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INSTITUTE OF BOTANY OF
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CANDIDA Berkhout YEASTS: DISTRIBUTION, BIOLOGICAL PECULIARITIES AND SEARCH FOR PREVENTIVE MEASURES AGAINST THEM

Summary of doctoral dissertation
Biomedical sciences, biology (01 B)
Microbiology, bacteriology, virology, mycology (B 230)

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VILNIAUS UNIVERSITETAS
GAMTOS TYRIMŲ CENTRO
BOTANIKOS INSTITUTAS

JURGITA ŠVEDIENĖ

CANDIDA Berkhout GENTIES MIELIŲ PAPLITIMAS, BIOLOGINIAI SAVITUMAI IR PREVENCINIŲ PRIEMONIŲ PRIEŠ JAS PAIEŠKA

Daktaro disertacijos santrauka
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INTRODUCTION

At present, more than 1500 yeast species belonging to 100 genera are described worldwide. *Candida* genus includes about 200 species. This genus is very heterogeneous; it contains species of complete and incomplete development. Among the *Candida* yeasts the species tolerant to osmosis, growing in extreme or anaerobic conditions are encountered. As commensals *Candida* yeasts are found on human and animal skin, mucus and digestive tract. Occurrence of *Candida* yeasts as causative agents of diseases is most widely studied (DRAGO et al., 2000; STAHMANN et al., 2000; SUH et al., 2005; KURTZMAN, PIŠKUR, 2006; SCHIRMER-MICHEL et al., 2008; TOFALO et al., 2009; LÓPEZ-MARTÍNEZ, 2010; NAVARATHNA, ROBERTS, 2010). However, the data on their abundance and species diversity in natural substrates, ambient air of residential and occupational surrounding is sparse.

Yeasts of this genus are used in biotechnology, agriculture, food and chemical industries. *Candida* yeasts are successfully employed in biocontrol of plant pathogens. *Candida intermedia* synthesize substances that inhibit the growth of pathogenic bacteria (GOTCHEVA et al., 2002; YEHUDA et al., 2003; WUCZKOWSKI, PRILLINGER, 2004; CHENG et al., 2005; MASSART et al., 2005; GOERGES et al., 2006; JACQUES, CASAREGOLA, 2008; KUNZE et al., 2009). Wider knowledge on biochemical and physiological properties of *Candida* yeasts is important for new opportunities of new species application as well as their biocontrol.

Candida yeasts can be the key agents of the skin and mucous membrane infections, opportunistic and systemic mycoses. Since 1963 *Candida albicans, C. parapsilosis, C. tropicalis* and *C. guilliermondii* have been described as medically important worldwide, and in 1995 this list already included 17 species. In the USA hospitals *Candida* yeasts are in the fourth place among all agents causing blood infections (HAZEN, 1995; ABIA-BASSEY, UTSALO, 2006; TORTORANO et al., 2006; PHALLER, DIEKEMA, 2007; WEIG, BROWN, 2007). In Lithuania the number of patients with various diseases caused by *Candida* yeasts is also increasing (PAŠKEVIČIUS, 2001; REINGARDIENĖ, 2002; ADUKAUSKIENĖ et al., 2009).

Rapid spread of *Candida* caused diseases promotes the development of new drugs and pharmaceutical forms and improvement of treatment. Currently many effective

medicinal substances for treatment of mycoses are already synthesized. But the majority of the medicines are chemically synthesized. Negative side effects and development of resistant microorganism strains are the disadvantages of chemical products. Therefore, one of the main modern challenges is to find biological substitutes for currently used antifungal medications. Peptides characterized by antifungal activity, substances present in medicinal and aromatic plants, essential oils and their components, metabolites produced by mycocinogenic yeast strains, extracts produced from sponges could become a valuable alternative to chemical products (GOLUBEV, 1998; SHAO et al., 2007; SOBRINO-LÓPEZ, MARTÍN-BELLOSO, 2008; SKOURI-GARGOURI, GARGOURI, 2008; MATUSEVIČIUS et al., 2008; VIUDA-MARTOS et al., 2008; EL-AMRAOUI et al., 2010). The data concerning the application of essential oils of plants growing in Lithuania or antagonistic microorganisms against *Candida* yeasts is not abundant.

Aim of the work was to determine the distribution of *Candida* yeasts in various substrates and the surrounding environment, to define their biological characteristics and preventive measures to reduce the pollution.

Main tasks:

1. To ascertain the distribution of *Candida* yeasts in natural substrates, food products and human surrounding.
2. To isolate and identify *Candida* yeasts that are pathogenic and opportunistically pathogenic to people.
3. To determine morphological, physiological, biochemical peculiarities of the isolated yeasts.
4. To investigate pathogenicity of most frequently isolated *Candida* yeasts to warm-blooded animals.
5. To conduct the search of chemical and biological fungicidal materials against pathogenic and opportunistically pathogenic *Candida* yeasts.

Scientific novelty. The dissertation presents data on the distribution of *Candida* yeasts in natural substrates, food products, human pathological material and the surrounding residential and occupational environments. For the first time in Lithuania a complex evaluation of morphological, physiological and biochemical peculiarities of *Candida*

yeasts isolated from various substrates was performed. Twenty-six *Candida* species were isolated, 4 of them are new to Lithuanian mycobiota – *C. magnoliae*, *C. saitoana*, *C. oleophila* and *C. sorboxylosa.*

Assessment of the impact of 11 disinfectants and 8 antifungal preparations on pathogenic *Candida* yeasts was conducted. For the first time in Lithuania investigations of the impact of *Thymus pulegioides*, *Carum carvi* essential oils on *Candida* yeasts were performed. The effects of *Pantoea citrea* (T_1x, T_2x, T_3x), *Streptomyces* sp. (Ux, Ux308) isolated from soil and spontaneous fruit and berry fermentations on *Candida* yeasts were determined. Acute toxicity/pathogenicity of single doses of *C. albicans* (C.A.4) and *C. parapsilosis* (C.P.1) administered *per os* or intraperitoneally to warm-blooded animals was ascertained.

Practical significance. The results on the distribution of *Candida* yeasts in different substrates (soil, water, food products, residential and occupational environment, pathological material) are important for the assessment of the diversity of yeast species in Lithuania. The accumulated complex data on morphological, physiological and biochemical properties of *Candida* yeasts could be used for the production of biologically active substances and creation of biopreparations. Investigations of the impact of essential oils of various plants as well as of *Pantoea citrea* (T_1x, T_2x, T_3x) and *Streptomyces* sp. (Ux, Ux308) on *Candida* yeasts are important for pharmaceutical research, creating a new generation of natural antifungal products or preventive measures. Results of the investigations on the impact of disinfectants on *Candida* yeasts are employed in dealing with sanitary issues in food processing and other manufacturing companies.

The statements for defense:

- *Candida* yeasts are usually present in food products, the surrounding environment and among patients with surface mycoses. *Candida* yeasts isolated from different substrates are characterized by different morphological, physiological and biochemical properties.

