Version of Record: https://www.sciencedirect.com/science/article/pii/S1164556319304698
Manuscript_758fad7cef5da2f36ef881afa3bbb9f4

Investigating some mechanisms underlying stress metal adaptations of two *Burkholderia* sensu lato species isolated from New Caledonian ultramafic soils

Alexandre BOURLES[a], Hamid AMIR[a], Simon GENSOUS[a], Flavie CUSSONNEAU[a], Valérie MEDEVIELLE[a], Farid JUILLOT[c], Alexis BAZIRE[d], Michaël MEYER[a], Valérie BURTET-SARRAMEGNA[a], Yvon CAVALOC[a], Philippe JOURAND[e], Linda GUENTAS[a,b,*]

[a]Institut des Sciences Exactes et Appliquées, Université de la Nouvelle-Calédonie, BR R4 98851 Nouméa, Nouvelle-Calédonie

[b]Université de Toulon, Laboratoire MAPIEM EA 4323, SeaTech-Ecole d'ingénieurs BP 20132 83957 La Garde Cedex, France

[c]UMR IRD 206 - Institut de Recherche pour le Développement, Centre IRD Nouméa, 101 Promenade Roger Laroque, BP A5 - 98848 Nouméa Cedex Nouvelle-Calédonie

[d]Laboratoire de Biotechnologie et Chimie Marines, EA 3884, LBCM, IUEM Université de Bretagne-Sud, 56100 Lorient, France

[e]IRD, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM UMR040), Campus International de Baillarguet, TA A-82/J, Montpellier F-34398 Cedex 05, France

[*]**Corresponding author**: linda.guentas@univ-nc.nc (L. Guentas), Université de la Nouvelle-Calédonie, BR R4 98851 Nouméa, Nouvelle-Calédonie. Phone Number: +687290262

© 2020 published by Elsevier. This manuscript is made available under the CC BY NC user license
https://creativecommons.org/licenses/by-nc/4.0/

Abstract

In New Caledonia, one third of the main island is covered by ultramafic soils characterized by high levels of metals and low levels of plant nutrients. In these soils, metal tolerant bacteria may play a role in plant adaptation to the edaphic constraints. Recently, two new bacteria species belonging to the genus *Burkholderia* sensu lato were isolated from rhizospheric roots of pioneer plant growing in New Caledonian ultramafic soils and were able to tolerate high metal concentrations. The purpose of this study was to determine the major mechanisms by which these two bacteria were able to tolerate metals and if they were able to reduce metal absorption by plants from which they were isolated. Bioaccumulation, adsorption on cell surface, ability to form biofilm and production of exopolysaccharides were assessed. A focus was carried out on Ni, generally the most toxic metal in New Caledonian ultramafic soils. All experiments were performed in comparison with a reference strain isolated from a non-ultramafic habitat, *Paraburkholderia caribensis* MWAP64^T. The two ultramafic bacterial species showed a high tolerance to Ni with a Ni–IC_{50} of 5 mM for *Caballeronia novacaledonica* STM10272^T and 30 mM for *Paraburkholderia ultramafica* STM10279^T, values significantly higher than those of *P. caribensis* MWAP64^T (2 mM). The major mechanism involved in the bacterial Ni tolerance was related to biofilm formation for *C. novacaledonica* STM10272^T and to acidic exopolysaccharide production for *P. ultramafica* STM10279^T. Both species alleviated metal contents in roots of *Tetraria comosa*, an endemic pioneer herbaceous species growing on New Caledonian ultramafic soils.

Key words: Metal tolerance, Biofilm; Exopolysaccharides; *Paraburkholderia*; *Caballeronia;* Plant metal alleviation

1 Introduction

Ultramafic soils (also known as serpentinic soils) are resulting from weathering and pedogenesis of ultramafic bedrocks [1]. These soils are characterized by (i) the dominance of iron oxides with high levels of potentially toxic metals including Ni, Co, Cr and Mn, (ii) low levels of major plant nutrients such as N, P, and K, and (iii) a strong unbalanced calcium/magnesium (Ca/Mg) ratio (<1) [1]. This *a priori* disadvantageous substrate constitutes a specific habitat, which frequently hosts unique plants communities and "clusters" of biodiversity consisting of restricted and endemic taxa [2]. In these extreme edaphic conditions, plants have developed several adaptive mechanisms such as (i) slower growth (ii) ability to limit Mg absorption, and (iii) strategies to tolerate high metal concentrations [2]. Among these strategies, the association with specific bacteria play a key role in plant adaptation to harsh edaphic conditions [3,4].

The study of microbial communities from ultramafic soils led to the identification of new metal tolerant strains and species [5,6]. Metal tolerant-bacteria show a wide panel of responses towards metals such as biosorption, bioaccumulation, precipitations, chelation, biofilm production, exopolysaccharides (EPS) production or metal transformation, thereby reducing metal toxicity towards themselves [7]. Moreover, metal tolerant bacteria have been shown to reduce the bioavailability of metals and thus to alleviate their toxicity to plants exposed to metal stress [8].

In New Caledonia, a tropical archipelago in the South Pacific Ocean, one third of the main island is covered by ultramafic soils. As a result, about 85% of the vascular flora growing on these soils are endemic [9]. However, ultramafic settings are exploited for economically valuable ores such as Ni, Co and Cr, disturbing ultramafic landscapes and classifying New Caledonia among the biodiversity hotspots [10]. New Caledonian ultramafic soils are characterized by high richness and phylogenetic bacterial diversity compared with other environment [11],however the interest for bacteria of these soils is recent [12]. In 2016, two new *Burkholderia* sensu lato (s.l.) species (*Burkholderia novacaledonica* and *Burkholderia ultramafica*), isolated by Gonin et al. (2013) [13] from roots of pioneers plants belonging to the Cyperaceae family growing on ultramafic soils in New Caledonia, have been characterized [6]. Recently, Dobritsa and Samadpour (2019) [14] have proposed the transfer of *B. novacaledonica* STM10272^T and *B. ultramafica* STM10279^T to the genus *Caballeronia* and *Paraburkholderia* respectively.

These two species showed tolerance to high levels of the metals found in New Caledonian ultramafic soils [13] suggesting the existence of mechanisms involved in bacterial adaptation to the ultramafic constraint. In this study, we investigated the mechanisms occurred in *Paraburholderia ultramafica* STM10279^T and *Caballeronia novacaledonica* STM10272^T in comparison with *Paraburkholderia caribensis* MWAP64^T isolated from a non-ultramafic soil [15]. We also investigated the implication of *P. ultramafica* STM10279^T and *C. novacaledonica* STM10272^T on the adaptation to ultramafic constraint of *Tetraria comosa*, an endemic pioneer Cyperaceae used in restoration programs [16].

2 Material and Methods

2.1 Bacterial species

For ultramafic species, plant sampling and bacterial strains isolation were performed by Gonin et al. (2013) [13]. Full description of *C. novacaledonica* STM10272^T and *P. ultramafica* STM10279^T including morphological, biochemical and molecular characterization was carried out by Guentas et al. (2016) [6] and are reported in Supplementary Table 1. According to Gonin et al. (2013) [13], the two species isolated from ultramafic soils, tolerate edaphic constraints such as low pH, unbalanced Ca/Mg ratio and high metals concentrations (Supplementary Table 2). Concerning *P. caribensis* MWAP64^T**,** bacterial strain was isolated from Vertisol microaggregates in Martinique by Achouak et al. (1999) [15].

2.2 *Test for metal tolerance (50% inhibition concentration; IC_{50})*

The metal tolerance was performed in aerobic conditions on microplates (sterile transparent 96 wells PS flat bottom, Greiner) in Lysogenic Broth Lennox medium (LB, tryptone: 10 g L^{-1}, yeast extract 5 g L^{-1}, NaCl: 5g L^{-1}; CONDA, Spain) supplemented with different metals concentrations (3 replicates by plate) as suggested by Chien et al. (2013) [17]. For these experiments, 1 M metal stock solutions were prepared from solid salts (Ni as $NiSO_4 \cdot 6H_2O$, Co as $CoCl_2 \cdot 6H_2O$, Cr as $K_2Cr_2O_7$, and Mn as $MnSO_4 \cdot H_2O$). To avoid metal precipitation in the assays, like suggested by Hartley et al. (1997) [18], these stock solutions were sterilized separately. Each well was filled with 20 µL of an overnight bacterial culture adjusted to an OD_{600} of 1, (which is approximately equal to 10^8 CFU mL^{-1}) and 180 µL of broth tested. The plate was incubated at 28°C under 125 r min^{-1} orbital shaking in a microplate incubator

reader (EPOCH 2 microplate reader; BioTek Instruments, Winooski, Vermont, USA). Cell growth was monitored by measuring the OD_{600} every hour for 48 h. The experimentations were done at least twice independently. According to the preliminary result of Gonin et al. (2013) [13], metals concentration tested ranged from 0.5 to 100 mM for Ni and Mn and from 0.01 to 50 mM for Co and Cr. The strain growth rates (μ) were determined for each concentration. A sigmoid dose–response curve was obtained by plotting growth rate against metal concentrations, and IC_{50} values were determined using GraphPad Prism software (version 6, GraphPad Software, La Jolla California USA).

