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

*Ružica Tomičić[1], Zorica Tomičić[2] and Peter Raspor[3]**

[1]Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, RS-21000 Novi Sad, Serbia

[2]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, RS-21000 Novi Sad, Serbia

[3]Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

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Summary

Yeast adhesion to and biofilm formation on surfaces 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 two common yeast genera involved in the spoilage of some food products. The aim of this study is to assess the potential of *Candida* and *Pichia* species to adhere to two types of wooden surfaces (smooth and rough), one of the materials typical for the food processing industry, and investigate the influence of surface roughness of wood on the degree of yeast adhesion. The adhesion of the cells to the wooden surfaces was determined by rinsing them from the surface, followed by methylene blue staining, and quantification after imaging under microscope by automatic counting of viable cells. 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 yeasts was not significantly affected by the roughness of the wood surfaces.

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

Introduction

Biofilms are defined as highly structured communities of microorganisms that attach to surfaces where they grow and produce extracellular polymeric substances (*1*). They are formed by yeast on different surfaces such as stainless steel, plastic, glass, wood and rubber, and present a great risk in the food industry (*2*). These are very serious issues because of the potential to cause cross-contamination, which leads to shorter shelf-life, food spoilage, and affects the consumer's health (*3,4*). Use of wood in industry as a food contact surface has been reduced because of the use of new plastic materials. Although wood as a porous material can entrap organic matter along with microorganisms (*5*), it is still in use in the developing and developed countries within food supply chain because it is readily available, cheap and easy to handle, and presents a sustainable resource (*6,7*). Problems caused by microbial fouling in food systems often occur due to increased resistance of sessile organisms to the existing disinfectants and sanitising agents (*8*).

Bacterial adherence to various surfaces is regularly studied; however, researchers have paid much less attention to the adhesion of yeasts, particularly *Candida* and *Pichia* species, which are usually contaminants isolated from biofilms on conveyor belts during canning and bottling in the beverage industry (*9–11*). Even though *Candida albicans* is the most significant and frequently isolated yeast pathogen (*12*), other species such as *Candida krusei*, *Candida glabrata* and *Candida parapsilosis* are often found as contaminants in the food industry (*13,14*). However, *C. albicans* is commonly found on human skin and can be transferred during handling by workers to food or food contact surfaces. Since it is frequently found in different food industry settings, its implications on sanitation issues in the food industry cannot be disregarded (*14,15*).

*Corresponding author: E-mail: peter.raspor@guest.arnes.si

Microbial adhesion to an abiotic surface is governed by complex interactions among the microorganisms, the substrate surface and the environmental conditions, involving physical, chemical and biochemical factors. However, the effects of all these factors on microbial adhesion have not yet been fully clarified, or they have sometimes been reported inconsistently. For example, opposing results have been reported for the effect of surface roughness of stainless steel on adhesion: positive correlation and no relation between microbial adhesion and surface roughness (*16,17*). Several studies have been conducted on microbial adhesion onto different types of food contact surfaces (*18,19*), but to our knowledge no information is available on the adhesion of *Candida* and *Pichia* species to wooden surfaces.

The purpose of this study is to assess the potential of *Candida* and *Pichia* species to adhere to two types of wooden surfaces (smooth and rough), material typical for the food processing industry in some developing countries, and investigate the influence of wood surface roughness on the degree of yeast adhesion.

Materials and Methods

Wooden surfaces

Smooth and rough wooden blocks (beech; length, width and thickness of 15.0, 7.0 and 1.0 mm, respectively) were used in this study. The surface roughness of the blocks was expressed in roughness parameter R_a. All wooden blocks were autoclaved at 121 °C for 15 min before use. The R_a for smooth and rough blocks were (44.0±1.5) and (5.8±1.5) μm, and for surface contact angle, describing wettability after 10 s, (72±10) and (41±7) μm, respectively.

Strains and growth conditions

A total of eight *Candida* and three *Pichia* strains were selected from ZIM Culture Collection of Industrial Microorganisms, Ljubljana, Slovenia (Table 1). All strains were stored in yeast peptone dextrose (YPD) medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 40 % glycerol at −80 °C. Prior to experiments, the strains were subcultured on malt extract agar (MEA; Merck KGaA, Darmstadt, Germany) for 24 h at 37 °C (*Candida* strains) or 27 °C (*Pichia* strains) for microbiological analyses.

