Biologically active feed preparations with the additional introduction of selenium are presently produced by culturing fodder yeasts and algae on various liquid sugar-containing medium, among which are wastes of the cellulose and food industries. Lactobacilli are also used in additives as sources of organic forms of essential trace elements (selenium and iodine).

The purposeful addition to the culture medium of inorganic salts of trace elements leads to their incorporation into the microorganisms being raised. As a result of “bioconversion,” the biomass becomes enriched with organic forms of the “embedded” trace element. with the selenium being incorporated mainly into Se-containing amino acids of peptides and proteins [2].

The purpose of this work was to study the biochemical composition and process of biomass accumulation of Trichoderma fungi on Se-containing media for assessing the possibility of using them as a selenium-containing feed additive.

**METHOD**

The objects of investigation were strains of fungi of four species of the genus *Trichoderma*: *T. asperellum* strains Mg-6, TH-5, TH-7, and TH-11; *T. harzianum* strain M99/5; *T. koningii* strains TSL-06 and TSG; and *T. viride* strains Lg-1 and Lg-2. The biochemical composition of the biomass and metabolites of the fungi were studied by the conventional methods: total protein content, by Kjeldahl’s method [6]; amino acid composition, on an A0326V2 automatic amino acid analyzer (Knauer, Germany); total tryptophan content, by the spectrophotometric method developed at the Bakh Biochemistry Institute, Russian Academy of Sciences; and content of lipids with their subsequent
separation into neutral and glyco- and phospholipids, by the Bligh and Dyer method [7].

To study the effect of Se on germination of spores of Trichoderma fungi, we prepared suspensions with a spore titer of 10^6 in Czapek nutrient medium with the addition of Se salts in a concentration of 15, 20, and 25 μg/mL. After 24 h, we determined in each of the suspensions the number of germinated and ungerminated spores/mL, using a Goryaev counting chamber.

The effect of Se on productivity of the biomass of mycelium by Trichoderma strains was studied by stationary and submerged culturing on Czapek medium of the following composition: 30 g sucrose, 2 g sodium nitrite, 0.5 g magnesium sulfate, 0.5 g potassium chloride, and 1.0 g dibasic potassium phosphate. The concentration of Se salt in the medium was 15, 20, and 25 mg/L. Czapek medium without Se was used in the control variant.

The Se concentration in mycelium of Trichoderma fungi was measured by the atomic absorption spectrometry method (Kvant-ZETA atomic absorption spectrometer). The procedure of mineralization of the samples in analytical autoclaves was done in conformity with methodological instructions MUK 4.1.98-00 [8].

The toxicity of the mycelium of T. asperellum Th–5 with Se or without it (control) was evaluated with the use of a Paramecium caudatum test culture. Paramecium caudatum infusoria were cultured in Petri dishes on a modified Lozin–Lozinskii medium, which was prepared in the following way. The food for P. caudatum was 2–3 granules of dry yeast introduced into the middle area of the Petri dish enclosed by a plastic dish. A day after the start of culturing and for the next 14 days, P. caudatum infusoria can be used for biotesting by means of a special device “Biolat” for introducing food. For this purpose, after 14 days, 4 mL of infusoria from the dish in which they were cultured and ground mycelium in a water medium are introduced into a clean Petri dish with a 30-mm-diameter plastic dish with holes along the perimeter of the side surface placed in it. To prepare the extract, a weighed amount of dry mycelium was covered with cold water in a 1:30 ratio. Extraction of mycelium by water was carried out for 24 h and then used for assessing the toxicity on protozoa. Disposable syringes were used for introducing the culture.

Digestibility of mycelium was carried out on an “artificial stomach” device developed at the State Applied Biotechnology University. To conduct the experiment, we used 0.5 g of dry mycelium of the fungus, which was obtained by culturing on Czapek with 20 mg/g medium with and without selenium. Egg white protein was used as the control. The degree of digestibility was investigated for 3 h; samples were taken every 30 min for measuring the optical density. The device allows conducting hydrolysis under conditions of continuous agitation of the medium and removal of low-molecular-weight products of protein hydrolysis through a semipermeable membrane. To determine the rate of protein digestion, we calculated the accumulation of protein hydrolysis products in dialysates in terms of tyrosine expressed in arbitrary units, μg/mL tyrosine, using a calibration graph of the dependence of tyrosine content in μg/mL on optical density of the dialysate stained as a result of reaction with Folin’s reagent on a photoelectric colorimeter at λ = 630 nm in a cuvette with a 10-mm working length (by Lowry’s method).

RESULTS AND DISCUSSION

Determination of proteins and lipids in biomass as the most valuable components of feeds participating in the most important functions of an organism is a mandatory condition for recommended micromycetes as producers of feed preparations and biologically active additives. However, information on the biochemical composition of the biomass of Trichoderma strains is scanty and fragmentary.

Investigations showed that the greatest amount of protein is contained in the biomass of T. asperellum TH–11 and TH–5, T. harzianum M99/5, and T. koningii TSL–06 strains (Table 1). These same strains of fungi accumulated more protein also in the culture liquid, from 1.26 to 1.54%. An amino acid analysis was performed for Trichoderma strains containing the greatest amount of total protein. The total amino acid content in the investigated strains was from 19.08 to 21.77% absolute dry weight (Table 2). Aspartic and glutamic acids dominated among nonessential amino acids. The total amount of essential amino acids out of the total sum of amino acids in a pure culture of mycelium of strains TH–5, TH–7, and TH–11 of species T. asperellum and M99/5 of T. harzianum was respectively 56.8, 48.18, 45.38, and 47.20%. Lysine, arginine, and leucine dominated among the essential amino acids in mycelium of the investigated strains. According to the literature data, these amino acids are contained in plant proteins in insignificant amounts; therefore, they are limited in animal feeds and often there is not enough of them for balanced nutrition. Furthermore, the mycelium of the strains contains the essential acid tryptophan in an amount comparable to the mycelium of basidiomycetes recommended as a source of food and feed protein [9].

An important place among biologically active substances, along with proteins, is occupied by lipids, and their value is higher, the more biologically important phospholipids in their composition [10].

The total content of lipids in the biomass of micromycetes of Trichoderma was 4.21–13.57%. Strains of T. asperellum TH–11, Mg–6, TH–7, and TH–5 contained their maximum amount (Table 2).