Biotechnology

Production of Protein-Rich, Easily Metabolizable Biomass Based on the Biotransformation of Apple Juice Industry Waste

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Bioconversion of apple juice industry waste of the Georgian company "Kula" into protein-rich, easily digestible biomass has been implemented. Non-pathogenic strain *Sporotrichum pulverulentum* G-11 from the collection of mycelial fungi of Durmishidze Institute of Biochemistry and Biotechnology (DIBB) of the Agricultural University of Georgia, which is characterized by fast growth on lignocellulosic substrates and high ability to accumulate protein in biomass, has been used as biotransformation agent. Mycoprotein-enriched, partially delignified, easily digestible biomass was obtained on the base of optimization of the strain cultivation conditions and nutrient medium composition. *© 2023 Bull. Georg. Natl. Acad. Sci.*

mycelial fungi, protein-rich biomass, solid-state fermentation, mycoprotein, apple juice industry waste

The global food crisis was further exacerbated by the record high food prices in 2020-2023. According to experts, this will inevitably increase the level of hunger and malnutrition and put millions of people at risk of extreme poverty. Production of food additives enriched with functional ingredients on the base of conversion of agro-industrial waste by the mycelial fungi is considered to be one of the most convenient, ecologically safe and fast-realizing technologies for filling food shortages [1]. That is why it is very important to search for a possible perfect protein producer, to optimize its cultivation conditions, and to develop environmentally friendly and efficient technologies for the food supplements production [2]. The aim of the presented study was to obtain protein-rich, easily metabolizable, non-toxic biomass based on the bioconversion of apple juice industry waste.

It should be noted that the microbial conversion of apple juice industry waste has not been implemented in Georgia until now. Therefore, the recycling of this type of waste will be beneficial for the companies, who, along with the utilization of the waste, will produce nutritional supplements rich in protein and biologically active compounds.

Materials and Methods

Thermophilic, non-pathogenic and non-toxic strain of *Sporotrichum pulverulentum* (*Phanerochaete chrysosporium*) G-11 from the collection of mycelial fungi of DIBB of the Agricultural University of Georgia was the object of the research. The residue of apple juice industry ("Kula" company) was used as a biotransformation substrate in the experiment.

The solid-state fermentation (SSF) of the waste was performed in a thermostat at 40ºC, during10 days. For this purpose, 6 g of absolutely dry weight (ADW) waste was placed in 100ml volume Erlenmeyer conical flasks, and added by 18ml of the following composition nutrient medium, (g/l): $NaNO_3$ – 3.0; KH_2PO_4 – 1.0; $MgSO_4x7H_2O$ – 0.5; $FeSO_4x7H_2O$ – 0.02. Flasks were sterilized by autoclaving at 1 atm. for 45 minutes. Two ml of fungal spores' suspension was placed in the cooled flasks.

After the cultivation was completed, the contents of the flasks were transferred with a spatula to a beaker adjusted to a constant weight. The beaker with the sample was placed in a thermostat at 105ºC, to be dried till the constant weight. The difference between the initial and final weights corresponded to the biomass obtained as a result of bioconversion.

In the biomass obtained by solid-state cultivation of mycelial fungi, the main constituents, namely, extractable matter, lignin, cellulose and hemicellulose were determined [3].

The percentage of true mycelial protein was determined in trichloroacetic acid-treated biomass, using Kjeldahl's modified photometric method with Nessler's reagent and a coefficient 6.25 [4].

Selection of the additional source of nitrogen and its optimal concentration was performed according to standard approach [5]. Cultivation conditions in which the producer accumulated the maximal amount of protein were considered as optimal.

A separate series of experiments were repeated three times. Average values of the results and their standard deviations are demonstrated in tables and graphs.

