# Use of Endophytic and Rhizosphere Bacteria To Improve Phytoremediation of Arsenic-Contaminated Industrial Soils by Autochthonous Betula celtiberica

Supplementary material

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**Supplemental material for Mesa *et al*., "Use of endophytic and rhizosphere bacteria to improve phytoremediation of arsenic-contaminated industrial soils by autochthonous *Betula celtiberica*"**

## A. Supplemental Methods

**The contaminated site.** The present study was conducted at the abandoned fertilizer industry, Nitrastur, located in Langreo, Asturias (NW Spain) (See Figure S1). Nitrastur was one of the main fertilizer plants in Spain for more than fifty years until its closure in 1997. The contaminated site has a total surface area of 20 ha (1). More than half of the surface corresponds to landfills between 4 and 5 m deep comprised of pyrite ashes and other iron and steel-type debris. A scheme of the soil location and working area is shown in Figure S1.

**DNA extraction and 454 pyrosequencing.** The primers 799F and 1391R were selected to minimize chloroplast contamination by providing considerable mismatches with chloroplast sequences (<0.1% of total sequences retrieved) (2). Since the concentration of bacterial DNA in comparison with the plant DNA was low, we chose a nested PCR strategy to amplify the samples. A first round of PCR amplification was conducted using primers without the Roche 454 pyrosequencing adaptors and sample-specific barcode. Products obtained from triplicate PCR reactions were pooled and purified using the QIAquick PCR purification kit (Qiagen Benelux B.V., the Netherlands), the quality of the amplicon pools was evaluated using an Agilent 2100 Bioanalyzer system (Agilent Technologies, Belgium), and DNA concentration of amplicon libraries was determined using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, CA, USA) and a Fluostar Omega plate reader (BMG Labtech, Germany) and pooled in equimolar concentrations.

**Identification and phylogenetic affiliation of cultivable *B. celtiberica*-associated bacteria.** Genomic DNA of the purified strains was extracted from bacterial colonies collected directly from Petri dishes using the GeneMATRIX bacterial and yeast Kit (EURx, Poland). For bacterial strain identification, the 16S rRNA gene was amplified by PCR using the universal bacterial primers 27f (5'-GAGTTTGATCACTGGCTCAG-3') and 1492r (5'-TACGGCTACCTTGTTACGACTT-3') and then partially sequenced from the 27f primer (3). Briefly, PCR amplicons were generated using AmpliTaq Gold 360 (Applied Biosystems®, Life TechnologiesTM, USA) and 100 ng $\mu l^{-1}$ of template DNA. The amplifications were performed in a MJ Mini Thermal Cycler (Bio-Rad, USA) with an annealing temperature of 56°C for 30 cycles. The PCR products obtained from separate reactions were pooled and purified using Ilustra GFX™ purification kit (GE Healthcare, UK) and DNA concentrations of the pools were determined via Qubit dsDNA assay kit (Invitrogen). Amplicons were sequenced according to the BigDye Terminator v3.1 sequencing kit protocol (Applied Biosystems®) adjusted to a final volume of 10 µL. PCR products were purified through Sephadex G-50 (Amersham Biosciences, UK), and subjected to capillary electrophoresis in an ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems®). The sequences were compared to the GenBank database using the BLAST algorithm (National Center for Biotechnology Information, USA). The phylogeny of the rhizosperic and endophytic cultivable bacteria associated with *B. celtiberica* were reconstructed using partial 16S rRNA gene sequences. Sequences were aligned using ClustalW (4) and phylogenetic analyses were conducted with MEGA v6 (5). The evolutionary distances were inferred using the Kimura-2 parameter model. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL), and then selecting the topology with superior log likelihood value.

The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 119 nucleotide sequences. All positions with less than 95% site coverage were eliminated. There were a total of 323 positions in the final dataset. The branches were tested with bootstrap analyses (1,000 replications). iTOL (6) was used to visualize and color tree branches based on taxonomy. The sequences reported in this study have been deposited in GenBank (http:// www.ncbi.nlm.nih.gov/Genbank/index.html) with accession numbers: KX881424 to KX881508.

**Characterization of Cultivable Isolates**. The Minimum Inhibitory Concentration (MIC) for Arsenic is defined as the lowest concentration of $As^{III}$ and $As^{V}$ that inhibited bacterial growth on agar medium. The bacterial isolates were streaked on LB agar plates supplemented with increasing concentrations of arsenite (2-20 mM) ($NaAsO_2$, Sigma-Aldrich, USA) or arsenate (1-100 mM) (Na2HAsO4 ˙ 7H2O, Sigma-Aldrich, USA) (7, 8). For each strain, the lowest concentration that inhibited visible growth at 30°C within 7 days was determined. Indole-3-Acetic Acid (IAA) is known to facilitate plant growth (9). Strains were assayed for their ability to produce IAA according to the methods described by (10) and (11). Isolates were grown in 20 mL Luria Bertani broth supplemented with 500 μg $mL^{-1}$ tryptophan as IAA precursor and incubated at 28°C for 3 days with 150 rpm shaking. After centrifugation, 1 mL of each culture supernatant was mixed with 2 mL Salkowski's reagent (2 ml 0.5M $FeCl_3$ + 49 ml water + 49 mL 70% perchloric acid), and incubated for 30 min in the dark. Absorbance at 530 nm was measured and IAA was quantified using a standard curve with known concentrations of pure IAA (Sigma-Aldrich, USA). Another important plant growth-promoting property (PGP) trait evaluated is 1-Amino-Cyclopropane-1-Carboxylic Acid (ACC) Deaminase Activity, which antagonizes ethylene synthesis cleaving ACC, the immediate precursor of ethylene (12). ACCD activity was determined by the presence of α-ketobutyrate, which is generated by ACC hydrolysis (13). Siderophores are low-molecular-weight Fe(III)-specific ligands which can solubilize and sequester iron from the soil and provide it to plant cells (14). Siderophore production was evaluated qualitatively (15). Bacterial isolates were streaked on blue agar plates containing Chromeazurol S (CAS; Sigma-Aldrich, USA), incubated at 27°C for 5 days, and the presence of orange halos around the colonies was then verified.

