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ORIGINAL PAPER

# Pathogens as potential hitchhikers on intercontinental dust

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Abstract Desert dust seeds distant lands and waters with minerals as well as micro-organisms raising the question of whether this ancient phenomenon also spreads pathogens across the globe. Severe dust storms require strong winds blowing over land-masses that are largely devoid of vegetation, effectively limiting the scope for winds to raise pathogens into the air. Nevertheless, changing patterns of land-use, often driven by belligerency, result in refugees spreading to areas that were previously deemed barely habitable. With the help of the International Committee of the Red Cross, a number of sand/dust samples were collected from the Republic of Chad, some near refugee camps, others further removed from human influence. In parallel studies, we documented the micro-organisms present in these samples and used a number of the isolates here to test the effect of

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Department of Pure and Applied Chemistry, Carl von Ossietzky University, Carl-von-Ossietzky-Straße 9-11, 26111 Oldenburg, Germany environmental constraints on their ability to survive intercontinental flight. We also added traditional pathogens to the palette of microbes and tested the effects of UV irradiation, desiccation and temperature on survival of both bacteria and fungi. A clear trend was obvious-those microbes that are coloured or able to form conidia or spores (in other words, those that are native to deserts) were well able to resist the imposed stresses. On the other hand, most pathogens were more sensitive to stresses than the environmental isolates. Toxin production in two species of Aspergillus was also investigated. Short-term desiccation (simulating environmental conditions during intercontinental travel) of sand amended with fungal spores containing sterigmatocystin leads to increased mycotoxin contents, but significant mycotoxin production was only possible under growth-permissive conditions, e.g. at higher humidity. It thus seems likely that

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an ever-decreasing fraction of the initial pathogen load survives as the dust recedes from its desert source and that those organisms that land on other continents are highly enriched in desert dwellers.

**Keywords** Chad · Desert sand · Sterigmatocystin · *Aspergillus sydowii* · *A. versicolor* 

## 1 Introduction

Most dust that is blown around the planet originates from deserts. Sparse vegetation particularly in hot, dry deserts allows winds to pick up surface particles and carry them aloft. Surface winds cause sand grains to hop (saltate) across exposed soils. Each time they hop, saltating grains dislodge other particles. Depending on wind speed, particle size, parent soil aggregate size distribution and so on, saltating grains may eject more than one grain when they land, causing logarithmic increases (or "avalanching"-Chepil 1957) in the numbers of particles ejected (Gillette et al. 1974, 1996; Gillette and Chen 1999; Grini and Zender 2004). Saltation thus provokes sand blasting, the particle-toparticle interactions that break up soil aggregates or the crystal structures within minerals (Gillette and Chen 1999). Together, saltation and sand blasting are the key processes in wind erosion of soil and rock surfaces (Gillette et al. 1974; Alfaro et al. 1998; Gorbushina 2007). Once aloft the larger grains settle out in a continuous fractionation process. Nevertheless, the smaller grains  $<20 \,\mu\text{m}$ —Kok (2011)[and typically <10 µm (Hwang and Ro 2006; Kalderon-Asael et al. 2009)] can be carried beyond the planetary boundary layer (that ranges anywhere from 100 to 3,000 m above the surface) into the free atmosphere. Once within the free atmosphere, they may be transported over long distances.

Most soils, including those in deserts, are covered by a crust. Crusts formed by living organisms and their by-products create a friable surface of soil particles bound together by organic materials. Cyanobacteria, green as well as brown algae, mosses and lichens predominate in crusts; liverworts, heterotrophic bacteria and fungi are also frequent habitants (Belnap and Gillette 1998). Generally, crusts cover all soil spaces not occupied by vascular plants and may make up 70% or more of the living cover (USGS Canyonlands Research Station 2006). Nevertheless, crusts are thin with much of the biomass concentrated in the top several millimetres of soil (Belnap and Gillette 1998; Valentin and Bresson 1992) and they are extremely vulnerable (Belnap and Gillette 1998). As soil-crust stability is directly affected by sand blasting and saltation, particles carrying soil crusts are abundant amongst the dislodged particles of the desert dust.

