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Differentiation between *Candida* species isolated from diabetic foot by fatty acid methyl ester analysis using gas chromatography

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Abstract

Gas chromatography (GC) was used to differentiate 100 isolates of *Candida* species (*Candida parapsilosis, Candida albicans, Candida tropicalis, Candida famata* and *Candida glabrata*) from 22 of 509 diabetic patients in whom the same species had been isolated from ulcer and interdigital spaces of the same and/or the other foot. All clinical isolates were identified by quantitative differences in the composition of six cell fatty acids (CFA). The values of the coefficients of variability (CV) of CFA show that the isolates from foot ulcers and interdigital spaces of the same diabetic patient probably belong to different chemotypes of the same *Candida* species.

Keywords: *Candida* species; Diabetic foot ulcers; Gas chromatography; Cell fatty acids

1. Introduction

On average, 10–15 years of diabetes can lead to the development of diabetic foot in 5–10% of the patients [1,2]. The pathogenesis of diabetic foot is highly complex, including polyneuropathy, peripheral vascular disease, and compromised immunity, slower wound healing, trauma and infection [1–3]. With increased incidence, *Candida* species colonize the skin on the body and feet of diabetic patients [4,5]. The same species of endogenous, low-pathogencity yeasts may be not only the infective agents, but also the secondary colonizers or contaminants of the diabetic foot ulcers [1]. An isolation of these yeast species from ulcer swabs is not a confirmation of their pathogenic role [4,5]. Finding fungal elements in the histopathology preparations of diabetic foot ulcer biopsies is confirming the pathogencity of isolated yeasts [4]. Present-day diagnosis of fungal infections is time consuming and labor intensive; as to the application of histopathology methods, it only adds to its complexity. Many authors have described an improvement in the species-specific identification of yeasts from clinical samples based upon characterization of cell fatty acids (CFA) by gas chromatography (GC) [6–15]. Some authors proposed GC as an easy method of epidemiologic typing (chemotyping) based on CFA analysis of several bacterial species (*Campylobacter* spp., coagulase-negative *Staphylococcus* spp., *Pseudomonas* spp.) [7]. The applicability of GC for identification to the subspecies level (typing) of yeast remains unstudied.

In consequence, the aims of the present study were: (i) to confirm the validity of the GC for the identification of isolates of different *Candida* species and, (ii) to look at the applicability of GC to the chemotyping of isolates of the same *Candida* species from foot ulcer and interdigital spaces of the same patient. The fact of these isolates belonging to the same or different chemotypes of the same *Candida* species (according

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to the CV values of the main identifying CFAs) can help in assessing the role of *Candida* isolates in the pathogenesis of diabetic foot ulcer infection as pathogenic micro-organisms or ulcer colonizers.

2. Patients and methods

2.1. Index patient details

The criterion for including diabetic patients in the present investigation was isolation of the same *Candida* species from foot ulcer samples (swabs and/or biopsy) and swabs of the interdigital spaces of an ulcerated and/or ulcer-free foot from the same patient.

Patient characteristics were as follows: 14 (63.6%) males, 8 (36.4%) females; their age range 48–79 years, mean age 62.1 years; 6 (27.3%) cases of Type-1 diabetes, and 16 (72.7%) cases of Type-2. The average duration of diabetes since diagnosed was 16.8 years (ranging from 0.5 to 45 years). All subjects had a foot ulcer each, infected with yeasts or with yeasts and bacteria. The average duration of foot ulcer infection was 7.6 weeks (range 2–20 weeks) (Table 1).

2.2. Culture, incubation period and standard biochemical identification

While still next to the patient, samples of foot ulcers biopsies were immediately inoculated in Sabouraud's broth [16]. Inoculation of interdigital foot space swabs and infected ulcer swabs made within 24 h on Sabouraud's agar with chloramphenicol which was added to inhibite the bacterial growth [4]. Liquid media were stored for 5–7 days at 37 °C and solid media for 5–7 days at 27 °C [4]. Whereas germination test was used to identify *Candida albicans*, the identification of other species proceeded by looking at physiologic and morphologic characteristics of the sugar assimilation and sugar fermentation tests, and by considering the appearance on corn meal agar [4].

