Colloids and Surfaces B: Biointerfaces 143 (2016) 293–300

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

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

Christine Faille[a,*], Ilyesse Bihi[b], Annette Ronse[a], Gilles Ronse[a], Michael Baudoin[b], Farzam Zoueshtiagh[b]

[a] UMR UMET: UMET, CNRS, INRA, Univ. Lille 1, 59650 Villeneuve d'Ascq, France
[b] IEMN, LIA LICS: Univ. Lille 1, UMR CNRS 8520, IEMN, 59652 Villeneuve d'Ascq, France

ARTICLE INFO

Article history:
Received 25 November 2015
Received in revised form 29 February 2016
Accepted 15 March 2016
Available online 16 March 2016

Keywords:
Bacillus spores
Adhesion
Detachment
Drying
Microspheres
Liquid bridge

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

* Corresponding author.
E-mail address: christine.faille@lille.inra.fr (C. Faille).

http://dx.doi.org/10.1016/j.colsurfb.2016.03.041
0927-7765/© 2016 Published by Elsevier B.V.

Table 1
Influence of drying conditions on the size of the adherent *Bacillus* spores and microspheres.

		Spore length (μm)		Spore width or microsphere diameter (μm)	
		2 h–25 °C	48 h–50 °C	2 h–25 °C	48 h–50 °C
B. cereus 98/4	Average value	1.64	1.74	0.80	0.82
	(standard deviation)	(0.24)	(0.19)	(0.24)	(0.04)
B. subtilis 98/7	Average value	1.49	1.55	0.75	0.75
	(standard deviation)	(0.12)	(0.09)	(0.05)	(0.05)
B. pumilus 98/6	Average value	1.12	1.14	0.62	0.60
	(standard deviation)	(0.08)	(0.10)	(0.03)	(0.03)
Microspheres	Average value	–	–	6.26	6.37
6 μm	(standard deviation)	–	–	(0.03)	(0.04)

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-

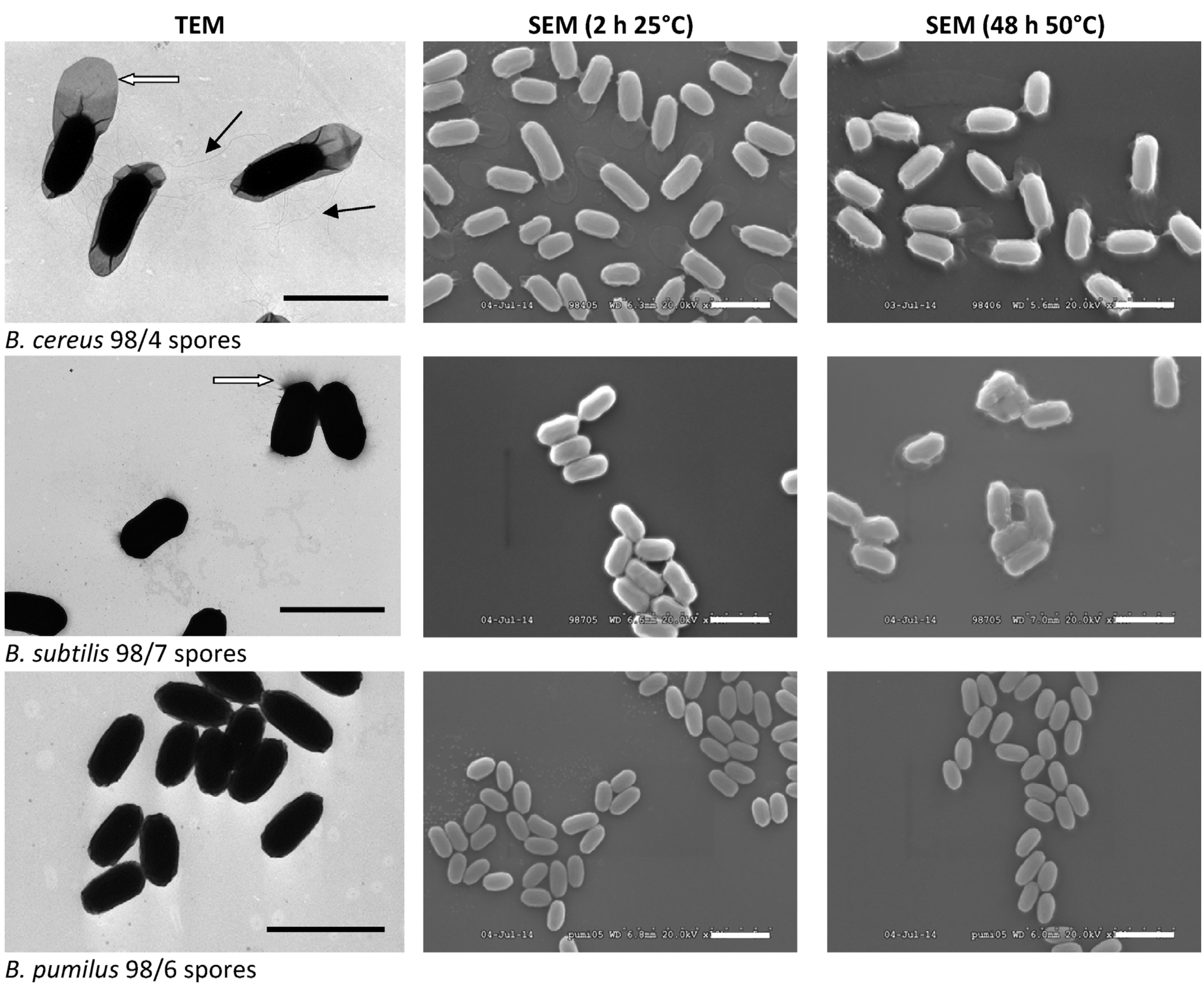

Fig. 1. Left column: transmission electron microscopy of *Bacillus* spores after negative staining. *B. cereus* 98/4 are surrounded by an exosporium (white arrow) and with appendages (black arrows), *B. subtilis* 98/7 spores are surrounded with a mucous layer (white arrow), and the surface of *B. pumilus* 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).