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Adhesion of *Candida* spp. and *Pichia* spp. to Wooden Surfaces

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Summary

Yeast adhesion to surfaces and the consequent biofilm formation is present in many different environments. In food industry, biofilms may be a source of contaminations, causing food spoilage and reducing quality of products. *Candida* and *Pichia* are the genera mainly involved in spoilage of products in food industry. The aim of this study was to assess the potential of *Candida* and *Picha* species to adhere to the two types of wooden surfaces (smooth and rough), material typical for the food processing industry, and investigate the influence of surface roughness of wood on the degree of yeasts adhesion. The concentration of the cells adhered to the wooden surfaces were determined using an innovative method for rapid viable-cell counting. The results showed that all *Candida* and *Pichia* strains were able to adhere to the wooden surfaces in a species- and strain-dependent manner. On the other hand, our data indicated that adhesion by these yeast were not significantly affected by the roughness of the wood surfaces.

Key words: adhesion, yeast, *Candida* spp., *Pichia* spp., wood surfaces

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Introduction

The term biofilm was created to describe the sessile form of microbial life, characterized by adhesion of microorganisms to biotic or abiotic surfaces, with consequent production of extracellular polymeric substances (*1*). Microbial adhesion and biofilms are of great importance for the food industry and occur on a high variety of food contact surfaces such as stainless steel, plastics, glass, wood and rubber surfaces (*2*). In the food industry, microbial adhesion to equipment surfaces and subsequent development of biofilm are very serious issues because of potential to cause cross-contamination, which leads to lowered shelf-life, food spoilage, and transmission of disease (*3,4*). Use of wood as a food contact surface has reduced in the developed countries because it is a porous and absorbent material where organic matter along with microorganisms can become entrapped, cross-contamination is a main concern (*5*) but wood is still in use in the developing countries for food processing because it is readily available and cheap. The increased resistance of sessile organisms towards disinfectants and sanitizing agents often exacerbates the problems caused by microbial fouling and can contribute to the inefficacy of cleaning in place systems (*6*).

Bacterial adherence to various surfaces is regulary studied, however researchers have paid much less attention to yeast as adhesion agents, particulary *Candida* and *Pichia* species which are usually contaminants isolated from biofilms on conveyor tracks and can and bottle warmers in packaging departments of the beverage industry (*7-9*). Even though *Candida albicans* is the most notable opportunistic fungal pathogen of medical importance (*10*), non-*albicans Candida* species, such as *Candida krusei*, *Candida glabrata* and *Candida parapsilosis* are often contaminants in the food industries (*11,12*). However, *Candida albicans* is commonly found on human skin and could be transferred to food or food contact surfaces by food handlers and are thus frequently found in food processing environments, its implications on sanitation issues in the food industry could not be disregarded (*12,13*).

Microbial adhesion to an abiotic surface is probably governed by complex interactions between the microorganism and the substrate surface involving physical, chemical, and biochemical factors. However, the effect of factors on microbial adhesion have not yet been fully clarified or sometimes have been reported with inconsistency. For example, opposing results have been reported for the effect of roughness of stainless steel surface on microbial adhesion: positive correlation and independence between microbial adhesion and surface roughness (*14,15*). Several studies have been conducted on microbial adhesion onto different types of food contact surface (*16,17*), but to our knowledge no information about the adhesion of *Candida* and *Pichia* species to wooden surfaces.

The aim of this study was to assess the potential of *Candida* and *Picha* species to adhere to the two types of wooden surfaces (smooth and rough), material typical for the food processing industry, and investigate the influence of surface roughness of wood on the degree of yeasts adhesion.

Materials and methods

Wooden surfaces

Two types of wooden (beech) discs (15.0 mm in length, 7.0 mm in width and 1.0 mm in thickness), smooth and rough, were obtained from Department of Wood Science and Technology, Biotechnical Faculty, University of Ljubljana. All wooden discs are autoclaved at 121 °C for 15 min before use.

