Short reports

Effect of desiccation on the ultrastructural appearances of *Acinetobacter baumannii* and *Acinetobacter lwoffii*

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Abstract

An *Acinetobacter baumannii* isolate survived desiccation beyond 30 days and an *Acinetobacter lwoffii* isolate up to 21 days. For both species, desiccation resulted in a significant increase in the proportion of round cells (*A baumannii*, 40% to 80%; *A lwoffii*, 51% to 63%) and a significant decrease in rod shaped cells (*A baumannii*, 58% to 13%; *A lwoffii*, 46% to 34%). Electronmicroscopic examination showed that there was also a corresponding significant increase in the cell wall thickness (*A baumannii*, up to 53%; *A lwoffii*, up to 26%). Desiccated *A baumannii* cells became more electron-dense and had significantly thicker cell walls (×1.3) than those of *A lwoffii*. Cell wall structures of *A baumannii* strains with different abilities to resist desiccation deserve further study.

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Acinetobacter baumannii, an important nosocomial pathogen, can survive desiccation for prolonged periods.[1-4] Digital image analysis of a continuous flow slide culture system of an *Acinetobacter* species revealed changes in morphology and attachment when irrigated with a poor nutrient.[5] We studied the ultrastructural appearances by electron microscopy of cells of isolates of *A baumannii* and *A lwoffii* before and after desiccation.

Methods

PREPARATION OF THE BACTERIAL SUSPENSION FOR DESICCATION

We studied an isolate of *A baumannii* (97.5% identification by API, bioMerieux) from a specimen of tracheal aspirate, and an isolate of *A lwoffii* (91.5% identification by analytical profile index) from a specimen of sputum. They were not outbreak strains. The cells of an overnight culture at 37°C in 40 ml of nutrient broth (Oxoid) were harvested by centrifugation and the deposit resuspended in 3 ml of sterile distilled water. For the desiccation experiment, 0.5 ml of the suspension was spread as a thin film on sterile Petri dishes (60×15 mm, Sterilin Laboratory). They were left in a 37°C incubator to dry for three hours and stored in a Dry Box (Toshiba) with humidity set at 45% in an air conditioned laboratory with room temperature maintained at 22–24°C. For bacterial counts at baseline (T_0) and other time intervals (T_n), the bacterial cultures in two plates were each reconstituted in 1.0 ml of distilled water with the aid of a sterile spatula. The contents were pooled, serially diluted, and enumerated on blood agar plates.

GROWTH CURVES

Growth curves of isolates before and after desiccation were obtained by using 10^2 to 10^4 colony forming units (cfu)/ml in 10 ml of nutrient broth (Oxoid, UK) as the initial inoculum. The culture was agitated at 37°C and enumerated by dilution at regular intervals.

TRANSMISSION ELECTRON MICROSCOPY

As control, we used the centrifuged deposit of an overnight culture (20 ml) in nutrient broth. To stabilise the bacterial glycocalyx, both the test and control cells were first incubated in Vicia faba lectin (Sigma) diluted in phosphate buffered saline (PBS) 20 μg/ml for 30 minutes. After a brief wash in PBS, the specimens were fixed in 2.5% glutaraldehyde for 30 minutes, postfixed in 1% OsO_4, dehydrated in acetone 30%, 50%, and 70% for five minutes and in 100% for 10 minutes, and embedded in Procure 812. Thin sections were cut with a Reichert Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Philips 100 CM transmission electron microscope using an acceleration voltage of 80 kV. For measurements, we obtained digital images with a Quantimet 500+ image analysis system (Leica Cambridge) linked to a Gatan 673 wide angle TV system (Gatan Inc). Cells with visible DNA profiles were chosen. The width (W) and the length (L) of rod shaped types and the diameter (D) of round types were measured. In addition, the shortest distance between the inner edge of visible cytoplasmic membrane and the outer edge of visible outer membrane was taken as the thickness of the wall (MT).

