Increased resistance to detachment of adherent microspheres and Bacillus spores subjected to a drying step

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Abstract

In various environments, including that of food processing, adherent bacteria are often subjected to drying conditions. These conditions have been shown to result in changes in the ability of biofilms to cross-contaminate food in contact with them. In this study, we investigated the consequences of a drying step on the further ability of adherent bacterial spores to resist detachment. An initial series of experiment was set up with latex microspheres as a model. A microsphere suspension was deposited on a glass slide and incubated at 25, 35 and 50 °C for times ranging from 1 h to 48 h. By subjecting the dried slides to increasing water flow rates, we showed that both time and temperature affected the ease of microsphere detachment. Similar observations were made for three Bacillus spores despite differences in their surface properties, especially regarding their surface physicochemistry. The differences in ease of adherent spore detachment could not be clearly linked to the minor changes in spore morphology, observed after drying in various environmental conditions. In order to explain the increased interaction between spheres or spores and glass slides, the authors made several assumptions regarding the possible underlying mechanisms: the shape of the liquid bridge between the sphere and the substratum, which is greatly influenced by the hydrophilic/hydrophobic characters of both surfaces; the accumulation of soil at the liquid/air interface; the presence of trapped nano-bubbles around and/or under the sphere.

1. Introduction

Pathogenic bacteria are commonly found to be associated with equipment surfaces in various industrial environments, including the agro-food sector where they are considered a major source of food contaminants, entailing both economic and health consequences. According to Haeghebaert et al. [1], equipment contamination would have been behind around 40% of food poisoning in France between 1996 and 1998. More recently, according to the French Institute for Public Health Surveillance, 62% of food infections in collective catering are induced by contamination of equipment surfaces (http://www.invs.sante.fr/content/download/36247/175238/version/2/file/tiac_donnees_2010.pdf). Similar observations have been made in homes, where kitchen surfaces are frequently contaminated [2–4]. Indeed, despite cleaning and disinfection procedures, some bacteria are still commonly found on the surfaces of food processing lines, mostly in the form of adherent spores, e.g. Bacillus spores in closed equipment [5,6] or in the form of biofilms, e.g. those partially composed of Pseudomonas spp. [7,8].

It has been shown that bacterial resistance to detachment is strongly affected by the surface properties of bacteria. In the case of Bacillus cereus spores for example, various works have described the role played by their hydrophobic nature [9–11], the presence of appendages [12] and the spore size [13]. The role of substratum properties has also been extensively investigated [14,15]. Conversely, the influence of conditions encountered by adherent bacteria between the contamination phase and the hygiene procedure, during which surface drying could occur, has been poorly investigated. However, many bacteria are able to withstand periods of desiccation, whether in the form of biofilms [16], or that of adherent cells [3] and spores [17]. Moreover, given the great variability of food contact surfaces, including utensils, cutting boards or closed equipment, adherent bacteria face a wide variety of environments and temperatures. One may rightfully wonder whether this drying phase might significantly influence the further resistance of bacteria to detachment. The few works relating to the drying of adherent
micro-organisms for extensive periods have mainly focused on cross-contamination between foods and surfaces [3,18,19]. Bacillus spores are of concern to the food industry as they are readily isolated from foods [20,21], highly resistant to heat-treatment and disinfection procedures [22], and often associated with food-borne diseases [23]. Moreover, Bacillus spores, particularly spores from strains belonging to the B. cereus group, firmly adhere to a wide variety of inert surfaces, such as those found on food processing premises [10,24]. Their resistance to cleaning procedures has been also reported [13,25]. The ability of Bacillus spores to adhere and to resist cleaning procedures is affected by both surface morphology and physico-chemistry, such as spore hydrophobicity [9,11], the presence of appendages or the size of the spores [11].