Strains and growth conditions

A total of eight *Candida* strains and three *Pichia* strains were used in the adhesion assays on smooth and rough surfaces of wood (beech). *Candida* and *Pichia* strains were obtained from the Collection of Industrial Microorganisms (ZIM) at the Biotechnical Faculty, Slovenia. The strains had been preserved in glycerol at −80 °C and were revitalized from frozen stocks by cultivation on Malt Extract agar for microbiology (MEA) (Merck KGaA, Darmstadt, Germany) for 24 h at 37 °C (*Candida* strains) and 27 °C (*Pichia* strains).

Subsequently, a loopful was inoculated into 4 mL of the Malt Extract broth for microbiology (MEB) (Merck KgaA, Darmstadt, Germany) individually for each microorganism and incubated for 18 h at 37 °C (*Candida* strains) and 27 °C (*Pichia* strains). After 18 h of incubation, 1 mL of culture diluted into 9 mL of fresh MEB to achieve the final cell concentration of 1×10^7 Colony Forming Units (CFU) per mL, and the cell concentration was determined by plate counting. These cell suspensions were used immediately for adhesion assay.

Table 1. Yeast strains used in the study and their origin

Species (strain)	**Origin**
Candida albicans (ATCC 10261)	Man, nail, of case of paronychia
Candida glabrata (ZIM 2367)	Traheal aspiration
Candida glabrata (ZIM 2369)	Bronchoalveolar wash (BAL)
Candida glabrata (ZIM 2382)	Urine from indwelling catheter
Candida parapsilosis (ATCC 22019)	Man, case of sprue
Candida parapsilosis (ZIM 2014)	Sputum
Candida parapsilosis (ZIM 2234)	Fruit juice concentrate
Candida krusei (ATCC 6258)	Man, sputum of bronchitic convict
Pichia pijperi (ZIM 1368)	Must of Refošk
Pichia membranifaciens (ZIM 2302)	Spoiled wine
Pichia membranifaciens (ZIM 2417)	White cheese of caws milk

Adhesion assay to smooth and rough surfaces of wood

Adhesion assays were performed on the two types of wooden discs, smooth and rough surfaces. Three discs (15.0 mm in length, 7.0 mm in width and 1.0 mm in thickness) of each type of wood were placed on the bottom of Petri plates. For each strain, 2 mL of cell suspension (1×10^7 CFU/mL) prepared as above was pipetted into each plate, covering the discs. The plates were incubated for 24 h at 37 °C (*Candida* strains) and 27 °C (*Pichia* strains). In blank control plates, the wooden discs were inoculated in an identical fashion with 2 mL of yeast-free MEB. After incubation period, non-adherent cells were removed by washing three times with the Phosphate Buffered Saline (PBS) (Oxoid, Hampshire, England), and the discs were than transferred to the Falcon tubes with 2 mL of PBS. An amount of yeast cells adhered to smooth and rough surfaces of wood was centrifuged at 1500 × *g* for 3 min. The concentrations of the cells were determined using methylene blue staining, a microscope with camera (Leica DFC290) and an image processing software ImageJ as described before (*18*).

Statistical analysis

All quantitative data are presented as means with error represented by standard deviation (SD) from two independent experiments. The resulting data were analyzed using Anova: Single Factor and Two-Factor with replication in Microsoft Office Excel. A p-value of <0.05 was considered as statistically significant.

