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Adhesion of *Penicillium italicum* and *Penicillium digitatum* spores to materials commonly used in the citrus packaging chain

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ABSTRACT

Purpose: The purpose of this study was to investigate the adhesion of *Penicillium italicum* and *Penicillium digitatum* spores on four materials commonly used in the citrus packaging chain (plastic, PVC, stainless steel, 316L and wood). **Research methods**: The physicochemical characterization of spores and material surfaces was carried out using the contact angle method. The number of adhered spores was estimated after being detached from supports in an ultrasonic bath. The results showed that all citrus materials processes were classified as hydrophobic except for the wood packaging. Surface spores of *P. digitatum* presented a relatively hydrophobic character, and surface spores of *P. italicum* presented a hydrophilic character. Both of the spores and all materials presented high electron donor/acceptor characters. **Findings**: The results showed that *P. digitatum* and *P. italicum* spores could adhere to all the studied substrates. Furthermore, the highest adhesion was observed by *P. italicum* and *P. digitatum* spores on wood packaging (58×10^6 CFU/cm^2) and (45×10^6 CFU/cm^2), respectively. The wood packaging was the least hygienic material concerning the adhesion ability of *P. digitatum and P. italicum* spores, followed by plastic packaging, PVC, and 316 L stainless steel. A correlation between substratum physicochemical properties and spore adhesion was also examined, while a good correlation was observed between spore adhesion and donor electron character. **Research limitations**: There were no limitations to this study. **Originality/value**: This research studied the adhesion of spores on materials commonly used in the citrus packaging chain.

INTRODUCTION

Due to fungal infections, postharvest diseases contribute to economic losses in the agriculture industry during storage, transportation, and markets (Klein & Lurie 1991). Citrus fruits deteriorate during harvest, handling, transport, storage, and marketing, providing entry points for different pathogens, mainly *P. digitatum* and *P. italicum*, causal agents of green and blue fungi respectively (Eckert, 1978). *P. digitatum* was the primary pathogen found in postharvest citrus fruits. It was responsible for 90% of fruit losses due to contamination during the storage period, leading to severe damages in commercialization (Singh et al., 2014). Spores adhere to the bark and, after germination, produce vegetative mycelium responsible for fruit rot.

Processing materials and packaging substrates can be the source of serious bio-contamination of citrus fruits by *P. digitatum* and *P. italicum.* These spores can attach to the substratum, then germinate and produce mycelia, causing consequential postharvest losses.

Prevention of this contamination begins by understanding the phenomenon leading to these spores' adhesion to processing and packaging materials. During storage and handling, citrus fruits are in contact with various types of surfaces, such as plastic, PVC, stainless steel, and the wood. Fungal spores are usually found on these materials, and their adhesion to the fruit leads to contamination.

It is known that the first and crucial step in bio-contamination is microbial adhesion, which depends on both substratum and microbial surface properties (Assaidi et al., 2018a; Hamadi et al., 2009). However, there have been very few studies fungal spores 'adhesion (Amiri et al., 2005; Barkai et al., 2015; El abed al. 2011; Newey et al., 2007). To our knowledge, no studies have been reported on fungal spore adhesion to materials commonly used in the citrus packaging chain.

To understand the microbial adhesion phenomenon. The knowledge of the physicochemical characteristics of spores and substrate surfaces is required to control postharvest citrus contamination. It is of paramount importance to identify materials that minimize the adhesion of the spores. The objective of this research is to study the adhesion of *P. digitatum* and *P. italicum* to all processing and packaging materials through their surface properties.

MATERIALS AND METHODS

Spores, growth conditions, and suspension preparation

The studied species were *P. digitatum* and *P. italicum* isolated from citrus fruit in the Laboratory of Biotechnology and Valorization of Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco. Preparation of the spore suspension began with cultivation on a Potato Dextrose Agar (PDA) medium at 25°C for seven days. The spores were harvested and filtered through glass wool and washed twice by centrifugation for 15 minutes (8400g) using a KNO_3 solution (0.1 M).

Substrate cleaning

Plastic, PVC, wood packaging, and 316 L stainless steel were used in this study, as these materials are often used in the citrus packaging industry. Each substratum was cut into 1cm^2 surfaces, soaked in an ethanol solution (95%) for 15 minutes, rinsed six times with sterile distilled water, then autoclaved for 15 minutes at 120°C, except for the plastic and PVC.

Physicochemical characterization of fungal spores and substrata by contact angle measurements

The contact angle measurements were performed using a goniometer (GBX instruments, France) using the sessile drop method. One to three drops of a liquid were placed on each solid material and bacterial filter (earlier described). Three measurements of the contact angle were made for each surface using three liquids (Water, Formamide, and Diiodomethane).

