IMMUNOLOGY AND MICROBIOLOGY

Comparative Study of *Saccharomyces cerevisiae* LPS

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LPS of yeast strains producing human epidermal growth factor were studied. Experiments demonstrated the absence of essential differences in the characteristics of these LPS and LPS of nonrecombinant *Saccharomyces cerevisiae* strains, which allowed us to develop a universal complex technology of simultaneous preparation of heterologous proteins and highly active immunomodulating LPS.

Key Words: *lipopolysaccharides; Saccharomyces cerevisiae; recombinant strains; immunomodulators*

In recent decades the sphere of application of *Saccharomyces cerevisiae* yeast in industrial biotechnology was markedly extended because of the use of recombinant strains (RS) of these microorganisms. The technology for obtaining human epidermal growth factor (EGF) based on use of *S. cerevisiae* RS secreting growth factor into culture medium was developed [3]. The biomass of yeast cells contains heterologous polypeptide in negligible amounts and is not used for obtaining EGF. On the other hand, the study of the composition and content of bioactive substances in this biomass and development of methods for their isolation for improving the efficiency of the technological process hold much promise.

A promising method of using biomass is isolation of pharmacologically active LPS similar to zymosan, pleiotropic immunomodulator with high clinical antitumor and antiinfection potential [8]. It is known that biological activity of LPS largely depends on the characteristics of yeast strains and parameters of culturing [1], which, in case of RS, are strictly determined by the conditions providing the maximum yield of the recombinant protein. We compared LPS derived from *S. cerevisiae* RS and nonrecombinant strains (NRS) in order to evaluate the possibility of isolating bioactive LPS from plasmid-containing microorganisms and their medical use.

MATERIALS AND METHODS

S. cerevisiae RS secreting EGF into culture medium, a gift from Laboratory of Yeast Genetics of Center of Bioengineering of Russian Academy of Sciences, and plasmid-free strains were used in the study. The strains were cultured on shakers at 26°C. Selective medium for RS culturing contained 0.67% Yeast Nitrogen Base (Difco), 0.5% Casein hydrolysate acids (Difco) without tryptophan, and 2% glucose (pH 6.0).

LPS were isolated from the biomass by the modified method of L. Pillemer [5] and tested for zymosan in accordance with Pharmacopoeia VFS-42-401-75 requirements. Components of LPS in their hydrolysate were qualitatively assayed by paper chromatography (Wathman No. 1) in a butanol-pyridine-water system (10:3:3). The content of reducing substances was evaluated by the method of Bertrane [2], total nitrogen was assayed after Kjeldahl [4]. LPS were tested for toxicity and pyrogenicity in accordance with the requirements of the State Pharmacopoeia [6]. Leukocytes in mouse peripheral blood were counted in a Goryaev chamber [9]. Serological activity of LPS was

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TABLE 1. Characteristics of LPS from Different *Saccharomyces cerevisiae* Strains (*M*±*m*)

Characteristics	NRS	RS
Weight loss after drying, %	4.8±0.4	4.9±0.5
Preparation output, %	9.7±0.5	9.8±0.4
Reducing substances, %	70.1±3.8	71.5±4.7
Total nitrogen, %	1.15±0.12	1.10±0.11
Glucose/mannose	6.57±0.61	6.60±0.52
Increase of leukocyte count in mouse peripheral blood, %	98.2±5.9*	101.2±6.4*
Phagocytic index, %	58.9±4.3*	59.3±3.9*
Phagocytic number	3.6±0.5*	3.5±0.5*
Number of rosette-forming cells, 10^{-4} per 10^6 splenocytes	2.27±0.30*	2.32±0.24*
Serological activity, mg/ml	1.15±0.10*	1.10±0.10*
Toxicity, number of dead mice	0*	0*
Maximum summary increase of body temperature, °C	1.3±0.1*	1.2±0.1*

Note. Means for RS and NRS groups are presented. *$p<0.05$ compared to the control.

evaluated by the least amount of the preparation inhibiting hemolysis by 50% [9]. Granulocyte phagocytic activity was evaluated using polystyrene latex [7], rosette-forming capacity of lymphocytes was assayed as described previously [12]. Control animals received equivalent volume of normal saline.

The results were statistically processed using Student's *t* test.

RESULTS

LPS isolated from RS and NRS are light-gray nonhygroscopic powders without odor, insoluble in water, ethanol, ether, acetone, and forming suspensions in normal saline stable for 30 min. Weight loss upon drying and the total yields were the same (Table 1).

