

Seed Microbiota: A Key Factor in Plant Adaptation to Arsenic Stress

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Abstract

Aim: Arsenic contamination represents one of the most critical anthropogenic stressors compromising organism resilience in the context of Global Change. However, some plant species can complete their life cycle in soils contaminated with this metalloid. Studies on plant–soil microbiome symbiosis have emphasized horizontal microbiome transmission (from soil to roots), while underestimating the role of vertically transmitted seed borne microbiomes. This work examines the seed borne endophytic bacterium *Acinetobacter radioresistens* MC 14, isolated from arsenic hyper resistant plants *Jasione montana* and known for its arsenic tolerance and plant growth promoting traits. The study investigates its capacity to modulate plant phenotypic traits and enhance adaptation under arsenic stress.

Methods: To this end, we evaluated the physiological responses of *Arabidopsis thaliana* exposed to As(III) concentrations following inoculation with *A. radioresistens* MC 14, which apoplastically colonizes roots and establishes a non invasive facultative symbiosis that improves plant survival under arsenic stress.

Results: *A. radioresistens* MC 14 improves plant fitness and ecological success, with optimal inoculum levels maximizing the benefits of the interaction while minimizing symbiotic costs. *A. radioresistens* MC 14 mitigates arsenic induced phytohormonal imbalances in roots during early development. This bacterium–plant association promotes root growth and reduces As(III) triggered oxidative stress by activating cellular recovery mechanisms. As a result, plants produce more roots, flowers, and leaves even under toxic conditions.

Conclusions: These findings indicate that plants exert selective pressure on their seed microbiome, driving co evolution and maintaining beneficial microbial reservoirs across generations, ultimately enhancing plant performance in stressful environments.

Highlights

- Seed-borne endophytes represent a potential reservoir that supports plant survival under environmental stress.
- Seed-borne *A. radioresistens* MC 14 apoplastically colonizes non-native roots, forming a non-invasive facultative symbiosis supported by key genomic traits that enable rhizosphere adaptation and plant association.
- *A. radioresistens* MC 14 carries key genes that facilitate root colonization and rhizosphere adaptation, providing strong bioremediation potential.
- Under high arsenic stress, seed-borne *A. radioresistens* MC 14 improves plant fitness, stress tolerance, and successful progression into reproductive stages.

Introduction

Pollution, climate change, and biodiversity loss are among the most critical global environmental challenges, with pollution alone contributing to approximately nine million deaths annually—one in six worldwide (Fuller et al., 2022). Plant ecosystems, essential to both natural and agricultural productivity, are increasingly affected by these stressors. Abiotic factors such as arsenic contamination, alongside biotic pressures, directly impair plant development and biodiversity, posing a serious threat to global food security (Zandalinas et al., 2021). Current projections for food production often neglect the compounded effects of climate change and arsenic contamination, which reduce crop yields and increase arsenic accumulation in seeds (Muehe et al., 2019). This issue is particularly severe in rice, a staple for over 350 million people in developing regions, due to its high capacity for arsenic bioaccumulation (Geng et al., 2023). Globally, arsenic contamination affects more than 140 million individuals across aquatic and terrestrial systems, frequently exceeding World Health Organization (WHO) safety thresholds (Babar & Tariq, 2018). In response to such environmental pressures, plants have evolved various acclimation strategies to withstand adverse conditions. Over recent decades, evidence has highlighted the pivotal role of plant-microbe interactions, especially in the rhizosphere, in enhancing resilience to abiotic stress (Berendsen et al., 2012). More recently, the endosphere has gained attention for its contribution to plant adaptability. In this context, seed endomicrobiota has a dual origin: horizontal transmission, mainly from soil and vertical transmission via maternal inheritance through seeds. Despite their biological importance, seed microbiomes—particularly in wild plants—have often been overlooked, despite exhibiting high taxonomic diversity throughout their life cycle (Chesneau et al., 2022). Although the environmental and biological stressors that shape the seed microbiota remain insufficiently characterized, the genetic composition of seed microbiomes is increasingly recognized as a key factor in modulating plant responses to environmental fluctuations and influencing the evolutionary trajectory of the plant metaorganism (Abdullaeva et al., 2021; González-Benítez et al., 2021; Matsumoto et al., 2021; Nelson, 2018; Wallace, 2023). Ecological selection has recently been identified as the principal mechanism influencing the succession of dominant taxa during the process of seed filling and maturation (Chesneau et al., 2022; Nelson, 2018). Although plant-bacterial interactions are increasingly recognised for enhancing resilience under arsenic stress (Molina et al., 2019; Singh et al., 2023), the role of maternally inherited seed microbiota in detoxification, adaptation, and evolution remains largely unexplored. Preliminary evidence suggests their importance; for example, *Rhodococcus rhodochrous*, a seed endophyte from *Jasione montana*, can modulate the phenotypic responses of arsenic-sensitive *J. sessiliflora*, conferring enhanced tolerance under arsenic exposure (González-Benítez et al., 2021). Sequencing the genomes of such endophytes offers a novel approach to understanding their functional potential, often overlooked in seed physiology studies. This strategy enables the identification of beneficial genes, their interactions with the plant genome, and mechanisms of stress resilience. It also supports biofertilizer development and biotechnological innovations for bioremediation.

Among seed-associated bacteria, *Acinetobacter* species—frequently found in ovules and seeds (Verma and White, 2019)—have shown notable arsenic-transforming capabilities. Certain strains can oxidize over 80% of As(III) to As(V), linked to the presence of the *aioA* gene (Basu et al., 2023; Dutta et al., 2023). A.

radioresistens, in particular, stands out for its phenotypic diversity, including plant growth promotion (Lafi et al., 2016; Zapata-Sifuentes et al., 2022), pollutant degradation (Cattuci et al., 2025; Hidalgo et al., 2025; Liu et al., 2020; Macaya et al., 2019; Xiang et al., 2023), and extremotolerance (McCoy et al., 2012), largely attributed to its genomic plasticity and mobile genetic elements (Gentilini et al., 2018; Walter et al., 2020). The MC-14 strain of *A. radioresistens*, isolated from *J. montanain* in an arsenic-contaminated area (García-Salgado et al., 2012), is cultivable under standard conditions and exhibits arsenate resistance (MIC = 200 mM), antifungal activity, and plant growth-promoting traits (Molina et al., 2019). These features make it an ideal model for exploring how seed microbiomes contribute to plant fitness and adaptation under arsenic stress, and for investigating the functional potential of the plant hologenome.

In this study, we propose the following objectives: (1) to perform a complete genome sequencing of the *A. radioresistens* MC-14 strain, with a particular emphasis on identifying genes implicated in detoxification and metabolic pathways related to arsenic and heavy metal stress, the presence of mobile genetic elements involved in horizontal gene transfer, and plant interaction genes; and (2) to assess and quantify the beneficial effects of this seed-derived strain on plant fitness and adaptation using *Arabidopsis thaliana* as a model host species for inoculation experiments. Genomic characterization will shed light on the functional contribution of maternally inherited microbiota to plant physiological adaptation under arsenic stress. This knowledge could inform the application of such bacteria in phytostimulation, and detoxification strategies, ultimately contributing to more sustainable agricultural practices and improved food quality.

Material and methods

Biological materials, culture media and growth conditions

Acinetobacter radioresistens MC-14 was previously isolated from seeds of *Jasione montana* plants (Molina et al., 2019), a highly arsenic tolerant plant species (Benson et al., 1981), collected from the vicinity of the Monica mine (Bustarviejo, Madrid) an area characterized by arsenic-contaminated soils with concentrations ranging from 0.3 to 30 g·kg⁻¹ (García-Salgado et al., 2012).

For whole-genome sequencing, *A. radioresistens* MC-14 was cultivated aerobically in LB medium at 30°C. Upon obtaining sufficient mass, genomic DNA was extracted and sequenced by MicrobesNG company (Birmingham, U.K) following a combination of Nanopore long-read sequencing (Clarke et al., 2009) and Illumina short-read sequencing (250-nt paired-end reads) to refine the genome sequence.

To assess resistance of *A. radioresistens* MC-14 to metals and metalloids, bacterial cultures were grown in sodium As(III) (NaAsO₂; 0–40 mM; Merck), as well as nickel chloride (NiCl₂), chromium(II) chloride (CrCl₂), cobalt(II) sulfate (CoSO₄), copper(II) sulfate (CuSO₄), lead(II) nitrate [Pb(NO₃)₂], and zinc sulfate (ZnSO₄) at concentrations ranging from 0 to 5 mM (Sigma-Aldrich). After 24 hours of incubation at 30°C

(end of exponential growth phase), bacterial growth was evaluated spectrophotometrically by measuring optical density at 600 nm (OD_{600nm}).

For bacterial inoculation experiments, *A. radioresistens* MC-14 was cultivated in LB broth until reaching OD_{600nm} of 0.07, 0.21, and 0.63 at 600 nm, as measured with a Spectronic Genesys-8 spectrophotometer (Spectronic Instruments Inc.). After sterilization and cooling of 50 mL of MS broth medium, 0.1 mL of the bacterial suspension at the desired concentration was added to the growth plates.

Although the *A. radioresistens* MC-14 strain originates from *J. montana*, the functional assays were conducted in *A. thaliana*, the reference model in plant biology due to its fully sequenced genome, the availability of numerous mutant and transgenic lines, and standardized protocols for microscopic and genetic analyses (Ortega-Villaizán et al., 2024). *Arabidopsis thaliana* seeds of the wild-type accession Columbia-0 (Col-0, stock N1092) were obtained from the Nottingham Arabidopsis Stock Centre (NASC).

A. thaliana was grown on sterile 0.5· Murashige & Skoog (MS) medium plates (Murashige & Skoog, 1962) supplemented with 0.5 g of MES monohydrate L^{-1} of medium and 1% of agar (w/v). The pH was adjusted to 5.8 using KOH. To sterilize *A. thaliana* seeds, they were first agitated in 70% ethanol for 2 minutes, followed by immersion in a sterilization solution containing 1% sodium dodecyl sulfate (w/v) and 2% sodium hypochlorite (w/v) in sterile distilled water for 12 min. The seeds were then washed five times with sterile MilliQ water. To confirm complete surface sterilization, the seeds were plated on Luria Bertani (LB) agar (Condalab). After 24 hours, the absence of bacterial growth confirmed sterilization.

Molecular and genomic, approaches to characterize *A. radioresistens* MC-14

A. radioresistens MC-14 was transformed with the pBAV1k-t5-gfp plasmid (Bryksin and Matsumura, 2010), which conferred the ability to produce a green fluorescent protein (GFP) and harbors kanamycin resistance. GFP expressing *A. radioresistens* MC-14 cells were grown at 28°C in LB medium supplemented with kanamycin at a concentration of 50 $\mu g m^{-1}$.

Plant Transformation and Co-Cultivation Conditions. *Arabidopsis* plants were transformed using *Agrobacterium tumefaciens* harboring the NPTII gene conferring resistance to kanamycin, following the floral dip method described by Clough and Bent (1998). The antibiotic kanamycin was included in the growth medium to prevent the loss of the GFP-encoding plasmid in *A. radioresistens* MC-14 during plant-microbe co-cultivation assays.

Gene prediction, genome annotation and sequence data analyses. The genome of *A. radioresistens* MC-14 was annotated using the Rapid Annotation using Subsystem Technology (RAST) pipeline (Overbeek et al., 2008). Functional annotation of predicted protein-coding genes was performed by comparison against several curated protein databases, including SwissProt, NCBI Protein, Clusters of Orthologous Groups (COG), Pfam, and SMART, using BLAST and RPS-BLAST algorithms (Altschul et al., 1997). Pairwise and multiple sequence alignments of proteins were conducted using the ClustalW algorithm (Thompson et al., 1994) via the EMBL-EBI server. Phylogenetic analyses were performed using the

Kimura two-parameter model (Kimura, 1980), and evolutionary trees were constructed with the neighbor-joining method (Saitou and Nei, 1987) implemented in the PHYLIP software package (Felsenstein, 1993). Comparative genomic analyses were conducted using Geneious software, version 10.0.2 (<https://www.geneious.com/>), and Venn diagrams were generated with EDGAR (Blom et al., 2009). The complete genome sequence and corresponding annotation of *A. radioresistens* MC-14 have been submitted to the NCBI GenBank database under accession number CP131483.

Effects of arsenic on *A. thaliana* colonization by *A. radioresistens*

To evaluate the impact of arsenic [As(III)] exposure and *A. radioresistens* MC-14 inoculation on *A. thaliana* phenotypic responses, a full-factorial experimental design was employed. The experiment comprised four arsenic concentrations (0, 3, 5, and 10 μM) and four bacterial inoculation levels, defined at OD₆₀₀ (0, 0.07, 0.21, or 0.63). Each treatment included three replicate plates, with six plants per plate (18 plants per treatment), resulting in a total of 24 plates (4×2×3), and a total of 144 plants.

Surface-sterilized *A. thaliana* seeds were sown on 0.5x MS plates and stratified in the dark at 4°C for two days. Thereafter, plants were transferred to treatment media containing either 0 (control), 3, 5, or 10 μM As(III), along with varying concentrations of *A. radioresistens* MC-14 (OD₆₀₀ = 0, 0.07, 0.21, or 0.63), depending on the specific experimental condition.