This study was designed to evaluate the influence of conditions encountered before the detachment of adherent spores (time and temperature of the drying phase) on their further ease of removal from the contaminated surfaces. As the spore surface properties are possibly affected by the conditions encountered during the drying step, which in turn might lead to changes in the interac-

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Spore length (µm)</th>
<th>Spore width or microsphere diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h–25 °C</td>
<td>48 h–50 °C</td>
</tr>
<tr>
<td><strong>B. cereus 98/4</strong></td>
<td>Average value</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>(standard deviation)</td>
<td>(0.24)</td>
</tr>
<tr>
<td><strong>B. subtilis 98/7</strong></td>
<td>Average value</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>(standard deviation)</td>
<td>(0.12)</td>
</tr>
<tr>
<td><strong>B. pumilus 98/6</strong></td>
<td>Average value</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>(standard deviation)</td>
<td>(0.08)</td>
</tr>
<tr>
<td><strong>Microspheres</strong></td>
<td>Average value</td>
<td>–</td>
</tr>
<tr>
<td><strong>6 µm</strong></td>
<td>(standard deviation)</td>
<td>–</td>
</tr>
</tbody>
</table>

**Fig. 1.** Left column: transmission electron microscopy of Bacillus spores after negative staining. B. cereus 98/4 are surrounded by an exosporium (white arrow) and with appendages (black arrows), B. subtilis 98/7 spores are surrounded with a mucous layer (white arrow), and the surface of B. pumilus 98/6 spores are devoid of any additional layer/material. Middle and right-hand columns: scanning electron microscopy of the same spores (Scale bars = 2 µm).
tion forces between spores and substrata, both *Bacillus* spores and latex microspheres were used as models. In order to investigate the underlying mechanisms, some additional experiments were performed to monitor the dynamics during drying of the liquid bridge at the interface between a sphere and the substratum, on which the sphere was placed.

2. Material and methods

2.1. *Bacillus* spores and microspheres

*B. cereus* 98/4 (highly hydrophobic, medium-sized spores), *Bacillus subtilis* 98/7 (hydrophilic, medium-sized spores), and *Bacillus pumilus* 98/6 (hydrophobic, small spores) were used in this study. Spores were produced as previously described [26]. Before each experiment, two further washes were performed and spores were subjected to a 2.5-min ultrasonication step in an ultrasonic cleaner (Branson 2510E-MT, 42 kHz, 100 W, Branson Ultrasonics Corporation, USA) to limit the presence of aggregates. Other experiments were performed with fluorescent latex microspheres, moderately hydrophobic, of 0.2 μm, 0.5 μm, 1 μm and 6 μm in diameter, which were washed twice in sterile water (Polybeads® Dyed Yellow Microspheres, Polysciences Inc.).

2.2. Influence of drying conditions on spore/microsphere size and morphology

Spores and microspheres deposited on glass slides were observed by scanning electron microscopy (SEM) after 2 h at 25 °C and after 48 h at 50 °C, after coating with gold–palladium for 1.5 min. Sample observations were performed through a Hitachi S–3000 SEM (Hitachi, Tokyo, Japan) operating at 15 kV. The spore length and width were measured on at least 70 individual spores and the microsphere diameter obtained by measuring over 40 individual beads.

2.3. Spores and microspheres adhesion and detachment

Hydrophilic (water contact angle, \( \theta = 11 \)) glass coupons (Superfrost®, ThermoScientific, 70 mm x 65 mm) were used in this study. These coupons were fouled by 150 μl of a spore or microsphere suspension. The suspension volume was spread onto the
3. Results

3.1. Spore/microsphere size and morphology

We first investigated whether the drying conditions would affect spore/microsphere size and morphology. For this purpose, spores and microspheres were rapidly observed under scanning electron microscopy without any dehydration steps in order to limit further changes in size (Fig. 1, middle and right-hand columns). As shown in Table 1, the length and width of the Bacillus spores were hardly affected by the drying conditions: the observed differences between drying conditions generally being below 2%. The length of B. cereus 98/4 spores was the only parameter which seemed to be affected by the temperature and/or time of the drying step, but astonishingly, spores were somewhat longer (about 7%) but not wider at high drying temperature. Concerning the microspheres (average values and standard deviations obtained from +/−40 microspheres), similar diameter values were obtained after both drying procedures.

We also investigated whether spore surface could be affected by the different drying conditions. As observed through transmission electron microscopy (TEM, Fig. 1, left column), B. cereus 98/4 spores are surrounded by an outermost membrane called exosporium (white arrow) and with numerous appendages of 10 nm in width (black arrows). After drying (Fig. 1, middle and right-hand columns), B. cereus 98/4 spores exhibited an intact exosporium even after 48 h at 50 °C. B. subtilis 98/7 spores, which are surrounded by a more or less regular mucous layer (left column, white arrow), also seemed only slightly affected by the drying conditions, the surface being less regular at 50 °C than at 25 °C. Lastly, the surface of B. pumilus 98/6 spores, which are devoid of any additional layer/material, as well as the 6-µm microspheres (data not shown) seemed relatively unaffected by the environmental conditions during the drying step.