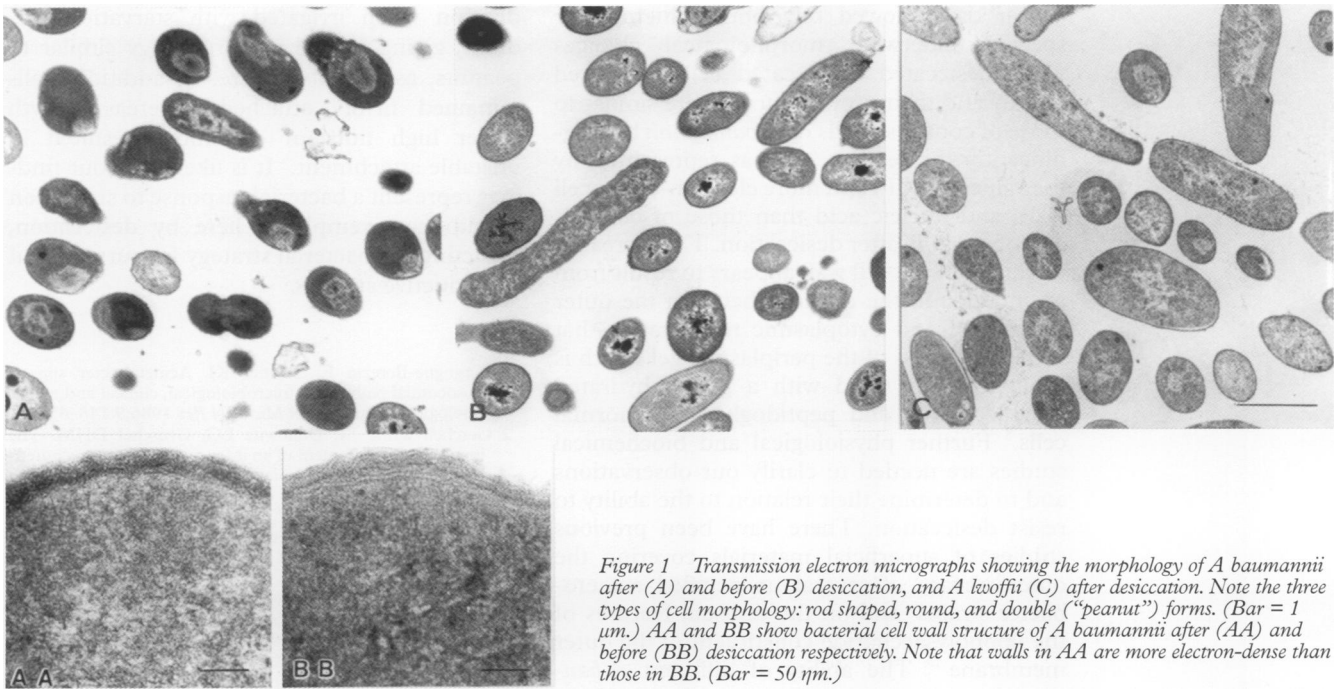

Figure 1 Transmission electron micrographs showing the morphology of A baumannii after (A) and before (B) desiccation, and A lwoffii (C) after desiccation. Note the three types of cell morphology: rod shaped, round, and double ("peanut") forms. (Bar = 1 μm.) AA and BB show bacterial cell wall structure of A baumannii after (AA) and before (BB) desiccation respectively. Note that walls in AA are more electron-dense than those in BB. (Bar = 50 ηm.)

Table 1 Comparison of the width (W), length (L), diameter (D), and cell wall thickness (MT) of rod shaped and round cells before (control) and after (test) dessication of isolates of Acinetobacter baumannii and Acinetobacter lwoffii

		Mean measurement (μm)						
Organism		*Control*	*n*	*SD*	*Test*	*n*	*SD*	*p Value †*
A baumannii								
Rod shaped	W	535.35	130	102.50	608.53	174	101.32	<0.001
	L	1597.25	92	750.92	1411.64	101	402.41	0.462
	MT	27.23	112	3.77	40.41	100	6.48	<0.001
Round	D	536.10	134	109.58	537.08	317	127.78	0.924
	MT	26.60	101	3.75	40.68	104	7.13	<0.001
A lwoffii								
Rod shaped	W	882.58	160	423.06	635.75	102	98.70	0.001
	L	1408.49	112	318.60	2181.38	118	811.77	<0.001
	MT	25.06	113	3.66	30.51	107	4.71	<0.001
Round	D	681.41	106	140.61	688.41	107	159.66	0.379
	MT	24.91	103	3.49	31.44	125	4.82	<0.001

†Two tailed p value by Mann–Whitney test.