**Determination of total non-protein thiol (NPT) compounds, arsenic content and speciation in plant tissues.** The extraction procedure for NPTs was conducted in a cold chamber (4°C); plant material (500 mg) was homogenized in 1% polyvinylpolypyrrolidone and 0.1 N HCl at a ratio 1:1.5 (w/v). The mixture was shaken for 30 sec and sonicated for 5 min. After centrifugation at 15,000 × g for 15 min at 4°C, the supernatant was collected with a syringe and filtered through a Millex-HV (0.45 μm diameter, Merck Millipore) filter. Finally, it was distributed in 150 μl aliquots in vials and immediately injected into a high-performance liquid chromatograph (HPLC). HPLC was performed using a Waters 600 chromatograph (Milford, MA) and the derivatized thiols were detected with a Waters 996 Photodiode-Array Detector (Milford, MA). The sample (100 μl) was injected into a Kromasil® C18 Column (Scharlau) and eluted with solvent A (acetonitrile: $H_2O$, 2:98 (v/v) to which 0.05% trifluoroacetic acid (TFA) was added). Post-column derivatization was conducted with Ellman's reagent at 0.5 mL $min^{-1}$ flow (16) and absorbance was measured at 412 nm. Standard samples of GSH and a mixture of PCs (PC2, PC3, and PC4) were run to identify peaks. PC concentrations were analyzed using the integration areas at 412 nm absorbance converted into nmoles of GSH equivalents $g^{-1}$ fresh weight.

Chemical As analysis were determined in leaves and roots, 0.2 g of powdered plant samples were dissolved with 8 mL of 50% nitric acid solution (Sigma, Aldrich, USA) using a microwave at 800W for 15 min (Multiwave3000, Anton Paar). The solutions were diluted to 50 mL with ultrapure water and filtered through a 0.45-µm polytetrafluorethylene (PTFE) filter prior to analysis. Plant samples were analyzed by ICP-MS as described above for soil samples (see plant material, rhizosphere soil sampling, and chemical analysis in Materials and Methods). For each sample, three independent replicates were measured. Concentration of As was expressed in mg $kg^{-1}$ dry weight (dw) of plant material. As speciation was determined in leaves, roots, and in the culture medium in which the plants grew.

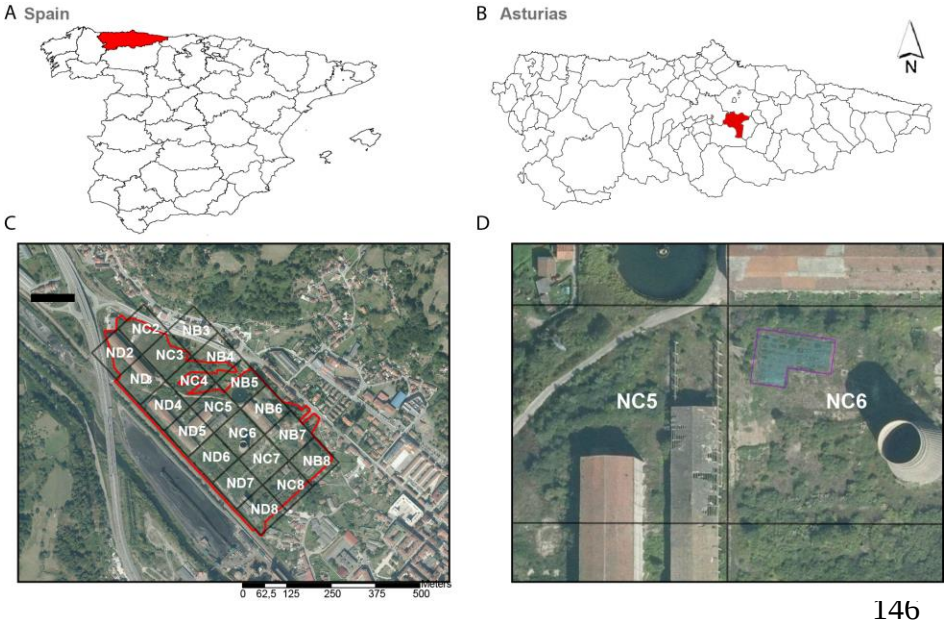
For As species determination in leaves and roots, 100 mg of finely-ground sample were extracted in 2.5 mL of nitric acid solution (0.3 M) at 95 °C for 90 min. The culture medium was directly extracted with the nitric acid solution. The extracts were centrifuged at 6,000 X g for 15 min and the supernatants were filtered through a 0.45-µm PTFE filter. The solutions were neutralized by adding NaOH. The As species ($As^{III}$, $As^{V}$, methylarsinic acid, and dimethylarsinic acid) were separated with a mobile phase of 2 M PBS (Phosphate Buffered Saline)/0.2 M EDTA (pH = 6.0) in a separation column with a 1260 Infinity HPLC coupled to a 7700 ICPMS (both from Agilent Technologies). Identification of As species was confirmed by spiking real extracts with a mixture of standard solutions.

