Original article

Adhesion of *Listeria monocytogenes* to materials commonly found in domestic kitchens

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Summary The aim of this work was to investigate the adhesion of *Listeria monocytogenes* ATCC 15313 to glass, granite, marble, polypropylene from a bowl (PPb), polypropylene from a cutting board (PPcb) and stainless steel (SS), which are materials commonly used in kitchens. Marble and granite were chosen because they are applied as kitchen bench covers and pavements in many countries and there are no literature reports on their behaviour in terms of microbial adhesion. The effect of surface hydrophobicity and roughness on the adhesion process was also analysed. The results showed that the highest extent of adhesion of *L. monocytogenes* occurred to stainless steel, followed by glass and in less extent to the other materials studied. However, it was not possible to establish a correlation between surface hydrophobicity or roughness and the extent of adhesion of *L. monocytogenes*. The adherence of *L. monocytogenes* should be dependent on other factors, like the presence of exopolymers and surface charge.

Keywords Adhesion, hydrophobicity, kitchen materials, *Listeria monocytogenes*, roughness.

Introduction

Food preparation can lead to the cross-contamination of many sites in kitchens by bacteria. Whyte *et al.* (2004) reported many food products including pork, lamb, beef and seafood as being significant sources of bacterial contamination. In Portugal, there are many products such as milk, meat, fish, flour and fresh cheese, which can be sources of foodborne pathogens, mainly *Listeria monocytogenes* (Mena *et al.*, 2004). When preparing food, any pathogen present can contaminate cooking utensils, such as the chopping board and knife, the food-processing surfaces and the equipment used to clean the surfaces, such as a dish cloth (Beumer & Kusumaningrum, 2003). The adhesion of bacteria to these materials is a potential source of contamination that may lead to the transmission of diseases. It is well known that even with acceptable disinfection procedures bacteria can remain on equipment surfaces (Dunsmore *et al.* (1981); Austin & Bergeron, 1995). It can therefore be concluded that domestic and industrial kitchens are an important source of foodborne infections.

Listeria monocytogenes is a foodborne pathogen of significant concern to the food industry (Wong, 1998). If ingested, it can cause infection leading to septicaemia, meningitis, abortions and stillbirths. An outbreak in California during 1985, involved 142 cases with forty-eight deaths (ICMSF, 1996). *Listeria monocytogenes* is able to grow at refrigeration temperatures (as low as −1.5 °C) and in environments of reduced water activity (Mena *et al.*, 2004), in salt concentrations up to 30% and at pH values below 5.0 (Bacon & Sofos, 2003). These characteristics contribute to its survival under conditions usually used to control the growth of pathogens in food.

The purpose of this study was to compare the adhesion ability of *L. monocytogenes* ATCC 15313 to granite and marble, two materials commonly used in kitchens of many countries, which are not referenced in the literature with other materials usually studied such as glass, polymers and stainless steel.

Materials and methods

Media and growth conditions

Listeria monocytogenes ATCC 15313 was grown in brain heart infusion agar (BHI; Merck, Germany) prepared according to the manufacturer's instructions. Then, *L. monocytogenes* was grown in 10 mL of trypticase soy broth (TSB; Merck) at 25 °C for 24 h. An aliquot of 100 μL of this culture was transferred to 30 mL of TSB and incubated overnight at 25 °C. The culture was then centrifuged at 4000 ***g*** at 4 °C for 15 min. The cell pellet was resuspended in a saline solution (0.9% NaCl

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prepared in distilled water) to a concentration of 1×10^8 cells mL^{-1} ($OD_{600\ nm} \cong 0.3$). These cell suspensions were used in the subsequent adhesion assays.

For contact angle determinations, a cell suspension was incubated overnight at 25 °C and the cells were harvested by centrifugation (at 4000 ***g*** at 4 °C for 15 min), washed twice in phosphate-buffered saline (PBS; pH = 7.0), twice in deionised water and resuspended in deionised water to a concentration of 1×10^8 cells mL^{-1}.