2.3. Strains

One hundred clinical isolates belonging to *Candida* genus and four reference stock cultures from American Type of Culture Collection (*Candida parapsilosis* (ATCC 22019), *C. albicans* (ATCC 60193), *Candida tropicalis* (ATCC 201380), *Candida glabrata* (ATCC 15126)), and one isolate of *Candida famata* from the Croatian National Institute of Public Health (CNIPH 312/03) were analyzed by GC. Single colonies were isolated and frozen until the clinical part of investigation was completed. They were re-identified by using standard biochemical identification methods prior to actual processing for GC.

2.4. Sample preparation

Methyl esters of the CFA yeasts were prepared using the Moss method [10]. Briefly, cells for fatty acid analysis were incubated at 27 °C for 48 h on slants of Sabouraud's agar. Cell removal from slants was by addition of 0.5 mL of sterile

Table 1
Index patient detail

No.	Sex	Age	Type of DM	Duration of DM (years)	Duration of infection (weeks)	Ulcerated foot		Ulcer-free foot
						Interdigital spaces	Ulcer	Interdigital spaces
1	F	58	2	6	8	*C. parapsilosis* 1[a]	*C. parapsilosis* 2[a]	*C. parapsilosis* 1[a]
2	M	56	1	27	2	*C. parapsilosis* 3[a]	*C. parapsilosis* 4[a]	
3	F	66	2	20	12	*C. parapsilosis* 1[a]	*C. parapsilosis* 4[a]	
4	M	56	1	27	4	*C. parapsilosis* 2[a]	*C. parapsilosis* 2[a]	*C. parapsilosis* 2[a]
5	M	77	2	2	2	*C. parapsilosis* 1[a]	*C. parapsilosis* 3[a]	
6	F	66	1	40	13	*C. parapsilosis* 1[a]	*C. parapsilosis* 2[a]	
7	F	58	2	15	6	*C. parapsilosis* 2[a]	*C. parapsilosis* 7[a]	
8	M	62	2	10	13	*C. parapsilosis* 3[a]	*C. parapsilosis* 4[a]	*C. parapsilosis* 4[a]
9	M	62	2	10	10	*C. parapsilosis* 3[a]	*C. parapsilosis* 5[a]	*C. parapsilosis* 1[a]
10	M	77	2	10	8	*C. parapsilosis* 2[a]	*C. parapsilosis* 1[a]	*C. parapsilosis* 1[a]
11	F	78	2	2	2	*C. parapsilosis* 1[a]	*C. parapsilosis* 2[a]	
12	M	71	2	20	14	*C. parapsilosis* 1[a]	*C. parapsilosis* 2[a]	
13	M	74	2	20	8	*C. parapsilosis* 3[a]	*C. parapsilosis* 4[a]	
14	F	58	2	6	20	*C. albicans* 1[a]	*C. albicans* 1[a]	
15	M	70	2	20	4	*C. albicans* 1[a]	*C. albicans* 2[a]	
16	M	48	2	0.5	2	*C. albicans* 2[a]	*C. albicans* 1[a]	*C. albicans* 1[a]
17	M	57	2	10	4	*C. tropicalis* 1[a]	*C. tropicalis* 2[a]	
18	F	54	1	32	8	*C. tropicalis* 1[a]	*C. tropicalis*, 1[a]	
19	M	71	2	10	6	*C. famata* 2[a]	*C. famata* 2[a]	*C. famata* 1[a]
20	M	66	1	45	2	*C. famata* 1[a]	*C. famata* 1[a]	
21	F	79	2	2.5	4	*C. glabrata* 1[a]	*C. glabrata* 1[a]	
22	M	65	1	35	14	*C. glabrata* 1[a]	*C. glabrata* 1[a]	

F: female, M: male; DM: Diabetes Mellitus.
[a] Number of clinical isolates.

