

Annals of Microbiology

Characterization of As(III) oxidizing *Achromobacter* sp. strain N2: effects on arsenic toxicity and translocation in rice

--Manuscript Draft--

Manuscript Number:	ANMI-D-17-01021R1
Full Title:	Characterization of As(III) oxidizing <i>Achromobacter</i> sp. strain N2: effects on arsenic toxicity and translocation in rice
Article Type:	Original Articles
Corresponding Author:	Lucia Cavalca, Ph.D. Università degli Studi di Milano Milano, ITALY
Order of Authors:	Anna Corsini Milena Colombo Claudio Gardana Sarah Zecchin Paolo Simonetti Lucia Cavalca, Ph.D.
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Università degli Studi di Milano
Corresponding Author's Secondary Institution:	
First Author:	Anna Corsini
First Author Secondary Information:	
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	<p><i>Achromobacter</i> sp. strain N2 was isolated from a pyrite-cinder-contaminated soil and presented plant-growth promoting traits (ACC deaminase activity, production of indole-3-acetic and jasmonic acids, siderophores secretion and phosphate solubilization) and arsenic transformation abilities. <i>Achromobacter</i> sp. strain N2 was resistant to different metals and metalloids, including arsenate (100 mM) and arsenite (5 mM). The strain was resistant to ionic stressors (i.e. arsenate and NaCl), whereas bacterial growth was impaired by osmotic stress. Strain N2 was able to oxidize 1.0 mmol L⁻¹ of arsenite to arsenate in 72 h. This evidence was supported by the retrieval of an arsenite oxidase AioA gene highly homologous to arsenite oxidases of <i>Achromobacter</i> and <i>Alcaligenes</i> species.</p> <p>Rice seeds of <i>Oryza sativa</i> (var. Loto) were bio-primed with ACCD-induced and non-induced cells in order to evaluate the effect of inoculation on rice seedlings growth and arsenic uptake. The bacterization with ACCD-induced cells significantly improved seed germination and seedling heights if compared with the seeds inoculated with non-induced cells and non-primed seeds. Enhanced arsenic uptake was evidenced in the presence of ACCD-induced cells, suggesting a role of ACCD activity on the mitigation of the toxicity of arsenic accumulated by the plant. This kind of responses should be taken into account when proposing PGP strains for improving plant growth in arsenic-rich soils.</p>
Response to Reviewers:	See attachment.

[Click here to view linked References](#)

1 **Characterization of As(III) oxidizing *Achromobacter* sp. strain N2: effects on arsenic toxicity and**
2 **translocation in rice**

3 Anna Corsini, Milena Colombo, Claudio Gardana, Sarah Zecchin, Paolo Simonetti, Lucia Cavalca

4

5
6
7
8
9 5 Università degli Studi di Milano - Department of Food, Environmental and Nutritional Sciences - DeFENS, -

10
11 6 Via Celoria 2, 20133 Milan, Italy

12
13
14 **7 Corresponding author:**

15
16 8 Lucia Cavalca

17
18 9 Università degli Studi di Milano - Department of Food, Environmental and Nutritional Sciences - DeFENS, -

19
20 10 Via Celoria 2, 20133 Milan, Italy

21
22 11 E-mail: lucia.cavalca@unimi.it

23
24 12 Phone: +39 02 50319116; Fax: +39 02 50319238

25
26
27
28
29 **13 ORCID numbers**

30
31 14 Anna Corsini: 0000-0002-4925-8200

32
33 15 Milena Colombo: 0000-0002-5730-7551

34
35
36 16

37
38 17 Claudio Gardana: 0000-0001-8722-4020

39
40 18 Sarah Zecchin: 0000-0003-2851-1702

41
42 19 Paolo Simonetti: 0000-0001-8092-5537

43
44 20

45
46 21 Lucia Cavalca: 0000-0002-3512-1705

47
48 **22 Acknowledgements**

49
50 23 The research was supported by Ministry of University and Research program PRIN (2010JBNLJ7-004).

51
52
53 24

25 **Abstract**

1
2 26
3
4 27 *Achromobacter* sp. strain N2 was isolated from a pyrite-cinder-contaminated soil and presented plant-growth
5
6 28 promoting traits (ACC deaminase activity, production of indole-3-acetic and jasmonic acids, siderophores
7
8 29 secretion and phosphate solubilization) and arsenic transformation abilities. *Achromobacter* sp. strain N2 was
9
10 30 resistant to different metals and metalloids, including arsenate (100 mM) and arsenite (5 mM). The strain was
11
12 31 resistant to ionic stressors (i.e. arsenate and NaCl), whereas bacterial growth was impaired by osmotic stress.
13
14 32 Strain N2 was able to oxidize 1.0 mmol L⁻¹ of arsenite to arsenate in 72 h. This evidence was supported by the
15
16 33 retrieval of an arsenite oxidase *AioA* gene highly homologous to arsenite oxidases of *Achromobacter* and
17
18 34 *Alcaligenes* species.
19
20 35 Rice seeds of *Oryza sativa* (var. Loto) were bio-primed with ACCD-induced and non-induced cells in order to
21
22 36 evaluate the effect of inoculation on rice seedlings growth and arsenic uptake. The bacterization with ACCD-
23
24 37 induced cells significantly improved seed germination and seedling heights if compared with the seeds
25
26 38 inoculated with non-induced cells and non-primed seeds. Enhanced arsenic uptake was evidenced in the presence
27
28 39 of ACCD-induced cells, suggesting a role of ACCD activity on the mitigation of the toxicity of arsenic
29
30 40 accumulated by the plant. This kind of responses should be taken into account when proposing PGP strains for
31
32 41 improving plant growth in arsenic-rich soils.
33