Microbes require a dust-like vehicle to travel long distances (USGS 2003). Most probably, electrostatic charges are involved in microbial adhesion, and the mineral particles themselves offer a measure of protection to the microbes during travel. It is for these reasons that we have likened the long-distance transport of microbes to "hitchhikers" on dust "vehicles". Other potential "vehicles" including, for example, water droplets and plant debris (though not pollen) lack both well-defined small sizes ( $<20 \mu m$ ) and the physical stability that would permit extended periods aloft. For all these reasons, desert dust is laden with living organisms, and although the dust/microbe vehicles can pick up further hitchhikers when they cross zones of turbulence, perhaps the majority of the microbes that "hitchhike" from continent to continent were present at the desert source.

We initiated a series of studies (see Gorbushina et al. 2007) to answer the following questions: (a) who are these hitchhikers? (b) are any of them known animal or plant pathogens? (c) which of them survive intercontinental travel? and (d) what impact do the survivors have on the landing zones? Our approaches to these questions include the following: (1) enumerating the micro-organisms that are present in the crusts/sands of African deserts that are the sources of the intercontinental dust that impinges on the Americas, Europe and the Middle East; (2) sampling the dust/microbe vehicles in-flight and (3) elucidating which of those micro-organisms that commence the journey survive (and therefore "seed" the places where they land). This latter aspect of the work is particularly important given the heightened interest in the spread of animal and plant pathogens throughout the world (e.g. Griffin 2007) and will be addressed here.

How well the microbial hitchhikers and their metabolic products (especially toxins) survive intercontinental journeys, obviously influences the environmental impact on the places where they land. Stresses high in the atmosphere include UV radiation, desiccation and temperature extremes. Here, we tested these effects on the survival of a range of microorganisms as well as long-term storage (under laboratory conditions) and the stability of a mycotoxin on sand under environmental conditions that correspond to those of intercontinental flight.

## 2 Materials and methods

## 2.1 Sand

Dust/sand samples from Chad were used to compare the number of survivors at the beginning and end of 1-year storage. All samples were collected by the International Committee of the Red Cross on our behalf (see Favet et al. 2012). Quartz sand was used to test survival of added micro-organisms using the methods described in Gorbushina et al. (2007).

## 2.2 Survival in dry sand

Survival was determined following storage of desert sand samples at room temperature ( $\approx 25^{\circ}$ C) and humidity ( $\approx 40-55\%$  RH) (in closed vials, in the dark) for 1 year. To do this, the numbers of viable microorganisms (including those that form spores) of all Chad samples (C1 to C9) were enumerated using the techniques described in Gorbushina et al. (2007) which mainly involved plating-out dilutions of the samples on a variety of media, isolating single colonies and typing them using PCR-based techniques.

Our second survival test included extended periods of desiccation in sand. Micro-organisms studied included Aspergillus sydowii, A. versicolor, Bacillus megaterium isolate B13 (Gorbushina et al. 2007), Candida albicans ATCC 10231, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 90927, P. fluorescens strain CHAO, Salmonella typhimurium ATCC 13311 and Staphylococcus aureus ATCC 6538 were obtained from either commercial sources or our own collections. To test whether the micro-organisms listed above are capable of surviving extended dry periods, they were inoculated into the following media: C. albicans (Sabouraud-dextrose) and S. aureus (Baird-Parker)-all other bacteria were inoculated into nutrient broth. To reduce the amount of organic matter in the conidia/spore mixture used to inoculate the sand, A. sydowii was grown on agar plates (amounts in g  $l^{-1}$  of tap water—potato powder 50; glucose 20; agar 10). After 13-d incubation (29°C in the dark), the conidia/spores were harvested and used to inoculate the sand.