Florescence and gas exchange parameters were measured at the end of the cultivation period (30 days) in 4 plants per plate (12 plants per treatment). Measurements were conducted between 7:00 and 10:00 h (solar time) using an infrared gas analyzer (LICOR 6400XT, Li-Cor Inc., Lincoln NE, USA). Environmental conditions were adjusted to match those of the growth chamber; air temperature inside the cuvette was set to 20°C, CO₂ concentration to 400 $\mu\text{mol mol}^{-1}$, vapor pressure deficit to 1.5, and photosynthetic photon flux density (PPD) to 800 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Initial measurements were performed under dark conditions (PPFD = 0). Once the net photosynthetic rate (A_{net}) stabilized, the maximum potential quantum efficiency of Photosystem II (Fv/Fm) and dark respiration rate (A_{dark}) were recorded. Subsequently, light was applied, and after a minimum of 10 min, to allow the plants for light acclimation, one A_{net} stabilized again, the following parameters were measured: effective quantum yield of PSII (Φ_{PSII}), photochemical quenching (qP), non-photochemical quenching (qN), and A_{net} .

The phenological stage of each plant was also recorded based on the following scale: 0, no visible inflorescence; 1, inflorescence present but peduncle not elongated; 2, elongated peduncle with discernible flower buds; 3, first flower bud opened, but most of them remained closed; 4, all flower buds open, with onset of wilting and abscission of stamens and petals. Additionally, plant survival was recorded for all individuals. A plant was considered dead when all leaves had turned brown and no signs of new growth were observed for a period of at least five consecutive days.

Then, plants were harvested and divided into leaves, inflorescence and roots. Leaf samples were immediately frozen at - 30°C and stored until analysis of photosynthetic pigment concentrations.

Pigment quantification was performed on the same plants used for gas exchange measurements.

Leaf subsamples (20 mg fresh weight) were extracted using pure dimethyl sulfoxide (DMSO) to determine the concentrations of chlorophyll *a* (cla), chlorophyll *b* (clb) and total carotenoids (xanthophylls + carotenoids; cars), following methodology described in Barnes et al. (1992). Pigment concentrations were calculated using the equations provided by Wellburn (1994). All pigment values were expressed on a dry mass per area basis. Finally, all plant organs (leaves, inflorescences, and roots) were oven-dried and weighed to determine dry biomass.

Bacterial colonization of plants. To visualize and localize *A. radioresistens* within root tissues, at least five plants of transgenic *A. thaliana* containing the NPII gene inoculated with *A. radioresistens* MC-14 harboring the plasmid pBAV1k-t5-gfp for GFP expression. Plants were grown under controlled conditions in a growth chamber with a 16 h light / 8 h dark photoperiod at 22/18°C (light/dark), and a photon flux density of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Visualization was performed at the post-germination stage, five days after sowing. Visualization was conducted using a Zeiss LSM 880 confocal laser microscope with excitation using an Argon multiline laser at 488 nm and a 494/596 nm broadband filter for GFP detection. Differential interference contrast (DIC) imaging was applied, and observations were processed using the maximum intensity projection technique to enhance spatial resolution.

Statistical analysis.

All analyses were performed using R version 4.3.0 (R Core Team, 2023). The fixed effects of *A. radioresistens* MC-14 inoculation (qualitative) and As(III) concentration (quantitative) were analyzed using linear mixed models including plate as a random factor. The influence of *A. radioresistens* MC-14 inoculation and As(III) concentration on plant phenological stage was evaluated using an ordinal multinomial analysis with the function *polr* from the “MASS” package (Venables and Ripley, 2002).

Results

Whole-genome analysis of *A. radioresistens* MC-14

The complete genome of *A. radioresistens* MC-14 was sequenced using a hybrid approach combining Illumina and Oxford Nanopore technologies, as conducted by the MicrobesNG (Birmingham, UK) genome sequencing service. The Sequencing generated a total of 319,201 reads, resulting in approximately 30 × genome coverage. The assembled genome consisted of a single circular chromosome with a predicted length of 3,161,054 base pairs, along with three plasmids measuring 21,734 bp (plasmid 1), 7,990 bp (plasmid 2), and 4,507 bp (plasmid 3), respectively (Fig. 1). Genome annotation predicted a total of 3,000 coding sequences and 98 RNA genes, including 77 tRNA and 21 rRNA genes.

Whole-genome comparison of *A. radioresistens* MC-14 with other *Acinetobacter radioresistens* strains revealed high average nucleotide identity (ANI) values: 99.64% with strain FDAARGOS_731 (GCA_014069095; 63.5% genome alignment), 98.47% with DSM 6976 (GCA_000248115; 63.55% aligned),

98.58% with LH6 (CP030031; 62.99% aligned), 98.57% with DD78 (65.84% aligned), and 98.62% with DSSKY-A001 (74.29% aligned). In contrast, comparisons with other *Acinetobacter* species such as *A. baylyi* ADP1 and *A. variabilis* RYU24 resulted in significantly lower ANI values of 73.95% (39.97% aligned) and 74.91% (38.94% aligned), respectively.

Analysis of the 16S rRNA gene sequence confirmed the close phylogenetic relationship between *A. radioresistens* MC-14 and other strains of the species, showing 100% identity with FDAARGOS_731, and > 99.7% identity with DSM 6976, DSSKY-A001, LH6, and DD18 (Figure S1). Notably, the GC content of the three plasmids identified in the MC-14 genome was markedly lower than that of the chromosome: 37.5% (plasmid 1), 34.1% (plasmid 2), and 31.6% (plasmid 3) (Table 1).

Table 1
Genome features of *A. radioresistens* MC-14 in comparison to those of strains FDAARGOS_731 and DSM 6976.

Genome features	MC-14	FDAARGOS_731	DSM 6976 ^T
Origin	<i>A. thaliana</i> seed	human skin	oil soil
Total size (bp)	3161054	3377444	3433938
GC% chromosome (bp)	41.7 (3126823)	41.7 (3218180)	41.8 (3038795)
GC% plasmid 1 (bp)	37.5 (21734)	37.5 (58767)	37.1 (240231)
GC% plasmid 2 (bp)	34.1 (7990)	37.1 (9483)	39.2 (77605)
GC% plasmid 3 (bp)	31.6 (4597)	34.9 (27601)	42.7 (29910)
GC% plasmid 4 (bp)	--	39.4 (61168)	36.2 (22527)
GC% plasmid 5 (bp)	--	39.1 (2245)	36.3 (9388)
GC% plasmid 6 (bp)	--	--	35.3 (5898)
GC% plasmid 7 (bp)	--	--	39.5 (5208)
GC% plasmid 8 (bp)	--	--	32.7 (4376)
Proteins	3000	3302	3330
RNA	96	97	81
Plasmids	3	5	8

The *A. radioresistens* strains included in the comparison originate from diverse habitats. Strain MC-14 was isolated from *J. montana* seeds collected from arsenic-contaminated soils; FDAARGOS_731 from human skin (Roca et al., 2024); LH6 from the avian intestinal tract (Crispen et al., 2018); DSM 6976 from cotton and gamma-sterilized soil (Nishimura et al., 1988); and DD78 from oil-contaminated sludge from the delta of the Aconcagua River. Ortholog analysis was performed to compare the genome of MC-14

with the closest strains. A Venn diagram (Fig. 2) illustrates the core and pan-genome composition of *A. radioresistens*. A total of 2,465 genes were shared among the four analyzed strains. MC-14 shared 2,474 genes with DD18, 2,654 with FDAARGOS_731, and 2,472 with LH6. Additionally, 202 genes were identified as unique singletons exclusive to the MC-14 genome (Fig. 2).

Genomic insights into heavy metal and arsenic resistance in *A. radioresistens* MC-14.

The genomic analysis of *A. radioresistens* MC-14 revealed a substantial number of genes encoding proteins that may confer resistance to heavy metals and metalloids (Table S1).

Minimum inhibitory concentration (MIC) assays indicated tolerance levels of 2.5 mM for CoSO_4 and NiCl_2 , > 5 mM for CrCl_2 , 5 mM for $\text{Pb}(\text{NO}_3)_2$ and ZnSO_4 , and 1 mM for CuSO_4 (Fig. 3). Remarkably, *A. radioresistens* MC-14 strain demonstrated substantial resistance to As(III), with growth sustained up to 20 mM and observable even at 25 and 30 mM concentrations (Fig. 4).

Genome annotation identified two distinct chromosomal gene clusters encoding arsenic resistance proteins (Fig. 5). Cluster 1 consists of the *arsHB1C1R1C2* operon, while cluster 2 contains *arsB2R2C3*. Both clusters are located on the chromosome. Comparative analysis with the closely related strains DSM6976 and FDAARGOS_731 revealed conserved *ars* operon architecture; however, the genomic positioning of these clusters varies. In DSM6976, a gene cluster analogous that resembles cluster 2 of *A. radioresistens* MC-14 is chromosomally located, whereas a cluster resembling MC-14 strain's cluster 1 is plasmid-encoded. A similar genomic arrangement was observed for FDAARGOS_731, with cluster 2-like genes chromosomally located and cluster 1-like genes present on a plasmid (Fig. 5). Interestingly, protein sequence comparisons revealed that the ArsR2 protein from MC-14 cluster 2 shares a higher degree of sequence identity with homologs encoded on plasmid-borne *ars* operons of DSM6976 and FDAARGOS_731 than with their chromosomal counterparts (Table S2). Phylogenetic analysis further supports this, showing that ArsR2 and other proteins encoded by *A. radioresistens* MC-14 cluster 2 group closely with plasmid-derived *ars* proteins from these related strains (Fig. 6). Notably, plasmid 2 of DSM6976 and plasmid 1 of FDAARGOS_731 harbor not only genes related to resistance to heavy metals and metalloids but also numerous genes associated with horizontal gene transfer, suggesting that these *ars* operons may have been acquired via mobile genetic elements.

Genomic determinants of an endophytic lifestyle.

After ten days of seedling growth, *A. radioresistens* MC-14 established a robust biofilm on the root surface, which was easily observable (Figure S2). Detailed microscopic examination further demonstrated that the bacteria were predominantly located within the apoplastic compartment, occupying intercellular spaces without penetrating plant cells, thus confirming their extracellular position within the root tissue. Notably, no evidence of intracellular was observed, indicating that *A. radioresistens* MC-14 adopts a non-invasive strategy, residing exclusively on the root surface and within intercellular spaces (Fig. 7).

We conducted a comprehensive analysis of the *A. radioresistens* MC-14 genome to identify genes potentially associated with traits relevant to plant-associated lifestyles. Genes related to bacterial motility were organized into distinct clusters encoding components of type IV pili and the chemotaxis apparatus (Table S3). The genome also harbors loci putatively involved in exopolysaccharide biosynthesis (Table S3), which are commonly implicated in biofilm formation and host interaction. Furthermore, we identified genes encoding enzymes such as catalases, superoxide dismutases, and peroxidases (Table S3), which are likely involved in reactive oxygen species (ROS) detoxification, a key feature for successful colonization of plant tissues. Genes orthologous to known osmolyte transporters, including those for choline and betaine uptake, were also present (Table S3), suggesting potential mechanisms for osmoregulation under plant-associated conditions. Notably, the genome encodes putative components of multiple secretion systems (types I, II, III, and VI) (Table S3). These systems are frequently associated with effector delivery, immune suppression, and promotion of intracellular colonization in plant hosts. The presence of gene clusters previously associated with plant-microbe interactions supports the hypothesis that *A. radioresistens* MC-14 possesses genetic traits consistent with an endophytic lifestyle.

Plant growth promoting effects under As(III)-induced stress

A. thaliana exhibited full survival in control conditions lacking As(III), irrespective of bacterial inoculum concentration. In contrast, non-inoculated plants showed a marked decrease in survival at As(III) concentrations $\geq 5 \mu\text{M}$, with only 36% of plants surviving exposure to $10 \mu\text{M}$ As(III). Notably, plant survival under As(III) stress was significantly influenced by the inoculation dose (interaction As(III) $\times$ inoculation $F = 11.99$; $p < 0.001$). Inoculation a low cell density ($\text{OD}_{600} = 0.07$) did not confer any protective effect; however, higher inoculum densities ($\text{OD}_{600} = 0.21$ or 0.63) resulted in complete plant survival even at the highest As(III) concentration tested ($10 \mu\text{M}$), highlighting a dose-dependent protective effect of *A. radioresistens* MC-14.

The phenological progression of *A. thaliana* under As(III) exposure was significantly influenced by the bacterial inoculum dose ($\chi^2 = 165$, $p < 0.001$; Fig. 8). In the absence of As(III), bacterial inoculation induced a slight delay in the development of the plants. Increasing As(III) concentrations markedly reduced the probability of plants reaching advanced flowering stages, with complete inhibition of reproductive development observed at the highest As(III) concentration. However, plants inoculated with *A. radioresistens* MC-14 at OD_{600} values of 0.21 or 0.63 demonstrated significantly improved progression to later phenological stages, even under the most severe As(III) stress. In absence of As(III) in the medium, inoculation did not result in significant differences in organ or plant mass. With increasing As(III) concentrations, a progressive reduction in both organ-specific and total biomass was observed. Importantly, these effects, particularly on inflorescence and root biomass, were significantly dependent on the inoculation dose (As(III) $\times$ inoculation interaction, $p < 0.021$ for all cases; Fig. 9). While low As(III) levels (e.g., $3 \mu\text{M}$) elicited minimal growth promotion, increasing the bacterial inoculum enhanced plant growth at higher As(III) concentrations, with maximal biomass observed at $\text{OD}_{600} = 0.21$.

