EVALUATION OF THE EFFECT OF OPTIMIZED KVASS WORT ON THE GROWTH OF DERMATOPHYTES DURING LIQUID-PHASE CULTIVATION

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Trychophytosis is widespread and causes significant economic losses by reducing the weight yield and the quality of leather raw materials, increasing the cost of medical and rehabilitation measures [2].

All known methods for manufacturing vaccines against mycoses are based on the cultivation of fungi by superficial mode [3, 4]. At the same time, on industrial scale, this method of growing microorganisms is considered low-tech and low-profit, comparing to modern requirements of biotechnology.

Analysis of the published literature reveals that only separate studies on the deep cultivation of *Tr. verrucosum* were previously conducted. At the same time the authors note that the debris of the trichophyton fungus obtained on the proposed liquid media had low immunogenicity. Thus, at present, the development of controlled liquid-phase cultivation of *Trichophyton spp*. and the selection of a nutrient medium is of current interest.

The goal of this research is to develop a technology for cultivating the Trichophyton fungus in deep layer cultivation based on the elaboration of a new medium and optimized conditions.

Material and methods. Fungi of the genus Trichophyton were used as an object: *Tr. verrucosum* No. 130 and *Tr. mentagrophytes* No. 135.

Kvass wort preparation. We used a concentrate of kvass wort containing 65% carbohydrates. The pH value was controlled potentiometrically in accordance with the instructions for the pH meter.

The carbohydrate content was set according to Balling using a sugar meter in accordance with the instructions attached to it.

Seed preparation. The inoculum is known to be one of the most important factor of any biotechnological process of cultivating microorganisms. The optimal dose of inoculum ensures a reduction in the lag phase, an increase in the productivity of the fungus and a reduction in the time of its growth.

Optimization of the method for obtaining the seed material of the trichophyton fungus will improve the manufacturability of the production of vaccines against trichophytosis. To obtain an inoculum, we used wort agar prepared according to an optimized method [1].

The pH of the medium after sterilization was 7.1–7.4. The culture of the trichophyton fungus was grown on wort agar for 15 days. At given temperatures of (20±2)ºC and (28±2)ºC the fungus was removed from the surface of wort agar under aseptic conditions and resuspended in a dilutor of a special composition to

a content of 50 million microconidia/cm^3. The inoculum was introduced into the liquid nutrient medium in a volume of 10%, 5% and 2.5%.

Preparation of the culture medium. We prepared kvass wort with a content of 1.5; 3.0 and 6.0% carbohydrates. The influence of physical parameters, temperature, quantity and ratio of trace elements, ionic power of the medium on the metabolism of carbohydrates has been unambiguously established.

To assess the effect of aeration on the development of the trichophyton fungus, different volumes of a liquid medium were introduced into the flasks and the air supply was regulated by calibrating the rotation of the stirrer in the fermentors (or the rocking platform). To work out the aeration mode, 100 cm^3 of the medium was introduced into 750 cm^3 flasks, the rotation speed of the rocking platform was 125 rpm. The quantity of fungal elements, their viability was determined according to technical specifications

Determination of mycelium dry mass. A suspension of the fungus culture in a volume of 10 cm^3 was centrifuged to separate the culture liquid. The fungus mycelium sediment was washed three times with a 10-fold volume of distilled water by centrifugation. The mycelium of the fungus was then transferred to a pre-weighed weighing bottle. The bottle with raw mycelium was dried at a temperature of (100–105)ºC to a constant weight, then placed in a desiccator for 2 hours to cool and be weighed.

Findings and their discussion. Due to the fact that until now there are just a few of isolated reports on deep cultivation of *Trichophyton spp*., we began our research by optimization of the kvass wort medium, the selection of cultivation modes and parameters in the liquid medium.

Different variations of media were prepared by adding various amounts of carbohydrates and mineral components. Their effectiveness was assessed by intensity of fungus development in them.

In preliminary studies, we have selected the optimal dose of inoculum. For this, the culture of the trichophyton fungus, grown on the optimized wort agar using a dilutor of a special composition, was removed after 15 days of growth and resuspended in a wort to a content of 50 million microconidia/cm^3. Kvass wort containing 6.3 and 1.5% carbohydrates was used as a medium.

The fungi *Tr. verrucosum* No. 130 and *Tr. mentagrophytes* No. 135 were grown at a temperature of 28°C for 72 hours and a rocking platform rotation speed of 125 rpm. The content of mycelium in percentage count, of microconidia in mln/cm^3 count and their viability in percentage rate was determined in the obtained samples of the fungus cultures.

From the data in tables 1-3, the growth of fungi is apparent to be affected by the concentration of sugars in the medium and the amount of inoculum added.

Table 1 – Influence of different amounts of inoculum on biomass yield and sporogenesis of *Tr. verrucosum* No. 130 in kvass concentrate wort medium with 1.5% carbohydrates

No of test	Amount of inoculum, %	Microconidia content, mln/cm^3	Dry mycelium concentration at the end of growth,%	Content of microconidia at the end of growth,mln/cm^3	Microconidia viability,%
1	9,88±0,42	4,9±0,21	0,45±0,025	7,5±1,68	19,5±3,36
2	5,0±0,42	2,5±0,21	0,32±0,017	6,3±1,26	13,0±2,52
3	2,5±0,21	1,25±0,11	0,24±0,025	5,8±1,68	12,3±2,1

Table 2 – Influence of different amounts of inoculum on biomass yield and sporogenesis of *Tr. verrucosum* No. 130 in kvass concentrate wort medium with 3% carbohydrates