34 42
35
36 43 **Keywords**

37
38 44 Arsenic; Arsenite oxidase; Plant growth promotion; ACC deaminase; Rice.
39
40 45
41

42 46 **Introduction**

43
44
45 47 Environmental stresses such as contaminants, drought and salinity are some of the limiting factors in crop
46
47 48 production due to their effect on plant functioning. Soil bacteria having plant growth promoting (PGP)
48
49 49 characteristics might be envisaged as enhancers for plant resistance to abiotic and biotic (phytopathogens)
50
51 50 stresses. Besides biological nitrogen fixation, important direct PGP mechanisms are: release of bacterial
52
53 51 siderophores that supply iron or phosphate to the plant (Burd et al., 2000; Abou-Shanab et al., 2003; Madhaiyan
54
55 52 et al., 2004), synthesis of phytohormones, such as indole-3-acetic acid (IAA) and jasmonic acid (JA), and
56
57 53 degradation of stress-related molecules. The expression in many plant-associated bacteria of the enzyme 1-
58
59 54 aminocyclopropane-1-carboxylic acid deaminase (ACCD) leads to a direct stimulation of plant growth due to the
60
61 55 catabolism of molecules related to the stress hormone ethylene (Glick et al., 1998). Under metal-stress
62
63
64
65

56 conditions, IAA and ethylene are released resulting in an increased uptake of metal ions (Dell'Amico et al.,
1 57 2005). However, the knowledge on plants hormone production by bacteria under stress conditions is still very
2 58 limited.

59 In rice fields, arsenic (As) contamination represents a public health issue, due to cultivation under flooded
60 conditions with As contaminated water in many parts of the world (Zhao et al., 2010). Such agronomic scheme
61 increments As mobility in soil solution resulting in higher As concentration in rice grains with respect to dry rice
62 (Spanu et al., 2012). In such anoxic conditions the predominant As species is arsenite [As(III)], more mobile and
63 toxic than arsenate [As(V)]. Italy is the first rice producer in Europe. Here, the cultivation of rice under flooded
64 conditions in soils with natural As levels (i.e. tot As < 20 mg kgdw⁻¹) (Mandal et al., 2002) leads to an average
65 metalloid concentration in grains that exceeds the European limits (Commission regulation (EU) 2015/1006) of
66 100 µg kg⁻¹ for baby food production (Meharg et al., 2009). Bacteria play a crucial role in As geochemical
67 cycling through microbial transformation processes, including reduction, oxidation, and methylation (Cavalca et
68 al., 2013). Recently, the role of As(III) oxidizing rhizobacteria in lowering As content in rice plant and in the
69 relief of As toxicity has been revealed (Yang et al., 2015). The presence of As(III) oxidizing bacteria on rice
70 roots iron plaques has been found to be correlated with As content in the plant. Since As(V) is bound to iron
71 minerals, As(III) oxidizing bacteria were actively catalyzing As transformation and greatly influencing metal
72 uptake by rice (Hu et al., 2015).

73 In this context, the aim of this study was to characterize PGP traits and As transformation abilities of a
74 rhizosphere bacterial strain isolated from a pyrite-cinders contaminated soil and to evaluate the effect of As on
75 PGP characteristics. The influence of strain inoculation was evaluated in relation to the growth of rice seedling
76 and As sensitivity.

78 **Materials and methods**

80 *Bacterial strain N2*

81 The bacterial strain N2 was isolated from sunflower (*Helianthus annuus*, L.) rhizosphere grown in an As pyrite-
82 cinder polluted soil (Torviscosa, Italy). The strain was identified by 16S rRNA nucleotide sequence analysis and
83 preserved in glycerol stocks at -80°C. Prior to use, the strain was grown to mid exponential phase in 1/10 Tryptic
84 Soy Broth (TSB) medium at 30°C with shaking.