Net photosynthetic rate (A_{net}) increased with rising As(III) concentrations, but this effect was restricted to non-inoculated seedlings (As(III) $\times$ inoculation interaction, Table 2). In contrast, dark respiration exhibited a positive correlation with As(III) concentration, and this response was amplified with increasing bacterial inoculum doses, indicating a significant interaction between As(III) exposure and inoculation. Chlorophyll a (C_{la}) and chlorophyll b (C_{lb}) contents decreased in response to As(III), although the reduction became statistically significant only at concentrations above 5 μ M. These changes were not modulated by bacterial inoculation. In contrast, carotenoid levels showed a clear dependence on both As(III) concentration and the inoculum dose of *A. radioresistens* MC-14 (As(III) $\times$ inoculation interaction). In non-inoculated plants, carotenoid levels increased in response to the highest As(III) concentration. However, in inoculated seedlings, carotenoid content was generally higher under control (As(III)-free) conditions compared to non-inoculated controls, and either remained stable (in the case of the lowest inoculum) or decreased with increasing As(III) concentrations, particularly at higher inoculum levels.

Table 2

Gas exchange parameters: A_{net} (Net photosynthetic rate) and A_{dark} (Dark respiration) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and photosynthetic pigments concentration: Cla (Chlorophyll *a*), Clb (Chlorophyll *b*) and cars (Carotenoids and xanthophylls) ($\mu\text{g gDM}^{-1}$) in plants of *A. thaliana* growing at different As(III) concentrations as a function of *A. radioresistens* MC-14 inoculation after 30 days of culture. Data are average $\pm$ SE ($n = 6$). The last three rows are the F and p values of the analyses.

As(III) (μM)	Inoculum (OD _{600nm})	A	Adark	Cla	Clb	Cars
0	0	4.8 $\pm$ 0.4	1.05 $\pm$ 0.10	16.1 $\pm$ 1.1	7.2 $\pm$ 0.5	2.0 $\pm$ 0.2
	0.07	5.1 $\pm$ 0.4	1.11 $\pm$ 0.13	15.4 $\pm$ 0.8	8.1 $\pm$ 0.5	2.8 $\pm$ 0.2
	0.21	4.5 $\pm$ 0.4	1.37 $\pm$ 0.40	16.3 $\pm$ 1.1	8.4 $\pm$ 0.9	2.4 $\pm$ 0.3
	0.63	4.8 $\pm$ 0.45	1.20 $\pm$ 0.08	17.1 $\pm$ 0.8	8.1 $\pm$ 0.5	2.8 $\pm$ 0.1
3	0	4.3 $\pm$ 0.6	1.74 $\pm$ 0.32	16.6 $\pm$ 0.7	9.5 $\pm$ 2.2	1.7 $\pm$ 0.7
	0.07	4.8 $\pm$ 0.4	1.26 $\pm$ 0.35	16.7 $\pm$ 1.0	7.4 $\pm$ 0.4	2.0 $\pm$ 0.2
	0.21	4.9 $\pm$ 0.3	0.88 $\pm$ 0.13	17.1 $\pm$ 0.9	8.4 $\pm$ 0.7	2.0 $\pm$ 0.3
	0.63	4.9 $\pm$ 0.6	1.08 $\pm$ 0.18	17.2 $\pm$ 0.8	8.0 $\pm$ 0.4	2.7 $\pm$ 0.2
5	0	5.6 $\pm$ 0.6	1.40 $\pm$ 0.37	16.4 $\pm$ 0.8	8.7 $\pm$ 1.5	1.9 $\pm$ 0.6
	0.07	4.2 $\pm$ 0.7	1.31 $\pm$ 0.17	16.5 $\pm$ 0.6	7.6 $\pm$ 0.4	2.3 $\pm$ 0.2
	0.21	4.8 $\pm$ 0.5	0.99 $\pm$ 0.11	17.2 $\pm$ 0.9	7.5 $\pm$ 0.4	2.1 $\pm$ 0.2
	0.63	4.2 $\pm$ 0.4	1.11 $\pm$ 0.12	15.5 $\pm$ 1.6	7.3 $\pm$ 0.9	1.6 $\pm$ 0.4
10	0	12.2 $\pm$ 1.2	2.4 $\pm$ 0.56	12.5 $\pm$ 0.4	6.0 $\pm$ 1.0	2.5 $\pm$ 0.3
	0.07	4.7 $\pm$ 1.4	1.93 $\pm$ 0.93	11.7 $\pm$ 1.1	4.7 $\pm$ 0.6	1.7 $\pm$ 0.2
	0.21	5.4 $\pm$ 0.6	1.48 $\pm$ 0.25	13.7 $\pm$ 0.8	6.8 $\pm$ 0.5	2.2 $\pm$ 0.2
	0.63	5.7 $\pm$ 0.4	2.96 $\pm$ 0.84	14.1 $\pm$ 0.5	6.5 $\pm$ 0.3	2.3 $\pm$ 0.1
As (III)		F = 11.3; p = 0.0014	F = 18.87; p < 0.001	F = 7.8; p = 0.005	F = 3.63; p = 0.014	F = 3.6; p = 0.014
Inoculation		F = 0.65; p = 0.58	F = 1.82; p = 0.18	F = 2.5; p = 0.47	F = 1.27; p = 0.29	F = 1.3; p = 0.29
As (III)×inoculation		F = 3.10; p = 0.036	F = 4.44; p = 0.036	F = 1.5; p = 0.66	F = 0.63; p = 0.77	F = 3.38; p = 0.019

Maximum quantum efficiency of PSII photochemistry (F_v/F_m) remained consistently high (> 0.79) across all treatment conditions, indicating the absence of severe photoinhibition (Table 3). In contrast, the effective quantum yield of PSII (Φ_{PSII}), photochemical quenching (q_P), and non-photochemical quenching (q_N) increased progressively with higher As(III) concentrations, particularly at the highest

concentration tested. Inoculation with *A. radioresistens* MC-14 further enhanced these fluorescence parameters at low As(III) levels, suggesting a stimulatory effect on photosynthetic efficiency and energy dissipation under mild stress. However, at elevated As(III) concentrations, bacterial inoculation moderated the increases in Φ PSII, qP, and qN observed in non-inoculated plants, indicating a buffering effect on photochemical stress responses (As(III) $\times$ inoculation interaction).

Table 3

Fluorescence parameters in plants of *A. thaliana* growing at different As(III) concentrations as a function of *A. radioresistens* inoculation after 30 days of culture. Data are average $\pm$ SE (n = 6). The last three rows are the F and p values of the analyses.

As(III) (μ M)	<i>Inoculum</i> (OD600nm)	Fv/Fm	Φ PSII	qP	qN
0	0	0.80 $\pm$ 0.01	0.12 $\pm$ 0.01	0.16 $\pm$ 0.01	0.44 $\pm$ 0.03
	0.07	0.79 $\pm$ 0.01	0.11 $\pm$ 0.01	0.16 $\pm$ 0.01	0.49 $\pm$ 0.01
	0.21	0.79 $\pm$ 0.01	0.12 $\pm$ 0.01	0.17 $\pm$ 0.01	0.51 $\pm$ 0.02
	0.63	0.80 $\pm$ 0.01	0.11 $\pm$ 0.01	0.15 $\pm$ 0.01	0.45 $\pm$ 0.02
3	0	0.79 $\pm$ 0.01	0.10 $\pm$ 0.01	0.15 $\pm$ 0.01	0.42 $\pm$ 0.02
	0.07	0.76 $\pm$ 0.03	0.11 $\pm$ 0.01	0.18 $\pm$ 0.03	0.50 $\pm$ 0.03
	0.21	0.80 $\pm$ 0.01	0.12 $\pm$ 0.01	0.17 $\pm$ 0.01	0.51 $\pm$ 0.02
	0.63	0.78 $\pm$ 0.01	0.12 $\pm$ 0.01	0.18 $\pm$ 0.02	0.49 $\pm$ 0.04
5	0	0.79 $\pm$ 0.01	0.13 $\pm$ 0.01	0.19 $\pm$ 0.02	0.55 $\pm$ 0.02
	0.07	0.78 $\pm$ 0.01	0.09 $\pm$ 0.01	0.13 $\pm$ 0.01	0.47 $\pm$ 0.02
	0.21	0.79 $\pm$ 0.01	0.11 $\pm$ 0.01	0.16 $\pm$ 0.01	0.52 $\pm$ 0.02
	0.63	0.79 $\pm$ 0.01	0.11 $\pm$ 0.01	0.15 $\pm$ 0.01	0.44 $\pm$ 0.03
10	0	0.80 $\pm$ 0.01	0.21 $\pm$ 0.01	0.3 $\pm$ 0.03	0.56 $\pm$ 0.06
	0.07	0.80 $\pm$ 0.01	0.12 $\pm$ 0.01	0.16 $\pm$ 0.02	0.39 $\pm$ 0.03
	0.21	0.79 $\pm$ 0.01	0.15 $\pm$ 0.01	0.21 $\pm$ 0.02	0.51 $\pm$ 0.02
	0.63	0.79 $\pm$ 0.01	0.16 $\pm$ 0.01	0.24 $\pm$ 0.01	0.56 $\pm$ 0.02

As(III) (μM)	<i>Inoculum</i>	Fv/Fm	ΦPSII	qP	qN
	(OD600nm)				
As (III)	F = 37.7; p = 0.92	F = 23.5; p < 0.001	F = 40.0; p = 0.007	F = 5.64; p = 0.018	
Inoculation	F = 41.5; p = 0.10	F = 1.78; p = 0.15	F = 45.3; p = 0.85	F = 0.84; p = 0.47	
As (III)×inoculation	F = 42.7; p = 0.85	F = 2.15; p = 0.095	F = 45.9; p = 0.26	F = 7.09; p < 0.001	

Discussion

Genome characterization, taxonomic confirmation, and mobile genetic elements in *A. radioresistens* MC-14

Whole-genome sequencing of *A. radioresistens* MC-14 confirmed its taxonomic identity, in agreement with previous classification based on 16S-ITS rDNA analysis (Molina et al., 2019). Average Nucleotide Identity (ANI) comparisons between *A. radioresistens* MC-14 and other *A. radioresistens* strains revealed values exceeding 99.5%, whereas comparisons with other *Acinetobacter* species yielded ANI values below 75%. These results are consistent with established phylogenetic boundaries within the genus (de Almeida et al., 2021), reinforcing the robust species-level classification of MC-14. The high degree of genomic similarity among *A. radioresistens* strains suggests for clonal dissemination, possibly facilitated by host transitions between humans and animals. This observation supports previous findings indicating interspecies transmission and genomic stability within this taxon (Roca et al., 2024).

The comparative genomic analysis of *A. radioresistens* MC-14 with closely related strains provides valuable insights into both the conserved and strain-specific features of this species. The identification of 2,465 core genes shared among the four *A. radioresistens* strains analyzed suggests a relatively stable genomic backbone that likely encodes essential functions for environmental survival and stress resistance, characteristic of *A. radioresistens*. The presence of 202 unique singleton genes in *A. radioresistens* MC-14, as reported by Zhao et al. (2023), suggests that these genes are primarily associated with environmental niche-adaptation functions. In our case, this may reflect adaptive genomic features linked to its specific ecological niche in *J. montana* seeds. These strain-specific genes could encode traits relevant to rhizosphere colonization, metal resistance, plant-microbe interactions, or metabolic specialization, and thus warrant further functional annotation and experimental validation. The relatively high number of shared genes with FDAARGOS_731, a strain isolated from human skin (Roca et al., 2024), suggests a degree of functional overlap that may reflect conserved stress response mechanisms. In contrast, the exclusivity of certain genes in *A. radioresistens* MC-14 may underpin its symbiotic association with plant hosts and its survival in heavy metal-polluted environments. Altogether, the ortholog analysis highlights the genomic plasticity within *A. radioresistens* and underscores the

potential of *A. radioresistens* MC-14 as a model for studying plant-associated bacteria with bioremediation capabilities.

As mentioned, our findings highlight the ecological adaptability and high propagation potential of *A. radioresistens* MC-14 across diverse environments, including human skin, animal gastrointestinal tracts, contaminated soils, adult plants, and seeds. This broad ecological range is consistent with the known plasticity of the *Acinetobacter* genus, which is characterized by a high prevalence of mobile genetic elements (Walter et al., 2020; Zhao et al., 2023). Such mobile elements facilitate horizontal gene transfer (HGT), contributing to both the functional diversification and taxonomic complexity of the genus. This supports the model of an open pangenome in *Acinetobacter*, in which phenotypic traits can be independently acquired in response to specific environmental pressures (de Almeida et al., 2021). Importantly, some mobile elements, particularly plasmids, appear to possess adaptive value. The presence of chromosomal orthologs in *A. radioresistens* MC-14 corresponding to genes typically located on plasmids in closely related strains suggests past events of horizontal gene transfer from plasmids to the chromosome, potentially followed by selection under specific environmental conditions (Walter et al., 2020). One such adaptation is arsenic resistance, a trait commonly associated with multiple copies of the *ars* operon (Andres and Bertin, 2016). In *A. radioresistens* MC-14, two distinct *ars* clusters were identified. Cluster 1 includes *arsHB1C1R1C2*, comprising: *arsH*, encoding an oxidative damage protector (Páez-Espino et al., 2020); *arsB1*, an As(III) efflux pump (Rosen, 2002); *arsC1*, a thioredoxin-dependent arsenate reductase (Mukhopadhyay et al., 2002); *arsR1*, a transcriptional repressor (Busenlehner et al., 2003); and *arsC2*, a glutathione-dependent reductase (Mukhopadhyay et al., 2002). Cluster 2 contains *arsB2R2C3*, with *arsB2* encoding a second As(III) efflux transporter, *arsR2* another transcriptional regulator, and *arsC3* a second glutathione-dependent reductase (Messens and Silver, 2006). Both clusters show high sequence identity with *ars* operons from other *A. radioresistens* strains, supporting the hypothesis of horizontal acquisition and chromosomal integration of these resistance determinants in *A. radioresistens* MC-14 (Walter et al., 2020). This genomic configuration likely contributes to the strain's high tolerance to arsenic-rich environments.

