### BRIEF COMMUNICATION

# Detailed identification of desert-originated bacteria carried by Asian dust storms to Japan

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Abstract Several halotolerant bacteria were isolated from dust allowed to settle passively on saline medium in Higashi-Hiroshima, Japan during Asia dust events in 2005–2006. The primary identification, based on the sequence similarity of the 16S rRNA gene, revealed that these isolates were strains of Bacillus subtilis, B. licheniformis, Staphylococcus epidermidis, Gracillibacillus sp., and Halomonas venusta. A parallel investigation carried out on desert sand collected directly from sand dunes in Dunhuang, Gobi Desert, China resulted in the revivification of seven bacterial strains that were highly identical to the B. subtilis and B. licheniformis strains obtained in Higashi-Hiroshima (99.7 and 100% of 16S rDNA

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sequence similarity, respectively). A subsequent genetic analysis on the group of B. licheniformis isolates based on the universally house-keeping genes, gyrB and parE, revealed high sequence similarities in both genes among the strains of both locations (99.0– 99.4%), which clustered them in a monophyletic line. Phenotype characterized by numerical taxonomy for 150 physiological tests confirmed the close relatedness between strains (similarity coefficient  $S_{SM}$  = 96.0%). The remarkable agreement between phenotype and genotype of the bacterial isolates allows us to conclude that there may have been an aerosolized dispersion of a Gobi Desert B. licheniformis by dust storms to Japan. This study provides evidence of microbial transport by yellow dust events in North-East Asia.

Keywords Bacillus licheniformis · Dust-borne bacteria · Gobi Desert · Halotolerant · Numerical taxonomy · Yellow dust

## 1 Introduction

The intercontinental transportation of millions of tons of desert dust annually influences ecosystems on a global scale (Duce et al. [1980;](#page-6-0) Parrington et al. [1983](#page-7-0); Betzer et al. [1988](#page-6-0); Uematsu et al. [2002](#page-7-0); Jickells et al. [2005\)](#page-7-0). The inorganic fertilization of ecosystems situated downwind may be a beneficial effect of this natural phenomenon; however, the pollutants and pathogenic microbes also carried with the desert dust may cause adverse effects to animals, plants, and humans (Schlesinger et al. [1990;](#page-7-0) Young et al. [1991](#page-7-0); Uematsu et al. [1992;](#page-7-0) Griffin et al. [2002](#page-6-0); Kwon et al. [2002;](#page-7-0) Griffin and Kellogg [2004;](#page-6-0) Griffin [2007](#page-6-0)). A large diversity of air-borne microorganisms has been identified to date; some are common inhabitants of soil, terrestrial, aquatic, and marine environments, others are present only in association with desert dust storms, while others are found in the aerosol at an altitude of 20,000 m (Bauer et al. [2002](#page-6-0); Griffin et al. [2003;](#page-6-0) Griffin [2004;](#page-6-0) Echigo et al. [2005;](#page-6-0) Prospero et al. [2005](#page-7-0); Griffin et al. [2006;](#page-6-0) Shivaji et al. [2006](#page-7-0); Brodie et al. [2007\)](#page-6-0). Several groups of researchers have studied dust-borne microorganisms in the African desert system and the ecological consequences of these on downwind regions (Shinn et al. [2000;](#page-7-0) Griffin et al. [2003,](#page-6-0) [2006;](#page-6-0) Kellogg et al. [2004](#page-7-0); Prospero et al. [2005\)](#page-7-0). In Asia, the yellow dust storms and their associated microbial communities are derived from the world's second largest desert system, which is located in China and Mongolia (Yongyi et al. [1993](#page-7-0); Choi et al. [1997;](#page-6-0) Ho et al. [2005](#page-6-0)). Numerical determinations of fungal spores in yellow dust-polluted air have been carried out in Taiwan and South Korea (Yeo and Kim [2002](#page-7-0); Wu et al. [2004\)](#page-7-0). Kwon et al. [\(2002](#page-7-0)) showed that public health in South Korea was so seriously affected by dust events that mortality increased by 1.7%. Japan is also impacted by yellow dust storms during the spring. Echigo et al. ([2005\)](#page-6-0) successfully isolated and identified many halophilic bacteria from soil surfaces of non-saline habitats around Tokyo and argued that endospores of these bacteria could had been distributed by Asian yellow storms. Although a significant number of taxa of dust-borne microorganisms have been isolated and characterized in both African and Asian desert systems, many of the studies carried out to date have been limited in their ability to identify isolates to the species level and, more importantly, to distinguish which isolates were of local origin and which were dust-borne.

