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EXPERIMENTAL ARTICLES

Study of Microbial Adhesion on Some Wood Species: Theoretical Prediction[1]

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Abstract—The initial interaction between microorganisms and substrata is mediated by physicochemical forces, which in turn originate from the physicochemical surface properties of both interacting phases. In this context, we have determined the physicochemical proprieties of all microorganisms isolated from cedar wood decay in an old monument at the Medina of Fez-Morocco. The cedar wood was also assayed in terms of hydrophobicity and electron donor–electron acceptor (acid–base) properties. Investigations of these two aspects were performed by contact angles measurements via sessile drop technique. Except *Bacillus subtilis* strain (Giwi < 0), all strains studied showed positive values of the degree of hydrophobicity (Giwi > 0) and can therefore be considered as hydrophilic while cedar wood revealed a hydrophobic character (Giwi = 58.81 mJ m^{-2}). All microbial strains were predominantly electron donor. The results show also that all strains were weak electron acceptors. Cedar wood exhibits a weak electron donor/acceptor character. Based on the thermodynamic approach, the Lifshitz-van der Waals interaction free energy, the acid-basic interactions free energy, the total interaction free energy between the microbial cells and six different wood species (cedar, oak, beech, ash, pine and teak) in aqueous media was calculated and used to predict which microbial strains have a higher ability to adhere to wooden surfaces. Except of weak wood, for all the situations studied, generalizations concerning the adhesion of the microbiata on wood species cannot be made and the microbial adhesion on wooden substrata was dependent on wood species and microorganisms tested.

Keywords: physicochemical proprieties, microbial adhesion, wood species, surface energy, prediction

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INTRODUCTION

As all inert surfaces, the materials are potential site for biofilm formation after initial attachment of microorganisms. Once established, the biofilm can be responsible for the spoilage of engineering materials and can lead to product contamination, and surface destruction [1, 2]. The capacity of microorganisms to adhere rapidly to surfaces such as plastics, polypropylenes, rubbers, stainless steel and glass is now well established. On wooden surfaces; very few studies have been focused on the interactions of wood and microorganisms (hygienic status of wood in food plants). The microbial characterization of a biofilm was first described in the study of Swaffield et al. [3], where bacteria (lactic and acetic acid bacteria) and yeasts were isolated from cider fermentation vats and the influence of their stable biofilm on the organoleptic profiles of ciders has also been demonstrated. In contrast, other than the use of this material in food industry, wood has a long tradition as a natural material used in heritage construction, to our knowledge; no works have been published on the interactions between microorganisms and wooden species in a heritage. It has been recognized that a better understanding of the interactions between microbial biofilms and the surfaces may play a significant role to control this problem and may help in the development of strategies to reduce their adherence to this type of substratum. The adherence of microorganisms to the surface is a complicated process that is affected by various physicochemical properties of both substrata and microbial surfaces. These interactions can be classified into three classes: Lifshitz-van der Waals interactions, electrostatic interactions [4, 5] and polar or Lewis acid-base interactions (i.e. electron-donor and electron-acceptor) [6, 8]. Reports in the literature have shown that parameters such as hydrophobicity [8, 9], surface charge [8, 10, 11] and electron donor–electron acceptor (acid–base) properties may have a significant effect on microbial adhesion. Therefore, there is a clear need for systematic investigation of the microbi-

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ological ecosystems on wooden surfaces and for quantifying total interaction free energy between them. The purpose of the present work was to predict microbial adhesion potential on various wood species using thermodynamic approach. The physicochemical proprieties of microbial isolated from cedar wood were also determined.