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

Species (strain)	Origin
Candida albicans (ATCC 10261)	human fingernail infection: paronychia
Candida glabrata (ZIM 2367)	tracheal aspiration
Candida glabrata (ZIM 2369)	bronchoalveolar lavage (BAL)
Candida glabrata (ZIM 2382)	urine from indwelling catheter
Candida parapsilosis (ATCC 22019)	human: sprue
Candida parapsilosis (ZIM 2014)	sputum
Candida parapsilosis (ZIM 2234)	fruit juice concentrate
Candida krusei (ATCC 6258)	human: bronchial secretion
Pichia pijperi (ZIM 1368)	Refošk must
Pichia membranifaciens (ZIM 2302)	spoiled wine
Pichia membranifaciens (ZIM 2417)	white cheese from cow's milk

Subsequently, a loop of each yeast biomass was inoculated into 4 mL of malt extract broth (MEB; Merck KGaA) for microbiological analyses and incubated for 18 h at 37 °C (*Candida* strains) or 27 °C (*Pichia* strains). After 18 h of incubation, 1 mL of culture was diluted in 9 mL of fresh MEB to achieve the final cell concentration of 10^7 colony forming units (CFU) per mL, and the cell count was determined by plate counting on MEA. These cell suspensions were used immediately for adhesion assay.

Adhesion assay

Adhesion assays were performed on the two types of wooden blocks, smooth and rough. Three blocks of each type were placed on the bottom of Petri dishes (30 mm in diameter). For each strain, 2 mL of cell suspension (10^7 CFU/mL) prepared as above were pipetted into each plate, covering the discs. The plates were incubated for 24 h at 37 °C (*Candida* strains) or 27 °C (*Pichia* strains). In control plates, the wooden blocks were inoculated with 2 mL of yeast-free MEB. After incubation period, non-adherent cells were removed by washing three times with phosphate-buffered saline (PBS; Oxoid, Hampshire, UK), and the blocks were then transferred to 15-mL Falcon tubes with 2 mL of PBS. Wood samples were centrifuged at 1500×*g* for 3 min to detach the adhering cells. The remaining adhering cells were determined by rinsing, followed by methylene blue staining, and their quantification after imaging under microscope using Leica Application Suite software v. 3.7.0 (Leica Microsystems AG, Heerbrugg, Switzerland) by automatic counting of viable cells (*20*). Briefly, 50 μL of cell suspension were diluted with methylene blue at a ratio of 1:1 (by volume), and Bürker–Türk haemocytometer (100 μm depth; Brand, Wertheim, Germany) was then filled with 20 μL of the stained suspension. After adjusting the settings of the microscope for counting only viable cells, images were analysed using ImageJ, v. 1.43u, image processing software (National Institutes of Health (NIH), Bethesda, MD, USA) as previously described (*20*).

Statistical analysis

All quantitative data are presented as mean values with error bars representing standard deviation (S.D.) from two independent experiments with three replicates. The analysis of variance (ANOVA) was used for statistical analysis. The results are considered significant at $p<0.05$.

Results and Discussion

Evaluation of adhesion of *Candida* spp. in the present study revealed that these yeasts possess the ability to adhere to wooden surfaces, although to different extents depending on the species and strains. Fig. 1 shows the number of cells (mean value±S.D.) of *Candida* strains adhered to the smooth and rough surfaces of wood. Statistical analysis showed that *C. albicans* ATCC 10261 and *C. glabrata* (ZIM 2367, ZIM 2369 and ZIM 2382) strains exhibited a much greater propensity for adherence to both types of wooden surfaces than *C. parapsilosis* (ATCC 22019, ZIM