The aim of this study was to isolate viable bacteria from an Asian dust storm and to determine their likely source of origin. The match in phenotypic and genotypic characteristics of bacterial isolates obtained in Higashi-Hiroshima, Japan and in Dunhuang, China provides evidence of the long-range atmospheric dispersion of Bacillus populations.

#### 2 Material and methods

#### 2.1 Sampling and bacterial culture

A strain of Bacillus licheniformis, strain L1 was isolated from a sand dune sample collected in Tottori prefecture, Japan in 2004. "Yellow sand" was collected directly in Dunhuang (40°10'00"N, 94°40'  $60''$ E). China in 2006.

Yellow dust storms occur during March and April and take approximately 3 days to move from the deserts of China to Japan. In this study, we coordinated our dust samplings with the forecasts of the Japan Meteorological Agency (map illustrated in Fig. 1). A passive sampling method was used to collect dust using a solid saline medium comprising Marine Broth (Difco, Sparks, MD) and 15% NaCl (150 g  $1^{-1}$ ). Agar and liquid media were prepared aseptically in petri dishes, 500-ml beakers, and 50-ml tubes ( $n = 10$  each, four times) and left open for sampling on the top of a building of Hiroshima University (Higashi-Hiroshima, 34°25′25″N, 132°44′46″E). During yellow storm events, the media was exposed to the atmospheric dust for approximately 24–36 h and then incubated at 37<sup>o</sup>C for 1–2 weeks. The cultures were subsequently streaked on agar medium, and single colonies were picked to produce pure cultures after at least three generations. A total of nine halotolerant bacteria were obtained from four dust samples (DstI–DstIV) collected from February 2005 to April 2006 (Table [1](#page-2-0)). Media used for all physiological tests comprised Bacto peptone (5 g  $1^{-1}$ ), Bacto meat extract (3 g  $1^{-1}$ ) (Becton,



Fig. 1 A meteorological map showing areas in Korea and Japan impacted by a yellow dust which originated in the Gobi Desert on March 31, 2007 (adapted from a predicted map of the Japan Meteorological Agency). Filled circles Sites affected by yellow dust. The sampling sites, Higashi-Hiroshima and Dunhuang, are indicated

<span id="page-2-0"></span>Table 1 Samples and bacterial isolates obtained in this study

Sampling sites		Sample Isolate	Closest bacteria		
			Species/strain <sup>a</sup>	16S rDNA accession no. (similarity, $\%$ )	Origin <sup>b</sup>
Higashi-Hiroshima DstI $(34^{\circ}25'25''N,$ 132°44'46"E)		DstI-1	Staphylococcus epidermidis (ATCC 12228)	AE015929 (100)	Human skin
		DstI-2	Bacillus sp. GCNB5	DO834373 (100)	Glycyrrhiza uralensis, China
			Bacillus subtilis (CICC 10034)	AY881640 (100)	China
			Bacillus sp. Ni36	DQ643186 (100)	China
			Clone B609	DQ290002 (100)	China
		$DstI-3$	Staphylococcus epidermidis (ATCC 12228)	AE015929 (100)	Human skin
		DstI-4	Bacillus licheniformis (DSM 603)	DQ082997 (99.9)	Soil, multiple
			Bacillus licheniformis (K19)	DQ351932 (99.9)	China
			Bacillus licheniformis (BPCRC 15413)	DO993676 (99.9)	China
			Bacillus licheniformis (CCBAU 10724)	EF405624 (99.9)	China
	DstII	$DstII-1$	Halomonas venusta (DSM 4743)	AJ306894 (99.7)	Hawaii, USA
		$DstII-2$	Halomonas venusta (DSM 4743)	AJ306894 (99.7)	Hawaii, USA
	DstIII	$DsIII-1$	Gracilibacillus sp. BH235	AY762980 (98.2)	China
			Gracilibacillus sp. EJ-15	AM040718 (98.0)	Saline lake, China
			Gracilibacillus sp. XH-63	AM040716 (98.0)	Saline lake, China
		$DstIII-2$	Like those for DstIII-1	Like those for DstIII-1	Like those for DstIII-1
	DstIV	$DstIV-1$	Like those for DstIII-1	Like those for DstIII-1	Like those for DstIII-1
Dunhuang $(40^{\circ}10'00''$ N, 94°40'60''E)	YS1	$YS1-4$	Like those for DstI-2	Like those for DstI-2	Like those for DstI-2
		$YS1-5$	Like those for DstI-4	Like those for DstI-4	Like those for DstI-4
	YS <sub>2</sub>	$YS2-1$	Like those for DstI-4	Like those for DstI-4	Like those for DstI-4
		<b>YS2-2</b>	Like those for DstI-4	Like those for DstI-4	Like those for DstI-4
		<b>YS2-3</b>	Like those for DstI-4	Like those for DstI-4	Like those for DstI-4
		$YS2-4$	Like those for DstI-4	Like those for DstI-4	Like those for DstI-4
		$YS2-5$	Like those for DstI-4	Like those for DstI-4	Like those for DstI-4