- Single doses of *Candida albicans* (C.A.4) and *C. parapsilosis* (C.P.1) suspensions isolated from food products administered *per os* and intraperitoneally are non-toxic and non-pathogenic to mice.
- Antifungal drugs of azole group (ketoconazole and clotrimazole) possess strong fungicidal activity against *Candida* yeasts. Disinfectants containing peracetic acid as their active substance are most effective against *Candida* yeasts.
- Essential oils of *Mentha* x *piperita* 'Zgadka', *Thymus pulegioides* thymol (T) and geraniol (G/G/N) chemotypes are characterized by strong fungicidal performance against *Candida* yeasts. Bacteria *Pantoea citrea* (T_1x, T_2x, T_3x), *Streptomyces* sp. (Ux, Ux308) possess fungicidal activity against *Candida* yeasts.

Approbation of the results. Material of the dissertation was presented at 4 international conferences and 5 conferences in Lithuania. The research results were published in 11 scientific articles and 9 conference abstracts.

Volume and structure of the dissertation. Dissertation manuscript consists of an Introduction, Literature Review, Material and Methods, Results, Summary and Conclusions, list of 350 references, list of scientific publications. Volume of the dissertation – 178 pages, 37 tables, 55 figures. The dissertation is written in Lithuanian, summary – in English.

2. MATERIAL AND METHODS

During the study 637 samples were examined (soil – 61 samples, water – 60, plant leaves – 84 residential indoor air – 60, industrial indoor air – 57, food products – 223, pathological material of arms, legs and skin – 92). Number of *Candida* yeasts in soil was tested following BILAJ, (1982); LST ISO 10381-6.1998; in water and phyllosphere – according to KVASNIKOV, ŠČELOKOVA, (1991); in the air of residential and occupational premises – VOL'PE, KUCHERENKO, (1970); JENSEN et al., (1992); NEVALAINEN et al., (1993); CROOK, (1995); in food products according to recommendations of LST ISO 6887-2:2005, LST ISO 6611:2004, LST ISO 8261:2001, KVASNIKOV, ŠČELOKOVA, (1991).

Yeasts isolated from various substrates were identified following KURTZMAN and FELL (1998), BARNETT, PAYNE, YARROW (2003). *Candida* yeasts were identified employing „Fungichrom®“ (International Microbio, France), „**api**® 20 C AUX“ (Biomérieux, France) and „Auxacolor® 2“ (Bio-Rad, France) diagnostic systems. Species of *Yarrowia*, *Candida*, *Debaryomyces*, *Clavispora* and *Pichia* yeasts isolated from various substrates were determined by polymerase chain reaction (PCR) using markers ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 (5' GGA AGT AAA AGT CGT AAC AAG G) (WHITE et al., 1990).

Antifungal drugs nystatin, fluconazole, flucytosine, miconazole, ketokonazole, econazole, itraconazole and clotrimazole (Liofilchem, Italy) were tested. Disinfectants F261 Kloriitti-forte, Pesetti antibact, Ipa-300 (FARMOS, Finland), Topax DD, Topax U (Ecolab, Germany), Divosan activ, Divosan Forte, Divodes FG, Suma Bac D10, Oxivir, Tego 2000 (JohnsonDiversey, USA) were examined. Essential oils of *Thymus pulegioides* linalool (L), geranial/geraniol/neral (G/G/N) and thymol (T) chemotypes, *Juniperus communis, Abies sibirica, Mentha* x *piperita, Monarda didyma, Carum carvi, Melaleuca alternifolia, Citrus limonum* var. *dulcis, Lavandula hybrida, Eucalyptus globulus, Citrus bergamia* were also explored. Fungicidal activity of antifungal medications, disinfectants and essential oils was assessed by disc diffusion method (KALEMBA, KUNICKA, 2003). Sensitivity of *Candida* yeasts to antifungal preparations was studied employing INTEGRAL SYSTEM LIEVITI (Liofilchem, Italy), FUNGITEST (BIO-RAD, France), ATB® FUNGUS 2 (Biomérieux, France) systems.

The impact of bacteria *Pantoae citrea* (T_1x, T_2x, T_3x), *Streptomyces* sp. (Ux, Ux308) on *Candida* yeasts was tested by the method of killer activity determination (GULBINIENĖ, 2002).

Pathogenicity and toxicity of *Candida* yeasts isolated from food products to mice was assessed according to the guidelines of microbial pesticide tests: Acute oral toxicity/pathogenicity (Microbial Pesticide Test Guidelines OPPTS 885.3050) and acute injection toxicity/pathogenicity (Acute Injection Toxicity/Pathogenicity/ Microbial Pesticide Test Guidelines OPPTS 885.3200).

The research data was processed with *SSPS 17* statistical program using analysis of variance (ANOVA) and least significant difference test (LSD). Data considered statistically significant at $p < 0.05$.

Distribution of *Candida* yeasts in various substrates

During the study 498 yeast isolates from various natural substrates (soil, water and plant phyllosphere), food products, ambient air of residential and occupational environment, human pathological material were isolated. Identified yeast isolates were ascribed to 21 genera and 63 species. *Candida* yeasts were recorded in all studied substrates (Fig. 1).

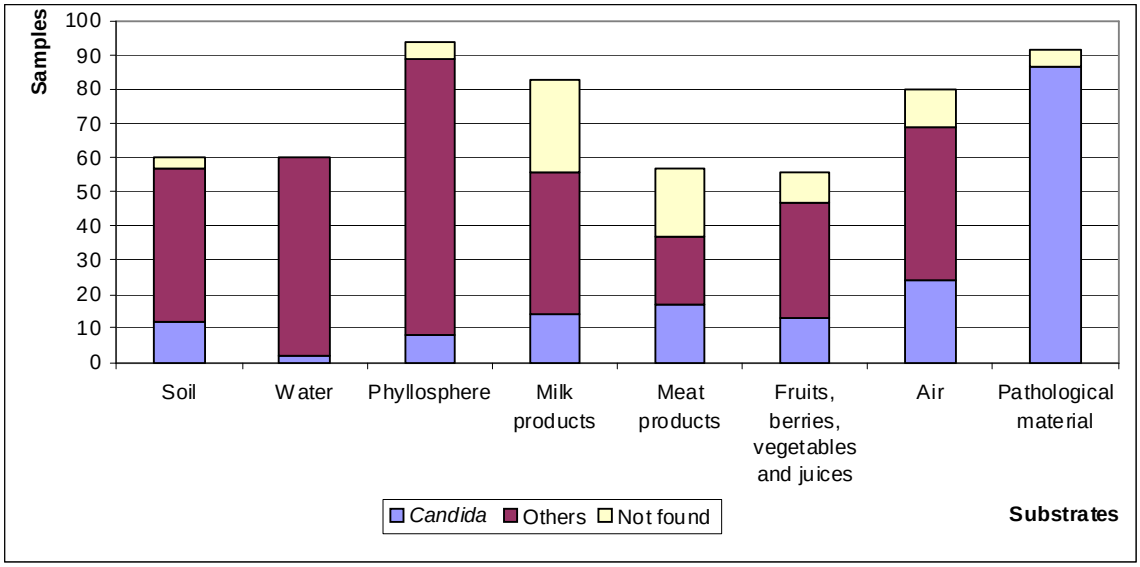


Fig. 1. Distribution of *Candida* yeasts in various substrates.