MATERIALS AND METHODS

Sampling, Isolation and Identification of Microorganisms

The micro-organisms used throughout this work were isolated from three sites of an old house built 450 years ago located in the former Derblamté in the Medina of the Fez, Morocco. This construction is made mainly by cedar wood. The samples damaged were scraped and were dissolved in 36 ml of sterile distilled water and shaken for 2 h. Serial dilutions were plated on different solid media: Malt Extract Agar and LB agar. The recovered bacterial cells were seeded on LB agar plates and incubated during 24 h or 48 h at 37°C. After that, isolates were picked from the agar plates and characterized by PCR-sequencing. The rDNA 16S regions were amplified using primers fD1 (5'AGAGTTTGATCCTGGCTCAG3') and Rs16 (5'TACGGCTACCTTGTTACGACTT3') [12]. Fungal were seeded also on Malt Extract Agar plates, incubated during 3 j or 7 j at 30°C and the rDNA ITS regions 1, 5.8S, and ITS region 2 were amplified using primers ITS1 and ITS4 [13]. Polymerase chain reaction amplification was performed with the following protocol: 94°C for 5 min; 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min followed by a final extension step of 72°C for 5 min. DNA sequencing was performed using ABI 3130; Applied Biosystems according to the manufacturer instructions. The GenBank BLASTN tools were used for sequence analysis.

Contact Angle Measurements and Surface Tension Components

Microbial cell surface characteristics were inferred from measured contact angles for all probe liquids including water, formamide and diiodomethane. In order to prepare microbial lawns suitable for contact angle measurements, microbial cell suspended in KNO_3 sterile solution were deposited on a cellulose acetate membrane filter (0.45 μm) by filtration of the suspension using negative pressure [14]. Filters containing the microorganisms (Bacteria and spores approximately 10^8 cell mm^{-2}) were placed to air dry for 30–60 min in order to obtain stable, so-called "plateau" contact angles. Contact angles were measured in triplicate with separately cultured microbes.

Once the contact angles were measured, the Lifshitz-van der Waals (γ^{LW}) and acid–base (γ^{AB}) surface tension components were obtained by the three equation system from the application of the Young–Dupré equation to each probe liquid [15]

$$\gamma_L(\cos\theta + 1) = 2[(\gamma_S^{LW}\gamma_L^{LW})^{1/2} + (\gamma_{S^+}\gamma_{L^-})^{1/2} + (\gamma_{S^-}\gamma_{L^+})^{1/2}],$$

where θ is the measured contact angle, γ^{LW} is the Van der Waals free energy component, γ^+ is the electron acceptor component, γ^- is the electron donor component and the subscripts (S) and (L) denote solid surface and liquid phases respectively.

The surface free energy is expressed as: $\gamma_S = \gamma_S^{LW} + \gamma_S^{AB}$ where $\gamma_S^{AB} = 2(\gamma_{S^-}\gamma_{S^+})^{1/2}$ is the acid-base free energy component.

The cell surface hydrophobicity was evaluated through contact angle measurements and by the approach of Van Oss and al. [15, 16]. In this approach, the degree of hydrophobicity of a given material (i) is expressed as the free energy of interaction between two entities of that material when immersed in water (w): ΔGiwi. If the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic (Giwi < 0); conversely, lor a hydrophilic material, ΔGiwi > 0. ΔGiwi is calculated through the surface tension components of the interacting entities, according to the following formula (1):

$$\Delta G\text{iwi} = -2\gamma_{iw} = -2[((\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2})^2 + 2((\gamma_i^+\gamma_i^-)^{1/2} + (\gamma_w^+\gamma_w^-)^{1/2} - (\gamma_i^+\gamma_w^+)^{1/2} - (\gamma_w^+\gamma_i^-)^{1/2})].$$

The total free energy of interaction between microbial cell (M) and substratum (S) through Water (W) is calculated as the sum of the LW and AB interactions as proposed by Van Oss [15]

$$\Delta G_{MLS}^{Total} = \Delta G_{MLS}^{LW} + \Delta G_{MLS}^{AB} \text{ Eq. (A.1)},$$

where

$$\Delta G^{LW} = ((\gamma_M^{LW})^{1/2} - (\gamma_S^{LW})^{1/2})^2 - ((\gamma_M^{LW})^{1/2} - (\gamma_L^{LW})^{1/2})^2 - ((\gamma_S^{LW})^{1/2} - (\gamma_L^{LW})^{1/2})^2 \text{ Eq. (A.2)},$$

and

$$\Delta G^{AB} = 2[(\gamma_L^+)^{1/2}[(\gamma_C^-)^{1/2} + (\gamma_S^-)^{1/2} - (\gamma_L^-)^{1/2}] + (\gamma_L^-)^{1/2}[(\gamma_C^+)^{1/2} + (\gamma_S^+)^{1/2} - (\gamma_L^+)^{1/2}] - (\gamma_L^-\gamma_S^+)^{1/2} - (\gamma_L^+\gamma_S^-)^{1/2}] \text{ Eq. (A.3)}.$$