Results and Discussion

It is known that high protein content in the biomass and the fast growth are the main parameters for the selection of mycoprotein producing strain [6]. One of the non-pathogenic strains of *Sporotrichum pulverulentum* G-11, from the collection of mycelial fungi of DIBB, Agricultural University of Georgia, which is distinguished by its high ability to degrade lignin and cellulose, as well as by the

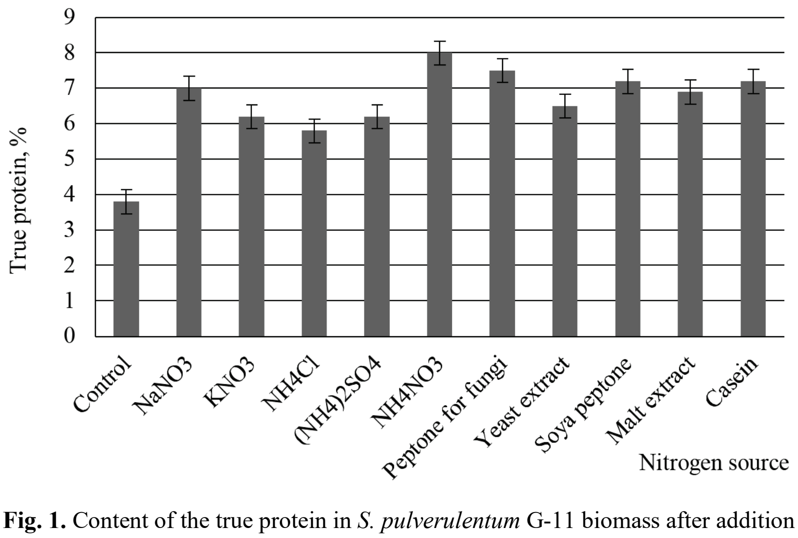

Fig. 1. Content of the true protein in *S. pulverulentum* G-11 biomass after addition of different sources of nitrogen.

fast growth and high content of protein in biomass, was selected as the leading agent for the apple pomace bioconversion in our experiments [7].

The SSF method was selected for the cultivation of *S. pulverulentum* G-11, which is similar to the natural conditions of mycelial fungi growth and allows the direct transformation of lignocellulosic substrates [8]. At the same time, SSF does not require special types of bioreactors, aeration and related energy costs and is economically efficient [9].

Since the selected substrate – pomace – contained a very small amount of nitrogen (3.8%), in order its further use in the bioconversion, supplying with the additional source of nitrogen of the strain nutrient medium was necessary. At the same time, it is known that an additional source of nitrogen can dramatically affect the bioconversion process of plant substrates. According to some authors, application of an additional source of nitrogen to the nutrient medium accelerates the utilization of polysaccharides and suppresses the delignification process, which leads to a decrease in biomass digestibility [10]. Accordingly, selection of the optimal nitrogen source for *S. pulverulentum* G-11 cultivation was aimed at the next stage of the research. For this purpose, the selected strain was cultivated on nutrient media with different nitrogen sources – $NaNO_3$, KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, peptone, yeast extract, soya peptone, casein and malt containing ones.

As seen from Figure 2, it is clear that in the case of ammonium nitrate or soybean peptone application as an additional source of nitrogen in the nutrient medium, the yield of true protein in biomass increased to 8%. Since ammonium nitrate is much cheaper than soya peptone, it was considered appropriate to use NH_4NO_3 in further research to reduce the cost of the target product. Influence of different concentrations of the selected nitrogen source on the growth and development of *S. pulverulentum* G-11 and, accordingly, on the protein content in the biomass was studied at next stage of experiment.

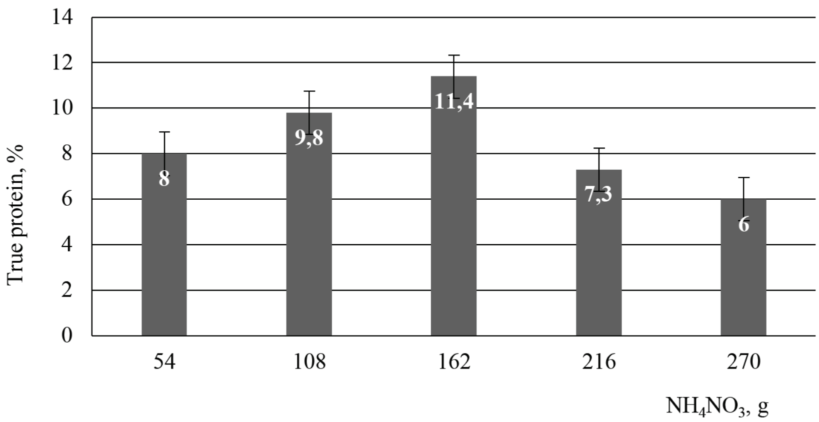

Fig. 2. Influence of different concentrations of the additional source of nitrogen on growth and development of *S. pulverulentum* G-11 under the SSF conditions on apple juice industry waste.

