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**SELECTION OF OPTIMAL NUTRIENT MEDIUM
FOR COLLAGENASE BIOSYNTHESIS BY ASSOCIATION
ASPERGILLUS AWAMORI 16 AND *ASPERGILLUS AWAMORI 22***

Abstract. Medium composition is the most important aspect to take into consideration when growing any microorganism. The culture medium should include all indispensable nutrients that microorganism requires. The production of collagenase enzyme have been affected by a variety of physical and chemical factors, such as inoculum concentration, time of incubation, pH, temperature, carbon, nitrogen and mineral sources etc. However, composition of the cultivation medium (carbon and nitrogen sources) play significant role in enzymes production. In this paper the effect of different carbon and nitrogen sources on collagenase production by fungal association *Aspergillus awamori 16* and *Aspergillus awamori 22* was investigated. Maximal collagenase activity (8.1 U/ml) was detected in media with 2% sucrose as a carbon source and 1% peptone as a nitrogen source. The medium had the following composition (%): KH_2PO_4 - 0.1; MgSO_4 - 0.05; KCl - 0.05; FeSO_4 - 0.001; peptone - 1.0; sucrose - 2.0. Selection of carbon and nitrogen sources for *A. awamori 22* and *A. awamori 16* allowed increase the extracellular collagenase biosynthesis from 6.8 U/ml to 8.1 U/ml.

Key words: collagenase, micromycetes, carbon and nitrogen sources.

Introduction. In the Kazakhstan market, the growing consumer demand for healthier products has stimulated the development of nutritionally enhanced meat foods. In order to achieve these nutritionally enhanced meat foods, changes such as the use of improved raw materials, reformulation of products, and technological processes are necessary [1-3]. From the initial characteristics of the meat (its tenderness or rigidity) depends the organoleptic properties of the final meat products. The highest consumer properties possess meat products, developed from parts of the carcass with a minimum content of connective tissue [4-7].

At the same time, the problem of processing raw meat containing an increased amount of connective tissue characterized by stiffness and dryness remains urgent. To prevent excessive stiffness in production of meat products different approaches of treatment of raw meat with a high content of connective tissue have been used, e.g. mechanical and biotechnological methods. In this regards, the use of collagenase enzyme that cause proteolysis of connective tissue proteins of collagen-containing raw materials is of particular interest [8, 9].

Despite the fact that among microorganisms that produce collagenase there are bacteria, fungi, yeasts and actinomycetes in the recent period micromycetes got wide application, particularly *Aspergillus* fungi because of high productivity [10-12]. The collagenase production has been affected by a variety of factors, such as nitrogen and carbon source, inoculum concentration, time of incubation, pH, temperature, salinity, etc. Present investigation involves studies on the effect of carbon and nitrogen sources on collagenase production by association of mixed fungi *Aspergillus awamori 16* and *Aspergillus awamori 22* in submerged fermentation.

Materials and methods. The object of the study was fungal association of *Aspergillus awamori* 16 and *Aspergillus awamori* 22. The culture was maintained on potato-dextrose agar medium and stored at 4°C. For investigation of effect of carbon and nitrogen sources on collagenase production following nutritional medium was used: NaNO₃ - 0,5%, sucrose - 1,0; KH₂PO₄ - 0, 1; MgSO₄ - 0, 05; KCL - 0,05; Fe SO₄ - 0,001.

As a carbon source, glucose, maltose, fructose, lactose, sucrose, galactose and starch were used at a concentration of 1.5%. Both inorganic and organic nitrogen sources - NH₄NO₃, NaNO₃, NH₄H₂PO₄, (NH₄)₂HPO₄, peptone, casein, soy flour, and yeast extract were used at a concentration of 0.5%. Fungal suspension at concentration of 2% was aseptically introduced into flasks with a nutrient medium and placed on a shaker at 210-230 rpm for 72 hours. After this time, the collagenase activity of culture broth of all variants was measured. Collagenase activity was assayed by spectrophotometric method [13].