2.3 Ni biosorption and bioaccumulation

The metal adsorbed onto the surface of the bacterial cell and the metal accumulated inside the cell were determined according to Giovanella et al. (2017) [19], with modifications as follows. A primary culture was done by inoculating 10 mL of LB Lennox medium with one colony of the chosen bacteria and then incubating for 24 h at 28°C and 125 r min^{-1}. A culture (25 mL) inoculated at an OD_{600} of 0.1 by a primary culture was incubated for 24 h at 28°C under rotary shaking at 125 r min^{-1}; then the culture was artificially contaminated by aseptically adding Ni such that Ni reached a final concentration of 1 mM, 5 mM and 50 mM (based on Ni-IC_{50} values). After 24 h, 1 mL samples were removed from the culture and centrifuged at 13,000 r min^{-1} for 10 min (Sigma, 2-16K). The pellet was washed thrice in sterile deionized water to remove free metal ions and finally suspended in 1 mL of sterile deionized water. The OD_{600} was read and the number of colony-forming units (CFU) per milliliter of the inoculum was carried out. To determine Ni accumulation in the cell walls and intracellular spaces of these bacteria, another aliquot (1 mL) was centrifuged at 13,000 r min^{-1} for 10 min; the pellet was washed with ethylenediaminetetraacetic acid (EDTA, 10 mM, pH: 6.5) for Ni desorption of from the cell surface, centrifuged, and re-suspended in 1 mL of sterile deionized water. Finally, the total amount of Ni accumulated intracellularly (bioaccumulation) and sorbed onto the cells (biosorption) was determined by digesting the 1 mL aliquots in a nitric acid (69%, 1 mL) and hydrogen peroxide (30%, 0.5 mL) solution for 1 night at room temperature. The digested samples were then adjusted to 10 mL with deionized water, and the Ni content in the final solution was quantified by Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES, Varian, Varian 730–ES, Palo Alto, USA) at Laboratoire des Moyens Analytiques (LAMA-US IMAGO-IRD, New Caledonia). The amount of Ni accumulated intracellularly (bioaccumulation) was determined by substracting the Ni content adsorbed onto the cell walls (biosorption) from the total Ni amount.

2.4 Biofilm formation assay in the presence of Ni

The biofilm formation assay was performed on a microtiter plate [20]. Aliquots of 90 µL of LB culture medium supplemented with different concentrations of Ni [(as $NiSO_4 \square 6H_2O$]) to reach 0.5, 1, 2.5, 5, 8, and 10 mM Ni concentration (based on Ni- IC_{50} values) were loaded into 96-well microtiter plates (sterile transparent 96 wells PS flat bottom, Greiner). Wells were inoculated with 10 µL of a 48 h primary culture adjusted to an OD_{600} of 1. For these experiments, a non-inoculated LB medium was used as the negative control, whereas an inoculated LB culture medium without Ni was used as the positive control. After, 24 h, 48 h, and 72 h of incubation at 28°C without agitation, the medium and the non-adhering bacteria biofilm were eliminated by simple inversion and each well was washed three times with 100 µL of sterile deionized water. The microplates were then dried at 50°C for 30 min. The cells from the adhering biofilm were dyed with 100 µL of 0.02% crystal violet (CV) solution (30 min at room temperature). The excess of dye was removed by simple inversion and the biofilm was washed three times with 100 µL of sterile deionized water at each wash. The microplates were dried at room temperature for 10 min before resuspending CV by adding 200 µL of 95% ethanol solution. After 10 min orbital shaking at 125 r min^{-1}, the content of each well was transferred into a new microplate and the OD_{590} was read with a microplate reader (EPOCH 2 microplate reader; BioTek Instruments, Winooski, Vermont, USA). The results obtained were then transformed into a quantification of biofilm formation by calculating the average absorbance of six replicates for each species and for each concentration and dividing by the average absorbance of the blank (without bacteria). The results expressed as this ratio can be considered as non-dependent from the non-specific coloring of the surface of the wells of the microplates by the CV. Following this procedure, biofilm formation was considered to have occurred when the ratio is greater than 2 [21].

2.5 Quantification of biofilm formation

Since the concentration of bioavailable Ni frequently reaches 1 mM in ultramafic soils, especially in the rhizosphere from New Caledonia [22], quantification of biofilm production without Ni and at 1 mM of Ni was carried out. The biofilm formation was visualized by confocal laser scanning microscopy (CLSM) after 24 h. For these experiments, a 24 h primary culture was first adjusted at a 0.1 OD_{600} in LB medium and used to inoculate a 24-

wells in glass bottom opaque black screening plate (SensoPlate, Greiner Bio-One) (1 mL per well). The microtiter dish was then incubated for 24 h and the biofilms were stained for 10 min with 1% of Syto 61 red fluorescent nucleic acid strain (5 mM, Molecular Probes, USA). CLSM observations were performed with a TCS-SP2 microscope (Leica Microsystems, Heidelberg, Germany), using a 63x immersion objective. The excitation and Emission wavelengths for Syto 61 Red were set at 633 nm and 645 nm, respectively. The images collected by confocal stacks were processed using Leica Confocal Software and the biovolume, thickness and substratum coverage of biofilms were quantified with COMSTAT [23].

2.6 Production and gross chemical composition of EPS

Regarding its ability to produce an exopolysaccharide [15], *P. caribensis* MWAP64^T was used as a positive control in order to approve the experimental design. EPS production was quantified after 96 h of culture at 28°C under 125 r min^{-1} orbital shaking in minimum broth medium (MB; $KH_2PO_4 \cdot 3H_2O$, 0.66 g L^{-1}; NaCl, 50 mg L^{-1}; $MgSO_4 \cdot 7\ H_2O$, 10 mg L^{-1}; $FeCl_3$, 4 mg L^{-1}; $CaCl_2$, 40 mg L^{-1}) supplemented with glucose (30 g L^{-1}). In order to produce biomass, 8 L of LB were inoculated using a 48 h grown culture (800 mL; 28°C, 125 r min^{-1} orbital shaking). After 72 h of incubation (28°C, 125 r min^{-1} orbital shaking), the biomass was recovered by centrifugation at 9,000 r min^{-1} (13,700 g) for 25 min (Sorvall RC5C Centrifuge, DuPont) and washed once with sterile deionized water. Cells were transferred at 28°C to 10 L reactors containing 8 L of MB medium supplemented with glucose. The bacteria were removed from the medium by centrifugation at 9,000 r min^{-1} (18,894 g) for 25 min. The supernatants were collected and the EPS were purified by ultrafiltration on a 100,000 molecular weight cut-off membrane from Sartorius and immediately freeze-dried. Protein content was determined by the Lowry method [24], modified by Smith et al. (1985) [25] and Brown et al. (1989) [26]. Sugar contents, was quantified by the method described by DuBois et al. (1956) [27] and completed by Chaplin (1986) [28], and uronic acid contents by the Blumenkrantz and Asboe-Hansen method [29], modified by Filisetti-Cozzi and Carpita (1991) [30]. Infrared spectroscopy was used to complete the biochemical characterization of EPS. The infrared spectrum of the freeze-dried EPS was recorded using a Fourier Transform Infrared (FT–IR) spectrometer Frontier TM (Perkin Elmer) at the spectroscopy lab of the IRD center in Noumea (New Caledonia). This spectrum corresponds to an average of four successive spectra that were

collected in Attenuated Total Reflectance mode (diamond crystal) over the 500 cm^{-1} – 3500 cm^{-1} range with a 4 cm^{-1} resolution.

2.7 Effect of microbial inoculation on Tetraria comosa plants under ultramafic conditions

The inocula for pot trial were prepared as follows: A pre-inoculum was performed as described previously in the section 2.3. After 2 days, the aerobic cultures were used to inoculate 100 mL of LB media at an $OD_{600\ nm}$ of 0.1. After 3 days at 28°C under orbital agitation (125 r min^{-1}), the cells were pelleted by centrifugation (SIGMA®, 2K15, 5,000 g, 20 min), washed twice with sterile deionized water and, then re-suspended in sterile deionized water at a bacterial concentration between 10^7 and 10^8 CFU mL^{-1} (corresponding to an OD_{600} of 0.5), as suggested by Larcher et al. (2003) [31]. The purity was checked and the concentration of the inoculum was carried out after a bacterial count on LB agar plate.

