Molecular Genetic Characterization of Pathogens Onychomycosis and Creation of a Collection of Microorganisms-producers of Specific Antigens

N.L.Shapekova, E.V.Kuchar
shapekova_nl@enu.kz
Eurasian national Gumilyov University, Astana, Kazakhstan

Object of research are antigens isolated from clinical strains of fungi - agents of onychomycosis person; immune serum-obtained by the use of these antigens.

The purpose of work - Molecular-genetic researches and development of a rapid test system for the detection of dermatomycetes antigens in order to improve the diagnosis of onychomycosis. At the intermediate stage: Obtaining of antigens and antibodies and checking of immunochemical characterization. To solve these problems: Obtain biomass, antigens isolated and purified, allocated the most frequent representatives of the fungi - agents from onychomycosis; get hyper-immune serum to specific antigens and to determine the diagnostic value of antibodies in different serological tests; examine the properties of the activity, specificity, and immunogenicity of the antigens, determine the working titer of the antigens in the different serological tests.

The methods of work – biochemistry, biotechnology, immunological and statistical methods of research have been used.

In the process of studies aimed obtaining biomass of dermatomycetes Trichophyton rubrum and Trichophyton interdigitale as the most frequent representatives of the fungi - agents of onychomycosis; the isolation and purification of antigens filtration methods of low-speed centrifugation, dialysis; obtaining Hyper sera to specific antigens and diagnostic evaluation of antibodies in various serological reactions: RA, RP, RAC, ELISA; analysis of the properties, activity, specificity and immunogenicity of antigens, determination of their working titer.

During the reporting period, the company conducted the cultivation of two strains of the underlying conditions for accumulation of biomass from which received 4 soluble antigen and 2 corpuscular antigen. Determined the concentration of the components of the antigens, polysaccharides and 0.125-0,500 mg/ml, proteins up to 0.6-0.25 mg/ml Concentration of polysaccharides of particular antigens reached values as 2.0 to 4.0 mg/ml Antigens used for immunization of laboratory animals and in the formulation of serological reactions.

The laboratory animals were immunized with various schemes and obtained immune serum. Testing of sera in various serological reactions allowed to find agglutination properties of particular antigens T.rubrum and T. interdigitale with a titer of antibodies in the range of 1:64-1:256. Statement of the precipitation reaction is finding precipitation properties of the protein antigen T.rubrum strain №146. Other antigens do not have precipitation properties. In ELISA analysis revealed a high activity of antigens with titers of specific antibodies to 1:1600-1:3200. Complement binding properties are defined due to the effect of anti-complement. The optimal concentration and the working titer of components for setting serological reactions: RMA - 100 thousand cells/ml, ELISA 10,0 mcg/ml.

As a result of research antigens obtained from two strains of pathogens onychomycosis T.rubrum and T. interdigitale performed checking of immunochemical characterization. It has been established that corpuscular antigens have agglutination properties, are active in the ELISA, do not have precipitation properties. Soluble polysaccharide and protein antigens are active in ELISA, identifys specific antibodies in high dilutions. Protein antigen T. rubrum has precipitation properties.

The essence of the novelty of the results of the study is that there have been antigens of T. rubrum and T. interdigitale and immune serum to them, carried out comprehensive studies of their immunochemical characteristics.

The major biotechnology indicators: the methods for the selection of antigens of agents of onychomycosis and the method of obtaining immune sera.

The degree of implementation - selected 10 strains dermatomycetes to create collections of microorganisms, which was the basis for the edition of the «Concise Atlas of mushrooms of the genus Trichophyton - typical agents of onychomycosis», 2 strains allocated antigens and immunochemical characterization was tested.

Field of application – biotechnology, medical and veterinary Mycology. The efficiency of development is determined by the application of the proposed method of obtaining the antigen from the agents of onychomycosis, which will be proposed as a component of the diagnostic test-systems.