To study survival of micro-organisms on dry sand, 1 g washed quartz sand was weighed into 3-cmdiameter watch glasses and autoclaved. Then, the watch-glasses containing sand were individually transferred to multi-well plates and inoculated with 400  $\mu$ l of 10<sup>9</sup> CFU ml<sup>-1</sup> bacterial cells or fungal spores. After 0-, 1-, 2-, 3-, 4- and 8-week incubation (at 26°C for bacteria and 30°C for fungi, first week at 100% relative humidity, thereafter at 20% relative humidity), two replicate watch-glasses per strain were removed to count the numbers of viable cells.

Both survival and toxin production by *A. sydowii* and *A. versicolor* were tested on a larger scale (10 g of sand per watch glass inoculated with 4.2 ml of  $10^9$  CFU ml<sup>-1</sup> spore suspension) and sub-sampled as follows: (i) 1 g for viable counts and (ii) three  $\times$  3 g for sterigmatocystin analysis. After 1- and 8-week incubation (at 25°C, 100% relative humidity, then left to dry), two replicate watch-glasses were removed to count the numbers of viable cells and spores.

#### 2.3 Detection of sterigmatocystin in A. versicolor

One gram of the dried sand-conidia mixture was loaded onto a chromatography column (2 cm diameter × 18 cm tall) filled with 7.5 g silica gel 60 (Merck KGaA, 64293 Darmstadt, Germany) that had been deactivated with 5% water. Sterigmatocystin was eluted with chloroform (Senkpiel et al. 2000). The fractions containing sterigmatocystin were combined and evaporated to dryness using a rotary evaporator. The residue was dissolved in 100 µl chloroform and 5 µl was applied to silica gel 60 plates (Merck KGaA). The plates were developed in toluene/ethyl acetate/90% formic acid (5:4:1) (Scott et al. 1970). Spots were visualised by spraying the TLC plates with a 20% (w/v) solution of AlCl<sub>3</sub> in 96% (v/v) ethanol and heating for 5 min at 110°C (Athnasios and Kuhn 1977). The resulting green fluorescent spots were quantified with a TLC scanner (Camag, 12169 Berlin, Germany) at an excitation wavelength of 366 nm and using a 400 nm cut-off filter for emission. Calibration was performed using standard sterigmatocystin solutions on each plate. Since the data were normally distributed (Kolmogorov-Smirnov test), significant differences were sought using pooled variance t tests, and the variances were equal (Bartlett test).

Calculations were made with WinStat (version 2009.1, R. Fitch Software—In der Breite 30, 79189 Bad Krozingen, Germany).

#### 2.4 Resistance to UV radiation

## 2.4.1 Bacteria tested

Earlier, we had isolated a number of different bacteria from dust samples that had been collected by Charles Darwin amongst others (Gorbushina et al. 2007). Selections of these were used to test resistance to UV irradiation (see Table 1). We also included a mixture of pigmented and non-pigmented environmental isolates that were collected from a monolith in the Great Australian Desert [(also listed in Table 1) (pigmented bacteria are indicated by an asterisk)]. Finally, Deinococcus radiodurans ATCC 13939, one of the most radiation-resistant organisms known was included as the reference strain. Resistance to irradiation  $[100 \text{ W} \text{ m}^{-2} \text{ sec}^{-1} \text{ at } 254 \text{ nm } (\text{UVC})]$  was tested for 1, 2, 4, 8 and 10 min following the recommendations in Bergey's Manual of Determinative Bacteriology (see Garrity et al. 2005).

## 2.4.2 Preparation of spore suspensions

All sporulating bacteria were inoculated on sporulation medium (meat extract 2.4 g, peptone 2.4 g, NaCl 1.2 g, MnSO<sub>4</sub>.4H<sub>2</sub>O 0.03 g, KH<sub>2</sub>PO<sub>4</sub> 0.25 g and agar 13 g) and incubated for 10 days at 25°C. Then, the bacteria were harvested and incubated for 30 min at 75°C in a water bath. Suspensions were adjusted to 10<sup>6</sup> CFU ml<sup>-1</sup> and 100  $\mu$ L was spread on PCA plates.