86 *Determination of plant growth promotion traits*

87 The isolate was qualitatively tested for its ability to produce 3-indoleacetic acid (IAA), abscisic acid (ABA) and
1 88 jasmonic acid (JA), growth on 1-aminocyclopropane-1-carboxylic (ACC) as the sole source of nitrogen,
2 89 siderophores secretion, phosphate mineralization, protein and chitin hydrolyzation, and as biocontrol agent.
3
4 90 IAA production was estimated according to Glickmann and Dessaux (1995). The strain was cultured for 4 days
5
6 91 at 30°C in flasks containing 20 mL of Dworkin and Foster (DF) mineral medium (Dworkin and Foster, 1958)
7
8 92 supplemented with 0.5 g L⁻¹ of L-tryptophan (Sigma-Aldrich). After incubation, 1 mL of cell suspension was
9
10 93 transferred into a tube and mixed vigorously with 2 mL of Salkowski's reagent and left at room temperature for
11
12 94 20 min. Development of pink colour indicated IAA production. JA and ABA production was determined by
13
14 95 Ultra performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS, Waters, Milford, MA)
15
16 96 after growing strain N2 for 72 h at 30°C in yeast mannitol medium (YEM).
17
18 97 The ability to use ACC as nitrogen source is a consequence of the enzymatic activity of ACCD. To test this trait,
19
20 98 strain N2 was cultured first in 1/10 TSB medium until mid-exponential phase and 5% (v/v) of inoculum was
21
22 99 transferred in DF medium containing 3.0 mM ACC (DF_ACC, Sigma–Aldrich) instead of (NH₄)₂SO₄ as N
23
24 100 source (Grichko et al., 2000). The culture was incubated at 30°C on a rotary shaker at 180 rpm for 48 h. The
25
26 101 ability to utilize ACC was verified by inoculating the strain in control tubes containing DF medium without any
27
28 102 N source, and incubating the tubes in the above-mentioned conditions for 10 days.
29
30 103 Siderophore secretion was determined as described by Schwyn and Neilands (1987) using blue agar plates
31
32 104 containing Chrome azurol S dye (CAS, Sigma–Aldrich). Orange halos around the colonies after 5 days
33
34 105 incubation at 30°C on blue agar were indicative of siderophore secretion.
35
36 106 The mineral P-solubilizing ability of the strain was assayed on agar plates containing insoluble Ca₃(PO₄)₂
37
38 107 according to Goldstein and Liu (1987). Strain N2 was streaked on agar medium (pH 7.2) containing glucose 10 g
39
40 108 L⁻¹, NH₄Cl 5 g L⁻¹, NaCl 1 g L⁻¹, MgSO₄·7H₂O 1 g L⁻¹ and Ca₃(PO₄)₂ 5 g L⁻¹. The plates were incubated at 30°C
41
42 109 for 5 days. The development of a clear zone around the colonies was indicative of P-solubilizing activity.
43
44 110 Proteolytic activity was tested by inoculation of the strain into skim milk agar medium containing 5 g L⁻¹
45
46 111 pancreatic digest of casein, 2.5 g L⁻¹ yeast extract, 1 g L⁻¹ glucose, 15 g agar and 100 ml L⁻¹ of 7% skim milk
47
48 112 solution. A clear zone around the cells on plates incubated at 30°C for 3 days indicated positive proteolytic
49
50 113 activity (Smibert and Krieg, 1994).
51
52 114 Chitinase activity was tested by streaking the strain on M9 chitin agar medium containing 1.62 g L⁻¹ nutrient
53
54 115 broth, 0.5 g L⁻¹ NaCl, 8 g L⁻¹ colloidal chitin and 15 g L⁻¹ agar (Kunz and Chapman 1981). The formation of
55
56 116 clear halos around the bacterial growth after 7 days incubation at 30°C indicated positive chitinase activity
57
58 117 (Sahoo et al., 1999).
59
60
61
62
63
64
65

118 Biocontrol activity of strain N2 against *Botrytis cinerea* ss177v and *B. cinerea* ss140t was determined by
119 inoculating fungal mycelium and strain N2 on plates containing CYA medium (Barka et al., 2002). After 7 days
120 of incubation at 30°C the growth of mycelium was measured and compared to that obtained without bacterial
121 inoculation (control).

122 All tests were performed in triplicate and repeated three times. All solutions were prepared with MilliQ water,
123 sterilized by membrane filtration (0.22 µm pore size, Millipore, Merck) and stored at 4°C in the dark. All
124 chemicals were of the highest purity available.

125

126 *Metal-resistance*

127 The strain was tested for its resistance to antimony (Sb) as SbCl₃, As(III) as (NaAsO₂), As(V) as (Na₂HAsO₄ x
128 7H₂O), cadmium (Cd) as CdCl₂, copper (Cu) as CuCl₂, chromium (Cr) as K₂Cr₂O₇, nickel (Ni) as NiCl₂, and
129 zinc (Zn) as ZnSO₄, (Sigma–Aldrich, St. Louis, MO, USA).

130 The growth of strain N2 was determined in liquid Tris mineral medium (TMM) at low phosphate content (0.12 g
131 L⁻¹ of Na₂HPO₄) to avoid metal precipitation (Sadouk and Mergeay, 1993) supplemented with a range of
132 concentrations of the different metals, added separately. Gluconate (0.6% w/v) was used as the carbon source in
133 TMM medium (TMMG) and the pH was adjusted to 7.0. Triplicate 100 mL flasks were inoculated with 1 mL of
134 an overnight culture of the strain grown on TMMG. Flasks were incubated for 5 days at 30°C and the growth
135 was measured as optical density at 600 nm (OD_{600nm}).

