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1. Introduction. 2. Biochemical properties of aminopeptidases. 3. Activation and regulation of the *C. albicans* leucine arylamidase. 4. Leucine arylamidase production by *C. albicans* and by other non-*albicans* yeast species. 5. Leucine arylamidase production and *C. albicans* yeast-to-hypha transition. 6. Conclusions

Abstract: *Candida albicans* is an opportunistic pathogen that frequently causes infections ranging from superficial mucosal lesions to disseminated or bloodstream infections. *Candida* infections are a problem of a growing clinical importance worldwide; therefore virulence factors of this pathogen have been extensively studied. This review focuses on the role and function of leucine arylamidase (Ape2) in *C. albicans*' virulence and pathogenesis in human infections. The L-leucine arylamidase is a member of metallo-peptidase group that removes the N-terminal L-leucine from peptide substrates. The hydrolytic enzymes play an important role in both colonization and invasion; moreover, it has been estimated that Ape2 facilitates penetration of *C. albicans* into the host tissue. Therefore, the comparison of Ape2 activity between *Candida* species might reveal their still unknown function during infection *in vivo*. As *C. albicans* can potentially cause superficial and systemic candidiasis with high mortality in immunocompromised patients, the involvement of this enzyme's activity in virulence (human tissues destruction) ought to be thoroughly evaluated in the future.

Rola arylamidazy leucynowej w wirulencji *Candida albicans*

1. Wstęp. 2. Biochemiczne właściwości aminopeptydaz. 3. Indukcja arylamidazy leucynowej w komórkach *C. albicans*. 4. Wytwarzanie arylamidazy leucynowej przez *C. albicans* i inne gatunki z rodzaju *Candida*. 5. Arylamidaza leucynowa a morfogeneza *C. albicans*. 6. Wnioski

Streszczenie: Oportunistyczny patogen *Candida albicans* jest przyczyną zakażeń skóry oraz błon śluzowych u ludzi jak również wieloukładowych kandydoz zagrażających życiu pacjentów. Zakażenia o etiologii *C. albicans* stanowią poważny problem kliniczny o wymiarze światowym. Częstość ich występowania, a także wysoka śmiertelność u chorych z immunosupresją spowodowały konieczność lepszego poznania czynników wirulencji *C. albicans*. Niniejsza praca przedstawia rolę arylamidazy leucynowej (Ape2) w wirulencji *C. albicans* (adhezji oraz inwazji do tkanek ludzkich). Enzymy hydrolityczne odgrywają rolę w procesie kolonizowania i inwazji morfotypów *C. albicans* do ludzkich tkanek. Według danych literaturowych arylamidaza leucynowa należy do grupy egzopeptydaz (trawi wiązanie w miejscu L-leucyny zlokalizowanej na N-końcu substratów białkowych). Dotychczas wykazano również udział Ape2 w inwazji ludzkich tkanek. Porównanie funkcji tego enzymu u różnych gatunków *Candida* pozwoli na dokładną analizę jego roli, jak dotąd nie poznanej, w patogenezie kandydoz. Jeśli ze względu na częste zakażenia o etiologii *C. albicans* obarczone wysoką śmiertelnością, patogen ten stanowi przedmiot licznych badań, to szczególną uwagę należy poświęcić jednemu z jego czynników wirulencji tj., arylamidazie leucynowej i jej roli w inwazji i destrukcji tkanek.

Key words: *Candida albicans*, leucine arylamidase, virulence

Słowa kluczowe: arylamidaza leucynowa, *Candida albicans*, wirulencja

1. Introduction

Candida albicans is a common commensal that is part of the normal microflora of the oral cavity, urogenital, gastrointestinal and vaginal tract of healthy individuals [34]. However in immunocompromised hosts this opportunistic pathogen frequently causes infections ranging from superficial mucosal lesions to disseminated or bloodstream infections [29]. The rapid medicine progress over the last 30 years as well as the development of new surgery and transplantology techniques have led to an enormous increase in the number of immunocompromised individuals that are more susceptible to opportunistic *Candida* infections