Since the active component of LPS is presented by β-(1,3)-glucan [11], the characteristics of polysaccharide components of LPS are essential. LPS from RS was characterized by high percentage of reducing substances and predominance of glucose over mannose in the monosaccharide composition. Preparations from RS and NRS did not differ statistically by these parameters (Table 1). The content of total nitrogen was low in all samples (<1.3%) due to hydrolysis of yeast cells with pronase [5].

For LPS from RS the maximum increase in peripheral blood leukocyte count in mice was observed after injection of 10 mg/kg LPS (Fig. 1, *a*). This parameter underwent biphasic changes (Fig. 1, *b*): initial leukopenia (76.2±5.1% of the control level was observed 20 min after LPS injection) was followed by

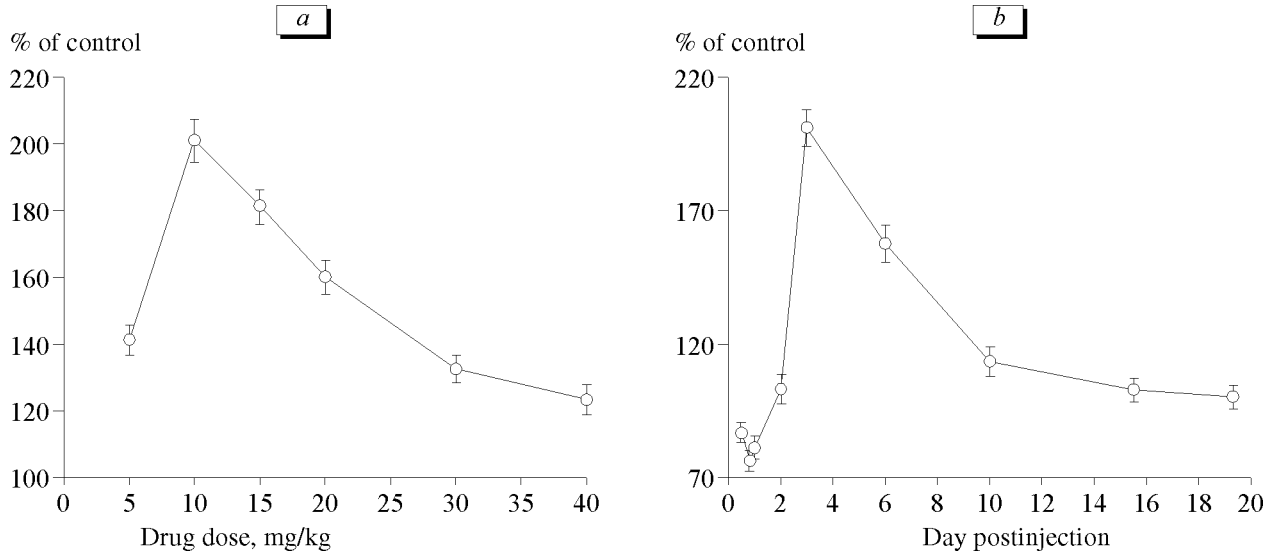

Fig. 1. Effect of LPS from recombinant *S. cerevisiae* strains on leukocyte count in mouse peripheral blood depending on the dose (*a*) and time postinjection (*b*).