**Field experiments.** Soil samples from each subplot were collected (at a depth of 25–30 cm), dried, mixed, and sieved (through a 2-mm sieve) and the metal(loid) content was determined by ICP-MS as described above. *B. celtiberica* plants were propagated *in vitro* for 30 days and acclimated to greenhouse conditions uncovering the vessels and placing them in a mist tunnel at 15-25°C and 90% humidity. After 3 days, the plants were transferred to 10-cm diameter pots filled with peat: vermiculite (1:1) and progressively decreasing the humidity to 60% for 15 days. After this period, plants were kept in the greenhouse for 10 days before being placed outside and translocated to the experimental plot. For field inoculation, bacteria were grown separately in 500 mL Erlenmeyer flasks containing 100 mL of GAE medium with continuous shaking at 30°C to reach $10^8$-$10^9$ cells per mL (24-48 h). Cells were collected by centrifugation at 8,000 X g during 10 min, washed and re-suspended with sterile distilled water. Bacterial suspensions (100 mL) were directly placed in contact with the roots for 20 min before covering with soil.

**As phytoextraction efficiency index determination.** Arsenic phytoextraction was assessed through the estimation of indices: i) Bioconcentration factor (BCF) calculated as the ratio of arsenic concentrations in plant tissues and soil ($BCF = C_{root}/C_{soil}$), values of BCF > 1 indicating the accumulation of a particular trace metal in roots; ii) mobility ratio (MR) calculated as the ratio of heavy metal in above-ground plant parts (shoots, branches, or leaves) to those in soil ($MR = C_{above\ ground\ plant\ part}/C_{soil}$), values of MR > 1 indicating that the plant is enriched with metals; iii) translocation factor (TF) calculated as the ratio of arsenic concentrations in above-ground plant parts to those in roots ($TF = C_{above\ ground\ plant\ part}=C_{root}$), values of TF > 1 indicating that the plant translocates metals effectively from root to shoot (17, 18).

## B. Supplemental Figures

**Supplementary Figure 1.** (**A** and **B**) Location of the fertilizer industry "Nitrastur" site in Spain (Asturias region, Langreo municipality). (**C**) Aerial view of the site where the red thread delimits the Nitrastur industry area and the black lines show the sampled grid delineating 100 X 100 m plots. (**D**) Zoom of the NC5 and NC6 sampling plots, the phytoremediation area is outlined in violet.

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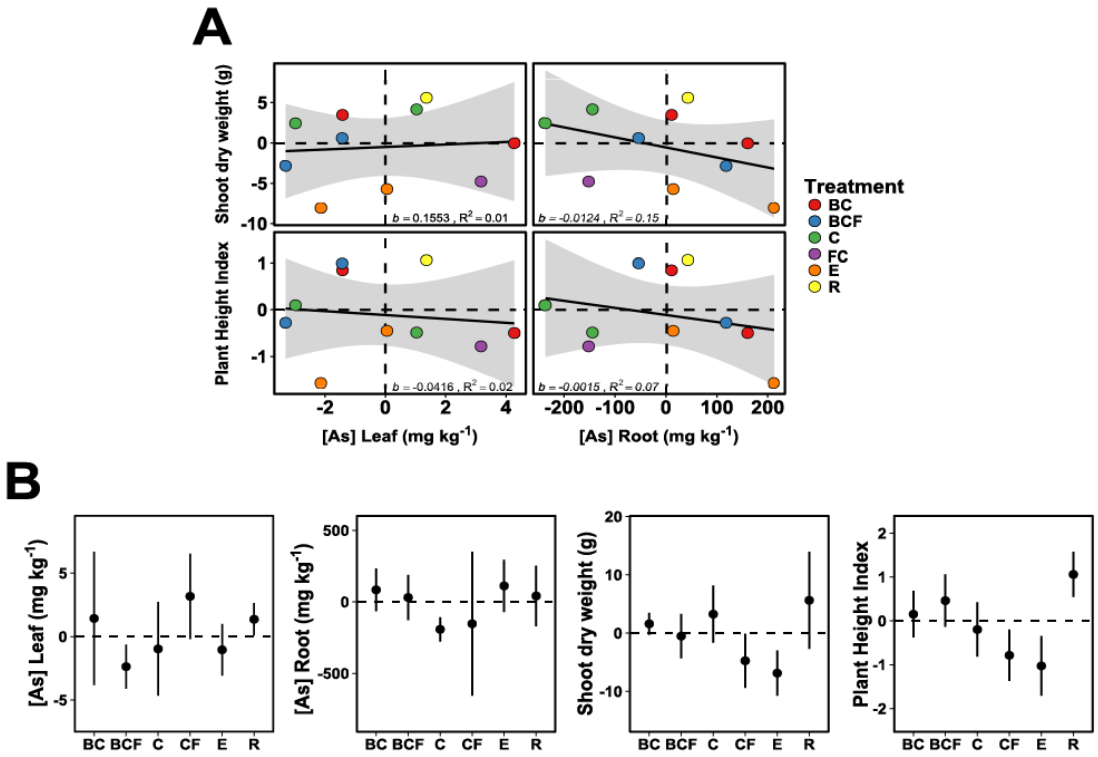
**Supplementary Figure 2.** (**A**) Correlation between the mean (per plot and treatment) of the residuals from the model $y = a + As\ soil\ + pH + \varepsilon$ (see Material and Methods in the main text) of the shoot biomass or plant height index and the mean of the residuals from the same model of leaf or root As concentrations of *Betula celtiberica* plants. Dot labels indicate whether plants were inoculated (E, with *Rhizobium herbae* strain 32E; R, with *Ensifer adhaerens* strain 91R; BC, with the bacterial consortium including *Variovorax paradoxus* strain 28EY and *Phyllobacterium myrsinacearum* strain 28EW and BCF, with the same consortium but with fertilizer) or not (C and FC for control and fertilized control, respectively). Dashed lines mark the zero value. The shaded areas represent the 95% confidence intervals of each linear relationship (black solid lines). The slope (b) and $R^2$ (proportion of variance explained) of each relationship are also shown. (**B**) Means and their corresponding 95% confidence intervals (dots and error bars) of the same residuals analyzed in (A), although only per treatment. Horizontal dashed lines mark the zero value.



