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CANDIDA ALBICANS BERTHOUT, 1923: HYDROLASES ACTIVITY AND OWN METHOD OF DIGESTIVE TRACT STRAINS BIOTYPING

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ABSTRACT. 112 strains of *Candida albicans* were isolated from oral cavity and ontocenoses of the upper digestive tract (endoscopy) of children (age: 5–17) with gastrointestinal disorders. Axenic strains were differentiated with API 20C AUX and API ZYM tests (bioMérieux). Then enzymograms and biotypes were determined for all the strains based on the activity of 19 hydrolases. The highest activity was noted for: e_2 – phosphatase alcaline, e_6 – leucine arylamidase, e_{11} – phosphatase acid, e_5 – lipase (c_{14}); e_7 – valine arylamidase, e_{12} – naphtol-AS-BI-phosphohydrolase, e_{16} – α-glucosidase and e_{18} – N-acetyl-β-glucosamidase, and the latter four were used for biotyping procedures. Our own system was based on the mathematical binominal distribution formula (1 : 4 : 6 : 4 : 1): all „+"; one „–", three „+"; two „–", two „+"; three „–", one „+"; all „–". We have found the following biotypes: A (16.1 ± 3.5%), B_1 (2.7 ± 2.53%), B_3 (8.0 ± 2.5%), B_4 (22.3 ± 3.9%), C_2 (1.8 ± 1.3%), C_3 (7.1 ± 2.4%), C_6 (30.4 ± 4.3%), D_3 (11.6 ± 3.0%).

Key words: biotypes, *Candida albicans*, hydrolases.

The aim of the presented study was to asses in vitro the activity of the hydrolases of strains isolated from the subsuequent ontocenoses of the alimentary tract.

MATERIALS AND METHODS

Between 1998–2000 the study was conducted with 96 children (50 boys and 46 girls) hospitalised at the Clinic, aged 5–17 years. The indication for endoscopy of the upper digestive tract were symptoms suggesting the inflammation of the mucous membrane in the stomach and/or duodenum. During the procedure a macroscopic appearance of the mucous membrane in the upper digestive tract was evaluated and biopsy samples were collected for histopathology (acc. to Sydney System) from the corpus and peripyrolic area of the stomach, duodenal bulb and extrabulbar area of the duodenum, and in justified cases also from the oesophagus.

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During endoscopy the contents of the oesophagus, stomach and duodenum were collected with catheters, and additionally washings from the oral cavity and anal swabs or samples of faeces were taken.

For the mycological examination the material collected consisted of rinsings, with a simultaneous application on selective media (Sabouraud, Czapek). It was then inoculated on Sabouraud's broth and incubated in temperature of 37°C for 24 h. The cultures were then left at room temperature for further 48 h. After that time direct specimes were prepared.

The cultures were then inoculated several times on Sabouraud's agar in order to isolate axenic fungi strains (Kurnatowska 1995). In the initial stage of differentiation macroscopic features of the colony (colour, shape, lustre, margins, surface structure, relation to agar surface, changes of its colour) were evaluated. Then biochemical properties of individual strains were investigated and the assesment of fermentation capacity (zymogram) and assimilation (auxanogram) of saccharide (glucose, galactose, lactose, maltose, saccharose, raffinose, trehalose, melibiose) was carried out. On the basis of tests results (API 20C and API 20C AUX, bioMérieux) allowing the assesment of fungi features, the strains were classified into a proper genus and species using our own system and the principle of numerical identification presented in the firm catalogue (Analytic of Profile Index 1995, bioMérieux, Lyon).