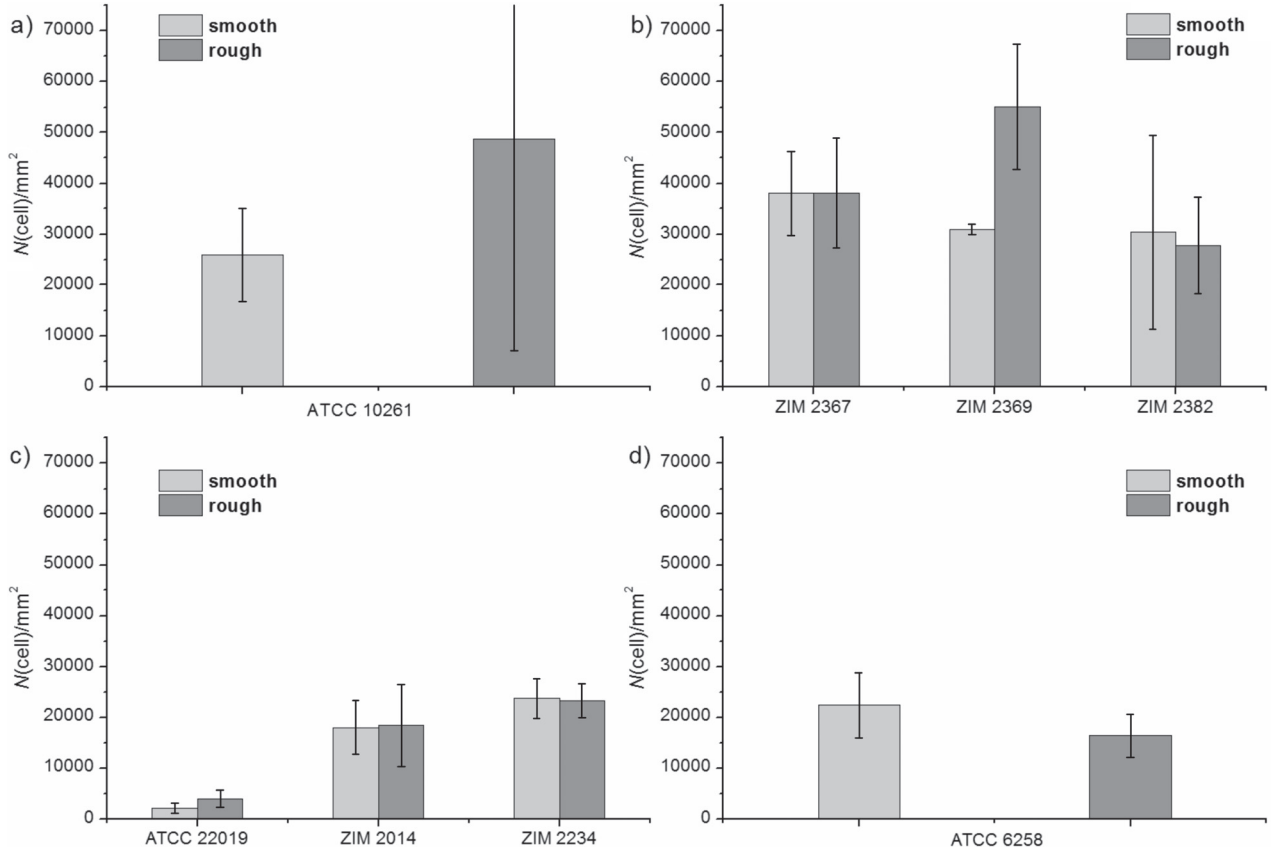

Fig. 1. Adhesion of *Candida* spp. to smooth and rough wooden surfaces: a) *C. albicans*, b) *C. glabrata*, c) *C. parapsilosis* and d) *C. krusei*. Each bar represents the mean value±standard deviation (S.D.)

2014 and ZIM 2234) strains and *C. krusei* ATCC 6258 ($p<0.05$). Considerable intraspecies variation was found for *C. parapsilosis*. *C. parapsilosis* ATCC 22019 has weaker capacity to adhere to wood ($p<0.05$) than *C. parapsilosis* ZIM 2014 and *C. parapsilosis* ZIM 2234. On the other hand, *C. glabrata* strains adhered in equivalent amount to wooden surfaces ($p>0.05$). Some previous studies also showed strain variation among *C. parapsilosis* regarding adherence to abiotic surfaces (*21,22*). This variation among species and strains reflects inherent physiological differences, and may have significance in relation to the pathogenic potential, since it is known that majority of pathogenic events start with adherence to the relevant surface. Nevertheless, we should mention that only a few strains were used in our study and other strains of the same species can have a greater potential for adhesion.