Results and discussion

Evaluation of adhesion by *Candida* spp. in present study revealed that these yeasts possess an ability for adherence on wooden surfaces, although to different extents depending on the species and strains. Fig. 1 shows number of cells (mean ± SD) adhered to the smooth and rough surfaces of wood by *Candida* strains. Statistical analysis showed that the reference strain *C. albicans* ATCC 10261 and *C. glabrata* (ZIM 2367, ZIM 2369 and ZIM 2382) strains exhibited a much greater propensity for adherence on both types of wooden surfaces in comparison with *C. parapsilosis* (ATCC 22019, ZIM 2014 and ZIM 2234) strains and the reference strain *C. krusei* ATCC 6258 ($p < 0.05$). Strain variation was particularly evident for *C. parapsilosis*, with *C. parapsilosis* ZIM 2014 and *C. parapsilosis* ZIM 2234 displaying the highest number of cells adhered to wood. This was significantly higher than for the reference strain *C. parapsilosis* ATCC 22019 ($p < 0.05$). On the other hand, *C. glabrata* strains adhered at equivalent levels to wooden surfaces ($p > 0.05$). This intra-species variation by *C. parapsilosis* in its adherence to different biomaterials has been reported previously (*19,20*). Such findings undoubtedly reflect inherent physiological differences between species and strains, and could have significance with respect to pathogenic potential. Nevertheless, it should be emphasized that only a few isolates were used and other isolates belonging to the same species may be able to more strongly adhere on the surfaces.