Genes coding for potential heavy metals and metalloids resistance.

The genomic identification of numerous putative resistance genes to metals and metalloids in *A. radioresistens* MC-14 underscores its ecological significance and points to its promising utility in biotechnological applications aimed at remediating environments contaminated with toxic metals. Although the strain displays moderate resistance compared to bacteria harboring specialized metal-resistance operons, its capacity to tolerate at least six distinct heavy metals aligns with the harsh geochemical conditions of its origin, the Monica mine, characterized by elevated concentrations of arsenic and other heavy metals (García-Salgado et al., 2012). Importantly, the high resistance of *A. radioresistens* MC-14 strain to both trivalent and pentavalent forms of arsenic suggest it can be classified as a hyper-resistant strain. This is particularly relevant in the context of plant-microbe interactions, as As(V) and As III are readily absorbed by plant roots and disrupt essential metabolic pathways. As(III), which is approximately 100-fold more toxic than As(V), exerts its toxicity primarily by

irreversibly binding to protein dithiols, thereby inactivating key enzymes (Williams and Silver, 1984). As(V), a phosphate analog, uncouples phosphorylation reactions, thereby impairing energy metabolism (Finnegan and Chen, 2012). The remarkable resistance profile of *A. radioresistens* MC-14 places it among a select group of arsenic hyper-resistant bacteria, comparable to strains such as *Pseudomonas putida* RS-5 [15 mM As(III), 500 mM As(V)] (Chang et al., 2008), *Serratia marcescens* [15 mM As(III), 500 mM As(V)] (Botes et al., 2007), and *Corynebacterium glutamicum* ATCC 13032 [10 mM As(III), 300 mM As(V)] (Ordóñez et al., 2005). These microbial models have served as the basis for various bioremediation strategies aimed at detoxifying arsenic-contaminated environments (González-Benítez et al., 2021). The unique resistance mechanisms of *A. radioresistens* MC-14 further reinforce its potential as a novel candidate for arsenic bioremediation technologies.

Genomic features associated with an endophytic lifestyle in *A. radioresistens* MC-14.

The genome of *A. radioresistens* MC-14 encodes multiple traits associated with plant colonization and adaptation to the rhizosphere environment. Notably, it includes genes responsible for the biosynthesis of type IV pili, a structure broadly conserved among plant-associated bacteria. These pili contribute to bacterial motility, specifically twitching motility, and chemotaxis toward plant-derived chemoattractants such as root exudates (Böhm et al., 2007). Beyond motility, type IV pili play an essential role in mediating adhesion to plant surfaces, a critical step in successful colonization (Mitter et al., 2013). In addition to motility-related genes, the genome harbors gene clusters potentially involved in the production of exopolysaccharides, which are important for surface adhesion and biofilm formation on root surfaces. These features likely facilitate effective root colonization and persistence in the dynamic rhizospheric environment, which is characterized by fluctuating osmolarity and the presence of reactive oxygen species (ROS) and phytotoxins (Böhm et al., 2007; Miller and Wood, 1996). The genome of *A. radioresistens* MC-14 contains genes encoding type I, II, III, and VI secretion systems (Table S5), which play a crucial role in plant-microbe interactions. These secretion systems are involved in mediating defense responses as well as facilitating microbial colonization (Bernal et al., 2018; Coulthurst, 2013; Entila et al., 2024; Sessitsch et al., 2012; Teulet et al., 2022; Tseng et al., 2009). Furthermore, the genome includes genes related to the biosynthesis and transport of the phytohormone indole-3-acetic acid (IAA), supporting prior evidence that *A. radioresistens* MC-14 produces auxin and may modulate host plant growth through hormonal signaling (Molina et al., 2019). Interestingly, while several canonical genes commonly found in plant growth-promoting bacteria (PGPB) are present, the genome lacks genes involved in nitrogen fixation, plant cell wall-degrading enzymes, and flagellum biosynthesis. The absence of these genes suggests that *A. radioresistens* MC-14 occupies a niche as a rhizosphere-dwelling bacterium rather than an obligate endophyte. Its genomic profile reflects an adaptation strategy in which symbiotic association is maintained without full intracellular colonization, supporting the hypothesis that some symbiotic bacteria undergo genome streamlining as they transition to specialized ecological roles (McCutcheon et al., 2024).

Bacteria colonization of roots and plant morpho-physiology.

In the current study, *A. radioresistens* demonstrated successful colonization of *A. thaliana* roots, with bacterial localization restricted exclusively to the apoplastic space. Microscopic analyses confirmed that the bacterium remained extracellular, occupying the intercellular spaces of root tissues without penetrating host cells. Although *A. radioresistens* MC-14 originates from *J. montana* seeds, its confinement to the apoplastic compartment of *A. thaliana* roots is consistent with the notion that bacteria colonize their native host more efficiently (Wippel et al., 2021), supporting a non-invasive, associative interaction characteristic of facultative symbiosis. This spatial arrangement allows *A. radioresistens* MC-14 to maintain close contact with host tissues—potentially influencing plant physiology—while avoiding the strong immune responses typically triggered by intracellular invasion.

Inoculation with *A. radioresistens* MC-14 significantly enhanced plant survival in substrates heavily contaminated with [As(III)], and notably promoted progression into advanced phenological stages associated with reproduction. This improved performance under arsenic stress may be attributed to the strain, eventually through their ability to resist high arsenic concentrations. The enhanced tolerance facilitated by *A. radioresistens* MC-14 not only supports vegetative growth under toxic conditions but also enables resource allocation to reproductive structures, a critical determinant of plant fitness and ecological success. These findings underscore the potential role of this bacterium as a microbial ally for sustaining plant reproduction and resilience in arsenic-contaminated environments.

Arsenic exposure has been widely reported to impair net photosynthetic rate (A_{net}) by compromising the structural and functional integrity of the photosynthetic apparatus, particularly photosystem II (Fv/Fm), at elevated As(III) concentrations (Arikan-Abdulveli, 2025; Tofan et al., 2025). However, in our experiments, Fv/Fm values remained consistently above 0.79 across all treatments, indicating that photoinhibition or structural damage to PSII did not occur under the experimental conditions. This observation is consistent with previous studies in other plant species that also exhibited limited or no impact on Fv/Fm under arsenic exposure (Vezza et al., 2022), suggesting a species-dependent tolerance threshold. Despite the stability of Fv/Fm, As(III) exposure led to a reduction in the total concentration of photosynthetic pigments, particularly chlorophylls. These pigments are known to be highly susceptible to oxidative stress (Agathokleous et al., 2020), a common consequence of arsenic toxicity. The observed decline in pigment content, in the absence of photoinhibition, suggests that As(III) primarily exerts its deleterious effects at the biochemical level rather than through direct disruption of PSII photochemistry. The reduction of photosynthetic pigments, primarily driven by oxidative stress, is a well-documented response in plants exposed to arsenic, including agriculturally important species such as *Vigna mungo* (Srivastava et al., 2017) and *Oryza sativa* (Rahman et al., 2007). In our study, the decline in pigment concentration likely reflects not only oxidative damage but also the impairment of other components of the photosynthetic machinery. This pigment degradation was accompanied by an increase in non-photochemical quenching (qN), a protective mechanism that enhances energy dissipation to prevent the formation of long-lived, redox-active compounds capable of inducing further oxidative damage (Heber, 2002; Miyake et al., 2002). Moreover, a significant increase in dark respiration rates was observed in response to elevated As(III) levels, likely reflecting heightened detoxification activity and enhanced cellular repair processes. These findings collectively indicate that arsenic-induced oxidative stress

triggers a multifaceted physiological response in plants. Interestingly, despite the oxidative burden, plants demonstrated an ability to maintain or even enhance photosystem II efficiency (Φ PSII) under high arsenic concentrations. This effect was likely mediated through increased photochemical quenching (qP) and other regulatory adjustments. However, the improved photosynthetic efficiency was insufficient to counterbalance the reduction in biomass accumulation. The concurrent increase in As(III) concentration and decrease in plant mass suggest that photoassimilates are being redirected toward detoxification and cellular maintenance rather than growth, highlighting a trade-off between survival and development under stress conditions.

Inoculation with *A. radioresistens* MC-14 significantly reduced dark respiration (A_{dark}) and non-photochemical quenching (qN), suggesting a lower metabolic cost associated with detoxification and cellular repair. This resource reallocation likely facilitates enhanced growth, particularly under stress conditions. The beneficial effects of this symbiosis were most pronounced at the highest As(III) concentration tested (10 μ M As(III)), where physiological indicators showed marked improvement. The genome of the *A. radioresistens* MC-14 seed strain encodes antioxidant defense enzymes such as peroxidases, catalases, and superoxide dismutases, which may directly contribute to the observed attenuation of oxidative damage. In addition, inoculated plants displayed elevated concentrations of carotenoids (cars) even in the absence of arsenic, a pigment class known for its radical scavenging properties and its central role in mitigating reactive oxygen species (ROS) (Xu & Rothstein, 2018). Interestingly, non-inoculated plants only exhibited a significant increase in the xanthophyll cycle pigments (C + X) at the highest As(III) concentration, whereas inoculated plants maintained elevated levels across all treatments. This constitutive enhancement of antioxidant pigment content in inoculated plants suggests a priming effect, whereby association with *A. radioresistens* MC-14 induces early activation of ROS defense mechanisms. This preemptive physiological adaptation may underlie the enhanced tolerance observed under subsequent oxidative stress conditions.

The beneficial effects of *A. radioresistens* MC-14 on plant performance in arsenic-contaminated environments were strongly dependent on the bacterial inoculum concentration. Under As(III) exposure, low inoculum levels (0.07 OD_{600nm}) failed to confer significant benefits to plant survival, growth, or reproductive success. In contrast, an intermediate inoculum concentration (0.21 OD_{600nm}) consistently maximized plant fitness, enhancing survival and progression to reproductive stages. However, a further increase in inoculum concentration (0.63 OD_{600nm}) did not lead to additional improvements and, in some parameters, even reduced the positive effects observed at the optimal intermediate dose. These results suggest a trade-off in the symbiotic interaction, wherein both insufficient and excessive bacterial colonization can limit the overall benefit to the host plant. At low colonization densities, the metabolic cost of maintaining the symbiont is minimal but potentially inadequate to elicit a meaningful physiological response. Conversely, high bacterial loads may impose excessive metabolic demands on the host or trigger defense responses, ultimately diminishing the benefits of the association. Similar findings have been reported by Liu et al. (2024), who noted that excessive microbial colonization can negatively impact host plants due to resource competition and immune activation. Thus, identifying and

applying an optimal inoculum concentration is critical to maximize the beneficial outcomes of plant–microbe symbioses while minimizing associated physiological costs.

Conclusions

The plant seed microbiome, despite its complexity and ecological relevance, has historically received limited attention in microbiome studies. Our findings underscore that seed-associated microbial communities are not passive passengers, but dynamic and selective systems shaped by host evolutionary pressures. The selective pressure exerted by plants on their seed microbiota has likely driven co-evolutionary processes, resulting in the persistence of beneficial microbial assemblages across generations. These microbial consortia provide adaptive advantages, particularly under environmental stress conditions.

Remarkably, even a single seed-isolated bacterium, such as *A. radioresistens* MC-14, carries a repertoire of functional genes encoded within its circular chromosome and associated plasmids. These genetic elements confer adaptive traits that may function independently or synergistically with plant regulatory pathways, enhancing plant survival and stress tolerance. This highlights the role of the seed microbiome not only as a source of symbiotic resilience but also as a potential evolutionary driver in plant adaptation to challenging environments.

The genome sequence of *A. radioresistens* MC-14 offers key insights into the adaptive strategies of a bacterium uniquely equipped to withstand high concentrations of [As(III)], modulate oxidative stress, and tolerate moderate levels of other heavy metals. This strain also displays vertical transmission potential via maternal inheritance, enhancing its ecological and evolutionary relevance. Genomic analysis reveals that horizontal gene transfer and the presence of mobile genetic elements have been fundamental in facilitating the adaptation of *A. radioresistens* MC-14 to diverse ecological niches and lifestyles. The absence of specific metabolic and structural genes typically present in free-living relatives further suggests a degree of genome reduction aligned with an endosymbiotic lifestyle, in which the bacterium relies on the host plant for certain cellular functions. Moreover, the broad ecological range observed among *A. radioresistens* strains, as contrasted with the *A. radioresistens* MC-14 seed-associated strain, points to a capacity for non-specific habitat colonization. This ecological versatility, together with the observed genomic plasticity and prevalence of mobile elements, supports the concept of an open pangenome within the *Acinetobacter* genus and helps to refine our understanding of its complex phylogenetic structure.

From a physiological perspective, the seed-borne bacterium *A. radioresistens* MC-14 alleviates auxin deficiencies induced by arsenic stress in the root during the early root development, supports the preservation of root structural integrity, reduces oxidative stress, and potentially activates cellular recovery mechanisms. These combined effects promote increased biomass allocation to both roots and leaves, contributing to improved vegetative growth under adverse conditions. This physiological advantage extends to later developmental stages, enhancing key fitness traits and increasing the

likelihood of reproductive success. In this context, the bacterium acts as a “germinal backpack”, endowing the seed with genetic elements advantageous for survival in stressful environments. Finally, genome mining revealed several gene clusters likely involved in plant-association relationships, paving the way for the utilization of this bacterium as a potential candidate for phytostimulation and biofertilization of plants exposed to metal-contaminated conditions, offering a promising tool for the development of sustainable agricultural strategies.