Majority of yeast species belonged to the genus *Candida* accounting for 31% of all yeast species .Twenty-six *Candida* species were isolated, 4 of them are new to Lithuanian mycobiota – *Candida magnoliae*, *C. saitoana*, *C. oleophila* and *C. sorboxylosa*. Somewhat less frequent were yeasts of the *Pichia* (12%), *Kluyveromyces* (8%), *Debaryomyces* (6%), *Rhodotorula* (5%), *Trichosporon* (6%), *Sporobolomyces* (4%), *Issatchenkia* (4%), *Dekkera* (3%), *Lipomyces* (3%) and other genera (18%) (Fig. 2).

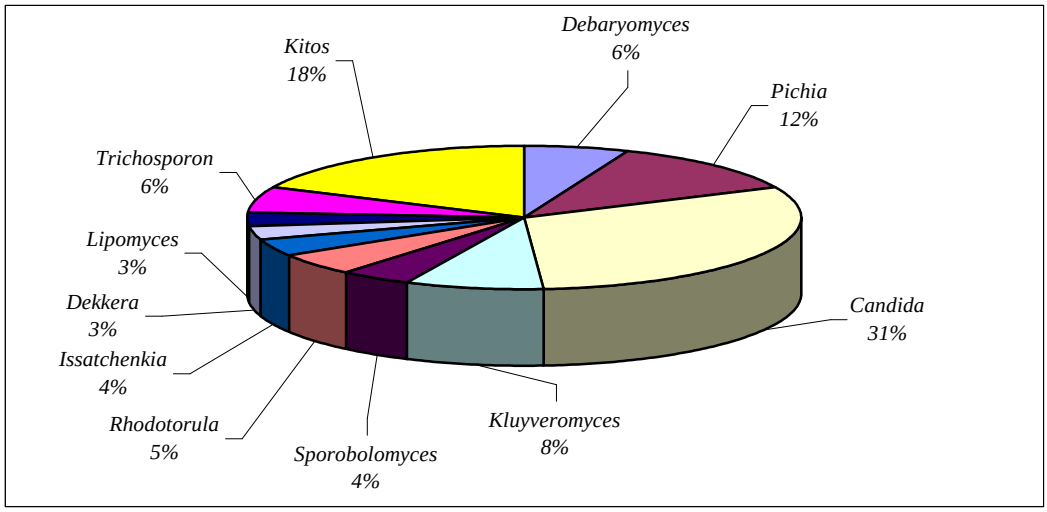


Fig. 2. Diversity of yeast genera.

Six *Candida* species were isolated from natural substrates (soil, water, plant leaves), it accounted for 22.7% of all isolated yeast species (Table 1). In natural substrates *C. catenulata*, isolated from soil samples, prevailed. Food products, with 16 detected *Candida* species, were characterized by the highest *Candida* species diversity. *C. sake* and *C. zeylanoides* dominated. *C. albicans*, *C. parapsilosis*, *C. rugosa*, *C. saitoana*, *C. magnoliae*, *C. vini*, etc. were also isolated from food products. In the air of residential and occupational environment *Candida* yeasts accounted for more than 40% of all isolated yeasts. *C. parapsilosis* yeasts, which have been detected in the air of both residential and industrial premises, prevailed. Additionally *C. sake* and *C. oleophila* were isolated from the air of residential premises. *C. utilis* yeasts were isolated only from the air of industrial premises.

Table 1. Distribution of *Candida* yeasts in various substrates

Species	Soil	Water	Phyllosphere	Food	Air of residential premises	Air of industrial premises	Pathological material
C. albicans	-	-	-	+	-	-	+
C. boidinii	-	+	-	-	-	-	-
C. catenulata	+	-	-	-	-	-	-
C. glabrata	-	-	-	-	-	+	+
C. guilliermondii	-	-	-	-	-	-	+
C. inconspicua	-	-	-	+	-	-	-
C. intermedia	-	-	-	+	-	-	+
C. krusei	-	-	-	-	-	-	+
C. lusitaniae	-	-	-	-	-	-	+
C. magnoliae	-	-	-	+	-	-	-
C. maltosa	+	-	-	+	-	-	-

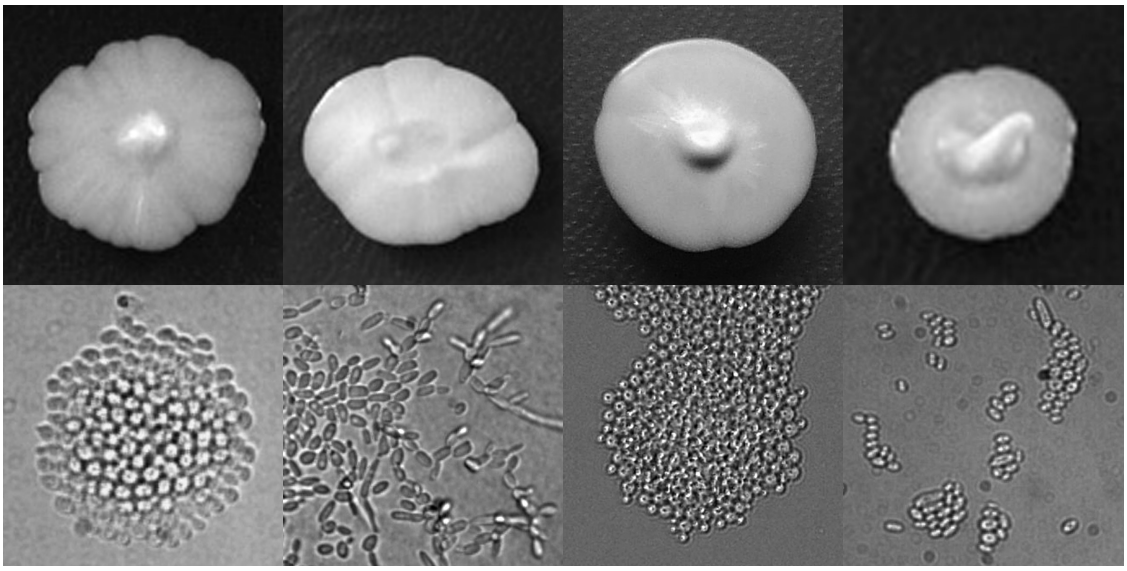
C. oleophila	-	-	+	-	+	-	-
C. parapsilosis	-	-	-	+	+	+	+
C. pararugosa	-	+	-	+	-	-	-
C. pseudotropicalis	-	-	-	-	-	-	+
C. pelliculosa	-	-	-	+	-	-	-
C. rhagii	-	-	-	+	-	-	-
C. rugosa	-	-	-	+	-	-	-
C. saitoana	-	-	-	+	-	-	-
C. sake	-	-	+	+	+	-	-
C. sorboxylosa	-	-	-	+	-	-	-
C. tropicalis	-	-	-	-	-	-	+
C. utilis	-	-	-	-	-	+	-
C. versatilis	-	-	-	+	-	-	-
C. vini	-	-	-	+	-	-	-
C. zeylanoides	-	-	-	+	-	-	+
Total	2	2	2	16	3	3	10

In cooperation with the staff of Vilnius University Hospital "Santariškių klinikos" Dermatovenerology clinic in 2005–2006 *Candida* yeasts were isolated from pathological material of 92 patients with surface mycoses. Among the patients with surface mycoses *C. albicans* (43%) and *C. tropicalis* (21%) yeasts were most common, less often *C. pseudotropicalis* (5%), *C. krusei* (5%), *C. parapsilosis* (6%), *C. lusitaniae* (6%) were diagnosed as major agents.