Materials

The materials assayed were glass, granite 'Pedras Salgadas' (Boticas, Portugal), white marble 'Sivec' (Greece), polypropylene from a kitchen bowl (PPb) and also from a cutting board (PPcb) and stainless steel 304 2R (SS). The materials were cut in sections of 3.0 cm × 2.0 cm. Prior to the tests, the sections were carefully washed in a 0.2% commercial detergent solution (Sonazol Pril, Alverca, Portugal) followed by immersion in 70% ethanol and then in sterile water.

Bacterial and substratum hydrophobicity

Hydrophobicity was evaluated through contact angle measurements and by using the approach of van Oss *et al*. (1987, 1988, 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:

$$\Delta G_{iwi} = -2\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 + 4\left(\sqrt{\gamma_i^+\gamma_w^-} + \sqrt{\gamma_i^-\gamma_w^+} - \sqrt{\gamma_i^+\gamma_i^-} - \sqrt{\gamma_w^+\gamma_w^-}\right)$$

where γ^{LW}accounts for the Lifshitz-van der Waals component of the surface free energy and γ^+ and γ^- are the electron acceptor and electron donor parameters, respectively, of the Lewis acid–base component (γ^{AB}), with $\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-}$.

The surface tension components of a solid material are obtained by measuring the contact angles of three pure liquids (one apolar and two polar), with well-known surface tension components, followed by the simultaneous resolution of three equations of the form:

$$(1 + \cos\theta)\gamma_l^{TOT} = 2\left(\sqrt{\gamma_i^{LW}\gamma_l^{LW}} + \sqrt{\gamma_i^+\gamma_l^-} + \sqrt{\gamma_i^-\gamma_l^+}\right)$$

where θ is the contact angle and $\gamma^{TOT} = \gamma^{LW} + \gamma^{AB}$, with the subscript *i* referring to the surface assessed (material or bacteria) and *l* to the liquid used in the contact angle measurements.

Contact angle measurement

Contact angle measurements (at least twenty-five determinations for each liquid and for each material and micro-organism) were performed by the sessile drop technique on bacterial layers and on the materials, using a contact angle measurement apparatus (model OCA 15 Plus; DataPhysics Instruments GmbH, Germany). All the measurements were performed at room temperature (20 °C). In the case of bacterial cells, the measurements were performed on bacterial layers deposited on membrane filters, according to the method described by Busscher *et al*. (1984). Briefly, a suspension was filtered through a 0.45-μM cellulose nitrate membrane to obtain a thick homogeneous cell layer. The membrane was air-dried, until the so-called plateau contact angle was attained. The three liquids used were ultra-pure water, α-bromonaphtalene and formamide, both of analytical grade. Their surface tension components were obtained from the literature (Janczuk *et al*., 1993).

Surface topography

Atomic force microscopy (AFM) was used to perform quantitative measurements of surface topography and roughness. The measurements were carried out with a Nanoscope III controller from Digital Instruments (DI), in the tapping mode under ambient conditions. The *Ra* value (which is the arithmetical mean deviation of the profile) is the most commonly used descriptor of surface roughness (Verran *et al*., 2000). AFM scans were made over 10 μm × 10 μm areas on each surface. Surface roughness was calculated from three different readings on each surface.

Adhesion assays

Sections (2×2 cm^2) of each material were placed in a six-well tissue culture plates containing 5 mL of a suspension of 1×10^8 cells mL^{-1} in saline solution. Initial adhesion to each substratum was allowed to occur for 2 h at 25 °C, in a shaker rotating at 120 r.p.m. Negative controls were obtained by placing the material squares in a saline solution without bacterial cells. The squares were then gently transferred to 100-mL glass beakers containing distilled water, and were allowed to rest there for approximately 10 s. After this, a new transfer was made to a different 100-mL glass beaker with distilled water, followed by a third transfer 10 s later. These washing steps were carefully performed in order to remove only the cells that were suspended in the

liquid interface formed along the surface, and to minimise cell detachment from the surface (Cerca *et al.*, 2004). The substrate squares with adhered cells were dried at 37 °C. All experiments were performed in triplicate, with three repeats.