## 2.4.3 Preparation of the other bacteria

Darwin's and the Australian isolates were inoculated onto R2A agar plates and the rest on PCA plates. When sufficient growth had accumulated, colonies were harvested and suspended to about  $10^6$  CFU ml<sup>-1</sup> for the test.

## 2.4.4 Irradiation

Six replicate Petri dishes (one of which served as the positive control) were inoculated and exposed to UV for 1, 2, 4, 8 and 10 min at 37°C for pathogens and 25°C for the others. The reduction in survival was

presented as *D* values (time for a tenfold reduction in the number of viable cells).

## 3 Results and discussion

#### 3.1 Survival on Chad sand

Our first question was how long can micro-organisms survive on desert dust/sand (i.e. at the origin of dust storms) without additional nutrients, water and so on. To do this, we stored sand from the deserts of Chad in 50-ml Falcon<sup>TM</sup> tubes for extended periods in the dark at 20°C. Surprisingly, the total number of bacteria was stable in all samples although the number of spores declined dramatically in sample C2 after 1-year storage (Fig. 1).

#### 3.2 Survival of pathogens in dry sand

We tested whether human pathogens can survive and grow on a dry, nutrient-poor substrate. Diluted growth media were used to stimulate the initial development of the pathogens. B. megaterium served as a control in these experiments since it is able to form spores. In the presence of an initial carbon/nitrogen source, B. megaterium, E. coli, P. fluorescens and S. aureus multiplied during the first few days of incubation but, after 1-2 weeks, the number surviving on sand declined (Table 2). Some were more sensitive than others (e.g. P. aeruginosa), while E. coli and S. aureus were amongst the most resistant. The yeast C. albicans was the most sensitive of all pathogens tested and was virtually extinct after the first-week incubation. Thus, despite species-specific variation, it seems that most pathogens do not survive extended periods of desiccation on nutrient-poor sand.

In contrast, fungi that are able to form spores have a much greater survival capacity. We verified this by performing similar experiments to those described above on conidia of *A. sydowii* and *A. versicolor* at 25°C and 30°C (Fig. 2). Both species easily survived 8-week incubation on dry sand suggesting that such micro-organisms could easily outlive intercontinental flight.

## 3.3 Sterigmatocystin stability

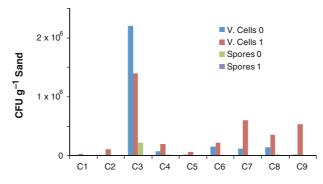
Having established that spore-forming fungal pathogens are capable of withstanding extended dry periods 
 Table 1
 List of bacteria