3.2. Microsphere resistance to detachment

Experiments in the parallel-plate flow chamber were performed at mean wall shear stresses of 25 Pa, 70 Pa, 140 Pa, and 260 Pa. As shown in Fig. 2A, a very limited microsphere detachment occurred at 25 Pa, even when contaminated coupons were kept at 25 °C. At higher shear stresses, the detachment increased and the resistance of the 6 µm-microspheres was greatly affected by the environmental conditions. When the microspheres were maintained at 25 °C, over 90% were detached at a shear stress of 260 Pa. At higher temperatures, the number of residual microspheres increased.

Variance analyses were performed to investigate the role of drying time and temperature on the microsphere detachment at 20 Pa (data not shown) and 260 Pa. As expected, temperature and time significantly affected the resistance of microsphere to high wall shear stresses (p value < 0.0001) but not to lower shear stress (p value = 0.1877). For example, the parameters jointly accounted for 67% of the whole variability observed at 260 Pa. Tukey’s grouping (Table 2) showed that the percentages of residual microspheres after the rinsing step at 260 Pa, was lower when soiled coupons were kept at 25 °C (Group B) than at 35 °C or 50 °C (Group A). Concerning the duration of the drying period, detachment of adherent microspheres was the easiest after only 1 h (Group C) and the most difficult after 48 h (Group A).

In order to investigate any impact of the size of the objects on the ease of removal, further experiments were performed with microspheres of different diameters (0.2 µm, 0.5 µm, 1 µm, 3 µm, 6 µm) dried for 1 h at 35 °C. The following wall shear stresses used for this set of experiments were 17 Pa, 60 Pa, 130 Pa, 191 Pa and 360 Pa. The diameter of the adherent microspheres were found to strongly impact their resistance to flow (Fig. 2B). Whatever the

Fig. 3. Shape of a liquid bridge between a 6 mm-sphere and a substratum with different wetting properties. θp = water contact angle of the particle surface. θs = water contact angle of the substratum surface.

coupon surface so that it covered the area analysed during the detachment step. The coupons were then dried at 25 °C, 35 °C or 50 °C for between 1 h and 24 h (Bacillus spores) or 48 h (microspheres). The concentration of the spore/microsphere suspension was chosen to allow the adhesion of several hundred spores per microscope field.

In order to monitor adherent spores/microspheres’ resistance to detachment, the contaminated coupons were inserted into a parallel-plate flow chamber with a rectangular flow channel (60 mm length by 4-mm width and 0.5-mm height), specially designed to withstand very high flow rates [26]. The flow channel was covered with a glass coverslip to allow the direct observation of spore/microsphere detachment under an optical microscope (Axioskop 2 plus, Zeiss). Observations were performed at a magnification of ×400. Images were recorded by camera (Olympus, DP21).

The contaminated coupons were subjected to 30-second steps of increasing flow rates of deionised water at room temperature. Images were acquired at T0 and at the end of each detachment step. The number of adherent spores before and after the rinsing step was measured, and the percentage of residual spores after rinsing was calculated (at least four replicates).

2.4. Influence of hydrophobic/philic properties on the liquid interface around a particle

In order to show the role played by the hydrophilic/hydrophobic property of surfaces in contact, on the liquid organisation, a sphere was positioned on a water droplet, deposited on a microscope slide and the shape of the liquid bridge made recorded with a CMOS-EOS 40D Canon camera (Fig. 3). Both sphere and slide surfaces were chemically-treated to be either hydrophilic (θ = 10°) or hydrophobic (θ > 100°).