Table 1. The list of examined hydrolytic enzymes and their substrates

Enzyme	Hydrolised substrate	pH	Classification*
e_2 Phosphatase alcaline	2-naphtylphosphate	8.5	3.1.3.1
e_3 Esterase (C4)	2-naphtylbutyrate	6.5	3.1.3.6
e_4 Esterase Lipase (C8)	2-naphtylcaprylate	7.5	3.1.1.3
e_5 Lipase (C14)	2-naphtylmyristate	7.5	3.1.1.3
e_6 Leucine arylamidase	L-leucyl-2-naphtylamide	7.5	3.4.11.14
e_7 Valine arylamidase	L-valyl-2-naphtylamide	7.5	3.4.11.14
e_8 Cystine arylamidase	L-cystyl-2-naphtylamide	7.5	3.4.11.14
e_9 Trypsin	N-benzoyl-DL-arginine-2-naphtylamide	8.5	3.4.4.4
e_{10} α-chymotrypsin	N-glutaryl-phenylalanine-2-naphtylamide	7.5	3.4.4.5
e_{11} Phosphatase acid	2-naphtylphosphate	5.4	3.1.3.2
e_{12} Naphtol-AS-BI-phosphohydrolase	Naphtol-AS-BI-phosphate	5.4	3.1.3.31
e_{13} α-galactosidase	6-Br-2-naphtyl-α-D-galactopyranoside	5.4	3.2.1.22
e_{14} β-galactosidase	2-naphtyl-β-D-galactopyranoside	5.4	3.2.1.23
e_{15} β-glucuronidase	Naphtol-AS-BI-β-D-glucuronide	5.4	3.2.1.31
e_{16} α-glucosidase	2-naphtyl-α-D-glucopyranoside	5.4	3.2.1.20
e_{17} β-glucosidase	6-Br-2-naphtyl-β-D-glucopyranoside	5.4	3.2.1.21
e_{18} N-acetyl-β-glucosaminidase	1-naphtyl-N-acetyl-β-D-glucosaminide	5.4	3.2.1.50
e_{19} α-mannosidase	6-Br-2-naphtyl-α-D-mannopyranoside	5.4	3.2.1.24
e_{20} α-fucosidase	2-naphtyl-α-L-fucopyranoside	5.4	3.2.1.51

*according to Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (1992)

The activity of hydrolytic enzymes of fungi strains was evaluated by bioMérieux API ZYM tests containing substrates for the detection of 19 hydrolases (Table 1). The suspension of fungal cells with a turbidity between McFarland No 5 and no 6 standard was prepared in distilled water. It was then inoculated on the cupules of the API ZYM strips. The strips were incubated for 4 h at 37°C. The results were read according to the intensity of colour reaction on the scale 1 – five, 2 – ten, 3 – twenty, 4 – thirty, 5 – forty and more, nanomoles.

Biotyping of *Candida* strains was done according to the Williamson classification (1986), modified by Kurnatowska and Kurnatowski (1998). In the modified system the mathematical binominal distribution formula was used. The distribution of „+" or „−" (positive or negative activity of particular enzymes) is expressed according to the ratio 1 : 4 : 6 : 4 : 1, involving the activity of 4 enzymes: valine arylamidase (e_7), naphtol-AS-BI-phosphohydrolase (e_{12}), α-glucosidase (e_{16}), N-acetylo-β-glucosamidase.

RESULTS AND DISCUSSION

In 86 out of 96 examined children (89.6%) inflammation of the upper digestive tract mucosa was demonstrated. In 12 children with gastritis or/and duodenitis associated changes in the mucous membrane of the oesophagus were seen. In the course of extensive diagnostics 24-hour pH-metry was done, which showed features of pathological gastroesophageal reflux in these children. Histopathological examination of the samples of mucous membrane led to the diagnosis of chronic inflammation in all children; in 56.5% it was mild and in 37.6% moderate.

For mycological examination the total of 234 samples was used. Among 96 children under study, the presence of fungi was found in 81 cases (84.3%). In the majority of children mycotic invasion was multifocal (82.0%), colonising 3–5 ontocenoses. All strains belonged to the *Candida* genus. The dominating species was *C. albicans* (91.5%); there were also *C. kefyr, C. famata, C. glabrata*, and *C. guilliermondi*. Among biochemical features which enabled the differentiation of the species, their ability to fermentation and assimilation of carbohydrates and use of other organic compounds as a source of carbon were the most important. In the *C. albicans* strains we found as many as 5 codes responsible for different biochemical properties. We analysed the activity of 19 hydrolases, evaluating of about 2500 enzymatic reactions. The appropriate enzymogram was determined in case of each *C. albicans* strain. Seven enzymograms, which differ in the number of detected enzymes (7–10) were described, among which acid phosphatate, an important marker of fungi pathogenicity, was prominent. Using 4 selected hydrolases we differentiated 6 biotypes of *C. albicans* according to Williamson and 6 new biotypes not reported in the literature so far.