 tested for resistance to U<sup>\*</sup>
 radiation

Origin	Strain	Family
Samples from Darwin	n Arthrobacter B67	Micrococcaceae
	Bacillus cereus 3IB13	Bacillaceae
	Bacillus circulans 2896-21	Bacillaceae
	Bacillus circulans 2896-40	Bacillaceae
	Bacillus funiculus 3IIB11	Bacillaceae
	Bacillus fusiformis 3IB8	Bacillaceae
	Bacillus licheniformis 2896-17	Bacillaceae
	Bacillus licheniformis 3IIB16	Bacillaceae
	Bacillus licheniformis B16	Bacillaceae
	Bacillus malacitensis B26	Bacillaceae
	Bacillus malacitensis B29	Bacillaceae
	Bacillus megaterium 3IIB12	Bacillaceae
	Bacillus megaterium B13	Bacillaceae
	Bacillus pumilus 3IB5	Bacillaceae
	Bacillus pumilus B23	Bacillaceae
	Bacillus pumilus B6	Bacillaceae
	Bacillus simplex 3IB3	Bacillaceae
	Bacillus simplex D38	Bacillaceae
	Bacillus simplex D1	Bacillaceae
	Bacillus simplex B38	Bacillaceae
	Bacillus subtilis B24	Bacillaceae
	Bacillus subtilis B37	Bacillaceae
	Paenibacillus agaridevorans B17	Paenibacillaceae
	Paenibacillus pabuli 2896-34	Paenibacillaceae
	Paenibacillus spp. B8	Paenibacillaceae
Australian samples	Arthrobacter N16	Micrococcaceae
	Cellulomonas* S1-18	Cellulomonadaceae
	Conexibacter* N1-M4	Conexibacteraceae
	Curtobacterium* S1-13	Microbacteriaceae
	Janthinobacterium* S1-1	Oxalobacteraceae
	Leifsonia W1-M7	Microbacteriaceae
	Massilia* W12	Oxalobacteraceae
	Modestobacter* W1-M10	Geodermatophilaceae
	Moraxella E1-9	Moraxellaceae
	Pseudomonas S1-10	Pseudomonadaceae
	Roseomonas* S1-M12	Acetobacteraceae
	Sphingomonas* N1-7	Sphingomonadaceae
Control	Deinococcus radiodurans	Deinococcaceae
	ATCC 13939	

\* Pigmented bacteria

under nutrient-poor conditions, it was also necessary to see whether their potential to produce toxins followed similar trends. Sterigmatocystin, a carcinogenic mycotoxin, is naturally produced in large amounts by strains of the common mould *Aspergillus versicolor* and under laboratory conditions by strains of *A. nidulans* and *Chaetomium* species (Frisvad and Thrane 2004). Thin-layer chromatographic methods



**Fig. 1** Changes in the viable numbers of bacteria and spores found in nine samples of sand (numbered C1 to C9) from the Republic of Chad (collected by the International Committee of

(Fig. 3a) were used to quantify the sterigmatocystin contents of *A. versicolor* inoculated onto sand and incubated for 2 months. Two isolates of *A. sydowii* did not produce detectable levels of the toxin under any condition. Humid conditions permitted both growth of the fungus and sterigmatocystin accumulation over the long term (Fig. 3b), which was to be expected since *A. versicolor* produces sterigmatocystin at water activities close to 1 (Nielsen 2003). After 1 week of desiccation, the toxin concentration increased, but thereafter declined significantly (Fig. 3).

## 3.4 UV irradiation

As noted in the Introduction, microbial hitchhikers are also subject to strong radiation in flight. To test the effects of UV, we exposed a large array of bacteria to radiation that had an emission peak of 254  $\mu$ m (UV-C). To simplify comparisons, the data are shown as *D* values, the exposure time required for a tenfold reduction in the number of viable cells.

Again, two groups of micro-organisms were apparent—those that form spores and the rest. *D* values for

the Red Cross) following storage at room temperature for a 1-year period. V. Cells = vegetative cells with two replicates (0, 1). The data for spores are also shown in two replicates (Spores 0, 1)

the former varied between 0.6 and 4.9 min (mean  $\approx 2.8$ ). Twenty-two (out of 28) species/isolates tested had *D* values over 2. Variation was also large within members of the same species with strains from the ATCC collection displaying lower tolerances as compared to the environmental isolates (Fig. 4—cf *B. pumilus* and *B. subtilis*). By way of comparison, the *D* value for *D. radiodurans* (an especially desiccation and radiation-resistant non-spore-forming bacterium) was 3.8, which is very high for a vegetative cell. The *D* values for the other bacteria were rarely more than 1 (Fig. 5).