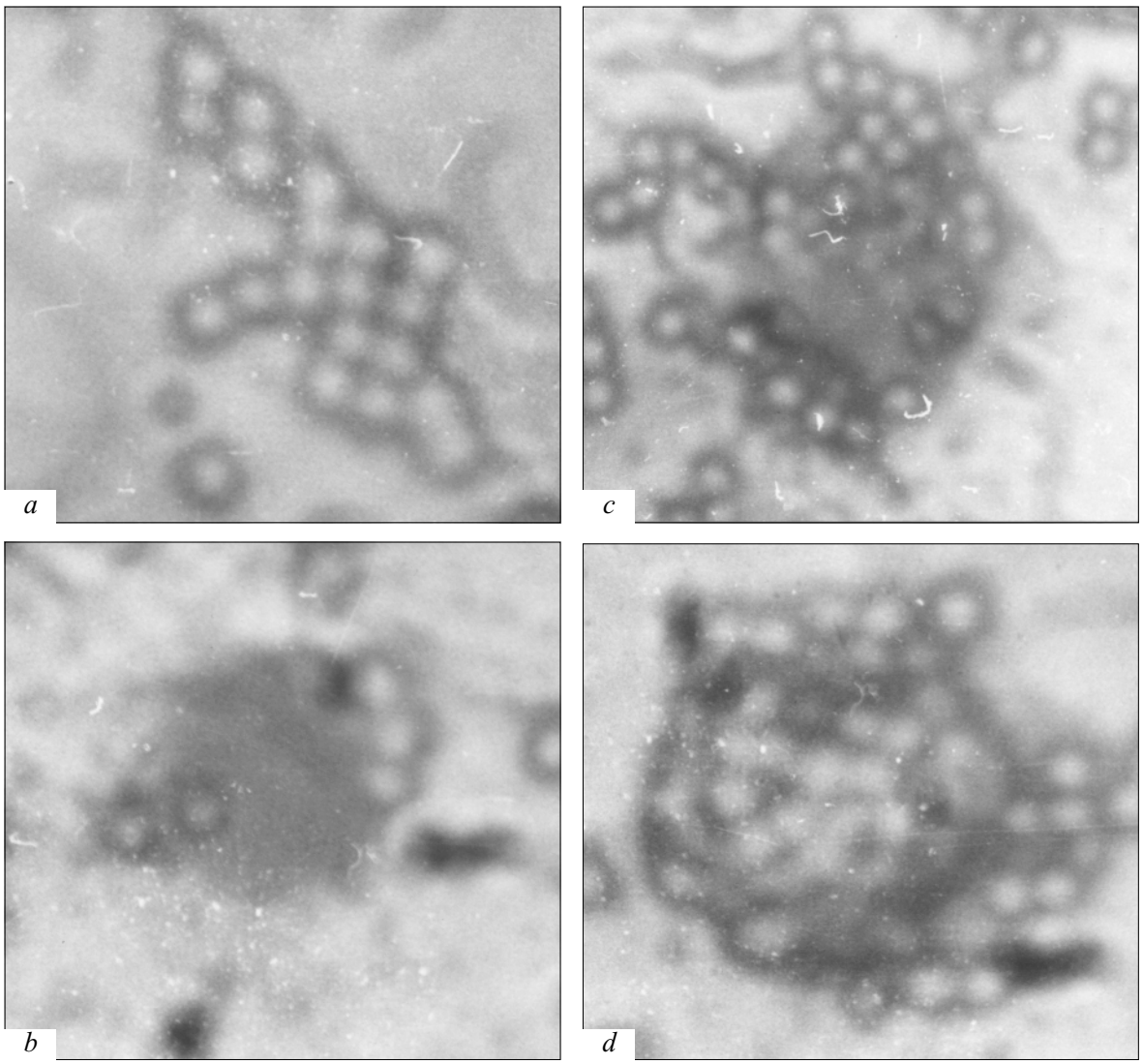

Fig. 2. Leukoconcentrate smear after incubation with latexes. Romanowskii-Giemza staining, ×900. *a*) latex particles before incubation; *b-d*) granulocytes with incorporated latex particles.

sharp leukocytosis, which peaked after 72 h and disappearing only by day 18 postinjection. These fluctuations are explained by the existence of two fragments of the alternative complement activation pathway: C5a induces neutropenia and C3a induces neutrophilia [8]. No essential differences between LPS from RS and NRS in the dose-dependent and time relationships were noted. High biological activity of LPS (2-fold increase of the leukocyte count in the peripheral blood of animals) was revealed. No statistically significant differences between LPS isolated from RS and NRS were detected (Table 1).

Evaluation of granulocyte phagocytic activity 72 h after LPS injection (latex test, Fig. 2) showed that the phagocytic index and phagocytic number increased by 25.9±1.8 and 25.2±2.0%, respectively, after injection of LPS from RS and by 59.1±5.1 and 63.6±5.7%, respectively, after injection of LPS from NRS (compared to the control). The absence of statistically significant differences (Table 1) attested to similar phagocytosis-stimulating activity of the studied agents.

The method of immune rosettes (Fig. 3) showed that injection of LPS from RS and NRS to mice immunized with sheep erythrocytes 2-fold increased the number of rosette-forming cells compared to the control (by 114.2±8.5 and 111.5±9.7%, respectively). No statistically significant differences in the stimulation of rosette-forming activity of lymphocytes between LPS from RS and NRS were detected (Table 1).

No essential differences in the serological activities of LPS isolated from RS and NRS were detected: all preparations were highly active towards C3 complement component (Table 1). Toxicity and pyrogenicity tests were negative with all LPS (Table 1).

