Chapter

High Incidence of an Emerging Opportunistic Pathogen *Candida parapsilosis* in Water-Related Domestic Environments

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Abstract

Candidiasis is one of the common fungal opportunistic infections, usually associated with diverse *Candida* species. *Candida albicans*, *C. glabrata* complex, *C. parapsilosis* complex, *C. tropicalis* and *C. auris* are often identified in affected patients. *Candida parapsilosis* sensu stricto is an emerging cause of hospital-acquired Candida infections, predominantly in Southern Europe, South America and Asia. Home environment is a less known source of infection despite frequent isolation of *C. parapsilosis* from kitchen surfaces and household appliances such as dishwashers, washing machines and refrigerators. *C. parapsilosis* is one of the first colonisers of novel dishwashers and a member of stable fungal communities on rubber seals worldwide in concentrations up to 10^2 CFU/cm^2. It colonises also drawers for detergents in washing machines and drainage channels in refrigerators. Tap water and groundwater act as vector for entrance of *C. parapsilosis* in the indoor environments. Within *C. parapsilosis*, four clinically relevant phenotypes can be distinguished. Experimental data on the prevalence of *C. parapsilosis* isolates phenotypes, obtained from indoor environments, will be presented. Smooth phenotype prevails in dishwashers and washing machines, while crepe and crater dominate in water. In conclusion, the ability to colonise diverse environments and accordingly switch phenotypes defines *C. parapsilosis* as a versatile, domestic environment-related opportunistic pathogen.

Keywords: emerging opportunistic pathogen, water, household appliances, phenotype occurrence in domestic environments

1. Introduction

Yeast *Candida parapsilosis* sensu stricto (Ascomycota, Saccharomycetes, Saccharomycetales, Debaryomycetaceae) is the most commonly isolated species from *C. parapsilosis* complex, followed by its closest relative *C. orthopsilosis* and *C. metapsilosis* [1]. Its primary natural habitat remains undefined to date although it was recently reported from different fresh water sources [2–4] as well as from pine trees [5]. On the other hand, the presence of *C. parapsilosis* in relation to humans is well documented [1, 6]. The species is one of the asymptomatic colonisers of

gastrointestinal and reproductive tract of most healthy humans [6]. In addition, it is commonly found on the skin and nails [1, 6]. Thus, the carriage and transfer of *C. parapsilosis* via hands of healthcare workers to patients have been for long recognised as a cause of opportunistic infections in hospitals [7]. The significance and prevalence of the yeast in clinical settings and samples dramatically increased during the past two decades, which ranks it among emerging opportunistic human pathogens [8]. *C. parapsilosis* is globally one of the most frequent non-albicans *Candida* (NAC) species causing a broad spectrum of infections from superficial to invasive candidiasis, including vulvovaginal infections, nosocomial bloodstream infections, pericarditis, endocarditis, endophthalmitis and sepsis [1, 9–12]. Individuals at the highest risk for severe infection include neonates and patients in intensive care units [8]. Infections with *C. parapsilosis* are often related to contaminated catheters, due to its remarkable ability to produce biofilms on plastic and silicone surfaces of catheter instruments [6, 8, 13]. Ability for successful biofilm formation was linked with observed phenotypic differences of *C. parapsilosis* strains [14]. Among four described phenotypes (smooth, crepe, crater and concentric), the yeastlike smooth phenotype reportedly formed less biofilm in comparison to the entirely filamentous concentric phenotype [14].

In our study we focused on little known phenotypic diversity of *C. parapsilosis* strains, isolated from clinical material in comparison to those isolated from human-made indoor environments, particularly related to tap water and household appliances, such as washing machines, dishwashers and refrigerators. In addition, we discuss the ability for biofilm formation among tested strains and possible sources of infection originating from the household environment.

2. Daily home-related activities pose an overlooked infection risk

The risk for infection caused by *C. parapsilosis* is reportedly the highest in hospitals and healthcare facilities, as *C. parapsilosis* is commonly transferred via hands of healthcare workers [1, 7]. However, recent discoveries reveal domestic environments as sites where people are exposed to this emerging pathogen on a daily basis. Exposure points include water and hygiene-related activities, cooking area and household appliances, like dishwashers, washing machines and refrigerators. *C. parapsilosis* was isolated in high frequencies from these areas, pointing towards its preference for indoor environment [4, 15–18].

