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Adhesion of *Staphylococcus aureus* and *Staphylococcus xylosus* to materials commonly found in catering and domestic kitchens

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ABSTRACT

The aim of this work was to investigate the adhesion of *Staphylococcus aureus* and *Staphylococcus xylosus* on marble, granite, polypropylene, stainless steel 304 and stainless steel 316. The results showed that *S. aureus* adhered to all substratums. The maximum was observed on marble (30 10^6 CFU/cm^2) and, on polypropylene (30,2 10^6 CFU/cm^2). The results showed also that *S. xylosus* revealed a high ability to adhere to all substratum. This strains adhere more on marble (32.8 10^6 CFU/cm^2) and granite (16,3 10^6 CFU/cm^2) than to others substratum. The highest extent of adhesion of *S. aureus* and *S. xylosus* occurred to marble, polypropylene and granite. A correlation between substratum physicochemical properties and bacterial adhesion was also examined. A good correlation was observed between *S. xylosus* adhesion and their acid-base character. The topography of substratum surface was investigated using AFM. A good correlation was obtained between roughness and bacterial adhesion.

1. Introduction

Staphylococcus xylosus, a common bacterial species from the skin microflora of mammals, It is part of coagulase-negative staphylococci and it is frequently isolated from milk, meat, and other food products such as cheeses and sausages (Kloos & Schleifer, 1986, pp. 1013–1035; Talon, Leroy-Se'trin, & Fadda, 2002). It was frequently isolated from soils and surfaces of food-processing plants (Kloos & Schleifer, 1986, pp. 1013–1035). Its ability to form a biofilm that can be source of infection often involved in the colonization of biotic and abiotic surfaces (Surpat et al., 2010).

Staphylococcus aureus was a gram positive which causes gastroenteritis resulting from the consumption of contaminated food. Food that is frequently responsible for staphylococcal food poisoning include meat products, poultry and eggs products, milk and dairy products. *S. aureus* is being occasionally found in food processing plant and have the ability to adhere to inert surface (Hamadi et al., 2005; Oulahal, Brice, Martial, & Degraeve, 2008) and consequently form biofilms (Marques et al., 2007).

Contamination of food stuff, during food preparation, due to bacteria present in kitchen surfaces is one of the main causes of foodborne outbreaks. Cells adhered to those surfaces of domestic kitchens and food processing are not easily removed by normal cleaning procedures. Therefore, they can be a source of contamination for other foods and objects (Teixeira, Silva, Araújo, Azeredo, & Oliveira, 2007; Silva, Andrade, Soares, & Frreira, 2003). Worldwide there is a concern about the impact of microbial foodborne diseases on the human behalf (White, Zhao, Simjee, Wagner, & McDermott, 2002). The importance of contaminated surfaces in spreading pathogenic microorganisms to foods is already well established in food processing, catering and domestic environment (Vasseur, Rigaud, Hébraud, & Labadie, 2001; Vautor, Abadie, Pont, & Thiery, 2008).

One of the most common ways for bacteria to live is adhering onto surfaces and forming organized communities named biofilms (Jenkinson & Lappin-Scott, 2001). The formation of biofilms on food-contact surfaces is known as a potential risk to the consumer's health, particularly, if the cross contamination of food occurs after a bactericidal procedure (Spoering & Lewis, 2001). Biofilm formation is a two stages process; it involves first attachment of cells to a solid

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surface and second the aggregation of cells and biofilm formation (Christensen et al., 1985). Bacterial adhesion is the key step to biofilm formation, it is governed by physicochemical interactions between the support and the bacterial surface including electrostatic, Van der Waals and Lewis acid–base interactions (Bellon_Fontaine, Rault, & van Oss, 1996; Briandet, Meylheuc, Maher, & Bellon-Fontaine, 1999; Krepsky et al., 2003; Pagedar, Singh, & Batish, 2010; Xu, Zou, Lee, & Ahn, 2010). Bacterial adhesion is a complex process regulated by diverse characteristics of support (roughness, physicochemical properties, nature …), bacterial cell surface, growth medium, and environmental conditions (pH, temperature, ionic strength) (Donlan, 2002; Bohinc et al., 2016, 2014,; Hamadi et al., 2005, 2004; Bengourram et al., 2009; Kouider et al., 2010). Many studies have reported that physicochemical properties (hydrphobicity, electron donor/electron acceptor character, surface charge) of bacterial and substratum surface play a crucial role in bacterial adhesion process. Moreover, the effect of surface roughness on bacterial adhesion have been also widely investigated by many researches (Flint, Brooks, & Bremer, 2000; Beck, Bobe, Gamer, Reiners, & Sommer, 2005; Bengourram et al., 2009; Kouider et al., 2010; Bohinc et al., 2014,2016). Some of these studies have reported that bacterial adhesion increases with increasing surface roughness, but others have found that bacterial adhesion is largely independent of the surface roughness.

