

***Candida Dubliniensis* in Dental Plaque of ECC Affected Children**

Language: English

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Introduction

The yeasts of *Candida* genus represent an important part of the normal oral microflora, including dental plaque. Their ability to produce organic acids during the fermentation of carbohydrates partakes in the cariogenic effect of dental plaque. The most frequent yeast in the oral cavity is *Candida albicans* but *Candida dubliniensis* is isolated relatively often from this location as well, especially in immunocompromised HIV+ patients. Its prevalence is highly variable and depends on the region, nationality, age, clinical status, etc.

Objectives

The study aims to evaluate the occurrence of *C. dubliniensis* in the dental plaque samples obtained from the children affected by Early Childhood Caries (ECC), and to assess reliability of the methods used for the differentiation between *C. dubliniensis* and *C. albicans*.

Material and Methods

Used strains:

The dental plaque samples (352) were obtained from children affected by Early Childhood Caries (ECC) and from caries-free children. The samples were cultivated on Sabouraud Agar (both HiMedia, Mumbai, India) at 30 oC for 24-72 hours. The identification of isolated strains was performed using typical growth on CHROM Agar (CHROM Agar, France), by conventional morphological analysis, especially micromorphology on rice agar, and by commercially available biochemical kits CANDIDA Test 21 (Pliva-Lachema) and/or ID 32C system (BioMérieux, Marcy-l'Etoile, France). As controls the strains *C. albicans* CCM 8261, CCM 8320 (Czech Collection of Microorganisms) and *C. dubliniensis* CCY29-177-1 (Culture Collection of Yeasts) were used.

Differentiation between *C. dubliniensis* and *C. albicans*:

CHROM Agar: cultivation at 30° C for 48 hours; *C. albicans* forms light-green colonies (Fig. 1), *C. dubliniensis* creates dark-green colonies (Fig. 2).

Growth at 45° C: *C. dubliniensis*, contrary to *C. albicans*, fails to grow on Sabouraud agar at 45° C.

Staib agar: The strains were inoculated on Staib agar (50g of *Guizotia abyssinica* seed (pulverized), 1g of glucose, 1g of KH₂PO₄, 1g of creatinine, and 15g of agar per litre) and incubated at 30° C for 72 h. *C. dubliniensis* strains form rough colonies with abundant hyphae and chlamydo spores on Staib agar (Fig. 6), whereas *C. albicans* isolates form smooth colonies without hyphae and chlamydo spores (Fig. 5).

Chlamydo spores formation on rice agar: *C. albicans* forms chlamydo spores separately (Fig. 3); *C. dubliniensis* forms clusters or grapes of chlamydo spores (Fig. 4).

Latex agglutination test to identify *C. dubliniensis*: BICHRO-Dubli (Fumoze Diagnostics)

