Variations of enzymatic activity and biotypes of the yeast like fungi strains isolated from cancer patients

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Abstract

Purpose: Determination of the enzymatic activity and enzymatic biotypes variations of the yeast like fungi strains isolated from cancer patients with oral candidiasis during last 5 years.

Material and methods: We evaluated enzymatic activity of 92 *Candida albicans* strains isolated from oral ontocenosis from cancer patients with candidiasis symptoms in 1999 and 2003. The enzymatic activity of the strains tested was assessed by the API ZYM (bioMerieux) method. Biotypes of the strains were determined according to Williamson's or Kurnatowska's and Kurnatowski's classifications.

Results: In 1999 *Candida albicans* 17 of 19 tested isolates had hydrolytic activity hydrolases and 87% of strains were assigned according to Wiliamson's. Only 8.7% of strains were classified according to Kurnatowska's and Kurnatowski's, but 4.3% strains according to Krajewska-Kułak et al. In 2003, 18 of 19 strains had hydrolytic activity and 93.5% of strains were classified according to Wiliamson's, but 4.3% according to Kurnatowska's and Kurnatowski's and 2.2% according to Krajewska-Kułak et al.

Conclusions: The results of present study indicate that most of tested strains were classified into Wiliamson's system. Our findings suggest that other Candida biotypes should be determined according to their different enzymatic activity and susceptibilities.

Key words: Candida albicans, API ZYM, biotypes.

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Introduction

Among the factors known to contribute to the pathogenicity of yeast, enzymes play a significant role, possibly being harmful to host tissues when they are liberated by the fungi. A correlation has been demonstrated between the amount of phospholipase produced and the virulence in *Candida albicans* strains and other yeast species [1,2]. There are still comparatively few data in the literature on the pathogenicity of yeast like fungi hydrolytic enzymes in women with Candida vaginitis.

Certain fungi such as: Mucor, Rhizopus, Aspergillus, Penicillium and Candida have the ability to release into the environment hydrolytic enzymes, which break down multimolecular compounds – polysaccharides, proteins, lipids, hydrocarbons [1,3]. Hydrolytic enzymes which break down cellulose are produced by some fungi pathological for humans and higher plants [1,4].

According to the Nomenclature Committee of The International Union of Biochemistry and Molecular Biology: the following enzyme nomenclature [1992] is valid: 1) esterases – hydrolase esters of the carboxyl group – lipase and phospholipase A2, hydrolases monoester phosphoric acid – alkaline phosphatase and acid hydrolases ester sulphuric – sulphatase; 2) glucosidases – α-glucosidase, β-glucosidase, α-mannosidase, N-acetyl-β-glucosaminidase; 3) peptidases – aminopeptidases, arylamidases, proteinases, elastases, collagenases, keratinases; 4) ureases [5,6].

Some enzymes in the pathological fungi *Candida albicans*, Candida inconspicua, Rhodotorula mulcilaginosa, Geotrichum candidum can be detected by the cytochemical method [1]. The activity of acid and alkaline phosphatase, adensine triphosphatase and lactate, succinate and 6-phosphoglycolysis dehydrogenase [1]. During the 1980s, a large number of typing methods for the strain differentiation of *Candida albicans* were described in the literature. Although these methods are based on a variety of physiological and genetic markers, none is ideal. Available typing methods for *Candida albicans* include serotyping, morphotyping, resistotyping, biotyping and killer