2.1 Water as a vector for transmission of *Candida parapsilosis* into household environment

In a modern society, microbiologically safe and potable water is not only one of the essential human rights but also remains one of the biggest concerns for the future [19]. Despite well-established water cleaning procedures, both, filamentous fungi and yeasts, are widely present in water intended for human consumption [19]. Except Swedish legislation, fungal parameters are not included in the present directives, and the lack of monitoring leaves out opportunistic and emerging fungal pathogens [19]. During the last 10 years, different water sources were identified as vectors for *C. parapsilosis*. Raw natural water, contaminated with *C. parapsilosis*, included streams [2], rivers [3], and groundwater [4]. Its presence positively correlated with the occurrence of dry season [3], the presence of middle-hard water type and nitrates [4, 18, 20]. Due to its ability to withstand filtration and chlorination process [21], *C. parapsilosis* is one of the building blocks in biofilms within municipal water systems, with the number of yeast cells in a range of 3.1–4.6 CFU/cm^2 [22].

Consequently, *C. parapsilosis* is regularly present in tap water at consumers' points, where it was isolated from 11 to 50% of samples [4, 17, 21, 23, 24]. Taps need thus to be taken into consideration as one of the important exposure points in households, where people may become infected with *C. parapsilosis* via drinking, food preparation and personal hygiene, like showering and bathing [19, 25].

2.2 Kitchens without dishwashers more likely host *Candida parapsilosis*

In every household, preparation and consumption of food cause dirty dishes, which can be cleaned manually or in a dishwasher. During the cleaning of kitchen utensils, the prewashing and washing steps are usually carried out using sponges in order to remove food residues. In due course, some food residues could adhere to the sponges and, together with retained humidity, tender a positive environment for growth and survival of pathogenic bacteria [26] and yeasts [27], including *C. parapsilosis*. From a microbiological point of view, kitchen surfaces are one of the most contaminated environments of our homes [17, 28–30]. Kitchen surfaces are not aseptic, but with proper cleaning, microorganisms may be reduced to the level that is generally recognised as safe. The most probable entryways of *C. parapsilosis* into domestic kitchen are water [4, 17] and human skin [15]. Adams et al. [15] reported that the highest incidence of C. parapsilosis is on the skin of the inhabitants (40%) and kitchen drains (25%) but the same yeast has a very low settle index on windowsills in kitchens (up to 2%). Zupančič et al. [17] reported the presence of *C. parapsilosis* on kitchen surfaces in high frequencies (up to 77% of tested kitchen surfaces were populated with *C. parapsilosis*). However, fungal diversity and occurrence varied considerably between kitchens containing dishwasher and kitchens without. The most significant difference was the presence of *C. parapsilosis*, which strongly dominated kitchens using handwashing only. The most contaminated sites in these kitchens were drain (43%), followed by dish drying rack and sink in the same occurrence (36%). Settlement index of *C. parapsilosis* on rubber seal in kitchen drain and kitchen counter did not exceed 25% [17].