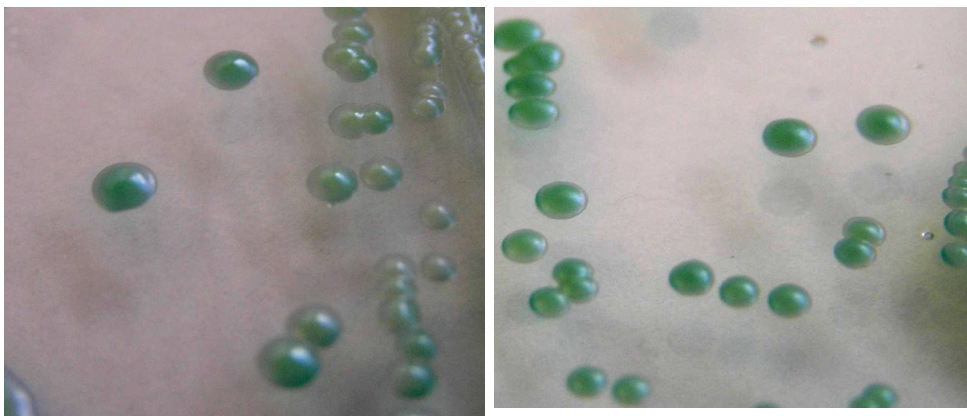


Fig. 1: Fig. 1-6 Growth on CHROMagar and Staib agar Fig. 2

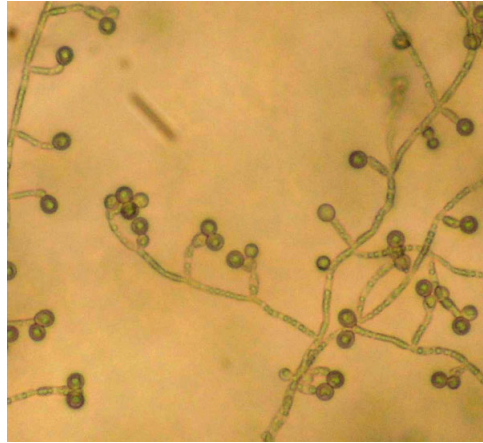
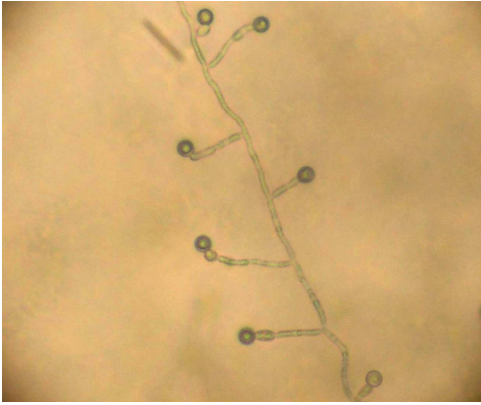


Fig. 3

Fig. 4



Fig. 5

Fig. 6

PCR-RFLP

The 50 µL PCR mixture contained HotStart Master Mix 1x (Quiagen, Hilden, Germany), 15 pmol UNF1 primer (5'ttg ata tgc tta agt tca gcg g 3'), 15 pmol UNF2 primer (5'ttg ata tgc tta agt tca gcg g 3'), 100µmol of dUTP (Sigma), 0,5 U of uracil-DNA-glycosylase (Sigma) for the elimination of cross-contamination and 5µL of isolated DNA sample. PCR reaction was performed in a thermocycler PTC-200 (BioRad).

Restriction analysis of amplified PCR products was performed by Sau3AI (New England Biolabs, Beverly, MA, USA). The product of PCR and restriction fragments were separated on 3% agarose gel with ethidium bromide, visualised by UV transilluminator (312 nm) and analysed by ULTRA LUM (Ultra-Lum, Inc., Clermont, CA, USA) gel detection system.

Capillary isoelectric focusing

Capillaries: Fused silica 0.1 mm I. D., 350 mm length, 200 mm to the detection window.

High-voltage supply: (-) 20kV.

Detection: on-column UV-Vis detector (λ = 280 nm)

Electrolyte solution: 3% (v/v) ethylalcohol, 0.3 %(w/v) PEG 10000 dissolved in 40 mM NaOH (catholyte) and 100 mM H3PO4 (anolyte).

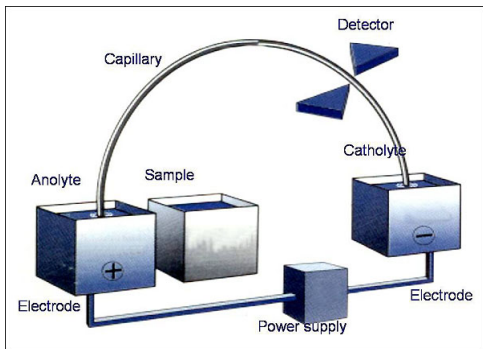


Fig. 7: Capillary isoelectric focusing

Results

We isolated 110 yeasts of *Candida* genus in 308 dental plaque samples from children affected by ECC and from caries-free children. 101 isolates grew as green colonies on chromogenic medium in CHROMagar *Candida* and formed pseudomycelium and chlamydo spores on rice agar. These strains were considered *C. albicans/dubliniensis*. Phenotypic methods (chlamydo spore arrangement, characteristic growth on CHROMagar *Candida* and Staib Agar, growth at 45° C and latex agglutination) were used for the differentiation between *C. dubliniensis* and *C. albicans*.

n = 101	CHROMagar			Growth at 45°C			Staib agar			Chlamydo spores			
	0	+/-	+	0	+/-	+	0	+/-	+	0	+/-	+	
Agglutination	0	76	7	2	65	14	6	61	24	0	74	11	0
	+	3	4	9	0	2	14	0	0	16	0	4	12

Table 1: Phenotypic methods

Identification of *C. dubliniensis* was confirmed by means of genotypic method (PCR-RFLP) (16). Simultaneously, the group of 31 *C. albicans* strains was examined using this method. Control strains *C. albicans* and *C. dubliniensis* were also included.

n = 49	PCR-RFLP	Agglutination	
		+	0
	+	17	0
	0	0	32

Table 2: Genotypic method

Capillary isoelectric focusing *C. albicans* and *C. dubliniensis*, pH gradient 2.0 - 4.7

The difference isoelectric points of *C. dubliniensis* (12) and *C. albicans* (16) strains was determined by means of Capillary Isoelectric Focusing (CIEF). All the *C. dubliniensis* strains focused near pI value 2.8, while the pI values of *C. albicans* strains were near 2.6.

