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Isolation and characterization of environmental bacteria with mercury extracellular biosorption capacities

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SUPPLEMENTAL MATERIAL

EXPERIMENTAL PROCEDURES

Scanning electron microscopy (SEM)

The bacteria were cultivated for 24 h at 30℃ on LB agar plates supplemented with glucose (3% w/v). A set of colonies were collected, placed in a microporous capsule (Electron Microscopy Sciences, Euromedex, Souffelweyersheim, France) and incubated for 1 h in 3% glutaraldehyde in phosphate buffer 0.2 mM pH 7.35. After washing in phosphate buffer 0.2 mM pH 7.35, the samples were dehydrated in a graded ethanol series diluted with water. The dehydrated bacteria were dried using the critical point method, fixed to SEM stubs using carbon adhesive tabs and gold coated. The SEM observations were performed on a JSM-840A instrument (JEOL, Croissy-sur-Seine, France).

Phylogenetic analysis

The 16S rRNA gene partial sequences obtained for the strain selected in this study, that of the reference strains *Bacillus cereus* GTC 02826 and *Bacillus subtilis* subsp. *subtilis* DSM 10, together with that of the nearest relatives obtained for each strain using BLASTN nucleotide alignment (2), were analyzed on the Phylogeny.fr web server (5), used in the advanced mode and performing the following steps: multiple alignment with MUSCLE (6), alignment curation with Gblocks (3), construction of the phylogenetic tree with PhyML (7) and visualisation of the phylogenetic tree with TreeDyn (4). The parameters used are provided in the figure legend.

TABLE S1. Characteristics of the used primers [a,b]

Name	*Gene*	*Sequence 5' – 3'*	*Reference*
27F	*16S*	GAGTTTGATCCTGGCTCAG	(9)
1385R	*16S*	CGGTGTGTRCAAGGCCC	(9)
BK1F	*16S*	TCACCAAGGCRACGATGCG	(10)
BK1R	*16S*	CGTATTCACCGCGGCATG	(10)

[a] The F and R letters in the primer names indicate forward and reverse, respectively.
[b] R denotes A/G nucleotide wobble.

FIG. S1.
Phylogeny of the selected strains (shown in red), nearest relatives, and strains used for comparison of the MIC and biosorption capacities (shown in blue). This unrooted tree was computed from the 16S rRNA gene partial sequences, on the Phylogeny.fr web server (5), using the default option of the advanced mode. The final alignment includes 410 ungapped positions. Numbers at branch points indicate bootstrap percentages, and branches have been collapsed for values < 50%.

FIG. S2.
TEM photographs of *Bacillus cereus* MM8 (A), *Lysinibacillus* sp. HG17 (B), *Bacillus* sp. CM111 (C), *K. rosea* EP1 (D), *M. oxydans* HG3 (E), *S. marcescens* HG19 (F) and *Ochrobactrum* sp. HG16 (G) incubated in the presence of 20 µM $HgCl_2$ (1, on the left), 100 µM $HgCl_2$ (2, middle), and 100 µM $HgCl_2$ after an autoclaving step (killed bacterial biomass) (3, on the right). Typical mercury accumulations, determined by XEDS, are indicated by arrows. The large dark granules observed for *Lysinibacillus* sp. HG17 (B1,B2) correspond to phosphate granules.

FIG. S3.
Cartography showing the Mα/β (A,C) and Lα (B,D) signals of mercury (in red) overlapped on the TEM photographs of *K. rosea* EP1 (D) after incubation in the presence of 100 µM $HgCl_2$ of the live (A,B) and killed bacterial biomass (C,D), respectively. The Mα/β X-ray emission peaks of mercury are more intense but less specific than that of Lα, due to overlapping with the Kα peak of the element S.

FIG. S4.
Hg(0) volatilization assay on the negative control (phosphate buffer culture medium) (A,G), positive control (culture of *E. coli* DU1040) (F,L), and cultures of *Microbacterium oxydans* HG3 (B), *Ochrobactrum* sp. HG16 (C), *Lysinibacillus* sp. HG17 (D,H), *Serratia marcescens* HG19 (E), *Kocuria rosea* EP1 (I), *Bacillus* sp. CM111 (J) and *Bacillus cereus* MM8 (K). The values indicated on the left correspond to $HgCl_2$ concentrations, in µM.

MOVIE S5.
Tomography experiment, carried out between -60 and +60° with 1° increments, showing the extracellular localization of mercury beads for the strain *Kocuria rosea* EP1 (bar = 1 µm).

FIG S6.
Scanning electron microscopy of *Bacillus* sp. CM111 showing the presence of a capsule (bar = 1 µm).

FIG. S7.
Characterization of the protein content of the EPS from *Lysinibacillus* sp. HG17. (A) Coomassie-stained SDS-PAGE showing the EPS extracted from the supernatant (1) and pellet (2) from *Lysinibacillus* sp. HG17. (B) Set of peptides identified by trypsin digestion followed by IDA LC-MS analysis (m/z exp: experimental monoisotopic *m/z*, Mw exp: experimental monoisotopic MW, Mw calc: calculated monoisotopic MW, ppm: ppm deviation between Mw exp and Mw calc, Miss: number of missed cleavages, Sequence: sequence of the peptides and neighboring amino acids, separated with dots). (C) Localization of the peptides identified on the amino acid sequence of SbpA, S-layer protein from *Lysinibacillus sphaericus* CCM2177 (Genbank AAF22978.1), in red. The SLH domains are highlighted in yellow. Numbers in angle brackets refer to the length in amino acids to the end of the sequence, a part of the protein with no matched peptides.

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