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ORIGINAL ARTICLE

Theoretical effect of cedar wood surface roughness on the adhesion of conidia from *Penicillium expansum*

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Abstract The influence of surface topography on microbial adhesion is an important regulatory issue. In this study, we use the potential adhesion of conidia from *Penicillium expansum* to cedar wood as a theoretical model to understand the effect of a roughness on physico-chemical attributes of spores, and their potential adhesive properties. The effect of roughness level ranging from 1.83 to 0.45 μm was investigated. Hydrophobicity, electron donor and acceptor character of the substrates was determined using contact angle measurements. Spore behavior switched from hydrophobic (99.2°), at an Ra value of 1.83 μm, to hydrophilic (60.02°), at an Ra value of 0.45 μm. It was difficult to find a relationship between the acid–base component of wood substrate and surface roughness over the range of Ra values tested. Prediction of the attachment tendencies of *Penicillium expansum*, was modeled using wood surfaces with different degrees of roughness according to the XDLVO approach. Maximum adhesion was predicted to occur at an Ra value of 1.83 μm. However, for Ra values less than 0.75 μm, the value of total interaction energy ranged from +4.17 mJ/m^2 (Ra=0.6 μm) to +6.58 mJ/m^2 (Ra=0.45 μm).

Keywords Cedar wood · Roughness · Physico-chemical properties · Adhesion · XDLVO · *Penicillium expansum*

Introduction

Despite the global use of wood as an invaluable structural and fabrication resource, the significance of adhesion phenomena and biofilm formation has only recently gained attention in wooden substrata (Swaffield et al. 1997; El abed et al. 2010a, b, 2011). The microbial characterization of biofilm on wood was first described in the study of Swaffield et al. (1997), where bacteria (lactic and acetic acid bacteria) and yeasts were isolated from cider fermentation vats and the influence of their stable biofilms on the organoleptic profiles of ciders was demonstrated.

The adhesion of microorganisms to a surface is one of the first stages in the development of biofilm and is believed to be influenced by a number of microbiological, physical, chemical, and material-related parameters. In particular, surface topography has been widely discussed as a parameter influencing microbial adhesion. Opinions vary as to the effect of surface characteristics on microbial adhesion. For example, a number of researchers have reported that there is a positive correlation between adhesion and increased

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surface roughness (Masurovsky and Jordan 1958; Hoffman 1983; Pedersen 1990; Leclercq-Perlat and Lalande 1994; Wirtanen et al. 1995), while others report no correlation between surface irregularities or roughness and the ability of microorganim to attach (Langeveld et al. 1972; Mafu et al. 1990; Vanhaecke et al. 1990; Flint et al. 1996). To our knowledge, no studies have reported the effect of wood roughness on adhesion phenomena. We have previously demonstrated the ability of fungal spores to adhere to cedar wood surfaces (El abed et al. 2010b). The aim of this study was to investigate the effect of wood surface roughness on these physico-chemical properties, including hydrophobicity and electron donor–electron acceptor characteristics, using measurements of *Penicillium expansum* and cedar wood to construct a theoretical model. Predicted adhesion to cedar wood surface with different roughness was also examined.

Materials and methods

The physico-chemical properties of *Penicillium expansum*

Physico-chemical properties incorporated in the model included the hydrophobicity and Lewis acid–base properties of *Penicillium expansum*: hydrophilic (θw=45.3°), electron donating (γ^-=52.2 mJ/ m^2) and weakly electron accepting (γ^+= 6.2 mJ/m^2) (El abed et al. 2010b).

Substratum surface

The substratum chosen for this study was cedar wood. The wood surface, cut into squares of 1 cm^2, was cleaned by soaking for 15 min in ethanol (50%) and rinsing six times (5 min each) with 10 ml of sterile distilled water. The blocks were subsequently autoclaved for 15 min at 120°C.

Wood roughness measurement

Surface roughness measurements were performed with a surface roughometer (Mitutoya SJ 301).