136

137 *Osmotic and ion toxicity tests*

138 To differentiate osmotic from ion toxicity effects, experiments were conducted under an osmotic potential $\Psi_0 = -$
139 1.5 MPa using three different sources. To achieve this osmotic potential, 175 mmol L⁻¹ of sodium As(V), 400
140 mmol L⁻¹ of NaCl and 26% (w/v) polyethylene glycol 6000 (PEG 6000) were separately added to Luria Bertani
141 (LB) medium (Sosa et al., 2005). Cells of N2 strain were grown at 30°C with 150 rpm shaking for 24 h and
142 inoculated in triplicate in the media. Bacterial growth was measured at successive incubation times (0-30 h) after
143 inoculation using a spectrophotometer at 546 nm (Forchetti et al., 2007). The ability of N2 strain to tolerate
144 different osmotic stresses was compared by calculating a resistance index (RI). The RI was defined as the ratio
145 of the exponential growth rate in the medium with stress to that in the control medium (Huang et al., 2010). The
146 closer RI was to 1, the smaller was the stress agent toxicity.

147

148 *Determination of stress-related phytohormones*

149 The production of stress-related phytohormones IAA, JA and ABA was determined in YEM in the absence and in
1 the presence of As(III) and As(V). In parallel experiments, the strain N2 was inoculated in YEM with L-
2 tryptophan 0.5 g L⁻¹ (YEMT) to check whether the strain utilized L-tryptophan as precursor to produce IAA.
3
4 151 Aliquots of a mid-exponential phase culture of strain N2 grown in YEM for 72 h were used to inoculate 60 mL
5
6 152 of YEM or YEMT (final cell number 10⁷ cells mL⁻¹) in the presence and in the absence of As(III) (3 mmol L⁻¹)
7
8 153 and As(V) (50 mmol/ L⁻¹), separately supplemented. Three replicates were used for each growth condition. The
9
10 154 flasks were incubated at 30°C under shaking at 150 rpm for 72 h. Bacterial suspensions were collected and
11
12 155 centrifuged at 8,000 rpm at 4°C for 15 min. From each supernatant, 50 mL were acidified with 0.25 mL of 12M
13
14 156 HCl and then extracted with ethylacetate (2 x 70 mL). The organic phase was dried under N₂ and the residue
15
16 157 dissolved in 0.5 mL of methanol (Merck, Darmstadt, Germany). The solution was centrifuged and 10 µL were
17
18 158 injected in an UPLC-MS/MS spectrometer and analyzed as described below.
19
20 159

21
22
23 160

24 25 161 *Arsenic transformation of strain N2*

26
27 162 The ability of strain N2 to oxidize As(III) or to reduce As(V) was tested both in growing cells or and resting
28
29 163 cells.

30
31 164 Cells grown to mid-exponential phase (growing cells) were inoculated into three flasks containing 20 mL of
32
33 165 TMMG with either 1 mmol L⁻¹ As(V) or As(III) to obtain an initial OD_{600nm} of about 0.05. In a parallel
34
35 166 experiment, As transformation capability was tested as ACCD-induced and uninduced growing cells by
36
37 167 inoculating cells grown to mid-exponential phase in DF and DF_ACC with either 0.05 mmol L⁻¹ As(V) or
38
39 168 As(III). Control flasks of TMMG, DF or DF_ACC without inoculum were incubated to check abiotic
40
41 169 transformation of As. Flasks without As were inoculated to compare the growth of the microorganisms in the
42
43 170 absence of As(V) or As(III). Three replicates per treatment were performed. All flasks were incubated at 30°C
44
45 171 under shaking at 150 rpm. After 72 h of incubation, aliquots of controls and cell suspensions were sampled to
46
47 172 measure cell growth by OD_{600 nm} and to determine Total As, As(V) and As(III) by Inductively Coupled Plasma
48
49 173 Mass Spectrometry (ICP-MS, Agilent Technologies, USA) analysis as described below.

50
51 174 As(III) oxidation and As(V) reduction ability of strain N2 was also tested as As(III)-induced or uninduced
52
53 175 resting cells. A pre-culture of N2 cells was established in TMMG in presence or in the absence of As for 24 h at
54
55 176 30°C under shaking at 150 rpm in the dark. After growth, cells were centrifuged at 12,857 g at 20°C for 15 min.
56
57 177 The cell pellet was washed three times with 500 mmol L⁻¹ CaSO₄ solution and resuspended in the same medium.
58
59 178 This cell suspension was inoculated in 60 mL of 500 mmol L⁻¹ CaSO₄ solution supplemented with 0.1 mmol L⁻¹
60
61 179 As(III), in order to obtain a final cell optical density OD_{600nm} of 0.35, corresponding to about 10⁷ cell mL⁻¹.

180 Resting cell experiment was carried out in triplicate for 48 h under shaking at 150 rpm in the dark at 30°C. At
181 the end of both growing and resting cells experiments, 10 mL of cell suspensions were collected, centrifuged and
182 syringe-filtered through 0.22 µm nitrocellulose membranes. Total As, As(III) and As(V) were determined by
183 ICP-MS analysis as described below.