[17, 34]. *Candida* infections are a problem of growing clinical importance worldwide, as the incidence of infections has increased dramatically over the past two to three decades, and this trend will inevitably continue into the 21st century [29]. By far, *Candida* remains the fourth most common hospital-acquired pathogen at the Intensive Care Units [34]. Both host and fungal attributes affect the development of *Candida* infections [36]. Moreover, factors such as the use of immunosuppressive agents, diabetes mellitus, AIDS, cancer chemotherapy, long-term catheterization, local disorders of the gastrointestinal tract, parenteral nutrition, mechanical ventilation, or organ transplantation enhance the risk of candidaemia [17, 34]. Most pathogens developed

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Table I

Virulence attributes of *Candida albicans*

Virulence attribute	Putative virulence role	References
Adhesins (e.g. Als family, Hwp1, Int1) ^a	Adhesion and colonization	[2, 29, 32, 49]
Hypha formation	Adhesion, invasion, tissue damage	[23, 29, 41–42, 46]
Extracellular hydrolytic enzymes (e.g., Sap families, Plb, Lip families, Ape2)	Nutrient acquisition, invasion, tissue damage, evasion of host response	[29, 36–37, 43–44]
Phenotypic switching	Adhesion, evasion of host response	[1, 6, 15, 29]

^a Als, agglutinin-like sequence; Hwp1, hyphal cell wall protein 1; Int1, integrin-like protein; Sap, aspartic protease; Plb, Phospholipase B; Lip, lipases; Ape2, leucine arylamidase 2.

an effective set of putative virulence factors and strategies that enhance their ability to colonize host tissues, overcome host defenses and cause diseases [30]. Despite many years of genetic, biochemical and microscopical analyses, factors contributing to *C. albicans* pathogenesis are not fully known [5]. Virulence factors identified so far include such phenomena as biofilm formation, adhesion, yeast to hyphae morphogenetic transition, surface recognition molecules, phenotypic switching, hydrolytic enzyme production and secretion [29–30]. The reader is guided to several excellent papers on the topics of virulence attributes of *C. albicans* listed in Table I.

Hydrolytic activity is considered as a key virulence factor of *C. albicans* [29]. It was confirmed that among extracellular hydrolytic enzymes of this pathogen phospholipases, lipases and secreted aspartyl proteinases are linked to virulence [22, 36–37]. These enzymes not only facilitate adherence and tissue penetration, but also enhance invasion of the host [36, 48]. Furthermore, similar role is suspected for other *C. albicans* hydrolytic enzymes such as acid and alkaline phosphatase, esterase, esterase-lipase and leucine arylamidase Ape2 [48]. So far, most attention has been paid to secreted aspartyl proteinases [29–31, 36–37], however, contribution of other hydrolytic enzymes to *C. albicans*' virulence is also important. Thus, this review focuses on the characteristics and function of leucine arylamidase, a recently identified member of zinc-metallo aminopeptidase group. Below, we discuss whether secretion of Ape2 varies depending on the *C. albicans*' morphology and whether the enzyme influences *C. albicans*' virulence and disease prevalence.

2. Biochemical properties and regulation of aminopeptidases

Aminopeptidases can be subdivided into three groups: I) aminopeptidases in the strict sense which hydrolyze the first peptide bond in the polypeptide chain with the release of a single amino acid residue (aminoacyl- and iminoacyl peptidases [EC 3.4.11]); II) aminopeptidases which remove dipeptides or tripeptides (dipeptidyl- and tripeptidyl peptidases [EC 3.4.14]) from polypeptide chains; III) aminopeptidases which hydrolyze only di- or tripeptides (dipeptidases [EC 3.4.15] and those which hydrolyze tripeptides [EC 3.4.14.4]) [35].