Identity of species composition determined using diagnostic systems Fungichrom®" (International Microbio, France), „**api**® 20 C AUX" (Biomérieux, France) and „Auxacolor® 2" (Bio-Rad, France) compared with phenotypic and polymerase chain reaction methods reaches 70% to 85%. It was ascertained that diagnostic systems used for yeast identification do not reflect the true systematic position of yeasts because it constantly changes, and manufacturers fail to immediately introduce systematic innovations into programs or sets of diagnostic systems.

Biological peculiarities of *Candida* yeasts

Macro- and micro- morphological peculiarities of *Candida* yeasts isolated from various substrates were studied. *Candida* yeasts were grown on yeast morphological agar; colony edge shape, color, texture and surface structure were described (Fig. 3).



A B C D

Fig. 3. Macro- and micro- morphological images of *Candida* yeasts new to Lithuanian mycobiota A – *Candida magnoliae*, B – *C. oleophila*, C – *C. saitoana*, D – *C. sorboxylosa*.

While yeasts were cultivated in a liquid glucose-peptone medium, formation of a film, ring or sediments were observed. The shape of individual yeast cells, their size and type of vegetative mycelium were determined.

Candida yeasts isolated from food products and ambient human environment exhibited a broader spectrum of carbohydrate fermentation than the yeasts isolated from natural substrates (Table 2). They fermented monosaccharides (D-glucose and D-galactose), disaccharides (sucrose and maltose) and trisaccharides (raffinose). None of the studied yeasts fermented lactose.

Table 2. Carbohydrate fermentations of *Candida* spp. detected in various substrates, %

Carbohydrate	Yeasts (N=22) from natural substrates	Yeasts (N=36) from food	Yeasts (N=24) from human and surrounding environment
	Fermenting strains, %		
D-glucose	100	61,1	100
D-galactose	72,7	38,8	66,6
Sucrose	59	36,1	58,3
Maltose	45,4	30,6	50
Lactose	0	0	0
Raffinose	0	5,5	12,5

Candida yeasts isolated from food products assimilated the major portion of the tested carbon sources (84.4%), while *Candida* isolated from natural substrates assimilated the lowest portion of the tested carbon sources (65.6%). Methanol myo-inositol, galactitol and D-gluconic acid as sole carbon sources were not assimilated by *Candida* yeasts isolated from various substrates. D-galactose, D-xylose, D-ribose, maltose, α-α-trehalose, melezitose, ribitol, D-mannitol, D-sorbitol, succinic, citric acid and D-gluconate were best assimilated by *Candida* yeasts isolated from natural substrates. Hexadecane was not assimilated by *Candida* yeasts isolated from food products. D-glucosamine and N-acetyl-D-glucosamine was assimilated by *Candida* yeasts detected in various substrates.

Candida yeasts isolated from natural substrates, human pathological material and the surrounding environment do not assimilate nitrates and nitrites. 33.3% of *Candida* yeasts detected in food products assimilate nitrites. A small amount of *Candida* yeasts (8.3%) isolated from food products assimilate nitrates. *Candida* yeasts isolated from various substrates do not release organic acids and do not form starch-like compounds. Only 2.7% of *Candida* yeasts isolated from food products exhibited proteolytic activity (Fig. 4).

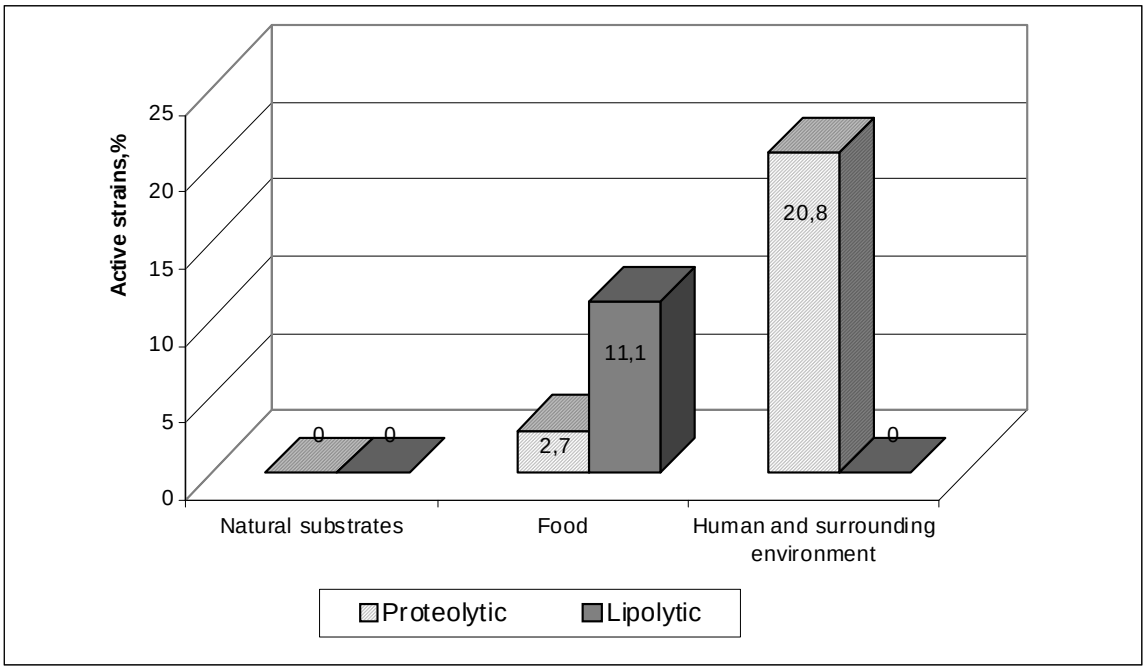


Fig. 4. Proteolytic and lipolytic activity of *Candida* spp. yeasts isolated from various substrates, %.

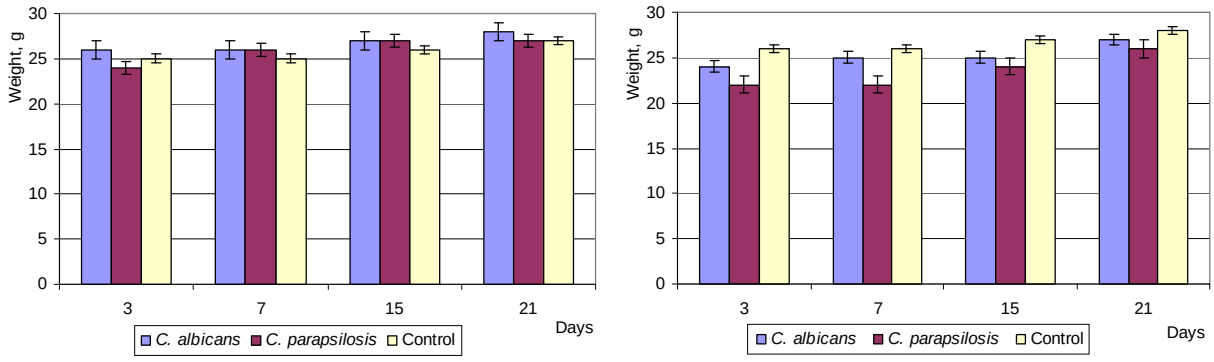
Lipolytic activity was characteristic of 11.1% of *Candida* yeasts detected in food products. 20.8% of *Candida* yeasts isolated from human pathological material and ambient air exhibited proteolytic activity.