184

185 *Rice germination tests*

186 Germination tests of rice (*Oryza sativa* L. var. Loto) seeds were conducted in triplicate in 120 mm Ø Petri dishes
187 containing filter paper moistened with 15 mL of CaSO₄ solution (Meharg and Hartley-Whitaker, 2002) either
188 supplemented with increasing concentrations of As(III) (0.05, 0.1 and 0.5 mM) or without As.

189 Prior to inoculation with *Achromobacter* sp. N2, rice seeds were surface-sterilized according the procedure from
190 Pandey et al. (2011). Rice seeds were rinsed in 1.5% sodium hypochlorite solution for 15 min, and then washed
191 three times with sterile deionized water. Seed sterility was verified by incubating 10 seeds onto LB agar plates at
192 30°C for 10 days. Bacterial cell suspension was prepared by growing strain N2 in 200 mL DF_ACC medium.

193 Cells were harvested by centrifugation (11,000 x g for 30 min) and suspended in 500 mmol L⁻¹ CaSO₄ until final

194 OD_{600nm} of 0.35 was achieved (corresponding to about 10⁸ CFU mL⁻¹). For inoculation, sterile seeds were soaked

195 in 40 mL bacterial suspension and gently stirred in the dark at room temperature for 2 h, after which they were

196 removed from the suspension by using sterile pliers. Twenty inoculated seeds were added to three Petri dishes

197 with or without 0.1 mM As(III), whereas addition of uninoculated seeds was used as control. Petri dishes were

198 incubated at 28°C per 10 days (3 days in the dark and 7 days in the light) according to Pandey et al. (2011). After

199 10 days of incubation, the percentage of germination, the root length, seedling dry weight (7 days at 100°C) and

200 height were measured.

201 Arsenic content of seedlings was determined in seedling biomass by digesting samples (0.5 g) in a mixture of

202 concentrated HNO₃ and HClO₄ (4:1, v/v). After digestion, the volume of each sample was adjusted to 20 mL

203 using deionized water. Arsenic content was determined by ICP-MS as specified below.

204

205 *Analytical methods*

206 *UPLC-MS/MS*

207 IAA, ABA and JA were determined by UPLC-MS/MS. Chromatographic system consisted of an UPLC mod.

208 Acquity (Waters, Milford, MA) coupled to a triple quadrupole mass spectrometer mod. Quattromicro (Waters).

209 A 1.7 µm C₁₈ BEH column (150x2.1 mm, Waters) was used for separation at a flow-rate of 0.5 mL/min. The

210 column was maintained at 50°C and the separation was performed by means of a linear gradient elution (eluent

211 A, 0.05% acetic acid; eluent B, 0.05% acetic acid in acetonitrile, Merck, Darmstadt, Germany). The gradient was
212 as follows: 20 to 60% B in 3 min, and then 60% B for 1 min. The capillary voltage was set to 3 kV, the cone
213 voltage and the collision energy was specific for each transition. The source temperature was 130°C, the
214 desolvating temperature was 350°C and argon was used at 2.0×10^{-3} mbar to improve fragmentation in the
215 collision cell. Masslynx 4.0 acquired data with Quan-Optimize option for fragmentation study. The fragmentation
216 transitions for the multiple reaction monitoring (MRM) was $(m/z)^{-} 263 \rightarrow 153$ for ABA, $(m/z)^{-} 209 \rightarrow 59$ for ABA,
217 $(m/z)^{+} 176 \rightarrow 130$ for 3-IAA, with a dwell time of 0.2 s.
218 The primary stock solutions of IAA, JA and ABA (0.1 mg mL^{-1} , Sigma-Aldrich) were prepared in methanol and
219 diluted to give working solutions in the range of $0.5\text{-}50 \text{ ng mL}^{-1}$. All stock solutions and the working solutions
220 were stored at -80°C and -20°C , respectively.

221

222 *ICP-MS*

223 Arsenic species in the samples were determined by ICP-MS analysis according to Kim et al. (2007). Specifically,
224 total As was determined in 5 mL of the sample previously acidified with HNO_3 to achieve a final concentration
225 of 2% (v/v). Inorganic As forms were determined in 5 mL of samples passed through a WATERS Sep-Pak®Plus
226 Acell Plus QMA cartridge (Waters). As(V) was retained in the cartridge while allowing As(III) to pass through
227 and collected. The cartridge was then washed with 0.16 M HNO_3 to extract As(V) from it. Total As, As(III) and
228 As(V) contents were determined by ICP-MS. Standards of As for concentrations ranging from 0 to 1 mg L^{-1}
229 were prepared from sodium As(III) NaAsO_2 (Sigma Aldrich, USA) solution.

230

231 *Molecular methods*

232 Genomic DNA was isolated from strain N2 using the Microbial DNA Extraction Kit (Mo Bio Laboratories Inc.,
233 Carlsbad, CA, USA) according to the manufacturer's protocol. The yield and quality of DNA were analysed by
234 agarose gel electrophoresis.