distilled water and by gentle scraping. The cell suspension was transferred to a test tube; 4 mL of a 5%-NaOH in 50% methanol were added, and the tube was sealed with a screw cap and placed in a boiling water bath for 1 h. Next, saponified material was cooled to room temperature, and the pH was adjusted to 2.0 by the addition of 6 N HCl. Then, free fatty acids were methylated by adding 4 mL of a 10%-boron trichloride-methanol reagent, and heated for 5 min in an 85 °C water bath. The mixture was cooled to room temperature, and the methyl esters were extracted by shaking with two successive 10 mL portions of a 1:1 mixture of diethyl ether-hexane. The ether-hexane layers were combined in a 50 mL beaker, and the volume was reduced to approximately 0.5 mL under a gentle stream of flowing nitrogen gas. Approximately 80 mg of Na_2SO_4 were added to remove residual moisture. The final volume was reduced to 0.1 mL and analyzed by GC.

2.5. Gas chromatography

CFA's composition was determined by GC [17] using an ATI Unicam instrument, model 610 (Cambridge, England) equipped with a split-injector and flame ionization detector (FID). Capillary column was a DB-23 (30 m × 0.25 mm × 0.25 μm). The temperature of the injector and detector was set at 250 °C. The initial oven temperature was 170 °C. This temperature was maintained for 8 min, and then increased to 190° at a rate of 2 °C/min, which was held for 7 min.

Helium was used as the carrier gas at a flow rate of 0.87 mL/min and injection volume was 1.0 μL.

The CFA esters' peaks were identified using standard methyl esters, supplied by Sigma Chemical Company (St. Louis, USA). The fatty acid composition is expressed as weight percentage of total FA (internal normalization method). Chromatography software (Unicam 4880 chromatography data system) was employed for data collection and processing.

2.6. Statistical analysis

Identification of *Candida* species is accomplished by comparing the CFA profiles of reference strains and reliably characterized clinical isolates using the Kruskall–Wallis test [18]. Chemotyping of clinical isolates of the same *Candida* species is based upon the calculation of the coefficient of variability (CV = standard deviation/mean × 100) of the main identifying CFAs of all *Candida* isolates from the different samples of the same patient.

3. Results

From 22 of 509 diabetic patients, the same species of *Candida* was isolated from their foot ulcer, as well as from interdigital spaces of the same and/or the other foot. In 13 (59.1%) patients, the isolates belonged to *C. parapsilosis*, and in 3 (13.6%) to *C. albicans*. Isolates of *C. tropicalis*, *C. famata* and *C. glabrata* were made from the samples obtained from 2 (9.1%) diabetic patients each (Table 1). Using GC method, we analyzed 100 clinical isolates of *Candida* species, including *C. parapsilosis* (75), *C. albicans* (9), *C. tropicalis* (5), *C. famata* (7) and *C. glabrata* (4). While 35 isolates of *Candida* species originated from interdigital spaces of ulcerated foot, 13 originated from these spaces of ulcer free foot, and 52 isolates were derived from foot ulcers.

3.1. Qualitative analysis of CFA

All *Candida* isolates contain saturated and unsaturated straight-chain CFA with C_{16} and C_{18} atoms. Neither capric ($C_{10:0}$) and lauric ($C_{12:0}$) acids, nor arachidic ($C_{20:0}$) and behenic ($C_{22:0}$) acids were found in any of the analyzed clinical and standard *Candida* isolates. The CFA retention time ranged from 5.95 to 14.0 min. Gas chromatogram of fatty acid methyl esters composition of one isolate of *C. parapsilosis* is showed on Fig. 1.