These three groups of aminopeptidases consists of exopeptidases that have the ability to hydrolyze the N-terminal amino acids of oligopeptides or/ and proteins [9]. It has been shown previously [11] that these enzymes are vital for metabolic pathway regulation, cell maturation and turnover of proteins, including utilization of exogenous proteins as nutrient substances and elimination of non-functional proteins. They usually utilize a zinc ion for activity, however other metal ions like Fe²⁺, Mn²⁺, Co²⁺, and Mg²⁺ might also be involved [9]. Moreover, it has been demonstrated that aminopeptidases exist in both soluble and membrane-bound forms and can be identified in different cellular compartments and in the extracellular environment [9]. Their localization in subcellular compartments include the cytoplasm, lysosomes and membranes, however, aminopeptidases can also be secreted extracellularly into the medium [35]. These enzymes play an important role in cell maintenance; therefore, it is not surprising that they are widely distributed throughout the animal, plant and microorganism kingdoms [11]. Moreover, some of microbial aminopeptidases seem to contribute to virulence [35; 48]. According to S a n z [35], microbial aminopeptidases can be divided according to their specificities into (I) general aminopeptidases showing broad specificity (PepN, PepC, LAP/APE and PepS); (II) aminopeptidases of narrow specificity that selectively hydrolyse certain amino acid residues such as acidic residues (PepA) and methionine (MAP) and D-amino acid residues (DppA) or peptide bounds containing proline (PepI and PepP); (III) dipeptidases hydrolysing peptide bounds containing proline (PepQ and PepR); (IV) dipeptidases (PepV and PepDA) and tripeptidases (PepT) of broad specificity that only hydrolyse dipeptides or tripeptides, respectively, and (V) dipeptidyl peptidases showing specificity for N-terminal X-Pro. Moreover, microbial aminopeptidase activity is strongly influenced by environmental factors i.e., pH and tem-

perature [33]. The optimal pH conditions for activity of aminopeptidases ranges from pH = 6 to 9, and the optimal temperature of enzyme assay coincides with the optimal growth temperature [33]. It was also described [33] that fungal aminopeptidases from *Aspergillus* sp. have high thermal stability.

Main types of microbial aminopeptidases include: PepN/Lysyl aminopeptidase; bleomycin hydrolase; aminopeptidase C (PepC); aminopeptidase A (PepA); PepS; CAP/PepA; leucine aminopeptidase/ arylamidase (Ape); aminopeptidase Y (yscI); aminopeptidase M (MAP); aminopeptidase P (PepP); proline iminopeptidase PepI; D-stereospecific aminopeptidase DppA [35]. The present review is restricted to studies addressing the relationship between leucine arylamidase production and *C. albicans* pathogenicity.

3. Activation and regulation of the *C. albicans* leucine arylamidase

L-leucine arylamidases are metallo-peptidases which remove the N-terminal L-leucine from peptide substrates [8, 33]. These enzymes are members of the M1 or M17 peptidase families and, therefore, their nomenclature is quite complex [28]. The leucine arylamidases are highly conserved at the amino acid level and monomers assembled into a homo-hexameric enzyme [26]. The leucine arylamidase enzymes consist of two unrelated functional domains: a unique N-terminal domain, which might play a regulatory role and a well-conserved catalytic C-terminal domain [13]. Moreover, leucine arylamidase is a ubiquitous enzyme that was the first cytosolic aminopeptidase to be identified [35]. The Apes have a preference for catalyzing the hydrolysis of leucine residues (Leu), however, they can exhibit a broader substrate range including proline (Pro) but not arginine (Arg) or lysine (Lys) [26, 28, 35]. In animals leucine arylamidase belong to exopeptidase group [9]. Contrariwise, most of the microbial leucine arylamidase are intracellular enzymes, yet extracellular enzymes are found in filamentous fungi [33]. These enzymes are homohexamers, comprising two trimers stacked on top of one another [7, 13]. Moreover, their activity is dependant on metal ions as enzymatic reaction will not proceed unless an enzyme-metal complex is formed to which the substrate can be bound [13]. All aminopeptidases of M17 family, including leucine arylamidase, bind two metal ions, both essential for catalysis [13], but those ions differ among organisms. Usually, a zinc ion is utilized, but a number of other metal ions, including Fe^{2+} , Mn^{2+} , Co^{2+} , and Mg^{2+} , can be involved [9]. In mammals, each monomer of leucine arylamidase enzyme binds two Zn^{2+} ions [7]. In plants Mn^{2+} -activated leucine arylamidase has been detected [12]. In