To 20 mg collagen from bovine tendon (Sigma) suspended in 3.8 ml Tris buffer (0.02 M Tris, 0.005 M CaCl₂, pH 7.4) was added 200 µl collagenase solution (1 mg/ml in Tris buffer) to make a total volume of 4.0 ml. The mixture was incubated at 40° C. for 3 hr or 70° C. for 30 min. The reaction mixtures were centrifuged in a microfuge for 10 min at 14,000 rpm. 1.5 ml of supernatant was mixed with 4.5 ml of 5 N HCl and kept in a drying oven at 110° C. for 16 hrs (overnight) for complete hydrolysis of soluble peptides. The hydrolysate was then analyzed for hydroxyproline content as follows: the hydrolysate was diluted 25 times with distilled water. To 1.00 ml of diluted hydrolysate 1.00 ml of chloramine-T solution is added and the mixture was allowed to stand at room temperature for 20 min. 1.00 ml of color reagent were added after this period and the reaction mixture is transferred to a 60° C. water bath and incubated for 15 min. Tubes were removed and allowed to cool down to room temperature. Absorbance at 600 nm was measured.

The fungal biomass (dry weight of mycelium) was determined as follows: the biomass obtained during cultivation of fungal association on a shaker in a liquid nutrient medium was filtered. After that the filter paper was placed in a drying oven at a temperature of 130°C for 40 min (to complete drying). The filters were transferred to a desiccator for 10-15 minutes and weighed on an analytical balance. The difference between the mass of the filter with dry mycelium and the mass of the empty filter is the mass of dry mycelium (X) formed during the period of cultivation of the fungus in the thermostat:

$$X = M_m - M_t,$$

where X is the mass of dry mycelium, g; M_f is the mass of the empty filter, g; M_m - the mass of the filter with dried mycelium.

All the analyses were performed in triplicate, and the results were expressed as mean SD values of the three sets of observations. The mean values and standard deviation will be calculated using STATISTICA 6 [14].

Results and discussion. Various carbon and nitrogen sources were supplemented in the production medium to study their effect on collagenase production. Effect of various carbon sources on collagenase production in *Aspergillus awamori* 22 and *Aspergillus awamori* 16 is shown in table 1.

As can be seen from the data presented in Table 1 enzyme production was maximal when sucrose was used as a carbon source. The activity of collagenase in medium with sucrose was 6.8 U/ml. All other monosaccharides and disaccharides used had a little effect on collagenase production. It is known that

Table 1 – The effect of various carbon sources on collagenase production in *A. awamori* 22 and *A. awamori* 16

Carbon sources	Biomass, g/100ml	Collagenase activity, U/ml
Sucrose	1,22	6,8±0,9
Glucose	1,18	3,2±0,6
Fructose	1,35	1,9±0,3
Galactose	1,0	1,2±0,4
Maltose	1,0	4,0±0,7
Lactose	1,14	1,6±0,6
Starch	1,26	1,5±0,6

disaccharides contain a higher content of carbon atoms (4.21 mol/l) than monosaccharides when used in the same concentrations. However, none of the disaccharides used, except of sucrose, did not affect on collagenase activity. The reason for this may be that the α -D-glucopyranosyl- β -D-fructofuranoside bond in sucrose makes carbon atoms more accessible to the fungus than other sugars.

Along with carbon an important factor for enzyme biosynthesis is nitrogen source. The effect of various nitrogen sources on collagenase production is summarized in table 2.