Certain *Candida* strains detected among yeasts from various substrates grew on vitamin-free medium: 41.6% of the tested *Candida* yeasts isolated from food products, 22.7% of yeasts isolated from natural substrates and 29.7% of yeasts isolated from the surrounding environment.

All *Candida* yeasts isolated from natural substrates and more than 70% of the tested yeasts from food products, human pathological material and the surrounding environment were growing in media of high osmotic pressure.

All tested *Candida* yeasts from natural substrates grew at 23 °C and 31 °C temperatures, but did not grow at 42 ºC. *Candida* yeasts isolated from food products and the human surrounding grew within 23–42 °C temperature range.

Tests on acute oral and injection toxicity/pathogenicity of *C. albicans* (C.A.4) and *C. parapsilosis* (C.P.1) isolated from food products showed that after administration of one dose *per os* or injection into the abdominal cavity no symptoms of toxicity and pathogenic were noted. Body weight gain was normal (Fig. 5).



A B

Fig. 5. BALB/c mice weight changes during acute oral (A) and injection (B) toxicity/pathogenicity studies of *Candida* yeasts.

During the test, none of the tested animal died. *Candida* yeast colonies were detected in 10% of the samples of test animal organs, but it caused no noticeable changes in the organs.

Assessment of the impact of substances characterized by fungicidal properties of chemical origin on *Candida* yeasts

The performed tests showed that for the tested *Candida* yeasts minimum inhibiting concentrations of 5-flucytosine, amphotericin B and fluconazole were 0.5 mg/l, while of itraconazole – 0.25 mg/l (Fig. 6).

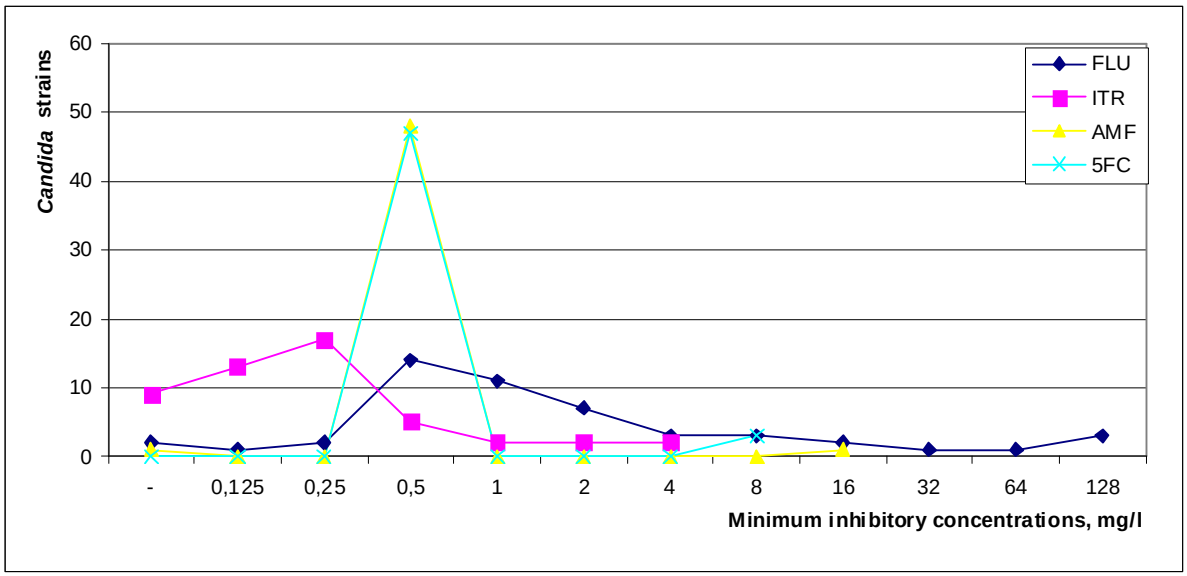


Fig. 6. Minimum inhibiting concentration of antifungal drugs for *Candida* yeasts causing surface mycoses (N=50) (FLU – fluconazole, ITR – itraconazole, AMF – amphotericin B, 5FC – flucytosine).

The tests on *Candida* yeast susceptibility to antifungal preparations performed with Integral Yeast Lieviti (Liofilchem, Italy) showed that all tested yeast strains were susceptible to fluconazole and ketoconazole and resistant to econazole.

The employment of FUNGITEST (Bio-Rad, France) system demonstrated that the tested yeast strains were most sensitive to 32 µg/ml concentration of 5-fluorocytosine. The tested yeasts were most resistant to 0.5 µg/ml concentration of miconazole and itraconazole. *C. krusei* PCKr1 yeasts were most resistant to all tested antifungal preparations at various concentrations.

Disk diffusion testing showed that the tested yeast strains were resistant to the impact of 1 µg/disk flucytosine. First-generation azoles (clotrimazole and ketoconazole) exhibited the strongest fungicidal activity against *Candida* yeast strains (Fig. 7). Other azole agents (miconazole and econazole) were less effective against *Candida* yeasts.

