SAT-LCR-2-BFT(R)-05

MILK-CLOTTING ACTIVITY OF HIGHER FUNGI FUNALIA TROGII

Master student Ekaterina Gannochka
Department of Technology of Microbiological Synthesis,
Saint Petersburg State Institute of Technology (Technical University), Russia
Tel.: +79992154382
E-mail: katenok_0496@mail.ru

Assist. Prof. Boris Kolesnikov
Department of Technology of Microbiological Synthesis,
Saint Petersburg State Institute of Technology (Technical University), Russia
Tel.: +79516582195
E-mail: kalelovo@mail.ru

Assoc. Prof. Mark Shamtsyan, PhD
Department of Technology of Microbiological Synthesis,
Saint Petersburg State Institute of Technology (Technical University), Russia
Tel.: +79602728168
E-mail: mark.shamtsyan@yandex.com

Abstract: *The purpose of the work was to study the milk-clotting activity of higher fungi. The object of the study was the submerged culture of the fungus Funalia trogii. It was submerged cultured on glucose-peptone medium. In the native solution the level of milk-clotting and total proteolytic activities were determined. The ultrafiltration method was used to purify and concentrate the enzyme.*

The milk-clotting activity of the enzyme in the ultrafiltrate was 94.19±4.67 U/mg, proteolytic activity was 0.11±0.02 U/mg. The ratio of MCA:PA of milk-clotting enzymes for high-quality cheese producing should exceed 800:1. For the obtained enzyme preparation the ratio of MCA /PA is 823:1. Further comparison with the rennin showed that the milk-clotting enzyme preparation obtained from fungal culture Funalia trogii is not inferior to it by the level of enzymatic activity.

Keywords: *Milk-clotting, Enzyme, Fungi, Submerged culture.*

INTRODUCTION

The process of cheese making is known from ancient times. But only in the 20th century, molecular level studies were carried out, which allowed a detailed study of the mechanism of milk clotting. One of the most important point of cheese production is the selection of the milk-clotting enzyme. The taste characteristics and the yield of the finished product will depend on this. When choosing an enzyme, it is necessary to take into account not only its milk-clotting activity, but also the total proteolytic activity. High proteolytic activity can cause bitterness in the cheese and reduce its yield (Gudkov, A.V., 2004).

Traditionally, rennet was used to produce cheese. Rennet is a group of milk-clotting preparations obtained from the young abomasum (stomachs of calves, lambs, kids). Deficiency of rennet and its high cost makes it important to search for its substitutes. Nowadays instead of the traditionally used rennin, enzymes of microbial origin are often used. However, most of them have a high total proteolytic activity and can not be used for making high quality cheeses (Karychev, R.Z., 2007). One of the most promising but still little studied sources of milk-clotting enzymes could be cultures of higher fungi.

EXPOSITION

The purposes of our research were:

- screening the cultures of basidiomycetes on milk-clotting activity;
- selection of medium for cultivation of fungi to increase the activity of the enzyme
- selection of enzyme purification method.

The objects of study were the cultures of Basidiomycetes *Pleurotus ostreatus*, *Ganoderma applanatum*, *Trametes ochracea*, *Trametes suaveolens*, *Funalia trogii*, *Coriolus hirsutus*. They were submerged cultured for 7 days at the temperature of 28-30°. A semisynthetic glucose-peptone medium of the following composition was used for cultivation (g/l): glucose - 10; peptone - 2.5; KH_2P0_4 - 0.6; K_2HP0_4 - 0.4; $MgSO_4$ - 0.05; NaCl – 0.5; yeast extract - 2.0. After cultivation, the native liquid solution was separated from the biomass by filtration. In the native solution the level of milk-clotting activity (MCA) and protein concentration were determined. To determine the milk-clotting activity, the Kawai-Mukai method was used (Kawai, M, Mukai, N., 1970). It is based on determining the time of formation of the milk clot under the action of the enzyme. Calculation of MCA was conducted according to the following formula (Tipograf, D. Ya., Petina, T.A., 1966):

$$\text{MCA} = \frac{40*100*K}{T*2} \text{ U/ml,} \quad (1)$$

K - enzyme dilution ratio;
T - time of milk clot formation, min;
40 - average time of clotting for 100 ml of milk, during the cheese production, min;
2 - amount of enzyme preparation, ml.