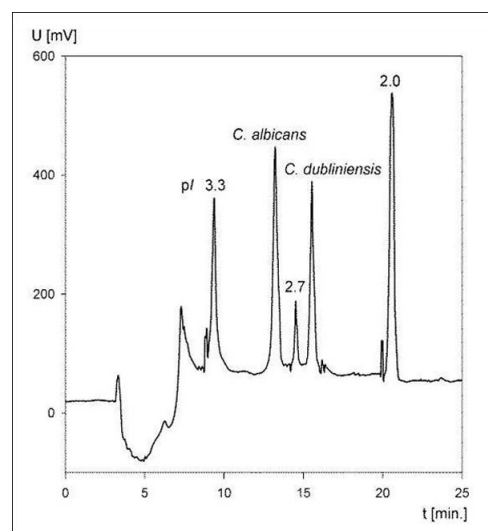


Fig. 8: Capillary isoelectric focusing

Conclusions

All the *C. dubliniensis* isolates originated from the dental plaque of ECC affected children. The reliability of simple phenotypic methods used for the differentiation between *C. dubliniensis* and *C. albicans* is limited. Therefore, these methods are suitable only for screening clinical strains. PCR-RFLP and latex agglutination are more reliable in comparison with the above mentioned method, but they are more expensive. Therefore they are suitable for the confirmation of results.

We have found that pI of *C. dubliniensis* differs from pI of *C. albicans*. Therefore CIEF is a suitable method for the differentiation between these species and isoelectric point is a useful criterion for the differentiation between these two similar species.

Acknowledgement

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This Poster was submitted by *Dr. Jarmila Kuklová*.

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Poster Faksimile:

***Candida dubliniensis* in dental plaque of ECC affected children**

Růžička F¹, Holá V. ¹, Horká M. ³, Kukletová M. ¹, Kuklová J. ², Žáčková L. ²

¹Faculty of Medicine, Masaryk University and St. Anna Faculty Hospital in Brno, ²Faculty Hospital in Brno, ³UIACH AVČR

The yeasts of *Candida* genus represent an important part of the normal oral microflora, including dental plaque. Their ability to produce organic acids during the fermentation of carbohydrates participates in the cariogenic effect of dental plaque. The most frequent yeast in the oral cavity is *Candida albicans* but *Candida dubliniensis* is isolated relatively often from this location as well, especially in immunocompromised HIV+ patients. Its incidence is highly variable and depends on the region, nationality, age, clinical status, etc. The study aims to evaluate the occurrence of *C. dubliniensis* in the dental plaque samples obtained from the children affected by Early Childhood Caries (ECC), and to assess reliability of the methods used for the differentiation between *C. dubliniensis* and *C. albicans*. **Material and methods.** In this study, 315 samples from ECC affected and caries free children (N=37) were examined. The species identification was established using: morphological characteristics, characteristic growth on CHROMagar and Stab Agar, growth at 45 °C, assimilation tests ID 32C and latex agglutination as well as genotypic method PCR-RFLP. **Results.** We identified 16 strains (4.5 %) of *C. dubliniensis* from the total amount of 110 isolated strains. All the *C. dubliniensis* isolates were from patients with ECC. The number of *C. albicans* represents 77.3 % (85) of isolates. **Conclusion.** *C. dubliniensis* is a frequent part of dental plaque in the ECC affected children. PCR-RFLP and latex agglutination are more suitable for the differentiation between *C. dubliniensis* and *C. albicans* in comparison with the other methods used.

Material methods

Used strains:

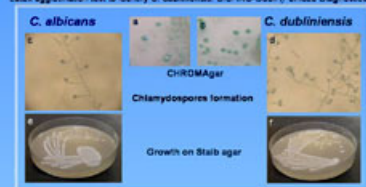
The dental plaque samples (N=302) were obtained from children affected by Early Childhood Caries (ECC, N=185) and from caries-free children (N=37). The samples were cultured on Sabouraud Agar (both Hi-Media, Mumbai, India) at 30 °C for 24 – 72 hours. The identification of isolated strains was performed using typical growth on CHROMagar (CHROMagar, France), by conventional morphological analysis, especially microscopically on rice agar, and by commercially available biochemical kits CANDIDAtest21 (Pivar, Lichern) and/or ID 32C system (bioMérieux, Marcy l'Etoile, France). As controls the strains *C. albicans* CCM 826, CCM 8320 (Czech Collection of Microorganisms) and *C. dubliniensis* CCY9-177-1 (Culture Collection of Yeasts) were used.

