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Distribution of Ca.LSU Intron and Acid Protease Production by *Candida albicans* Strains Isolated from Gastrointestinal Tract of Diabetes Children

U. NAWROT[1], J. SKALA[2], A. NOCZYNSKA[3], N. POTOCKA[2], K. KOCZOCIK[1] and E. BARAN[4]

[1] Department of Microbiology, Medical University of Wrocław
[2] Department of Genetics, Institute of Microbiology, University of Wrocław
[3] Department of Children Endocrinology, Medical University of Wrocław
[4] Department of Dermatology, Venereology and Allergology, Medical University of Wrocław
Wrocław, Poland

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A b s t r a c t

The association between the presence of self-splicing intron Ca.LSU and proteolytic activity of *Candida albicans* isolates was tested. Study included 95 *C. albicans* strains isolated from gastrointestinal tract of diabetes children. The strains with the intron (genotype B) displayed a significantly higher proteolytic activity (385.2 ± 192U/L) than did strains without intron (genotype A) (119 ± 115U/L) (p = 0.0000048).

K e y w o r d s: *Candida albicans*, proteolytic activity, Ca.LSU intron

One of the method used in genotyping of *C. albicans* strains is based on the presence of self-splicing intron group I in 25S rDNA (Mercury *et al.*, 1993; McCullough *et al.*, 1999). The strains are differentiated in three genotypes: A – without intron, B – with intron, and C – with intron present partially. The correlation between presence of intron and susceptibility to 5-fluorocytosine and pentamidine was described by Mercury *et al.* (1993) and Zhang *et al.* (2002). Recently, Sugita *et al.* (2002) showed statistically higher protease and phospholipase production by *C. albicans* blood isolates belonging to genotype B, compared to genotype A and C. In present study we search for correlation between the proteolytic activity and the presence of intron in non-pathogenic *C. albicans* isolates and our results also indicated high proteolytic activity of genotype B. We tested 95 strains of *C. albicans* and 3 strains of *C. dubliniensis* isolated previously from the faeces (53) and oral cavity (45) of 58 children with diabetes (outpatients) and without symptoms of any infection. The strains were identified using the ID32C tests (BioMérieux) and stored at –70°C. For DNA extraction yeast cells were grown in a YPG medium (2% yeast extract, 2% peptone and 2% glucose) at 30°C for 18 hours. Cellular DNA was extracted according to Rose *et al.* (1990). The PCR assay was performed with the primer pair CA-INT-L (5'-ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3') and CA-INT-R (5'CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3'), described previously by Mercury *et al.* (1993) and McCullough *et al.* (1999). The reactions were performed with a thermal cycler DNA ENGINE PTC-200 (JM Research, USA). DNA samples were denaturated at 94°C for 3 min before 30 cycles of 94°C for 1 min, 65°C for 1 min, 72°C for 4 min and a final extension at 72°C for 4 min following the last cycle. The primers pair using results in the expected PCR products of 450 bp, 840 bp and 1080 bp for genotypes A, B, and D (*C. dubliniensis*), respectively. Genotype C yields two bands of a weight of 450 bp and 840 bp, respectively. For assay proteolytic activity the *Candida* isolates were cultured on Staib's medium with 5% casein at 28°C for 7 days. The supernatants were removed by centrifugation (3000 rpm, 30 min), adjusted to pH 6.5 (using 0.1 M NaOH), sterilised by filtration and stored frozen at

Corresponding author: Dr Urszula Nawrot, Department of Microbiology, Medical University of Wroclaw, Chalubinskiego 4, 50-368 Wrocław; e-mail: nawrot@mbio.am.wroc.pl