Table 1. **Hydrolytic enzymes and their substates assayed using API ZYM test**

No	Enzyme assayed	Substrate
E1	Phosphatase alkaline	2-naphtylophosphate
E2	Esterase (C4)	2-naphtylbutyrate
E3	Esterase lipase (C8)	2-naphtylcapylate
E4	Lipase (C14)	2-naphtylmyristate
E5	Leucine arylamidase	L-leucyl-2-naphthylamide
E6	Valine arylamidase	L-leucyl-2-naphtylamide
E7	Cystine arylamidase	L-cystyl-2-naphthylamide
E8	Trypisn	N-benzoyl-DL-arrginine-2-naphthylamide
E9	Chymotripsin	N-glutaryl-phenylalanine-2-naphthylamide
E10	Phosphatase acid	2-naphthylphosphate
E11	Naphtol-AS-BI-phosphohydrolase	Naphthyl-AS-BI-phosphate
E12	α-galactosidase	6-Br2-naphthyl-αD-galactopyranoside
E13	β-galactosidase	2-naphthyl-βD-galactopyranoside
E14	β-glucuronidase	Naphthol-AS-BI-βD-glucuronide
E15	α-glucosidase	2-naphthylyl-αD-glucopyranoside
E16	β-glucosidase	6-Br-2-naphthyl-βD-glukopyranoside
E17	N-acetyl-β-glucosaminidase	1-naphthyl-N-acetylo-βD-glucosaminide
E18	α-mannosidase	6-Br-2-naphthyl-αD-mannopyranoside
E19	α-fucosidase	2-naphthyl-α-L-fukopiranoza

yeast typing. Electrophoretic methods include immunoblotting, isoenzyme analysis, analysis of DNA restriction fragment length polymorphism, karyotyping and the use of DNA probes. The application of these methods to epidemiological research the investigation of outbreaks of disease, and the study of virulence is described. The potential impact of the phenomenon of phenotypic switching on the reproducibility of these typing methods is discussed. It is concluded that many of that several have only a poor discriminatory power or reproducibility [7-11].

API ZYM (bioMerieux) by the use of standard test is possible to determine various species of fungi from ontocenosis in various organs characteristic for their enzymograms.

Aim of the study was determination of the enzymatic activity and enzymatic biotypes variations of the yeast like fungi strains isolated from cancer patients with oral candidiasis during 5 years.

Material and methods

The present study was carried out on 92 *Candida albicans* strains isolated from oral ontocenosis of cancer patients with candidiasis symptoms treated with cytostatics since several months to years in 1999 and 2003. Biotypes were assessed according to Williamson's or Kurnatowska's and Kurnatowski's classifications.

The enzymatic activity of the strains tested was assessed by the API ZYM (bioMerieux) method. We used the 5 degree Mc Farland scale to assess the 24 hour incubation with density suspension for *Candida albicans* strains. Test API ZYM is a semiquantitative method of determining the activity on micro scale. The API ZYM stripes consist of 20 microprobes which enable contact between enzymes and non-soluble substrates (*Tab. 1*). The stripes with microprobes are placed in special chambers filled with water (so-called moist chambers). The results were read according to the instructions provided by the producer. The activity of the enzymes was expressed in nanomols of hydrolysed substrate – according to the intensity of the color reaction on a 5 step scale: 0 – no reaction, 1–5 nanomols, 2–10 nanomols, 3–20 nanomols, 4–30 nanomols, 5–40 nanomols and more.

Biotyping of the Candida strains was done according to Williamson's classification (1986) [1,12]. He described 8 biotypes (from A to H) based on analysis of the activity of 5 selected hydrolases: esterase, valine arylamine, naphtol-AS-BI-phosphohydrolase, α-glucosidases and N-acetyl-β-glucosaminidase [1]. The classification of Kurnatowska and Kurnatowski described 6 biotypes [J to N] but additional biotypes described [1] Krajewska-Kułak et al. In 2001 they determined new biotypes O-T [13]. Details are presented in (*Tab. 2*).

The data were analysed by the Wilcoxon matched-pairs signed-ranks test and Chi^2 test with the Statistica 6.0 program.

Results

In 1999 year, 17 of 19 *Candida albicans* tested isolates had hydrolytic activity, the highest activity had leucine arylamidase, esterase, and cystine arylamidase. In 2003 year, 18 of 19 *Candida albicans* isolates had hydrolytic activity. The highest enzymatic activity had leucine arylamidase, esterase lipase, and esterease (*Tab. 3*). No significant differences in the enzymatic activity between 2003 and 1999 year were found.