More than one closest species/strains which first found in China are listed

<sup>b</sup> References taken from sequence databases, GenBank/EMBL/DDBJ

Dickinson and Co., Baltimore, MD), 0.1 g  $1^{-1}$  MnSO<sub>4</sub>  $\times$ H2O (DSMZ medium 1) and varying amounts of NaCl  $(0-200 \text{ g } l^{-1})$ , in distilled water, pH 7.0. A standard strain, Bacillus licheniformis DSM 603, was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ strains; Braunschweig, Germany) and used as a reference.

#### 2.2 Physiological characterization

To determine the phenotypic identity of strains the B. licheniformis group, we carried out detailed quantitative taxonomic analyses on isolates DstI-4, YS2-1 and L1, and strain DSM 603. These were tested at different temperatures (10–60 $\degree$ C; 5 $\degree$ C interval) and NaCl concentrations (0–20%; 2% interval; 37°C). Oxidase and urease activity and starch hydrolysis were determined using the methods described by Smibert and Krieg ([1981\)](#page-7-0). Single carbon utilization of 95 substrates was assayed using GP2 microplates (Biolog, Hayward, CA) (Garland and Mills [1991](#page-6-0)). Acid production and hydrolysis of polymers were determined using 50-well API 50CH kits (Biomerieux, Marcy l'Etoile, France). These tests were conducted in triplicate at 0% NaCl, and 24 h to 1 week results were recorded and numerically coded for analyses with SPSS software (SPSS, Chicago, IL), applying simple  $(S<sub>SM</sub>)$  and Jaccard  $(S<sub>J</sub>)$  coefficients.

#### 2.3 Genetic analysis

Genomic DNA extraction followed the method described by Wilson [\(1995\)](#page-7-0). The primers and PCR procedures used to amplify a region of the 16S rRNA gene were those of DeLong ([1992\)](#page-6-0). The universal single-copy conserved  $gyrB$  gene encoding the DNA gyrase  $\beta$  subunit and the *parE* gene encoding the DNA topoisomerase IV subunit B were also amplified from genomic DNA with newly designed primers YF3 (5'-TAT AAR GTN TCN GGH GGN YTR CAC-3') (nucleotides 325-348; E. coli K-12 numbering) and YR3 (5'-YTT NGC NGA NCC NCC NGC NGA RTC-3<sup>'</sup>) (nucleotides 1299–1264; E. coli K-12 numbering). The thermocycler profile used to amplify these genes consisted of an initial denaturation at  $95^{\circ}$ C for 3 min, followed by 30 cycles of 94 °C for 30 s (denaturation),  $57$  °C for 40 s (annealing), and 72-C for 1 min (elongation), and terminated with a final elongation at  $72^{\circ}$ C for 10 min. The PCR products were cloned using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA). The amplified 16S rDNA (approx.1400 bp) and clones of the gyrB and  $parE$ fragments (approx.1000 bp and 1220 bp, respectively) were sequenced by Macrogen [\(http://www.](http://www.macrogen.com) [macrogen.com](http://www.macrogen.com)). Sequences of the genes were identified and analyzed with tools of the European Molecular Biology Laboratory (EMBL [http://www.](http://www.ebi.ac.uk/embl/) [ebi.ac.uk/embl/\)](http://www.ebi.ac.uk/embl/). Phylogenetic analysis was conducted with MEGA 3.1 (Kumar et al. [2004\)](#page-7-0). DNA sequences were deposited in GenBank/EMBL/DDBJ under accession numbers of AB305265–AB305277 and AB307800–AB307807.