2.3 *Candida parapsilosis* is the first coloniser of new dishwashers

In modern societies, dishwashers are a permanent utility in kitchens facilitating residents' daily tasks. Washing in a dishwasher is usually carried out at high temperatures of 55–65°C, followed by a shorter hot water rinse cycle (~85°C) and the use of alkaline detergents. The mechanical power of water jets cleans the vessels [31]. The dishwashers do not disinfect the dishes, but reduce the number of microorganisms to a level that is considered safe [32]. The number of bacteria on the vessels is partly reduced due to high pH and temperature [33]. Recent studies have shown that under these unfavourable conditions, such as high temperature, wet and dry periods, high and low pH, presence of high concentrations of salt (NaCl) and water shearing forces, a certain group of microorganisms—polyextremotolerant ones—are enriched [34]. These unfavourable circumstances can defy also the opportunistic pathogenic species like *C. parapsilosis* [17], which seems to be one of the first colonisers of new dishwashers [20], providing a biotic surface for the construction of mixed bacterial-fungal biofilms [35]. *C. parapsilosis* forms together with *Exophiala dermatitidis*, *Exophiala phaeomuriformis*, *Rhodotorula mucilaginosa*, *Aureobasidium melanogenum*, *Bisifusarium dimerum* (formerly *Fusarium dimerum*), *Fusarium oxysporum* and *Saprochaete clavata*, a stable microbiota of dishwasher rubber seals worldwide [17, 34, 36, 37]. It is globally present on rubber seals of dishwashers [34, 36] with settlement up to 10^2 CFU/cm^2 [17]. It can be found in high frequencies also on dishwasher doors and walls. Drains, cutlery racks and side nozzles are less exposed [17]. Higher dishwasher frequency of use (7–14 times per week) and connection to tap water system with moderately

hard tap water hardness (1.5–2 mmol/l $CaCO_3$) significantly affect the incidence of *C. parapsilosis* [20]. *C. parapsilosis* can be released from dishwashers via waste water, cleaned vessels and hot aerosols, formed at the end of the washing cycle [17].

2.4 The use of softeners increases the likelihood of *Candida parapsilosis* settlement inside washing machines

Knowledge on washing machines' microbiomes is relevant particularly in hospitals and other healthcare facilities due to the possible transfer of pathogenic microorganisms between clothes being washed at the same time [38, 39]. Washing cycles at elevated temperatures may prevent cross-contamination lowering the number of microorganisms, but recent energy-saving trends promote washing with biodegradable detergents and usage of eco-programmes with temperatures of washing not exceeding 40°C [16]. These features favour microbial growth and propagation, resulting in persistent odour of textiles and elevated risk for infections [39, 40]. The main worries remain the bacteria of the genera *Pseudomonas* and *Staphylococcus*, together with dermatophyte fungi [38]. However, recent studies conducted globally reported *C. parapsilosis* as one of the most common fungi in washing machines, colonising 8–25% of sampled machines [16, 18, 41]. It was isolated mainly from biofilms at water-entry points, drawers for detergent and softener and rubber seals [16, 18, 41]. Its presence in washing machines positively correlated with the regular use of commercial softeners and washing temperatures ≤40°C [16]. Forty-eight percent of tested *C. parapsilosis* strains from washing machines showed a remarkable ability of biofilm formation, while none of the tested strains grew on 0.1% cycloheximide [18].

2.5 *Candida parapsilosis* colonises refrigerators' rubber and moist parts

Primarily basidiomycetous yeasts but to a lesser extent also ascomycetous yeasts have been reported from extremely cold natural environments, including *C. parapsilosis* [42]. Extremely cold environments are also present indoors, in the form of refrigerators and freezers. Until date, there are no reports of yeasts, isolated from freezers, and few are reporting their isolation from refrigerators. Yeasts have been isolated from plastic refrigerator vegetable compartments, rubber seals, walls and water dispensers [43, 44]. *Candida* species have been isolated most frequently, with *Pichia kudriavzevii* prevailing in refrigerator air [45]. Our preliminary results showed the presence of *C. parapsilosis* on the shelves and in drainage channel of domestic refrigerators.

3. Phenotypic diversity of *Candida parapsilosis* in domestic environments

Phenotypic diversity of *C. parapsilosis* was first described by Enger et al. [46] who identified five different phenotypes originating from one isolate (crepe, concentric, snowball, rough and smooth) [46]. They were later reidentified into four groups, crepe, concentric, smooth and crater, with a described ability to switch from one phenotype into another [14]. Phenotypic differences of the strains were linked with micromorphological features, growth rate and the ability to form biofilm [14]. The yeast cells of smooth phenotype grow most rapidly but form less biofilm in comparison to the crepe or crater phenotype. On the other hand, concentric phenotype produces entirely filamentous cells and forms biofilm most successfully (**Table 1**) [14].