Contact angle measurements and surface tension components

Surface free-energy characteristics of wood were inferred from measured contact angles using the sessile drop technique (Blanco et al. 1997). Three to six contact angle measurements were made on wood surface for all probes using three pure liquids with known energy characteristics: formamide, distilled water, (>99%), and diiodomethane (>99%) (van Oss 1996). The surface-free energy of γs substrates, electrostatic van der Waals components, electron donor (or Lewis-base), and electron acceptor (or Lewis acid) parameters, was calculated using the Young–van Oss equation (Absolom et al. 1983):

$$\gamma_{\mathrm{L}}(\mathrm{Cos}\theta + 1) = 2(\gamma_{\mathrm{s}}{}^{\mathrm{LW}}\gamma_{\mathrm{L}}{}^{\mathrm{LW}})^{1/2}/\gamma_{\mathrm{L}} + 2(\gamma_{\mathrm{s}}^{+}\gamma_{\mathrm{L}}^{-})^{1/2}$$
$$/\gamma_{\mathrm{L}} + 2(\gamma_{\mathrm{s}}^{-}\gamma_{\mathrm{L}}^{+})^{1/2}$$

Extended DLVO theory

The total free energy of interaction between microbial cells (M) and substratum (S) through water (W) is calculated as the sum of the LW and AB interactions as proposed by Van Oss (1996)

$$\Delta\mathrm{G}_{\mathrm{MLS}}{}^{\mathrm{Total}} = \Delta\mathrm{G}^{\mathrm{LW}}{}_{\mathrm{MLS}} + \Delta\mathrm{G}^{\mathrm{AB}}{}_{\mathrm{MLS}}$$

where

$$\begin{aligned}\Delta\mathrm{G}^{\mathrm{LW}} &= ((\gamma_{\mathrm{M}}{}^{\mathrm{LW}})^{1/2} - (\gamma_{\mathrm{S}}{}^{\mathrm{LW}})^{1/2})^2 \\ &\quad - ((\gamma_{\mathrm{M}}{}^{\mathrm{LW}})^{1/2} - (\gamma_{\mathrm{L}}{}^{\mathrm{LW}})^{1/2})^2 \\ &\quad - ((\gamma_{\mathrm{S}}{}^{\mathrm{LW}})^{1/2} - (\gamma_{\mathrm{L}}{}^{\mathrm{LW}})^{1/2})^2\end{aligned}$$

and

$$\begin{aligned}\Delta\mathrm{G}^{\mathrm{AB}} &= 2[(\gamma_{\mathrm{L}}{}^{+})^{1/2}[(\gamma_{\mathrm{S}}{}^{-})^{1/2} + (\gamma_{\mathrm{S}}{}^{-})^{1/2} - (\gamma_{\mathrm{L}}{}^{-})^{1/2}] \\ &\quad + (\gamma_{\mathrm{L}}{}^{-})^{1/2}[(\gamma_{\mathrm{C}}{}^{+})^{1/2} + (\gamma_{\mathrm{S}}{}^{+})^{1/2} - (\gamma_{\mathrm{L}}{}^{+})^{1/2}] \\ &\quad - (\gamma_{\mathrm{L}}{}^{-}\gamma_{\mathrm{S}}{}^{+})^{1/2} - (\gamma_{\mathrm{L}}{}^{+}\gamma_{\mathrm{S}}{}^{-})^{1/2}]\end{aligned}$$

From a thermodynamic point of view, adhesion or attraction between two interacting surfaces occurs when the total energy $\mathrm{G}^{\mathrm{XDLVO}}$ is negative, and repulsion occurs when $\mathrm{G}^{\mathrm{XDLVO}}$ is positive.

Statistical analysis

Statistical analysis was performed using ANOVA. The MINITAB 16 for Windows Statistical program was used to determine the meanand standard deviation and evaluate the significance of the data in the tests.

Results and discussion

The effect of roughness on the hydrophobicity of cedar wood

It has been hypothesized that microorganisms preferentially adhere to rougher surfaces for three reasons: (1) a larger surface area may be available for attachment, (2) there may be protection from shear forces, and (3) there may be chemical changes that cause preferential physico-chemical interactions (Scheuerman et al. 1998). The relationship between the roughness of surfaces and physico-chemical properties of spores is poorly understood. This is the first report of an observed correlation between surface roughness of wood and hydrophobicity characteristics. Surface hydrophobicity of cedar wood was dramatically influenced by surface roughness of the wood (Ra), and ranged from 1.83 to 0.45 μm. Hydrophobicity of the cedar blocks decreased when decreasing the roughness level of wood (Fig. 1). According to Vogler (1998), hydrophobic surfaces exhibit water contact-angle values higher than 65°, whereas hydrophilic ones exhibit water contact-angle values lower than 65°. The water contact measurement changed from a hydrophobic character (99.2° at an Ra of 1.83 μm) to a hydrophilic value (60.02° at an Ra of 0.45 μm; Table 1; Fig. 1). Furthermore, maximum hydrophilicity was expressed when Ra equaled 1.83 μm. Inversely, cedar wood blocks exhibited hydrophilic behavior at a roughness level of Ra <0.75 μm. These results corroborate the observations of Abdelsalam et al. (2005), where topography was seen to be important in assessing contact-angles, and where it was possible to change from a hydrophilic surface to a hydrophobic surface purely through control of surface topography. However, contradicting results concerning correlation between surface hydrophobicity and roughness for glass have been reported. Bengourram et al. (2009) found that glass surfaces display hydrophilic characteristics at all roughness levels (3.55–0.46 μm).