SIRAS Pacifique (Noumea, New Caledonia) provided seeds of *Tetraria comosa*. Germination occurred on a compost amended ultramafic soil composed of (i) 2 mm sieved colluvial lateritic soil (ferralsol) sampled in Plum area in New Caledonia (22°16'59''S, 166°39'12''E) and (ii) commercial compost (4:1 *v:v*). Characteristics of the ferralsol are: coarse sand, 39.4%; fine sand, 22.1%; silt-clay, 37.2%; pH H_20, 5.9; pH KCl, 5.6; total C, 42.1 g kg^{-1}; total N, 2.2 g kg^{-1}; total P, 147 mg kg^{-1}; available P (Mehlich), 3 mg kg^{-1}; total Ca, 1.06 g kg^{-1}; total Mg, 5.08 g kg^{-1} and Ca/Mg, 0.207. The total metals contents of the ferralsol measured after an alkaline fusion are as follows: Co, 0.87 g kg^{-1}; Cr, 22 g kg^{-1}; Fe, 348 g kg^{-1}; Mn, 10 g kg^{-1}; Ni, 5.6 g kg^{-1}. The available metals extracted by dimethylene triaminopentaacetic acid (DTPA) are as follows: Co_{DTPA}, 68 mg kg^{-1}; Cr_{DTPA}, 0.17 mg kg^{-1}; Fe_{DTPA}, 71 mg kg^{-1}; Mn_{DTPA}, 1.1 g kg^{-1}; Ni_{DTPA}, 130 mg kg^{-1}. The composition of commercial compost is as follows: N, 1.7 mg g^{-1}; P_{total}, 150 mg kg^{-1}; P_{Olsen}, 7 mg kg^{-1}; K,139 mg kg^{-1} (Terreau universel, Agrofino, France). The compost amended-ultramafic soil was autoclaved 3 times at 120°C for 1 h, with an interval of 24 h before being used in the greenhouse experiment. Although, *T. comosa* was able to develop on ultramafic soil in natural condition, the addition of this commercial compost to the ultramafic soil was necessary because the growth of the plantlets of *T. comosa*

could be inhibited under greenhouse condition in a pure sterile lateritic topsoil [32]. After two months, roots seedlings were washed with sterile water to remove any contaminants and were transplanted into one-liter plastic containers filled with the compost amended-ultramafic soil described above. A 5 mL bacterial inoculum was applied on roots of each plantlet during transplantation in 1 L pots. The same volume of sterilized water was applied to a control set of plants. A set of 15 plants was inoculated per treatment. The plants were grown under greenhouse conditions as reported by Lagrange et al. (2011) [33]. After 15 months of growth, the plants were harvested. Shoot and root biomasses were determined by measuring the dry mass tissue (3 days at 60°C). The 15 samples of plant tissues were randomly pooled by five and ground to powder. The contents of P, K, Ca, Mg, Co, Cr, Mn, and Ni in tissues were quantified by ICP OES with a Varian 730–ES spectrometer after digestion in a mixture (4:1, v/v) of HNO_3 (69%) and H_2SO_4 (37%) at the Laboratoire des Moyens Analytiques (LAMA-US IMAGO-IRD, New Caledonia).

2.8 Statistical analysis

All statistical analysis were performed with the R software version 3.3.1 [34]. All data were analyzed with parametric tests (one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test; $P < 0.05$) or with non-parametric tests (Kruskal-Wallis test; followed by the Fisher's least significant difference (LSD) test; $P < 0.05$) when data did not follow the assumptions of a parametric test.

3 Results

3.1 Test for metal tolerance

The metals IC_{50} determined for the three species are reported in Table 1. *P. ultramafica* STM10279^T and *C. novacaledonica* STM10272^T showed a significant higher tolerance to Co, Mn and Ni compared to *P. caribensis* MWAP64^T. For instance, *P. ultramafica* STM10279^T Ni-IC_{50} value was 16 times significantly higher and *C. novacaledonica* STM10272^T Ni-IC_{50} value was 2.5 times significantly higher than Ni-IC_{50} value of *P. caribensis* MWAP64^T. The two ultramafic species showed significantly different metal tolerance profiles for Cr and Ni. Indeed, the Ni IC_{50} of *P. ultramafica* STM10279^T was 6.4 times higher compared to Ni-IC_{50} value of *C. novacaledonica* STM10272^T. Conversely, the Cr IC_{50} of *P. ultramafica* STM10279^T was significantly lower than Cr-IC_{50} value of *C.*

novacaledonica STM10272^T. No significant differences between the two ultramafic species were observed for the tolerance to Co and Mn.

3.2 Ni biosorption and bioaccumulation

Biosorption of Ni onto the cell surface and intracellular compartment were evaluated. Results expressed in ng (10^7CFU)$^{-1}$ are reported in Fig. 1. The fraction of Ni inside the cells and onto cell wall surface were significantly lower for *P. ultramafica* STM10279^T and *C. novacaledonica* STM10272^T than for *P. caribensis* MWAP64^T. Moreover, significant differences are observed between *P. ultramafica* STM10279^T and *C. novacaledonica* STM10272^T. Major differences appeared at 5 mM of initial Ni concentration, the mean intracellular Ni content in *P. ultramafica* STM10279^T (0.76 ± 0.02 ng (10^7CFU)$^{-1}$) was 430 times lower than the value of *C. novacaledonica* STM10272^T and was 11,000 times lower compared to the value of *P. caribensis* MWAP64^T. Ni content on cell surface of *P. ultramafica* STM10279^T (3.45 ± 0.34 ng (10^7CFU)$^{-1}$) was 86 times lower than the value of *C. novacaledonica* STM10272^T and was significantly 675 times lower compared to the value of *P. caribensis* MWAP64^T.

3.3 Biofilm formation assay in the presence of Ni

Biofilm formation by the three species studied in the presence of different Ni concentrations and their variation with time are presented in Fig. 2. At 24 h increase of Ni concentration decreased biofilm formation for all the strains then, biofilm formation increased with time particularly for *C. novacaledonica* STM10272^T. At 48 h and 72h biofilm formation occurred until 2.5 mM of Ni initial concentration for *P. ultramafica* STM10279^T and until 1 mM of Ni initial concentration for *C. novacaledonica* STM10272^T and *P. caribensis* MWAP64^T. The amount of biofilm formed by *C. novacaledonica* STM10272^T was significantly higher than those formed by *P. ultramafica* STM10279^T and *P. caribensis* MWAP64^T. The differences found ranged from 1.8 times, 2.6 times and 3 times higher at respectively 0, 0.5 and 1 mM initial Ni concentration at 48 h and 72 h, in comparison to the control. At 48 h and 72 h initial Ni addition did not influence biofilm formation by *C. novacaledonica* STM10272^T until 2.5 mM. At the opposite, biofilm formation by *P. ultramafica* STM10279^T and *P. caribensis* MWAP64^T decreased with the increase of Ni concentration until 2.5 mM and 1 mM of Ni for *P. ultramafica* STM10279^T and *P. caribensis* MWAP64^T respectively.

3.4 *Quantification of biofilm production*

The biovolume, thickness and substratum coverage of the biofilms produced by the three species studied are presented in Fig. 3. The biovolume, the thickness average and the substratum coverage of the biofilm produced by *P. ultramafica* STM10279^T and *C. novacaledonica* STM10272^T were significantly higher compared to the biofilm of *P. caribensis* MWAP64^T. Moreover, the addition of 1 mM of Ni to the medium has significantly reduced the substratum coverage by *P. caribensis* MWAP64^T biofilm from 60% to 39%. For *C. novacaledonica* STM10272^T, the addition of Ni has significantly enhanced the thickness average of the biofilm from 21 µm to 26 µm.

3.5 *EPS production and its gross chemical composition*

Under the experimental conditions used in this study, *P caribensis* MWAP64^T was able to produce EPS at a production yield around 1.2 g L^{-1}. Regarding ultramafic species, only *P. ultramafica* STM10279^T was able to produce EPS at a production yield around 140 mg L^{-1}. FT–IR spectroscopy was employed to detect the main functional groups in the EPS produced by *P. ultramafica* STM10279^T. The FT–IR spectrum obtained (Fig. 4) showed a broad and large absorbance band centered at 3276 cm^{-1} that was assigned to hydroxyl groups or hydrogen bonds. It also showed two peaks at 2925 and 2868 cm^{-1} that were respectively assigned to CH-stretching of methylene (-CH_2) and methyl (-CH_3) groups. Several absorbance peaks between 1500 cm^{-1} and 1760 cm^{-1} that were assigned to the stretching modes of C=O bonds were also visible. The peak at 1719 cm^{-1} indicated the presence of carboxyl of uronic acid groups, the peak at 1248 cm^{-1} is assigned to the deformation vibration of C=O bonds and the peak at 1032 cm^{-1} is assigned to the stretching modes of C-O from intra-molecular C-O-C ring. This latter peak thus strongly suggested that the monosaccharides in the EPS have pyranose rings. In addition, the two weak peaks near 841cm^{-1} and 896 cm^{-1} could be respectively assigned to the two α- and β-ends of carbon-glucoside bonds. Finally, two additional peaks could be seen at 783 and 701 cm^{-1} but they could not be unambiguously assigned to specific molecular groups. The colorimetric test carried out (Supplemental Table 3) has shown that the ratio of carbohydrates to proteins (C/P) was almost 15, with values of 61% carbohydrates to 4% of proteins residues and the presence of uronic acid (17%).