## C. Supplemental Tables

**Supplementary Table 1** Physical-chemical characteristics and metal(loid) content (mg $kg^{-1}$) for rhizosphere soils sampled from five *B. celtiberica* trees. Results are an average of three samples with a standard error < 5%.

*SSLs (Soil Screening Levels established by Spanish law) for Industrial (Ind) and Urban (Urb) soil use.

| Tree sample ID no./ Plot | Texture | pH Rhizosphere soil | Organic matter (%) | Organic carbon (%) | Water content (%) | Cr | Ni | Cu | Zn | As | Cd | Hg | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 N-C6 | Sandy loam | 6.19 | 9.20 | 5.34 | 34.76 | 45.41 | 22.57 | 1,168 | 1,912 | 1,940 | 6.36 | 11.57 | 6,567 |
| B2 N-C5 | Sandy loam | 6.41 | 16.36 | 9.49 | 31.40 | 46.74 | 22.16 | 844 | 2,894 | 865 | 9.49 | 18.63 | 1,880 |
| B3 N-C6 | Sandy loam | 6.97 | 10.31 | 5.98 | 16.89 | 39.51 | 19.42 | 509 | 1,127 | 1,643 | 4.75 | 25.62 | 2,336 |
| B4 N-C6 | Sandy loam | 6.20 | 9.11 | 5.73 | 35.22 | 41.52 | 17.22 | 577 | 1,389 | 1,701 | 4.83 | 19.14 | 2,569 |
| B5 N-C6 | Sandy loam | 6.01 | 9.00 | 5.1 | 35.12 | 12.37 | 16.91 | 964 | 1,524 | 3,349 | 8.20 | 5.92 | 13,055 |
| *SSLs Ind | | | | | | 10,000 | 6,500 | 4,000 | 10,000 | 200 | 200 | 100 | 800 |
| *SSLs Urb | | | | | | 10,000 | 4,150 | 400 | 4,550 | 40 | 20 | 10 | 400 |

**Supplementary Table 2** Pyrosequencing data and Goods coverage obtained from the endosphere (E) and rhizosphere (R) associated with *Betula celtiberica* trees

| Sample | No. of sequences | | No. of trimmed sequences[a] | | No. of OTUs | | Goods coverage | |
|---|---|---|---|---|---|---|---|---|
| | E | R | E | R | E | R | E | R |
| **B1** | 4,286 | 14,565 | 3,179 | 9,518 | 119 | 881 | 0.99 | 0.98 |
| **B2** | 4,628 | 11,028 | 1,939 | 7,063 | 595 | 934 | 0.92 | 0.97 |
| **B3** | 4,571 | 15,662 | 2,640 | 5,634 | 320 | 1463 | 0.96 | 0.96 |
| **B4** | 4,464 | 13,951 | 2,718 | 7,657 | 256 | 1267 | 0.99 | 0.95 |
| **B5** | 2,149 | 17,524 | 1,571 | 9,110 | 190 | 798 | 0.97 | 0.99 |

[a]Reads after trimming and chimera removal

**Supplementary Table 3.** Phylogenetic affiliation, MIC (minimal inhibitory concentration), indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD), and siderophore (SID) production of endophytes and rhizobacteria. Phylogenetic affiliations were based on 16S rRNA gene sequences of about 800 bp length. Strains in boldface were selected for further analysis. IAA data are means ± SD (n= 2).