The effect of roughness on the acid–base component of cedar wood

It was difficult to find a relationship between the acid–base component of wood substrate and surface roughness over the range of Ra values tested (Figs. 2 and 3; Table 2). However, they can be divided into two groups with different electron donor properties versus Ra values. When Ra<0.75 μm, the wood surface appeared to be weakly electron-donating, ranging from 0 mJ/m^2 (Ra=1.83 μm) to 1.98 mJ/m^2 (Ra=0.8 μm). In contrast, when Ra≥0.75 μm, the cedar wood blocks exhibited a more electropositive than surfaces with Ra< 0.75 μm. Maximum electronegativity was recorded at an Ra value of 0.75 μm, indicating that charge was significantly affected by changes in surface roughness. The wood surface was weakly electron accepting, ranging from 0 mJ/m^2 (Ra= 1.83 μm) to 3.90 mJ/m^2 (Ra=1.5 μm). These findings indicate that the surface properties of wood vary with roughness, a result in agreement with several authors (Bengourram et al. 2009; Kouider et al. 2010).

Table 1 Contact angle with water (θw), formamide (θ_F) and diiodomethane (θ_D) on cedar wood surface with different roughness

Ra (μm)	Liquid contact angles		
	θw (°)	θ_F (°)	θ_D (°)
Ra=1.83 (0.09)	99.2 (1.75)	65.8 (3.5)	24.9 (1.5)
Ra=1.74 (0.1)	88.2 (2.5)	54.6 (1.9)	39.5 (1.2)
Ra=1.5 (0.07)	79.0 (2.0)	38.6 (0.1)	45.6 (1.8)
Ra=1.21 (0.05)	78.4 (1.3)	39.7 (0.5)	16.6 (3.7)
Ra=0.8 (0.03)	74.7 (1.9)	32.3 (3.1)	32.0 (2.30)
Ra=0.75 (0.05)	67.9 (0.6)	45.0 (0.8)	31.2 (1.50)
Ra=0.6 (0.08)	62.5 (1.0)	28.4 (1.4)	16.8 (0.9)
Ra=0.45 (0.05)	60.02 (0.9)	23.5 (1.1)	14.7 (0.5)

Prediction of adhesion the *Penicillium expansum* with level roughness of wood

Several works have evaluated the potentiality of adhesion in various surfaces with level roughness (Bengourram et al. 2009; Kouider et al. 2010; Flint et al. 2000; Whitehead et al.