3.6 *Effect of microbial inoculation in Tetraria comosa under ultramafic conditions*

Most significant data variations were observed for the contents of metal in plant tissue (Table 2). In shoot, no significant differences were measured between the inoculated plants and the control, but in roots, the three strains reduced metal contents even if no significant differences were observed with the inoculation of *C. novacaledonica* STM10272^T for Co and Mn contents compared with control. The values of the 4 elements were about two times lower in presence of *P. ultramafica* STM10279^T and *P. caribensis* MWAP64^T in comparison with control plants. The effect of *C. novacaledonica* STM10272^T on root metal contents was less pronounced but the differences with *P. ultramafica* STM10279^T were not significant. The inoculation of *T. comosa* plants with *P. ultramafica* STM10279^T and *P. caribensis* MWAP64^T did not induce significant changes in plant biomass (Supplemental Table 4) and main nutritional element contents in plant organs (Supplemental Table 5). However, the inoculation with *C. novacaledonica* STM10272^T significantly decreased the shoot and root biomasses compared to the other treatments (50% lower in average).

4 Discussion

In our experiments, *C. novacaledonica* STM10272^T and *P. ultramafica* STM10279^T showed a higher metal tolerance than *P. caribensis* MWAP64^T a non-ultramafic species isolated from Vertisol in Martinique [15]. These results are in agreement with previous studies, which have shown that ultramafic soils are known to hold bacterial communities more adapted to metal stress [4]. For instance, a set of *Burkholderia* s.l. strains isolated from Ni-rich ultramafic soil, tolerated up to 15 mM Ni, 10 mM Co and 5 mM Cr [35]. In New Caledonia, Stoppel and Schlegel (1995) [36] reported the occurrence of *Burkholderia* s.l. strains that could tolerate up to 30 mM Ni which is comparable to *P. ultramafica* STM10279^T Ni-IC_{50}.. Differences in profiles of metal tolerance were observed between the two ultramafic species and more particularly for Ni suggesting that different mechanisms are involved depending of the species.

Intracellular sequestration has already been reported as a mechanism of Ni tolerance for *Burkholderia* s.l. species [37]. However, our data indicated a preferential biosorption of Ni onto the cell surface of the three species studied. This result suggests that the tolerance mechanism used by these three species was to limit the movement of Ni across the cell envelope. *Burkholderia* s.l. species are gram-negative bacteria that exhibit a complex cell-membrane

291 structure composed of numerous phosphorylated biomolecules (i.e. phospholipids, lipoprotein, lipopolysaccharide)
292 located on the external membrane and involved in metal chelation [38]. In fact, the various components (proteins,
293 exopolysaccharides layers, extracellular capsules) of the surface of bacteria cells are known to provide non-specific
294 sorption capacity toward metals [39]. This non-specific binding capacity of the cell wall has been already
295 demonstrated to prevent the entry of nickel into the bacteria cell and thus to hinder its interference with essential
296 cellular components [7].

297 Biofilm formation is known to be involved in bacterial tolerance to Ni [17,40]. In our study, biofilm production
298 occurred until 1 mM for *C. novacaledonica* STM10272^T and *P. caribensis* MWAP64^T and until 2 mM for *P.*
299 *ultramafica* STM10279^T. It is not surprising that biofilm production was reduced at concentrations below the IC_{50}.
300 Indeed, Vega et al. (2014) [41] have shown that Ni inhibited the *Quorum Sensing* and biofilm formation without
301 affecting viability in *Burkholderia multivorans.* Confocal analysis clearly demonstrated the negative effect of nickel
302 on the formation of biofilm in *P. caribensis* MWAP64^T suggesting that another mechanism must be involved in the
303 metal tolerance. Until 1 mM of Ni, biofilm formation appeared to be an important mechanism for *C. novacaledonica*
304 STM10272^T in view of the large quantities of biofilm found and the enhancement of thickness average with the
305 addition of Ni. Since the concentration of bioavailable Ni frequently reaches 1 mM in New Caledonian ultramafic
306 soils, especially in the rhizosphere [22], we can conclude that Ni-concentration in these soils could induce biofilm
307 formation. These results corroborate those obtained by Perrin et al. (2009) [42] who showed *in vitro* biofilm
308 stimulation by nickel for *Escherichia coli*.

309 In order to go further into the mechanisms developed by the three *Burkholderia* s.l. species, we investigated their
310 ability to excrete EPS. Indeed, the high molecular sugar-based polymers that constitute these bacterial extracellular
311 substances have already been described to play a role in *Burkholderia* s.l. adaptation to different stress conditions
312 [43]. Bacterial EPS are known to play a significant role in the complexation of metals and subsequent decrease in
313 their mobility in soils [39]. Under our experimental conditions, *P. ultramafica* STM10279^T and *P. caribensis*
314 MWAP64^T were able to produce EPS. *P. caribensis* MWAP64^T is a well-known EPS-producing bacterium [15].
315 Structural analysis of the polysaccharide excreted by *P. caribensis* strain MWAP71, a strain belonging to the *P.*
316 *caribensis* cluster [15] has shown the presence of 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) [44]. Anionic groups
317 such as carbonyl, carboxyl and hydroxyl groups have been reported to scavenge several metals and to be involved in

318 metal biosorption [39,45]. Colorimetric assays showing that carbohydrates were the main component in the crude
319 EPS produced by *P. ultramafica* STM10279^T and the FT-IR peaks found suggest that the EPS of *P. ultramafica*
320 STM10279^T is a polysaccharide containing pyranose and uronic acid groups [46–48]. All the features on the FT-IR
321 spectrum of the EPS of *P. ultramafica* STM10279^T indicated that (i) carbohydrates were the main component in the
322 crude EPS produced by *P. ultramafica* STM10279^T and (ii) the EPS produced by *P. ultramafica* STM10279^T was a
323 polymer of a repeating unit presenting uronic acid residues. The pyranose and uronic acid groups that are expected to
324 constitute the polysaccharides are considered involved in the metal binding process. Such a behavior has already
325 been shown for *Rhizobium etli,* which was able to chelate Mn in these EPS [49].

326 The two ultramafic species clearly showed different metal tolerance patterns and mechanisms. *C. novacaledonica*
327 STM10272^T was found to develop a biofilm to protect from metals, whereas *P. ultramafica* STM10279^T excreted
328 EPS to sequester metals, although it was able to develop a biofilm. Finally, our results are in agreement with the
329 literature, as biofilm formation has already been reported for *Paraburkholderia* [50] and *Caballeronia* species [51],
330 while EPS production was only observed in the *Paraburkholderia* genus [15,52,53]. However, other mechanisms
331 may occur in metal tolerance of *P. ultramafica* STM10279^T and *C. novacaledonica* STM10272^T. Gonin et al. (2013)
332 [13] have shown that these bacteria possess cnr (Co–Ni resistance) and nre (Ni resistance) genes involved in metal
333 exclusion and active removal. Such mechanisms may operate in *C. novacaledonica* STM10272^T and in *P.*
334 *ultramafica* STM10279^T.

335 All these mechanisms put in place by bacteria to protect themselves can be also involved in the variations of
336 metal absorption by inoculated plants [8]. Alleviation of metal contents in plants by metal tolerant *Burkholderia* s.l.
337 have been reported in the literature. Madhaiyan et al. (2007) [54] showed a reduction of Ni uptake and translocation
338 in tomato after inoculation with a *Burkholderia* s.l. strain. These authors suggested that this reduction might be
339 attributed to the biosorption of Ni by the bacterial cells, which would have prevented the metal from being
340 transported into the plant. Kuffner et al. (2010) [55] reported that a *Burkholderia* s.l. strain had the capacity of
341 reducing Zn and Cd accumulation in the roots of *Salix caprea* by favoring the immobilization of these metals in the
342 soil. Our results on the effect of *P. ultramafica* STM10279^T and *C. novacaledonica* STM10272^T are in agreement
343 with these studies. However, the alleviation of metals observed in *T. comosa* were more pronounced with *P.*

344 *ultramafica* STM10279^T than *C. novacaledonica* STM10272^T, in possible relation to the differences of their metal
345 profile tolerance.

346 Although it is important to underline that an increase of the plant biomass is not the unique aspect of plant health
347 and adaptation [56], the lack of positive effect of bacterial inoculation on the biomass of *T. comosa* plants suggests
348 that metal alleviation had no influence on *T. comosa* plant growth. Other adaptation mechanisms inherent in the plant
349 may explain this result. Indeed, Gonin et al. (2013) [13] using sorghum, a plant non-native of ultramafic maquis,
350 reported a significant enhancement of plant biomass when inoculated by *P. ultramafica* STM10279^T. It must be
351 outlined that *Tetraria* species are considered as oligotrophic (with low nutrition requirements) and their low growth
352 rate is an adaptive trait to ultramafic constraints [57], which can also explain the absence of positive effect of
353 bacterial inoculation on their biomass production. Concerning the detrimental effect *of C. novacaledonica*
354 STM10272^T on plant biomass, it can be possibly explained by their ability to produce high level of biofilm. Although
355 biofilm plays a major role in the attachment of bacteria to plant roots, known biofilm-forming rhizosphere
356 *Burkholderia* s.l. are reported to inhibit root development [58].