Phenotype properties	Phenotypes			
	Crepe	**Crater**	**Concentric**	**Smooth**
Micromorphology	Pseudohyphae	Elongated, yeastlike	Wide, pseudohyphae	Small, yeastlike
Chitin distribution	Cell wall	Cell wall, bud neck	Cell wall, bud neck	Bud scar
Growth rate	Medium	Medium	Low	High
Biofilm formation ability	Medium	Medium	High	Low

Table 1.
The main differences between four phenotypic groups of ***C. parapsilosis*** *according to Laffey and Butler (2005) [14].*

3.1 Smooth phenotype of *Candida parapsilosis* prevails in domestic environment

One-hundred and eighty-four strains of *C. parapsilosis* sensu lato, deposited in Ex Culture Collection of the Infrastructural Centre Mycosmo, MRIC UL, Slovenia: http://www.ex-genebank.com/, at the Department of Biology, Biotechnical Faculty, University of Ljubljana, were included in the present study. Tested strains originated from clinical material (N = 7), groundwater (N = 2) and domestic environment, like tap water (N = 23), bathrooms (N = 14), washing machines (N = 16), kitchens (N = 22), dishwashers (N = 96) and refrigerators (N = 4). All strains were plated onto malt extract agar and incubated at 30°C for 4 weeks. Phenotypic diversity of the strains (**Figure 1**) was evaluated weekly (**Table 2**).

Identification of yeasts from the *C. parapsilosis* complex can often be false or incorrect, since the species *C. parapsilosis*, *C. metapsilosis* and *C. orthopsilosis* are genetically very similar. Commercially available reagents currently do not allow accurate distinction within the *C. parapsilosis* complex [47]. One of the methods

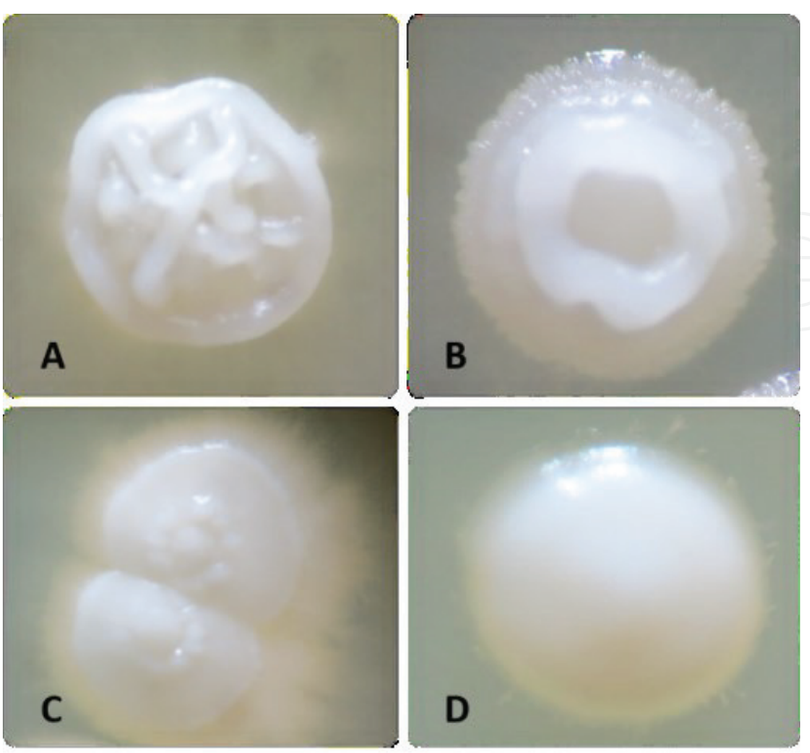

Figure 1.
C. parapsilosis phenotypes in domestic environment. (A) Crepe phenotype, (B) concentric phenotype, (C) crater phenotype and (D) smooth phenotype.

