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Introduction

Yeasts are ascomycetous or basidiomycetous fungi, which in vegetative stage propagated by gemmation or division that resulted in single-celled growth; their sexual structures do not put in fruit bodies [1]. Now it is known more than 200 species of yeasts, and some of them are clinically important. Number of yeast genera/species is to be found in clinical practice progressively increase: In 1980 it was 4 genera of 10 species [2], in 1987 – 5 genera of 18 species [3], in 1998 – 9 genera of 36 species [4], and in 2011 – 19 genera of 80 species [5]. Such situation can be explain on the one hand by increasing (discovering) of new species partly due to creating new methods of fungal identi cation, but on the other hand by extension of individuals with depressed immunity. It is important however those 19 genera may be reducing to 7 basic genera of yeasts, because most of them are teleomorphs (sexual stage) of these 7 genera: Candida, Malassezia, Rhodotorula, Cryptococcus, Trichosporon, Geotrichum and Saccharomyces. Type species of these genera are C. albicans, M. furfur, R. mucilaginosa (syn. R. rubra), Cr. neoformans, T. cutaneum, G. candidum, and S. cerevisiae.

e data concerning of habitats and diseases associated with

Genus, species	Habitats		Diseases (mycoses)		Allergic diseases
	In nature	In healthy human organism	Deep	**Superficial**	
Candida C. albicans	Soil, water, plants, birds faeces	Mucous membranes, intestine, vaginal tract, skin	Candidosis of internal organs, peritonitis, osteomyelitis, endocarditis, sepsis, granuloma	Vaginal and oral candidosis, onichomycosis, keratitis	Candidosis, atopic dermatitis, allergic broncho-pulmonary mycosis
Cryptococcus Cr. neoformans	Soil, dry birds faeces, [illegible]	Mucous membranes (rare)	Meningitis (usually in patients with disturbed function of T-cells)	Skin mycosis (secondary in the case of deep mycosis)	No data
Geotrichum ***G. candidum***	Soil, sewage, deteriorate vine, cheeses	Respiratory tract, intestine	Candidosis of internal organs, sepsis (rare)	No data	No data
Rhodotorula R. rubra (mucilaginosa)	Soil, water (including the sea water)	Respiratory tract, skin, vaginal tract	Candidosis of internal organs Rare: Meningitis endocarditis, sepsis, endophtalmitis	Keratitis	“Summer-type hypersensitivity pneumonitis”
Malassezia M. furfur	Animal skin	Skin	Sepsis in the case of [illegible]	Pityriasis versicolor, folliculitis otitis	“Head-neck” type atopic dermatitis
Trichosporon T. cutaneum	Sewage, soil, sea and pools water	Hair, skin	Sepsis (in the case of leucaemia and organs transplantation), endocarditis, meningitis, pneumonia, peritonitis	White piedra, skin mycosis, onicho-mycosis, keratitis	“Summer-type hypersensitivity pneumonitis”
Saccharomyces S. cerevisiae					

Development of immunological diagnostic preparations from yeast Candida albicans and Cryptococcus neoformans was started many years ago and constantly improved [14,15]. Recently the commercial kit with antigens of yeast Malassezia has appeared. So only for three yeast genera of 7 the diagnostic kits exist.

The known methods of antigens extraction are based on the mechanic or ultrasonic disruption of yeasts cells. This approach coupled with the presence of cross reactive determinants exists in many fungi. Moreover the main method of yeasts cells growing now is the periodic cultivation on solid media in Petri dishes. It is rather archaic approach, because in this case it is not known exactly the physiological stage (growth phase) of resulted culture. Growth phase is very important because it is known that more specific antigens are characteristic for exponential growth phase (young culture), but cross-reactive antigens – for stationary phase (old cells) [16].

Thus the aim of this work was to study of yeasts growth phases in liquid defined media for further determination of specific antigens localization.

Materials and Methods

Yeasts strains were obtained from institute collection- Candida albicans 927, Geotrichum candidum 1206, Malassezia furfur 1451, Rhodotorula mucilaginosa 132, Cryptococcus neoformans 3465, Trichosporon cutaneum 18; and Saccharomyces cerevisiae 375 from Russian Collection, Pushchino.