235 PCR amplification of 16S rRNA and of As genes was performed in a final volume of 25 μL containing: 10 ng of
236 genomic DNA, 1.5 U of *Taq* polymerase, 0.4 μM of each primer, 0.2 mM of dNTPs, 1.75 mM MgCl_2 , and 1x
237 PCR buffer. All reagents were obtained from Invitrogen.

238 An universal primer pair for bacterial 16S rRNA gene was used: P27f and P1495r (Weisburg et al., 1991). DNA
239 amplification conditions were: initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 1 min, 55°C for 40
240 sec, 72°C for 1 min 40 sec followed by a final extension step at 72°C for 10 min. Genes for As(V) reductase

241 (*arsC*), As(III) efflux pump (*arsB*) and As(III) oxidase (*aioA*) were amplified using the primer pairs listed in
1
2 242 Table 1.
3
4 243 PCR reactions were performed on a T-Gradient Biometra apparatus (Germany). PCR products were checked on
5
6 244 2% (w/v) agarose gel containing 0.01% (v/v) GelRed™ (Biotium, CA, USA) and visualized using the GelDoc
7
8 245 image analyzer system (Biorad, CA, USA).

9
10 246

11 247 *Sequence analyses*

12 248 Amplified genes were sequenced with the respective primers using the Taq Dye-Deoxy Terminator Cycle
13
14 249 Sequencing kit (Life Technologies Co., USA). The forward and reverse samples were run on a 310A sequence
15
16 250 analyzer (Life Technologies Co., USA). Sequences were edited and aligned using MEGA software version 6
17
18 251 (Tamura et al. 2007), translated into amino acid sequences and compared with the entire GenBank database
19
20 252 (BlastX). Obtained amino acid sequences and reference sequences were then aligned with ClustalX and trees
21
22 253 were built with MEGA6 using the neighbor-joining distance method based on p-distance. A total of 1000
23
24 254 bootstrap replications were calculated. Sequences obtained in the present work were deposited to GenBank-
25
26 255 EMBL databases under the following accession numbers: KY344276 and KY293395.

27
28 256 Strain N2 was identified as *Achromobacter* sp. according to 16S rRNA nucleotide sequence analysis (100%
29
30 257 homology to KT992330).

31
32 258

33 259 *Statistical analyses*

34
35 260 Obtained data were subjected to one-way analysis of variance (ANOVA) with Bonferroni tests using SPSS
36
37 261 Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY). All analyses were performed at the $p < 0.05$
38
39 262 level.

40
41 263

42
43 264

44 265 **Results**

45
46 266

47 267 *Characterization of Achromobacter sp. strain N2*

48 268 Metals and metalloid resistance and plant growth promotion traits of *Achromobacter* sp. strain N2 are shown in
49
50 269 Table 2.

51
52 270 Strain N2 was resistant to high concentrations of As(V) (100 mM), SbIII (10 mM), As(III) (5 mM) and CuII (5
53
54 271 mM) and to CrVI, NiII, CdII and ZnII (Table 2). The isolate was also resistant to ionic stress under -1.5 MPa

272 generated by 400 mmol L⁻¹ NaCl and 175 mmol L⁻¹ of sodium As(V) (Figure 1). On the contrary, it was affected
1
2 273 by non-ionic osmotic stress induced by 26% (w/v) PEG6000. The resistance index (RI) for As(V)-induced stress
3
4 274 was 0.7, followed by NaCl and PEG6000 with a mean RI of 0.4.

5
6 275 *Achromobacter* sp. strain N2 was able to produce IAA and JA, utilize ACC as the sole N source, secrete
7
8 276 siderophores, solubilise phosphate and hydrolyse proteins. The strain produced 0.068±0.008 nmol mL⁻¹ IAA
9
10 277 without the addition of L-tryptophan and 0.62±0.08 nmol mL⁻¹ with the addition of tryptophan, evidencing that
11
12 278 the amino acid was utilized as precursor. In the presence of 3 mM As(III) and of 50 mM As(V), IAA production
13
14 279 increased to 1.85±0.09 and 1.14±0.13 nmol mL⁻¹, respectively. JA production of strain N2 was equal to
15
16 280 0.17±0.03 pmol mL⁻¹, and it was not affected by the presence of As(III) and As(V), being 0.16± 0.02, 0.13±0.01
17
18 281 pmol mL⁻¹, respectively.

19
20 282 Biocontrol activity of the strain towards *B. cinerea* ss 177v and *B. cinerea* ss 140t was also evidenced.
21

22 283

23 24 284 *Arsenic metabolism and As-related genes*

25
26 285 Due to the relevance of bacterial metabolism in cycling As in plant rhizosphere and in influencing plant growth,
27
28 286 the ability of *Achromobacter* sp. strain N2 to transform inorganic As forms was further characterised.