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

The surface free energy could not be measured directly. Instead, contact angle measurements with test liquids deposited on a solid surface were performed to calculate surface energy. The approach of van Oss and Chaudhury (acid–base theory) was used in this study (Van Oss et al., 1988).

According to Van Oss (1995), the degree of hydrophobicity of a given material (i) was 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 was stronger than the interaction of each entity with water, the material was considered hydrophobic (ΔGiwi < 0); however, for a hydrophilic material, ΔGiwi >0. ΔGiwi was simply related to the interfacial tension between i and water, ΔGiwi, as:

$$\Delta G_{iwi} = -2\gamma_{iw} = -2\left[\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 + 2\left(\sqrt{\gamma_i^+\gamma_i^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_i^+\gamma_w^-} - \sqrt{\gamma_w^+\gamma_i^-}\right)\right]$$

The interfacial free energy between entities i and water (w) can be expressed as:

$$\gamma_{iw} = \left[\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 + 2\left(\sqrt{\gamma_i^+\gamma_i^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_i^+\gamma_w^-} - \sqrt{\gamma_w^+\gamma_i^-}\right)\right]$$

Therefore, the free energy of interaction (ΔG_{iwi}) between surface molecule immersed in water (w) can be expressed as:

$$\Delta G_{iwi} = -2\gamma_{iw} = -2\left[\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 + 2\left(\sqrt{\gamma_i^+\gamma_i^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_i^+\gamma_w^-} - \sqrt{\gamma_w^+\gamma_i^-}\right)\right]$$

Three to six contact angle measurements were made on each filter, for all probe liquids including water, formamide and diiodomethane.

Experimental adhesion of fungal spores and evaluation using the ultrasonic method

One milliliter of a spore suspension containing 108 CFU/ml was incubated on a plate containing the tested coupons. After two hours of contact, the coupons were rinsed gently three times with sterile distilled water to remove non-adhesive spores. The coupons were then immersed in a test tube containing 9 mL of physiological water (NaCl: 9 g/l). Spores were detached from the inert substratum using a sonication bath for five minutes. The adhered CFU were counted by serial dilution of the spore suspension obtained after sonication. Counts were determined on the PDA medium after incubation for two days at 25°C using the plate count. Each experiment was repeated three times (Azelmad et al., 2017; El Abed et al., 2010; Hamadi et al., 2005; Herald & Zottola, 1988), and the results were expressed in CFU/cm^2.

RESULTS

Spores and substratum surface characterization

The surface free energy characteristics of the different substratum surfaces and both spores are presented in Table 1. The results showed that all citrus materials processes were classified as hydrophobic except for the wood packaging, which was found to be relatively hydrophilic (θw = 51.06°). All materials tested in this study presented a relatively high electron donor character (high value of γ-) and a weak electron acceptor character (low value of γ+). As shown in Table 2, we observed that spores surfaces of *P. digitatum* presented a relatively hydrophobic character (θw = 70.7°) and spores surfaces of *P. italicum* showed a hydrophilic character (θw= 32.7°). Both of the spores showed high electron donor/acceptor characters.

Table 1. Contact angles and standard deviation (°), surface energy value, and standard deviation (mJ/m^2) of materials commonly used in the citrus packaging chain

Substratum	Contact angle (°)			Surface tension: Components and parameters (mJ/m^2)			ΔG_{iwi} (mJ/m^2)
	Diiodometane	Formamide	water	γ^{LW}	γ^{+}	γ^{-}	
Wood packaging	51.9 (3.42)	79.76 (5.45)	51.06 (2.05)	33.1 (1.82)	10.03 (0.80)	94.6 (0.4)	32.4 (2.82)
Plastic packaging	41.8 (1.8)	47.2 (2.09)	66.4 (2.32)	0.8 (1.01)	2 (0.06)	39.4 (1.03)	-10.2 (1.89)
PVC	91.8 (0.7)	76.2 (1.4)	83.3 (0.9)	12.01 (0.2)	3.03 (0.67)	11.41 (0.06)	-24.70 (0 .78)
Stainless steel 316L	51.9 (2.1)	55.8 (1.2)	67.9 (1.9)	33.2 (2.03)	0.51 (0.99)	6.9 (1.07)	-44.20 (1.79)

Notes: γ^{LW}: The Lifshitz-Van der Waals components of the surface tension. γ−: electron donor components of the surface tension. γ+: electron acceptor components of the surface tension. γ^{AB}: the Lewis acid-base surface tension component. ΔGiwi: the free energy of interaction between two entities of that material when immersed in water. Notes: Standard deviation was given in parentheses.

