose (b) used as source of carbon on amilolytic activity of the *Aspergillus awamori* ICCF 165 strain](image)
Both the tested fungal strains registered a similar dynamics regarding the amylolytic enzymes accumulation, which was maximum for 2% starch, respectively 0.5% maltose added in the culture medium.

### 1.3. Testing of some natural ingredients as carbon source

Some natural ingredients (corn flour, corn starch and wheat flour) were tested because of their lower costs in comparison with the soluble starch used in the mentioned experiments.

Therefore the two tested fungal strains were cultivated on the M2a culture medium (starch 2%, soy meal 0.5%) in which starch was replaced with the mentioned natural ingredients as source of carbon.

The analyses of the obtained results (table 3) indicated that the presence of the corn starch in the culture medium determined an increase of the amylase biosynthesis with 15% for the Aspergillus awamori ICCF 165 strain, and with 10% for Aspergillus niger F2T strain in comparison with the results obtained on the medium containing soluble starch. The determinations of the amylolitic activities were made to 60 hours old fungal cultures.

<table>
<thead>
<tr>
<th>Source of carbon</th>
<th>Aspergillus awamori ICCF 165</th>
<th>Aspergillus niger F2T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AE(U/ml/min)</td>
<td>AES(U/mg)</td>
</tr>
<tr>
<td>starch</td>
<td>4.2</td>
<td>2.7</td>
</tr>
<tr>
<td>corn starch</td>
<td>4.6</td>
<td>3.1</td>
</tr>
<tr>
<td>corn flour</td>
<td>4.2</td>
<td>2.8</td>
</tr>
<tr>
<td>wheat flour</td>
<td>3.9</td>
<td>2.6</td>
</tr>
</tbody>
</table>

### 2. Influence of the source of nitrogen on the biosynthesis of the fungal amylases

#### 1.1. Selection of the optimum nitrogen source

Study of the influence of the nitrogen source on the amylase biosynthesis of the fungal strains Aspergillus awamori ICCF 165 and Aspergillus niger F2T was made on the M2a culture medium (starch 2%, soy meal 0.5%) which was modified. So, some different variants of culture medium were used, in which the source of nitrogen was replaced with: soy waste (1%), sunflower waste (2%), yeast extract (1%) and peptone (1%).

The activities of the amylolytic enzymes (A.E.) and the specific amylolytic activities (A.E.S.) of the fungal strains were analysed after 60 hours of culture on the mentioned media, when the cell concentration is high and the maximum accumulation of the enzymes in the culture medium occurred (table 4 and table 5).

Table 4. Effect of source of nitrogen on the amylolitic activities of Aspergillus awamori ICCF 165 strain

<table>
<thead>
<tr>
<th>Source of nitrogen</th>
<th>AE (U/ml/min)</th>
<th>AES (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy waste</td>
<td>4.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Sunflower waste</td>
<td>3.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.2</td>
<td>2.0</td>
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<tr>
<td>Peptone</td>
<td>3.9</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Influence of the culture medium on the biosynthesis of the amilolytic enzymes obtained from *Aspergillus* strains

<table>
<thead>
<tr>
<th>Source of nitrogen</th>
<th>AE (U/ml/min)</th>
<th>AES (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy waste</td>
<td>4,6</td>
<td>3,2</td>
</tr>
<tr>
<td>Sunflower waste</td>
<td>3,2</td>
<td>2,0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>4,1</td>
<td>3,1</td>
</tr>
<tr>
<td>Peptone</td>
<td>3,7</td>
<td>2,3</td>
</tr>
</tbody>
</table>

These results indicated that the most appropriate sources of nitrogen for the *Aspergillus awamori* ICCF 165 strain were soy waste and peptone, which determined the maximum accumulation of amylase: the values registered for the enzymatic activities were 4,3 U/ml/min, respectively 3,9 U/ml/min after 60 hours of cultivation.

For *Aspergillus niger F;T* strain, the most significant increases of the amilolytic activities after 60 hours of cultivation were registered on the medium containing soy waste, respectively yeast extract (4,6 U/ml/min, respectively 4,1 U/ml/min).

Considering these results, soy waste, peptone and yeast extract were selected for further experiments.

1.2. Selection of the optimum concentration of nitrogen source

The optimum concentration of the nitrogen source was established by testing different amount of these ingredients added in the composition of the culture medium for the fungal strains used. So, the M2a medium (starch 2%, soy meal 0,5%) was used in some experimental variants with supplements of different concentrations of soy waste, respectively yeast extract: 0,5%; 1%; 1,5%; 2%, and also different concentrations of peptone: 0,25%; 0,5%; 0,75%; 1%.

![Figure 3](image1.png)

**Figure 3.** Influence of concentration of soy waste (a) and peptone (b) on the amilolytic activity of *Aspergillus awamori* ICCF 65 strain

The analysis of the obtained results shows that for achievement of high amilolytic activities of the fungal strain, the optimum concentration of the soy waste was 1%. Beside soy waste, peptone can also be used as nitrogen source for *Aspergillus awamori* ICCF 165 strain. The optimum value for the concentration of peptone was 0,75%, but significant results were obtained even with 0,5% peptone added in the culture medium (fig. 3).