29
30 287 *Achromobacter* sp. strain N2 completely oxidized 1.0 mmol L⁻¹ of As(III) to As(V) in 72 h when incubated in
31
32 288 chemoorganotrophic condition. The strain did not oxidize As(III) in chemolitho-autotrophic conditions,
33
34 289 indicating that the oxidation was a detoxification rather than an energy generating process. As(III) was oxidized
35
36 290 to an extent of 5.4% when the strain grew in DF medium, and of 20% when the strain grew in DF_ACC
37
38 291 medium. As(III) oxidation did not occur with strain N2 resting cells.

39
40 292 In accordance with the ability to oxidize As(III), a gene fragment corresponding to *AioA* was retrieved in strain
41
42 293 N2. The deduced amino acid sequence of the fragment had 99% homology to the alpha subunit of As(III)
43
44 294 oxidase of different *Achromobacter* (acc. num. AEL22195 and AOS87742) and *Alcaligenes* species (acc. num.
45
46 295 ABY19322, ABY19321 and ADF47192). Phylogenetic analysis of deduced amino acid sequences (Figure 2)
47
48 296 indicated that the sequence of strain N2 clustered together with *Betaproteobacteria* members of *Alcaligenaceae*
49
50 297 family retrieved in different As-contaminated environments.

51
52 298 Strain N2 was not able to reduce As(V) in any of the tested conditions and *arsC* and *arsB* genes for As(V)
53
54 299 reductase and As(III) efflux pump, respectively, were not detected.

56 300

57 58 301 *Effect of Achromobacter sp. N2 on rice growth and arsenic uptake*

59
60
61
62
63
64
65

302 The effect of *Achromobacter* sp. N2 on rice growth and As uptake was determined by germination tests
1 performed at increasing concentrations of As(III).
2 303
3
4 304 Rice seedling biomass production was significantly ($p<0.05$) affected in the presence of 0.05 and 0.1 mmol L⁻¹
5
6 305 As(III) (Table 3). As(III) 0.5 mmol L⁻¹ completely inhibited seeds germination.
7
8 306 The PGP effect of *Achromobacter* sp. strain N2 was tested by inoculating rice seeds with ACCD-induced and
9
10 307 ACCD-uninduced bacterial cells (Table 4). Although seed germination was not significantly affected by the
11
12 308 presence of the ACCD-uninduced cells, root length and seedling height of samples inoculated with ACCD-
13
14 309 induced inoculum were significantly ($p<0.05$) higher. This data demonstrated that the growth conditions of the
15
16 310 inoculum had an effect on the displacement of the PGP activity and that PGP traits might not be visible in a plant
17
18 311 unless induced during inoculum preparation.
19
20 312 In order to evidence the effect of ACCD activity of *Achromobacter* sp. strain N2 on rice under As pressure,
21
22 313 germination tests were performed with rice seeds inoculated with ACCD-induced and ACCD-uninduced cells in
23
24 314 the presence As(III) 0.1 mmol L⁻¹. When in the presence of As(III), ACCD-induced cells significantly promoted
25
26 315 seed germination (Figure 3) and seedling height with respect to ACCD-uninduced cells (Table 5). The specific
27
28 316 As content of seedlings was determined after 10 days of incubation (Table 5). In rice inoculated with ACCD-
29
30 317 induced cells the total As content was significantly higher than in rice inoculated with ACCD-uninduced cells
31
32 318 and in the non-inoculated ones. This indicated that As uptake did not impair seedling germination.
33
34 319

320 Discussion

321 In the present work, PGP activities and As metabolism of *Achromobacter* sp. strain N2 was described and its
322 capability to promote rice growth and As uptake was evidenced.
323 The strain was able to oxidise As(III) in chemoorganotrophic growth conditions. This ability was strongly
324 impaired when the strain grew in the absence of a nitrogen source or in the presence of ACC as sole source of
325 nitrogen. The presence of As stimulated IAA production in accordance with recent evidences (Mendoza-
326 Hernandez et al., 2016). On the contrary, JA, signalling compound involved in plant growth, development and
327 response to (a)biotic stress factors (Cuyper et al., 2011) was not affected. The strain was characterised by an
328 As(III) oxidase gene with high degree of amino acid conservation with known As(III) oxidase of *Achromobacter*
329 and *Alcaligenes* species. Particularly, the high conserved motif HNRPAYNSE (Quemeneur et al., 2008) was
330 present in the amino acid sequence of strain N2.
331 Arsenic exposure significantly affected the normal growth and development of tested rice seedlings. In
332 accordance with previous study conducted in similar conditions (Choudhury et al., 2009), As toxicity in rice was

333 displayed at 0.5 mmol L⁻¹ As(III). The rate of root length inhibition was stronger than seedling height inhibition,
334 suggesting that the increment of As has negative effects on the area of the seedlings directly in contact with the
335 metalloid (Choudhury et al., 2009; Shri et al., 2009). Total biomass was confirmed to be a less sensitive
336 parameter of As toxicity, in accordance with Liu et al. (2005) and Williams et al. (2005).

