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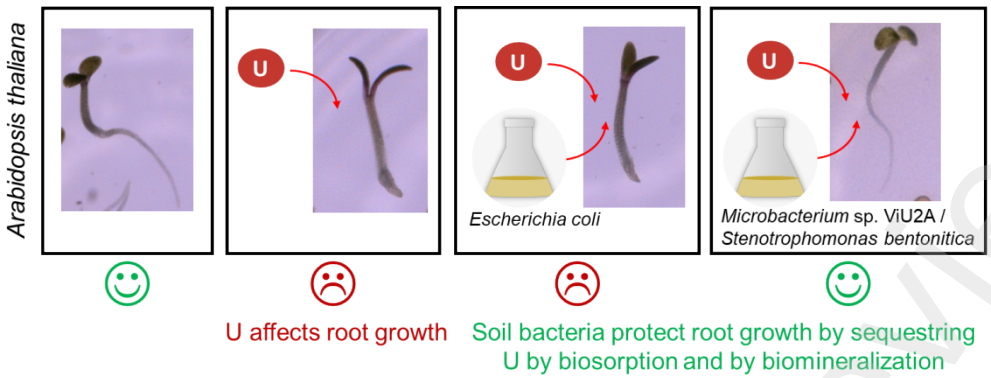
GRAPHICAL ABSTRACT



HIGHLIGHTS

- U strongly affects Arabidopsis seedling growth but much less seed germination
- Soil bacteria alleviate the toxic effects of U on seedlings by efficiently sequestering U
- Active bacterial molecular processes are involved in the protection of roots from U
- The U-binding protein UipA from *Microbacterium* protects seedlings from U toxicity

ABSTRACT

We investigated the impact of uranium (U) on *Arabidopsis thaliana* seed germination and on the early stages of seedling development. Using an *in vitro* assay, we demonstrated that U has a very low impact on Arabidopsis seed germination but has a drastic effect on the development of Arabidopsis seedlings.

We showed that the two soil bacterial strains *Microbacterium* sp. ViU2A and *Stenotrophomonas bentonitica* BII-R7, which are able to tolerate high concentrations of U, strongly reduced the toxic effects of the metal on the seedling development. This protective effect is specific to these soil bacteria, as *Escherichia coli* is not able to protect seedlings. The analysis of U distribution between Arabidopsis seedlings and soil bacteria showed that the protective effect of the bacteria is due to their ability to sequester U, either by biosorption at the level of the cell surface and/or by intracellular or extracellular biomineralization.

This study reveals that these bacteria are very good candidates for use in phytoremediation strategies in the case of phytostabilisation of U-polluted soils. They would be also useful to limit the contamination of the food chain by U because they would limit the entry of this toxic element in crop plants.

STATEMENT OF "ENVIRONMENTAL IMPLICATION"

Uranium (U) contamination of soils and water represent a threat for ecosystems, agrosystems and therefore human health.

We showed that two uranium-tolerant soil bacteria were able to protect seedling development of *Arabidopsis thaliana* from U pollution in such a way that it can no longer have harmful effects on the plant.

The use of these soil bacteria could be very promising for phytoremediation purposes in the case of phytostabilisation of U-polluted soils and also to investigate and understand plant-bacteria interactions at the molecular level in the context of U pollution.

INTRODUCTION

Uranium (U) is a naturally occurring radionuclide present at an average concentration of 2 to 6 ppm in the earth's crust [1]. Anthropogenic activities, including U and phosphate mining, nuclear industry, military activities and agriculture with the use of phosphate fertilizers, are mainly responsible of its redistribution on earth [2, 3]. Although it is not an essential nutrient for life, U in its soluble form can be taken up by plants and can therefore be transferred into the food chain [4]. Uranium can have both chemical and radiological toxic effects on all living organisms [5]. Only enriched U poses a radiological risk, whereas its chemical toxicity is predominant in compounds containing natural U [6]. In recent years, significant progress has been made in understanding the molecular and cellular mechanisms that lead to U toxicity in plants (for a review see [7]). The bioavailability of U and consequently its toxicity is highly related to its speciation, which in turn depends on pH, redox potential, soil composition and biotic factors such as microbial activity [8-12]. Uranium is known to affect different processes in plant development, physiology and metabolism, including photosynthesis [13-17]. Uranium also induces oxidative stress leading to the expression of genes involved in stress signaling and oxidative stress response [17-26]. Uranium interferes with mineral nutrition by changing the concentrations of some essential elements and altering their distribution between roots and shoots [15, 20, 27-29].