| Isolate | Plot | Most closely related species[a] (NCBI accession number) | Sequence similarity (%) | MIC (mM) As (V) | MIC (mM) As (III) | IAA (± SD) (µg mL$^{-1}$) | SID | ACCD |
|---|---|---|---|---|---|---|---|---|
| | | ***Root Endophytic Bacteria*** | | | | | | |
| 28EY | NC6 | ***Variovorax paradoxus* S110 (KX881445.1)** | 100 | 100 | <2 | 10.29 (± 0.51) | + | - |
| 28EW | NC6 | ***Phyllobacterium myrsinacearum* NBRC 100019 (KX881446.1)** | 98 | 100 | 2 | 13.77 (± 0.22) | + | - |
| 29E | NC6 | ***Neorhizobium alkalisoli* ZY-4s (KX881444.1)** | 99 | 100 | <2 | 13.26 (± 0.38) | ++ | - |
| **31E** | NC6 | *Bacillus* sp. FRC_Y9-2 (KX881432.1) | 99 | 1 | 2 | 13.26 (± 0.15) | - | - |
| 32E | NC6 | ***Rhizobium herbae* CCBAU 83011 (KX881443.1)** | 99 | 100 | <2 | 13.06 (± 0.43) | ++ | - |
| **68E** | NC6 | *Bacillus* sp. PK-2013-B11 (KX881434.1) | 99 | 1 | 2 | 13.15 (± 1.8) | ++ | - |
| **69E** | NC6 | *Caulobacter* sp. Cra15 (KX881433.1) | 99 | 20 | 5 | 9.79 (± 0.29) | ++ | - |
| **70E** | NC6 | *Phyllobacterium* sp. T1293 (KX881431.1) | 89 | 100 | 5 | 11.12 (± 0.29) | ++ | - |
| **71E** | NC6 | *Rhizobium* sp. CCBAU 83515 (KX881487.1) | 99 | 50 | 20 | 11.95 (± 0.47) | ++ | - |
| **72E** | NC6 | *Leuconostoc mesenteroides* PON271 (KX881430.1) | 99 | 10 | 15 | 9.89 (± 0.29) | - | - |
| **73E** | NC6 | *Streptomyces* sp. SAUAS2-3 (KX881429.1) | 99 | 20 | 20 | 15.15 (± 0.36) | ++ | ++ |
| **75E** | NC6 | *Bacillus* sp. DB124(2010) (KX881428.1) | 99 | 1 | 5 | 13.11 (± 0.46) | - | - |
| **105E** | NC6 | *Rhizobium* sp. L48C631A00 (KX881490.1) | 99 | <1 | <2 | 10.35 (± 0.23) | - | - |
| **106E** | NC6 | *Pseudomonas frederiksbergensis* IHB B 7114 (KX881482.1) | 100 | 1 | 20 | 11.22 (± 0.42) | ++ | - |
| **107E** | NC6 | *Paenibacillus* sp. IMP09 (KX881481.1) | 99 | 100 | 15 | 13.57 (± 0.16) | - | - |
| **108E** | NC6 | Bacillus sp. SKNB1 (KX881480.1) | 99 | 20 | 5 | 18.01 (± 0.31) | - | - |
| **109E** | NC6 | *Burkholderia phytofirmans* PSB48 (KX881479.1) | 99 | 50 | 5 | 12.49 (± 0.38) | - | ++ |
| **110E** | NC6 | *Serratia* sp. F6 (KX881478.1) | 99 | 50 | 5 | 10.91 (± 0.17) | - | - |
| **111E** | NC6 | *Pseudomonas moraviensis* WW1 (KX881477.1) | 100 | 100 | 10 | 11.22 (± 0.32) | - | - |
| **112E** | NC6 | *Rhodococcus erythropolis* MS 70 (KX881476.1) | 99 | 20 | 15 | 10.45 (± 0.63) | - | - |
| **113E** | NC6 | *Lysinibacillus sphaericus* JOS59-1 (KX881475.1) | 99 | 100 | 5 | 14.89 (± 0.22) | - | - |

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|---|---|---|---|---|---|---|---|---|
| **114E** | NC6 | *Flavobacterium* sp. FlavoEI_ST1r2CH8_F08 (KX881474.1) | 98 | 1 | 5 | 10.30 (± 0.18) | - | - |
| **116E** | NC6 | *Pseudomonas* sp. BA-74-09 (KX881473.1) | 100 | 20 | <2 | 11.93 (± 0.66) | ++ | - |
| **117E** | NC6 | *Pseudomonas* sp. R-42071 (KX881491.1) | 98 | 1 | <2 | 8.82 (± 0.36) | + | - |
| **119E** | NC6 | *Variovorax boronicumulans* E2B5 (KX881472.1) | 99 | <1 | <2 | 9.48 (± 0.27) | - | - |
| **120E** | NC6 | *Luteibacter* sp. UR 2-03 (KX881471.1) | 99 | <1 | <2 | 9.64 (± 0.21) | ++ | - |
| **147E** | NC5 | *Bacillus thioparans* JB3 (KX881457.1) | 97 | 1 | <2 | 9.99 (± 0.33) | - | - |
| **148E** | NC6 | *Flavobacterium* sp. 976H-09 (KX881455.1) | 93 | <1 | 5 | 8.92 (± 0.63) | - | - |
| **149E** | NC6 | *Dyella yeojuensis* rif200829 (KX881503.1) | 99 | <1 | <2 | 9.99 (± 0.17) | | - |
| **150E** | NC6 | *Serratia proteamaculans* Moyola (KX881454.1) | 99 | 100 | 5 | 11.32 (± 0.11) | - | - |
| **151E** | NC5 | *Bacillus* sp. 17880 (KX881453.1) | 99 | 100 | 5 | 12.24 (± 0.95) | - | - |
| **152E** | NC5 | *Microbacterium phyllosphaerae* 0511TES6F7 (KX881452.1) | 99 | <1 | 2 | 10.25 (± 0.65) | - | - |
| **153E** | NC6 | *Flavobacterium* sp. H43(2010) (KX881451.1) | 93 | <1 | 20 | 9.79 (± 0.14) | - | - |
| **154E** | NC5 | *Bacillus simplex* IHB B 15619 (KX881450.1) | 99 | 100 | 10 | 9.23 (± 0.69) | - | - |
| **155E** | NC6 | *Bacillus pumilus* 1Z1 (KX881449.1) | 99 | 20 | <2 | 11.78 (± 0.47) | - | - |
| **156E** | NC6 | *Bacillus mycoides* 0911MAR9I2 (KX881448.1) | 99 | 50 | 10 | 9.43 (± 0.21) | - | - |
| **158E** | NC5 | *Bacillus weihenstephanensis* 22U1 (KX881506.1) | 99 | 20 | 2 | 9.48 (± 0.74) | - | - |
| **159E** | NC6 | *Burkholderia* sp. HC87 (KX881507.1) | 99 | 100 | 20 | 12.19 (± 0.32) | - | - |
| **160E** | NC6 | *Bacillus weihenstephanensis* R22U3 (KX881508.1) | 94 | <1 | <2 | 9.79 (± 0.18) | - | - |
| *Rhizospheric Bacteria* | | | | | | | | |
| **26R** | NC6 | *Bacillus cereus* BRL02-31 (KX881447.1) | 99 | 20 | <2 | 11.09 (± 0.39) | - | - |
| **42R** | NC6 | *Rhodococcus erythropolis* Lb13 (KX881456.1) | 99 | 100 | 20 | 10.40 (± 0.12) | ++ | - |
| **43R** | NC6 | *Leifsonia xyli* LB-G (KX881442.1) | 99 | 100 | 2 | 15.07 (± 0.47) | - | ++ |
| 44R | NC6 | ***Rhodococcus erythropolis* TS-TYKAKK-12 (KX881441.1)** | 99 | 100 | 20 | 12.39 (± 0.36) | ++ | ++ |
| **45R** | NC6 | *Flavobacterium pectinovorum* AD-R2 (KX881483.1) | 99 | 20 | 2 | 11.11 (± 0.36) | - | + |
| **46RY** | NC6 | Caulobacter henricii ATCC 15253 (KX881484.1) | 99 | 20 | 2 | 9.64 (± 0.28) | + | - |
| **46RW** | NC6 | *Tardiphaga robiniae* R-45977 (KX881440.1) | 99 | 20 | <2 | 9.64 (± 0.11) | ++ | ++ |
| **47R** | NC6 | *Flavobacterium* sp. 25AGV (KX881485.1) | 98 | 20 | <2 | 11.85 (± 0.47) | - | - |
| **50R** | NC6 | *Williamsia* sp. SBA 19 (KX881439.1) | 99 | 1 | 2 | 10.09 (± 0.43) | - | ++ |