the case of microorganisms, Kuo et al. [24] proposed to classify microbial Apes into three groups depending on the ion used for catalysis; I) the leucine arylamidase enzymes containing at least one Zn^{2+} per monomer and displaying the characteristic peptide motif HEXXH i.e., the PepN aminopeptidases from *E. coli* and lactic acid bacteria; II) enzymes binding two closely located Zn^{2+} per monomer whose activity is strongly inhibited by bestatin; III) the leucine arylamidase enzymes containing two Co^{2+} per monomer i.e., the prolidase of *Lactococcus lactis* subsp. *Cremonis*, aminopeptidase-A of *L. lactis* subsp. *lactis*, a dipeptidase of *Lactobacillus sake*, and leucine arylamidase II of *B.(G.) stearothermophilus*.

While similar in primary structure, the animal, plant and prokaryotic leucine arylamidase enzymes appear to have distinct functions [12]. In microorganisms these enzymes might be involved in virulence [8]. The study by Carroll et al. [8] demonstrated that *Staphylococcus aureus* strains containing a mutation in the pepZ gene encoding leucine arylamidase are severely attenuated in virulence. Moreover, the same authors [8] described that *S. aureus* leucine arylamidase is required for survival inside human macrophages which also contribute to virulence. The microbial leucine arylamidase have been described in both Gram-positive and Gram-negative bacteria as well as in filamentous fungi [8, 11, 33]. Based on the present data (see the section below) it is highly probable that the main role of the *C. albicans* arylamidases is to provide nutrition to the cells aiding penetration and invasion.

4. Leucine arylamidase production by *Candida albicans* and by other non-*albicans* yeast species

The presence of leucine arylamidase (Ape) in both *C. albicans* and culture medium was first described by Kim et al. [19]. In this early study [19] both endogenous and exogenous Ape activities were evaluated. The exogenous Ape activity was shown to be much lower in comparison to the endogenous enzyme activity by ratios of 1:8 to 1:20, when referred to the protein concentration of the aliquots used for the assay. According to Kim et al. [19] the lower exogenous Ape activity in comparison to endogenous Ape may possibly reflect the liberation of the enzyme from dead or dying organisms rather than active enzyme secretion for purposes of extracellular digestion of nutrient substrates, but this conclusion was based on activity level only. Later studies [33] on Ape activity in filamentous fungi revealed their ability of extracellular Ape production. Moreover, Imbert et al. [16] demonstrated *C. albicans* metallo-peptidase activity in culture medium, suggesting that the enzyme can be secreted. Furthermore, Kinke et al. [20] described secretion mechanism for

arginine/alanine/leucine-specific metallo-aminopeptidase isolated from *C. albicans* indicating extracellular function of this enzyme. The authors [20] also ruled out the possibility CaApe2 plays a role in the degradation of human dentin and other collagen-based extracellular structures. The biological role of leucine arylamidase in yeast and factors regulating Ape activity are still not completely understood [13]. Kim et al. [19] indicated that Ape activity in *C. albicans* varies with the type of culture medium and growth phase, since Ape activity was higher in Sabouraud dextrose agar than in the liquid medium. Moreover, according to Bautista-Muñoz et al. [5] highest enzyme activity was found in the medium with proline as sole nitrogen source, which indicates higher enzyme production during nutritional stress since proline is considered non-preferential nitrogen source. The study of Kim et al. [19] revealed also that Ape activity was increased up to 24 hours of incubation during the active growth phase of the colonies. Those results stay in agreement with Bautista-Muñoz et al. [5] who indicated highest enzyme activity during logarithmic (6 h) and early stationary phases (12 h) of growth. The studies of Herrera-Camacho et al. [13] and Klinke et al. [20] indicated that Ape activity is strongly inhibited by bestatin (a peptide produced by *Streptomyces olivoreticuli*), while EDTA also displayed considerably high inhibition effect. Moreover, the results of Glowacka et al. [10] showed that Fir Siberian essential oil lowers *C. albicans* Ape activity as proved in api° Zym test.