Wooden Species and Surface Characterization

The material employed was cedar wood. The wood plate was characterized using angle contacts measure-

Table 1. Energy characteristics (mJ m^{-2}) of pure liquids used to measure contact angles (Van Oss 1996) [15]

Liquid	γ^{LW} (mJ m^{-2})	γ^{+} (mJ m^{-2})	γ^{-} (mJ m^{-2})
Water (H_2O)	21.6	25.4	25.4
Formamide (CH_3NO)	38.7	2.3	39.4
Diiodomethane (CH_2I_2)	50.5	0.7	0.0

ments (CAM) by the following dimensions: length 20 mm, thickness 1 mm, and height 10 mm. Contact angles (θ) were measured using Diiodomethane (99 per cent) and formamide (99 per cent) and distilled Water [15]. Each experiment was repeated five times and θ values obtained were averaged. The data of surface characterization for the other wood species typically used in the construction and building monuments historic (Oak; teak, beech, pine and ash) was obtained in the literature (Table 3).

RESULTS AND DISCUSSIONS

Microorganisms Surface Characterization

Several techniques are usually employed to assess cell surface properties, Cell surface hydrophobicity was evaluated by hydrophobic interaction chromatography [17], bacterial adhesion to hydrocarbon [18], salting out [19] and water contact angle [20, 14] and Three methods have been used to assess the acid–base properties of cell surface including contact angle measurement [14] combined with equation of Van Oss et al. [16], microbial adhesion to solvents [7] and acid–base titration [21]. In the present study, we used contact angle measurements techniques to determine cell and substrate surface characteristics.

The surface hydrophobicity of all samples was analyzed from the water contact angle and is listed in Table 2. According to Vogler [22], hydrophobic surfaces exhibit a water contact angle values higher than 65°, whereas hydrophilic ones exhibit water contact angle values lower than 65°. However, with this approach it is only possible to assess hydrophobicity qualitatively Oliveira et al. [23]. Using the approach of Van Oss and co-workers [15, 16], it is possible to determine the absolute degree of hydrophobicity of any substance (i) vis-a-vis water (w), which can be precisely expressed in applicable System International (Formula 1).

Concerning the hydrophobicity of the microbial population (Table 2), *Bacillus subtilis* (numbered as SS2) was the only hydrophobic bacterial strains. In contrast, the results show similar hydrophilic character for all strains. *Penicillium commune*, *Penicillium crustosum* and *Penicillium chrysogenum* spores (numbered as SS10, SS8, SS11) demonstrated the highest hydrophilicity. *Penicillium chrysogenum* spores (numbered as

Table 2. Contact angles of water (θ_w), formamide (θ_F), diiodomethane (θ_D), Lifshitz-van der Waals (γ^{LW}), electron-donor (γ^{-}), electron-acceptor (γ^{+}) parameters obtained with all strains