Abbreviations

Absorbance at 600 nm: OD_{600nm} .

Arsenate: As(V).

Arsenite: As(III).

Aerobic arsenite oxidase gene: *aioA*

Chlorophyll a: Cla.

Chlorophyll b: Clb.

Total carotenoids: cars

Dark respiration rate: A_{dark} .

Green florescent protein: GFP

Horizontal gene transfer (HGT).

Maximum potential quantum efficiency of Photosystem II: Fv/Fm

Minimum inhibitory concentration: MIC

Murashige and Skoog broth: MS

Net photosynthetic rate: A_{net}

Non-photochemical quenching: qN

Photochemical quenching: qP

Photosynthetic photon flux density: PPD

Quantum yield of photosystem II: Φ_{PSII}

Wild-type phenotype Columbia-0: Col-0

2-(N-morpholino) ethanesulfonic acid monohydrate: MES monohydrate

Statements and Declarations

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Natalia González-Benítez: Writing – original draft, Writing – review & editing, , Validation, Formal analysis, Data curation, Conceptualization. M^a Carmen Molina: Writing – review, Methodology, Formal analysis. Stephan Pollman: Writing – review & editing. Mercedes Uscola: Writing – review & editing, Methodology, Data curation. Visualization. Gonzalo Durante-Rodríguez: Writing – review, Methodology, Formal analysis. Jesús Vicente Carbajosa: Validation, Data curation, Cristina Serrano-Pelejero: Methodology, Formal analysis, Validation, Data curation. Irene Cano: Methodology, Formal analysis, Validation, Weikang Huang: preliminary analysis, Manuel Carmona: Writing – original draft, Visualization, Validation, Methodology, Data curation.

Data Availability

The complete genome sequence and corresponding annotation of *A. radioresistens* MC-14 have been submitted to the NCBI GenBank database under accession number CP131483. The rest of datasets generated during the current study are available from the corresponding author on reasonable request.

References

1. Abdullaeva, Y., Ambika Manirajan, B., Honermeier, B., Schnell, S., & Cardinale, M. 2021. Domestication affects the composition, diversity, and co-occurrence of the cereal seed microbiota. *Journal of Advanced Research*, 31, 75-86. doi: /10.1016/j.jare.2020.12.008
2. Agathokleous, E., Feng, Z., & Peñuelas, J. 2020. Chlorophyll hormesis: are chlorophylls major components of stress biology in higher plants?. *Science of the Total Environment*, 726, 138637. doi: 10.1016/j.scitotenv.2020.138637
3. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389-3402. doi:/10.1093/nar/25.17.3389

4. Andres, J., and Bertin, P.N. 2016. The microbial genomics of arsenic. *FEMS Microbiol Revs* 40: 299–322. doi: 10.1093/femsre/fuv050
5. Arian-Abdulveli. 2025. Acetylcholine treatments enhance growth, photosynthesis efficiency and stress resilience in maize seedlings exposed to arsenic. *Turkish Journal of Botany*, 49(1), 13-24. Doi:10.55730/1300-008X.2838
6. Babar, M. M., & Tariq, A. 2018. Status of arsenic toxicity in the world. *Mechanisms of Arsenic Toxicity and Tolerance in Plants*, 457-481. Doi:10.1007/978-981-13-1292-2_18
7. Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S., & Davison, A. W. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environmental and Experimental Botany*, 32, 85-100. Doi:10.1016/0098-8472(92)90034-Y
8. Basu, S., Dutta, A., Mukherjee, S. K., & Hossain, S. T. 2023. Isolation and characterization of an As(III) oxidizing bacterium, *Acinetobacter* sp. TMKU4 from paddy field for possible arsenic decontamination. *Journal of Hazardous Materials Advances*, 10, 100289. Doi:10.1016/j.hazadv.2023.100289
9. Benson, L. M., Porter, E. K., & Peterson, P. J. 1981. Arsenic accumulation, tolerance and genotypic variation in plants on arsenical mine wastes in S.W. England. *Journal of Plant Nutrition*, 3(1-4), 655-666. doi: 10.1080/01904168109362868
10. Berendsen, R. L., Pieterse, C. M., & Bakker, P. A. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17(8), 478-486. Doi:10.1016/j.tplants.2012.04.001
11. Bernal, P., Llamas, M. A., & Filloux, A. 2018. Type VI secretion systems in plant-associated bacteria. *Environmental Microbiology*, 20(1), 1-15. Doi:/10.1111/1462-2920.13956
12. Blom, J., Albaum, S. P., Doppmeier, D., Pühler, A., Vorhölter, F. J., Zakrzewski, M., & Goesmann, A. 2009. EDGAR: a software framework for the comparative analysis of prokaryotic genomes. *BMC Bioinformatics*, 10, 154. Doi:10.1186/1471-2105-10-154
13. Böhm, M., Hurek, T., & Reinhold-Hurek, B. 2007. Twitching motility is essential for endophytic rice colonization by the N₂-fixing endophyte *Azoarcus* sp. strain BH72. *Molecular Plant-Microbe Interactions*, 20, 526-533. Doi:10.1094/MPMI-20-5-0526
14. Botes, E., Van Heerden, E., & Litthauer, D. 2007. Hyper-resistance to arsenic in bacteria isolated from an antimony mine in South Africa: research in action. *South African Journal of Science*, 103(7), 279-281.
15. Bryksin, A. V., & Matsumura, I. 2010. Rational design of a plasmid origin that replicates efficiently in both Gram-positive and Gram-negative bacteria. *PLoS ONE*, 5(10), e13244. Doi: 10.1371/journal.pone.0013244
16. Busenlehner, L. S., Pennella, M. A., & Giedroc, D. P. 2003. The SmtB/ArsR family of metalloregulatory transcriptional repressors: structural insights into prokaryotic metal resistance. *FEMS Microbiology Reviews*, 27, 131-143. Doi:10.1016/S0168-6445(03)00054-8
17. Chang, J.S., Kim, Y.H. & Kim, K.W. 2008. The *ars* genotype characterization of arsenic-resistant bacteria from arsenic-contaminated gold–silver mines in the Republic of Korea. *Appl Microbiol*

18. Chesneau, G., Laroche, B., Prévieux, A., Marais, C., Briand, M., Marolleau, B., & Barret, M. 2022. Single seed microbiota: assembly and transmission from parent plant to seedling. *MBio*, 13(6), e01648-22. Doi: 10.1128/mbio.01648-22.
19. Clarke, J., Wu, H. C., Jayasinghe, L., Patel, A., Reid, S., & Bayley, H. 2009. Continuous base identification for single-molecule nanopore DNA sequencing. *Nature Nanotechnology*, 4, 265-270. Doi:10.1038/nnano.2009.12
20. Clough, S. J. and Bent, A. F. 1998. Floral dip: a simplified method for *Agrobacterium* mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16(6): 735-743. Doi: 10.1046/j.1365-313x.1998.00343.x
21. Coulthurst, S. J. 2013. The Type VI secretion system – a widespread and versatile cell targeting system. *Research in Microbiology*, 164, 640-654. Doi:10.1099/mic.0.000789
22. de Almeida, O. G. G., Furlan, J. P. R., Stehling, E. G., & De Martinis, E. C. P. 2021. Comparative phylo-pangenomics reveals generalist lifestyles in representative *Acinetobacter* species and proposes candidate gene markers for species identification. *Gene*, 791, 145707. Doi:/10.1016/j.gene.2021.145707.
23. Dutta, A., Mukherjee, S. K., & Hossain, S. T. 2023. Characterization of As(III)-oxidizing bacteria *Acinetobacter* sp. TMKU7 having plant growth promoting features for possible application in arsenic-contaminated crop field. *Bioremediation Journal*, 1-15. Doi:10.1080/10889868.2023.22
24. Entila, F., Han, X., Mine, A., Schulze-Lefert, P., & Tsuda, K. 2024. Commensal lifestyle regulated by a negative feedback loop between *Arabidopsis* ROS and the bacterial T2SS. *Nature Communications*, 15(1), 456. Doi:10.1038/s41467-024-44724-2
25. Felsenstein, J. 1993. PHYLIP (phylogenetic inference package) version 3.5.1. Seattle, W.A: University of Washington.
26. Finnegan, P. M., & Chen, W. 2012. Arsenic toxicity: the effects on plant metabolism. *Frontiers in physiology*, 3, 182. Doi:10.3389/fphys.2012.00182
27. Fuller, R., Landrigan, P. J., Balakrishnan, K., Bathan, G., Bose-O'Reilly, S., Brauer, M., & Yan, C. 2022. Pollution and health: a progress update. *The Lancet Planetary Health*, 6(6), e535-e547. Doi:1016/S2542-5196(22)00090-0.
28. Fulsundar, S., Kulkarni, H. M., Jagannadham, M. V., Nair, R., Keerthi, S., Sant, P., Pardesi, K., Bellare, J., & Chopade, B. A. 2015. Molecular characterization of outer membrane vesicles released from *Acinetobacter radioresistens* and their potential roles in pathogenesis. *Microbial Pathogenesis*, 83-84, 12-22. Doi:10.1016/j.micpath.2015.04.005
29. García-Salgado, S., García-Casillas, D., Quijano-Nieto, M. A., & Bonilla-Simón, M. M. 2012. Arsenic and heavy metal uptake and accumulation in native plant species from soils polluted by mining activities. *Water, Air, & Soil Pollution*, 223, 559-572. Doi:10.1007/s11270-011-0882-x
30. Geng, A., Lian, W., Wang, X., & Chen, G. 2023. Regulatory mechanisms underlying arsenic uptake, transport, and detoxification in rice. *International Journal of Molecular Sciences*, 24(13), 11031.

31. Gentilini, F., Turba, M. E., Pasquali, F., Mion, D., Ramagnoli, N., Zambon, E., Terni, D., Peirano, G., Pitout, J. D. D., Parisi, A., Sambri, V., & Zanoni, R. G. 2018. Hospitalized pets as a source of carbapenem-resistance. *Frontiers in Microbiology*, 9, 2872. Doi:/10.3389/fmicb.2018.02872
32. González-Benítez, N., Martín-Rodríguez, I., Cuesta, I., Arrayás, M., White, J. F., & Molina, M. C. 2021. Endophytic microbes are tools to increase tolerance in *Jasione* plants against arsenic stress. *Frontiers in Microbiology*, 12, 664271. Doi:10.3389/fmicb.2021.664271
33. González Ortega-Villaizán, A., King, E., Patel, M. K., Pérez-Alonso, M. M., Scholz, S. S., Sakakibara, H., Pollmann, S. 2024. The endophytic fungus *Serendipita indica* affects auxin distribution in *Arabidopsis thaliana* roots through alteration of auxin transport and conjugation to promote plant growth. *Plant, Cell & Environment*, 47(10), 3899-3919. Doi:10.1111/pce.14989
34. Heber, U. Irrungen, Wirrungen? The Mehler reaction in relation to cyclic electron transport in C3 plants. *Photosynth. Res.* 2002, 73, 223–231
35. Hidalgo, K. J., Centurion, V. B., Lemos, L. N., Soriano, A. U., Valoni, E., Baessa, M. P., & Oliveira, V. M. 2025. Disentangling the microbial genomic traits associated with aromatic hydrocarbon degradation in a jet fuel-contaminated aquifer. *Biodegradation*, 36(1), 7. Doi:/10.1007/s10532-024-10100-6
36. Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120. Doi: 10.1007/BF01731581.
37. Lafi, F. F., Alam, I., Bisseling, T., Geurts, R., Bajic, V. B., Hirt, H., & Saad, M. M. 2016. Draft genome sequence of the plant growth-promoting rhizobacterium *Acinetobacter radioresistens* strain SA188 isolated from the desert plant *Indigofera argentea*. *Genome Announcements*, 5, e01708-16. Doi:10.1128/ genomeA.01708-16.
38. Liu, Y., Xu, Z., Chen, L., Xun, W., Shu, X., Chen, Y., ... & Zhang, R. 2024. Root colonization by beneficial rhizobacteria. *FEMS Microbiology Reviews*, 48(1), fuad066. Doi:10.1093/femsre/fuad066
39. Macaya, C. C., Méndez, V., Durán, R. E., Aguila-Torres, P., Salvà-Serra, F., Jaén-Luchoro, D., Moore, E. R. B., & Seeger, M. 2019. Complete genome sequence of hydrocarbon-degrading halotolerant *Acinetobacter radioresistens* DD78, isolated from the Aconcagua river mouth in central Chile. *Microbiology Resource Announcements*, 8 (33). 10-1128. Doi: 10.1128/MRA.00601-19
40. Matsumoto Haruna, Xiaoyan Fan, Yue Wang, Peter Kusstatscher, Jie Duan, Sanling Wu, Sunlu Chen, Kun Qiao, Yiling Wang, Bin Ma, Guonian Zhu, Yasuyuki Hashidoko, Gabriele Berg, Tomislav ernava, Mengcen Wang. 2021. Bacterial seed endophyte shapes disease resistance in rice. *Nat. Plants* 7, 60–72. Doi:10.1038/s41477-020-00826-5
41. McCoy, K. B., Derecho, I., Wong, T., Tran, H. M., Huynh, T. D., La Duc, M. T., Venkateswaran, K., & Mogul, R. 2012. Insight into the extremotolerance of *Acinetobacter radioresistens* 50v1, a gram-negative bacterium isolated from the Mars Odyssey spacecraft. *Astrobiology*, 12, 854-862. Doi:10.1089/ast.2012.0835