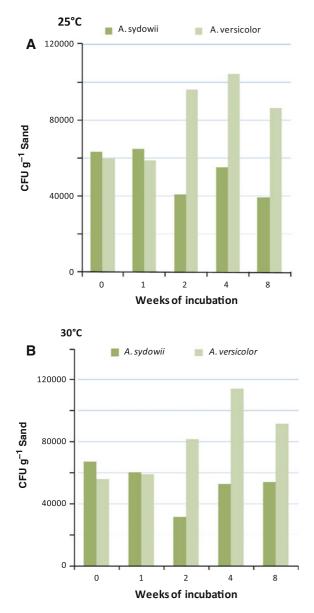
In all strains of spore-forming bacteria, UV sensitivity began only after 4 min of irradiation. In contrast, those bacteria that are unable to form spores were strongly affected after 2-min exposure (data not shown). Eight of the 13 resistant strains are pigmented, and seven of them were the more resistant Actinobacteria. Commensal and pathogenic bacteria were particularly sensitive and were unable to survive the shortest exposure time (1 min) (Fig. 5).

A simple and still true answer to the fears of pathogen spread via desert dust is that strong winds

Table 2 Survival of various bacterial pathogens and the yeast C. albicans on dry sand over a 1-month period

	Onset	1 Week	2 Weeks	4 Weeks
Candida albicans	$6.9 \times 10^{4}$	n.g.	n.g.	n.g.
Bacillus megaterium spores	$1.8 \times 10^{3}$	$3.0 \times 10^{7}$	$1.6 \times 10^{6}$	$1.8 \times 10^{7}$
Escherichia coli	$1.2 \times 10^{6}$	$4.6 \times 10^{6}$	$1.4 \times 10^{7}$	$2.4 \times 10^{5}$
Pseudomonas aeruginosa	$8.8 \times 10^{5}$	$2.4 \times 10^{3}$	$5.1 \times 10^{2}$	n.g.
Pseudomonas fluorescens	$9.9 \times 10^{5}$	$4.6 \times 10^{8}$	$1 \times 10^{5}$	$1.6 \times 10^{3}$
Salmonella typhimurium	$1.2 \times 10^{6}$	$1 \times 10^{4}$	$3.4 \times 10^{5}$	$2.8 \times 10^{3}$
Staphylococcus aureus	$1.5 \times 10^{6}$	$3.4 \times 10^{7}$	$6.1 \times 10^{6}$	$9 \times 10^5$

The spore-forming, non-pathogenic bacterium Bacillus megaterium was used as a positive control, n.g. no growth



**Fig. 2** Survival of fungal spores on sand at two different temperatures over an 8-week period (means of three replicates). Viable counts were used to assess the survival capacity of conidia after incubation on quartz sand. Further sub-samples of the same sand inoculated with *Aspergillus versicolor* were used to quantify sterigmatocystin production

have always shaped the environment (see Gorbushina et al. 2007). Only a change of land-use at the source would materially alter the outcome (Mulitza et al. 2010). And as large quantities of dust are really only blown aloft from true deserts (i.e. regions in which evaporation exceeds rainfall; where vegetation is sparse to almost non-existent; in truly remote areas), this seems unlikely (see Prospero et al. 2002). Nevertheless, human belligerence has driven large numbers of refugees to inhabitable places where their activities could, potentially, load the dust with for example, enteric pathogens.

Simple calculations suggest that at a wind speed of say 20–30 km  $h^{-1}$ , it would take only about 2 weeks for dust to travel from Chad to the east coast of the Americas. While in flight, the microbial hitchhikers would be subject to a variety of more or less severe stresses. Amongst these are drastic changes in humidity and temperature, solar and UV radiation. Thus, the question we posed here is what effect would these stresses have on pathogens associated with human activity in comparison with those of the desert environment.