Strains		Contact angles (°)			Surface tension: components and parameters (mJ m^{-2})			
		θ_w	θ_F	θ_D	γ^{LW}	γ^{+}	γ^{-}	ΔGiwi
Bacillus sp.	SS1	52.6 (1.80)	50.2 (4.9)	89.0 (2.9)	13.1	6.9	31.6	3.3
Bacillus subtilis	SS2	68.9 (1.03)	56 (3.01)	63.3 (3.05)	26.7	1.3	15.3	−18.3
Pseudomonas pseudoalcaligenes	SS3	40.3 (3.90)	44.4 (2.73)	79.5 (1.58)	18.7	4	45	20
Klebsiella sp.	SS4	34.2 (1.04)	40.6 (2.38)	84 (2.35)	15.5	6.4	49.1	18.6
Acinotobacter lwoffi	SS5	33.5 (3.52)	43.7 (4.10)	93 (3.32)	11.4	8.1	52.5	16
Oceanobacillus picturae	SS6	42.5 (1.37)	49.4 (4.02)	91 (4.13)	12.0	6.5	46.1	14.6
Aspergillus niger	SS7	47.3 (1.71)	62 (2.79)	96 (3.70)	10	3.6	53.1	23.7
Penicillium crustosum	SS8	11.9 (1.98)	17 (0.35)	75 (3.29)	20.1	7.9	53.5	20.2
Penicillium granulatum	SS9	36.5 (0)	39.3 (5.13)	77.1 (4.31)	19	5	45.3	18.7
Penicillium commune	SS10	17.9 (0.75)	15.8 (0.77)	87.5 (4.45)	13.8	13.6	48.2	8.5
Penicillium chrysogenum	SS11	10.4 (4.54)	13.0 (2.44)	81.8 (3.39)	16.5	11.2	52.2	6.3
Penicillium expansum	SS12	45.3 (1.50)	59.0 (3.65)	102.6 (5.30)	7.8	6.2	52.2	15.2

Note: Standard deviation was given in parentheses.

Table 3. Contact angle values, surface energies and their components of wood species

Wood species	Contact angles (°)			Surface tension: components and parameters (mJ m^{-2})				Reference
	θ_w	θ_F	θ_D	γ^{LW}	γ^+	γ^-	ΔGiwi	
Cedar	82.5 (3.92)	61.8 (5)	44.5 (4.8)	37.3	0	5.5	−58.8	This work
Cedar*	69 (2)	–	–	47	1.7	4.7	−52.8	[25]
Beech*	54.5	–	–	49.5	9.1	1.5	−42.4	[32]
Pine*	55.4	–	–	47.6	1.2	24.2	−12.0	[32]
Ash*	68	–	–	45.2	2	6.1	−37.8	[33]
Oak*	81	–	–	42.9	3.5	0.3	−64.5	[33]
Teak*	18	–	–	42.6	0.2	59.3	−41.9	[33]

Notes: Standard deviation was given in parentheses.
* Surface free energy data from the literature.

SS11) were the most hydrophilic among all the strains. The microbial surface properties depend essentially on the chemical compositions of the cell surface. For spores; the hydrophilic character is notably correlated with protein/carbohydrate ratios. In this regard, extracts of the more hydrophobic spores tended to have greater protein/carbohydrate ratios. Indeed, spores with rugose surfaces were hydrophobic, whereas hydrophilic spores had smooth surfaces [24]. In addition, Table 2 provides the values of formamide and diiodomethane contact angles. On the other hand, the lifshitz-van der Waals (γ^{LW}) component as well as electron donor (γ^-) and electron acceptor (γ^+) are also presented (Table 2). The result show that all strains were predominantly electron donor (high value of γ^-) ranging from 53.10 mJ m^2 to 31.6 mJ m^{-2}. The results show also that all strains were weak electron acceptors ranging from 13.6 mJ m^{-2} to 1.3 mJ m^{-2}.

Substratum Surface Characterization

As for the microbial characterization, Table 3 presents the water, formamide, and diiodomethane contact angles on cedar wood. Also, some data of the literature are presented (Table 3). The contact angle water measurements show that the cedar wood surface is hydrophobic. This fact is corroborated by the results presented by De Meijer [25], who found a value of contact angle measurement water ($\theta w = 69°$) when examining the variation in wettability and surface energy between different wood species. Furthermore, our results show that the cedar wood is weakly accepting donating ($\gamma^- = 5.5$ mJ m^{-2}; $\gamma^+ = 0$ mJ m^{-2}). The value of the acid-base component is in accordance with studies of De Meijer [25]. He reported rather similar values for cedar wood species ($\gamma^- = 4.7$ mJ m^{-2}) and ($\gamma^+ = 1.7$ mJ m^{-2}). On the other hand, when all wood species were compared, it appears clearly that the electron donor of oak and beech wood were low that all species. Table 3 show also that teak and pine wood exhibits more electron donating ($\gamma^- = 59.3$ mJ m^{-2}) and ($\gamma^- = 24.2$ mJ m^{-2}) respectively.