42. McCutcheon, J. P., Garber, A. I., Spencer, N., & Warren, J. M. 2024. How do endosymbionts work with so few genes? *PLoS Biology*, 22(4), e3002577. Doi:10.1371/journal.pbio.3002577
43. Messens, J., & Silver, S. 2006. Arsenate reduction: thiol cascade chemistry with convergent evolution. *Journal of Molecular Biology*, 362, 1-17. Doi:10.1016/j.jmb.2006.07.002
44. Miller, K. J., & Wood, J. M. 1996. Osmoadaptation by rhizosphere bacteria. *Annual Review of Microbiology*, 50, 101-136. Doi:10.1146/annurev.micro.50.1.101
45. Mitter, B., Petric, A., Shin, M. W., Chain, P. S., Hauberg-Lotte, L., Reinhold-Hurek, B., Nowak, J., & Sessitsch, A. 2013. Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. *Frontiers in Plant Science*, 4, 120. Doi:10.3389/fpls.2013.00120
46. Miyake, C.; Yonekura, K.; Kobayashi, Y.; Yokota, A. Cyclic electron flow within PSII functions in intact chloroplasts from spinach leaves. *Plant Cell Physiol.* 2002, 43, 951–957. Doi:10.1093/pcp/pcf113
47. Molina, M. C., White, J. F., Kingsley, K. L., & González-Benítez, N. 2019. Seed Endophytes of *Jasione montana*: Arsenic Detoxification Workers in an Eco-friendly Factory. En S. K. Verma & J. F. White, Jr (Eds.), *Seed Endophytes* (pp. 365-384). Springer International Publishing.
https://doi.org/10.1007/978-3-030-10504-4_17
48. Muehe, E. M., Wang, T., Kerl, C. F., Planer-Friedrich, B., & Fendorf, S. 2019. Rice production threatened by coupled stresses of climate and soil arsenic. *Nature Communications*, 10(1), 4985. Doi:/10.1038/s41467-019-12946-4.
49. Mukhopadhyay, R., Rosen, B. P., Phung, L. T., & Silver, S. 2002. Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiology Reviews*, 26, 311-325. Doi:10.1111/j.1574-6976.2002.tb00617.x.
50. Murashige, T., & Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, 15(3). Doi:10.1111/j.1399-3054.1962.tb08052.x
51. Nelson, E. B. 2018. The seed microbiome: Origins, interactions, and impacts. *Plant and Soil*, 422(1-2), 7-34. <https://doi.org/10.1007/s11104-017-3289-7>
52. Ordóñez, E., Letek, M., Valbuena, N., Gil, J. A., & Mateos, L. M. 2005. Analysis of genes involved in arsenic resistance in *Corynebacterium glutamicum* ATCC 13032. *Applied and environmental microbiology*, 71(10), 6206-6215. Doi:10.1128/AEM.71.10.6206-6215.2005
53. Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A. R., Xia, F., & Stevens, R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Research*, 42, D206-D214. Doi:10.1093/nar/gkt1226.
54. Rahman, M. A., Hasegawa, H., Rahman, M. M., Islam, M. N., Miah, M. M., & Tasmen, A. 2007. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere*, 67(6), 1072-1079. Doi:10.1016/j.chemosphere.2006.11.061
55. Roca, I., Espinoza, K., Irigoin-Lovera, C., Piquet, M., Palomino-Kobayashi, L. A., Castillo, A. K., Ruiz, J. 2024. Clonal dissemination of *Acinetobacter radioresistens* among Humboldt penguins (*Spheniscus*

- humboldti*) inhabiting a barren northern Peruvian island. *European Journal of Microbiology and Immunology*, 14(2), 210-218. Doi:10.1556/1886.2023.00066.
56. Rosen, B. P. 2002. Biochemistry of arsenic detoxification. *FEBS Letters*, 529, 86-92. Doi: 10.1016/s0014-5793(02)03186-1.
 57. Saitou, N., & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425. Doi: 10.1093/oxfordjournals.molbev.a040454
 58. Sessitsch, A., Hardoim, P., Döring, J., Weilharter, A., Krause, A., Woyke, T., Mitter, B., Hauberg-Lotte, L., Friedrich, F., Rahalkar, M., Hurek, T., Sarkar, A., Bodrossy, L., van Overbeek, L., Brar, D., van Elsas, J. D., & Reinhold-Hurek, B. 2012. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. *Molecular Plant-Microbe Interactions*, 25, 28-36. Doi:10.1094/MPMI-08-11-0204.
 59. Singh, N., Ma, L. Q., & Srivastava, M. 2023. Role of plant-associated bacteria in arsenic detoxification: Mechanisms and applications. *Journal of Hazardous Materials*, 436, 129282. Doi:10.1007/978-3-031-37561-3_12
 60. Srivastava, S., Sinha, P., & Sharma, Y. K. 2017. Status of photosynthetic pigments, lipid peroxidation and anti-oxidative enzymes in *Vigna mungo* in presence of arsenic. *Journal of Plant Nutrition*, 40(3), 298-306.
 61. Teulet, A., Camuel, A., Perret, X., & Giraud, E. 2022. The versatile roles of type III secretion systems in rhizobium-legume symbioses. *Annual Review of Microbiology*, 76(1), 45-65. Doi:10.1146/annurev-micro-041020-032624
 62. Thompson, J. D., Higgins, D. G., & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673-4680.
 63. Tofan, A., Gurkan, E., Arikan-Abdulveli, B., Balci, M., Yildiztugay, E., & Ozfidan-Konakci, C. 2025. Curcumin, a Secondary Metabolite, Activates the Defense System by Regulating Water Status, PSII Photochemistry, and Antioxidant Capacity in *Lactuca sativa* Exposed to Lead and/or Arsenic Stresses. *Food and Energy Security*, 14(1), e70027. Doi:10.1002/fes3.70027.
 64. Tseng, T. T., Tyler, B. M., & Setubal, J. C. 2009. Protein secretion systems in bacterial–host associations, and their description in the gene ontology. *BMC Microbiology*, 9, S2. Doi:10.1186/1471-2180-9-S1-S2
 65. Venables, W. N., & Ripley, B. D. 2002. *Modern Applied Statistics with S*. Fourth Edition. Springer, New York. ISBN 0-387-95457-0. Doi:10.1007/978-0-387-21706-2
 66. Verma, S. K., & White, J. F. 2019. *Seed endophytes*. Springer, Cham, Switzerland. Doi:10.1007/978-3-030-10504-4.
 67. Wallace, J. G. 2023. Maize seed endophytes. *Molecular Plant Pathology*, 24(7), 801-810. Doi:10.1111/mpp.13278

68. Walter, T., Klim, J., Jurkowski, M., Gawor, J., Köhling, I., Slodownik, M., & Zielenkiewicz, U. 2020. Plasmidome of an environmental *Acinetobacter lwoffii* strain originating from a former gold and arsenic mine. *Plasmid*, 110, 102505. Doi:10.1016/j.plasmid.2020.102505
69. Wang, T., Costa, V., Jenkins, S. G., Hartman, B. J., & Westblade, L. F. 2019. *Acinetobacter radioresistens* infection with bacteremia and pneumonia. *IDCases*, 16, e00495. Doi:10.1016/j.idcr.2019.e00495.
70. Williams, J. W., & Silver, S. 1984. Bacterial resistance and detoxification of heavy metals. *Enzyme and microbial technology*, 6(12), 530-537. Doi:10.1016/0141-0229(84)90081-4
71. Wippel, K., Tao, K., Niu, Y., Zgadzaj, R., Kiel, N., Guan, R., Garrido-Oter, R. 2021. Host preference and invasiveness of commensal bacteria in the Lotus and Arabidopsis root microbiota. *Nature microbiology*, 6(9), 1150-1162. <https://doi.org/10.1038/s41564-021-00941-9>
72. Xiang, P., Zhang, Y., Zhang, T., Wu, Q., Zhao, C., & Li, Q. 2023. A novel bacterial combination for efficient degradation of polystyrene microplastics. *Journal of Hazardous Materials*, 458, 131856. Doi:10.1007/s40520-022-02325-3
73. Xu, Z., & Rothstein, S. J. 2018. ROS-Induced anthocyanin production provides feedback protection by scavenging ROS and maintaining photosynthetic capacity in Arabidopsis. *Plant signaling & behavior*, 13(3), 1364-77. Doi:10.1080/15592324.2018.1451708.
74. Zandalinas, S. I., Fritschi, F. B., & Mittler, R. 2021. Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. *Trends in Plant Science*, 26(6), 588-599. Doi: 10.1016/j.tplants.2021.02.011.
75. Zapata-Sifuentes, G., Hernandez-Montiel, L. G., Saenz-Mata, J., Fortis-Hernandez, M., Blanco-Contreras, E., Chiquito-Contreras, R. G., & Preciado-Rangel, P. 2022. Plant growth-promoting rhizobacteria improve growth and fruit quality of cucumber under greenhouse conditions. *Plants*, 11, 1612. Doi:10.3390/plants11121612
76. Zhao, Y., Wei, H. M., Yuan, J. L., Xu, L., & Sun, J. Q. 2023. A comprehensive genomic analysis provides insights on the high environmental adaptability of *Acinetobacter* strains. *Frontiers in Microbiology*, 14, 1177951. Doi:10.1007/s40520-022-02325-3

Figures

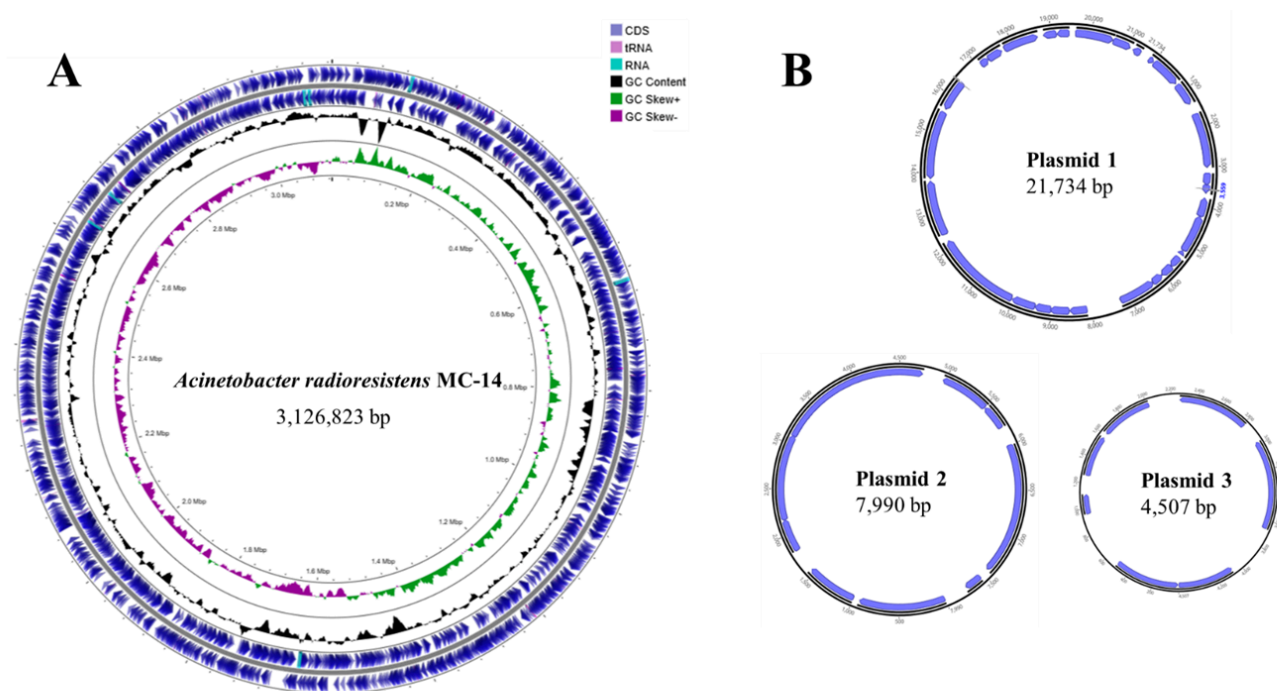


Figure 1

Figure 1

Graphical representation of the genome of *A. radioresistens* MC-14 containing their chromosome and plasmids 1, 2 and 3, indicating their size in bp.