The results of the determination of the enzymatic activity for the studied cultures are presented in the Table 1.

Table 1. Characteristics of milk-clotting activity of the studied species of Basidiomycetes

Characteristic / Fungal culture	Time of milk coagulation, min	Milk-clotting activity, U/ml
Trametes suaveolens	52.00±3.27	3.86±0.25
Funalia trogii	22.25±1.30	9.02±0.52
Coriolus hirsutus	117.11±1.85	1.71±0.03
Trametes ochracea	25.67±2.05	7.74±0.60
Ganoderma applanatum	>79	<2.53
Pleurotus ostreatus	87.38±1.58	2.29±0.04

The results show that *Funalia trogii* have the highest level of MCA. We also determined the total proteolytic activity of the native solution of this fungus. It was carried out according to the standard method recommended by GOST "20264.2-88". The results showed that the studied culture has a low proteolytic activity. Further work was carried out with this culture of the fungus.

The activity of the enzyme is greatly influenced by the amount and ratio of carbon and nitrogen sources in the medium. We studied 9 variants of media with different levels of glucose and peptone. The mineral compositions of all media were identical. The results are shown in Table 2.

Table 2. Time of milk coagulation under the action of enzyme of *Funalia trogii*,growing on different media

Number of medium	Glucose concentration, g/l	Peptone concentration, g/l	Time of milk coagulation, min
1	5	1	22,75±4,82
2	5	2,5	9,75±1,87
3	5	4	10,00±0,82
4	10	1	18,50±0,50
5	10	2,5	23,00±1,00
6	10	4	19,00±1,58
7	15	1	37,25±1,92
8	15	2,5	7,00±0,82
9	15	4	4,25±0,43

From the results it can be seen that the shortest time of milk clotting (4,25 min) is observed when using a culture grown on the medium number 9. Figure 1 shows the difference in the MCA level of the fungus *Funalia trogii* grown on different media.