Image analysis

Before image observation and enumeration of adhered cells, the dried substrate squares were stained with a 0.01% 4′,6-diamino-2-phenylindole solution (DAPI; Sigma-Aldrich Inc., Sintra, Portugal), for better image contrast. After 30 min, each square was rinsed with distilled water in order to remove excess stain, left to air dry and kept in the dark. Direct bacterial counts were performed using an epifluorescence microscope (Zeiss, Germany) coupled to a three color capture device (CCD) video camera (Axiocam HRC, Zeiss, Germany) that acquired images with 820 × 560 pixels resolution at a magnification of 1000×. With this magnification 1 cm^2 was equivalent to 1.24×10^4 captured images (as determined by a Neubauer chamber). For each surface analysed, fifty images were taken. Cells were counted using automated enumeration software (Sigma Scan Pro, Chicago, IL, USA).

Statistical analysis

The resulting data were analysed using the Statistical Package for the Social Sciences Software (SPSS, Inc., Chicago, USA). The comparison was performed through a one-way analysis of variance (ANOVA) by applying the Bonferroni analysis as a post-hoc test (Benjamini & Hochberg, 1995). All tests were performed with a confidence level of 95%.

Results and discussion

Contact angles (in degrees) and the degree of hydrophobicity of the tested materials (ΔG_{iwi}) are shown in Table 1. The values of the water contact angle of *L. monocytogenes* (25.9°) is in agreement with those reported by Sinde & Carballo (2000) (25–25.7°), Mafu *et al.* (1991) (26.3°) and Absolom (1988) (25°).

Water contact angles of the materials were statistically different ($P < 0.05$), with the exception of granite and glass ($P > 0.05$). Both types of polypropylene displayed the highest values followed by SS, marble and granite. Using the approach of van Oss *et al.* (1989), it is possible to determine the absolute degree of hydrophobicity of any substance (1) vis-à-vis water (w), which can be precisely expressed in applicable SI units. According to this criterion, *L. monocytogenes* is hydrophilic and all the materials, with the exception of granite (ΔG_{iwi} = 8.1 mJ m^{-2}), are hydrophobic. The surface of the cutting board was the most hydrophobic one, while granite was the least hydrophobic material studied.

Table 1 Contact angle (in degrees) and the materials degree of hydrophobicity, ΔG_{iwi}, in mJ m^{-2}

Bacterium material	θ_W	θ_F	$\theta_{\alpha\text{-B}}$	ΔG_{iwi}
Listeria monocytogenes	25.9 ± 2.0	29.3 ± 1.7	32.9 ± 1.5	32.3
Glass	49.7 ± 2.0	22.0 ± 2.2	34.2 ± 1.3	−14.8
Granite	49.2 ± 2.3	43.0 ± 2.6	32.7 ± 2.3	8.1
Marble	76.0 ± 2.7	53.8 ± 3.0	28.0 ± 1.2	−48.4
Polypropylene (kitchen bowl)	109.6 ± 3.5	105.7 ± 3.5	58.4 ± 3.2	−30.9
Polypropylene (cutting board)	95.5 ± 3.6	79.6 ± 2.0	43.7 ± 2.8	−56.3
Stainless steel 304	81.3 ± 2.6	62.5 ± 3.3	35.5 ± 2.5	−52.2

θ_W, contact angle of water; θ_F, contact angle of formamide; $\theta_{\alpha\text{-B}}$, contact angle of α-bromonaphthalene.