## 3 Results and discussion

#### 3.1 Yellow dust-borne bacteria

A total of nine isolates (two strains of staphylococci, two strains of halomonads, and five strains of bacilli) were obtained from four samples collected in 2005– 2006 (DstI–IV; Table [1\)](#page-2-0). The bacteria were all extremely halotolerant and were able to grow both in the absence of salt and at salt concentrations up to 16% (Kushner [1978\)](#page-7-0). Of these, five strains were endospore-formers belonging to family Bacillaceae (isolates/strains DstI-2, DstI-4, DstIII-1, DstIII-2, and DstIV-1), two (DstI-1 and DstI-3) were relatives of the widely distributing bacterium, Staphylococcus epidermidis (100%), and two others (strains DstII-1 and DstII-2) were strains of marine-originated species, Halomonas venusta type strain DSM 4743 (99.7%). Very few bacterial isolates were able to grow in the medium containing 15% NaCl used for screening for halophiles. The small number of isolates obtained in this study could also reflect low survival rates caused by atmospheric sources of stresses, such as UV light, dessication, and temperature (Griffin [2005\)](#page-6-0). Sources of microbes carried by wind may depend on the extent and direction of the storm, terrestrial topology, and distance. The halomonads found in this study could have been transported from salt lakes in China and South Korea or from regional seafog. Staphylococcus epidermidis, which is a wellknown human pathogen that is distributed widely throughout the atmosphere according to many reports, was also isolated in this study (Yongyi et al. [1993](#page-7-0); Rupp and Archer [1994;](#page-7-0) Kumer et al. [1996](#page-7-0); Montacutelli et al. [2000;](#page-7-0) Zhang et al. [2003\)](#page-7-0).

#### 3.2 Bacteria isolated from Gobi Desert

Dunhuang is an oasis located in the Gobi Desert and is famous for its large sand dunes. Dry sand collected there was subjected to the same treatment analyses for the recovery of halotolerant bacteria as those carried out on sand collected in Higashi-Hiroshima. Isolates from the Dunhuang sand were identified as individuals of two groups, with six isolates (YS1-5 and YS2-1 $\sim$ 5) being close relatives of *B*. *licheni*formis and one isolate (strain YS1-4) being a member of the  $B$ . *subtilis* group (Table [1](#page-2-0) and Fig. [2](#page-4-0)). Within each group, these isolates were almost identical in terms of their 16S rDNA sequence (99–100%). Interestingly, they also matched 99.7–100% with our strains isolated from Asian dust obtained in Higashi-Hiroshima. Although no strain of Gracilibacillus spp. was found in these samples, as was the case in the atmospheric dust samples (strains DstIII-1, DstIII-2, and DstIV-1), homology showed that their

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Fig. 2 Neighbor-joining tree showing phylogenetic clusters of dust-borne and desert-related isolates with other selected bacteria. Bootstrap values of 1000 replications greater than 50% are shown at the nodes of tree. Names in bold indicate strains identified in this study. 16S rRNA gene sequence accession numbers are in parenthesis. Scale bar: 0.02 nucleotide substitution per site. Bacillus licheniformis cluster includes isolates DstI-4 (AB305269), YS1-5 (AB305271), YS2-1 (AB305272), YS2-2 (AB305273), YS2-3 (AB305274),

closest relatives (98%) have been isolated from salt lakes in Inner Mongolia, China (Table [1\)](#page-2-0).

## 3.3 Genotypic and phenotypic evidence for desert origin of the dust-borne B. licheniformis strains

The six strains of *B. licheniformis* isolated from desert sand were 99.9–100% identical to the dustderived strain DstI-4 and strain DSM 603 in terms of the 16S rDNA sequence and they clustered into a monophyletic line in a phylogenetic tree (Fig. 2). To solve the ambiguity in differentiating them based

YS2-4 (AB305275), YS2-5 (AB305276), L1 (AB305265), and reference strains DSM 603 (DQ081997), DSM 13 (NC00 6322), CICC 10107 (DQ112220), K19 (DQ351932), BCRC 15413 (DQ993676), CICC 10181 (AY842871), CCBAU 10724 (EF405624); Bacillus subtilis cluster includes isolates DstI-2 (AB305268), YS1-4 (AB305270), AU30 (EF032678), clone B609 (DQ290002), Ni36 (DQ643186), GCNB5 (DQ834373), CICC 10034 (AY881640)

solely on the 16S rRNA gene, we turned to the variable universal protein coding genes gyrB and  $parE$ . Sequence similarity of the  $gyrB$  gene has been used in earlier studies for identifying and classifying bacteria; for example, a sequence similarity of 88.3– 99.1% was found for interspecies comparisons of genera Salmonella, Shigella, Escherichia, Enterobacter, and Klebsiella in the family Enterobacteriaceae (Fukushima et al. [2002\)](#page-6-0). Similarly, within the genus *Bacillus*, the *gyrB* gene shows a  $68.9-77.9\%$  similarity between species and a 99.1–100% intra-species (unpublished observations). In this study, sequences of the gyrB and parE genes of three strains  $-$  DSM 603, DstI-4, and YS2-1 – showed a 99.2%–99.4%

match with each other and were highly divergent to those of strain L1 (about 95% and 60%, respectively). A phylogenetic tree based on combined sequence sets of gyrB and parE genes (Fig. 3a) suggests that the strains are very similar.