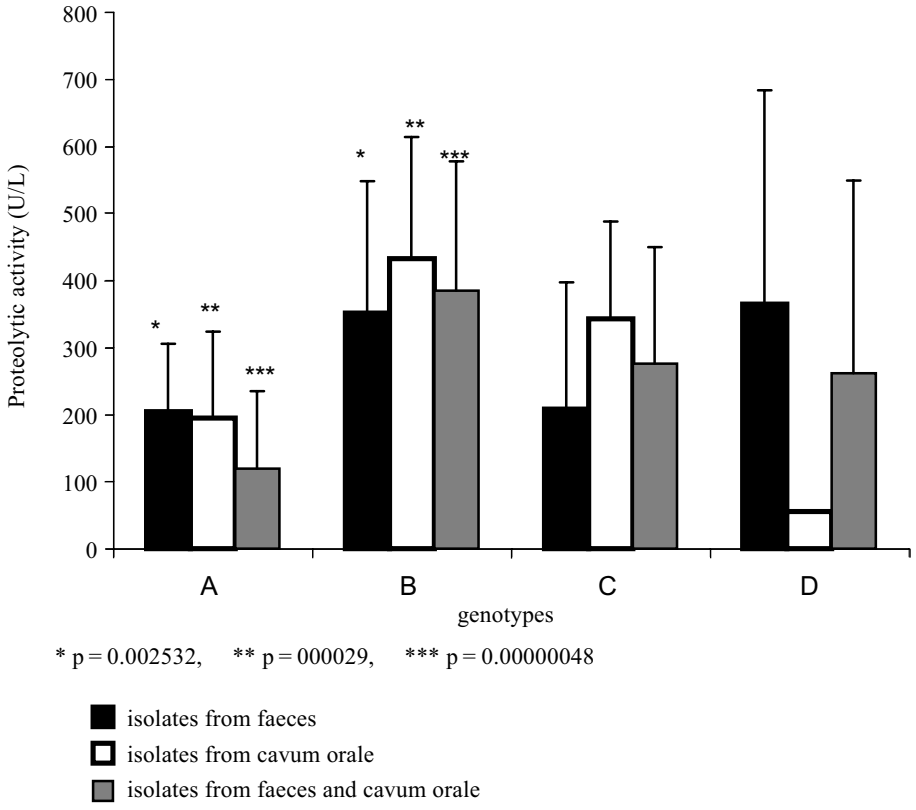

Fig. 1. Proteolytic activity of *Candida* strains of genotypes A, B, C, and D (medium value +/–SD)

–20°C. 500 µl of 0.2 M sodium citrate-HCl buffer (pH 3.3) and 700 µl of 0.5% hemoglobin (used as a substrate) were added to 200 µl of the supernatant (for each strain in triplicate). After 1 hour of incubation at 37°C the reaction was stopped by adding 5% TCA. At the same time control tests were carried out with buffer, TCA (added at the beginning of incubation), hemoglobin and appropriate supernatant. After incubation the samples were centrifuged at 4000 rpm for 30 min and protein concentrations were measured spectrophotometrically at 280 nm (Remold *et al.*, 1968). One arbitrary unit of enzyme activity was defined as 0.1 extinction increase at 280 nm and was calculated for 1 liter of the medium.The Student's test was used to determine the significant differences in the proteolytic activity of the investigated strains.

Among the investigated strains genotype A (24 /53%/ isolates from oral cavity and 23 /43%/ from faeces) and genotype B (15 /33%/ from oral cavity and 23 /43%/ from faeces) were dominant. We found only 10 strains with genotype C (5 from faeces and 5 from oral cavity) and 3 with genotype D. It is interesting to note that the percentage of genotype B we found was higher than in other studies on invasive and non-invasive isolates reported by other authors. For example, Millar *et al.* (2002) found 16.7% genotype B in non-invasive strains and 9.5% in invasive and Tamura *et al.* (2001) found 21% and Sugita *et al.* (2002) 27% genotype B in Japan clinical isolates. These findings indicate geographically related differences in *C. albicans* genotypes distribution and suggest no correlation between presence of Ca.LSU intron and invasiveness.

Acid proteases produced by *Candida albicans* are considered as virulence factors, which play role in adhesion, penetration and damage of tissues, and also in interaction with immune system (Hube, 2000; Monod *et al.* 2002). In our study (Fig. 1), the genotype B had a significantly higher activity (385.2 ± 192U/L) than did genotype A (119 ± 115U/L) ($p = 0.0000048$). This difference was greater between genotypes A and B isolated from oral cavity ($p = 0.000029$) than between the same genotypes isolated from faeces ($p = 0.002532$). The proteolytic activities of ten strains classified as genotype C averaged 275 ± 174U/L and those of three strains defined as genotype D (*C. dubliniensis*) were 261.66 ± 287.5U/L. These data suggest that there is a association between the presence of intron and the stronger proteolytic activity. However, this does not mean that all strains of the genotype B under study produced high levels of acid protease. Of the 38 strains classified as genotype B, seven (18%) showed a low activity (<200U/L). Among the 47 strains of genotype A, three (6%) were protease-negative and 19 (40%) displayed activity below 200U/L.