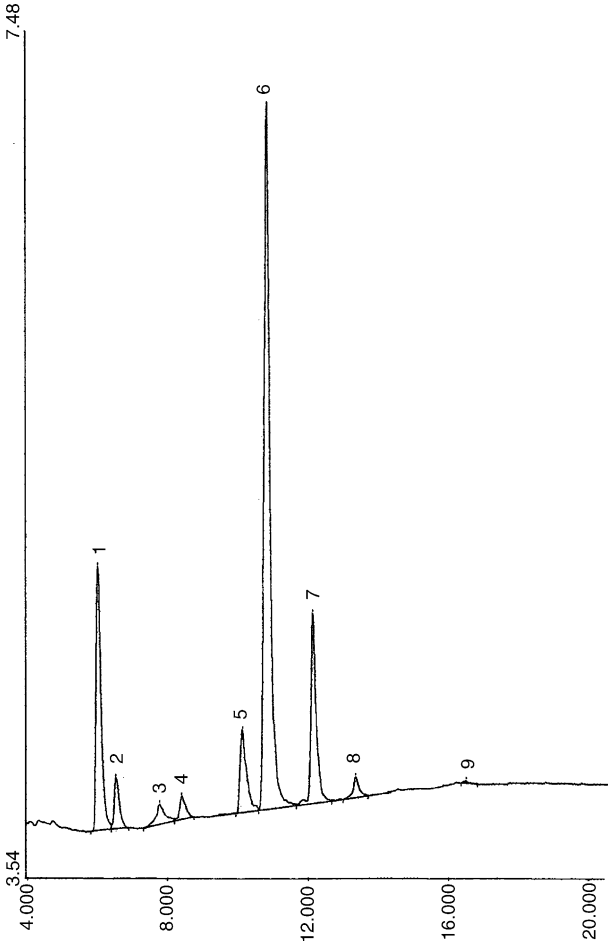

Fig. 1. Gas chromatogram of fatty acid methyl esters in one isolate of *Candida parapsilosis* (peak 1—$C_{16:0}$, peak 2—$C_{16:1}$, peak 3—$C_{17:0}$, peak 4—$C_{17:1}$, peak 5—$C_{18:0}$, peak 6—$C_{18:1}$, peak 7—$C_{18:2}$, peak 8—$C_{18:3}$, peak 9—$C_{20:1}$).

Table 2
Distribution of major fatty acids of five species of *Candida*, mean and (range)

Fatty acids	*Candida parapsilosis* 76[a]	*Candida albicans* 10[a]	*Candida tropicalis* 6[a]	*Candida famata* 8[a]	*Candida glabrata* 5[a]
$C_{16:0}$ 5.95–6.1[b]	15.70 (7.1–24.8)	17.36 (14.2–18.9)	14.73 (13.7–16.2)	16.67 (12.7–20.3)	6.93 (2.6–10.7)
$C_{16:1}$ 6.48–6.65[b]	5.13 (0.5–25.7)	13.21 (7.3–20.0)	12.20 (3.1–39.2)	5.51 (2.3–11.2)	29.54 (7.7–43.7)
$C_{18:0}$ 9.98–10.1[b]	4.86 (1.4–10.9)	6.28 (3.3–8.7)	5.52 (3.9–7.0)	4.24 (2.9–5.1)	7.18 (4.4–9.0)
$C_{18:1}$ 10.7–11.16[b]	51.32 (41.3–61.3)	42.51 (35.0–46.5)	48.12 (36.5–55.5)	49.19 (41.6–54.1)	50.43 (39.3–69.3)
$C_{18:2}$ 12.0–12.3[b]	14.62 (6.4–34.3)	12.12 (7.5–21.9)	12.21 (4.2–20.0)	13.90 (7.1–21.1)	5.72 (1.4–12.3)
$C_{18:3}$ 13.3–14.0[b]	1.31 (0.11–3.7)	1.19 (0.94–2.3)	1.24 (0.62–2.1)	3.47 (2.3–5.4)	0

[a] No. of tested isolates.
[b] Retention time.

3.2. Quantitative analysis of CFA

The mean relative percentages (MRP) of CFA that composed the individual species of *Candida* tested are listed in Table 2. In computing the mean, account was taken of the relative composition of CFA of all isolates of *Candida* species tested. The average percentage obtained from three replicates of each isolate of all species tested was calculated. The CV of triplicate assays of a single isolate never exceeded 5%.