Although there are many studies related to the impact of U on plant physiology and development, research investigating the effect of this radionuclide on seed germination and early seedling development remains limited. The toxicity of U on germination depends on several factors such as the nature of seeds [30, 31], U concentrations [32, 33] and the nature of soils [34]. Depleted U has no significant effect on the germination of two grass seeds, purple three-awn (*Aristida purpurea*) and bermuda (*Cynodon dactylon*), up to the concentration of ca. 5256 ppm of soil [31]. Also, no impact of U was observed on the germination of *Cleome amblyocarpa* seeds up to the concentration of 300 ppm (≈1.26 mM solution) [30] and on the germination of radish (*Raphanus sativus*) seeds up to 100 µM [35]. In contrast, Soudek et al. [33] showed that U has an inhibitory effect on seed germination of the five plants tested, and the EC_{50} was determined. The EC_{50} value ranged from 0.10 mM for *Lactuca sativa* to 0.71 mM for *Cucumis sativus*. Uranium also has a toxic effect on the germination of six common edible vegetable seeds tested on highly contaminated soils, with EC_{50} ranging

from 273 ppm of dry soil for *Purple kohlrabi* to 1978 ppm for *Cucumis sativus* [32]. The EC_{50} for *Zea mays* was found to be above 1000 ppm of dry soil [36]. The effect of U on Arabidopsis seed germination and early seedling development stages has not yet been investigated.

Bacteria represent an important fraction of the soil where they play a pivotal role. By interacting with metals and radionuclides, bacteria can change their speciation and solubility [12]. For instance, U can be immobilized through bacterial-mediated mechanisms such as enzymatic reduction, sorption, precipitation and biomineralization [37]. These properties have been described in several bacterial species from various phyla, in particular in U-tolerant bacteria, and some underlying mechanisms have been identified [38-40]. Thus, soil bacteria can greatly affect U bioavailability and U transfer to other soil organisms, including plants.

In order to develop strategies for the phytoextraction and phytostabilisation of U present in U-polluted soils, the role of the plant-microorganisms association needs to be considered [7, 41]. A closer look at the literature on the interaction between U, soil bacteria and plants reveals a limited number of reports. Langella et al (2014) [42] carried out one of the first microbiologically assisted phytoremediation study of soils contaminated with several chemical elements including U. Using two different microbial consortia, the element uptake determined in harvestable biomass of different plant species did not significantly improve U extraction by plants. Also, treatment with *Streptomyces* bacteria, with or without mycorrhiza, did not affect the U content of *Sorghum bicolor* shoot [43]. In the studies conducted by Tan et al (2020) [44], the addition of *Leifsonia* sp. to the pot soil in which Typha seedlings were grown decreased the accumulation of U in the roots and shoots of the plants, presumably by immobilizing U in an oxidized form on the surface of the bacterial membrane. In contrast, in *Leptochloa fusca* grown in U and lead-contaminated soil, the presence of three chromium-resistant endophytic bacteria (*Pantoea stewartii* ASI11, *Enterobacter* sp. HU38 and *Microbacterium arborescens* HU33) enhanced U accumulation in roots by 53-88%, but the radionuclide strongly affected plant biomass [45]. More recently, inoculation of different mixtures of plant-growth-promoting bacteria composed of *Bacillus* and *Citrobacter* in a certain proportion in U-polluted soils (20 to 150 ppm U in soil dry weight) slightly increased the U uptake capacity of three grass species and their biomasses [46]. Greenhouse and field experiments with couch grass (*Elytrigia*

repens), plantain (*Plantago major*) and wheat (*Triticum aestivum*) showed that inoculation of seeds with the phosphate-mobilizing *Cellulomonas* bacteria resulted in a significant increase of U uptake and translocation to shoots [47].

The objective of our study was to investigate the impact of U on *Arabidopsis thaliana* seed germination and early seedling development stages and to analyze the interactions between plant seedlings and soil bacteria in the presence of U. To this aim, we selected two soil bacteria, the actinobacterium *Microbacterium* sp. strain ViU2A [48, 49] and the proteobacterium *Stenotrophomonas bentonitica* BII-R7 [50-52], both highly tolerant to U. These bacterial strains are able to biomineralize U as phosphate mineral phases with a structure similar to that of m-autunite and sequester this radionuclide, thus potentially influencing its impact on the early development of Arabidopsis. In addition, together with Bacteroidetes, Proteobacteria and Actinobacteria are preferentially colonizing the rhizosphere of *A. thaliana* in natural soils [53].