Results

For *A lwoffii*, the viable count at T_0 was 2×10^9, at T_{17} (day 17) 3×10^6, and at T_{21} < 10 cfu/ml. For *A baumannii*, the viable count at T_0 was 3×10^9, at T_{12} 6×10^9, at T_{24} 3×10^9, and at T_{30} 1×10^9 cfu/ml. Desiccated cells of *A lwoffii* at day 17 and of *A baumannii* at day 30 were used to obtain growth curves. They appeared similar to those obtained with their respective controls.

The same batch of desiccated cells was also used for electron microscopic examination. For both species, three morphotypes were observed: the round, the rod shaped, and the double form, the last resembling peanuts (fig 1). After desiccation, for *A baumnnii*, the proportion of rod shaped cells decreased from 57.3% to 12.9% (c^2 test, $p < 0.001$; 95% confidence intervals, 6.01 to 13.75), round forms increased from 40.4% to 79.7% ($p < 0.001$; 4.03 to 8.38), and double forms from 2.3% to 7.4% ($p < 0.001$; 1.40 to 8.29). For *A lwoffii*, after desiccation, rod shaped cells decreased from 46.3% to 34% ($p < 0.001$; 1.26 to 2.23), round forms increased from 50.5% to 63.4% ($p < 0.001$; 1.28 to 2.26), but double forms remained the same (2.6% to 3.1%) (p=0.76; 0.52 to 3.00).

Compared with *A lwoffii* after desiccation, rod shaped cells of *A baumannii* were significantly shorter and the round forms significantly smaller (table 1). The cell wall thickness as represented by MT values increased by 21–26% for *A lwoffii* and 48–53% for *A baumannii* after desiccation. The MT values of *A baumannii* were significantly larger than those of *A lwoffii* before and after desiccation. Furthermore, in the majority of *A baumannii* cells, the space between the outer membrane and the cytoplasmic membrane and nucleic acid became more electron-dense, resulting in darker cells when compared with controls (see AA, BB in fig 1).

Discussion

Compared with other Gram negative rods, *Acinetobacter spp* are more resistant to dry conditions.[3 6 7] They can survive in the environment and cause nosocomial infections.[1 2] Others have also shown that *Acinetobacter calcoaceticus var anitratus* (*A baumannii*) survived better than *var lwoffii* on dry surfaces[3 4 6] and on the tips of fingers.[4] This might explain why *var anitratus* is more commonly implicated in outbreaks caused by cross infection.[4]

Our data showed that both acinetobacter species underwent morphological changes when desiccated. Desiccated cells recovered rapidly and their growth curves were similar to those of controls. Cells of *A baumannii* had significantly thicker (by 30%, as represented by the values of MT) and more electron-dense cell walls and nucleic acid than those of controls and of *A lwoffii* after desiccation. The increased thickness in the cell wall appears to result from an increase in the distance between the outer membrane and cytoplasmic membrane—that is, the thickness of the periplasmic gel which is thought to be filled with a gel of hydrated polysaccharide and peptidoglycan in normal cells.[8] Further physiological and biochemical studies are needed to clarify our observations and to determine their relation to the ability to resist desiccation. There have been previous studies of superficial materials covering the outer surface of several unspecified acinetobacter strains[9] and on the unusual features of the lipopolysaccharide constituent of the outer membrane.[10] The ability of different *A baumannii* strains to survive on dry surfaces appears to vary but correlates with the source of the organism. Strains isolated from dry sources, for example inanimate environments, survive better than those isolated from wet sources, for example urine.[7] It will be interesting to determine whether strains that survive better on dry surfaces have different cell wall contents from those that do not.

Digital image analysis of starvation responses of an acinetobacter isolate showed that rod shaped cells changed to cocci by reduction division when irrigated with starvation medium, giving rise to a morphology similar to peanuts, as described here. In addition, cells remained firmly attached, whereas growth under high nutrient conditions resulted in unstable attachment.[5] It is likely that our findings represent a bacterial response to starvation conditions, exemplified here by desiccation, reflecting the bacterial strategy for survival and reproductive success.

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