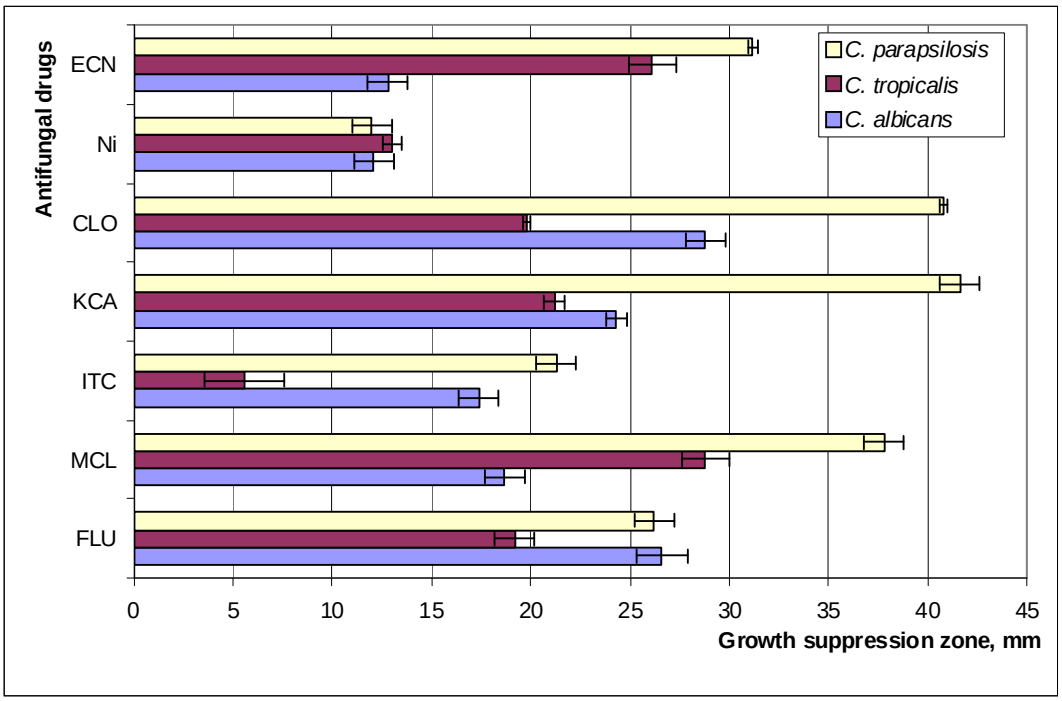


Fig. 7. Impact of antifungal drugs on *Candida albicans* (N=3), *C. tropicalis* (N=3) and *C. parapsilosis* (N=3) yeasts, which are most frequently causing superficial mycoses: ECN – econazole 10 μg/disc, Ni – nystatin 100 IU/disc, CLO – clotrimazole 50 μg/disc, KCA – ketoconazole 10 μg/disc, ITC – itraconazole 50 μg/disc, MCL – miconazole 10 μg/disc, FLU – fluconazole 100 μg/disc.

The tested disinfectants produced unequal effect on pathogenic *Candida* yeasts. According to the susceptibility to disinfectants, *Candida* yeasts were grouped into susceptible, resistant and moderately susceptible to the impact of the tested disinfectants (Fig. 8). *Candida* yeasts were most susceptible to the impact of Divosan Forte disinfectant, the resulting fungicidal zones ranged from 17.7 to 52.2 mm ($p<0.05$). *C. parapsilosis* PTCP1.2 was characterized by the strongest susceptibility to Divosan Forte, inhibitory zone size was more than 50 mm ($p <0.05$). A rather high proportion of the tested *Candida* yeasts (62.5–81.2%) remained unaffected by F261 Kloriitti forte, Topax U and Divodes FG disinfectants. These disinfectants exhibited a weaker effect on *Candida* yeasts than antifungal preparation nystatin used as a control. *Candida* yeasts were moderately susceptible to Pesetti antibact, Divosan activ, Suma Bac D10, Tego 2000, Oxivir and IPA-300 disinfectants.

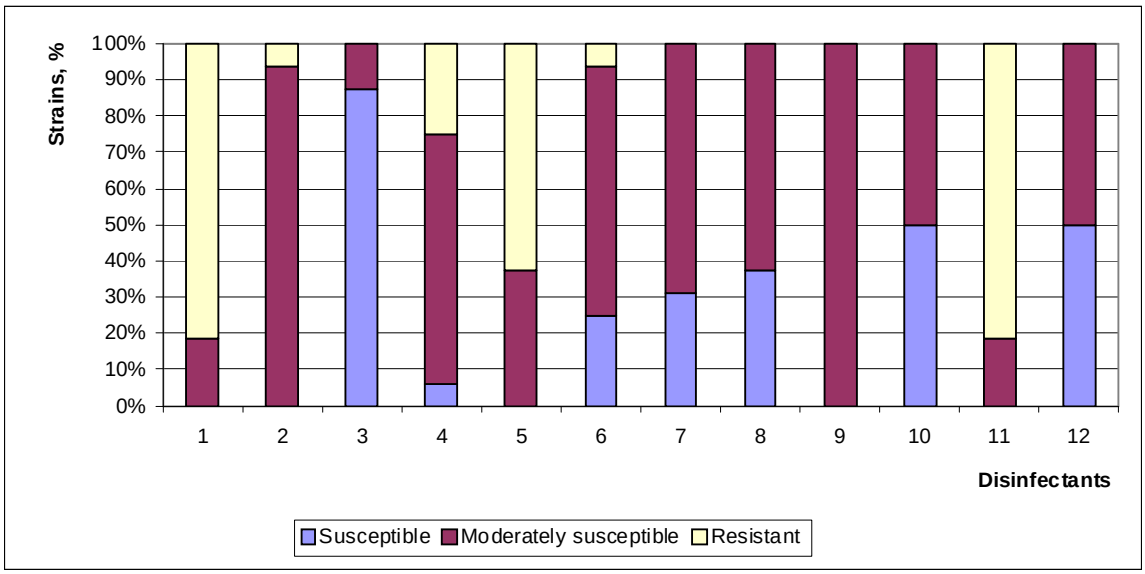


Fig. 8. Susceptibility of *Candida* yeasts to disinfectants: 1 – Divodes FG; 2 – Divosan activ (4%); 3 – Divosan Forte (2%); 4 – Ipa-300; 5 – F261 Kloriitti forte (2,5%), 6 – Oxivir (2,5%); 7 – Pesetti antibact (1%), 8 – Suma Bac D10 (2%), 9 – Tego 2000 (1%), 10 – Topax DD (5%), 11 – Topax U (5%), 12 – nystatin as a control (100 IU).

Disinfectant Divosan Forte strongly inhibited the growth of *Candida* yeasts, its active ingredient is 15% peracetic acid. This disinfectant is suitable in order to reduce the contamination with *Candida* yeasts. The impact of disinfectants Divodes FG and U Topax against *Candida* yeasts was very weak; therefore they should not be used against the yeasts of this genus.

Search for substances characterized by fungicidal properties of biological origin against *Candida* yeasts

Tests of the impact of essential oils of 11 plants on pathogenic *Candida* yeasts revealed that yeasts were susceptible, resistant or moderately susceptible to the effects of tested essential oils. *Candida* yeasts were most susceptible to the impact of essential oils of *Mentha* x *piperita* 'Zgadka', *Citrus limonum* var. *dulcis*, *Thymus pulegioides* geranial/geraniol/neral (G/G/N) and thymol (T) chemotypes (Fig. 9). These essential oils were characterized by a broader spectrum of activity and a stronger effect on yeasts than antifungal preparation nystatin used as a control. The results showed that *Candida* yeasts were resistant to the impact of essential oil of *Juniperus communis* needles and berries. *Candida* yeasts resistant to the impact of *Lavandula hybrida*, *Eucalyptus globulus* and

Citrus bergamia, *Monarda didyma* essential oils made 31.2–37.5% of the tested yeasts. *Candida* yeasts were moderately susceptible to the effects of essential oils of *Melaleuca alternifolia*, *Carum carvi*, *Abies sibirica*, *Thymus pulegioides* linalool (L) chemotype.