Fig. 3 clearly demonstrates that the maximal amount of protein (11.4%) was accumulated by the fungal culture when nutrient medium was enriched with 162mg of NH_4NO_3 per 6g of the substrate. The increase of fungal protein in this variant made 7.6% (as compared to control). According to obtained results it was concluded that 162 mg of NH_4NO_3 per 6 g of substrate is optimal for the obtaining of protein-rich biomass from the pomace.

It is known that the digestibility of lignocellulosic substrates is inversely related to their lignin content. In order to determine the possibility of application of the strain-produced biomass as animal food additive, the component composition of the product obtained by the biotransformation of apple pomace was studied at the last stage of the experiment; the digestibility of the product was evaluated on the base of obtained data.

appropriate studies, in particular, excluding its pathogenicity/toxicity it is quite possible to recommend it as a feed additive in animal and poultry diet.

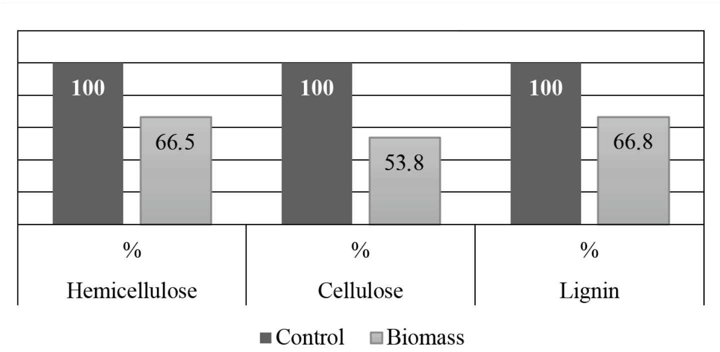

Fig. 3. Content of hard-to-degrade biopolymers (cellulose and lignin) in the biomass obtained by the SSF of pomace.

From Fig. 4 it is evident that *S. pulverulentum* G-11 prefers cellulosic component from the biopolymers of pomace. 46.2% of the initial amount of cellulose was metabolized by the fungus during 10 days of cultivation. As for hard-to-degrade non-carbohydrate biopolymer – lignin, its content was reduced by 33.2%, which significantly increases the digestibility of biomass. Thus, on the basis of step-by-step experiments easily metabolizable, partially delignified biomass was obtained under the SSF of apple juice industry residues with *S pulverulentum* G-11, in which the true protein content increased by 7.6% as compared to the control.

The obtained biomass may be stored in a dry state, in the form of flour. After the completion of

Conclusions

1) Biotransformation of the residue of apple juice industry – apple pomace into the mycoprotein-rich, easily digestible biomass is possible.
2) After the determination of nutritional value of the protein-rich biomass obtained from the bioconversion of apple juice industry waste and excluding its pathogenicity/toxicity, the product may be recommended as a food additive in animal and poultry diet.

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ბიოტექნოლოგია

ცილით მდიდარი, ადვილად მეტაბოლიზებადი ბიომასის მიღება ვაშლის წვენის წარმოების ნარჩენების ბიოტრანსფორმაციის საფუძველზე

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განხორციელებულია ქართული კომპანია „კულას“ ვაშლის წვენის წარმოების ნარჩენების ბიოკონვერსია ცილით მდიდარ, ადვილადმონელებად ბიომასად. ბიოტრანსფორმაციის აგენტად გამოყენებულია საქართველოს აგრარული უნივერსიტეტის, დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტის მიცელიური სოკოების კოლექციის არაპათოგენური შტამი – ***Sporotrichum pulverulentum*** G-11, რომელიც ხასიათდება ლიგნოცელულოზურ სუბსტრატებზე სწრაფი ზრდითა და ბიომასაში პროტეინის დაგროვების მაღალი უნარით. შტამის კულტივირების პირობებისა და საკვები არის შემადგენლობის ოპტიმიზაციის საფუძველზე, მიღებულია მიკოპროტეინით გამდიდრებული, ნაწილობრივ დელიგნიფიცირებული, ადვილადმონელებადი ბიომასა.

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