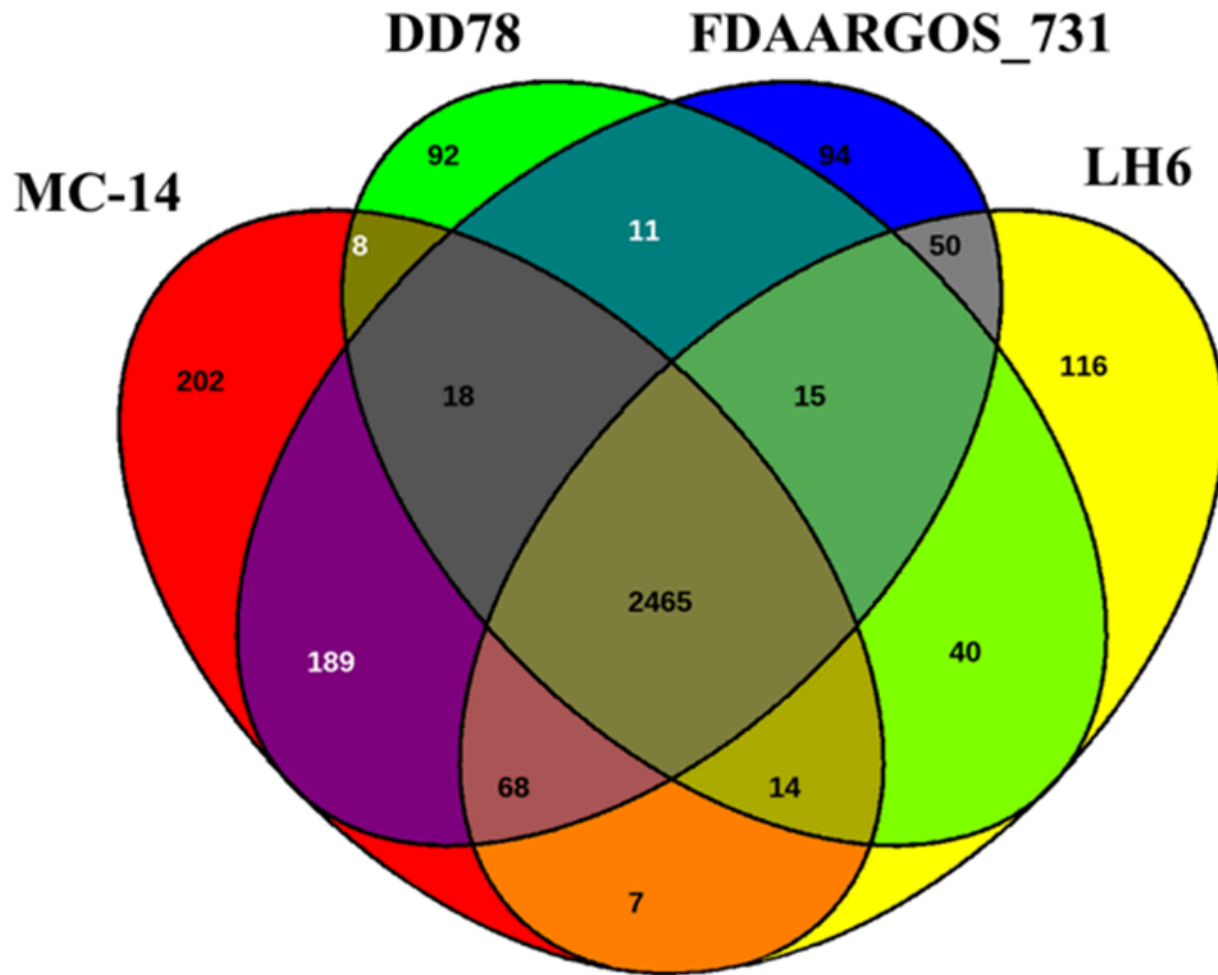


Figure 2

Figure 2

Venn diagram representing the core genome and pan genome of the sequenced *A. radioresistens* MC-14 strain, DD78, FDAARGOS_731 and LH6. The core and pan genome were calculated with the EDGAR program by iterative pairwise comparison of all genomes, taking as reference genome that of strain *A. radioresistens* MC-14. The Venn diagram shows the number of reciprocal best hits between a subset of genomes. Therefore, the number of exclusive genes in each genome is always higher than the number of singletons (a gene without any hits against any other genome) for each strain.

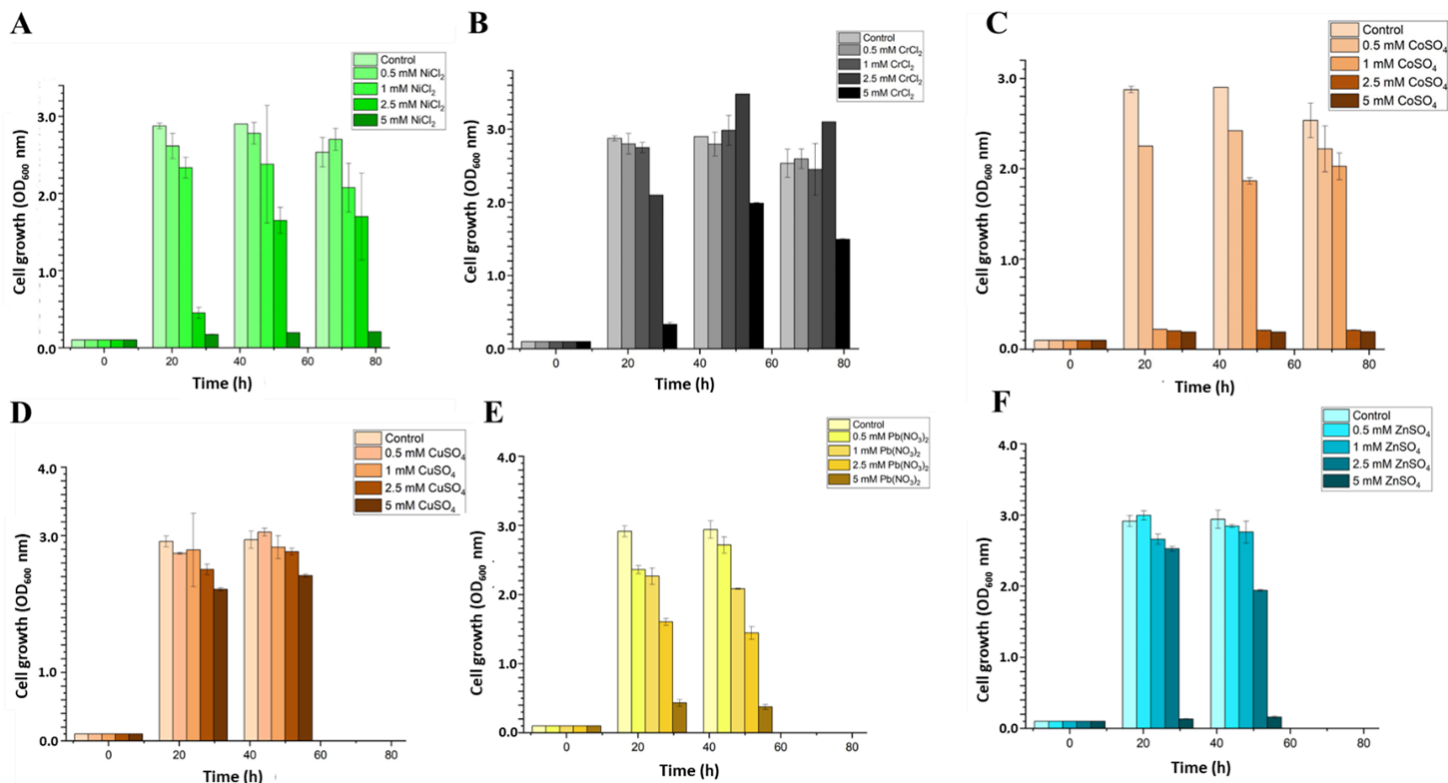


Figure 3.

Figure 3

Cell growth of *A. radioresistens* MC-14 expressed as absorbance at 600 nm (OD_{600nm}) at 24 h of incubation (end of exponential phase) in the presence of increasing concentrations of NiCl₂ (A), CrCl₃ (B), CoSO₄ (C), CuSO₄ (D), Pb(NO₃)₂ (E), or ZnSO₄ (F). Error bars indicate the standard deviations of three independent experiments.

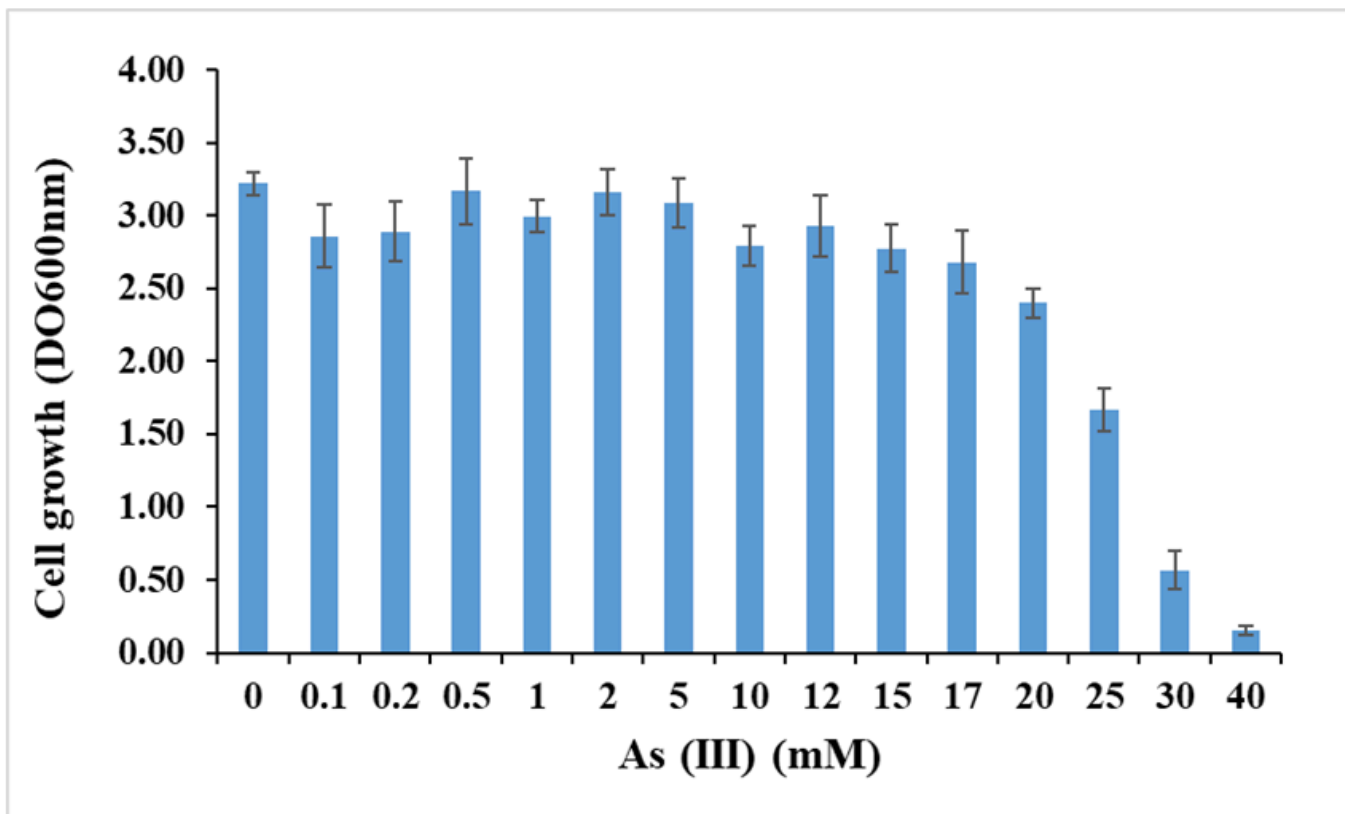
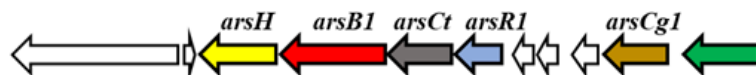


Figure 4

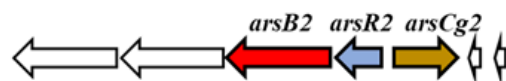
Figure 4

Cell growth of *A. radioresistens* MC-14 expressed as absorbance at 600 nm (OD_{600nm}) at 24 h of incubation (end of exponential phase) in the presence of increasing concentrations of As(III). Error bars indicate the standard deviations of three independent experiments.

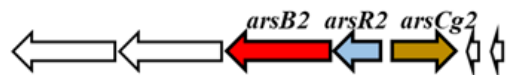
A. radioresistens MC-14 Cluster 1



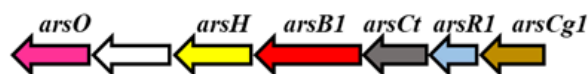
A. radioresistens MC-14 Cluster 2



A. radioresistens DSM6976 Chromosome



A. radioresistens DSM6976 Plasmid 2



A. radioresistens FDAARGOS_731 Chromosome



A. radioresistens FDAARGOS_731 Plasmid 1

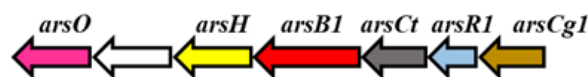


Figure 5

Organization of the *ars* clusters in *A. radioresistens* MC-14, *A. radioresistens* DSM6976 and *A. radioresistens* FDAARGOS_731. Genes are represented by arrows as follows: *arsH*, oxidoreductase; *arsB*, As(III) transporter; *arsCt*, glutaredoxin-dependent arsenate reductase, *arsR*, transcriptional regulator; *arsCg*, thioredoxin-dependent arsenate reductase; *arsO*, monooxygenase. Location of each cluster in chromosome or plasmid is indicated. White arrows represent genes of unknown function.