EXPERIMENTAL

Plant Material

Seeds of *Arabidopsis thaliana* ecotype Columbia (Col-0) were chlorine gas sterilized according to Lindsey et al [54]. Seed germination and seedlings growth were then performed in 48-well cell culture plates (Thermo Scientific). Fifteen Arabidopsis seeds were placed in each well containing 400 µl of buffer composed of 1 mM MES (pH 5.0) and 10 mM NaCl supplemented with 2, 5, 10, 15, 20, 50, 100, 200 and 500 µM uranyl nitrate ($UO_2(NO_3)_2$), in the presence or absence of bacteria. A minimum of four replicates were performed for each condition. Plates were then incubated for four days on a shaker in a plant growth chamber in a controlled environment (8 h of light period at 22°C, 110 µmol photons m^{-2} sec^{-1} followed by a 16 h-dark period at 20°C).

Bacterial strains and culture conditions

Microbacterium sp. ViU2A strain (hereafter abbreviated as *M.* sp. ViU2A) was isolated from a natural uranium-rich soil collected in Bessines-sur-Gartempe near Limoges, France [49]. *Stenotrophomonas bentonitica* BII-R7 was isolated from bentonite formations located in southern Spain [50-52]. Soil strains and *Escherichia coli* DH5α cells (control bacterial strain) were grown in lysogeny broth (LB) medium, with agitation of 190 rpm, at 28°C for soil bacteria and at 37°C for *E. coli*. Bacteria were cultured in 100 ml of LB inoculated with an overnight culture until A_{600} has reached 1.0 corresponding to a concentration of 4-6 x 10^7 cells/ml for *M.* sp. ViU2A and *S. bentonitica* and to 4-6 x 10^8 cells/ml for *E. coli*. After centrifugation, the cells were washed twice with 10 mM MES (pH 5.0), 100 mM NaCl and diluted in the same buffer. The bacterial cells were then incubated in the 48-well cell culture plates in the presence of Arabidopsis seeds at the concentrations of 0.8-1.2 x 10^7 and 4-6 x 10^7 cells/ml depending on the test.

Dead bacterial cells were obtained by treatment with 70% (v/v) isopropanol according to the method of Stiefel et al. [55]. To estimate cell survival after isopropanol treatment, cells were washed with 10 mM MES (pH 5.0), 100 mM NaCl, plated on LB agar plates and incubated for 48 h at 28°C. Isopropanol-treated cells failed to grow on LB agar plates.

Microscope observation and image analyses

Four days after imbibition, the seedlings were observed with a Keyence VHX digital microscope. Images of each well were taken to calculate the germination rate and to measure the length of roots and hypocotyls using the ImageJ software.

Production and purification of the extracellular domain of recombinant UipA protein

The production and the purification of the soluble domain of the UipA recombinant protein were performed according to the method described by Gallois et al (2022) [49].

U quantification by inductively coupled plasma-mass spectrometry (ICP-MS)

To quantify U in Arabidopsis seedlings in the absence of bacteria, the medium was first collected from the wells of the 48-well cell culture plates and acidified with 0.65 % (w/v) nitric acid before ICP-MS analysis. Seedlings were washed with MilliQ water and then dried overnight at 80°C. In the presence of bacteria, the media collected from the wells were centrifuged for 4 min at 15,000 g. Supernatants were collected and acidified with 0.65 % (w/v) nitric acid before ICP-MS analysis. Cell pellets were dried overnight at 80°C. Dried samples were resuspended in 100 µL of 65% (w/v) HNO3 and mineralized for 2 hours at 90°C.

Samples diluted with 0.65% (w/v) nitric acid were analyzed using an iCAP RQ quadrupole mass instrument (Thermo Fisher Scientific GmbH, Germany). ^{238}U concentration was determined using a standard curve and corrected using the internal standard 172Yb (standard acquisition mode).

Statistical analyses

Non-parametric statistical analysis was done on our datasets that typically contain small sample sizes ($n<30$) and do not pass normality test, as determined using the Shapiro–Wilk test (alpha=0.05). Mann-Whitney tests were conducted and the confidence level was set at 95% ($p<0.05$). Statistical analyses were performed using GraphPad Prism (version 9.5.1).

RESULTS

Effect of U on *Arabidopsis thaliana* seed germination and seedling development

To assess the impact of U on seed germination and early stages of seedling development, *Arabidopsis thaliana* seeds were incubated for four days in the presence of uranyl nitrate at concentrations ranging from 0 to 500 µM in 48-well cell culture plates. Seed germination (radicle emergence), root and hypocotyl growth were determined from microscope images. Figures 1A and 1B clearly show that U had a very low impact on Arabidopsis seed germination. A significant effect on germination was only observed at the highest U concentration tested (500 µM). However, at this concentration, the germination rate was still above 90%. In contrast, U severely inhibited root development. Root growth was drastically reduced at 20 µM U and fully arrested from 50 to 500 µM U (Fig. 1C). Hypocotyl growth was less affected by U than root growth (Fig. 1D). Hypocotyl development was significantly reduced from 50 µM U and inhibition was drastic at 200 and 500 µM U. Noteworthy, U had a small but significant positive effect on hypocotyl growth at the lowest concentration tested (20 µM).