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|---|---|---|---|---|---|---|---|---|
| **51R** | NC6 | *Paenibacillus* sp. DSL09-3 (KX881438.1) | 99 | 20 | 2 | 10.70 (± 0.50) | - | - |
| **52R** | NC6 | *Bacillus simplex* JP44SK13 (KX881437.1) | 99 | 50 | 5 | 17.52 (± 0.03) | - | - |
| **62R** | NC6 | *Bacillus* sp. Y81_1 (KX881486.1) | 99 | 1 | 2 | 13.93 (± 0.81) | - | + |
| **64R** | NC6 | *Pseudomonas* sp. FGS9 (KX881436.1) | 95 | <1 | <2 | 9.86 (± 0.32) | ++ | - |
| **65R** | NC6 | *Janthinobacterium* sp. HH106 (KX881435.1) | 99 | 50 | 5 | 11.85 (± 0.39) | ++ | - |
| 89R | NC6 | ***Aminobacter aminovorans* LZ1304-3-1 (KX881427.1)** | 99 | 100 | <2 | 23.92 (± 0.91) | ++ | ++ |
| **90R** | NC6 | *Streptomyces phaeochromogenes* HBUM173331 (KX881488.1) | 99 | 100 | 2 | 16.67 (± 0.64) | ++ | - |
| 91R | NC6 | ***Ensifer adhaerens* Sx1 (KX881426.1)** | 99 | 100 | 5 | 30.01 (± 0.82) | ++ | + |
| **92R** | NC6 | *Streptomyces* sp. H (KX881489.1) | 99 | 20 | 2 | 10.78 (± 0.46) | - | ++ |
| **93R** | NC6 | *Arthrobacter nicotinovorans* T258 (KX881425.1) | 99 | 100 | 10 | 11.80 (± 0.39) | ++ | - |
| **94R** | NC6 | *Arthrobacter* sp. WR2 (KX881424.1) | 99 | 100 | 15 | 11.55 (± 0.32) | ++ | - |
| **121R** | NC6 | *Streptomyces* sp. THG-e117 (KX881492.1) | 99 | 20 | <2 | 10.10 (± 0.04) | - | - |
| **122R** | NC5 | *Streptomyces ciscaucasicus* HBUM175135 (KX881493.1) | 99 | 20 | <2 | 9.48 (± 0.38) | - | + |
| **123R** | NC6 | *Stenotrophomonas maltophilia* FGS4-1 (KX881470.1) | 100 | 50 | 2 | 9.99 (± 0.02) | - | - |
| **124R** | NC6 | *Bacillus* sp. HH57 (KX881494.1) | 99 | 20 | 5 | 10.51 (± 0.05) | - | - |
| **125R** | NC5 | *Streptomyces* sp. INBio_4517K (KX881495.1) | 99 | 20 | <2 | 10.96 (± 0.61) | - | - |
| **126R** | NC6 | *Chitinophaga arvensicola* MRP-16 (KX881469.1) | 98 | 1 | 15 | 11.22 (± 0.11) | - | - |
| **127R** | NC5 | *Pseudomonas jessenii* LAM 1 (KX881468.1) | 99 | 50 | <2 | 9.99 (± 0.04) | + | - |
| **128R** | NC6 | *Streptomyces* sp. S-4-11 (KX881496.1) | 99 | 20 | <2 | 9.74 (± 0.57) | - | ++ |
| **129R** | NC6 | *Pseudomonas* sp. R48 (KX881497.1) | 100 | 100 | <2 | 14.33 (± 0.27) | + | - |
| **130R** | NC6 | *Rhodococcus erythropolis* Dr10 (KX881498.1) | 99 | 100 | 20 | 10.05 (± 0.67) | - | - |
| **131R** | NC5 | *Bacillus* sp. YC6907 (KX881467.1) | 99 | 20 | 5 | 13.92 (± 0.41) | - | - |
| **132R** | NC6 | *Streptomyces prunicolor* B430 (KX881499.1) | 99 | 100 | 10 | 12.39 (± 0.71) | ++ | - |
| **133R** | NC5 | *Bacillus megaterium* VC2 (KX881466.1) | 100 | 100 | 5 | 12.24 (± 0.04) | - | - |
| **134R** | NC6 | *Streptomyces* sp. MR-I16 (KX881465.1) | 99 | 20 | <2 | 11.47 (± 0.63) | + | + |
| **135R** | NC6 | *Streptomyces xanthophaeus* SLG-1 (KX881500.1) | 99 | 20 | <2 | 10.81 (± 0.14) | - | ++ |
| **136R** | NC6 | *Bacillus* sp. e (2013) (KX881501.1) | 99 | 100 | 10 | 12.34 (± 0.74) | - | - |
| **138R** | NC5 | *Arthrobacter* sp. 7B-214 (KX881464.1) | 99 | 100 | 5 | 11.27 (± 0.09) | - | + |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **139R** | NC5 | *Flavobacterium* sp. LW53 (KX881463.1) | 98 | 20 | <2 | 10.61 (± 0.21) | - | - |
| **140R** | NC5 | *Flavobacterium* sp. FlavoEI_ChT7r1CD2_C07_51 (KX881462.1) | 99 | 20 | <2 | 12.04 (± 0.34) | - | - |
| **141R** | NC6 | *Streptomyces tauricus* T2Z2 (KX881502.1) | 99 | 100 | <2 | 9.84 (± 0.14) | - | - |
| **142R** | NC6 | *Pedobacter panaciterrae* Tibetlhz-40 (KX881461.1) | 98 | 20 | 15 | 10.20 (± 0.04) | - | - |
| **143R** | NC6 | *Dyella* sp. GR28 (KX881460.1) | 99 | 1 | 2 | 9.08 (± 0.78) | ++ | - |
| **144R** | NC6 | *Pseudomonas* sp. B3ZZ (KX881459.1) | 98 | 100 | <2 | 16.63 (± 0.39) | + | - |
| **145R** | NC6 | *Streptomyces* sp. LZ4S2 (KX881458.1) | 98 | 20 | <2 | 10.76 (± 0.71) | - | - |
| **163R** | NC6 | *Bacilli bacterium* DP_00088 (KM274109.1) | 99 | <1 | <2 | 11.88 (± 0.31) | - | - |
| **164R** | NC6 | *Pseudomonas frederiksbergensis* BDR1P1B1 (KJ567113.2) | 99 | <1 | 2 | 9.23 (± 0.14) | - | - |