In the modified system of 112 *C. albicans* strains biotyping based on the mathematical binominal distribution formula was used; biotypes of these strains are presented in Table 2.

Table 2. Biotypes of *Candida albicans* strains (Williamson classification, modified by Kurnatowska and Kurnatowski 1998)

Group	Biotype	e_7	e_{12}	e_{16}	e_{18}*
A	A	+	+	+	+
B	B_1	−	+	+	+
	B_2	+	−	+	+
	B_3	+	+	−	+
	B_4	+	+	+	−
C	C_1	−	−	+	+
	C_2	−	+	−	+
	C_3	−	+	+	−
	C_4	+	−	−	+
	C_5	+	−	+	−
	C_6	+	+	−	−
D	D_1	−	−	−	+
	D_2	−	−	+	−
	D_3	−	+	−	−
	D_4	+	−	−	−
E	E	−	−	−	−

*e_7 – valine arylamidase, e_{12} – naphtol-AS-BI-phosphohydrolase, e_{16} – α-glucosidase, e_{18} – N-acetyl-β-glucosaminidase

The numbers correspond to subgroups in particular biotypes, i.e 1 – group A: all „+"; 4 – group B: one „−", three „+"; 6 – group C: two „−", two „+"; 4 – group D: three „−", one „+"; 1 – group E, all „−".

The occurence of *C. albicans* strains of various biotypes in particular ontocenoses of the digestive tract is presented in Table 3.

The highest number of strains belonged to biotype C_6 (30.4 ± 4.3%), B_4 (22.3 ± 3.9%) and A (16.1 ± 3.5%).

The choice of these 4 hydrolases for biotyping studies allowed to perform mathematical analysis on binominal distribution with 16 different possible configurations of biotype. It must be added that biotyping model proposed by Williamson et al. (1986) for *C. albicans* and *C. tropicalis* (isolated only from oral cavity) biotyping, which is also based on hydrolase activity, could not be applied in our study to compare intraspecific characteristics of *Candida albicans* strain in particular ontocenoses of the digestive tract. Also the lack of mathematical rationale for created biotypes did not allow to analyse the data in multifocal infection by *Candida* spp. strains isolated from oral cavity ontocenosis and the ontocenoses of the upper part of the digestive tract (Kurnatowska 1971, Kurnatowska and Kurnatowski 1998, Kurnatowski et al. 1999).

Table 3. Occurence of *Candida albicans* strains of various biotypes in particular ontocenoses of the digestive tract

Bio-type	Ontocenoses					Strains total	
	oral cavity	oesophagus	stomach	duodenum	rectum	n	% ± s
A	5	3	5	3	2	18	16.1 ± 3.5
B_1	2	0	0	1	0	3	2.7 ± 1.53
B_2	0	0	0	0	0	0	0
B_3	1	5	1	1	1	9	8.0 ± 2.5
B_4	3	6	8	5	3	25	22.3 ± 3.9
C_1	0	0	0	0	0	0	0
C_2	2	0	0	0	0	2	1.8 ± 1.3
C_3	3	3	1	1	0	8	7.1 ± 2.4
C_4	0	0	0	0	0	0	0
C_5	0	0	0	0	0	0	0
C_6	10	6	8	6	4	34	30.4 ± 4.3
D_1	0	0	0	0	0	0	0
D_2	0	0	0	0	0	0	0
D_3	2	2	5	2	2	13	11.6 ± 3.0
D_4	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0
AU:	28	25	28	19	12	112	100

CONCLUSIONS

(1) In the modified system of *Candida albicans* strains biotyping based on the analysis of selected 4 hydrolases, the mathematical binominal distribution formula was used.

(2) The modified system enables the classification of all the new biotypes of *Candida albicans* strains isolated during our experiment.

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