Effect of soil bacteria *Microbacterium* sp. ViU2A and *Stenotrophomonas bentonitica* BII-R7 on *Arabidopsis thaliana* seed germination and seedling development in the presence of U

Arabidopsis thaliana seed germination and seedling development were analyzed under U stress in the presence of two U-mineralizing bacteria, *M.* sp. ViU2A and *S. bentonitica*, inoculated at the concentration of 4-6 x 10^7 CFU/ml. Figures 2A and 2B show that the presence of bacteria had no statistically significant impact on the germination rate of Arabidopsis seeds, in the absence or presence of U. We also analyzed the influence of bacteria on root development (Fig. 2C). Interestingly, the presence of bacteria in the incubation medium abolished the negative effect of U on root growth. Figure 2C clearly shows that in the presence of *M.* sp. ViU2A or *S. bentonitica*, the addition of 20, 50 and 100 µM U in the medium had no or little impact on the growth of Arabidopsis roots, which is in marked contrast to the situation observed in the absence of bacteria. The protective effect of bacteria was less efficient in the presence of 200 and 500 µM U. Protection by bacteria was also observed at the hypocotyl growth level (Fig 2D). Both soil bacteria were able to protect hypocotyl

development up to the highest concentration of U (500 µM). However, at 500 µM U, the protection was less efficient and *M.* sp. ViU2A appeared to be more effective than *S. bentonitica*.

Uranium distribution between Arabidopsis seedlings and *Microbacterium* sp. ViU2A or *Stenotrophomonas bentonitica* bacteria

We examined first the bioaccumulation of U in Arabidopsis seedlings in the absence of bacteria after a four-day treatment with different U concentrations (Fig. 3, Control). The amount of U accumulated in the seedlings and remaining in the medium was determined by ICP-MS. The seedlings accumulated most of the U initially present in the medium. Uranium accumulated in the seedlings represented 90%, 87% and 82% of the total U after exposure to 20, 50 and 100 µM U, respectively. This percentage dropped to 62% and 58% upon exposure to 200 and 500 µM U. When the experiment was performed in the presence of bacteria (at 4-6 x 10^7 CFU/ml), U accumulated massively in the microorganisms rather than in the Arabidopsis seedlings (Fig. 3, *M.* sp. ViU2A and *S. bentonitica*). In the case of *M.* sp. ViU2A, for example, 78% and 82% of total U was found in the bacteria when the treatments were done with 20 and 50 µM U, respectively. Under these conditions, U accumulated in the seedlings averaged only 16% and 13%, and 5% and 6% of total U remained in the medium, respectively. At 200 µM U, the distribution of U between bacteria, seedlings and the remaining medium was 87, 10 and 3%, respectively. When *S. bentonitica* was incubated with Arabidopsis seeds in the presence of 20 to 200 µM U, the amount of U associated with bacteria accounted for 71 to 84%, and that associated with seedlings was 4 to 27%. At the highest concentration (500 µM U), the amount of U present in the bacteria dropped to 20%, while 53% was accumulated in the seedlings and 27% remained in the medium.

Impact of bacteria on Arabidopsis root growth at low U concentrations

As a concentration of 20 µM U significantly inhibited Arabidopsis root development (Fig. 1), we decided to analyze the impact of U on root growth in the presence of bacteria at concentrations below this value. Arabidopsis root length was measured on seedlings treated with 2, 5, 10, 15 and 20 µM uranyl nitrate and observed under the microscope (Supplemental Fig. 1). Figure 4 shows that U concentrations up to 5 µM had no effect on root length. The radionuclide at concentrations of 10 µM and above had a negative impact on root development. In these conditions, we also analyzed the

protective effect of U-mineralizing soil bacteria and used *E. coli* as a control bacterium. Bacteria were inoculated at the concentration of 4-6 x 10^7 CFU/ml. We observed a protective effect of *M.* sp. ViU2A and *S. bentonitica* on the root growth while *E. coli* had no effect (Fig. 4). We also analyzed the distribution of U between seedlings and bacteria under these conditions (Supplemental Fig. 2). In the absence of bacteria, most of U (74 to 85%) was accumulated in the seedlings (Supplemental Fig. 2, Control). In the presence of *E. coli*, the accumulation of U in the seedlings remained very high (87% to 96%) (Supplemental Fig. 2, *E. coli*). In the presence of *M.* sp. ViU2A, U present in the seedlings dropped to 28% to 40% of the total U, while 53% to 67% was associated with *M.* sp. ViU2A cells (Supplemental Fig. 2, *M.* sp. ViU2A). In the presence of *S. bentonitica*, the majority of U was associated to the bacterial cells (79% to 95%), while a very low percentage (<2%) was found in the seedlings (Supplemental Fig. 2, *S. bentonitica*).