No of test	Amount of inoculum, %	Microconidia content, mln/cm^3	Dry mycelium concentration at the end of growth,%	Content of microconidia at the end of growth,mln/cm^3	Microconidia viability,%
1	9,88±0,42	4,9±0,21	0,68±0,034	12,0±1,26	22,5±3,36
2	5,0±0,42	2,5±0,21	0,52±0,029	12,0±1,68	19,8±1,68
3	2,5±0,21	1,25±0,11	0,33±0,025	6,0±1,68	14,0±2,1

Table 3 – Influence of different amounts of inoculum on biomass yield and sporogenesis of *Tr. verrucosum* No. 130 in kvass concentrate wort medium with 6% carbohydrates

No of test	Amount of inoculum, %	Microconidia content, mln/cm^3	Dry mycelium concentration at the end of growth,%	Content of microconidia at the end of growth,mln/cm^3	Microconidia viability,%
1	9,88±0,42	4,9±0,21	0,70±0,042	14,3±1,68	23,8±2,9
2	5,0±0,42	2,5±0,21	0,56±0,021	17,8±2,9	19,0±2,1
3	2,5±0,21	1,25±0,11	0,44±0,013	12,3±2,1	20,5±1,68

The data from table 3 shows that, in case of the *Tr. verrucosum* No. 130, the highest mycelium accumulation (0.56-0.7%), sporulation (14.3-17.8%) and

spore viability (19.0-23.8%) were observed in the kvass wort containing 6% sugars as well as after adding 5-10% of the seed.

The medium containing 3% sugars (table 2) and after addition of 5-10% *Tr. verrucosum* No. 130 inoculum yielded a 0.52–0.68% mycelium accumulation, spore formation reached 12 million/cm^3 with a viability of 19.8–22.5%. After addition of 2.5% *Tr. verrucosum* No. 130 inoculum to the medium with 3% sugars the mycelium accumulation reached 0.33%.

A poor growth rate of *Tr. verrucosum* № 130 mycelium was seen in kvass wort containing 1.5% sugars (table 1), when 2.5%, 5% and 10% of inoculum was added, which amounted to 0.24, 0.32 and 0.45%, respectively. In this medium, regardless of the volume of inoculum, minimal sporogenesis was also observed and amounted to 5.8–7.5 million/cm^3 with a viability of microconidia 12.3–19.5%.

Analysis of the results of cultivation of *Tr. mentagrophytes* No. 135 rendered similar data as for Tr. verrucosum No. 130 (tables 4-6).

Table 4 – Influence of different amounts of inoculum on biomass yield and sporogenesis of *Tr. mentagrophytes* No. 135 in kvass concentrate wort medium with 1.5% carbohydrates

No of test	Amount of inoculum, %	Microconidia content, mln/cm^3	Dry mycelium concentration at the end of growth,%	Content of microconidia at the end of growth,mln/cm^3	Microconidia viability,%
1	9,88±0,42	4,9±0,21	0,42±0,017	4,5±0,84	14,8±2,52
2	5,0±0,42	2,5±0,21	0,33±0,025	4,8±1,68	12,3±2,1
3	2,5±0,21	1,25±0,11	0,26±0,013	4,0±1,26	9,0±2,1

Table 5 – Influence of different amounts of inoculum on biomass yield and sporogenesis of *Tr. mentagrophytes* No. 135 in kvass concentrate wort medium with 3% carbohydrates

No of test	Amount of inoculum, %	Microconidia content, mln/cm^3	Dry mycelium concentration at the end of growth,%	Content of microconidia at the end of growth,mln/cm^3	Microconidia viability,%
1	9,88±0,42	4,9±0,21	0,63±0,025	12,5±1,68	21,5±3,36
2	5,0±0,42	2,5±0,21	0,54±0,029	13,0±1,26	18,3±1,68
3	2,5±0,21	1,25±0,11	0,41±0,017	7,5±2,1	13,0±2,5

Table 6 – Influence of different amounts of inoculum on biomass yield and sporogenesis of *Tr. mentagrophytes* No. 135 in kvass concentrate wort medium with 6% carbohydrates

No of test	Amount of inoculum, %	Microconidia content, mln/cm^3	Dry mycelium concentration at the end of growth,%	Content of microconidia at the end of growth,mln/cm^3	Microconidia viability,%
1	9,88±0,42	4,9±0,21	0,66±0,021	13,8±1,68	21,8±1,68
2	5,0±0,42	2,5±0,21	0,67±0,021	15,0±1,68	22,0±2,52
3	2,5±0,21	1,25±0,11	0,44±0,025	11,8±1,26	14,8±1,68

Conclusion. 1. The most technologically advanced liquid-phase cultivation of *Trichophyton spp*. is stated to be in kvass wort with 3% sugars.

2. Adding of at least 5% of *Tr. verrucosum* No. 130 and *Tr. mentagrophytes* No. 13 seed material to the medium from the kvass wort is highly advisable.

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ANALYSIS OF THE CONTENT OF CESIUM-137 IN AGRICULTURAL PRODUCTS OF MINSK DISTRICT IN THE PERIOD 1990–2019

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As a result of the accident at the 4th power unit of the Chernobyl NPP about 70% of the radioactive substances emitted from the destroyed reactor into the atmosphere and fell to the territory of Belarus. At the same time 23% of the territory of the republic (46.5 thousand km^2) with 3668 settlements was contaminated with cesium-137 more than 37 kBq/m^2. After the accidental release a significant part of the radionuclides accumulated in the upper soil