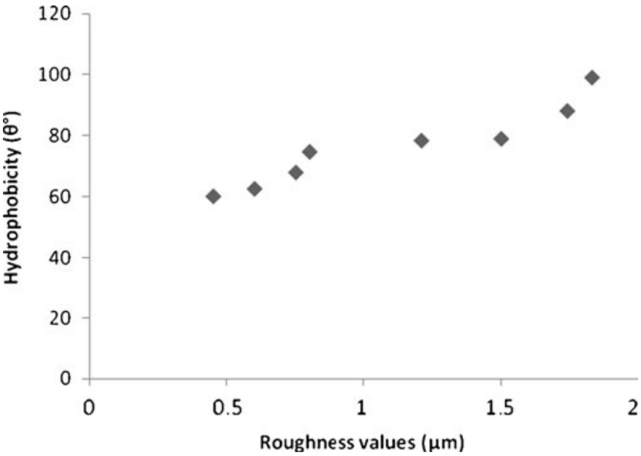

Fig. 1 Variation of hydrophobicity of cedar wood according to roughness

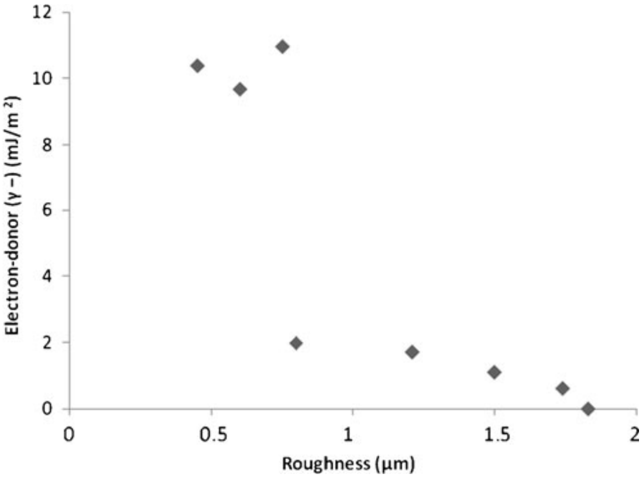

Fig. 2 Electron-donor characteristics of cedar wood according to roughness

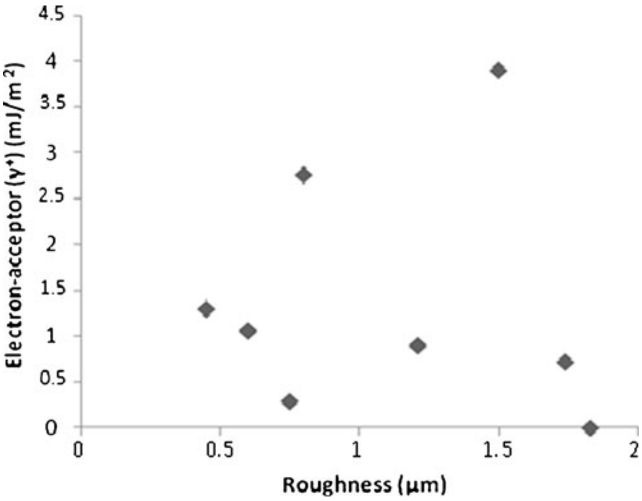

Fig. 3 Electron-acceptor characteristics of cedar wood according to roughness

2006). Despite the fact that wood is a widely used natural resource, crucial in building construction, food-processing, etc., no studies have investigated the potentiality of adhesion of microorganisms to wooden substrata with roughness levels ranging from 1.83 to 0.45 µm. Thus, one of the objectives proposed in this study was to use a theoretical model to predict the ability of spores from fungi such as *Penicillium expansum* to adhere to wooden surface with different roughness. In order to do this, the total interactive free-energy of adhesion processes must be calculated (Table 3). From the LW and AB interactions, electrical interactions can be very important in suspending liquids with low ionic strength. Since the suspending liquid employed in this work (aqueous KNO_3) has a high ionic strength (0.1 M), we neglected electrical interactive-free energy versus the sum of ΔG_{LW} and ΔG_{AB}, in line with the experiments of Gallardo-Moreno et al. (2003) and Riijinart et al. (1999). In our study, it was difficult to find a relationship between the theoretical adhesion of *Penicillium expansum* to wood and surface roughness over the range of Ra values between 1.83 and 0.75 µm (Fig. 4). Total interaction energy varied from −6.62 mJ/m^2 to −17.01 mJ/m^2. Maximum adhesion was predicted at an Ra value of 1.83 µm. However, when Ra was less than 0.75 µm, the positive value of total interaction energy ranged from +4.17 mJ/m^2 (Ra=0.6 µm) to +6.58 mJ/m^2 (Ra=0.45 µm), indicating unfavorable adsorption in this range of roughness.

Table 2 The Lifshitz–van der waals (γ^{LW}) (mJ/m^2) and electron-donor (γ^{-}) (mJ/m^2) and electron-acceptor (γ^{+}) (mJ/m^2) parameters of cedar wood surfaces with different roughness

Ra (µm)	γ^{LW}	γ^{-}	γ^{+}
Ra=1.83 (0.09)	46.09 (0.3)	0 (0)	0 (0)
Ra=1.74 (0.1)	39.77 (0.7)	0.62 (0.4)	0.72 (0)
Ra=1.5 (0.07)	36.61 (1.3)	1.11 (0.8)	3.90 (1)
Ra=1.21 (0.05)	48.60 (0.6)	1.71 (0.5)	0.90 (0.7)
Ra=0.8 (0.03)	43.28 (1)	1.98 (0.9)	2.76 (0.1)
Ra=0.75 (0.05)	43.62 (0)	10.97 (0.1)	0.29 (1.6)
Ra=0.6 (0.08)	48.55 (0.1)	9.68 (0.6)	1.06 (1.9)
Ra=0.45 (0.05)	49.04 (0.2)	10.39 (0.3)	1.30 (1.7)