[a]Phylogenetic affiliations were based on 16S rRNA gene sequences of about 800 bp length

**Supplementary Table 4.** Ratio NPTs Leaves/NPTs Roots (L/R) of non-inoculated (Control) or inoculated *B. celtiberica* plants in *in vitro* experiments. 29E: *Neorhizobium alkalisoli*; 32E: *Rhizobium herbae*; 44R: *Rhodococcus erythropolis*; 89R: *Aminobacter aminovorans*; 91R: *Ensifer adhaerens*; BC: bacterial consortium including *Variovorax paradoxus* strain 28EY and *Phyllobacterium myrsinacearum* strain 28EW.

| | **0 µM $As^V$** | **150 µM $As^V$** |
|---|---|---|
| **Control** | 4.79 | 0.46 |
| **29E** | 4.96 | 1.49 |
| **32E** | 0.88 | 0.75 |
| **44R** | 5.52 | 0.31 |
| **89R** | 5.71 | 2.78 |
| **91R** | 5.96 | 0.28 |
| **BC** | 7.59 | 0.52 |

**Supplementary Table 5.** pH and metal(loid) concentrations (mg $kg^{-1}$) in soil samples taken in the experimental phytoextraction area. Values are the average of three samples with a standard error < 5%. Plots are named according to subsequent treatment: plants inoculated with *Rhizobium herbae* strain 32E (E), *Ensifer adhaerens* strain 91R (R), or bacterial consortium composed of *Variovorax paradoxus* strain 28EY and *Phyllobacterium myrsinacearum* strain 28EW (BC and BC+F correspond to bacterial consortium without or with fertilizer, respectively) and non-inoculated plants (control, C and fertilized control, FC).