357 **5 Conclusions**

358 The results of the present study clearly demonstrated that the two ultramafic bacterial species used different
359 mechanisms to cope with metal toxicity such as cell wall absorption, biofilm formation and EPS production. These
360 mechanisms can explain the decrease of metal contents in the plant and underlined the importance to consider the
361 plant/rhizobacteria interaction in ultramafic soils in order to understand the mechanisms occurred in plant adaptation
362 to the extreme edaphic constraints. Although no improvement in plant growth could be noticed, the ability of *P.*
363 *ultramafica* STM10279^T to produce an EPS can create a more hospitable environment for other organisms that will
364 promote plant growth. It will then be interesting to experiment the co-inoculation of the studied bacteria with
365 arbuscular mycorrhizal fungi, to combine effects on metal alleviation in plant organs and improvement of plant
366 mineral nutrition.

367 **Disclosure statement**

368 Declaration of interest: none.

369 **Acknowledgments**

The authors thank J.L. Duprey and L. Jamet from the LAMA laboratory (LAMA-US IMAGO-IRD, New Caledonia) for chemical analyses and E. Chalkiadakis from BIOTECAL Company for the technical support. This work was supported by the Grand Observatoire du Pacifique Sud (ARBRE project); Province Sud of New Caledonia (Prix d' Encouragement à la Recherche: Thesis Grant and Vivaldi program: Master internship allowance).

Appendix A. Supplementary data

References

[1] R.R. Brooks, Serpentine and its vegetation: a multidisciplinary approach, Dioscoride, Portland, OR, 1987. http://refhub.elsevier.com/S0723-2020(16)00044-8/sbref0270.

[2] E. Kazakou, P.G. Dimitrakopoulos, A.J.M. Baker, R.D. Reeves, A.Y. Troumbis, Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level, Biol. Rev. (2008) 495–508. doi:10.1111/j.1469-185X.2008.00051.x.

[3] M. Rajkumar, N. Ae, H. Freitas, Endophytic bacteria and their potential to enhance heavy metal phytoextraction, Chemosphere. 77 (2009) 153–160. doi:10.1016/j.chemosphere.2009.06.047.

[4] A. Mengoni, H. Schat, J. Vangronsveld, Plants as extreme environments? Ni-resistant bacteria and Ni-hyperaccumulators of serpentine flora, Plant Soil. 331 (2010) 5–16. doi:10.1007/s11104-009-0242-4.

[5] A. Pal, G. Wauters, a. K. Paul, Nickel tolerance and accumulation by bacteria from rhizosphere of nickel hyperaccumulators in serpentine soil ecosystem of Andaman, India, Plant Soil. 293 (2007) 37–48. doi:10.1007/s11104-007-9195-7.

[6] L. Guentas, S. Gensous, Y. Cavaloc, M. Ducousso, H. Amir, B. De Georges de Ledenon, L. Moulin, P. Jourand, *Burkholderia novacaledonica* sp. nov. and *B. ultramafica* sp. nov. isolated from roots of *Costularia* spp. pioneer plants of ultramafic soils in New Caledonia, Syst. Appl. Microbiol. 39 (2016) 151–159. doi:10.1016/j.syapm.2016.03.008.

[7] M.R. Bruins, S. Kapil, F.W. Oehme, Microbial resistance to metals in the environment, Ecotoxicol. Environ. Saf. 45 (2000) 198–207. doi:10.1006/eesa.1999.1860.

[8] H. Etesami, Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: Mechanisms and future prospects, Ecotoxicol. Environ. Saf. 147 (2018) 175–191. doi:10.1016/j.ecoenv.2017.08.032.

[9] S. Isnard, L. L'Huillier , F. Rigault, T. Jaffre, How did the ultramafic soils shape the flora of the New Caledonian hotpsot?, Plant Soi.l 403 (2016) 53-76. doi :10.1007/s11104-016-2910-5.

[10] N. Myers, Biodiversity hotspots revisited, Bioscience. 53 (2003) 916–917. doi:10.1641/0006-3568(2003)053[0916:BHR]2.0.CO;2.

[11] L. Bordez, P. Jourand, M. Ducousso, F. Carriconde, Y. Cavaloc, S. Santini, J.M. Claverie, L. Wantiez, A. Leveau, H. Amir, Distribution patterns of microbial communities in ultramafic landscape: a metagenetic approach highlights the strong relationships between diversity and environmental traits, Mol. Ecol. 25 (2016) 2258–2272. doi:10.1111/mec.13621.

[12] H. Amir, M. Ducousso, Les bactéries et les champignons du sol sur roches ultramafiques, in: L. L'Huillier, T. Jaffré, A. Wulff (Eds.), Mines Environ. En Nouv. Les Milieux Sur Substrats Ultramafiques Leur Restaur., IAC Ed, Noumea, 2010: pp. 129–145. http://www.documentation.ird.fr/hor/fdi:010057561.

[13] M. Gonin, S. Gensous, A. Lagrange, M. Ducousso, H. Amir, P. Jourand, Rhizosphere bacteria of *Costularia* spp. from ultramafic soils in New Caledonia: diversity, tolerance to extreme edaphic conditions, and role in plant growth and mineral nutrition, Can. J. Microbiol. 59 (2013) 164–174. doi:10.1139/cjm-2012-0570.

[14] A.P. Dobritsa, M. Samadpour, Reclassification of *Burkholderia insecticola* as *Caballeronia insecticola* comb. nov. and reliability of conserved signature indels as molecular synapomorphies, Int. J. Syst. Evol. Microbiol. (2019) 1–7. doi:10.1099/ijsem.0.003431.

[15] W. Achouak, R. Christen, M. Barakat, M.-H. Martel, T. Heulin, *Burkholderia caribensis* sp. nov., an exopolysaccharide-producing bacterium isolated from vertisol microaggregates in Martinique, Int. J. Syst. Bacteriol. 49 (1999) 787–794. doi:10.1099/00207713-49-2-787.

[16] A. Wulff, L. L'Huillier, C. Véa, T. Jaffré, Espèces indigènes utilisables en revégétalisation, in: L. L'Huillier, T. Jaffré, A. Wulff (Eds.), Mines Environ. En Nouv. Les Milieux Sur Substrats Ultramafiques Leur Restaur.,

IAC Ed, Noumea, 2010: pp. 231–344. http://www.documentation.ird.fr/hor/fdi:010057563.

[17] C.C. Chien, B.C. Lin, C.H. Wu, Biofilm formation and heavy metal resistance by an environmental *Pseudomonas* sp., Biochem. Eng. J. 78 (2013) 132–137. doi:10.1016/j.bej.2013.01.014.

[18] J. Hartley, J.W.G. Cairney, A.A. Meharg, Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment?, Plant Soil. 189 (1997) 303–319. doi:10.1023/A:1004255006170.

[19] P. Giovanella, L. Cabral, A.P. Costa, F.A. de Oliveira Camargo, C. Gianello, F.M. Bento, Metal resistance mechanisms in Gram-negative bacteria and their potential to remove Hg in the presence of other metals, Ecotoxicol. Environ. Saf. 140 (2017) 162–169. doi:10.1016/j.ecoenv.2017.02.010.

[20] G.A. O'Toole, Microtiter Dish Biofilm Formation Assay, J. Vis. Exp. (2011) 10–11. doi:10.3791/2437.

[21] F. Brian-Jaisson, Identification et caractérisation des exopolymères de biofilms de bactéries marines, Université de Toulon, 2014.

[22] H. Amir, R. Pineau, Relationships between extractable Ni, Co, and other metals and some microbiological characteristics of different ultramafic soils from New Caledonia, Aust. J. Soil Res. 41 (2003) 215. doi:10.1071/SR02040.

[23] A. Heydorn, A.T. Nielsen, M. Hentzer, C. Sternberg, M. Givskov, B.K. Ersbøll, S. Molin, Quantification of biofilm structures by the novel computer program comstat, Microbiology. 146 (2000) 2395–2407. doi:10.1099/00221287-146-10-2395.

[24] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent., J. Biol. Chem. 193 (1951) 265–75. http://devbio.wustl.edu/InfoSource/ISPDFs/Lowry 1951.pdf.

[25] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Measurement of protein using bicinchoninic acid, Anal. Biochem. 150 (1985) 76–85. doi:10.1016/0003-2697(85)90442-7.

[26] R.E. Brown, K.L. Jarvis, K.J. Hyland, Protein measurement using bicinchoninic acid: elimination of interfering substances, Anal. Biochem. 180 (1989) 136–139. doi:10.1016/0003-2697(89)90101-2.

443 [27] M. DuBois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of
444 sugars and related substances, Anal. Chem. 28 (1956) 350–356. doi:10.1021/ac60111a017.