As expected, micro-organisms that are native to the crusts/sands of deserts easily survive extended desiccation (Gorbushina et al. 2007). Many of the microbes associated with humans do not form spores, however, and some of these (e.g. C. albicans and P. aeruginosa) rapidly die when water is removed from the environment (Table 1). Others like E. coli are more resistant to desiccation but are probably incapable of surviving long. As asexual reproduction in the Ascomycetes involves conidia formation, these fungi are also resistant to many of the stresses associated with deserts. Our results show that the numbers of Aspergillus sydowii and A. versicolor are little affected by temperature or long-term storage on dry sand (Fig. 2). As a result, conidia are always present in the air in environments containing Ascomycetes, but it is unlikely that either of these two species is present in the dust/sand of the Chadian deserts (Favet et al. 2012). While sterigmatocystin content in the sand increases after 1 week of incubation, its further stability and production is strongly dependent on the environment. Furthermore, toxin contents of A. versicolor decrease under dry conditions (Fig. 2), suggesting that even if present at the desert source Aspergillus sp. would be much less harmful after intercontinental flight. Nevertheless, the conidia could germinate on landing and re-synthesise the toxins if more humid conditions were encountered (Fig. 2b).

UV radiation is another major environmental hurdle that microbial hitchhikers encounter on their travels. Our data show that only those micro-organisms that form conidia or spores or are coloured, show appreciable resistance to the shorter UV wavelengths (Fig. 4 and 5). Exceptions exist but UV resistance is known to

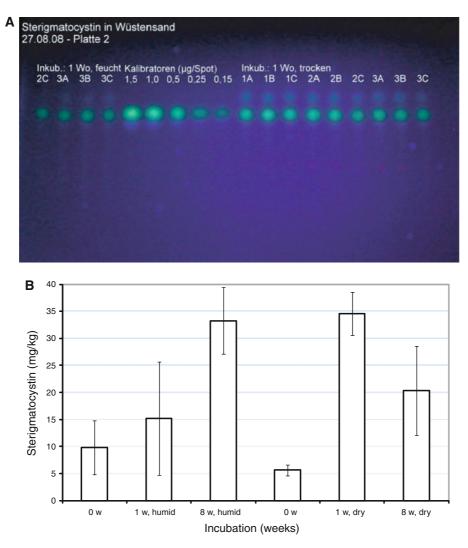


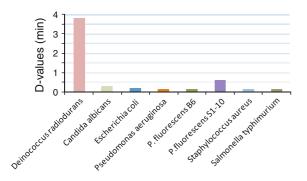
Fig. 3 Stability of the mycotoxin sterigmatocystin produced by the fungus *Aspergillus versicolor* in wet or dry sand over an 8-week (w) period. (a) shows a developed thin-layer

chromatography plate that was scanned to yield the quantitative data shown in (b). Values are given  $\pm$  SD from the mean

be confined to highly pigmented bacteria (González-Toril et al. 2008) and those micro-organisms that possess structures capable of resisting multiple stresses (Ryan 2001; Griffin 2007). Amongst the latter, *Deinococcus radiodurans*, *Kineococcus radiotolerans*, *Methylobacterium* spp. and *Rubrobacter* spp. are present in the sands of Chad (Favet et al. 2012).

Of course, the dust itself also attenuates the radiation load. Microscopic analyses of historic samples, including dust that Charles Darwin collected, show microbes anchored in the crevices of the dust particles (Gorbushina et al. 2007). In many orientations vis-à-vis the sun, the microbes would thus be in

the shade. Even with constant tumbling, significant protection could be provided this way. It should also be remembered that most of the dust that crosses continents is raised from the desert floors by gale-force winds. Large clouds of dust form as a result and research by the National Aeronautics and Space Administration of the United States of America (NASA) has shown that the particle load in large dust clouds can attenuate UV by more than 50% (see Griffin 2007). Most probably, our use of germicidal UV-C overestimates the effect of UV radiation on survival because the shorter UV wavelengths would normally be attenuated by ozone in the atmosphere.