Protection of Arabidopsis seedlings from the effects of U by dead vs living soil bacteria

To further characterize the mechanisms of seedling protection by soil bacteria, we performed experiments in the presence of dead or live bacteria. Soil bacteria were inoculated at 0.8-1.2 x 10^7 and 4-6 x 10^7 CFU/ml in the presence of 20, 50, 100 and 200 µM uranyl nitrate (Supplemental Fig. 3 and 4). Figure 5 shows the impact of *M.* sp. ViU2A on Arabidopsis root length under the different culture conditions. At both bacterial concentrations, live *M.* sp. ViU2A had an effective protective effect up to 100 µM U. Dead *M.* sp. ViU2A protected seedlings efficiently only in the presence of 20 µM U, the highest bacterial density being the most effective. At higher U concentrations, very low protection of the seedling was observed by dead *M.* sp. ViU2A. The same type of experiment was performed with *S. bentonitica* (Fig. 6). At the lowest bacterial concentration (0.8-1.2 x 10^7 CFU/ml), dead or living *S. bentonitica* were able to protect the seedlings efficiently only at 20 µM U. Very low protective effect was observed at higher U concentrations. At the bacterial concentration of 4-6 x 10^7 CFU/ml, both dead and live *S. bentonitica* played a role in protecting seedlings at 50 µM U, while at 100 µM U only living *S. bentonitica* was effective. A very low bacterial protection was observed at 200 µM U.

Impact of the Uranium-induced protein A (UipA) from *Microbacterium* sp. ViU2A on Arabidopsis root growth in the presence of U

The Uranium-induced protein A (UipA) has been recently characterized as a uranyl-binding protein potentially involved in U tolerance in *M.* sp. ViU2A [49]. UipA is a single-pass membrane protein that appears to be specific to U-tolerant *Microbacterium* strains and is highly produced in response to uranyl exposure in the *Microbacterium* ViU2A strain. To test whether UipA could contribute to the protection of *Arabidopsis thaliana* seedlings by *M.* sp. ViU2A, we analyzed the impact of U on Arabidopsis root growth in the presence of increasing amounts of pure recombinant UipA protein soluble domain which possesses the U-binding sites (Fig. 7). In the presence of 20 µM U, we observed a dose-dependent protective effect of UipA on root growth inhibition, with partial protection at 6.25 µg (0.81 µM) and 12.5 µg (1.62 µM) and full protection at 25 µg (3.26 µM) of protein. With a treatment at 100 µM U, UipA was not able to effectively protect seedling development regardless of the amount of protein tested (result not shown).

DISCUSSION

In this study, we showed that the U-tolerant soil bacteria *M.* sp. ViU2A and *S. bentonitica* inoculated with *Arabidopsis thaliana* seeds were able to alleviate the toxic effect of the radionuclide on the development of young seedlings. This protective effect was due to the specific properties of these bacteria to cope with a large amount of U.

First, we set up a biological assai that allowed analyzing the impact of U on seed germination (radicle emergence) and on the early stages of development of Arabidopsis seedlings (root and hypocotyl growth). We also analyzed the influence of the U-tolerant soil bacteria *M.* sp. ViU2A [48, 49] and *S. bentonitica* [51, 52] on the early stages of development of Arabidopsis seedlings in a U pollution context. We showed that U had a very low impact on Arabidopsis seed germination. The negative impact of U was only visible from 500 µM U with a germination rate still above 90% four days after U exposure (Fig. 1B). Our results are very similar to those obtained with *Aristida purpurea* and *Cynodon dactylon* seeds [31], *Raphanus sativus* seeds [35] and *Cleome amblyocarpa* seeds [30]. No impact of U was observed on the germination of *Cleome* amblyocarpa seeds up to 1.26 mM U. Other studies [33] revealed that some seeds such as those from *Lactuca sativa*, *Brassica oleracea*, or *Lepidium sativa* are less tolerant to U, suggesting that the inhibitory effect on seed germination depends on the nature of the seeds. This is supported by the idea that the permeability of the seed coat depends of its morphology [56, 57]. These differences can also be explained by the different treatment conditions (metal concentrations, pH, presence of ligands…) that lead to different speciations of U and consequently modify the bioavailability and toxicity of U. Nevertheless, it is important to note that the inhibitory effects of U on germination occur at quite high concentrations (EC_{50} between 100 and 710 µM U) [33]. This suggests that U is not very mobile in seeds compared to others metals like Ni [56]. In our experiments, we analyzed the speciation of U using the VISUAL MINTEQ software (Supplemental Table 1) under the different treatment conditions. At very low U concentrations (2- 20 µM), the main species predicted in the medium is the uranyl ion (UO_2^{2+}). At higher U concentrations, the U speciation is dominated by positively charged U hydroxyl species. Most of the uranyl and U hydroxyl species are probably immobilized in the seed coat by interacting with negatively charged functional groups (carboxyl, phosphate, hydroxyl groups) present in the seed coat, thus mitigating the inhibitory effects of U on germination.