Several studies have examined the bacterial adhesion on many substratum surfaces such as: stainless steel, glass, rubber, granite, marble, polymers (polypropylene, polyethylene …), etc …. , that can be contaminated either by spoilage or pathogenic bacteria. These bacteria can adhere to these surfaces and Therefore form biofilm (Silva, teixeira, oliveira, & azeredo, 2008; Teixeira, Lima, Azeredo, & Oliveira, 2008; Careli, Andrade, & Soares, 2009; Oliveria et al., 2006; Teixeira & Oliveira, 1999). The adhesion of *S. aureus* to substratum surfaces (stainless steel, glass) was widely investigated by many works (Hamadi et al., 2005, 2009, 2014; Teixeira et al., 2007; Mousavi, Kennedy, & Fanning, 2014; Bohinc et al., 2014, 2016; Kouider et al., 2010) but *S. xylosus adhesion was* weakly studied (Planchon et al., 2006; Surpat et al., 2010). Also the adhesion of *S. xylosus* and *S. aureus* to granite and marble, two materials commonly used in kitchens of many countries, were not investigate right now.

The aim of this research is to investigate the adhesion of *S. aureus* and *S. xylosus* to five food surfaces used in the food industry, domestic kitchens, and restaurants: granite, marble, polypropylene, stainless steel 304 and stainless steel 316. The effect of hydrophobicity, Lewis acid–base properties and material roughness on the adhesion of *S. aureus* and *S. xylosus* was also examined.

2. Materials and methods

2.1. Bacterial strains, growth conditions and preparation of microbial suspension

The Strains used in this study were *S. aureus* and *S. xylosus* isolated from food services surfaces. The incubation of cells was at 37 °C for 24 h. The cells were harvested by centrifugation for 15 min at 8400xg and were washed twice with, and resuspended in, KNO_3 solution with ionic strength (0.1 M).

2.2. Preparation of substratum surface

The materials used were marble, granite, polypropylene, stainless steel 304and stainless steel316. The materials were cut into 1 cm^2 squares (10 × 10 × 2 mm coupon-tests), and these surfaces were cleaned by soaking them in ethanol solution 95%, for 15 min and were rinsed six times with distilled water. Finally the substrates were autoclaved for 20 min at 120 °C.

2.3. Contact angle measurement

Contact angle measurements were performed using a goniometer (GB instruments, France) by the sessile drop method. Contact angles were measured in triplicate with separately cultured bacteria. Three to six contact angle measurements were made on each substratum surface for all probe liquids including formamide (99%), diiodométhane (99%) and distilled water (van Oss, Good, & Chaudhury, 1988).

The Lifshitz-Van der Waals (γ^{LW}), electron donor (γ^-) and electron acceptor (γ^+) components of the surface tension of bacteria and for the solid substrates were estimated from the approach proposed by van Oss et al. (1988). In this approach the contact angles (θ) can be expressed as:

$$\mathrm{Cos}\theta = -1 + 2\left(\gamma_S{}^{LW}\gamma_L{}^{LW}\right)^{1/2}/\gamma_L + 2\left(\gamma_S{}^{+}\gamma_L{}^{-}\right)^{1/2}/\gamma_L + 2\left(\gamma_S{}^{-}\gamma_L{}^{+}\right)^{1/2}/\gamma_L$$

θ is measured by contact angle. (S) and (L) denote solid surface and liquid phases respectively.

Lewis acid-base surface tension component is defined by: $\gamma_S{}^{AB} = 2(\gamma_s{}^{-}\gamma_S{}^{+})^{1/2}$

The method for measuring contact angles on bacterial layers has been described by Busscher et al. (1984). Briefly, a suspension of cells in KNO_3 solution was deposited onto a 0.45μm cellulose acetate filter (Sartorius) by a first washing of the filter with 10 ml of distilled water for wetting, and then 10 ml of the cell suspension was added to obtain a thick lawn of cells after filtration using of negative pressure. The wet filters were placed carefully on a glass support with double-sided sticky tape and were allowed to air dry until so-called stable "plateau contact angles" could be measured. For each strain, three independently grown cultures were used, from which three filters of each were prepared and measured. Three to six contact angle measurements were made on each filter, for all probe liquids including water, formamide and diiodomethane. The solid substrates were allowed to air dry and the contact angle measurements were carried out.

The cell surface hydrophobicity was evaluated through contact angle measurements and using the approach of van Oss and coworkers (van Oss et al., 1988; Van Oss, 1997). 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) ΔG_{iwi}: If the interaction between the two entities is stronger than the interaction of each entity with water $\Delta G_{iwi} < 0$ the material is considered hydrophobic. Conversely, if $\Delta G_{iwi} > 0$ the material is hydrophilic. ΔG_{iwi} can be calculated through the surface tension components of the interacting entities, according to:

$$\Delta G_{iwi} = 2\gamma_{iw} = -2\left[\left(\left(\gamma_i{}^{LW}\right)^{1/2} - \left(\gamma_w{}^{LW}\right)^{1/2}\right)^2 + 2\left(\gamma_i{}^{+}\gamma_i{}^{-}\right)^{1/2} + \left(\gamma_w{}^{+}\gamma_w{}^{-}\right)^{1/2} - \left(\gamma_i{}^{+}\gamma_w{}^{-}\right)^{1/2} - \left(\gamma_w{}^{+}\gamma_i{}^{-}\right)^{1/2}\right]$$

2.4. Adhesion experiments and counting adhered cells using the plate count method

Ten millimeters of bacterial suspension containing 10^8 CFU/ml