		Environment							
		Clinical material	**Groundwater**	**Tap water**	**Bathroom**	**Washing machine**	**Kitchen**	**Dishwasher**	**Refrigerator**
Phenotypes	Crepe	EXF-10095; EXF-10098; EXF-10192; EXF-10193;	EXF-8460; EXF-8247	EXF-8404; EXF-8405; EXF-8452; EXF-9873; EXF-10048	EXF-12615; EXF-12765	EXF-6337; EXF-8239	EXF-9920 EXF-9924 EXF-9925 EXF-9954 EXF-9574	EXF-8850; EXF-8854; EXF-8862; EXF-8907; EXF-8908; EXF-8909; EXF-8931; EXF-9096; EXF-9103; EXF-9105; EXF-9108; EXF-9112; EXF-9113; EXF-9190; EXF-9193; EXF-9207; EXF-9220; EXF-9251; EXF-9259; EXF-9311; EXF-9354; EXF-9355; EXF-9384; EXF-9386; EXF-9490; EXF-9491	EXF-9596
	Crater	EXF-10096		EXF-9872; EXF-10133; EXF-10144; EXF-10179; EXF-10240; EXF-9623; EXF-9693	EXF-12671; EXF-9692		EXF-9915 EXF-9960 EXF-12806	EXF-8892; EXF-8901; EXF-8905; EXF-8914; EXF-9081; EXF-9092; EXF-9106; EXF-9110; EXF-9330; EXF-9334	
	Concentric	EXF-10099		EXF-5670; EXF-8411; EXF-8248	EXF-6998	EXF-6334	EXF-9941; EXF-9952; EXF-9953; EXF-10087; EXF-8104	EXF-6078; EXF-8903; EXF-8937; EXF-8938; EXF-9045; EXF-9048; EXF-9099; EXF-9260; EXF-9280; EXF-9281; EXF-9283; EXF-9326; EXF-9342; EXF-9361; EXF-9389; EXF-9475; EXF-9496	

		Environment							
		Clinical material	**Groundwater**	**Tap water**	**Bathroom**	**Washing machine**	**Kitchen**	**Dishwasher**	**Refrigerator**
	Smooth	EXF-10097		EXF-8406; EXF-9899; EXF-10058; EXF-10067; EXF-10174; EXF-9691; EXF-9694; EXF-9697	EXF-6342; EXF-6356; EXF-7001; EXF-12776; EXF-9696; EXF-8101; EXF-8146; EXF-8149	EXF-5667; EXF-5730; EXF-5731; EXF-8288; EXF-8289; EXF-8290; EXF-8296; EXF-9781; EXF-9782; EXF-6335; EXF-6336; EXF-6338; EXF-8399	EXF-9907; EXF-9916; EXF-9928; EXF-9944; EXF-9955; EXF-9956 EXF-8111; EXF-9556 EXF-9557	EXF-8251; EXF-5540; EXF-5545; EXF-5547; EXF-5659; EXF-5717; EXF-5722; EXF-5723; EXF-5726; EXF-5728; EXF-6088; EXF-6102; EXF-6112; EXF-6120; EXF-6126; EXF-8849; EXF-8866; EXF-8867; EXF-8894; EXF-8919; EXF-9052; EXF-9054; EXF-9088; EXF-9095; EXF-9102; EXF-9200; EXF-9203; EXF-9206; EXF-9224; EXF-9275; EXF-9278; EXF-9340; EXF-9364; EXF-9370; EXF-9371; EXF-9395; EXF-9504; EXF-9509; EXF-9514; EXF-9535; EXF-9537; EXF-9769; EXF-9211	EXF-11755; EXF-12203; EXF-12266

Table 2.
Phenotypic diversity of ***C. parapsilosis*** *sensu stricto strains. EXF refers to culture collection strain designation.*

used for genetic differentiation between the complex species is also the analysis of the restriction polymorphism of the secondary alcohol dehydrogenase (*SADH*) gene [48]. After DNA extraction, identification based on the whole internal transcribed spacer (ITS) region and partial 28S rDNA, D1/D2 domains, was performed. All tested strains were checked for accurate identification of *C. parapsilosis* species complex by RFLP analyses of the *SADH* gene fragment. *SADH* amplicons obtained with the primer set S1F and S1R [49] were digested with the restriction enzyme *Ban*I. All tested strains belonged to *C. parapsilosis* sensu stricto group.