It is well known that surface roughness impedes hygiene and cleaning procedures. The most common parameter used to define surface roughness is *Ra* and it is generally known that an increase in surface *Ra* value will cause a corresponding increase in microbial retention on that surface (Whitehead *et al.*, 2004). This has been confirmed by the work of several authors (Yamauchi *et al.*, 1990; Verran *et al.*, 1991; Tebbs *et al.*, 1994), which showed that the greatest extent of adhesion occurred to surfaces with *Ra* values ranging from 1.12 to 1.29 μm. This increase in adhesion may be because of the protection afforded to the cells from shear forces by microscopic niches (Quirynen & Bollen, 1995). According to Flint *et al.* (1997) *Ra* values of 0.8 μm or less are generally used to describe a hygienic surface. From the values of Fig. 1, it can be concluded that all the materials assayed are hygienic surfaces. SS is the material with the highest surface roughness, with a value almost double that of the *Ra* for granite. The results for the polymers were very similar and marble and glass displayed the lowest roughness. These results are illustrated by the images obtained by AFM observation of the surface of the materials studied (Fig. 1). The adhesion results clearly demonstrate that SS 304 and glass are more prone to bacterial colonisation than the other materials as can be observed in the fluorescence microscopic images displayed in Fig. 2. The average values and the corresponding standard deviation of *L. monocytogenes* adhered to each type of material are presented in Fig. 2. The number of cells adhered to SS 304 and to glass were statistically different from the number of cells adhered to the other materials ($P < 0.05$). However, between SS 304 and glass no statistical difference was encountered ($P > 0.05$). Beresford *et al.* (2001) studied the adherence of *L. monocytogenes* to several materials such as, SS, polymers, metals and rubbers. They verified that the extent of

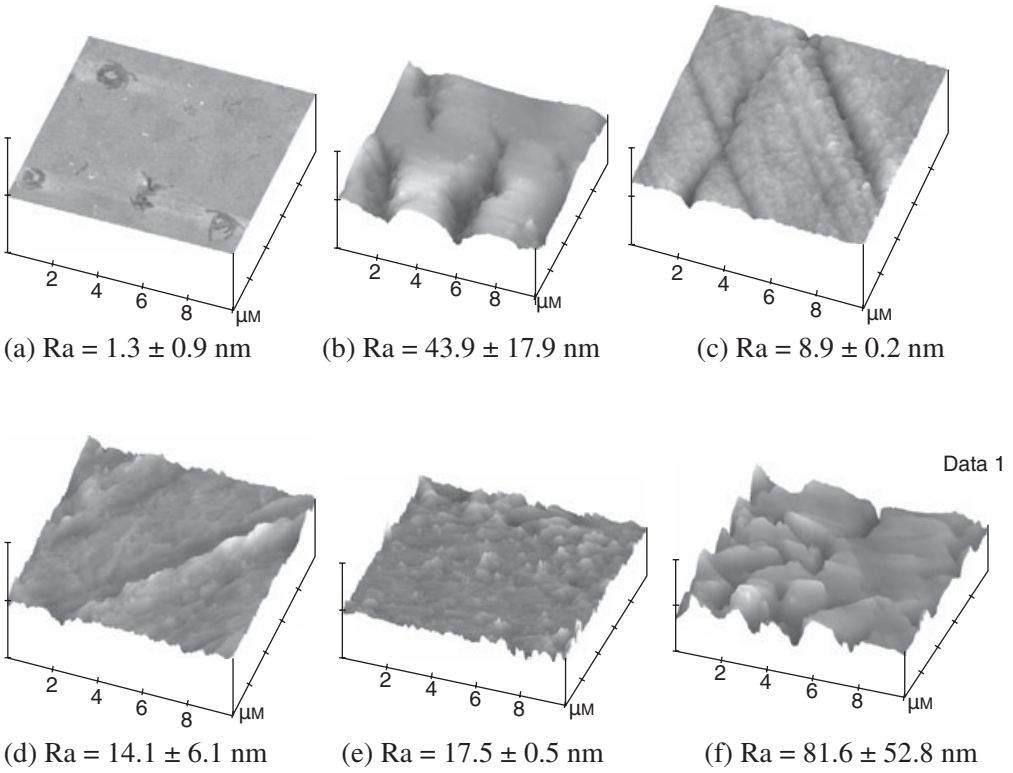

Figure 1 Atomic force microscopic three-dimensional images (surface topography) of: (a) glass, (b) granite, (c) marble, (d) polypropylene from a bowl, (e) polypropylene from a cutting board and (f) stainless steel (*X*, 0.2 µm div^{-1}; *Z*, 500 nm div^{-1}) and the respective mean roughness values (*Ra*) in nm.