Even when strains show a high level of genotypic identity, those from different origins may exhibit different phenotypic features. To assess these potential phenotypic differences, we carried out a battery of 150 physiological tests on the B. licheniformis group. Significant differences between the four strains tested were found for carbon utilizations and acid production, yielding numerical taxonomic similarity coefficients  $(S_{SM})$  of 96.0% between strain DstI-4 and YS2-1, and 90.6 and 87.2% between these and strains L1 and DSM 603, respectively. In this analysis, strain DSM 603 was far removed from the dust-related isolates DstI-4 and YS2-1 (Fig. 3b). The most notable differences between these two bacteria and other Bacilli and other strains of B. licheniformis were their growth at salinity as high as 2.7 M and at an incubation temperature of  $60^{\circ}$ C (Palmisano et al.

[2001\)](#page-7-0). The extreme tolerances of DstI-4 and YS2-1 may reflect adaptation to desert conditions.

One Bacillus species commonly observed in arid areas, such as the Sahara, Mojave, Sonoran, and Gobi Deserts (Duncan et al. [1994](#page-6-0); Roberts and Cohan [1995](#page-7-0); Palmisano et al. [2001](#page-7-0)), is *B. sonorensis*, the most closely related species to B. licheniformis. Yongyi et al. ([1993\)](#page-7-0) reported that the most abundant populations in the airborne bacterial community of Beijing were Bacillus populations along with isolates of the genus Staphylococcus. Our results support this finding.

In the phylogenetic tree based on 16S rRNA sequences (Fig. [2](#page-4-0)), the *Gracilibacillus*-clustered strains (DstIII-1, DstIII-2, and DstIV-1) were closely related to the previously reported arid Chinese isolates, including BH235, EJ-15, XH-63 (GenBank accession numbers of AY762980, AM040718, and AM040716, respectively). The *B. subtilis*-clustered strains (DstI-2 and YS1-4) were closely related to the arid Chinese strains/clone GCNB5, CICC 10034, Ni36, and B609 (DQ834373, AY881640, DQ643186, and DQ290002, respectively). The B. licheniformis-clustered strains DstI-4 and YS2-1

Fig. 3 a A Unweighted Pair Group Method with Arithmetic mean (UPGMA) tree showing a monophyletic line of isolates DstI-4. YS2-1 and strain DSM 603 were compared to other related bacteria based on composites of sequences of the gyrB and parE genes. Bootstrap values of 1000 replications greater than 50% are placed at the nodes. Scale bar indicates nucleotide sequence similarity  $(\%)$ . **b** An average linkage dendogram inferred from similarity coefficients ( $S_{SM}$  and  $S_J$ ) of the numerical taxonomic analysis based on data of 150 physiological tests. Names and cell micrographs (bar: 10 μm) of strains are noted in the end of the dendogram



<span id="page-6-0"></span>were almost identical to Chinese isolates K19, BPCRC 15413, and CCBAU 10724 (DQ351932, DQ993676, and EF405624, respectively). These relationships indicate a potential point of origin for these isolates which were recovered in our study.

Bacteria and fungi responsible for diseases in human, animals, and plants have been identified from airborne desert dust samples in Africa and Asia (Kwon et al. [2002](#page-7-0); Kellogg et al. [2004](#page-7-0); Kellogg and Griffin [2006\)](#page-7-0). Although not the focus of this work, non-halotolerant bacteria, viruses, and fungi may be common constituents of Asian dust storms. The increasing frequency of dust storms in Asia is thought to result from conditions of decreasing precipitation and prolonged drought which, in turn, will enhance desertification rates (Quian [2002\)](#page-7-0). Consequently, Asian desert dust storms and their air-borne constituents have the potential to become a major public and ecosystem health concern as they may occur more frequently and in increasingly more severe manifestations in the coming years.

## 3.4 Conclusions

Our data demonstrate that Asian dust storms originating in China transport viable microorganisms to Japan. Dust-borne bacteria isolated in this study were salt- and temperature-tolerant. The populations of B. subtilis and B. licheniformis that were found in both the Gobi Desert and atmospheric dust impacting Japan had similar genetic identities. Our data indicate that the two isolates have similar traits and, based on the relatedness of their isolation (dust event and dust source region), a potential similar lineage.

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