The leucine aminopeptidase seems to be a new potential factor in the yeast pathogenicity. Therefore, in the last years attempts were made [5, 13, 20] to evaluate the proteolytic potential of yeasts by identifying novel aminopeptidases able to cleave leucine residues. The leucine arylamidase of *S. cerevisiae* has been described by Hirsch et al. [14]. In 2007 Herrera-Camacho et al. [13] reported the identification of new leucyl aminopeptidase in fission yeast *Schizosaccharomyces pombe*. The enzyme was characterized as a hexameric manganese-dependent metallo-exopeptidase, named leucyl aminopeptidase yspII (LAP yspII) and classified as cytosolic leucyl aminopeptidase [13]. Moreover, the location of the gene coding LAP yspII enzyme was estimated to be in chromosome I of *S. pombe* [13]. The identified LAP yspII enzyme cleaved synthetic aminoacyl-4-nitroanilides at an optimum of pH 8.5 and preferred leucine and methionine as N-terminal amino acids. Although arginine and lysine were also recognized as substrates, they were cleaved at a much reduced rate [13]. Furthermore, a clear dependence on Mn^{2+} ion was found, however Mg^{2+} and Ni^{2+} also enhanced aminopeptidase activity [13]. As LAP enzymes are usually Zn^{2+} dependent [9], Herrera-Camacho et al. [13] reported that for LAP yspII from

S. pombe Zn^{2+} is among ions that had inhibitory effect on enzyme activity. The ability to inhibit cell surface leucine aminopeptidase of various species has been described for bestatin [47]. Herrera-Camacho et al. [13] tested the effect of protease inhibitors on *S. pombe* LAP yspII enzyme activity. As expected bestatin behaved as a competitive inhibitor of LAP yspII, and chelating agents such as chloroquine, EDTA and phenanthroline also reduced enzyme activity [13].

Imbert et al. [16] indicated that *C. albicans* metallo-peptidase located in the cell wall can degrade some of host extracellular matrix components, indicating its potential role in pathogenesis. In 2008 Klinke et al. [20] reported the identification of arginine/alanine/leucine-specific metallo-aminopeptidase from *C. albicans* (CaApe2). This enzyme was isolated from the cell wall as a result of secretion and acted on N-terminal arginine, alanine and leucine residues as substrates for hydrolysis. Moreover, the secretion of this novel enzyme suggested its extracellular function [20]. The optimal conditions for CaApe2 activity were estimated to be pH = 7.2 and temperature of 30°C [20]. Furthermore, according to Klinke et al. [20] the enzyme should exhibit 85% of its maximum catalytic activity at the host body temperature 37°C, which suggests its role during infection. Similarly as in the study of Herrera-Camacho et al. [13] bestatin proved to be effective on CaApe2 activity even in micromolar concentrations [20]. Moreover, EDTA and phenanthroline also strongly inhibited CaApe2-dependant peptide hydrolysis. Contrariwise, the pepstatin A and PMSF – the known inhibitors of aspartic and serine proteases respectively, had no effect on CaApe2 activity [20]. The CaApe2 enzyme has a broader substrate specificity when compared to the *Saccharomyces cerevisiae* aminopeptidases *S. cerevisiae* two main aminopeptidases (API) – yscI and yscII encoded by *API1* and *API2* genes, respectively [14, 21]. The API yscI is an alanine/arginine specific aminopeptidase which lacks the ability to cleave leucine residues [21], while the API yscII has the activity of leucine/lysine specific aminopeptidase [14]. These distinct functions might be the result of the whole-genome duplication event in evolution history of *Saccharomyces* [50]. The most common outcome of duplication event is subfunctionalization of proteins encoded by the duplicated genes [50] only small fraction of the genes is retained in duplicate and thus. In contrast to aminopeptidases of *S. cerevisiae*, CaApe2 combines the substrate specificities of those aminopeptidases [20]. This broader substrate specificity might be the result of the fact that *C. albicans* is unlikely to have undergone whole genome duplication [25]. The extracellular role of Ape2 in *C. albicans* is still unknown, however this enzyme may support fungal growth and proliferation by providing free amino acids required for metabolism [20].