Almost 87% of the strains were classified into biotypes F (33.7%) and A (30.4%) according to Wilimason's, in 1999 year. Only 8.7% of the strains were determined as K and M biotypes according to Kurnatowska's and Kurnatowski's classification. Four strains (4.3% of tested strains) were classified into Krajewska-Kułak et al. classification (*Tab. 4*). In 2003

Table 2. **Biotyping of the strains on the basis of their enzymatic activity**

BIO-TYPE	Enzyme E 2 Esterase	E 6 Valine arylami-dase	E 11 Naphtol-AS-BI-phos-phohydrolase	E 15 α-gluco-sidase	E 17 N-acetyl-β--glucosa-minidase
Biotype vs Williamson's 1996					
A	+	+	+	+	+
B	+	-	+	+	+
C	+	+	+	-	+
D	+	+	-	+	+
E	+	+	+	-	-
F	+	+	+	+	-
G	+	-	+	+	-
H	+	+	-	-	-
Biotype vs Kurnatowska and Kurnatowski 1998					
I	-	-	-	-	+
J	-	-	-	+	+
K	+	+	-	+	-
L	+	-	+	-	+
M	+	-	+	-	-
N	+	-	-	-	+
Biotype vs Krajewska-Kułak et al. 2001					
O	+	-	-	-	-
P	+	-	-	+	-
R	-	+	+	+	+
S	+	+	-	-	+
T	+	-	-	+	+

year, 93.5% of the tested strains were classified into biotypes A (67,4%) and F (18,5%) according to Wilimason's. Only 4.3% of the strains were determined as K and M biotypes according to Kurnatowska's and Kurnatowski's classification. Two strains (2.2% of tested strains) classified into biotypes according to Krajewska-Kułak et al. (*Tab. 4*).

No significant differences of the enzymatic activity between 2003 and 1999 year were found among Wilimason's biotypes.

Disscusion

The release of hydrolytic enzymes into the environment by dermatophytes and the yeast like fungi strains is an important factor in their pathogenicity and tissue destruction. The fungal enzymatic activity and their character is a susbstantial factor in virulence and adaptation [14-18].

These findings may suggest that drug – resistant strains have a higher enzymatic activity than sensitive strains. The API ZYM offers a useful method for the biotyping of *Candida albicans*. We assessed 19 hydrolytic enzymes by this method.

Some strains isolated from the vagina needed new classification and we designated them as a biotype O. Lane and Garcia have demonstrated (*in vitro*) that *Candida albicans* phospholipase (responsible for hydrolysis of phospholipids – the main component of membrane cells) is a factor in their virulence and resistance to animycotics [19]. Białasiewicz et al., have shown different hydrolytic activities of yeast like strains independent of the species using the API ZYM test. These authors have

Table 3. **Enzymatic activity (in score scale) of the *Candida albicans* strains isolated from cancer patients with candidiasis during 5 last years**