Additionally, under the conditions of our study all assayed *Pichia* strains were able to adhere to wooden surfaces, as shown in Fig. 2. *P. pijperi* ZIM 1368 and *P. membranifaciens* ZIM 2302 had a better ability to adhere to the smooth surfaces than *P. membranifaciens* ZIM 2417 ($p<0.05$). Therefore, the ability of *Candida* spp. and *Pichia* spp. to adhere to wooden surfaces is important in different food industry settings because these microorganisms can be a source of food contamination.

Our study has confirmed previous findings that *Candida* species are capable of adhering to abiotic surfaces (*23,24*). Nevertheless, to the best of our knowledge, this is the first time the adhesion of *Candida* and *Pichia* species to

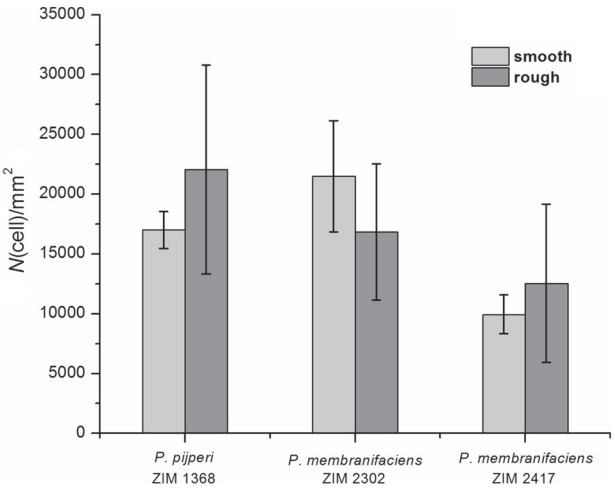

Fig. 2. Adhesion of *Pichia* spp. to smooth and rough wooden surfaces. Each bar represents the mean value± standard deviation (S.D.)

wooden surfaces has been shown. Considerable differences in adhesion ability among *Candida* species were observed. *C. albicans* and *C. glabrata* strains adhered better than *C. parapsilosis* and *C. krusei* (Fig. 1). These results confirm previous findings, which showed that *C. albicans* had good biofilm growth on the surface of PVC catheter discs (*25*) and on silicone elastomer discs (*26*). Furthermore, Shin *et al.* (*27*) observed that biofilm formation by isolates

of *C. parapsilosis* (73 %) followed by *C. glabrata* (28 %) and *C. albicans* (8 %) on polystyrene was most frequent. The reason for these contradictory findings could be the fact that microbial adhesion is also influenced by the properties of the different substrates, contact medium and methods used to quantify adhesion.

It is well known that the surface properties of materials, such as surface roughness, can significantly influence the quality and quantity 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 (*28*,*29*). 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 (*30*). The promoting effect of surface roughness on microbial adhesion may also be related to the difficulties in surface cleaning (*31*), resulting in rapid regrowth of a biofilm. In contrast, in our study the roughness of wooden surfaces did not have significant influence on the adhesion of *Candida* and *Pichia* strains ($p>0.05$), as shown in Figs. 1 and 2. Such result is in agreement with previous studies which showed that bacterial adhesion was not influenced by surface roughness of different materials (*32*,*33*). The effect of surface roughness on adhesion could be attributed to species- and strain-specific cell surface characteristics.

Conclusions

The present study indicates that *Candida* and *Pichia* strains readily adhered to smooth and rough surfaces of wood, which are nowadays frequently used in food processing environments in some countries. *C. albicans* and *C. glabrata* adhered better to wooden surfaces than *C. parapsilosis* and *C. krusei*, while all tested *Pichia* strains adhered in a strain-dependent manner. Additionally, adhesion of these yeasts was not significantly affected by the roughness of the wooden surfaces. Therefore, as yeast adhesion is the first step of biofilm formation, which may be responsible for contamination and adulteration of food products, the potential ability of contaminant yeast to adhere to wood must be taken into account to prevent undesirable biofilm formation in food processing environment.