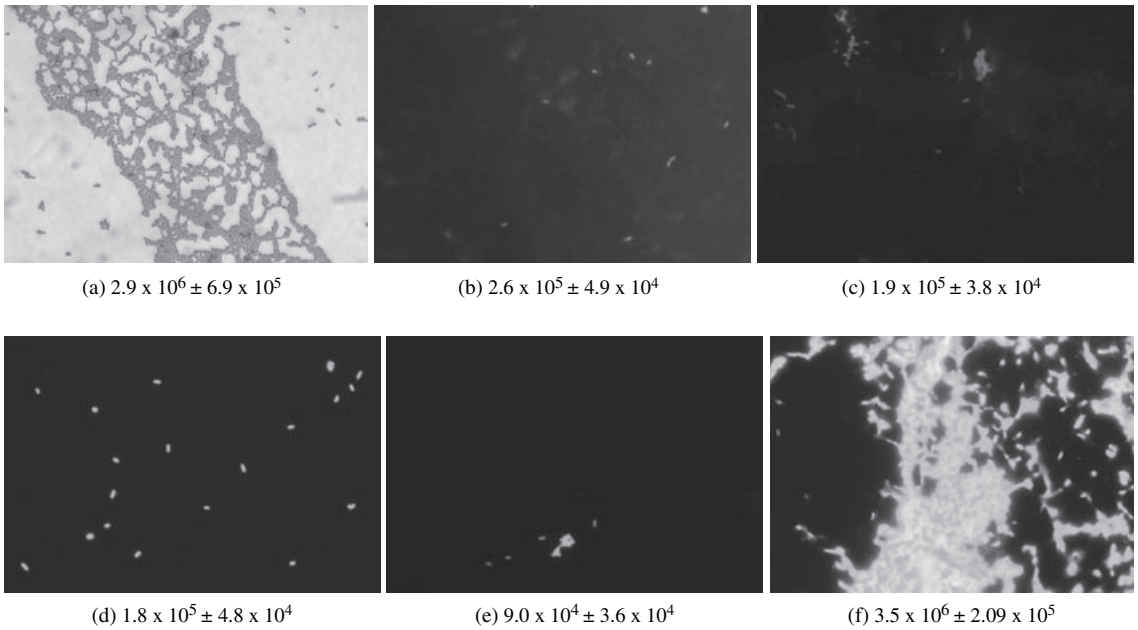

Figure 2 Images obtained by epifluorescence microscopy of cells of *Listeria monocytogenes* adhered to: (a) glass, (b) granite, (c) marble, (d) polypropylene from a bowl (e) polypropylene from a cutting board and (f) stainless steel (magnification of 100×) and the number of adhered cells per cm^2 average.

adhesion, like in the present work, was higher on SS than on polypropylene.

From this research no correlation was found between the number of cells that was adhered to the different materials and their surface properties. In fact, adhesion occurs to a great extent to the material with higher roughness (SS) and with similar extent to the smoothest material (glass). Probably, the higher number of adhered cells to SS is because of its surface topography as bacteria can be entrapped in the valleys (Fig. 1a). In the case of glass, its greater smoothness (Fig. 1f) provides a higher contact surface with bacteria favouring adhesion. As far as the effect of hydrophobicity is concerned, it can be seen that adhesion is higher to a relatively hydrophobic material and to an almost hydrophilic one, but because of their different roughness hydrophobicity might not be the determinant factor. Several authors had the same difficulty in establishing a relationship between surface properties and the extent of adhesion (Mafu *et al*., 1991; Reid *et al*., 1994; Flint *et al*., 1997; Sinde & Carballo, 2000); therefore, other factors, such as surface charge and the presence of exopolymers should also be taken into account, likewise the listerial adhesins (Beresford *et al.,* 2001).

When concerning the colonisation by *L. monocytogenes*, SS seems to be the less advisable material to be used in kitchens. However, it must be noted that this happens when dealing with new materials. Regarding 'used' materials, the formation of craves, fissures and other surface imperfections can significantly change the ability of those materials in terms of bacterial adhesion (data not shown). Nevertheless, granite and marble are materials less prone to develop worn out defects than polymers and SS and thus less susceptible to microbial colonisation.

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