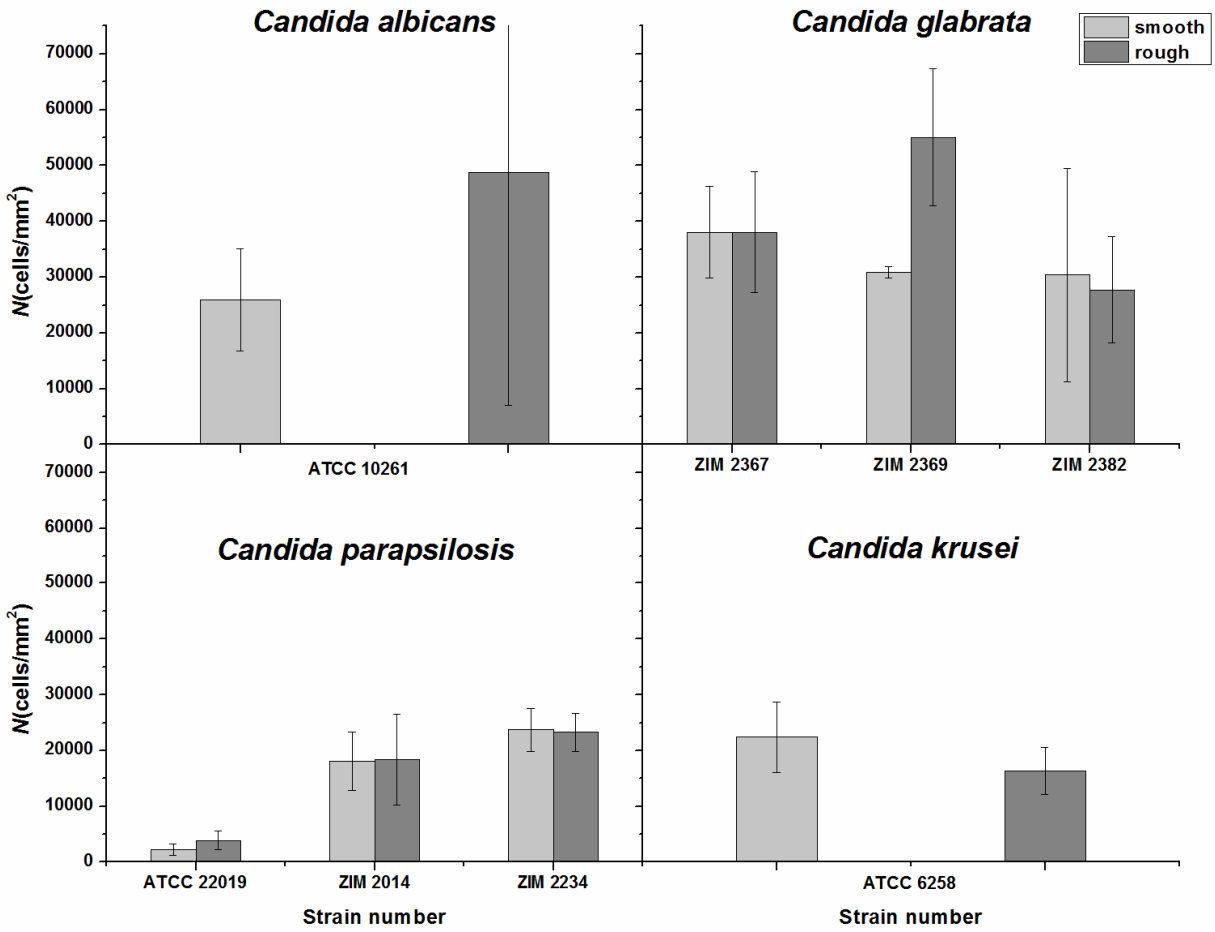

Fig. 1. Adhesion of *Candida* spp. to the two types of wooden surfaces (smooth and rough). Each bar represents the mean ± standard deviation (SD).

Additionally, under the conditions of our study all assayed *Pichia* strains have been able to adhere to wooden surfaces as shown in the Fig. 2. The results present the numbers of cells (mean ± SD) adhered to smooth and rough surfaces of wood by *Pichia* strains. Statistical analysis indicated that there was significant difference among strains in their ability to adhere on both types of wooden surfaces ($p < 0.05$). We observed that *P. pijperi* ZIM 1368 and *P. membranifaciens* ZIM 2302 showed a higher ability to adhere to wood than *P. membranifaciens* ZIM 2417.