Enzymatic activity has been recognized as one of the factors influencing the course of *Candida* infection [3]. As hydrolytic enzymes play an important role in both colonization and invasion, comparison of hydrolytic activity between *Candida* species might enlighten their role during infection [3]. Therefore, in last decade many authors examined hydrolytic activity of *C. albicans* and others non-*albicans Candida* strains using the api[®]Zym test [3, 10, 18, 22, 27, 39, 48]. Moreover, the activity of candidal leucine arylamidase has received more attention in the recent studies (Table II, Table III). According to Ł u k a s z u k et al. [27], 89–94% of tested *C. albicans* clinical isolates, displayed hydrolytic activity. Further-

more, leucine arylamidase, esterase lipase and esterase were enzymes with the highest activity in strains isolated from cancer patients [27]. These results are in agreement with other studies [4, 22] which also showed high activity of these enzymes in *C. albicans* clinical isolates indicating their role in *Candida* spp. virulence. Moreover, according to V i d o t t o et al. [48], leucine arylamidase facilitates penetration of *C. albicans* into the host tissue. The recent studies of *Candida* sp. enzymatic activity conducted on clinical isolates have shown that *C. albicans* Ape2 activity is higher (Table II) than in other non-*albicans Candida* species (Table III). Moreover, *C. albicans* Ape2 activity varied among isolates

Table II

Comparison of *Candida albicans* leucine arylamidase activity estimated by apiZym test in various studies

Material used (no. of strains tested)/ strain isolation source	Activity in apiZym scale	Statistic method	Mean activity [nmoles]	Reference
Sputum (48)/ LC, COPD patients	4,5 ± 0,6	Mann-Whitney test	30–40	[3]
Swab (27)/ OO of HIV patients	4,96			[48]
Urether (65)	3,75±0,13	Wilcoxon test	20–30	[22]
Swab (92)/ OO of cancer patients	4±1		30	[27]
Swab (92)/ OO of cancer patients	3,9±0,9		20–30	
Swab (6)/ pharyngeal mucosa, swab (1)/ tongue, peritoneal fluid (3), sputum (4), ear secretion (1), urine (4), stools (2), blood (1), after-surgery wound secretion (2)	4,04		30–40	[10]
Blood (6), swab (5)/ drain, swab (9)/ OO, swab (6)/ vagina, pancreas samples (1), aspirate samples (1), swab (4)/ wound, stool (3), urine samples (1)	3–5	Cohen s kappa coefficient	20–40	[39]

Activity in apiZym 0–5 scale, where 0 is 0 nmol; 1 is 5 nmol; 2 is 10 nmol; 3 is 20 nmol; 4 is 30 nmol; and 5 is ≥ 40 nmol; LC, lung cancer; COPD, chronic obstructive pulmonary disease; OO, oral ontocenosis; HIV, human imunodeficiency virus.

Table III

Comparison of non-*albicans* spp. leucine arylamidase activity estimated by apiZym test in various studies

<i>Candida</i> species	Material used (no. of strains tested)/ strain isolation source	Activity in apiZym scale	Statistic method	Mean activity [nmoles]	Reference
<i>glabata</i>	Urether (10)	1,2±0,13	Wilcoxon test	5–10	[22]
	Urether (4)	1,75±0,75		5–10	
<i>krusei</i>	Swab (1)/ pharyngeal of LTR	5	–	≥40	[18]
	Swab (1)/ nose of LTR				
	Swab (1)/ drain of LTR				
	Feces (1) of LTR				
	Swab (1)/ drain of LTR				
	Bile (1) of LTR				
	Fluid (1)/ abdominal cavity of LTR	3		20	
<i>tropicalis</i>	Urether (2)	4,5 ± 0,5	Wilcoxon test	30–40	[22]
<i>kefyr</i>	Urether (1)	0		0	
<i>rugosa</i>	Urether (1)	4,5 ± 0		30–40	
<i>dubliniensis</i>	Swab (26)/ OO of HIV patients	4,7	Mann-Whitney test		[48]