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References

1. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002;8:881–90. http://dx.doi.org/10.3201/eid0809.020063
2. Marques SC, Rezende JGOS, Alves LAF, Silva BC, Alves E, de Abreu LR, Piccoli RH. Formation of biofilms by Staphylococcus aureus on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. Braz J Microbiol. 2007;38:538–43. http://dx.doi.org/10.1590/S1517-83822007000300029
3. Shi X, Zhu X. Biofilm formation and food safety in food industries. Trends Food Sci Technol. 2009;20:407–13. http://dx.doi.org/10.1016/j.tifs.2009.01.054
4. Srey S, Jahid IK, Ha SD. Biofilm formation in food industries: a food safety concern. Food Control. 2013;31:572–85. http://dx.doi.org/10.1016/j.foodcont.2012.12.001
5. Lauzon HL. The suitability of materials used in the food industry, involving direct or indirect contact with food products. Project P 98076 'Wood in the Food Industry' Nordic industrial fund. Center for innovation and commercial development and the industry partners in Denmark, Iceland, Norway and Sweden; 1998. p. 5.
6. Fink R, Filip S, Oder M, Jevšnik M. Wood in food industry – potential applications and its limitations. In: Mendez-Vilos A, editor. Microbial pathogens and strategies for combating them: science, technology and education, vol. 4. Badajoz, Spain: Formatex Research Center. 2013; p. 188–94.
7. Aviat F, Gerhards C, Rodriguez-Jerez JJ, Michel V, Le Bayon I, Ismail R, Federighi M. Microbial safety of wood in contact with food: a review. Compr Rev Food Sci Food Saf. 2016;15: 491–505. http://dx.doi.org/10.1111/1541-4337.12199
8. Simões M, Simões LC, Vieira MJ. A review of current and emergent biofilm control strategies. LWT – Food Sci Technol. 2010;43:573–83. http://dx.doi.org/10.1016/j.lwt.2009.12.008
9. Brugnoni LI, Cubitto MA, Lozano JE. Candida krusei development on turbulent flow regimes: biofilm formation and efficiency of cleaning and disinfection program. J Food Eng. 2012;111:546–52. http://dx.doi.org/10.1016/j.jfoodeng.2012.03.023
10. Loureiro V, Malfeito-Ferreira M. Spoilage yeasts in the wine industry. Int J Food Microbiol. 2003;86:23–50. http://dx.doi.org/10.1016/S0168-1605(03)00246-0
11. Salo S, Wirtanen G. Disinfectant efficacy on foodborne spoilage yeast strains. Food Bioprod Process. 2005;83:288–96. http://dx.doi.org/10.1205/fbp.04317
12. Li J, Hirota K, Goto T, Yumoto H, Miyake Y, Ichikawa T. Biofilm formation of Candida albicans on implant overdenture materials and its removal. J Dent. 2012;40:686–92. http://dx.doi.org/10.1016/j.jdent.2012.04.026
13. Koc AN, Silici S, Mutlu-Sariguzel F, Sagdic O. Antifungal activity of propolis in four different fruit juices. Food Technol Biotechnol. 2007;45:57–61.
14. Dworecka-Kaszak B, Krutkiewicz A, Szopa D, Kleczkowski M, Biegańska M. High prevalence of Candida yeast in milk samples from cows suffering from mastitis in Poland. Sci World J. 2012;2012:Article ID 196347. http://dx.doi.org/10.1100/2012/196347
15. Mhone TA, Matope G, Saidi PT. Detection of Salmonella spp., Candida albicans, Aspergillus spp., and antimicrobial residues in raw and processed cow milk from selected smallholder farms of Zimbabwe. Vet Med Int. 2012;2012:Article ID 301902. http://dx.doi.org/10.1155/2012/301902
16. Flint SH, Brooks JD, Bremer PJ. Properties of the stainless steel substrate, influencing the adhesion of thermo-resistant streptococci. J Food Eng. 2000;43:235–42. http://dx.doi.org/10.1016/S0260-8774(99)00157-0
17. Ortega MP, Hagiwara T, Watanabe H, Sakiyama T. Factors affecting adhesion of Staphylococcus epidermidis to stainless steel surface. Jap J Food Eng. 2008;9:251–9.
18. Mafu AA, Plumety C, Deschênes L, Goulet J. Adhesion of pathogenic bacteria to food contact surfaces: influence of pH

of culture. Int J Microbiol. 2011;2011:Article ID 972494. http://dx.doi.org/10.1155/2011/972494