Obtained results showed differences between abundance of phenotypes in clinical strains in comparison to the environmental strains (**Figure 2**). The prevalent phenotype among clinical strains was crepe (57.1%), while the others were evenly distributed (14.3%). The results are similar to already reported by Laffey and Butler [14]. Among environmental strains, the crepe phenotype was the only one observed in strains isolated from groundwater (2/2). It was represented in a lesser extent in household appliances, with the highest incidence on kitchen surfaces (22.7%) and in dishwashers (27.1%), and the lowest in washing machines (12.5%).

C. parapsilosis strains isolated from groundwater-derived tap water mostly formed smooth (34.8%) or crater (30.4%) phenotypes, followed by crepe (21.7%) and concentric (13.0%) phenotype. Tap water serves as a vector for fungi entering water-related niches in households [4], where environmental pressure leads to the selection of the most tolerant strains [17], even on the phenotypic level. Room interior and household appliances that are usually present in these rooms (bathroom and washing machine, kitchen and dishwasher) show similar phenotype distribution (**Figure 3**). In addition, co-occurrence of different phenotypes from

Figure 2.
Prevalence of C. parapsilosis phenotype in indoor environments and among clinical isolates. Prevailing indoor phenotype of C. parapsilosis is the smooth one; crepe phenotype is a predominant phenotype in clinical isolates.

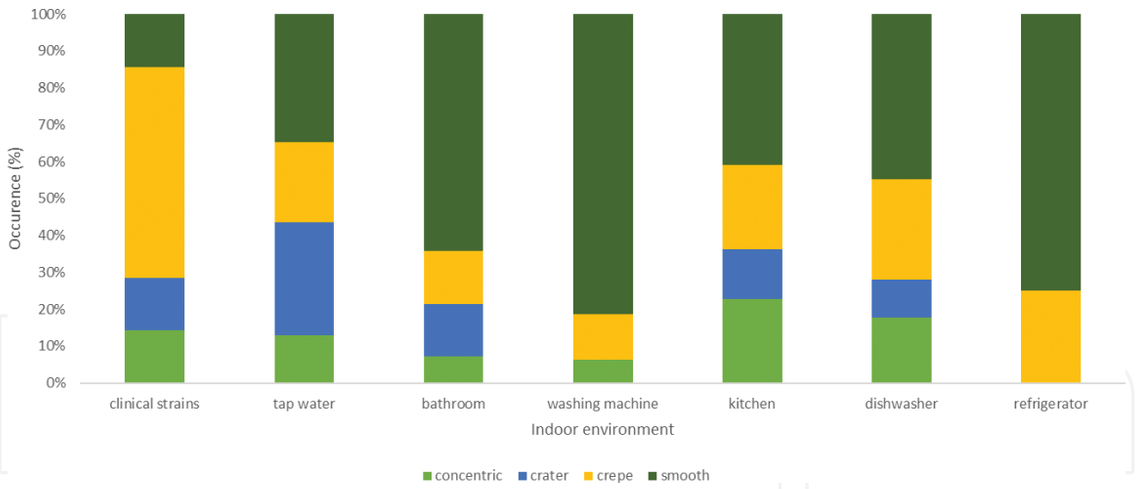

Figure 3.
Distribution of C. parapsilosis phenotypes in indoor environments. In clinical strains, crepe phenotype was prevailing, while in household appliances, such as washing machines, dishwashers and refrigerators, the predominant phenotype was smooth. Crepe phenotype was present to a lesser extent.

the same sampling spot was observed. Smooth phenotype was positively selected in all appliances, washing machine, refrigerator and dishwasher, with 81.3, 75.0 and 44.8%, respectively. Slightly positive selection was observed also for concentric phenotype in kitchens (22.7%) and inside dishwashers (17.7%) in comparison to bathrooms (7.1%) and washing machines (6.3%). On the other hand, negative selection was observed for crater phenotype, which was among all tested habitats most commonly found in tap water (30.4%), but its presence was low on kitchen (13.6%) and bathroom (14.3%) surfaces, with total absence in washing machines and refrigerators.