2.5. Analysis of data and statistical analysis

Data were analysed by general linear model procedures using SAS V8.0 software (SAS Institute, Cary, NC, USA). Variance analysis was performed first to determine the role of temperature and time on the ease of removal 1) of adherent latex microspheres, 2) of adherent Bacillus spores. These analyses were followed by multiple comparison procedures using Tukey’s test (Alpha level = 0.05).
Table 2

Influence of drying conditions (temperature and time) on the percentage of residual adherent microspheres (mean values) after detachment at a shear stress of 260 Pa.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Percentage of residual adherent microspheres</th>
<th>Tukey Grouping*</th>
<th>Time</th>
<th>Percentage of residual adherent microspheres</th>
<th>Tukey Grouping*</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 °C</td>
<td>6.4</td>
<td></td>
<td>1 h</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>35 °C</td>
<td>25.3</td>
<td>A</td>
<td>3 h</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>50 °C</td>
<td>21.0</td>
<td>A</td>
<td>5 h</td>
<td>16.8</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>24 h</td>
<td>25.7</td>
<td></td>
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<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>48 h</td>
<td>27.6</td>
<td></td>
</tr>
</tbody>
</table>

* Tukey grouping. Groups with common letters are not significantly different.

shear stress, less than 5% detachment was observed for the smallest microspheres (0.2 μm- and 0.5 μm-diameter). Conversely, the largest microspheres could be removed from the glass coupons and the level of residual spheres decreased with the diameter. For example, after 30 s at 191 Pa, only 10% of the 6-μm microspheres were still counted on the surface, compared to 47% of the 1-μm microspheres.

3.3. Spore resistance to detachment

Further experiments were performed with *Bacillus* spores at mean wall shear stresses of 25 Pa to 447 Pa (Table 3). Whatever the strain in use, spore resistance to flow was significantly higher than that of microspheres. Indeed, at the highest flow velocity used in these experiments (447 Pa vs 260 Pa for the microspheres), over 40% of the adherent spores resisted detachment when drying occurred at 35 °C or 50 °C. Even in the mildest conditions (3 h at 25 °C), between 14 and 61% of the spores were still counted after the detachment step. Furthermore, differences were observed between strains, primarily at 25 °C but also to a lesser extent in the other drying conditions. When dried at 25 °C, *B. cereus* 98/4 spores were the most resistant to detachment, while *B. pumilus* were the most resistant after 1 h and 3 h at 35 °C and 50 °C. Conversely, *B. subtilis* 98/7 spores were relatively easily removed from the surface whatever the drying conditions. Taking into account the data obtained at 447 Pa for the three strains, the three parameters: strain, time and temperature of drying significantly affected the resistance of spores to detachment (p-values < 0.0001), while the trial did not. Tukey’s grouping (Table 4) confirmed that: firstly, the three strains had different adhesive behaviours (or responses to drying); secondly, incubation at 35 °C and 50 °C resulted in a stronger interaction between spores and substratum than observed at 25 °C; thirdly, longer incubations resulted in stronger interaction forces.

4. Discussion

In previous studies conducted on PHM on *Bacillus* spore detachment during cleaning-in-place (CIP) procedures, the data analysis sometimes revealed a wide variability in the percentage of spores resistant to a CIP or a rinsing procedure [26]. Beside the various factors known to play a role in spore detachment (spore morphology, material and spore surface physicochemistry...), we hypothesized that the time between the contamination step and the detachment procedure could play a significant role in determining the ease with which adherent spores could be removed from contaminated surfaces. If that is the case, this parameter could play a key role in the efficacy of the hygiene procedures in real environments, such as the food industry, where very different environmental conditions are found. For example, bacteria are repeatedly isolated from many surfaces, such as closed (inner surfaces of pipes, valves, pumps...) or open surfaces (cutting boards, conveyor belts...), in more or less humid environments and at various temperatures (cold rooms, cheese ripening facilities, processing lines...). Consequently, the soil properties would change differently over time depending on the contaminated area. For example, adherent bacteria on splashing areas will be subjected to a rapid desiccation, whereas bacteria located on the low points of pipes or pieces of equipment are not. However, this drying step has been the subject of very few studies except regarding its consequences for bacteria transfer from contaminated surfaces to food in contact. Rodriguez and his colleagues, for instance, showed that the transfer rate of *Listeria monocytogenes* biofilms from contaminated surfaces to food decreased with drying [27,28]. They postulated that the cell-surface adhesion was weakened during drying. Other reported experiments were performed on bacterial cells directly deposited on surfaces. The same authors [27] failed to produce any evidence of the influence of a 1 h-drying step at 37 °C on the transfer rate of *L. monocytogenes* cells from stainless steel to foods. Conflicting results have been also reported with *Salmonella* and *Escherichia coli* cells, whose transfer rates to vegetables were often lowered following drying [18]. An important point to note here is that, along with the presence of a residual film of water on surfaces, desiccation could also greatly affect bacterial metabolism and surface properties that, in turn, alter the interaction between bacteria and materials [29].
Therefore, we decided to set up a series of experiments with latex microspheres, which have been shown to be poorly affected by the various drying conditions. Microspheres were spread onto the glass surfaces and dried without any washing step. This approach would mimic what happens on splashed areas, as any water is removed by evaporation. As suggested by Rodriguez and McLandersborough [27], this procedure would favour hydrophobic interaction between bacteria and the solid surfaces. The first set of experiments was performed with microspheres of 6 μm-diameter. They first exhibited a relatively high flow resistance with remaining adherent microspheres still observed after 30 s at 260 Pa. A high resistance of adherent objects to water rinsing has been previously reported in the literature. For example, the percentage of *Saccharomyces* yeasts removed from stainless steel was negligible under the threshold value of 15 Pa, above which the yeast detachment increased with the wall shear stress to reach 80% at 100 Pa [30]. According to other authors, the resistance to mechanical detachment was even more strikingly low with only 10% of yeasts detached at 80 Pa [31]. Concerning the influence of the time and temperature of drying, both parameters clearly affected the subsequent ease of removal of adherent microspheres. In general, the percentage of residual microspheres after rinsing increased with drying time. The temperature also affected interactions between microspheres and glass, but the differences observed were only significant between 25 °C and 35 °C.