The amount of palmitic acid ($C_{16:0}$) detected is almost constant for *C. parapsilosis*, *C. albicans*, *C. tropicalis*, and *C. famata*, with the exception of *C. glabrata*. In every species the concentration of palmitoleic acid ($C_{16:1}$) was different, the amounts being the highest in *C. glabrata*, and the lowest in *C. parapsilosis* and *C. famata*. The amount of stearic acid ($C_{18:0}$) was low and similar in all clinical and standard isolates of *Candida* species. The oleic acid ($C_{18:1}$) proved to be the predominant component in the CFA pool in the species tested so far, tending to be higher in *C. parapsilosis* and lower in *C. albicans*. The linoleic acid ($C_{18:2}$) was common in all *Candida* species tested and MRP of this CFA varied between 5.7 and 14.62%. In every species tested, the amount of linolenic acid ($C_{18:3}$) was very low. The absence of this CFA is characteristic of *C. glabrata*. Whereas in *C. parapsilosis*, *C. albicans*, *C. tropicalis* and *C. famata* the ratio of $C_{16:1}$ to $C_{16:0}$ acids was less than 1.0, in *C. glabrata* it was 4 or greater. Yet another difference between isolates of different species was the relative amounts of $C_{16:0}$ and $C_{18:0}$ acids. In *C. famata*, *C. parapsilosis*, *C. albicans* and *C. tropicalis*, the ratio of $C_{16:0}$ to $C_{18:0}$ acids was 2.7 or greater, but in *C. glabrata* it was less than 1.0.

The values of CV of identifying CFA show there to be bigger or smaller quantitative differences in the content of each of these acids among analyzed clinical isolates of the

Table 3
Coefficient of variability values for six identifying acids of clinical isolates of *Candida* species isolated from ulcers and interdigital spaces of both feet in 22 diabetic patients

Yeast species	Patient	No. isolates	Coefficient of variability					
			$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
	1	4	10.2	20.3	27.8	1.8	4.5	9.8
	2	7	14.8	28.7	10.1	7.5	31.5	44.1
	3	5	11.8	31.4	13.7	13.4	52.6	27.5
	4	6	9.9	36.0	19.7	5.0	35.2	43.6
	5	4	19.7	21.0	11.3	6.3	25.3	37.4
	6	3	17.8	4.9	7.2	9.6	5.9	24.7
Candida parapsilosis (75)	7	9	17.2	37.0	16.5	7.6	45.8	31.8
	8	11	27.4	50.3	24.8	8.2	25.0	72.4
	9	9	9.8	36.9	24.1	8.4	17.2	26.8
	10	4	6.3	24.8	28.2	4.9	6.8	9.1
	11	3	6.4	14.8	18.6	10.3	15.1	36.3
	12	3	11.6	22.7	26.0	2.3	31.4	20.1
	13	7	15.7	93.0	33.7	11.5	31.5	57.9
	14	2	4.1	1.1	2.7	5.9	4.2	141.4
Candida albicans (9)	15	3	6.5	11.2	31.0	3.4	25.3	23.9
	16	4	11.7	19.0	18.9	6.0	26.0	67.7
Candida tropicalis (5)	17	3	4.4	9.4	11.3	2.7	31.2	13.9
	18	2	4.7	5.0	27.0	1.5	13.4	9.7
Candida famata (7)	19	5	9.0	66.0	11.6	9.1	44.5	29.2
	20	2	2.3	39.9	0.9	10.7	0.5	9.2
Candida glabrata (4)	21	2	55.7	2.0	10.3	10.5	22.5	0
	22	2	33.4	34.9	10.6	7.3	110.9	0

same *Candida* species in all 22 diabetic patients (Table 3). The CV of $C_{18:3}$ was the highest in all analyzed isolates, ranging from 9.1 to 141.4%. As to $C_{18:2}$ and $C_{16:1}$, they too had high CV values. For $C_{16:0}$ and $C_{18:0}$, CV ranged between 0.9 and 55.7%. In all clinical isolates of *Candida* the lowest CV values found were in $C_{18:1,}$ ranging from 1.8 to 20.1%.