Survival of microorganisms invading household niches is higher due to biofilm formation [17]. Next-generation sequencing of dishwasher biofilm community and further usage of several statistical models showed that *Candida* (*C. parapsilosis*) is one of the first colonisers of rubber seals in dishwashers [20].

4. Conclusions

C. parapsilosis is a commonly known opportunistic pathogen, particularly in a connection with hospital care, as a natural coloniser of health workers' hands and skin. Superficial or invasive infections usually occur via catheters, due to yeast's biofilm formation ability. Recent studies revealed human-made indoor environments as a previously unrecognised hot spot of their occurrence. This completely new aspect enables many possible routes for infection with this emerging opportunistic pathogen. *C. parapsilosis* is commonly present in tap water, bathrooms, washing machines, kitchens surfaces, dishwashers and refrigerators. While tap water carried all four phenotypes of the species, with a slight preference for the crater phenotype, selection inside household appliances clearly promoted the smooth phenotype. In accordance, the smooth phenotype showed the most abundant biofilm formation on polystyrene. On the other hand, tested clinical strains mainly formed the crepe phenotype, which was isolated also from all sampled indoor niches, with the highest incidence in kitchens, dishwashers and refrigerators. In the future, household environments where people maintain and prepare food and personal hygiene should be taken into consideration as possible routes for infection with *C. parapsilosis*.

4.1 Objectives

There are four different phenotypes of *C. parapsilosis* strains, smooth, crepe, crater and concentric. As *C. parapsilosis* is commonly present in domestic environment, we were interested in occurrence and prevalence of these phenotypes in different indoor environments.

4.2 Experimental methods used

All tested strains, stored in deep frozen stock (−80°C), were inoculated with a loop on malt extract agar plates (MEA) and incubated for 4 weeks at 30°C. Phenotype check-up was made after 1, 2, 3 and 4 weeks of incubation. Results of *C. parapsilosis* phenotype occurrence after 4 weeks are presented in **Table 2**.

4.2.1 Extraction and molecular characterisation of DNA

Pure fungal cultures were revived from deep frozen stock of EX culture collection by inoculation on a fresh malt extract agar medium. After 3 days of incubation at 30°C, the DNA was extracted using PrepMan Ultra reagent (Applied Biosystems), according to the manufacturer instructions.

Identification was based on amplification and sequencing of the large subunit ribosomal DNA sequences (LSU; partial 28S rDNA, D1/D2 domains), using the NL1 and NL4 primer set [50]. A fragment of the rDNA including internal transcribed spacer (ITS) region 1, 5.8S rDNA and ITS2 was also amplified and sequenced for identification, using the ITS5 and ITS4 primer set [51]. The ITS and LSU nucleotide sequences were determined by direct PCR sequencing, performed by Microsynth AG, Switzerland. BigDye terminator cycle sequencing kits were used in the sequence reactions (Applied Biosystems, Foster City, CA, USA). The sequences were obtained using an ABI Prism 3700 Big Dye Sequencer (Applied Biosystems). The sequences were assembled using FinchTV 1.4 (Geospiza, PerkinElmer, Inc.) and automatically and manually aligned using the Molecular Evolutionary Genetics Analysis (MEGA) software, version 6.06 [52]. The assembled DNA sequences were examined using the BLAST software of the National Center for Biotechnology Information (NCBI) database and were compared to the appropriate sequences of the reference and type strains. All strains, included into this research, were sequenced as *C. parapsilosis* sensu lato.

4.2.2 Determination of Candida parapsilosis species complex

Amplification of *SADH* gene was performed using S1F and S1R primer set according to [49]. After the final amplification, PCR products were treated with restriction enzyme *Ban*I (*Bsh*NI) (Thermo Fisher Scientific™, USA) according to the manufacturer instructions. After restriction the obtained fragments were checked on 1% agarose gel (Sigma-Aldrich) for 20 minutes at 120 V. The expected fragment length for *Candida metapsilosis* was 400 bp, for *Candida orthopsilosis* was 700 bp and for *Candida parapsilosis* was 550 bp [49]. After restriction profile, all tested strains were determined as *Candida parapsilosis* sensu stricto.

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Conflict of interest

Authors declare no conflict of interest.

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