In contrast to seed germination, we showed that U has a drastic effect on the development of Arabidopsis seedlings, confirming the fact that seedling growth is generally more sensitive to metal toxicity than seed germination [56]. The root was the most U-sensitive tissue. Its growth was impaired at 10 µM U, drastically affected at 20 µM and completely stopped at 50 µM (Fig 1C). The negative effect of U on the hypocotyl growth was only observed from 50 µM U with a more pronounced effect at higher concentrations. This difference in sensitivity can be explained by the fact that the toxic effects of U on root growth occur through the inhibition of the cell division at the apex [17] whereas in the case of the hypocotyl, growth occurs by cell elongation with almost no contribution from cell division [58, 59].

Interestingly, the addition to the incubation medium of the soil bacteria *M.* sp. ViU2A and *S. bentonitica*, which are able to tolerate high concentrations of U, strongly reduced the toxic effects of the metal on seedling development. In the presence of bacteria and up to 100 µM U, the young plant was able to grow in the same way as the plant grown in the absence of U (Fig. 2CD). We also showed that this protective effect is specific to the soil bacteria, as *E. coli* was not able to protect seedlings at U concentrations above 10 µM.

The analysis of the distribution of U between Arabidopsis seedlings, soil bacteria and the medium showed that the protective effect of bacteria was due to their ability to sequester U. Indeed, we showed that in the absence of bacteria, Arabidopsis seedlings captured the majority of U (e.g. 82% at 100 µM). When soil bacteria were present with the seeds, they accumulated the majority of U (70 to 88 %). This indicates that the affinity of the bacteria for U is higher than that of the seedlings. The bacteria were able to biosorb a large amount of the radionuclide present in the medium, thus protecting the seedlings from the toxicity of U. Our results are similar to those of Tan et al (2020) [44] conducted with the gram-positive bacterium *Leifsonia sp*. and Typha seedlings. By immobilizing U in an oxidized form on the surface of their membrane, these bacteria are able to decrease the accumulation of U in the roots and shoots of the plants [44]. Our experiments with dead bacteria suggest that some of the U trapped by the bacteria was due to the adsorption properties of the bacterial membranes. Indeed, even dead, cells of *M.* sp. ViU2A or *S. bentonitica* were able to mitigate the toxicity of U on root development at low U concentration (20 µM) (Fig. 5 and 6).

At higher concentrations of U, the behavior of the two bacteria was different. At 50 and 100 µM U, only living *M.* sp. ViU2A cells were able to efficiently protect Arabidopsis seedlings (at both bacterial concentrations of 0.8-1.2 x 10^7 and 4-6 x 10^7 cells/ml). This suggests that under these conditions, the bacteria triggered active molecular processes necessary to cope with important U concentration in the medium. Interestingly, it has been shown recently that *M.* sp. ViU2A challenged with U induced a U and iron-binding membrane protein, UipA. UipA has a high affinity for uranyl (within a nanomolar range) and is associated with U tolerance of the bacterium [49]. In the present work, we produced and purified the soluble part of UipA, which contains the U binding site, and showed that its presence in the medium protects Arabidopsis seedlings from the toxicity of U (Fig. 7). This confirms that this protein contributes to the bacterial defense mechanisms involved in U tolerance. It is also clear that another bacterial defense process is involved in bacterial tolerance to U since at high U concentrations, UipA alone is not able to cope with the radionuclide. This is in line with previous results showing that *M.* sp. ViU2A was capable of intracellular biomineralization of U, removing more than 80 % of the radionuclide present in the medium [49].