Scale	Number of enzyme / number of strains E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19
Year 1999																			
0	22	2	0	40	0	17	16	78	74	12	10	86	76	92	16	80	60	88	92
1	40	14	6	46	4	26	14	12	12	38	44	6	6	0	27	10	14	4	0
2	16	39	12	6	4	25	17	2	4	32	19	0	2	0	20	2	4	0	0
3	10	27	42	0	14	18	25	0	2	10	15	0	2	0	14	0	5	0	0
4	2	3	20	0	36	4	11	0	0	0	4	0	0	0	6	0	3	0	0
5	2	7	12	0	34	2	9	0	0	0	0	0	6	0	9	0	6	0	0
Mean values of the enzymatic activity																			
N=92	1.3 ±1.1	2.4 ±1.1	3.2 ±1	0.6 ±0.6	4 ±1	1.7 ±1.2	2.3 ±1.5	0.2 ±0.4	0.3 ±0.6	1.4 ±0.6	1.6 ±1	0.07 ±0.2	0.5 ±1.3	0 ±0	1.9 ±1.5	0.2 ±0.4	0.9 ±1.5	0.04 ±0.2	0 ±0
Total mean – 1.19±1.1																			
Year 2003																			
0	10	1	0	32	0	6	9	68	71	5	4	89	83	92	7	57	28	66	90
1	61	8	4	51	1	24	17	23	18	34	38	3	2	0	22	31	10	26	2
2	14	37	26	9	5	40	38	1	2	35	39	0	1	0	36	4	6	0	0
3	5	42	48	0	26	19	21	0	1	18	9	0	1	0	19	0	12	0	0
4	1	1	8	0	34	2	5	0	0	0	2	0	2	0	4	0	15	0	0
5	1	3	6	0	26	1	2	0	0	0	0	0	3	0	4	0	21	0	0
Mean values of the enzymatic activity																			
N=92	1.2 ±0.8	2.5 ±0.8	2.8 ±0.9	0.8 ±0.6	3.9 ±0.9	1.9 ±0.9	2.02 ±1.1	0.3 ±0.4	0.3 ±0.6	1.7 ±0.8	1.6 ± 0.8	0.03 ±0.2	0.3 ±1.1	0 ±0	2.03 ±1.1	0.6 ±0.6	2.4 ±2	0.3 ±0.5	0.02 ±0.1
Total mean – 1.3±1.1																			

***Table 4*. Enzymatic biotyping of the strains isolated from oral cavity of cancer patients**

BIOTYPE	Year 1999	Year 2003
Biotype vs Williamson 1999 year – 87% 2003 year – 93.5%		
A	28 (30.4%)	62 (67.4%)
B	2 (2.2%)	1 (1.1%)
E	6 (6.5%)	3 (3.3%)
F	31 (33.7%)	17 (18.5%)
G	9 (9.8%)	2 (2.2%)
H	4 (4.3%)	1 (1.1%)
Biotype vs Kurnatowska i Kurnatowski 1999 year – 8.7% 2003 year – 4.3%		
K	4 (4.3%)	2 (2.2%)
M	4 (4.3%)	2 (2.2%)
Biotype vs Krajewska-Kułak et al. 1999 year – 4.3% 2003 year – 2.2%		
O	2 (2.2%)	1 (1.1%)
R	2 (2.2%)	1 (1.1%)

also reported an enzymatic activity of 15 out of 19 hydrolases of *Candida albicans* mouth isolates [20,21].

Kurnatowska and Kurnatowski determined the activity of 19 hydrolytic enzymes for 146 strains of *Candida albicans* from patients with various stomatological diseases (gingivitis, adult periodontitis, juvenile periodontitis, glossitis, leukoplakia, stomatitis prothetica, stomatitis atrophica) using the API ZYM [1]. According to their capacity for releasing hydrolytic enzymes 12 biotypes characteristic of *Candida albicans* were found [1]. Of 146 strains of Candida determined from the oral cavity only 60% complied with Williamson's classification [1].

In our earlier studies on 993 *Candida albicans* strains isolated from oral cavity (92 patients with symptoms of candidiasis and 63 healthy people), from vagina (607 patients with candidiasis and 95 healthy people), from urethra (83 patients with candidiasis), from skin (15 patients), nails (18 patients), ulcera (10 patients) and from stomach (10 patients) with different stomach disease were assessed and 132 of *Candida albicans* strains isolated from patients with candidiasis of urethra and 160 from vagina were evaluated. We found that isolates from patients with oral candidiasis more frequently were assigned the biotypes F (33.7%) and A (30.4%). Overall 89.1% of strains were classified into Williamson's classification but only 4.3% had new signs of biotypes. However, 35% of isolates from healthy subjects were assigned to the new biotypes.

Conclusions

The results of present study indicate that most of tested strains were classified into Wiliamson's system. Our results suggest that other Candida biotypes need to be determined according to their different enzymatic activity and susceptibilities.

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