445 [28] M.F. Chaplin, Monosaccharides, in: M.F. Chaplin, J.F. Kennedy (Eds.), Carbohydr. Anal. A Pract.
446 Approach, IRL Press, Oxford, UK, 1986: pp. 1–36.

447 [29] N. Blumenkrantz, G. Asboe-Hansen, New method for quantitative determination of uronic acids, Anal.
448 Biochem. 54 (1973) 484–489. doi:10.1016/0003-2697(73)90377-1.

449 [30] T.M.C.C. Filisetti-Cozzi, N.C. Carpita, Measurement of uronic acids without interference from neutral
450 sugars, Anal. Biochem. 197 (1991) 157–162. doi:10.1016/0003-2697(91)90372-Z.

451 [31] M. Larcher, B. Muller, S. Mantelin, S. Rapior, J.-C. Cleyet-Marel, Early modifications of *Brassica napus*
452 root system architecture induced by a plant growth-promoting *Phyllobacterium* strain, New Phytol. 160
453 (2003) 119–125. doi:10.1046/j.1469-8137.2003.00862.x.

454 [32] A. Lagrange, L. L'Huillier, H. Amir, Mycorrhizal status of Cyperaceae from New Caledonian ultramafic
455 soils: Effects of phosphorus availability on arbuscular mycorrhizal colonization of *Costularia comosa* under
456 field conditions, Mycorrhiza. 23 (2013) 655–661. doi:10.1007/s00572-013-0503-1.

457 [33] A. Lagrange, M. Ducousso, P. Jourand, C. Majorel, H. Amir, New insights into the mycorrhizal status of
458 Cyperaceae from ultramafic soils in New Caledonia, Can. J. Microbiol. 57 (2011) 21–28. doi:10.1139/W10-
459 096.

460 [34] R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical
461 Computing, Vienna, Austria. URL https://www.R-project.org/.

462 [35] R.A.I. Abou-Shanab, P. van Berkum, J.S. Angle, Heavy metal resistance and genotypic analysis of metal
463 resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the
464 rhizosphere of *Alyssum murale*, Chemosphere. 68 (2007) 360–367. doi:10.1016/j.chemosphere.2006.12.051.

465 [36] R.D. Stoppel, H.G. Schlegel, Nickel-resistant bacteria from anthropogenically nickel-polluted and naturally
466 nickel-percolated ecosystems, Appl. Environ. Microbiol. 61 (1995) 2276–2285.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1388466.

[37] M.N. Dourado, M.R. Franco, L.P. Peters, P.F. Martins, L.A. Souza, F.A. Piotto, R.A. Azevedo, Antioxidant enzymes activities of *Burkholderia* spp. strains—oxidative responses to Ni toxicity, Environ. Sci. Pollut. Res. 22 (2015) 19922–19932. doi:10.1007/s11356-015-5204-1.

[38] A. Sessitsch, M. Kuffner, P. Kidd, J. Vangronsveld, W.W. Wenzel, K. Fallmann, M. Puschenreiter, The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils, Soil Biol. Biochem. 60 (2013) 182–194. doi:10.1016/j.soilbio.2013.01.012.

[39] P. Gupta, B. Diwan, Bacterial exopolysaccharide mediated heavy metal removal: A Review on biosynthesis, mechanism and remediation strategies, Biotechnol. Reports. 13 (2017) 58–71. doi:10.1016/j.btre.2016.12.006.

[40] S. Koechler, J. Farasin, J. Cleiss-Arnold, F. Arsène-Ploetze, Toxic metal resistance in biofilms: diversity of microbial responses and their evolution, Res. Microbiol. 166 (2015) 764–773. doi:10.1016/j.resmic.2015.03.008.

[41] L.M. Vega, J. Mathieu, Y. Yang, B.H. Pyle, R.J.C. McLean, P.J.J. Alvarez, Nickel and cadmium ions inhibit quorum sensing and biofilm formation without affecting viability in *Burkholderia multivorans*, Int. Biodeterior. Biodegrad. 91 (2014) 82–87. doi:10.1016/j.ibiod.2014.03.013.

[42] C. Perrin, R. Briandet, G. Jubelin, P. Lejeune, M.-A. Mandrand-Berthelot, A. Rodrigue, C. Dorel, Nickel promotes biofilm formation by *Escherichia coli* K-12 strains that produce curli, Appl. Environ. Microbiol. 75 (2009) 1723–1733. doi:10.1128/AEM.02171-08.

[43] A.S. Ferreira, Insights into the role of extracellular polysaccharides in *Burkholderia* adaptation to different environments, Front. Cell. Infect. Microbiol. 1 (2011) 1–9. doi:10.3389/fcimb.2011.00016.

[44] C. Vanhaverbeke, A. Heyraud, W. Achouak, T. Heulin, Structural analysis of the exopolysaccharide from *Burkholderia caribensis* strain MWAP71, Carbohydr. Res. 334 (2001) 127–133. doi:10.1016/S0008-6215(01)00176-8.

491 [45] P.M. Joshi, A.A. Juwarkar, In vivo studies to elucidate the role of extracellular polymeric substances from
492 *Azotobacter* in immobilization of heavy metals, Environ. Sci. Technol. 43 (2009) 5884–5889.
493 doi:10.1021/es900063b.

494 [46] R. Sun, J. M. Fang, A. Goodwin, J. M. Lawther, A. J. Bolton, Fractionation and characterization of
495 polysaccharides from abaca fibre, Carbohydr. Polym. 37 (1998) 351–359. doi:10.1016/S0144-
496 8617(98)00046-0.

497 [47] C. Su, X. Xu, D. Liu, M. Wu, F. Zeng, M. Zeng, W. Wei, N. Jiang, X. Luo, Isolation and characterization of
498 exopolysaccharide with immunomodulatory activity from fermentation broth of *Morchella conica*, DARU J.
499 Pharm. Sci. 21 (2013) 5. doi:10.1186/2008-2231-21-5.

500 [48] X. Liu, Y. Huang, Y. Chen, Y. Cao, Partial structural characterization, as well as immunomodulatory and
501 anti-aging activities of CP2-c2-s2 polysaccharide from *Cordyceps militaris*, RSC Adv. 6 (2016) 104094–
502 104103. doi:10.1039/C6RA23612J.

503 [49] W. Pulsawat, N. Leksawasdi, P.L. Rogers, L.J.R. Foster, Anions effects on biosorption of Mn(II) by
504 extracellular polymeric substance (EPS) from *Rhizobium etli*, Biotechnol. Lett. 25 (2003) 1267–1270.
505 doi:10.1023/A:1025083116343.

506 [50] A. Zúñiga, R.A. Donoso, D. Ruiz, G.A. Ruz, B. González, Quorum-sensing systems in the plant growth-
507 promoting bacterium *Paraburkholderia phytofirmans* PsJN exhibit cross-regulation and are involved in
508 biofilm formation, Mol. Plant-Microbe Interact. 30 (2017) 557–565. doi:10.1094/MPMI-01-17-0008-R.

509 [51] S. Yoshida, N. Ogawa, T. Fujii, S. Tsushima, Enhanced biofilm formation and 3-chlorobenzoate degrading
510 activity by the bacterial consortium of *Burkholderia* sp. NK8 and *Pseudomonas aeruginosa* PAO1, J. Appl.
511 Microbiol. 106 (2009) 790–800. doi:10.1111/j.1365-2672.2008.04027.x.

512 [52] R. V Serrato, G.L. Sassaki, L.M. Cruz, F.O. Pedrosa, P.A.. Gorin, M. Iacomini, Culture conditions for the
513 production of an acidic exopolysaccharide by the nitrogen-fixing bacterium *Burkholderia tropica*, Can. J.
514 Microbiol. 52 (2006) 489–493. doi:10.1139/w05-155.

515 [53] A. Silipo, T. Ierano, R. Lanzetta, A. Molinaro, M. Parrilli, The structure of the O-Chain polysaccharide from

the Gram-negative endophytic bacterium *Burkholderia phytofirmans* strain PsJN, European J. Org. Chem. 2008 (2008) 2303–2308. doi:10.1002/ejoc.200800135.

[54] M. Madhaiyan, S. Poonguzhali, T. Sa, Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.), Chemosphere. 69 (2007) 220–228. doi:10.1016/j.chemosphere.2007.04.017.

[55] M. Kuffner, S. De Maria, M. Puschenreiter, K. Fallmann, G. Wieshammer, M. Gorfer, J. Strauss, A.R. Rivelli, A. Sessitsch, Culturable bacteria from Zn- and Cd-accumulating *Salix caprea* with differential effects on plant growth and heavy metal availability, J. Appl. Microbiol. 108 (2010) 1471–1484. doi:10.1111/j.1365-2672.2010.04670.x.

[56] S.E. Smith, F.A. Smith, Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth, Mycologia. 104 (2012) 1–13. doi:10.3852/11-229.