In the case of *S. bentonitica*, at high concentration of U (100 µM), only living bacteria (at 4-6 x 10^7 cells/ml) are able to efficiently protect seedlings from U toxicity (Fig. 6). This also suggests that, under this condition, *S. bentonitica* triggered active molecular mechanisms to cope with the high U content in the medium. Indeed, Pinel-Cabello et al [52] proposed that, in the presence of U, *S. bentonitica* activates a biphasic process made of a sorption of U on cell wall and its precipitation as U-phosphate at the membrane and extracellular levels. The precipitation is enhanced by the induction of phosphatases. Interestingly, we noticed that brown precipitates were visible in our experiments in the presence of *S. bentonitica* (but not with *M.* sp. ViU2A, Fig. 2). These precipitates were previously observed and identified as U-phosphate minerals by high-angle annular dark field scanning transmission electron microscopy coupled with energy dispersive X-ray spectroscopy analyses [52].

At 50 µM U and at the lower concentration of bacteria (0.8-1.2 x 10^7 cells/ml), *S. bentonitica* was no longer able to protect Arabidopsis seedlings, suggesting that the active mechanism of *S. bentonitica* was not efficient enough to deal with the U in the

medium. We could explain this behaviour by the fact that the phosphatase activity was not sufficient to precipitate most of the U initially present in the medium.

At 50 µM U, both dead and live bacteria at the concentration of 4-6 x 10^7 cells/ml were able to protect seedlings, suggesting that the adsorption capacities of *S. bentonitica* cells were high and greater than those of *M.* sp. ViU2A, since at this concentration *M.* sp. ViU2A was no longer able to protect seedlings. At the highest U concentration (200 µM U), both bacterial U-scavenging mechanisms are overwhelmed, resulting in a detrimental effect of U on root development.

CONCLUSION

In the present work, we showed that the two bacterial strains *Microbacterium* sp. ViU2A and *Stenotrophomonas bentonitica* BII-R7 were able to protect seedling development of *Arabidopsis thaliana* from U pollution. This protection is achieved thanks to the ability of these bacteria to alleviate U toxicity, either by biosorption at the cell surface and/or by intracellular or extracellular biomineralization, in such a way that it can no longer have harmful effects on the plant.

This study paves the way to investigate and understand plant-bacteria interactions at the molecular level in the context of U pollution and to determine whether there is some mutualistic relationship between the two organisms, and whether these bacteria can be considered as plant growth-promoting bacteria.

In the future, the use of these soil bacteria could be very promising to limit the contamination of the food chain by U because they would limit the entry of this toxic element in crop plants. These bacteria could also be used for phytoremediation purposes in the case of phytostabilisation of U-polluted soils.

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456 FIGURE LEGENDS

457

458 **Figure 1: Effect of U on seed germination and seedling development of**
459 ***Arabidopsis thaliana*.**
460 Arabidopsis seeds were incubated four days in the presence of different concentrations
461 of uranyl nitrate in a 48-well culture plate (15 seeds/well) and visualized with a Keyence
462 VHX digital microscope (scale bar 1 mm) (A). Germination rates (B), root length (C)
463 and hypocotyl length (D) were determined at different U concentrations. See 'Materials
464 and Methods' and supplemental data 1 for statistical analyses.
465

466 **Figure 2. Effect of soil bacteria *Microbacterium* sp. ViU2A and**
467 ***Stenotrophomonas bentonitica* on *Arabidopsis thaliana* seed germination and**
468 **seedling development in the presence of U.** Arabidopsis seeds (15 seeds/well) were
469 incubated for four days in the presence of different concentrations of uranyl nitrate, in
470 the presence or absence of bacteria, and visualized with a Keyence VHX digital
471 microscope (scale bar 1 mm). Control: without bacteria, Micro: with *M. sp. ViU2A* (4-6
472 x 10^7 PFU/ml) and Steno: *S. bentonitica* (4-6 x 10^7 PFU/ml) (A). Germination rates (B),
473 root length (C) and hypocotyl length (D) determined at different U concentrations, in
474 the absence of bacteria (Control) or in the presence of *M.* sp. ViU2A (Micro) or *S.*
475 *bentonitica* (Steno). See 'Materials and Methods' and supplemental data 1 for
476 statistical analyses.
477

478 **Figure 3. Distribution of U between Arabidopsis seedlings, soil bacteria**
479 **(*Microbacterium* sp. ViU2A or *Stenotrophomonas bentonitica* BII-R7T) and**
480 **medium.** Arabidopsis seeds (15 seeds/well) were incubated for four days in the
481 presence of different concentrations of uranyl nitrate, in the presence or absence of
482 bacteria as mentioned in Fig. 2. ^{238}U was quantified by ICP-MS. Control: distribution of
483 U between seedlings (green) and medium (grey) in the absence of bacteria.
484 Microbacterium: distribution of U between *M.* sp. ViU2A (red), seedlings (green) and
485 medium (grey). *Stenotrophomonas:* distribution of U between *S. bentonitica* (blue),
486 seedlings (green) and medium (grey). Numbers represent the % of U present in the
487 fraction. See 'Materials and Methods' and supplemental data 1 for statistical analyses.