Microsphere properties being relatively impervious to the drying conditions used in this study, it can be assumed that interactions between microspheres and surfaces would be influenced by the amount of surrounding water, which decreases through evaporation. Along with the amount of residual liquid at the interface, the hydrophobicity of both particle and substratum surfaces may further affect interaction forces. Indeed, the shape taken by a liquid bridge between a sphere and a substrate differs greatly with the surface properties (Fig. 3). As this shape determines the pressure inside the liquid, it may therefore affect the type of force felt by the particle (attraction or repulsion to the substrate).

Another phenomenon suspected of influencing interactions during the drying process is that the triple contact line that determines the frontier between gas, solid and liquid may accumulate soils or submicron particles (e.g. food residues) on wetting surfaces. This may give rise to the formation of commonly called ‘coffee stains’ [22]. Indeed, depending on the evaporation rate, nanoparticles can accumulate on liquid-drying areas [33]. The accumulation of these submicron particles near and around a spore can greatly contribute to changes in the interaction force. Fig. 4 highlights this effect at the contact point between a 6 mm-diameter sphere and a microscope slide. After the evaporation of a deionized-water bridge, the accumulation of soil trapped by the liquid bridge is clearly observed. The position of these accumulated submicron particles would also strongly depend on the wetting characteristics of both the particle and the substratum.

Lastly, the authors believe that, as observed on hydrophobic surfaces [34], nano or micro bubbles may form around (or under) dried hydrophobic spheres or spores when they are wetted. The presence of such trapped bubbles may greatly increase the required detachment force, since the spore contact point with the substrate may then consist of multiple liquid bridges. The effect of the surrounding environment on the adhesion force is currently being investigated.

We then investigated the behaviour of *Bacillus* spores, which are very stable in response to various environments. Previous works have shown that drying could result in significant changes in the *Bacillus* spore morphology. The dimension of the *Bacillus atrophaeus* spores, for example, significantly decreased with air-drying (by around 12%) and concomitant modifications of the spore coat morphology were clearly observed by atomic force microscopy (AFM) [35]. Other observations indicated that spores of *Bacillus thuringiensis* would swell or shrink in response to a change in relative humidity [36]. In this study, we noted only minor changes in the spore size following the various drying procedures. It is unlikely that these changes would account for the significant changes observed in the spore resistance to detachment. We also examined whether the spore surface features were affected by the drying step. Indeed, spores from strains belonging to various *Bacillus* species are surrounded by different external layers and sometimes by appendages. *B. subtilis* spores, surrounded by a mucous layer and *B. pumilus* spores, devoid of any surface features [31] were only slightly if not at all affected, by the drying conditions. *B. cereus* 98/4 spores are surrounded by a balloon-like layer called an exosporium, which is loosely bound to the underlying spore. In liquid environments, fluid is adsorbed through the exosporium, which becomes swollen as shown on spore sections of fully-hydrated spores observed by TEM [11,37]. Conversely, the exosporia of air-dried spores collapse (Fig. 1), thereby increasing the exchange surface area between spores and substratum. However, this collapse was observed whatever the drying condition (data not shown) and therefore could not be connected to changes in interface phenomena.