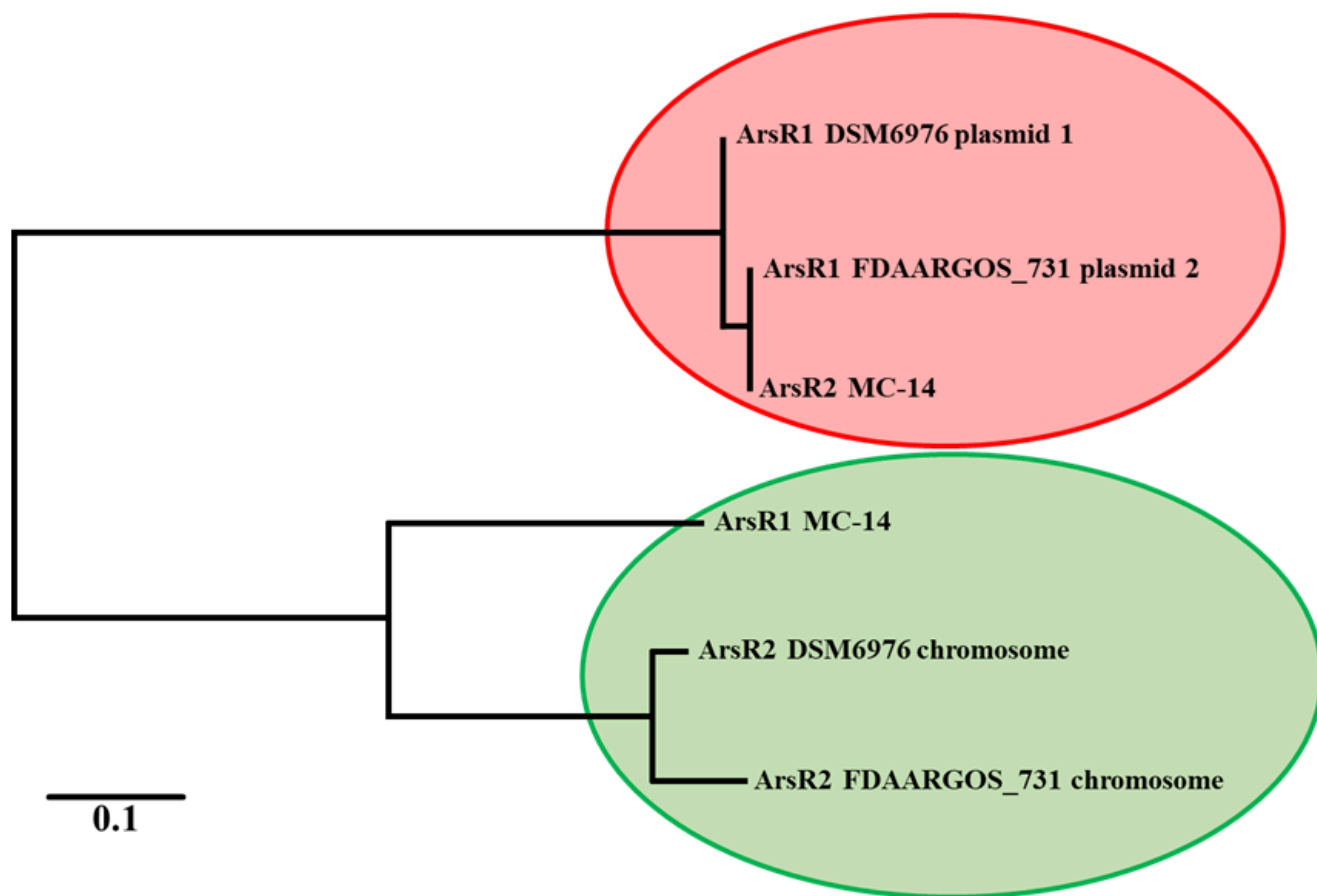


Figure 6.

Figure 6

A cladogram tree is built from the multiple amino acid sequence alignment of the ArsR proteins using the program Phylogeny. The clustering of each ArsR from *A. radioresistens* MC-14 is indicated in red and green areas.

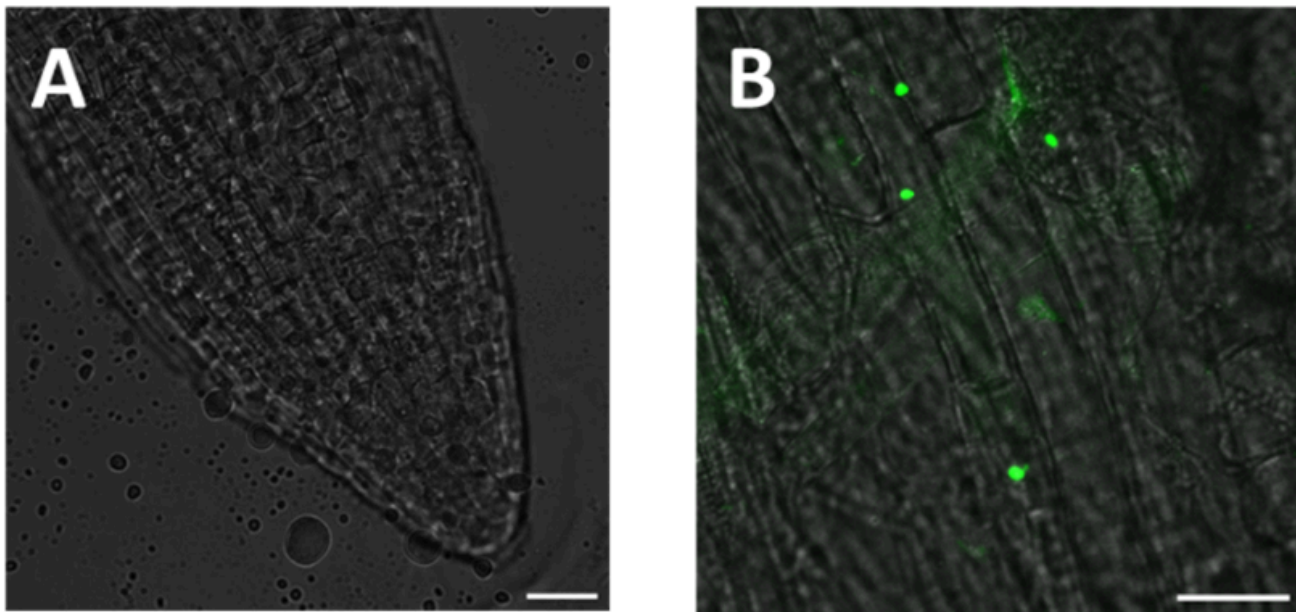


Figure 7.

Figure 7

Roots of *A. thaliana* under confocal laser microscopy after five days of germination and inoculation: (A) root appendage of control plant, and (B) root colonized by *A. radioresistens* transformed with the pBAV1k-t5-gfp plasmid. Scale bar = 25 μm. Arrows indicate fluorescence emitted by bacteria.

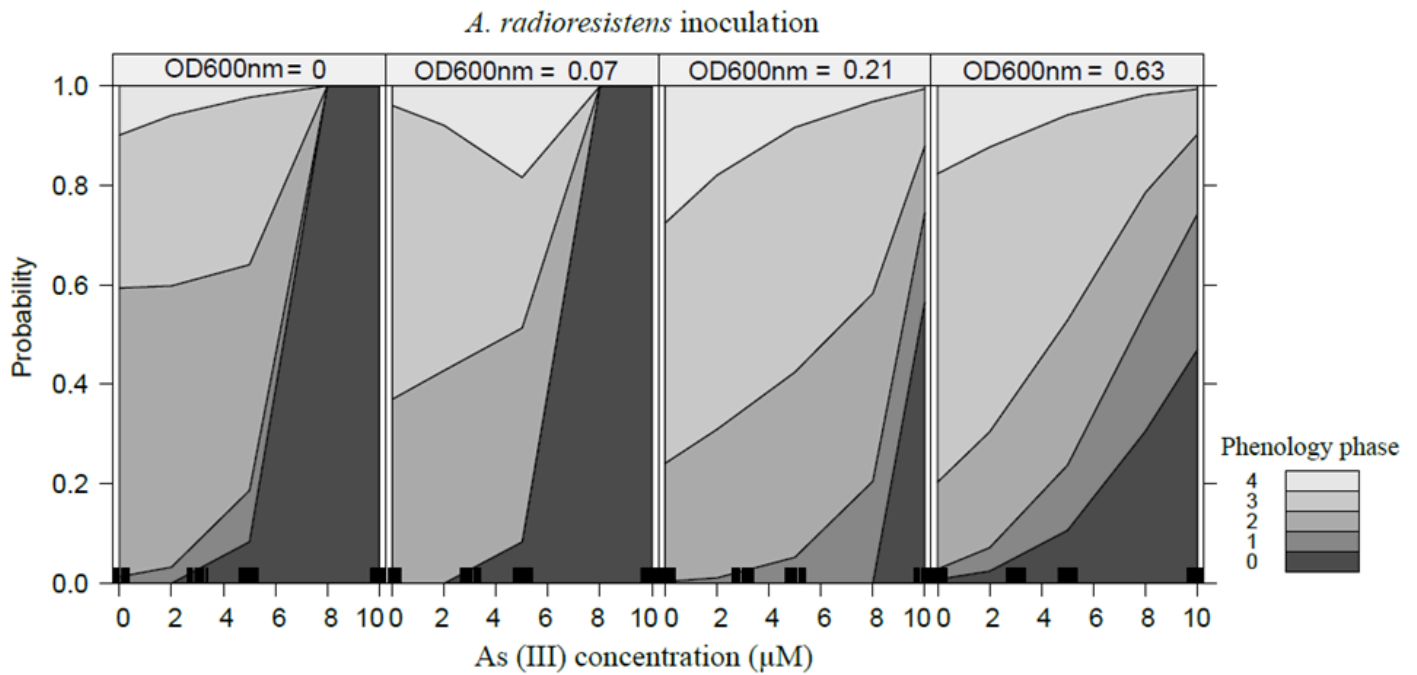


Figure 8.

Figure 8

Probability of each phenology phase of *A. thaliana* plants growing at different As(III) concentrations as a function of the inoculum concentrations of *A. radioresistens* (line type) after 30 days of culture. Note: 0 indicate no inflorescence perceptible; 1, inflorescence present but peduncle not elongated; 2, flower buds determined, and peduncle elongated; 3, first flower bud flourish, but most of them were not opened; 4, all flower buds already flourish, and some wilting of stamens and petals, which undergo abscission.

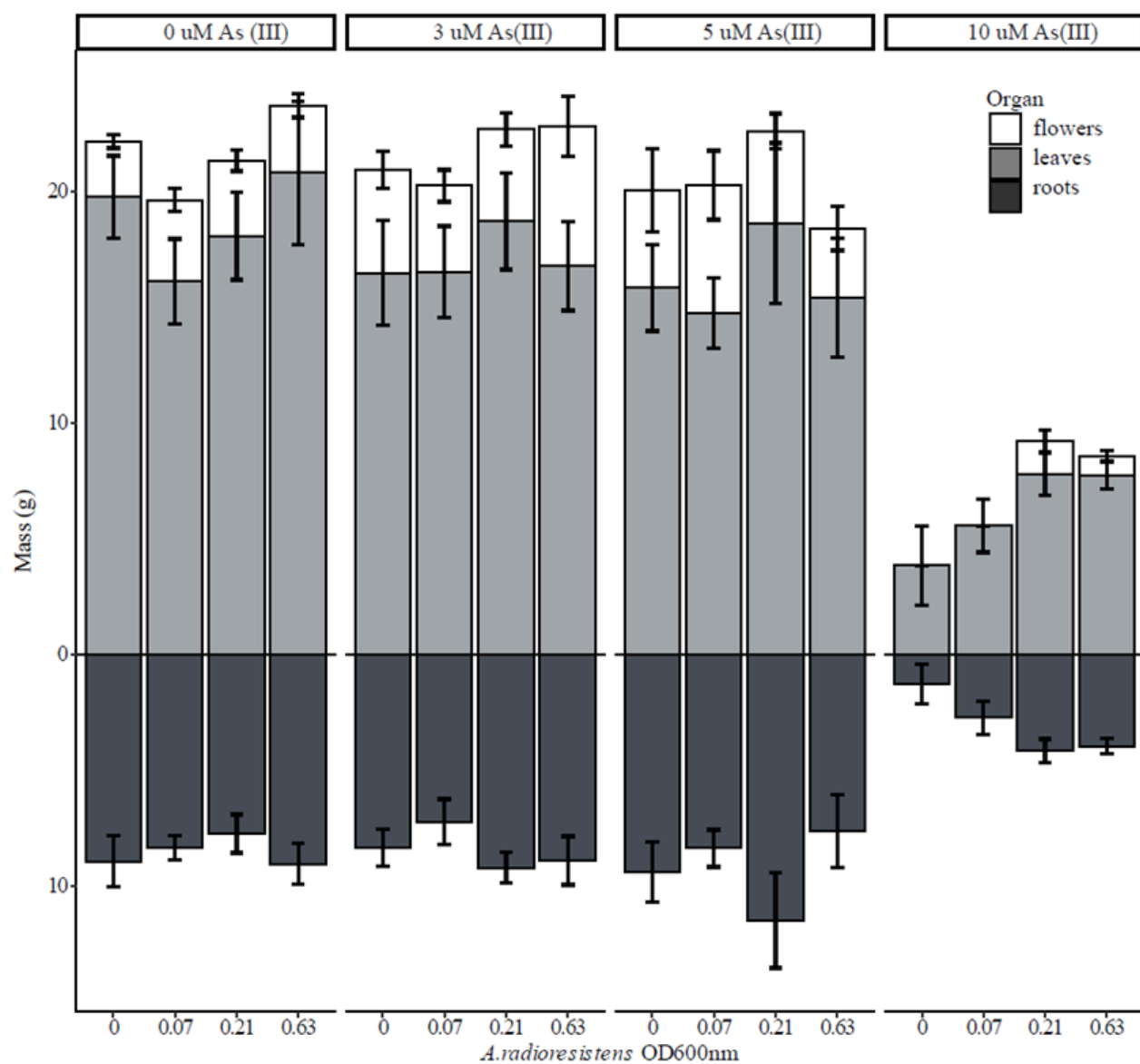


Figure 9.

Figure 9

Mass (mean $\pm$ SE, n = 12) by fractions of *A. thaliana* plants growing at different As(III) concentration as a function of the inoculum concentrations of *A. radioresistens* after 30 days of culture