19. Silva S, Teixeira P, Oliveira R, Azeredo J. Adhesion to and viability of Listeria monocytogenes on food contact surfaces. J Food Prot. 2008;71:1379–85. http://dx.doi.org/10.4315/0362-028X-71.7.1379
20. Zupan J, Avbelj M, Butinar B, Kosel J, Šergan M, Raspor P. Monitoring of quorum-sensing molecules during minifermentation studies in wine yeast. J Agric Food Chem. 2013;61: 2496–505. http://dx.doi.org/10.1021/jf3051363
21. Panagoda GJ, Ellepola ANB, Samaranayake LP. Adhesion of Candida parapsilosis to epithelial and acrylic surfaces correlates with cell surface hydrophobicity. Mycoses. 2001;44:29–35. http://dx.doi.org/10.1046/j.1439-0507.2001.00611.x
22. De Bernardis F, Mondello F, Millàn RS, Pontòn J, Cassone A. Biotyping and virulence properties of skin isolates of Candida parapsilosis. J Clin Microbiol. 1999;37:3481–6.
23. Estivill D, Arias A, Torres-Lana A, Carrillo-Muñoz AJ, Arévalo MP. Biofilm formation by five species of Candida on three clinical materials. J Microbiol Methods. 2011;86:238–42. http://dx.doi.org/10.1016/j.mimet.2011.05.019
24. Silva S, Negri M, Henriques M, Oliveira R, Williams D, Azeredo J. Silicone colonization by non-Candida albicans Candida species in the presence of urine. J Med Microbiol. 2010; 59:747–54. http://dx.doi.org/10.1099/jmm.0.017517-0
25. Hawser SP, Douglas LJ. Biofilm formation by Candida species on the surface of catheter materials in vitro. Infect Immun. 1994;62:915–21.
26. Kuhn DM, Chandra J, Mukherjee PK, Ghannoum MA. Comparison of biofilms formed by Candida albicans and Candida parapsilosis on bioprosthetic surfaces. Infect Immun. 2002; 70:878–88. http://dx.doi.org/10.1128/IAI.70.2.878–888.2002
27. Shin JH, Kee SJ, Shin MG, Kim SH, Shin DH, Lee SK, et al. Biofilm production by isolates of Candida species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. J Clin Microbiol. 2002;40:1244–8. http://dx.doi.org/10.1128/JCM.40.4.1244-1248.2002
28. Verran J, Maryan CJ. Retention of Candida albicans on acrylic resin and silicone of different surface topography. J Prosthet Dent. 1997;77:535–9. http://dx.doi.org/10.1016/S0022-3913(97)70148-3
29. Radford DR, Sweet SP, Challacombe SJ, Walter JD. Adherence of Candida albicans to denture-base materials with different surface finishes. J Dent. 1998;26:577–83. http://dx.doi.org/10.1016/S0300-5712(97)00034-1
30. Boulangé-Petermann L, Rault J, Bellon-Fontaine MN. Adhesion of Streptococcus thermophilus to stainless steel with different surface topography and roughness. Biofouling. 1997; 11:201–16. http://dx.doi.org/10.1080/08927019709378331
31. Korber DR, Choi A, Wolfaardt GM, Ingham SC, Caldwell DE. Substratum topography influences susceptibility of Salmonella enteritidis biofilms to trisodium phosphate. Appl Environ Microbiol. 1997;63:3352–8.
32. Hahnel S, Rosentritt M, Handel G, Bürgers R. Surface characterization of dental ceramics and initial streptococcal adhesion in vitro. Dent Mater. 2009;25:969–75. http://dx.doi.org/10.1016/j.dental.2009.02.003
33. Li B, Logan BE. Bacterial adhesion to glass and metal-oxide surfaces. Colloid Surf B: Biointerfaces. 2004;36:81–90. http://dx.doi.org/10.1016/j.colsurfb.2004.05.006