Theoretical Prediction of Adhesion on Wood Species

Several works have evaluated the potentiality of adhesion in various surfaces using thermodynamic approach [26–28]. Despite that wood are renewable natural resource and the use crucial in building construction monuments and food-processing. In our knowledge, no studies have investigated in potentiality of adhesion in wooden substrata. Thus, one of the objectives proposed in this study was to predict the ability of microorganisms to adhere to wooden surfaces and to have indications on adhesion potentials of microbiata onto wood species. In order to do this, total interactions free energy of adhesion process must be calculated (Table 4). Besides the LW and AB interactions, the electrical interactions can be very important in suspending liquid with low ionic strength. Since the suspending liquid (KNO_3 solution) employed in this

work has high ionic strength (0.1 M), we neglected electrical interactions free energy versus the sum of ΔG^{LW} and ΔG^{AB} as done by [29, 30].

As previously mentioned, all the micro-organisms were isolated from the cedar wood substrata; however, there are other wood species in the construction and also building historic monuments, being oak, beech, ash, pine and teak commonly used. The results emphasizes that generalizations concerning the adhesion of the microbiata on wood species shall not be made.

The theoretical adhesion of all wood species studied show that this phenomenon was completely different on the different wood species and microorganisms tested (Table 4). Several reports have already mentioned that the interaction of microorganisms and wood depend of the inoculated bacterium and type of wood. An excellent example thereof is already described by Milling et al. [31] when studying the hygienic performance of wood use of wood in food processing. They showed that pine and oak wood exhibit substantially better hygienic performance. For all the case studied (all microorganisms and all wood species), the bacteria cells have a higher ability to adhere in wood species than fungal spores (Fig. 1). Furthermore, for all spore fungi, it can be seen that the positive values of the ΔG^{Total} (Table 4) indicate unfavorable adhesion to some wood species like teak, pine and cedar teak, from a thermodynamical point of view. On the opposite, we can see that the bacterial adhesion was dependent on bacteria studied on those surfaces. For other types of wood (oak, beech, ash), a large variation can be observed. For example, adhesion process of the *Klebsiella* sp. (SS4) is unfavorable to beech. In contrast, the same strains show favorable adhesion to oak and beech. Also, only of *Penicillium commune* (SS10) and *Penicillium chrysogenum* (SS11) spores, it must be noted that the adhesion process is not thermodynamically favorable to any of the wood species studied ($\Delta G^{Total} > 0$). Interestingly, it should be expected that teak exhibit a better proprieties than the others studied to use in construction and also in building historic monuments because the results show unfavorable adhesion in all microorganism tested. In terms of thermodynamic adhesion potential, we can classify wood species as follows: oak > beech > ask > cedar > pine > teak (Fig. 2).

CONCLUSION

For the first time, the potential adhesion of microorganisms on wood species was investigated employing thermodynamic approach. The results revealed that the microbial adhesion on wooden substrata was dependent on two factors: wood species and microorganisms tested. These findings have important potential in the patrimonies applicability as they suggest that

Table 4. Lifshitz-van der Waals ΔG^{LW} (mJ m^{-2}), acid–base ΔG^{AB} (mJ m^{-2}) and total ΔG_{Tot} (mJ m^{-2}) interaction free energy for the adhesion between microorganisms and wood species