Activity in apiZym 0–5 scale, where 0 is 0 nmol; 1 is 5 nmol; 2 is 10 nmol; 3 is 20 nmol; 4 is 30 nmol; and 5 is ≥ 40 nmol; LC, lung cancer; COPD, chronic obstructive pulmonary disease; HIV, human imunodeficiency virus; LTR, liver transplant recipients; OO, oral ontocenosis; – none statistic method used.

from different sources. Still the knowledge about these enzymes and their role in yeasts, especially in *Candida* spp. is limited. Thus, the construction of *ape2Δ* mutant strains is expected to provide an experimental tool which is required to analyze the enzyme activity in the virulence of *C. albicans*. Therefore, the *in vivo* and *in vitro* studies of CaApe2 activity in the future should shed more light on morphotypes strategies and adaptation to the host niches.

5. Leucine arylamidase production and yeast-to-hypha transition

Among the factors contributing to the pathogenicity of yeast, hydrolytic enzymes play a significant role during infection [27]. One of the most extensively studied virulence trait of *C. albicans* is its ability to form true hyphae helping to invade the host tissues and cause deep-seated infections [23]. Our recent studies have provided evidence for true hyphae formation under human serum influence at human body temperature. We established that hyphae are essential elements providing the structural integrity of fungal-ball model, which may be expected to play a role in bloodstream infections [39–42]. Furthermore, our comparative analysis of morphogenesis mutants and clinical isolates showed that Efg1 is required for human serum-induced cell growth and morphological switching (forthcoming publication). Our current studies shed light on the role of *C. albicans* hyphal morphologies in biofilm formation at the surface of intestinal epithelial cells (Fig. 1). Moreover, our data strongly suggest that the epithelial cell type (cell line Caco-2, ATTC) influences the behaviour of *C. albicans* hyphal cells attached to their surface. Previous studies [38–39] have shown that the high invasiveness of hyphal forms is associated with the expression of numerous hydrolytic enzymes regarded as virulence factors. Also, it has been confirmed that a distinct set of SAP genes is expressed during candidal infection [36]. Moreover, aspartic protease isoenzymes Sap1-3, Sap4-6 exhibit a higher expression level in true hyphae compared with blastoconidial cells [38]. However, detailed information on leucine arylamidase gene (*APE*) expression in different pleomorphic forms of *C. albicans* is still not available. Significantly, an intriguing phenomenon has recently been revealed by us [39], namely satisfactory agreement ($\kappa = 0.770$) between the levels of Ape activity for the two examined pleomorphic (blastoconidial and hyphal) forms was observed. By contrast, results obtained by B a u t i s t a - M u ñ o z et al. [5] suggested the participation of this enzyme during dimorphism as its activity increased during the formation of germ tube and pseudohyphae. Yet, the highest enzyme activity was detected during the true hyphae formation [5]. Indeed,