Figure 4. Impact of bacteria on Arabidopsis root growth in the presence of low concentration of U. Arabidopsis seeds were incubated for four days in the presence of different concentrations of uranyl nitrate, in the presence or absence of bacteria (*E. coli*, *M.* sp. ViU2A and *S. bentonitica*) as mentioned in Supplemental Fig. 1. Bacteria were inoculated at the concentration of 4-6 x 10^7 CFU/ml. See 'Materials and Methods' and supplemental data 1 for statistical analyses.

Figure 5. Impact of dead and living *Microbacterium* sp. ViU2A on Arabidopsis root growth in the presence of different concentration of U. Arabidopsis seeds were incubated for four days in the presence of uranyl nitrate as mentioned, in the absence of bacteria (control) or in the presence of dead and living *Microbacterium* inoculated at two different concentrations: 0.8-1.2 x 10^7 and 4-6 x 10^7 CFU/ml. See 'Materials and Methods' and supplemental data 1 for statistical analyses.

Figure 6. Impact of dead and living *Stenotrophomonas bentonitica* on Arabidopsis root growth in the presence of different concentrations of U. Arabidopsis seeds were incubated for four days in the presence of uranyl nitrate as mentioned, in the absence of bacteria (control) or in the presence of dead and living *S. bentonitica* inoculated at two different concentrations : 0.8-1.2 x 10^7 and 4-6 x 10^7 CFU/ml. See 'Materials and Methods' and supplemental data 1 for statistical analyses.

Figure 7. Impact of the Uranium induced protein A (UipA) from *Microbacterium* sp. ViU2A on Arabidopsis root growth in the presence of U. Arabidopsis seeds were incubated for four days in the presence of 20 µM uranyl nitrate and different quantities of UipA (0, 6.25, 12.5 and 25 µg), and visualized with a Keyence VHX digital microscope (scale bar 0.5 mm). Samples are labeled with reference to U and UipA concentrations, respectively: U0-UipA0 (control), U20-UipA0, U20-UipA6.25, U20-UipA12.5 and U20-UipA25. See 'Materials and Methods' and supplemental data 1 for statistical analyses.

Supplemental Figure 1. Impact of bacteria on Arabidopsis root growth at low U concentrations (<20 µM)

Arabidopsis seeds were incubated for four days in the presence of different concentrations of uranyl nitrate, in the presence or absence of bacteria, and visualized with a Keyence VHX digital microscope (scale bar 0.5 mm). Control: in the absence of bacteria; *Micro*: in the presence of *M.* sp. ViU2A*; Steno*: in the presence of *S. bentonitica, E. coli*: in the presence of *E. coli.* Bacteria were inoculated at the concentration of 4-6 x 10^7 CFU/ml.

Supplemental Figure 2. Distribution of U between Arabidopsis seedlings, bacteria (*Microbacterium* sp. ViU2A, *Stenotrophomonas bentonitica* or *E. coli*) and medium. Arabidopsis seeds were incubated for four days in the presence of different concentrations of uranyl nitrate, in the presence or absence of bacteria (see also supplemental Fig. 1). ^{238}U was quantified by ICP-MS. Control: distribution of U between seedlings (green) and medium (grey) in the absence of bacteria. Microbacterium: distribution of U between *M. sp. ViU2A* (red), seedlings (green) and medium (grey). *Stenotrophomonas:* distribution of U between *S. bentonitica* (blue), seedlings (green) and medium (grey). *E. coli: d*istribution of U between *E. coli* (yellow)*,* seedlings (green) and medium (grey). Numbers represent the % of U present in the fraction. See 'Materials and Methods' and supplemental data 1 for statistical analyses.

Supplemental Figure 3. Protection of Arabidopsis seedlings from exposure to U by dead and living soil *Microbacterium* sp. ViU2A

Arabidopsis seeds were incubated for four days in the presence of different concentrations of uranyl nitrate and two different concentrations of live or dead *M.* sp. ViU2A (Micro). Seedlings were visualized with a Keyence VHX digital microscope (scale bar 0.5 or 1 mm).

Supplemental Figure 4. Protection of Arabidopsis seedlings from exposure to U by dead or living soil *Stenotrophomonas bentonitica*

Arabidopsis seeds were incubated for four days in the presence of different concentrations of uranyl nitrate and two different concentrations of live or dead *S.*

551 *bentonitica* (Steno). Seedlings were visualized with a Keyence VHX digital microscope
552 (scale bar 0.5 or 1 mm).
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