Microorganisms	Interaction free energy ΔG_{Tot} (mJ m^{-2})		
	Cedar	Beech	Pine
Bacillus subtilis (SS1)	−34.10	−36.92	−12.27
Bacillus sp. (SS2)	−4.30	−11.23	8.59
Pseudomonas pseudoalcaligenes (SS3)	1.27	−14.92	13.90
Klebsiella sp. (SS4)	−12.74	2.11	−21.90
Acinotobacter lwoffi (SS5)	14.05	−11.63	13.23
Oceanobacillus picturae (SS6)	7.50	−6.40	18.45
Aspergillus niger (SS7)	9.94	−6.98	23.96
Penicillium expansum (SS8)	13.47	−1.90	24.87
P. granulatum (SS9)	3.14	−13.19	13.97
P. commune (SS10)	14.50	1.81	18.89
P. chrysogenum (SS11)	19.11	6.11	21.99
P. crustosum (SS12)	11.25	−7.08	18.12
	Interaction free energy ΔG_{Tot} (mJ m^{-2})		
	Ash	Oak	Teak
Bacillus subtilis (SS1)	−30.52	−44.34	8.37
Bacillus sp. (SS2)	−4.02	−14.22	22.02
Pseudomonas pseudoalcaligenes (SS3)	−2.23	−15.59	32.75
Klebsiella sp. (SS4)	−10.53	−0.53	35.68
Acinotobacter lwoffi (SS5)	1.21	−9.10	28.71
Oceanobacillus picturae (SS6)	4.67	−6.95	33.73
Aspergillus niger (SS7)	6.65	−7.91	43.09
Penicillium expansum (SS8)	10.27	−2.20	40.53
P. granulatum (SS9)	−0.99	−13.45	31.57
P. commune (SS10)	10.67	3.37	28.20
P. chrysogenum (SS11)	14.87	8.33	29.88
P. crustosum (SS12)	5.64	−5.10	33.37

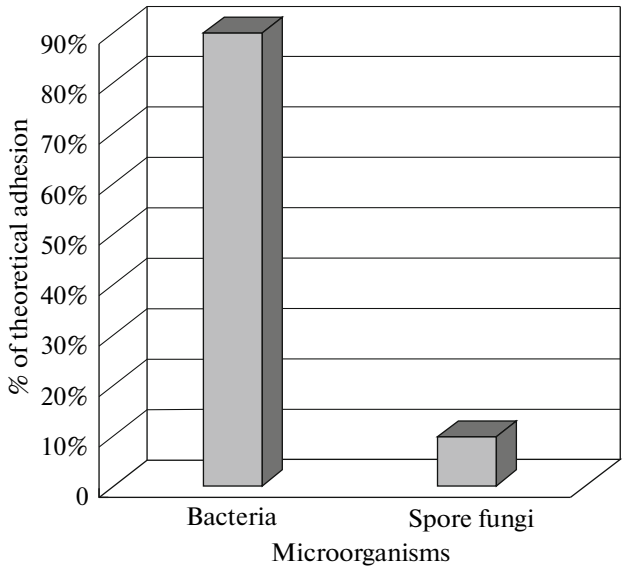

Fig. 1. Percentage of theoretical adhesion of all microorganisms in all wood species.

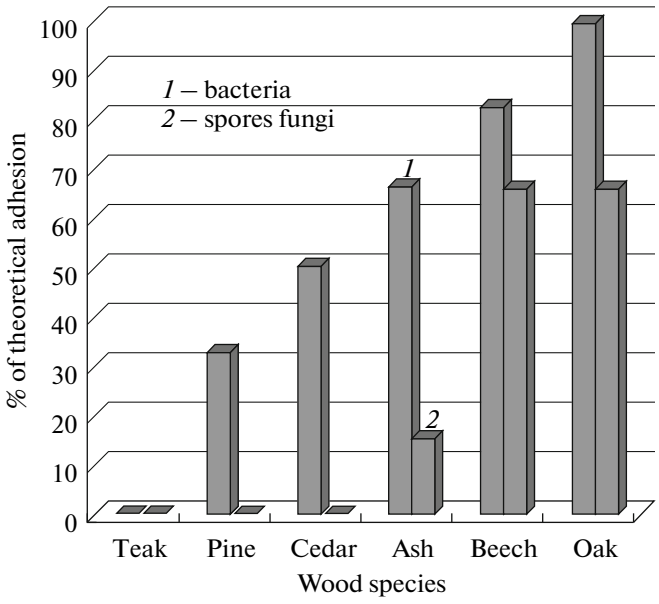

Fig. 2. Potentiality of adhesion on some wood species.

teak wood have better proprieties to use in construction and also in building historic monuments and can used as negative control to study adhesion on wood.

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