Table 2. Contact angles and standard deviation (°), surface energy values and standard deviation (mJ/m^2) of *P. digitatum* and *P. italicum* spores

Spores	Contact angle (°)			Surface tension: Components and parameters (mJ/m^2)			ΔG$_{iwi}$ (mJ/m^2)
	Diiodometane	Formamide	water	γ^{LW}	γ^{+}	γ^{-}	
P. italicum	44.86 (1.80)	78.73 (0.64)	70.7 (0.64)	35.36 (1.18)	3.6 (0.43)	36.34 (1.18)	9 (0.36)
P. digitatum	95.03 (4.5)	27.8 (2.4)	32.7 (2.5)	14 (2)	16.26 (1.9)	42.6 (1.34)	4.2 (0.1)

Notes: γLW: The Lifshitz-Van der Waals components of the surface tension. γ−: electron donor components of the surface tension. γ+:electron acceptor components of the surface tension. γAB: the Lewis acid–base surface tension component. ΔGiwi: the free energy of spores.

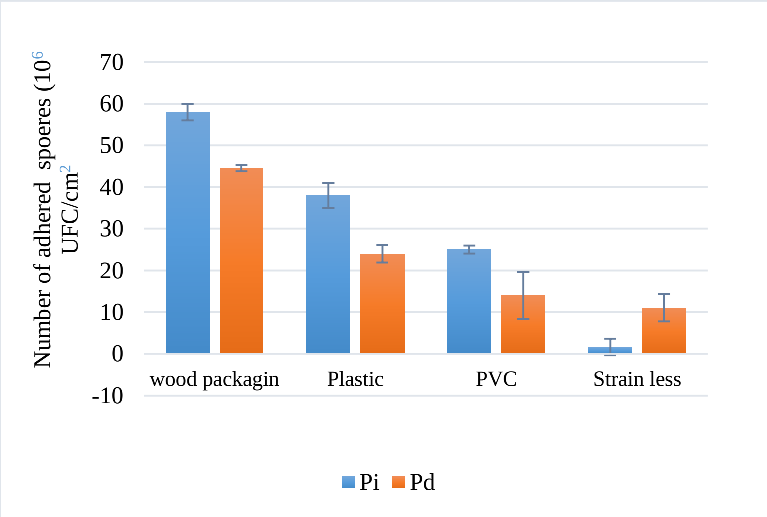

Fig 1. The number of *P. italicum* (Pi) and *P. digitatum* (Pd) spores adhered to different materials

Adhesion of fungal spores to substrates

P. digitatum and *P. italicum* spores adhesion to materials commonly used in the citrus packaging chain was studied. The results presented in Fig 1 showed that *P. digitatum* and *P. italicum* spores were able to adhere to all materials. The highest spore adhesion rate was observed on wood (44.5×106 CFU/cm^2) and plastic (24×106 CFU/cm^2). The lowest spore adhesion rates were observed in 316L stainless steel (11×106 CFU/cm^2). *P. italicum* spores also adhered significantly to wood (58×106 CFU/cm^2) and plastic (38×106 CFU/cm^2). The wood packaging was the most promising of all materials spore adhesion.

DISCUSSION

In this work, we attempted to assess the ability of spores to adhere to materials commonly used in the citrus packaging chain. Some studies reported that the ability of fungal spores to attach to a substratum. It is controlled by hydrophobins proteins (Wösten et al., 1994; Wösten & Wessels, 1997). These proteins favored the adhesion of fungal structures to hydrophobic surfaces and could be involved in the adhesion of *P. digitatum* and *P. italicum* to the studied surface substratum. Plastic, PVC, and stainless steel, which had a relatively hydrophilic character, were less occupied by fungi. However, wood was the most appropriate for spore adhesion.

The adhesion results, demonstrated clearly that fungal spores were better able to adhere to wood and plastic materials than to stainless steel and PVC (Fig. 2). Many studies have described adhesion in terms of physicochemical parameters such as hydrophobicity (Rosenberg & Kjelleberg, 1986).The surface energy also (Absolom et al., 1983; Van der Mei et al., 1997) and electrostatic interactions of the cell particles with supports (Bayer & Sloyer Jr 1990; Pedersen, 1981; van Loosdrecht et al., 1990). In this study, we also looked at *P.italicum* and *P. digitatum* spores and different substrata surface properties about the adhesive ability of spores on the studied substrata.

