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ISOLATION AND CHARACTERIZATION OF ANTIGENS FROM *Pseudomonas aeruginosa* IN EXHAUSTIVELY CULTIVATED BIOMASS*

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Biotechnology for producing biomass and controlling biosynthetic processes of desired complex polysaccharides is developed. The isolated polysaccharides exhibit protective activity in a test for active protection. Various polysaccharide fractions induce the production of antibodies with different degrees of passive protection. The most active antibodies are obtained from the blood of animals immunized with the first fraction of polysaccharides from biomass strain 263. Polysaccharides of the first fraction are analyzed by HPLC. The amount of nucleic acids and proteins in the preparation is quantitatively estimated.

The wide distribution of *Bacillus* infection with frequently generalized cases and the high degree of mortality from it are due to resistance to a whole range of chemical and physical agents and the partial resistance to antibiotics [1, 2].

We prepared biomass with controlled biosynthesis of the desired products, complex polysaccharides, in order to isolate the protective agents of *Bacillus*, the basis of the immunopreparations. We used *Bacillus* strains Nos. 8, 263, and 868 to produce them. Three polysaccharide fractions were isolated from the supernatant of strain biomass. The protective activity of the resulting preparations was exhibited in a test of active protection. Thus, the ED_{50} of polysaccharide fractions 1-3 from biomass of strain 263 consisted of 0.001, 0.005, and 0.002 mg; of strain 868, 0.002, 0.005, and 0.008 mg; of strain 8, 0.005, 0.01, and 0.01 mg, respectively.

The protective properties of the polysaccharides were studied by i.p. and s.c. injection of rabbits with subsequent determination of the antibody titer in serum of experimental animals by reactive passive hemagglutination (RPHA) with an estimate of the preventive activity. A high titer of antibody is found in the serum of rabbits immunized with fraction 1 of strain 263. The inverse geometric-average antibody titer in RPHA was 41,686 ± 1.7. The inverse geometric-average antibody titers in RPHA in sera of animals immunized with fractions 1 and 2 of strain 263 and fractions 1-3 of strain 8 and 868 were from 9120 ± 1.4 to 27,542 ± 1.4.

The preventive activity of rabbit hyperimmunized sera was estimated in a test for passive activity. The indices of effectiveness (IE) of sera from rabbits immunized with polysaccharide fractions 1-3 of strain 263 were 32, 20, and 24 ED, respectively. The IE of sera from rabbits immunized with fractions 1-3 of strains 8 and 868 were from 11 to 23 ED.

The results demonstrated that different polysaccharide fractions from *Bacillus* induce the production of preventative antibodies with various degrees of passive protection. The most active antibodies are obtained from the blood of animals immunized with fraction 1 of strain 263. Polysaccharides of fraction 1 of strains 8 and 868 are less effective for inducing antibody production.

The physicochemical properties of fraction 1 antigens from strain 263 biomass were studied. The protein and nucleic-acid contents were determined [3]. The results indicated that this preparation contains no protein but does contain nucleic acids at 4.7% of the dry weight. HPLC analyses provided a quantitative determination of the preparation composition and the mass of the polysaccharides [4]. The antigenic preparation of fraction 1 consists of three polysaccharides A, B, and C. The MM of these polysaccharides and their percent contents are 28 (67.3%), 33 (17.3%), and 85 kDa (15.4%), respectively.

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EXPERIMENTAL

Stationary strains of *Bacillus* 8 and 868 were obtained from the museum collection at the I. I. Mechnikov Scientific-Research Institute of Vaccines and Sera, Russian Academy of Medical Sciences. Freshly isolated strain 263 came from the blood of a patient with *Bacillus* sepsis. All cultures were standardized in Hottinger and Marten classical nutrient media.

Microbe biomass was grown with periodic exhaustive cultivation in a reactor. Cultures were grown for 12 h at 37°C with constant stirring and continuous supply of sterile air. Sterile glucose solution (40%) calculated to be 0.1-0.2% of the substrate volume was added during the cultivation in order to stimulate the multiplication of the microbes and to maintain the pH at 6.8-7.2.

Strains of *Bacillus* accumulated biomass during the cultivation and produced a large quantity of polysaccharides in the supernatant. The first fraction of polysaccharides precipitates during clarification of the supernatant. The complex is released during dialysis of the precipitated substances. The polysaccharide biopolymers transfer into solution but the highly polymerized polysaccharides form a precipitate.

The isolated polysaccharide fractions from the clarified supernatant were precipitated by ethanol cooled to -30°C. The polysaccharides were isolated from the ethanol at -10°C or below. The precipitate was dialyzed against distilled water. All polysaccharide fractions were lyophilized.

The protective properties of the polysaccharides in tests of active and passive protection were determined according to the literature method [5].

Chinchilla rabbits of mass 2-2.5 kg were used to produce hyperimmune sera. The animals were i.p. immunized with the tested preparations three times at one week intervals. The entire blood pool was collected seven days after the last injection.

Reactive passive hemagglutination followed the usual method [6] with use of erythrocytic diagnostics constructed by us on the basis of sensitins isolated from the supernatant of *Bacillus* cell biomass.

The presence of proteins in the preparation was determined by the Warburg and Christian method. The nucleic-acid content was determined according to Spirin. Polysaccharides were separated by gel-filtration HPLC on a 1×250 mm microcolumn packed with TSK-G4000SW gel. The eluent was doubly distilled water. The linear elution rate was 19 mm/min. A Millichrome chromatograph with a UV spectrophotometric detector operating at 210 nm was used. Polysaccharide markers were dextrans T20, T40, and T110.

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