4. Discussion

Although microbiologic laboratories began using the GC in 1950, the method gained momentum as late as 1970s owing to technological improvements (introduction of capillary columns) and computer interpretation of results [6–8]. Abel et al. were the first to describe (in 1963) GC as a method permitting identification and chemotaxonomy of different micro-organism types according to their chemical composition and products of metabolism [7]. The variable properties that make an organism's CFA composition distinctive include quantitative differences in CFA content and the presence of other CFAs [7]. Yeast (eukaryotic cells) contains some CFAs that are unique, i.e., not generally found in bacteria (prokaryotes). Branched-chain and cyclopropane-containing CFAs characterize Gram-positive and Gram-negative bacteria, respectively, but are not found in fungi. Conversely, the polyunsaturated fatty acids found in higher organisms (yeast) tend to be absent in aerobic bacteria [7].

Many researchers have tested the value of GC in yeast identification by comparing the results of this method with those of standard mycological methods for yeast identification [6–15]. GC attains a high accuracy (94.7%) of identification [11] and its results become available very quickly (2 h), giving it an advantage over the classic morphological and physiological tests [10–15] for the identification of yeasts (48–72 h).

For all *Candida* species analyzed by GC in present study the results of the qualitative and quantitative analyses of CFAs are in agreement with the findings obtained by other authors using the same method [10–15]. Six CFAs proved to be sufficient for the successful identification of the following *Candida* species: *C. parapsilosis*, *C. albicans*, *C. tropicalis*, *C. famata* and *C. glabrata*. In this study, the Kruskall–Wallis test confirmed that there was statistically significant difference between five tested *Candida* species for each of CFAs ($p < 0.05$). The results of statistical analysis for $C_{16:0}$ was H (4, $N = 102$) = 20.882, $p = 0.00003$; for $C_{16:1}$ was H (4, $N = 102$) = 35.915, $p = 0.00001$; for $C_{18:0}$ was H (4, $N = 102$) = 13.927, $p = 0.0075$; for $C_{18:1}$ was H (4, $N = 102$) = 17.462, $p = 0.016$; for $C_{18:2}$ was H (4, $N = 102$) = 11.769, $p = 0.0192$ and for $C_{18:3}$ was H (4, $N = 101$) = 29.431, $p = 0.0001$.

C. parapsilosis was significantly different from the other four species of the *Candida* genus by its relative concentrations of each CFA measured by GC method. From the other species, *C. albicans* differed significantly in regard of the $C_{16:1}$ and $C_{18:0}$ amounts, differing from *C. parapsilosis*, *C. tropicalis* and *C. glabrata* in regard of its $C_{16:0}$ concentration, by its $C_{18:1}$ content only from *C. parapsilosis*, by its $C_{18:2}$ level from *C. parapsilosis* and *C. glabrata*, and by its $C_{18:3}$ content from *C. parapsilosis*, *C. famata* and *C. glabrata.* While by its $C_{16:0}$ and $C_{16:1}$ contents *C. tropicalis* differed significantly from *C. parapsilosis* and *C. albicans*, differing from *C. parapsilosis* by its $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ relative amounts, and by $C_{18:3}$ concentration from *C. parapsilosis*, *C. famata* and *C. glabrata*. By its $C_{16:1}$ and $C_{18:2}$ contents, *C. famata* was significantly different from *C. parapsilosis* and *C. glabrata*, thus differing by its $C_{16:1}$ and $C_{18:0}$ concentrations from *C. parapsilosis* and *C. albicans*, by its $C_{18:1}$ content from *C. parapsilosis*, and by its $C_{18:3}$ amount from *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. *C. glabrata* was statistically significantly different in regard of the $C_{16:0}$ and $C_{18:2}$ concentrations from *C. parapsilosis*, *C. albicans* and *C. famata*, by its $C_{16:1}$ content from *C. parapsilosis* and *C. albicans*, by its $C_{18:0}$ and $C_{18:1}$ contents from *C. parapsilosis*, and by its $C_{18:3}$ amount from any other species of the *Candida* genus analyzed by GC.