Differentiation between *C. dubliniensis* and *C. albicans*.

CHROMagar: cultivation at 30° C for 48 hours. *C. albicans* forms light-green colonies (Fig. a) *C. dubliniensis* creates dark-green colonies (Fig. b).
 Growth at 45° C: *C. dubliniensis* contrary to *C. albicans* fails to grow on Sabouraud agar at 45° C.
 Stab agar: The strains were inoculated on Stab agar (50g of Guizote adyessence seed (solvented), 1g of glucose, 1g of K₂HPO₄, 1g of creatinine, and 15g of agar per liter) and incubated at 30° C for 72 h. *C. dubliniensis* strains form rough colonies with abundant hyphae and chlamydozoospores on Stab agar (Fig. f), whereas *C. albicans* isolates form smooth colonies without hyphae and chlamydozoospores (Fig. g).

Chlamydozoospore formation on rice agar: *C. albicans* forms chlamydozoospores separately (Fig. d), *C. dubliniensis* chlamydozoospores mixes clusters of grapes (Fig. e).

Latex agglutination test to identify *C. dubliniensis*: BICHRO-Dubi (Fumzeo Diagnostica)



PCR-RFLP

The 50 µl PCR mixture contained HotStar Master Mix 1x (Qiagen, Hilden, Germany), 15 pmol UHF-1 primer (5'ttg ata tgc tta agt tca ggg g 3'), 15 pmol UHF-2 primer (5'ttg ata tgc tta agt tca ggg g 3'), 100µmol of dUTP (Sigma), 0.5 U of uracil-DNA-glycosylase (Sigma) for the elimination of cross-contamination and 5µl of isolated DNA sample. PCR reaction was performed in a thermal cycler PTC-200 (BioRad).
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Capillary isoelectric focusing

Capillaries: Fused silica 0.1 mm I. D., 350 mm length, 200 nm to the detection window.
 High-voltage supply (-) 20kV
 Detection: spectrometric UV-VIS detector (λ = 280 nm)
 Electrolyte solution: 3% (w/v) ethylalcohol, 0.3 % (w/v) PEG 10000 dissolved in 40 mM NaOH (analytika) and 100 mM H3PO4 (analytika)

Sample composition at segmental injection

- 1) Yeast sample: tested strain in PSS (8 x 10⁷ cells/ml) + PEG 10 000 2%
- 2) Spacer segment: L-aspartic acid + HEPES simple ampholytic electrolyte + 20 mM NaOH,
- 3) ampholytic segment: low-molecular-mass pI markers/institute of Analytical Chemistry, Czech Republic), carrier ampholyte BioLyte pH 3 – 10 (Bio-Rad laboratories, CA, USA), pH gradient 2.0 – 3.3



Results:

We isolated 110 yeasts of *Candida* genus in 315 dental plaque samples from children affected by ECC and from caries-free children (N=37). 101 isolates grew as green colonies on chromogenic medium in CHROMagar *Candida* and formed pseudomycelium and chlamydozoospores on rice agar. These strains were considered *C. albicans/dubliniensis*. Phenotypic methods (chlamydozoospore arrangement, characteristic growth on CHROMagar *Candida* and Stab Agar, growth at 45° C and latex agglutination) were used for the differentiation between *C. dubliniensis* and *C. albicans*.

Agglutination	CHROMagar		Growth at 45°C		Stab agar		Chlamydozoospores					
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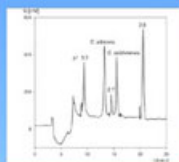
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C. dub PCR	Agglutination	
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Capillary isoelectric focusing *C. albicans* and *C. dubliniensis*, pH gradient 2.0 – 4.7

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Conclusions

All the *C. dubliniensis* isolates originated from the dental plaque of ECC affected children.

The reliability of simple phenotypic methods used for the differentiation between *C. dubliniensis* and *C. albicans* (too) is limited. Therefore, these methods are suitable only for screening clinical strains. PCR-RFLP and latex agglutination are more reliable in comparison with the above mentioned method, but they are more expensive. Therefore they are suitable for the confirmation of results.

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