| **Treatment** | **Plot** | **pH** | **Cr** | **Ni** | **Cu** | **Zn** | **As** | **Cd** | **Hg** | **Pb** |
|---|---|---|---|---|---|---|---|---|---|---|
| C | C-1 | 4.25 | 32.81 | 29.99 | 1,402 | 2,565 | 1,767 | 6.72 | 62.86 | 4,255 |
| | C-2 | 4.75 | 30.34 | 24.83 | 1,130 | 2,101 | 1,219 | 6.8 | 19.37 | 4,335 |
| FC | FC-1 | 4.4 | 32.53 | 35.82 | 1,369 | 2,460 | 2,235 | 9.24 | 16.64 | 9,945 |
| E | E-1 | 5.25 | 31.03 | 32.12 | 1,146 | 2,044 | 1,699 | 6.49 | 40.78 | 4,505 |
| | E-2 | 5.1 | 21.62 | 21.96 | 1,416 | 2,458 | 859 | 8.33 | 20.26 | 4,664 |
| R | R-1 | 5.56 | 25.48 | 25.87 | 1,443 | 266 | 1,264 | 8.76 | 16.32 | 5,211 |
| BC | BC-1 | 2.56 | 18.41 | 22.68 | 1,192 | 1,861 | 2,020 | 6.33 | 16.68 | 6,536 |
| | BC-2 | 2.91 | 21.36 | 18.19 | 1,168 | 1,946 | 3,034 | 7.09 | 14.89 | 9,176 |
| BC+F | BC+F-1 | 3.26 | 24.28 | 23.46 | 1,173 | 1,771 | 1,342 | 6.42 | 15.25 | 4,651 |
| | BC+F-2 | 4.01 | 19.05 | 23.48 | 1,338 | 2,019 | 1,626 | 6.64 | 16.62 | 5,995 |

**Supplementary Table 6.** ANOVA analysis in *in vitro* culture and field experiments.

| ***In vitro* culture** | | | | | | |
|---|---|---|---|---|---|---|
| | | **Sum of squares** | **Degrees of freedom** | **Mean square** | **F** | **Sig.** |
| [As] Leaf | Within groups | 503,737 | 6 | 83,956 | 0.66 | 0.67 |
| | Between groups | 1,758,435 | 14 | 125,603 | | |
| | Total | 2,262,173 | 20 | | | |
| [As] Root | Within groups | 134,965,177 | 6 | 22,494,196 | 0.15 | 0.98 |
| | Between groups | 1,984,560,266 | 14 | 14,1754,305 | | |
| | Total | 2,119,525,443 | 20 | | | |
| Leaf dry weight | Within groups | 0.041 | 6 | 0.007 | 6.22 | 0.00 |
| | Between groups | 0.015 | 14 | 0.001 | | |
| | Total | 0.056 | 20 | | | |
| Root dry weight | Within groups | 0.005 | 6 | 0.001 | 0.70 | 0.65 |
| | Between groups | 0.016 | 14 | 0.001 | | |
| | Total | 0.021 | 20 | | | |
| **Field experiments** | | | | | | |
| | | **Sum of squares** | **Degrees of freedom** | **Mean square** | **F** | **Sig.** |
| [As] Leaf | Within groups | 26,326 | 5 | 5,265 | 1.23 | 0.29 |
| | Between groups | 817,696 | 192 | 4,259 | | |
| | Total | 844,021 | 197 | | | |
| [As] Root | Within groups | 64,817,895 | 5 | 12,963,579 | 0.98 | 0.43 |
| | Between groups | 2,536,855,888 | 192 | 13,212,791 | | |
| | Total | 2,601,673,783 | 197 | | | |
| Plant Height Index (PHI) | Within groups | 457,564 | 5 | 91,513 | 21.22 | 0.00 |
| | Between groups | 827,863 | 192 | 4,312 | | |
| | Total | 1,285,427 | 197 | | | |
| Shoot dry weight | Within groups | 878,365 | 5 | 175,673 | 3.33 | 0.00 |
| | Between groups | 10,107,898 | 192 | 52,645 | | |
| | Total | 10,986,264 | 197 | | | |

**Supplementary Table 7.** Akaike information criterion (AIC) and $R^2$ for the different variables and three different models.

| **Treatment** | **pH** | | **As** | | **As and pH** | |
|---|---|---|---|---|---|---|
| | **AIC** | **$R^2$** | **AIC** | **$R^2$** | **AIC** | **$R^2$** |
| [As] Leaf | 110.72 | 0.43 | 111.16 | 0.42 | 108.77 | 0.52 |
| [As] Root | 294.99 | 0.22 | 286.63 | 0.47 | 288.46 | 0.47 |
| Shoot dry weight | 433.64 | 0.25 | 434.56 | 0.24 | 430.95 | 0.31 |
| Plant Height Index (PHI) | 709.63 | 0.31 | 741.48 | 0.17 | 710.24 | 0.31 |

**Supplementary Table 8.** Effects of different bacteria and experimental conditions on the final arsenic content of *Betula celtiberica* leaves and roots. BCF, bioconcentration factor; MR, mobility ratio; TF, translocation factor. E: *Rhizobium herbae* strain 32E; R: *Ensifer adhaerens* strain 91R; BC: bacterial consortium including *Variovorax paradoxus* strain 28EY and *Phyllobacterium myrsinacearum* strain 28EW; BC+F: bacterial consortium with fertilizer; C: non-inoculated control; FC: fertilized control.

| Treatment | BCF | MR | TF |
|---|---|---|---|
| Control (C) | 0.05 | 0.003 | 0.06 |
| Control + Fertilizer (FC) | 0.18 | 0.007 | 0.04 |
| Endophyte (E) | 0.14 | 0.001 | 0.01 |
| Rhizobacteria (R) | 0.18 | 0.003 | 0.02 |
| Consortium (BC) | 0.47 | 0.009 | 0.02 |
| Consortium + Fertilizer (BC+F) | 0.12 | 0.001 | 0.01 |

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