[57] T. Jaffré, L. L'Huillier, La végétation des roches ultramafiques ou terrains miniers, in: L. L'Huillier, T. Jaffré, A. Wulff (Eds.), Mines Environ. En Nouv. Les Milieux Sur Substrats Ultramafiques Leur Restaur., IAC Ed, Noumea, 2010: pp. 45–103.

[58] A. a Angus, A.M. Hirsch, Biofilm formation in the rhizosphere: multispecies interactions and implications for plant growth, in: F. J (Ed.), Mol. Microb. Ecol. Rhizosph., John Wiley, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2013: pp. 701–712. doi:10.1002/9781118297674.ch66.

Fig. 1.

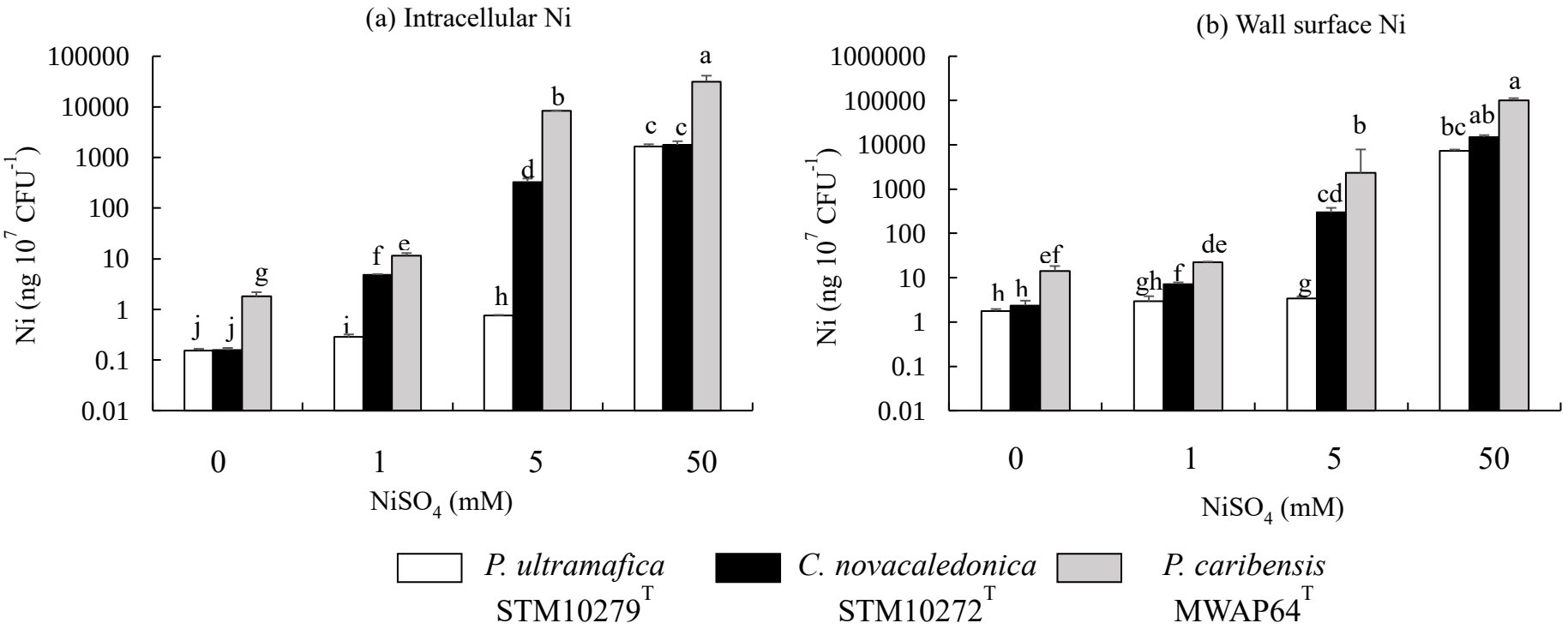

(a) Intracellular Ni
Ni (ng 10^7 CFU^-1)
100000
10000
1000
100
10
1
0.1
0.01
j j g
i f e
h d b
c c a
0
1
5
50
NiSO4 (mM)
(b) Wall surface Ni
Ni (ng 10^7 CFU^-1)
1000000
100000
10000
1000
100
10
1
0.1
0.01
h h ef
gh f de
g cd b
bc ab a
0
1
5
50
NiSO4 (mM)
P. ultramafica STM10279T
C. novacaledonica STM10272T
P. caribensis MWAP64T

Fig. 2.

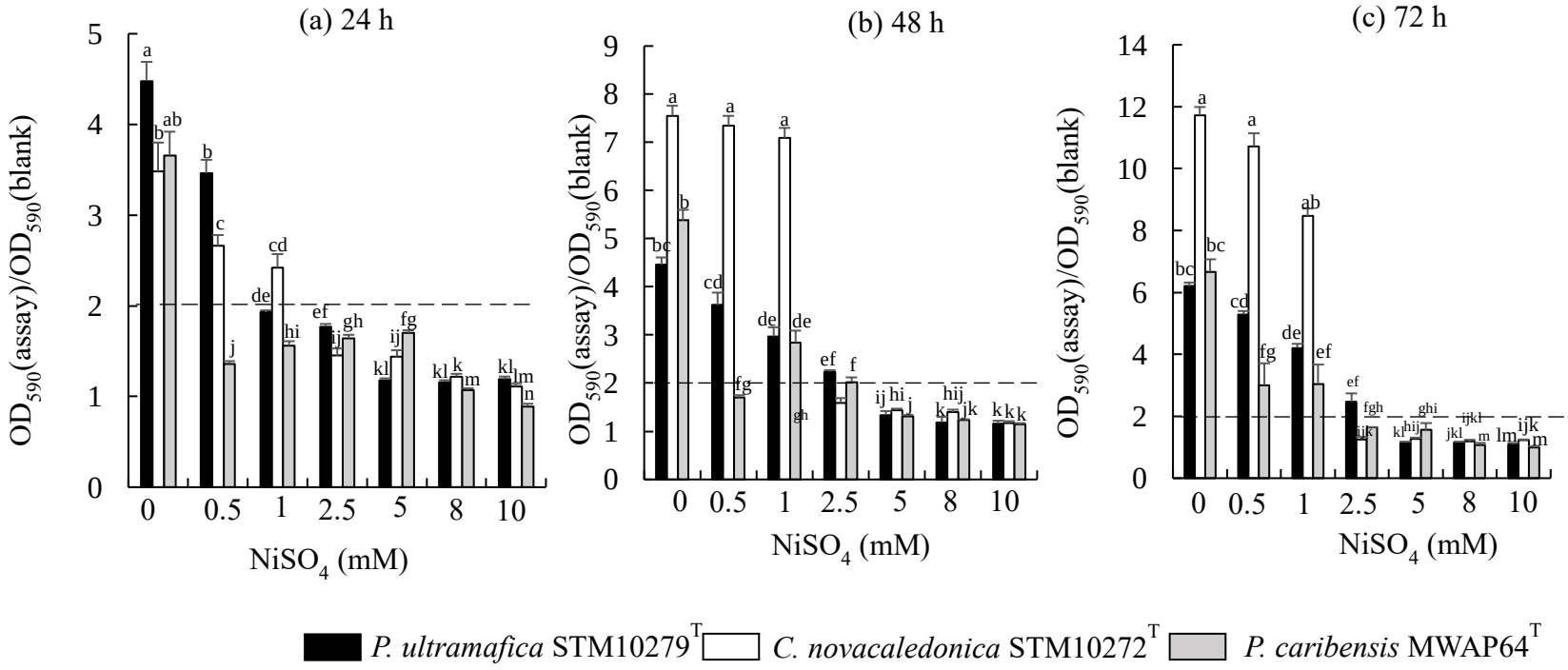

(a) 24 h
(b) 48 h
(c) 72 h
OD_{590}(assay)/OD_{590}(blank)
$NiSO_4$ (mM)
0 0.5 1 2.5 5 8 10
P. ultramafica STM10279T
C. novacaledonica STM10272T
P. caribensis MWAP64T

Fig. 3.