Таблица 2 – The effect of various nitrogen sources on collagenase production in *A. awamori* 22 and *A. awamori* 16

Nitrogen sources	Biomass, g/100ml	Collagenase activity, U/ml
(NH ₄) ₂ SO ₄	1,3	6,6±0,6
(NH ₄) ₂ HPO ₄	1,2	5,8±1,6
NH ₄ NO ₃	1,1	3,5±1,4
KNO ₃	1,2	1,9±0,8
Yeast extract	1,5	3,8±1,1
Peptone	1,5	7,1±0,6
Casein	1,3	5,8±0,6
Gelatin	1,4	3,3±0,4

As can be seen from the data presented in table 2, among nitrogen sources studied peptone supported moderate growth and collagenase production in *A. awamori* 16 и *A. awamori* 22.

For the selection of optimal concentrations of carbon and nitrogen sources for collagenase production 64 nutrient media with different concentration of sucrose and peptone were used (table 3).

Table 3 – Effect of various concentrations of sucrose and peptone on collagenase biosynthesis in *A. awamori* 22 and *A. awamori* 16

Sucrose	Peptone	Collagenase activity, U/ml
1	2	3
0,25%	0,25%	2,2±0,8
	0,5%	2,1±1,5
	0,75%	3,3±1,2
	1,0%	2,9±1,3
	1,25%	3,3±1,6
	1,5%	2,5±1,1
	1,75%	3,1±1,2
	2,0%	3,2±0,9
0,5%	0,25%	3,3±1,8
	0,5%	2,9±0,7
	0,75%	3,2±0,5
	1,0%	3,7±0,6
	1,25%	3,8±1,5
	1,5%	2,9±1,2
	1,75%	3,6±1,1
	2,0%	4,0±0,9
0,75%	0,25%	4,2±0,9
	0,5%	4,9±0,7
	0,75%	4,5±1,5
	1,0%	5,5±1,3

Продолжение таблицы 3		
1	2	3
0,75%	1,25%	5,6±1,2
	1,5%	5,3±0,8
	1,75%	6,0±0,4
	2,0%	4,8±1,1
1,0%	0,25%	5,9±1,4
	0,5%	4,9±0,8
	0,75%	5,5±1,7
	1,0%	5,5±1,8
	1,25%	4,2±0,6
	1,5%	5,3±1,1
	1,75%	5,0±0,4
	2,0%	5,4±1,1
1,25%	0,25%	4,5±1,0
	0,5%	5,5±1,8
	0,75%	4,9±0,6
	1,0%	5,0±0,7
	1,25%	5,1±0,5
	1,5%	4,8±0,5
	1,75%	5,3±0,9
	2,0%	4,9±1,1
1,5%	0,25%	5,5±1,3
	0,5%	4,7±1,1
	0,75%	5,2±1,0
	1,0%	5,0±0,8
	1,25%	4,6±1,0
	1,5%	5,9±0,9
	1,75%	6,7±0,5
	2,0%	7,1±0,6
1,75%	0,25%	6,8±0,7
	0,5%	6,2±0,4
	0,75%	6,4±0,5
	1,0%	7,9±1,0
	1,25%	7,2±1,8
	1,5%	7,4±1,2
	1,75%	6,9±1,1
	2,0%	8,1±1,2
2,0%	0,25%	6,8±0,9
	0,5%	7,2±0,4
	0,75%	6,9±0,4
	1,0%	8,1±0,6
	1,25%	7,7±1,2
	1,5%	7,5±1,1
	1,75%	7,3±1,5
	2,0%	7,9±0,9

Studying the effect of sucrose and peptone concentrations from 0.25 to 2.0% on the biosynthesis of protease and collagenase showed that the highest enzyme activity (8.1 U/ml) was observed in the variant with sucrose and peptone at concentration of 2.0 and 1.0%, respectively. The collagenase activity was 8.1 U/ml. In other variants, collagenase activity ranged from 2.1 to 7.9 U/ml.

In conclusion, the optimal carbon and nitrogen sources in the nutrient medium were selected. Maximal collagenase activity was detected in media with 2% sucrose as a carbon source and 1% peptone as a nitrogen source. The medium had the following composition (%): KH_2PO_4 - 0.1; MgSO_4 - 0.05; KCl - 0.05; FeSO_4 - 0.001; peptone - 1.0; sucrose - 2.0. Selection of carbon and nitrogen sources for fungal association of *A. awamori* 22 and *A. awamori* 16 allowed increase the extracellular collagenase biosynthesis from 6.8 to 8.1 U/ml.