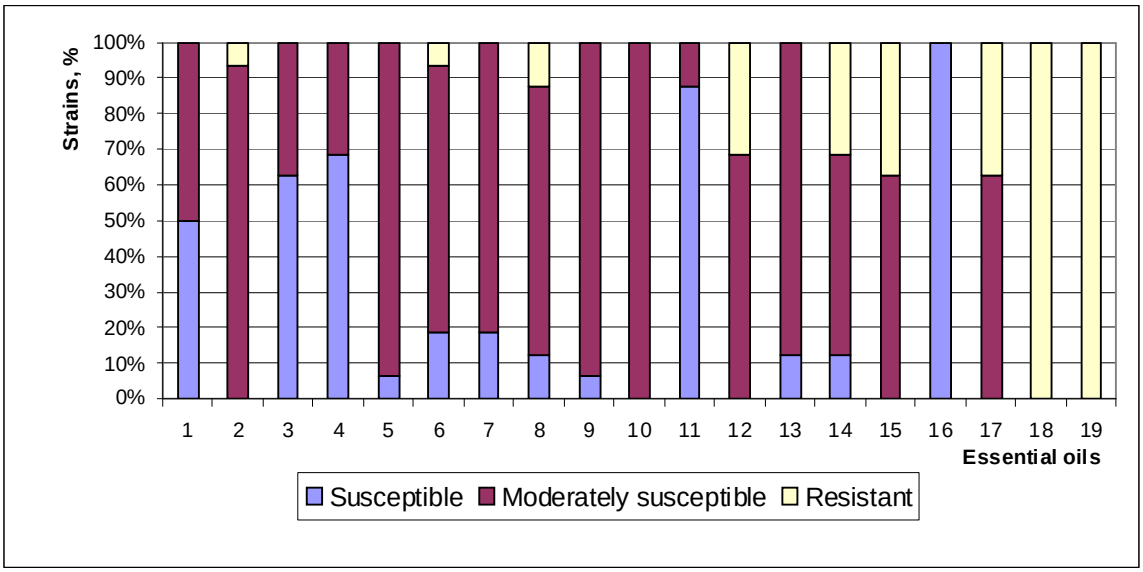


Fig. 9. Susceptibility of *Candida* yeasts to various essential oils: 1 – nystatin 100 IU (control), 2 – *Thymus pulegioides* linalool (L) chemotype, 3 – *T. pulegioides* thymol (T) chemotype, 4 – *T. pulegioides* geraniol G/G/N chemotype, 5–9 – *Carum carvi*, 10 – *Melaleuca alternifolia*, 11 – *Citrus limonum* var. *dulcis*, 12 – *Lavandula hybrida*, 13 – *Abies sibirica*, 14 – *Eucalyptus globulus*, 15 – *Citrus bergamia*, 16 – *Mentha* x *piperita* 'Zgadka', 17 – *Monarda didyma*, 18 – *Juniperus communis* needles, 19 – *Juniperus communis* berries.

Essential oils of *Citrus limonum* var. *dulcis*, *Thymus pulegioides* thymol (T) and geraniol (G/G/N) chemotypes and *Mentha* x *piperita* 'Zgadka' exhibited the strongest inhibitory effects on pathogenic *Candida* yeasts. These essential oils could be used for creation of natural and effective antifungal products or preventive measures.

Tests of bacteria *Pantoea citrea* (T_1x, T_2x, T_3x), *Streptomyces* sp. (Ux, Ux308) activity revealed that substances released by these bacteria have inhibitory effects on pathogenic *Candida* yeasts and those isolated from food products. *Streptomyces* sp. (Ux308) were characterized by strong inhibitory effect on *Candida* yeasts; zone size ranged from 4 to 21.3 mm (Fig. 10). Control standard *Sacharomyces cerevisiae* mycocinogenic strains K7, M437, MS300, Rom-K100, DBY, K28 produced no effect on these yeasts.

Table 2. Killer effect of bacterial strains on pathogenic *Candida* yeasts

Candida strains	*Pantoea citrea*			*Streptomyces* sp.	
	T_1x	T_2x	T_3x	Ux	Ux308
	Lysis zone, mm				
C. albicans PTCA3	7.0±0.1	5.0±0.1	5.0±0.1	10.0±0.2	6.0±0.4
C. famata PCF1	6.0±0.3	7.0±0.2	4.0±0.1	8.0±0.4	4.0±0
C. glabrata PTCG2	8.0±0.1	6.0±0.3	6.0±0.1	6.0±0	8.0±0.2
C. kefyr PCK1	5.0±0.1	6.0±0.1	5.0±0.1	7.0±0.1	10.0±0.5
C. lusitaniae PCLu1	8.0±0.2	7.0±0.3	7.0±0.1	0	0
C. tropicalis PTCT3	6.0±0	5.0±0.1	5.0±0.1	0	10.0±0.5
C. parapsilosis PTCP1.2	7.0±0.2	5.0±0.1	6.0±0.2	10.0±0.5	8.0±0.4

Only *C. lusitaniae* PCLu1 were resistant to the impact of *Streptomyces* sp. (Ux308). Pathogenic yeasts were more susceptible to substances released by *Pantoea citrea* (T_1x, T_2x, T_3x) compared to those isolated from food products.

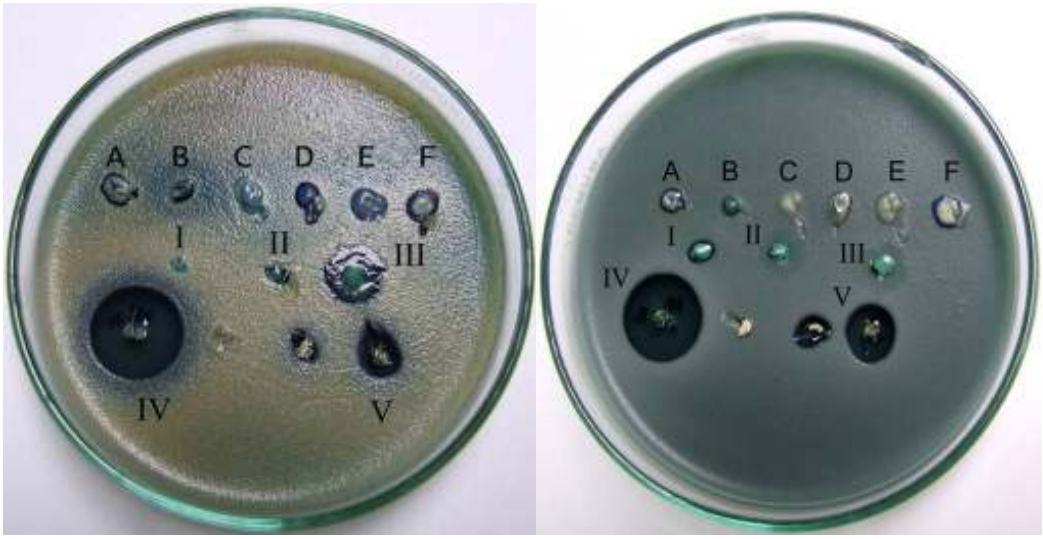


Fig. 10. Killer effect of bacteria: **I** – *P. citrea* T_1x; **II** – *P. citrea* T_2x; **III** – *P. citrea* T_3x; **IV** – *Streptomyces* sp. Ux; **V** – *Streptomyces* sp. Ux308 and standard *S. cerevisiae* mycocinogenic strains (**A**–MS300; **B**–DBY; **C**–K7; **D**–K28; **E**–Rom-K100; **F**–M437) on *Candida rhagii* C.Rh.1 and *Candida zeylanoides* C.Z.4.

The studied *P. citrea* (T_1x, T_2x, T_3x), *Streptomyces* sp. (Ux, Ux308) exhibited inhibitory effect upon the tested pathogenic *Candida* yeasts and those isolated from food products. These bacterial strains may be used for creating new generation of natural antifungal preparations, preventive measures or disinfectant tools.