These spores were then subjected to increasing shear stresses in the parallel-plate flow chamber. The phenomena observed were similar to those of microspheres in that their resistance to detachment increased with temperature and drying time, although

<table>
<thead>
<tr>
<th>Strain</th>
<th>% of residual spores</th>
<th>Tukey Grouping</th>
<th>Temperature</th>
<th>% of residual spores</th>
<th>Tukey Grouping</th>
<th>Time</th>
<th>% of residual spores</th>
<th>Tukey Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cereus 98/4</td>
<td>84.9</td>
<td>A</td>
<td>25 °C</td>
<td>56.7</td>
<td>B</td>
<td>1 h</td>
<td>71.8</td>
<td>AB</td>
</tr>
<tr>
<td>B. pumilus 98/6</td>
<td>74.5</td>
<td>B</td>
<td>35 °C</td>
<td>78.8</td>
<td>A</td>
<td>3 h</td>
<td>61.6</td>
<td>B</td>
</tr>
<tr>
<td>B. subtilis 98/7</td>
<td>61.1</td>
<td>C</td>
<td>50 °C</td>
<td>84.1</td>
<td>A</td>
<td>5 h</td>
<td>75.0</td>
<td>A</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>24 h</td>
<td>80.7</td>
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<td>-</td>
<td>-</td>
<td>48 h</td>
<td>78.8</td>
<td>A</td>
</tr>
</tbody>
</table>

* Tukey grouping. Groups with common letters are not significantly different.
spores proved significantly more resistant to removal. Almost no detachment occurred at higher temperatures and longer times, yet clear differences exist between strains. Indeed, B. subtilis spores are much less resistant to removal than the other two strains. Such differences could be accounted for by the different hydrophilic/hydrophobic character of the three spores. As we have previously shown [11], B. cereus 98/4 spores were highly hydrophilic and B. subtilis spores highly hydrophilic. B. pumilus spores have intermediate properties. However, the 6 μm-microspores are moderately hydrophobic and their ease of removal was even more marked than that of B. subtilis spores. This would imply that another parameter (or other parameters) play a major role in their resistance to shear. Among the properties known to affect the resistance of adherent objects to shear, the object’s size plays a major role under dynamic conditions [38]. The 6 μm-microspores used in this study are around ten times larger than spores. Consequently, it is likely that these spheres reach the external region of the boundary layer [26] and are subjected to the effect of large-scale motion, which might explain their poor resistance to detachment. This hypothesis is confirmed by the very high resistance to shear observed with the smallest microspores (0.5 μm and 0.2 μm).

Irrespective of the spores’ properties, the same trends applied to their resistance to detachment i.e. an increase in time and/or temperature of drying clearly resulted in a decrease in spore detachment by shear. This cannot be attributed to the presence of an exosporium, appendages or a mucous layer around the spores. This observation suggests once again the key role played by the liquid film in interface phenomena, as suggested by previous results, which showed that partial drying increased the resistance to shear of Bacillus spores [39]. However, the increase in the resistance to detachment was somewhat more pronounced in the case of B. cereus spores (e.g. when drying occurred at 35 °C). These spores being hydrophobic, one can reasonably suspect the presence of a residual liquid film (or of air bubbles) between the exosporium and the substratum, which may affect the hydrophobic interaction between spores and substratum.

5. Conclusion

As a closing remark, it is important to point out that, in most environments, many bacterial soils undergo a drying step. This is particularly true in the food industry, on open contaminated surfaces, such as conveyor belts or walls, but also at the air-liquid interface on surfaces of partly-filled equipment, including tanks, pumps, valves, or piping systems, whether during the production phase or after a hygiene procedure. According to our results, these drying conditions would result in increased resistance of adherent bacteria to detachment, which, at least in the case of bacterial spores is much higher than could have been reasonably expected. Therefore, the presence of dried bacterial soils should be a concern in the food industry.

Acknowledgements

Jean François Migdal and Laurent Waquier, from PIHM, are thanked for their valuable assistance.

References