The genomic analysis of high number of *C. albicans* isolates performed by use DNA fingerprinting probe Ca3 by Lott and Effat (1999), Lott *et al.* (2001) and Blignaut *et al.* (2002) revealed presence of intron in particular clades and absence in others. So, the authors mentioned above considered intron as valuable marker in investigations of evolutionary relationships. Sugita *et al.* (2002) found correlation between the presence of Ca.LSU and high protease activity in pathogenic, blood isolates. Now, we obtained the same result in non-pathogenic, gastrointestinal isolates. These findings indicated that association between proteolytic activity and presence of Ca.LSU possibly reflect evolutionary relationships and is not connected with stronger invasiveness.

Literature

Blignaut E., C. Pujol, S. Lockhart, S. Joly and D.R. Soll. 2002. Ca3 fingerprinting of *Candida albicans* isolates from human immunodeficiency virus-positive and healthy individuals reveals a new clade in South Africa. *J. Clin. Microbiol*. **40**: 826–36.

Hube B. 2000. Extracellular proteinases of human pathogenic fungi. p. 126–137. In: J.E. Ernst and A. Schmidt (eds), Dimorphism in Human Pathogenic and Apathogenic Yeasts. Contrib. Microbiol. Basel. Karger.

Lott T.J. and M.M. Effat. 2001. Evidence for a more recently evolved clade within a *Candida albicans* North American population. *Microbiology* **147**: 1687–92.

Lott T.J., B.P. Holloway, D.A. Logan, R. Fundyga and J. Arnold. 1999. Towards understanding the evolution of the human commensal yeast *Candida albicans*. *Microbiology* **145**: 1137–43.

McCullough M.J., K.V. Clemons and D.A. Stevens. 1999. Molecular and phenotypic characterisation of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea*. *J. Clin. Microbiol*. **37**: 417–421.

Mercury S., S. Montplaisir and G. Lemay. 1993. Correlation between the presence of a self-splicing intron in the 25S rDNA of *C. albicans* and strains susceptibility to 5-fluorocytosine. *Nucleid Acids Res.* **21**: 6020–6027.

Millar B.C., J.E. Moore, J. Xu, M.J. Walker, S. Hedderwick and R. McMullan. 2002. Genotypic subgrouping of clinical isolates of *Candida albicans* and *Candida dubliniensis* by 25S intron analysis. *Lett. Appl. Microbiol*. **35**: 102–6.

Monod M., S. Capoccia, B. Lechenne, C. Zaugg, M. Holdom and O. Jousson. 2002. Secreted proteases from pathogenic fungi. *Int. J. Med. Microbiol*. **292**: 405–19.

Remold H., H. Fasold and F. Staib. 1968. Purification and characterisation of proteolytic enzyme from *Candida albicans*. *Biochim. Biophys. Acta*. **167**: 399–406.

Rose M.D., F. Winston and P. Hietr. 1990. Methods in yeast genetics. A laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Sprin Harbor, NY.

Sugita T., S. Kurosaka, M. Yajitate, H. Sato and A. Nishikawa. 2002. Extracellular proteinase and phospholipase activity of three genotypic strains of a human pathogenic yeast, *Candida albicans*. *Microbiol. Immunol*. **46**: 881–3.

Tamura M., K. Watanabe, Y. Mikami, K. Yazawa and K. Nishimura. 2001. Molecular characterization of new clinical isolates of *Candida albicans* and *C. dubliniensis* in Japan: analysis reveals a new genotype of *C. albicans* with group I intron. *J. Clin. Microbiol*. **39**: 4309–15.

Zhang Y., Z. Li, D.S. Pilch and M.J. Leibowitz. 2002. Pentamidine inhibits catalytic activity of group I intron Ca.LSU by altering RNA folding. *Nucleic Acids Res*. **30**: 2961–71.