The role of the physicochemical properties of both fungal spores was investigated. It is generally admitted that the physicochemical properties of spores and substratum surfaces. There are the main factors mediating spore adhesion. However, few works have studied physicochemical proprieties of spores and substrates surfaces to explain this phenomenon (El abed et al. 2013; Van der Mei et al., 1997) and relate the adhesion results to the surface physicochemical characteristics (Garrett et al., 2008). In this work, the electrostatic force contribution was neglected since the experiments were performed at high ionic strength (0.1 M). The surface free energy (ΔG_{iwi}) components and qualitative hydrophobicity (θw) were determined through contact angle measurements. The surfaces of all the substrata had a hydrophobic character, except for the wood packaging. Our findings were in agreement with previous studies that reported a wood was hydrophilic (El Abed et al., 2010). These results were also in agreement with an earlier study, which reported that PVC was classified as a hydrophobic material (Boutaleb et al., 2008; Teixeira & Oliveira, 1999). Stainless steel was also considered hydrophobic by several authors (Assaidi et al. 2018b; Chamberlain & Johal 1988; Hamadi et al. 2014; Oliveira et al., 2006). In terms of electron donor/acceptor character, our results were in agreement with those reported by Assaidi et al. (2018b), who found that stainless steel 316 L and PVC had high electron donor/acceptor character.

It is well known that microbial adhesion to inert surfaces depends on both bacterial cell and substratum surface properties, such as electron donor/acceptor properties. To get insight into the process of *P. digitatum* and *P. italicum* adhesion to substrates, we examined the role of physicochemical properties in this process. While a positive correlation between electron donor/acceptor properties and bacterial adhesion was previously reported (Hamadi et al., 2005), other researchers could not establish a relation between bacterial adhesion and physicochemical properties (Oliveira et al., 2006).

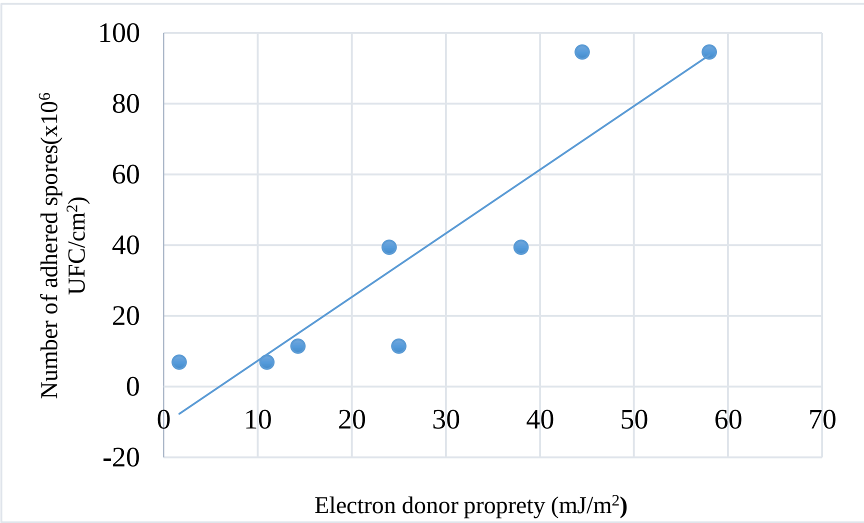

Fig 2. Correlation between *P. digitatum* and *P. italicum* spore adhesion and electron donor property of substratum surface (r = 0.90)

Our results showed a good correlation between *P. digitatum* and *P. italicum* spore adhesion to the studied materials and their electron donor character (r=0.90) (Fig. 2). These findings suggested that this adhesion could be controlled by electron donor properties. Therefore, we concluded that spore adhesion was governed by physicochemical properties. Our results were in line with previous reports (Hamadi et al., 2005; Hamadi et al., 2009; Silva et al., 2003), which also observed a good relation between bacterial adhesion and physicochemical properties.

CONCLUSION

Understanding the adhesion of spores to materials commonly used in the citrus packaging chain is essential to find ways to prevent postharvest citrus contamination. From the obtained results, we could conclude that *P. italicum* and *P. digitatum* adhesion spores was related to the surface donor properties of the studied substrates. Spores of *P. italicum* and *P. digitatum* were able to adhere to all the studied materials. Wood was the most favorable for spore adhesion, followed by plastic, PVC, and stainless steel. The latter presented the weakest adhesion rates. According to our findings, wood packaging was less hygienic concerning adhesion ability. Identifying surfaces with the most appropriate characteristics could be crucial for controlling the adhesion process.

Conflict of interest

The authors have no conflict of interest.

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