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# ISOLATION OF BIOLOGICALLY ACTIVE POLYPEPTIDE FROM SACCHAROMYCES CEREVISIAE YEAST

F. S. Nabieva
Samarkand State Medical University

G. A. Dushanova
Institute of Biochemistry at Sharof Rashidov
Samarkand State University Samarkand, Uzbekistan

**The aim of the work.** To isolate and study the characteristics of biologically active polipeptides of Saccharomyces cerevisiae.

**Material and methods.** The object of the research was dry yeast Saccharomyces cerevisiae. Determination of protein content in the studied objects was carried out after preliminary mineralization of the sample with sulfuric acid, followed by determination of protein nitrogen with Nessler's reagent. Extraction was carried out with 0.2% NaOH for 3 hours, 370 $C^0$, at a water ratio of 1:5, then ultrasound treatment for 5 minutes, which ensures the maximum protein yield. Then, ammonia sulfate was used at 80% sediment to obtain low-molecular polipeptides. The 80% sediment extract was centrifuged and the resulting low-molecular oligopeptide precipitate was desalted on a Sephadex G25 column. The electrophoretic mobility of polypeptides was studied.

**Research results.** In the studies to determine the protein content, the following devices were used: spectrophotometer, analytical scales with an accuracy of 0.0001 g, filter paper (white tape), conical funnels, 50 ml measuring flasks, sodium hydroxide, Rochelle salt, Nessler reagent, distilled water, concentrated sulfuric acid, concentrated hydrogen peroxide. Next, optimal conditions for protein extraction were developed. Extraction was performed with 0.05 Tris HCl (pH 8.5), 2% Na2CO3, 0.2% NaOH. The hydromodule was selected (1:5, 1:10, 1:15), and the extraction time was 60-120-180-240 minutes, for complete destruction of cells, the homogenate was treated with ultrasound at an oscillation frequency of 34 kHz.

Extraction was carried out with 0.2% NaOH for 3 hours, 370C$^0$, at a water ratio of 1:5, then ultrasound treatment for 5 minutes, which ensures the maximum protein yield. After extraction, centrifugation was performed for 30 min at 8000 rpm. The next purification step was fractionation with dry ammonium sulfate. To remove high-molecular proteins, ammonium sulfate was used at 20-40-60% precipitation. Ammonium sulfate was then used at 80% precipitation to obtain low molecular weight polipeptides. The 80% sediment extract was centrifuged and the resulting precipitate of low molecular weight polypeptides was desalted on a Sephadex G25 column. Gel filtration on Sephadex G25 was performed with collection of 4 ml of eluate. The concentration of the obtained desalted protein fractions was determined using a Cary 60 spectrophotometer (Germany) at a wavelength of 280 and 260 nm. The resulting desalted protein fraction was collected and lyophilized under high vacuum (-870C) using a CHRIST Alpha 2-4 LSCbasic instrument (Germany). The yield of purified (polipeptides) was 5.7%. Fractions were analyzed using electrophoresis in 10% polyacrylamide gel. The standard Novex™ Sharp pre-stained Protein Ladder (Invitrogen) was used as markers. Electrophoresis was performed under denaturing conditions. Samples were applied at 20 µl per well. During the migration of samples in the stacking gel (6%), the voltage was maintained at 50 V. When moving the front in the separating gel (10%), the voltage was increased to 80 V. Electrophoresis was performed until bromophenol blue was released from the gel. Gels were stained with 0.25% Coomassie G250 solution (Sigma, USA) in a mixture of water: ethanol: acetic acid:water (1:1:2).

## Conclusion

According to electrophoresis data, purified polipeptides have an identical fractional composition with a molecular weight from 8 kDa to 40 kDa.