Some authors have attempted to explain the clinical importance of *Candida* isolations from the diabetic foot ulcers by assessing the healing of lesions through the application of antimycotics [19]. The explanation that we would offer is chemotyping of isolates of the same species of *Candida* from foot ulcer and interdigital spaces of the same and/or the other foot by using GC method. In the literature, we found Gangopadhyay's study [13] claiming that CV of none of the six main CFA among isolates from the same species of yeast exceeded the value of 22%. Our CV results for acid $C_{18:1}$ among isolates of the same *Candida* species from the same patient were less than 22%. However, CV values for the other five main identifying CFA are evidence of the existence of significant variations in the content of these CFAs. Namely, we believe they are evidence of the existence of different chemotypes of the same *Candida* species isolated from ulcerated and/or ulcer-free foot in the same diabetic patient.

The chemotyping of *Candida* isolates supported by mycological finding (isolation in pure culture and/or large colony count, and/or repeat isolation from the same ulcer), length of infection and the progression in the clinical finding despite surgical treatment, as well as long-term antibiotic therapy are helpful in diagnosing a fungal infection of diabetic foot ulcers. The existence of different chemotypes in isolates of the same *Candida* species from the ulcer and interdigital spaces of the same and/or the other foot of the same patient substantiate the assumption that yeast species exerts a pathogenic action in the etiology of ulcer infection. Furthermore, our results suggest that the GC analysis of clinical isolates of yeast is a complementary to histopathological findings for distinguishing an infection from colonization and contamination of diabetic foot ulcers. Further research is needed on a larger number of clinical isolates for a final assessment of the effectiveness of yeast typing and for the assessment of the application of GC in

the routine diagnosis and epidemiological monitoring of the infections due to *Candida* species.

References

[1] C.R. Kahn, G.C. Weir, Joslin's Diabetes Mellitus, Williams Wilkins, Media, 1994.
[2] D.R.R. Williams, in: A.J.M. Boulton, H. Connor, P.R. Cavanaugh (Eds.), The Foot in Diabetes, Chichester Wiley, Philadelphia, 2000, p. 3.
[3] P. Rich, J. Am. Acad. Dermatol. 5 (2000) S130.
[4] P.R. Murray, E.J. Baroby, M.A. Phaller, F.C. Tenover, R.H. Yolken, Manual of Clinical Microbiology, American Society for Microbiology, Washington, DC, 1999.
[5] F.C. Odds, Candida and Candidosis, Bailliere Tindall, London, 1988.
[6] M.V. Novotny, Science 246 (1989) 51.
[7] D.F. Welch, Clin. Microbiol. Rev. 4 (1991) 422.
[8] C.W. Moss, in: E.H. Lennette, A. Ballows, W.J. Hausler, H.J. Shadomy (Eds.), Manual of Clinical Microbiology, American Society for Microbiology, Washington, DC, 1985, p. 1029.
[9] M. Sasser, M.D. Wichman, in: A. Ballows, W.J. Hausler, K.L. Herrmann, H.D. Isenberg, H.J. Shadomy (Eds.), Manual of Clinical Microbiology, American Society for Microbiology, Washington, DC, 1991, p. 111.
[10] C.W. Moss, T. Shinoda, J.W. Samuels, J. Clin. Microbiol 6 (1982) 1073.
[11] K. Marumo, Y. Aoki, J. Clin. Microbiol. 7 (1990) 1509.
[12] M. Gunasekaran, W.T. Hughes, Mycologia 72 (1980) 505.
[13] P.K. Gangopadhyay, H. Thadepalli, I. Roy, A. Ansari, J. Infect. Dis. 6 (1979) 952.
[14] I. El Menyawi, M. Wögerbauer, H. Sigmund, H. Burgmann, W. Graniger, J. Chromatogr. B 742 (2000) 13.
[15] I. Brondz, I. Olsen, M. Sjostrom, J. Clin. Microbiol. 12 (1989) 2815.
[16] D.H. Larone, Medically important fungi, American Society for Microbiology, Washington, DC, 2002, p. 345.
[17] International Standard (1990) Animal and Vegetable Fats and Oils-Analysis by Gas Chromatography of Methyl Esters of Fatty Acids, ISO-5508.
[18] S. Siegel, N.J. Castellan, Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill Book Company, New York, 1988, p. 206.
[19] A.H. Heald, D.J. O'Halloran, K. Richards, F. Webb, S. Jenkins, S. Hollis, et al., Diabet. Med. 18 (2001) 567.