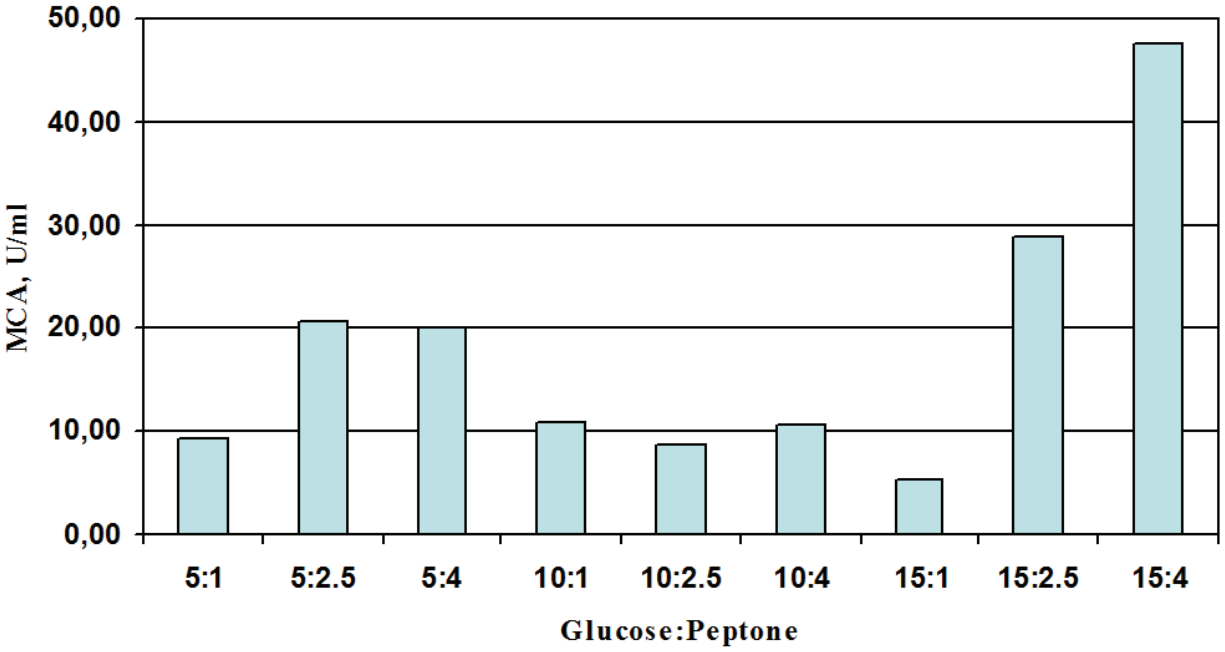

Fig. 1. MCA of *Funalia trogii*, growing on different media

As can be seen, the largest value of MCA is observed when growing the fungus on a medium with a glucose and peptone content of 15 and 4 g/l respectively.

A further aim of study was purification and concentration of the enzyme. We used several methods of purification, such as sedimentation with organic solvents, ultrafiltration, dialysis. The results showed that the highest level of MCA is achieved when the enzyme is purified by ultrafiltration with 4-fold concentrating. For this the ultrafiltration cell with membrane "MIFIL-PA-20" was used. In the ultrafiltrate the level of milk-clotting and total proteolytic activities, as well as the protein concentration, were determined. Obtained enzyme preparation was compared with commercial preparation of a rennet enzyme. The results are shown in Table 3.

Table 3. Comparative characteristics of the milk-clotting preparation of *Funalia trogii* and commercial rennet

Characteristics Preparation	Specific milk-clotting activity, U/mg	Specific proteolytic activity, U/mg	MCA/PA
Native liquid	51.19±4.67	0.064±0.002	808.59±19.83
Ultrafiltrate	94.19±4.67	0.11±0.02	823.64±3.64
Standard rennet preparation GOST 9225-84	72.8	0.082	887

It can be seen from the results that the milk-clotting enzyme preparation obtained from the Funalia trogii culture has a higher level of MCA than the commercial rennet and low level of total proteolytic activity. According to the technological requirements for milk-clotting preparations intended for high-quality cheeses, the ratio of MCA:PA should exceed 800:1. For the obtained enzyme preparation the ratio of MCA/PA is 823:1.

CONCLUSION

As a result of the work, a fungus-producer of highly active milk-clotting enzyme was found. The enzyme isolated from the native liquid of fungal culture has a high milk-clotting activity in combination with low total proteolytic activity. According to its characteristics, the enzyme is not inferior to the commercial rennet. This makes it promising for use in the cheese producing industry.

REFERENCES

Gudkov, A.V. (2004). Syrodeliye: Tekhnologicheskiye, biologicheskiye i fiziko-khimicheskiye aspekty. M.: Deli print, 2004, p.804.

Karychev, R.Z., Sokolova O.M. (2007). Molosvertyvayushchiye fermenty dlya syrodeliya. *Pererabotka moloka*, 10, pp.12-13.

Kawai, M., Mukai N. (1970). Studies on milk clotting enzymes produced by Basidiomycetes.I. Screening test of Basidiomycetes for the production of milk clotting enzymes. *Agr. Biol. Chem*, 34(2), pp.159-163.

Tipograf D. Y. A., Petina T. A. (1966). Usloviya kul'tivirovaniya Aspergillus candidus, sht. 111 i yego fermentativnyye kompleksy. *Prikladnaya biokhimiya i mikrobiologiya*, 2(4), pp.417-424.