CONCLUSIONS

1. During the study 498 yeast isolates from soil, water, phyllosphere, food products, human body and the surrounding residential and occupational environment were isolated and identified. It was revealed that they belong to 21 genera and 63 species. *Candida* yeasts comprised 31% of all isolated yeasts; new to Lithuanian mycobiota species were recorded: *C. magnoliae, C. saitoana, C. oleophila* and *C. sorboxylosa.*
2. In natural substrates *Candida* yeasts comprised 22.7% of all recorded yeasts, in food products – 27.9%, while in residential and occupational environment – over 40%. *C. catenulata* dominated in soil, *C. zeylanoides* and *C. sake* – in food products, *C. parapsilosis* – in residential and occupational environment. In Lithuania *C. albicans* (43%) and *C. tropicalis* (21%) prevailed among patients with surface mycoses.
3. It was revealed that *Candida* yeasts isolated from food products assimilated 84.4% of the tested carbon sources, while those isolated from human body and the surrounding environment – 73.8%, yeasts from natural substrates – 65.6%. *Candida* yeasts from natural substrates possess no lipolytic and proteolytic activity.
4. Single doses of *Candida albicans* (C.A.4) and *C. parapsilosis* (C.P.1) suspensions isolated from food products administered *per os* and intraperitoneally are non-toxic and non-pathogenic to mice.
5. It was found that antifungal preparations clotrimazole and ketoconazole possessed strong fungicidic activity against agents of surface mycoses, while flucytosine had no effect on their growth. For the tested *Candida* yeasts minimum inhibiting concentration of 5-flucytosine, amphotericin B and fluconazole was 0.5 mg/l, while of itraconazole – 0.25 mg/l. Disinfectants Divosan activ and Divosan forte, with 5–15% of peracetic acid as active substance, effectively inhibited the growth of pathogenic *Candida* yeasts.
6. Essential oils of *Mentha* x *piperita* 'Zgadka', *Thymus pulegioides* thymol (T) and geraniol (G/G/N) chemotype, *Citrus limonum* exhibited strong fungicidal activity against pathogenic *Candida* yeasts. Essential oils of *Juniperus communis* needles and berries did not inhibit the growth of *Candida* yeasts.
7. It was revealed that pathogenic *Candida* yeasts were more susceptible to substances released by bacteria *Pantoea citrea* (T1x, T2x, T3x) than those isolated from food

products. *Streptomyces* sp. (Ux, Ux308) were characterized by fungicidal activity against both pathogenic *Candida* yeasts and those isolated from food products.

REZIUMĖ

Tyrimų metu iš dirvožemio, vandens, filosferos, maisto produktų, gyvenamosios ir darbo aplinkos oro, žmogaus patologinės medžiagos buvo išskirti ir identifikuoti 498 mielių izoliatai. *Candida* genčiai priklausančios mielės sudarė 31% visų mielių rūšių. Išskirtos 26 *Candida* rūšys, iš jų 4 naujos Lietuvos mikobiotai – *C. magnoliae*, *C. saitoana*, *C. oleophila* ir *C. sorboxylosa*.

Iš gamtinių substratų (dirvožemio, vandens, augalų lapų) buvo išskirtos 6 *Candida* rūšys. Maisto produktai išsiskyrė didžiausia *Candida* rūšių įvairove, juose buvo aptikta 16 *Candida* rūšių. Gyvenamosios ir darbo aplinkos ore *Candida* mielės sudarė daugiau negu 40% visų išskirtų mielių. Tarp sergančiųjų paviršinėmis mikozėmis labiausiai paplitusios buvo *C. albicans* (43%) ir *C. tropicalis* (21%).

Ištirti iš įvairių substratų išskirtų *Candida* mielių makro- ir mikro- morfologiniai savitumai. Auginant *Candida* mieles ant agarizuotos morfologinio agaro terpės, aprašyta kolonijos krašto forma, spalva, konsistencija ir paviršiaus struktūra. Kultivuojant mieles skystoje gliukozės – peptono terpėje stebėtas plėvelės, žiedo ar nuosėdų susidarymas. Nustatyta atskirų mielių ląstelių forma, dydis, bei vegetatyvinio micelio tipas.

Daugiausiai (84,4%) tirtų anglies šaltinių įsisavina iš maisto produktų išskirtos *Candida* mielės, mažiausiai (65,6%) gamtiniuose substratuose aptinkamos *Candida* mielės. *Candida* mielės išskirtos iš gamtinių substratų ir žmogaus patologinės medžiagos bei jį supančios aplinkos nitratų ir nitritų neasimiliuoja. Nitratus ir nitratus asimiliuoja tik nedidelė dalis iš maisto produktų išskirtų *Candida* mielių. Įvairiuose substratuose aptinkamų *Candida* mielių tarpe esama padermių augančių terpėje be vitaminų, osmotinėmis bei 42ºC temperatūroje. Iš įvairių substratų išskirtos *Candida* mielės organinių rūgščių neišskiria ir nesudaro junginių panašių į krakmolą. Nedidelis kiekis *Candida* mielių išskirtų iš maisto produktų pasižymėjo proteolitiniu ir lipolitiniu aktyvumais.

Iš maisto produktų išskirtų *Candida* rūšių ūmaus oralinio ir injekcinio toksiškumo/patogeniškumo tyrimų metu nustatyta, jog po *C. albicans* (C.A.4) ir *C. parapsilosis* (C.P.1) suspensijų vienkartinės dozės įvedimo į skrandį ar suleidimo į pilvo

ertmę ir visą bandymo laikotarpį šiltakraujams gyvūnams ūmaus toksiškumo ir patogeniškumo požymiai nepasireiškė.

Antigrybiniai preparatai klotrimazolas ir ketokonazolas pasižymi stipriu fungicidiniu poveikiu paviršinių mikozių sukėlėjams, o flucitozinas *Candida* mielių augimui įtakos neturi. Patogeninėms *Candida* mielėms antigrybinių preparatų 5-flucitozino, amfotericino B ir flukonazolo minimali inhibuojanti koncentracija yra 0,5 mg/l, o itrakonazolo – 0,25 mg/l.

Dezinfekcinės priemonės Divosan forte (2%) ir Divosan activ (4%), kurių veiklioji medžiaga 5–15% peracto rūgštis, efektyviai slopina *Candida* mielių augimą. Labai silpnas fungicidiniu poveikiu *Candida* mielėms pasižymi priemonės Topax DD (5%), Topax U (5%) ir Divodes FG, kurių veiklioji medžiaga amfoteriniai junginiai arba alkoholiai.

Stipriu fungicidiniu veikimu patogeninėms *Candida* mielėms pasižymi *Mentha* x *piperita* 'Zgadka', *Thymus pulegioides* timolio (T) ir geraniolio (G/G/N) chemotipo, *Citrus limonum* eteriniai aliejai. *Candida* mielių augimo neslopino *Juniperus communis* spyglių ir uogų eteriniai aliejai. *Pantoea citrea* (T_1x, T_2x, T_3x), *Streptomyces* sp. (Ux Ux308) pasižymi fungicidiniu poveikiu *Candida* mielėms.

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