Cultivation of non-lipophilic yeasts was held at 30°C and speed of rotation 150 per min in 500 ml flasks contained 100 ml of defined medium (g /l): glucose–20, asparagine- 15, $(NH_4)_2HPO_4$ – 1.4, $MgSO_4 \times 7H_2O$ – 0.5, NaCl-0.1, $CaCl_2$-0.02; 10 ml of 1.2 M phosphate buffer ph 5.5; trace elements, antibiotic [17]. Lipophilic yeast Malassezia furfur was cultivated in the same flasks in liquid defined medium, created earlier and contained (g/l): Tween 40–10, asparagin-1, sodium taurocholic -10; the same salts, buffer and antibiotic (see above) [18].

Periodically probes were sterile collected and optical density (OD) was measured at wave-length 530 nm and cuvette thickness 0.5 cm. Maximal growth rate (μ_{max}) and doubling time (T_d) was calculated using formula [19]:

$\mu_{max} = (dx/dt) \times (1/x)$; 1/h

$dx = x_2 - x_1$; OD units

$dt = t_2 - t_1$; h

$x = (x_1 + x_2)/2$; OD units

$T_d = 0.693/\mu_{max}$; h

Maximal OD corresponded to OD in stationary phase.

Immunization of mice

Yeasts cells from the end of exponential phase were harvested by centrifugation, suspended in 0.02 % sodium mertiolate at different concentrations, exposed 2 hours at room temperature for killing cells and frozen at -25°C untill immunized. For one experiment for each yeast strain 10 white outbred mice were used (in sum – 70 mice), and 10 such mice were used as a control. As a whole 3 immunization experiments were held. Altogether 5 immunization cycles were carried out within each experiment: at first step each mouse was intraperitonealy introduced by 0.5 ml of yeast suspension with concentration 250 μg protein /ml; at second step – a 1 week later - 500 μg protein /ml; at third step – 1 week later – 1000 μg protein/ml; at forth step – 1 week later – 2000 μg protein/ml; at fifth step – 1 week later – again 2000 μg protein/ml; 1 week after total mice antisera were collected and pooled according to yeasts strains and controls.

Dot-blot analysis

Antigen preparations 1 were obtained from cells of the late exponential growth phase by rapid extraction with 1 % sodium dodecyl sulfate (SDS) solution during 10 min at 40°C – in this case cell walls were not disrupted, and extract consisted in general from super cial proteins.

extract reacted better with corresponding antiserum, however it is clear that some preparations reacted with other antisera too. is may be explained in the following way: such treatment with SDS leads to partial extraction of some cross-reactive (may be internal) proteins. Moreover the preparation from M. furfur reacted with control sera, maybe because of this yeast is the resident of healthy animal skin.

We expected that speci c proteins secreted to the extracellular medium during the yeast growth, therefore decided to collect exponential cultures, eliminate cells and carry out the dot blot analysis with cell-free cultural liquids and mice antisera (see above – preparation 2). e appropriate results demonstrated on Figure 2B. Within the limits of sensitivity of present method we can establish that each antigen preparation 2 reacted only with corresponded mice antisera, except the M. furfur. Most likely Tween 40, which was the constituent of de ned medium for this fungus, prevented the interaction between yeast proteins and nitrocellulose. In this case one should carry out special puri cation of the preparation 2, or use the preparation of late stationary phase then all Tween 40 will be consumed by cells (although in this case the cross-reacted proteins may be secreted to the medium).

Summarize the results we conclude that clinical yeast growth was studied and comparative estimation of growth phases, growth rates and biomass accumulation was done. Moreover the most convenient approach for subsequent development of speci c yeasts diagnostic preparations was selected – it is the use of cell-free cultural liquids derivable a er cultivation of yeasts in de ned liquid media.

Acknowledgement

$XWKRUV H[SUHVV WKHLU WKDQNV WR 7DPDUD $UW for technical support and permanent care.

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