For *Aspergillus niger F;T* strain soy waste added in 1% concentration and yeast extract in concentrations of 1-2% determined the obtaining of high values of the amilolytic activities (fig. 4).

![Figure 4](image2.png)

**Figure 4.** Influence of concentration of soy waste (a) and yeast extract (b) on the amilolytic activity of *Aspergillus niger F;T* strain
1.3. Testing of some natural ingredients as source of carbon and nitrogen

Many authors reveal that some natural products, such as wheat bran and rice bran, can be used as source of nitrogen for the cultivation of the fungal strains. So, we performed further researches in order to identify other performing sources of carbon and nitrogen for improvement the biosynthesis of amilolytic enzymes of the tested fungal strains.

The experiments were performed by using *Aspergillus awamori* ICCF 165 and *Aspergillus niger* F 2T strains cultivated on M 2a medium (starch 2%, soy meal 0,5%) by replacing the carbon and the nitrogen sources with wheat bran (2%) and rice bran (2%).

The obtained results (table 6) show that using rice bran as complex source of carbon and nitrogen determined lower amilolytic activities in comparison with using wheat bran, which led to increased biosynthesis of fungal amylases for both the tested strains.

This effect of supplementation of the culture medium with wheat bran on the amilolytic activities may be explained by the richer content in water soluble vitamins (B 2, B6, B9) of wheat bran, as these vitamins are growing factors for the *Aspergillus* fungus.

### Table 6. Effect of some complex sources of carbon and nitrogen on the amilolytic activities of the *Aspergillus awamori* ICCF 165 and *Aspergillus niger* F 2T strains.

<table>
<thead>
<tr>
<th>Source of carbon and nitrogen</th>
<th><em>Aspergillus awamori</em> ICCF 165</th>
<th><em>Aspergillus niger</em> F 2T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran</td>
<td>AE(U/ml/min) 4,4 AES(U/mg) 2,9</td>
<td>AE(U/ml/min) 4,6 AES(U/mg) 3,2</td>
</tr>
<tr>
<td>Rice bran</td>
<td>AE(U/ml/min) 1,7 AES(U/mg) 0,8</td>
<td>AE(U/ml/min) 1,5 AES(U/mg) 0,6</td>
</tr>
</tbody>
</table>

In order to establish the optimum concentrations of wheat bran added in the culture medium, some tests were performed with different concentration of this ingredient (0,5%; 2%; 3,5%; 5% wheat bran in the medium) for the preparations of the medium M 2a used for cultivation of *Aspergillus awamori* ICCF 165 and *Aspergillus niger* F 2T strains.

The analysis of the obtained results reveals that both fungal strains registered a similar dynamics of the biosynthesis of the amylases (fig. 5 and fig. 6). The enzymatic activities increased in the culture medium containing between 0,5% and 5% wheat bran.

![Figure 5](image-url) Effect of concentration of wheat bran added in the medium on the amilolytic activity of *Aspergillus awamori* ICCF 165 strain.

![Figure 6](image-url) Effect of concentration of wheat bran added in the medium on the amilolytic activity of *Aspergillus niger* F 2T strain.

The highest amilolytic activity was obtained at 3,5% wheat bran added in the medium, both for *Aspergillus awamori* ICCF 165 strain, which reach a value of 2,9 U/mg for the
Influence of the culture medium on the biosynthesis of the amilolytic enzymes obtained from Aspergillus strains

Specific amilolytic activity, and for Aspergillus niger $F_3T$ strain, which registered a value of 3.2 U/mg of specific amilolytic activity after 60 hours of cultivation.

Conclusions

The researches performed in order to determine the influence of the source of carbon on the biosynthesis of fungal amylases showed that:

- Starch is the most appropriate source of carbon for the cultivation of the fungal strains, followed by maltose and glycogen; lactose and maltose determine moderate values of the amilolytic activities;
- Using the starch as source of carbon determined values of 4.3 U/ml/min for Aspergillus awamori ICCF 165 strain, respectively 4.5 U/ml/min for Aspergillus niger $F_3T$ strain of amilolytic activity;
- For obtaining of maximum activity of amylases the optimum concentration of the starch in the culture medium was established at 2%, value which is according to researches performed also by other authors;
- Using maltose as source of carbon in concentration of 0.5% in the culture medium determined the obtaining of good results concerning also the activities of fungal amylases;
- Replacing of the soluble starch with some natural ingredients (corn flour, corn starch and wheat flour) determined an increase of amylase biosynthesis with 15% for Aspergillus awamori ICCF 165 strain and with 10% for Aspergillus niger $F_3T$ strain.

The researches performed in order to determine the influence of the source of nitrogen on the biosynthesis of the fungal amylases showed that:

- The most appropriate sources of nitrogen for Aspergillus awamori ICCF 165 strain were soy waste in concentration of 1% and peptone in concentration of 0.75%, which determined values of amilolytic activity of 4.3 U/ml/min, respectively 3.9 U/ml/min;
- The most appropriate sources of nitrogen for Aspergillus niger $F_3T$ strain were soy waste and yeast extract in concentration of 1-2%, which determined values of amilolytic activity of 4.6 U/ml/min, respectively 4.1 U/ml/min;
- Using of some natural sources of nitrogen (wheat bran and rice bran) led to an increase of the amylase biosynthesis for both the tested fungal strains.

References