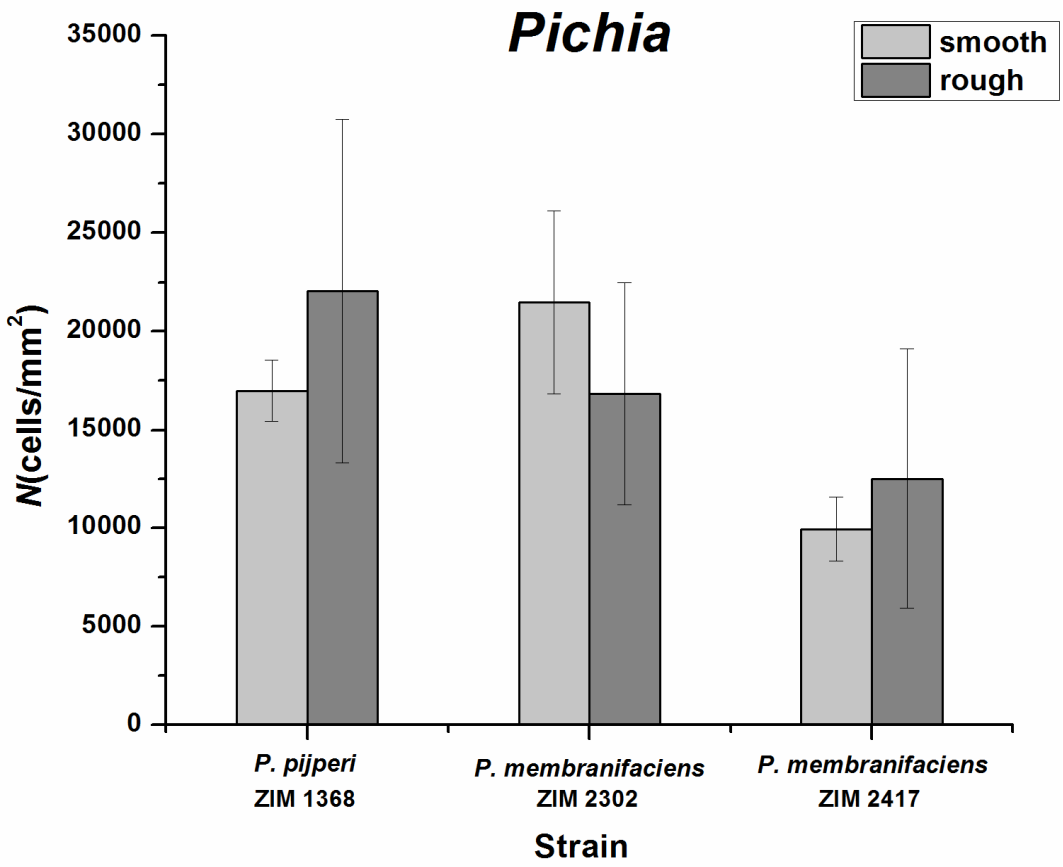

Fig. 2. Adhesion of *Pichia* spp. to the two types of wooden surfaces (smooth and rough). Each bar represents the mean ± standard deviation (SD).

As it has been observed with the wooden surfaces in our study, others have also noted that *Candida* species are capable adhering to abiotic surfaces (*21,22*). Nevertheless, to our knowledge we have shown for the first time the adhesion of *Candida* and *Pichia* species to wooden surfaces. We found that considerable differences in ability for adherence existed among *Candida* species. It is important to note that *C. albicans* and *C. glabrata* strains adhered to a higher extent than *C. parapsilosis* and *C. krusei* (Fig. 1). Hawser and Douglas (*23*) demonstrated that isolates of *C. parapsilosis* and *C. glabrata* made significantly less biofilm on the surface of PVC catheter discs than *C. albicans*, which is more pathogenic. The finding that *C. albicans* isolates consistently produce more biofilm in vitro than non-*C. albicans* isolates was confirmed recently (*24*). On the other hand, Shin, *et al.* (*25*) observed that biofilm formation on polystyrene was most frequent for isolates of *C. parapsilosis* (73 %) followed by *C. glabrata* (28 %) and *C. albicans* (8 %). The reason for these contradictory findings could be the fact that microbial adhesion is also influenced by the properties of the different cultivation substrates, contact medium and methods used to quantify adhesion.

It is well known that the surface properties of materials, such as surface roughness, are significantly influence the quantity and quality of fungal adhesion. Evaluation of *C. albicans* adhesion to denture base resin with different surface roughness has revealed greater adhesion

to rough surfaces than to smooth ones (*26,27*). This phenomenon is understandable since a rough surface is irregular, has an extended surface area, and likely to possess more binding sites for adhering microorganisms (*28*). The promoting effect of surface roughness on microbial adhesion may also be related to the difficulties in surface cleaning (*29*), resulting in rapid re-growth of a biofilm. In contrast, in the our study (Fig. 1 and Fig. 2) adhesion of *Candida* and *Pichia* strains was not significantly influenced by the roughness of the wooden surfaces ($p > 0.05$). The effect of surface roughness on yeast adhesion is still far from being fully understood. There are also some studies which showed that the surface roughness has no effect on the microbial adhesion. Hahnel *et al.* (*30*) showed that the surface roughness of different cheramic materials have no significant influence on bacterial adhesion. Also Li and Logan (*31*) reported that there was no significant effect of surface roughness (glass and metal–oxide) on bacterial (*P. aeruginosa* and *E. coli*) adhesion. The effect of surface roughness might depend on the microbial species, possibly due to difference in adhesion manner and/or cell surface characteristics.

Conclusions

The results of our studies indicate that the *Candida* and *Pichia* strains readily adhered on smooth and rough surfaces of wood, which are nowadays frequently used in food-processing environments. It was shown that *C. albicans* and *C. glabrata* strains exhibited higher colonization of wooden surfaces compared with *C. parapsilosis* and *C. krusei*. While in the case of *Pichia*, all tested strains were adhered in a strain-dependent manner. Additionally, adhesion by these yeast was not significantly affected by the roughness of the wooden surfaces. Therefore, as yeast biofilm formation is responsible for contamination and alteration of food products, the potential ability of contaminant yeast to adhere to wood must be taken into account with a view to preventing biofilms in food processing environment.

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