

# Studying the Iroteolytic Activity of the Mushroom *Aspergillus Oryzae*

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**Annotation:** The article is devoted to proteases, which are industrially important enzymes and are successfully used in the food, pharmaceutical and many industries. And pepsin is an enzyme of animal origin, characterized by a number of properties, but the production of which is dependent on the supply of animal rennet. The study of microbial producers, the resulting proteases like pepsin, is a very relevant and highly effective area both in enzymology and in practical terms.

**Keywords:** proteaza, ferment, pepsin, enzimologiya.

Proteases are industrially important enzymes that are successfully used in food, pharmaceutical and many manufacturing industries. In this regard, enzymatic hydrolysis of protein-containing substrates of various origins (plants, animals and their protein-containing wastes) to their constituent amino acids and their mixtures still remains the most important area of research in biotechnology, where to improve biotechnological processes it is necessary to search for highly active proteolytic and accompanying enzymes, especially when grinding cereals and leguminous crops are used, which contain, in addition to proteins, a high content of starch, cellulose, hemicellulose, pectin, etc.

Pepsin is an enzyme of animal origin, characterized by a number of properties, but the production of which is dependent on the supply of animal rennet. But, the study of microbial producers, the resulting proteases like pepsin, is a very relevant and highly effective direction both in enzymology and in the practical aspect. The main criterion for the successful creation of new, domestic drugs and technologies should be the selection and use of comprehensively studied microorganisms, the study of their enzyme system, as well as the identification of active producers among environmentally confined - local strains of microorganisms.

Proteases (EC.-3.4.1.) have a significant effect on the activity of many, especially the enzymes that accompany them. Using the example of alcohol factories of the Republic, it was found that the production efficiency due to proteases decreases noticeably for the following reasons: firstly, they can inhibit the actions of amylolytic enzymes, leading to proteolysis, on the other hand, they hydrolyze milled wheat proteins into amino acids, which can later be consumed by yeast during fermentation, which leads to a reduction in fermentation time and the concentration of nitrogen-containing reagents for mother yeast cultures. Unfortunately, some of these melted amino acids can serve as the basis for fusel biosynthesis, i.e. higher alcohols, which have a negative impact on the quality of alcohol and the productivity of factories. Therefore, the study of proteases and increasing their activity in a complex amylase preparation for production and in the pharmaceutical industry for the creation of drugs against diseases of the gastrointestinal tract, etc.

Based on the above, the purpose of this study was to study the component composition of enzymes in the culture liquid (CL) of the fungus *Aspergillus oryzae*, isolated from potato pulp, and to study the dynamics of the formation of neutral protease during the growth of the fungus.

### Experimental part and results

At the first stage of the research, a qualitative analysis was carried out to identify the ability of the fungus to form a protease using 10-15% gelatin and gliadin (wheat protein) on agar media, both on Petri dishes and on test tubes with a solid medium, by developing a zone of hydrolysis, liquefaction and clarification. Comparative protease activities were noted, and in all cases the protein glycolyzing ability of the culture was detected within 48-96 hours.

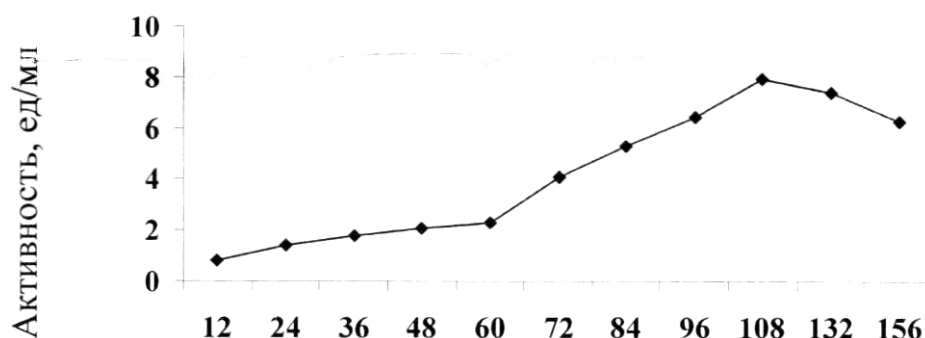
Further, for deep cultivation of the fungus and assessment of extracellular protease activity, natural carbon sources were used, such as wheat bran, starch, wheat, soy and corn flour, as well as an easily metabolized carbohydrate - glucose in various concentrations. As a source of nitrogen, in addition to flour proteins, low concentrations of mineral salts (urea, ammonium sulfate), macro and microelements (calcium, magnesium, potassium, sodium salts, as well as phosphorus in the form of diammonium phosphate) in various concentrations were added.

By studying the dynamics of growth, development and biosynthesis of protease, it was found that the local strain of the fungus *ASPERGILLUS ORYZAE*-5 is not only an active producer of extracellular hydrolytic enzymes, but also proteases that differ in the time of synthesis. The active stage of enzyme formation for protease (neutral pH value) is 84-108 hours, i.e. it is formed by *ASPERGILLUS ORYZAE*-5 in the post-stationary growth phase of the fungus.

A study of the dynamics of growth and accumulation of neutral protease activity during fungal growth showed that the protease of the fungus *A. ORYZAE*, strain -5 depends on the pH nutrient medium and the reaction mixture.

Due to the fact that the use of the highly active CL *ASPERGILLUS ORYZAE* -5, namely glucoamylase, in the production conditions of alcohol factories is carried out in a neutral pH environment, we studied the dynamics of the formation of neutral protease by this culture.

The data shown in Fig. shows that the formation of protease begins slowly from 24 to 48 hours (1.4 units/ml), then by 72 hours growth doubles, while by 96 hours it is 3.5-4.0 times and is 7.4 units/ml. So, a nutrient medium containing 3% wheat bran and 2% wheat flour with the addition of low concentrations of mineral salts is favorable for the synthesis of protease by the fungal culture under study.



Cultivation time, hour Fig. Dynamics of neutral protease production by the fungus *Aspergillus oryzae*-b.

Thus, by regulating the components of the nutrient medium and the cultivation time, it is possible to achieve that the local strain of the fungus *ASPERGILLUS* is a producer of three important enzymes simultaneously.

Similar data on protease synthesis were obtained by other authors in studies with bacterial cultures, that the accumulation of the maximum amount of intracellular protease (neutral) occurred at 36-48 hours, and release into the extracellular space occurred in two stages, 60 and 84 hours of growth of *B. subtilis* -150, i.e. secretion of protease occurred during the stationary phase of culture growth.

Thus, it was found that *ASPERGILLUS ORYZAE*, strain -5 can simultaneously serve as a producer of protease and other hydrolytic enzymes, the activity and maximum accumulation of which can be regulated by the components of the nutrient medium and the cultivation time in deep conditions for cultivating the fungus.

#### Literature:

1. Morozova K.A., Gracheva I.M., Rimareva L.V., Overchenko Selection of the *A. oryzae* strain to obtain an active producer of proteolytic and amylolytic enzymes // Abstracts of doc. All-Russian scientific and technical conference-exhibition M.: MGUPP, 2004, pp. 143-145.
2. Yakhyaeva M.A., Akhmedova Z.R. Proteolytic enzymes of the local strain of the fungus *Aspergillus oryzae*-5 and prospects for their use. Sat. abstracts of the International conference "Problems of introducing innovative ideas. Technologies and projects into production", Tashkent, 2012, pp. 514-516.
3. O.H. Lowry, N.J. Rosenbrough, Farr A.L., Randell R.I. Protein measurement with Folin phenol reagent // J. Biol. Chem. 1951. V. 193. №1. P. 265-275.