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ASPERGILLUS AWAMORI 16 ЖӘНЕ ASPERGILLUS AWAMORI 22 АССОЦИАЦИЯСЫМЕН КОЛЛАГЕНАЗАНЫ БИОСИНТЕЗДЕУ ҮШІН ОҢТАЙЛЫ ҚОРЕКТІК ОРТАНЫ ТАҢДАУ

Аннотация. Микроорганизмдерді өсіру процесінде қоректік орта құрамы үшін негізгі талап - оның өндірушінің өсуіне және мақсатты өнімнің синтезін қамтамасыз етуге пайдасы. Қоректік орта микроорганизмнің өсуі үшін қажетті барлық қоректік заттарды қамтуы керек. Коллагеназа ферментінің биосинтезіне инокуляция концентрациясы, инкубация уақыты, рН, температура, көміртек, азот көздері, минералды көздер және т.б. физикалық және химиялық факторлар әсер етеді. Алайда, осы факторлардың арасында ферменттердің биосинтезінде қоректік орта құрамы (көміртегі мен азот көздері) аса маңызды рөл атқарады. Бұл мақалада біз түрлі көміртек және азот көздерінің *Aspergillus awamori* 16 және *Aspergillus awamori* 22 микромицетті ассоциациясының коллагеназаны биосинтездеуіне әсерін зерттедік. Коллагеназдың максималды белсенділігі (8.1 U/ml) вариантта көміртегі көзі ретінде 2% сахароза және азот көзі ретінде 1% пептон бар. Коллагеназаның максималды белсенділігі (8.1 U/ml) көміртегі көзі ретінде 2% сахароза және азот көзі ретінде 1% пептон бар вариантта көрсетілген. Қоректік ортаға көміртегі мен азоттың оңтайлы көздері таңдап алынды (%): KH_2PO_4 - 0.1; MgSO_4 - 0.05; KCl - 0.05; FeSO_4 - 0.001; пептон - 1.0; сахароза - 2.0. *A. awamori* 22 және *A. awamori* 16 ассоциациялық дақылы оңтайлы көміртекті және азот көздерін таңдау клетка сыртылық коллагеназаның түзілуін 6,8 U /мл-ден 8,1 U /мл-ге дейін арттыруға мүмкіндік берді.

Түйін сөздер: коллагеназа, микромицеттер, көміртегі мен азот көздері.

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ПОДБОР ОПТИМАЛЬНОЙ ПИТАТЕЛЬНОЙ СРЕДЫ ДЛЯ БИОСИНТЕЗА КОЛЛАГЕНАЗЫ АССОЦИАЦИЕЙ ASPERGILLUS AWAMORI 16 И ASPERGILLUS AWAMORI 22

Аннотация. Основным требованием, предъявляемым к составу питательной среды в процессе культивирования микроорганизмов, является ее полноценность для роста продуцента и обеспечения синтеза целевого продукта. Питательная среда должна включать все питательные вещества, которые необходимы для роста микроорганизма. На биосинтез фермента коллагеназы влияют различные физические и химические факторы, такие как концентрация инокулята, время инкубации, рН, температура, источники углерода, азота, минеральные источники и т.д. Однако, среди этих факторов состав питательной среды (источники углерода и азота) играют значительную роль при биосинтезе ферментов. В настоящей статье было исследовано влияние различных источников углерода и азота на биосинтез коллагеназы ассоциацией микромицетов *Aspergillus awamori* 16 и *Aspergillus awamori* 22. Максимальная активность коллагеназы (8,1 Ед/мл) отмечена в варианте, содержащем в качестве источника углерода 2% сахарозу и 1% пептон в качестве источника азота.