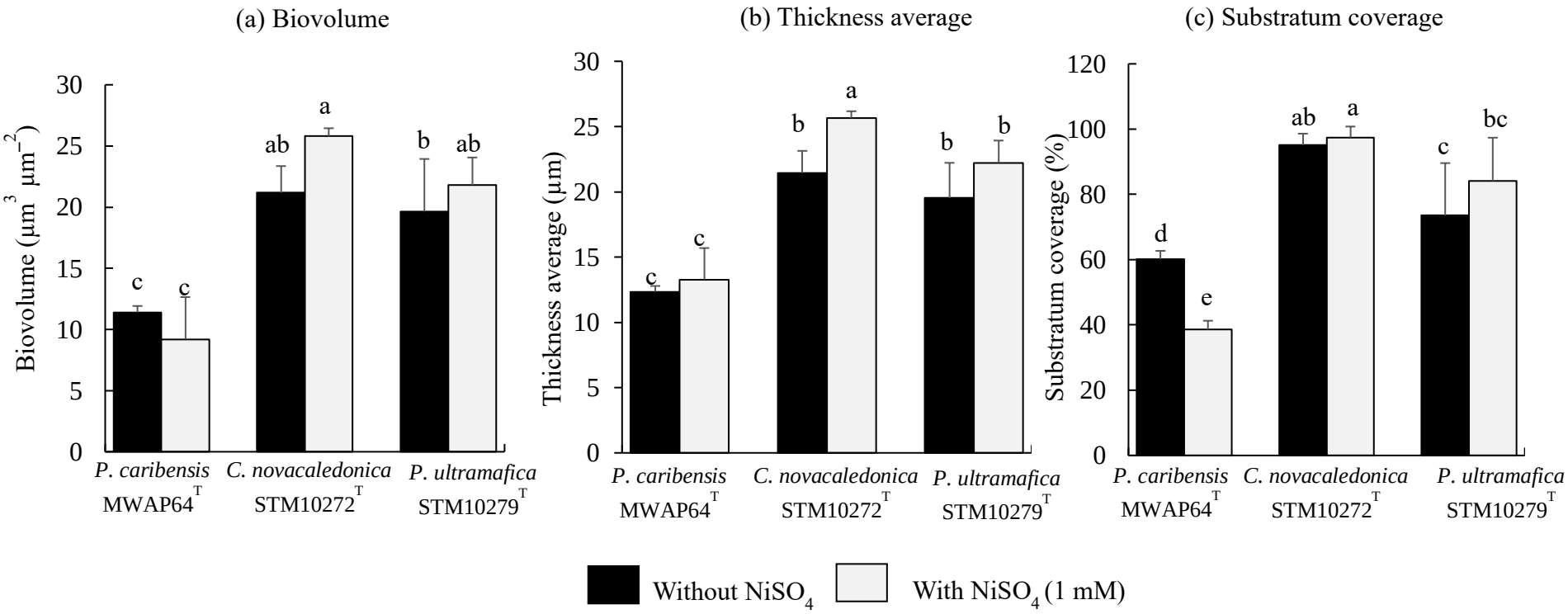

(a) Biovolume
Biovolume (µm³ µm⁻²)
30
25
20
15
10
5
0
c
c
ab
a
b
ab
P. caribensis MWAP64T
C. novacaledonica STM10272T
P. ultramafica STM10279T
(b) Thickness average
Thickness average (µm)
30
25
20
15
10
5
0
c
c
b
a
b
b
P. caribensis MWAP64T
C. novacaledonica STM10272T
P. ultramafica STM10279T
(c) Substratum coverage
Substratum coverage (%)
120
100
80
60
40
20
0
d
e
ab
a
c
bc
P. caribensis MWAP64T
C. novacaledonica STM10272T
P. ultramafica STM10279T
Without NiSO4
With NiSO4 (1 mM)

Fig. 4.

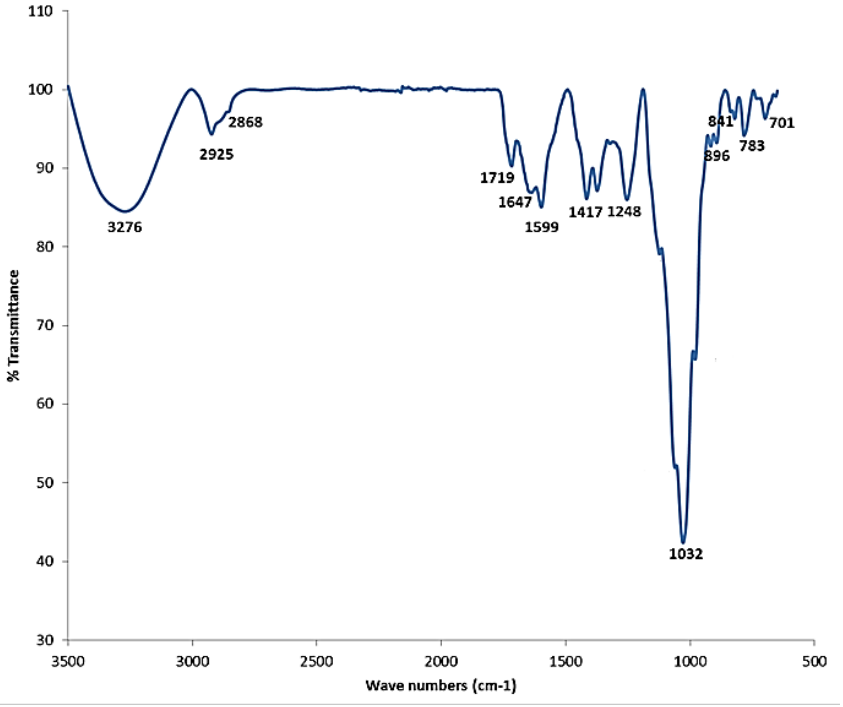

% Transmittance
Wave numbers (cm-1)
110
100
90
80
70
60
50
40
30
3500
3000
2500
2000
1500
1000
500
3276
2925
2868
1719
1647
1599
1417
1248
1032
896
841
783
701

Figure Captions

Fig. 1. Intracellular Ni (a) and wall surface Ni (b) in *Paraburkholderia ultramafica* STM10279^T, *Caballeronia novacaledonica* STM10272^T and *Paraburkholderia caribensis* MWAP64^T as a function of the Ni concentration in the growing medium. Bars represent means, and error bars represent standard errors (3 replicates). Value with the same lower case letters are not significantly different at $P < 0.05$ using Fisher's LSD test.

Fig. 2. Formation of biofilm by *Paraburkholderia ultramafica* STM10279^T, *Caballeronia novacaledonica* STM10272^T and *Paraburkholderia caribensis* MWAP64^T in response to nickel stress and as a function of the exposure time 24 h (a), 48 h (b) and 72 h (c). Bars represent means, and error bars represent standard errors (6 replicates). Biofilm production occurs when the ratio is greater than two (dotted line on the graphic). Value with the same lower case letters are not significantly different at $P < 0.05$ using Fisher's LSD test.

Fig. 3. Biovolume (a), Thickness average (b) and substratum coverage (c) for *Paraburkholderia caribensis* MWAP64^T, *Caballeronia novacaledonica* STM10272^T and *Paraburkholderia ultramafica* STM10279^T with and without nickel in the growing medium. Bars represent means, and error bars represent standard errors (6 replicates). Value with the same lower case letters are not significantly different at $P < 0.05$ using Tukey's HSD test or Fisher's LSD test when data did not follow the assumptions of a parametric test.

Fig. 4. Fourier Transform Infrared (FT-IR) spectrum of the *Paraburkholderia ultramafica* STM10279^T EPS in the 500-3500 cm^{-1} range showing absorbance bands or peaks characteristic of polysaccharides.

Table 1 Metals IC_{50} (mM) for *Paraburkholderia ultramafica* STM10279^T, *Caballeronia novacaledonica* STM10272^T and *Paraburkholderia caribensis* MWAP64^T (Mean ± Standard Error, n = 6).

Species	IC_{50} (mM) Ni	Co	Cr	Mn
P. caribensis MWAP64^T	1.9 ± 0.9c	1.1 ± 0.1b	0.2 ± 0.1b	11.1 ± 0.9b
P. ultramafica STM10279^T	30.7 ± 2.8a	3.9 ± 0.9a	0.5 ± 0.1b	32.4 ± 1.6a
C. novacaledonica STM10272^T	4.8 ± 0.5b	5.8 ± 0.9a	1.5 ± 0.3a	29.1 ± 5.7a

Value with the same lower case letters within a column are not significantly different at $P < 0.05$ using Fisher's LSD test.

Table 2 Metals contents in the shoots and roots of *Tetraria comosa* as a function of various treatments (mean ± SE n = 3).

Plant part	Treatment	Ni (mg Kg^{-1})	Co (mg Kg^{-1})	Cr (mg Kg^{-1})	Mn (mg Kg^{-1})
Shoot	Control	132 ± 18a	13.5 ± 1.8a	191 ± 29ab	415 ± 29a
	P. caribensis MWAP64^T	159 ± 18a	11.2 ± 1.0a	242 ± 12a	380 ± 9a
	P. ultramafica STM10279^T	155 ± 12a	9.4 ± 1.0a	191 ± 13b	388 ± 19a
	C. novacaledonica STM10272^T	154 ± 10a	15.2 ± 0.5a	227 ± 16ab	468 ± 7a
Root	Control	1377 ± 111a	284 ± 26a	2168 ± 29a	3351 ± 232a
	P. caribensis MWAP64^T	582 ± 54c	126 ± 13b	811 ± 12b	1806 ± 150c
	P. ultramafica STM10279^T	766 ± 68bc	155 ± 13b	1158 ± 13b	2097 ± 159b
	C. novacaledonica STM10272^T	966 ± 81b	206 ± 19ab	1414 ± 16b	2698 ± 165ab

Value with the same lower case letters within a column for each plant part are not significantly different at $P < 0.05$ using Tukey's HSD test or Fisher's LSD test when data did not follow the assumptions of a parametric test.