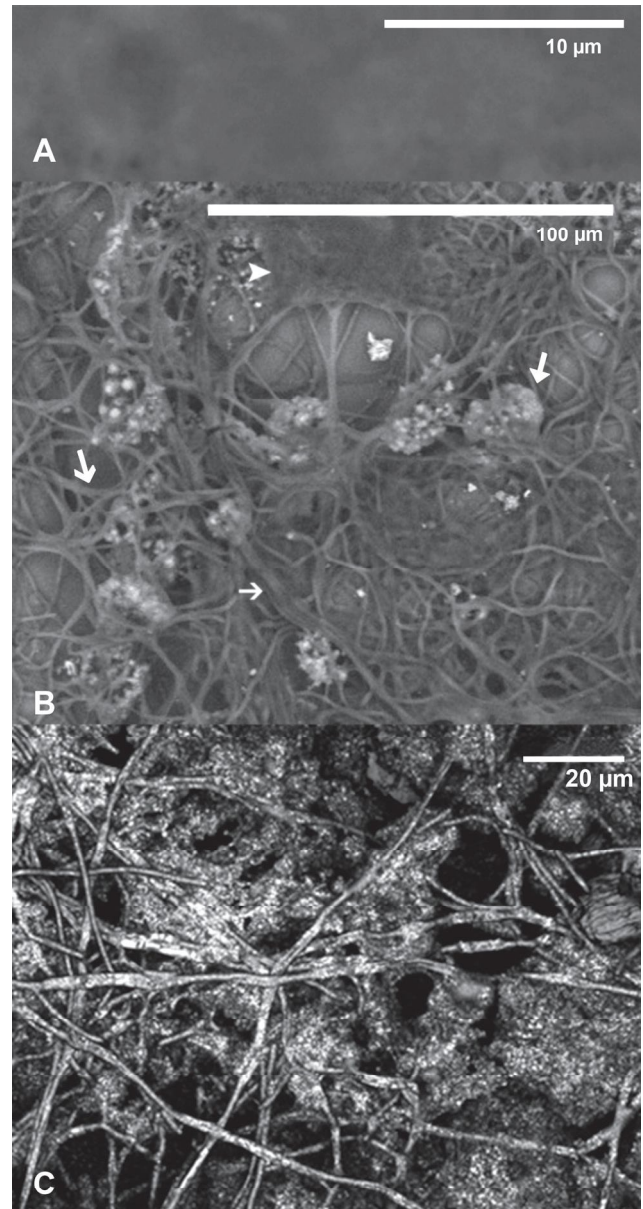


Fig. 1. The architecture of mono-species biofilm (18 h) of *Candida albicans* at the surface of Caco-2 cell monolayer covering glass slides in 24-well plates. Adherence pattern of *C. albicans* strains showing forms of uniform morphology (true hyphae). (A) Scanning electron microscopy (SEM) image of Caco-2 monolayer with no biofilm (control). (B, C) The cell line treated with blastoconidial cells resulted in hyphal biofilm covering cell line. (B) SEM image of *C. albicans* strain SC5314 mature biofilm (18 h). *C. albicans* hyphal forms (open arrows) appear adherent to Caco-2 monolayer. Adherent filamentous forms are able to form aggregate (arrow) at the surface of intestinal cells. The extracellular material is visible (arrowhead) (C) Confocal laser scanning electron (CLSM) image of *C. albicans* mutant strain $\Delta sap1-2$ biofilm grown for 18 h on Caco-2 monolayer. True hyphal forms (open arrows) and extracellular material (arrowhead) are condensed

we suggest that it may be related to the role of the various morphotypes in particular steps of pathogenesis. Such pattern shared by two pleomorphic forms may be virulence a marker of both morphotypes. For clinical

benefits, this phenomenon ought to be explored in further studies. Moreover, according to Vi d o t t o et al. [48], Ape facilitates penetration of *C. albicans* into the host tissue. Taking into consideration the Ape activity, it seems that both morphotypes play a similar role in virulence. Whether Ape affects morphogenesis in *C. albicans* or plays an essential role in virulence still needs to be analyzed. In our opinion, elucidation of factors that influence leucine arylamidase activity could lead to a deeper understanding of the mechanisms that underpin pathogenicity of *C. albicans*. Moreover, we have undertaken further experiments based on the quantification of the *APE2* gene expression level in clinical samples from patients suffering from *Candida* infection. These studies should shed some light on the possible role of these proteins as determinants of *Candida* virulence and will also allow for the assessment of Ape contribution in the pathogenesis process. Furthermore, better understanding of the role and function of leucine arylamidase in *C. albicans* infections will help to design new arylamidase inhibitors with potential anticandidal activity.

6. Conclusions

The leucine arylamidase (Ape) belongs to metallopeptidases which remove the N-terminal L-leucine from peptide substrates [33]. These hydrolytic enzymes are widely distributed throughout the animal, plant and microorganism kingdoms [11]. In microorganisms the enzymes may play a role in virulence [8]. Biological role of Ape in yeasts is still not fully understood [13], however this enzyme might be involved in candidal pathogenesis. According to some authors leucine arylamidase facilitates penetration of *C. albicans* into the host tissue and for that reason further studies are required.

Since *C. albicans* infections are associated with high mortality in immunocompromised patients, they remain a serious problem worldwide. Therefore, the role of leucine arylamidase activity in virulence (human tissue destruction) ought to be evaluated in future.

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