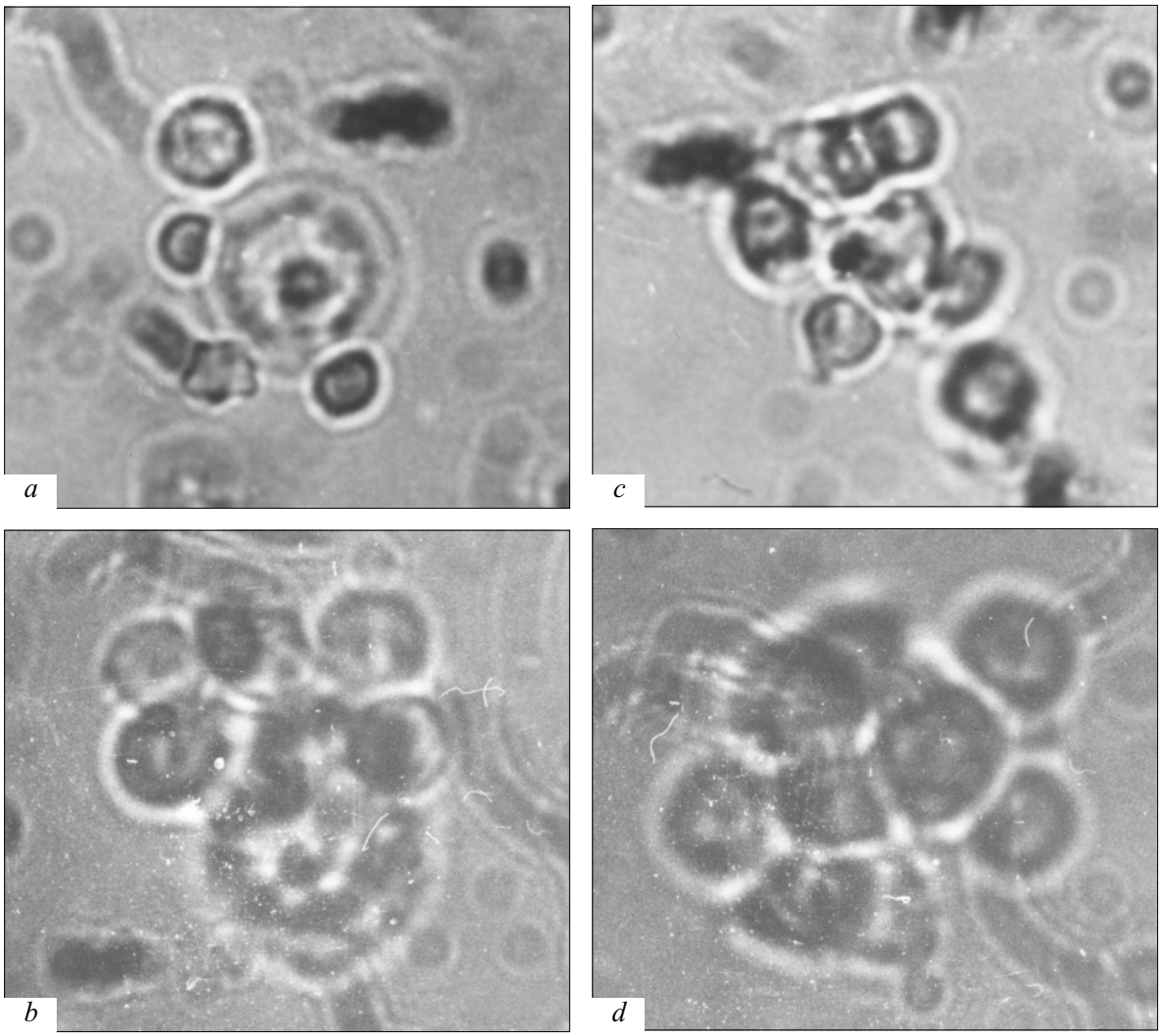

Fig. 3. Different erythrocytic rosettes. Romanowskii-Giemza staining ×900. *a*) initial stages of rosette formation; *b*) polar rosette; *c*) incomplete rim of erythrocytes around splenocyte; *d*) morule-shaped rosette.

Thus, LPS isolated from *S. cerevisiae* RS and NRS possess the same physicochemical characteristics and produce similar stimulatory effects on the immune system. Hence, the biomass of recombinant yeast strains can be used for isolation of highly active immunomodulating LPS. The detected regularities will help to improve the efficiency of technological process using RS producers due to simultaneous isolation of several bioactive substances.

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