Table 3 Theoretical Lifshitz–van der Waals (ΔG^{LW}) and acid–base (ΔG^{AB}) and total ΔG^{Total} interaction-free energies corresponding to the adhesion of *Penicillium expansum* to wood with different roughness

Ra (µm)	ΔG^{LW}	ΔG^{AB}	ΔG^{Total}
Ra=1.83 (0.09)	−2.67	−3.94	−6.62
Ra=1.74 (0.1)	−2.97	−14.04	−17.01
Ra=1.5 (0.07)	−2.29	−12.11	−14.40
Ra=1.21 (0.05)	−1.93	−15.01	−16.94
Ra=0.8 (0.03)	−3.22	−8.89	−12.11
Ra=0.75 (0.05)	−2.67	−11.21	−13.88
Ra=0.6 (0.08)	−2.71	6.88	4.17
Ra=0.45 (0.05)	−3.27	9.84	6.58

These results indicate an important role for surface roughness in the adhesion process. Reported data clearly indicate an the effect of surface roughness on *Penicillium expansum* adhesion on wood, but there are differences of opinion as to whether there is a minimum value below which the retention of microorganisms is unaffected. Within the food processing industry, regulations specify that hygienic surfaces require a Ra value of ≤0.8 µm (Flint et al. 1997). However, work on surfaces with lower Ra values has led to suggestions that surface roughness has a significant effect on the rate of dental plaque formation if the Ra

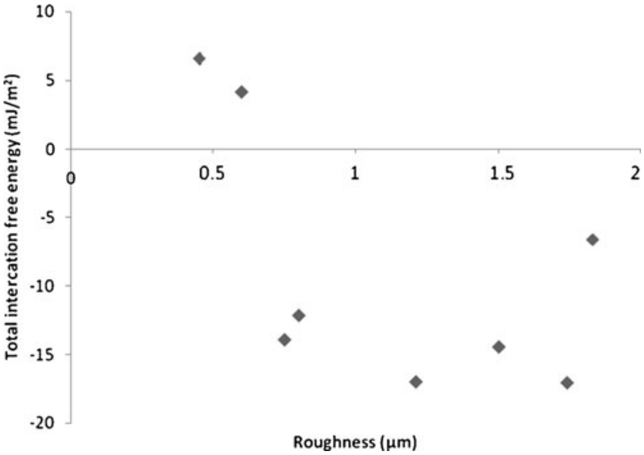

Fig. 4 Predicted interaction of conidial free energy versus surface roughness for conidia of *P. expansum* adhering to cedar wood

exceeds 0.2 μm (Quirynen et al. 1990, 1996; Bollen et al. 1996). Bengourram et al. (2009) suggest that a range of Ra values between 0.48 and 1.47 μm should be considered as a critical level of glass roughness to predict adhesion. The apparent conflict in these opposing observations is probably related to the degree of surface roughness studied, the microbial species tested, and the physico-chemical parameters of the surface. Our findings suggest that a value of Ra >0.75 μm could be considered a critical range to predict adhesion on wood substrata. Indeed, the contribution of Lifshitz–van der Waals interactions to the total interaction-free energy is higher than that of acid–base interactions, which means, from a theoretical point of view, that the adhesion process would be governed by long range forces .Some researchers have concluded that Lifshitz–van der Waals interactions are the main factor affecting cell-surface adhesion. For this study, for Ra values less than 0.75 μm, the theoretical adhesion of *Penicillium expansum* was only mediated by Lifshitz–van der Waals interactions, since AB components of adhesion-free energy were positive.

Conclusion

The substrate is essential in the development of a biofilm; an understanding of how substrate properties affect adhesion of microbial cells may assist in designing or modifying substrates inhibitory to microbial adhesion. Our study demonstrated that cedar wood surface physico-chemical proprieties were dramatically influenced by surface roughness of wood ranging from 1.83 to 0.45 μm. Moreover, it appears that the adhesive properties of conidia from *Penicillium expansum* would be influenced predominantly by degree of cedar wood surface roughness. Our findings suggest that Ra values >0.75 μm could be considered as a critical range to predict adhesion on cedar wood substrata.

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