**Fig. 4** Effect of UV radiation (wavelength—254  $\mu$ m) on survival of spore-forming environmental bacteria in comparison with two strains from the American Type Culture Collection (ATCC). *Deinococcus radiodurans* was included as a non-spore-forming, but radiation-resistant control. Data are expressed in terms of *D* values (time for a tenfold reduction in the number of viable cells)

Only at altitudes above about 20 km would the shorter wavelengths play an important role, but most intercontinental transport of dust occurs below this limit.

From take-off to landing, a single word encapsulates the fate of desert micro-organisms-fractionation. As mentioned in the Introduction, the size of the surface particles carried aloft decreases as a function of the distance from the origin. Heavier particles fall back to Earth first and with them the larger organisms or clumps of organisms. Similar processes affect the micro-organisms that are destined to travel longer distances. Microbes that are native to desert soils are normally in the form of bio-films. In contrast, those that have recently gained access to the same habitat do not adhere to the sand grains in the same way. For this reason, they are most likely to be ejected first. Furthermore, those that are coloured, or are in the form of conidia or bacterial spores (in other words, those that are native to the desert soils), will survive longest. Foreign microbes, especially human-associated pathogens, are less resistant to the rigours of the flight and will slowly die (or be "fractioned out") as they travel.

Even then, the danger of seeding distant lands with desert pathogens is probably remote. According to the "International Classification Schemes for Microorganisms Based on their Biological Risks" (Directives de l'Office Fédéral de l'environnement, des forêts et du paysage, Bern 2003), most of the bacteria present in the dust/sand samples from Chad belong to Class 1 (no risk or not classified) (Favet et al. 2012). Yet, some Enterobacteriaceae (Class 2 or 3) and fungal pathogens were also present. Generally, the fungi include opportunistic pathogens or potent allergens (belonging to class 2) which are more harmful. Dust clouds can also pick up new hitchhikers when they pass over air masses containing other microbes. For example, during their journey towards the Middle East, the dust clouds pass over deserts in Libya, Egypt and the Sinai (Schlesinger et al. 2006). When travelling above oceans, marine micro-organisms aerosolised by wave action can also be gathered up (Kellogg and Griffin, 2006).

Yet perhaps the most important risk is that associated with micro-organisms and viruses that are spread as aerosols (Griffin 2007). Human populations in the flight path of desert storms are first subjected to the abrasive effects of dust which augment such respiratory problems as allergies, asthma and silicosis. These are the very real problems associated with inhaling microbe-laden dust particles. Mechanical abrasion is probably at the origin of meningitis outbreaks in the Sahel region of North Africa (Griffin 2007). Although we did not study viral transmission, vast bodies of evidence show that it is exactly this type of human pathogen that is least likely to

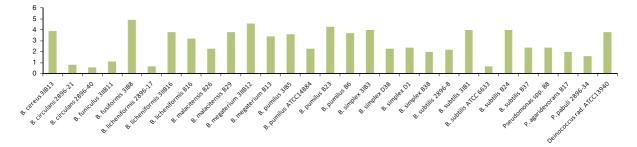


Fig. 5 Effect of UV radiation (wavelength—254 µm) of survival of various commensal and pathogenic bacteria and fungi. *Deinococcus radiodurans* was included as a non-spore-

forming, but radiation-resistant control. Data are expressed in terms of D values (time for a tenfold reduction in the number of viable cells)

survive long-distance transport. It is for this reason that disease associated with dust storms is mostly confined to regions around the origin of the storm.

The data presented above have answered some questions and posed others. For example, what proportion of microbes is transported out of Africa on dust as opposed to other means? Travel is an obvious alternative route as is export of agricultural and other products. At the present moment there is no easy way to answer such questions but given the magnitude of dust raised in Africa [1,088 Tg  $(=1.08 \times 10^9 \text{ metric tonnes})$ —Su and Toon (2011)], the transport of microbes on dust undoubtedly dwarfs all other ways. What effect will the increasing numbers of refugees that are driven from their homelands by extended droughts have on the pathogen load in dust? Again, this question is impossible to answer but our data suggest that the impact on other continents will be minimal.

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