337 The presence of *Achromobacter* sp. strain N2 increased As content in the seedlings. Arsenic uptake by plants in
338 relation to the presence of As(III) oxidizing strains in rhizosphere is still debated in the literature. In a recent
339 work, Das and colleagues (2016) observed a decrement of total As content in rice straw and grain and an
340 increment of root As content in the presence of As(III) oxidizing strains. On the contrary, in a molecular ecology
341 study it was demonstrated that higher number of As(III) oxidase genes in rice rhizosphere corresponded to lower
342 As content in plant (Hu et al., 2015). With this regard, a possible explanation of contrasting results could be the
343 variety of rice used in these studies, as previously evidenced by Yang et al. (2015). The detoxification ability of
344 strain N2 did not rely on oxidation of As(III) to As(V), since resting and ACCD-induced cells of
345 *Achromobacter* sp. strain N2 were impaired in this ability, but more likely on IAA production and ACCD
346 activity that improved seedling development.

347 ACCD activity is recognized to protect plants against trace element toxicity when growing on contaminated soils
348 (Glick, 2003). Plants inoculated with bacterial strains carrying ACCD activity can regulate their ethylene levels,
349 thus presenting a more extensive root system that leads to enhanced uptake of heavy metals (Arshad et al., 2007)
350 by modification of root structure (Zhang et al., 2008). In accordance with previous studies (Rahman et al., 2007;
351 Pandey et al., 2013), seed priming with *Achromobacter* sp. strain N2 increased root growth and As uptake, thus
352 demonstrating that this PGP trait is involved in plant relief.

353

354 **5 Conclusion**

355 *Achromobacter* sp. strain N2 exhibited As(III) oxidation activity and several PGP traits. The ACCD activity of
356 the strain was involved in rice germination relief in the presence of As and determined an increased As content
357 of seedlings. This kind of responses should be taken into account when proposing PGP strains for ameliorating
358 plant growth in As-rich soils. Nevertheless, the assessment of *Achromobacter* sp. strain N2 as a potential
359 inoculum of non-food plants in phytoremediation processes is under way.

360

361 **References**

362 Abou-Shanab R, Angle JS, Delorme TA, Chaney RL, Van Berkum P, Moawad H, Ghanem K, Ghazlan HA
363 (2003) Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. *New phytologist*
364 158: 219–224

365

1

2 366 Achour AR, Bauda P, Billard P (2007) Diversity of arsenite transporter genes from arsenic-resistant soil bacteria.

3

4 367 Res Microbiol 158: 128–137

5

6 368

7

8 369 Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation.

9

10 370 TRENDS Biotechnol (25) 356–362

11

12 371

13

14 372 Bachate SP, Cavalca L, Andreoni V (2009) Arsenic-resistant bacteria isolated from agricultural soils of

15 373 Bangladesh and characterization of arsenate-reducing strains. J Appl Microbiol 107: 145–156

16

17

18 374

19 375 Barka A, Gognies ES, Nowak J, Audran JC, Belarbi A (2002) Inhibitory effect of endophyte bacteria on *Botrytis*

20 376 *cinerea* and its influence to promote the grapevine growth. Biol Control 24:135–142

21

22

23 377

24

25 378 Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in

26 379 plants. Can J Microbiol 46: 237–245.

27

28

29 380

30

31

32 381 Cavalca L, Corsini A, Zaccheo P, Andreoni V, Muyzer G (2013) Microbial transformations of arsenic:

33 382 perspectives for biological removal of arsenic from water. Future Microbiol 8: 753–768

34

35

36 383

37

38 384 Choudhury B, Chowdhury S, Biswas AK (2009) Regulation of growth and metabolism in rice (*Oryza sativa* L.)

39 385 by arsenic and its possible reversal by phosphate. J Plant Interact 6: 15–24

40

41

42 386

43

44 387 Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards

45 388 maximum levels of inorganic arsenic in foodstuffs. [http://eur-lex.europa.eu/legal-](http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32015R1006)

46 389 [content/EN/TXT/?uri=CELEX:32015R1006](http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32015R1006)

47

48

49

50 390

51

52 391 Cuypers A, Smeets K, Ruytinx J, Opendakker K, Keunen E, Remans T, Horemans N, Vanhoudt N, Van Sanden

53 392 S, Van Belleghem F, Guisez Y, Colpaert J, Vangronsveld J (2011) The cellular redox state as a modulator in

54 393 cadmium and copper responses in *Arabidopsis thaliana* seedlings. J Plant Physiol 168: 309–316

55

56

57

58 394

59

60

61

62

63

64

65

395 Das S, Jiin-Shuh J, Mon-Lin C, Jagat R, Chia-Chuan L (2016). Arsenite-oxidizing bacteria exhibiting plant
1 396 growth promoting traits isolated from the rhizosphere of *Oryza sativa* L.: Implications for mitigation of arsenic
2 397 contamination in paddies. *J Hazard Mater* 302: 10–18
3
4
5 398
6
7 399 Dell’Amico E, Cavalca L, Andreoni V (2005) Analysis of rhizobacterial communities in perennial graminaceae
8 400 from polluted water meadow soil, and screening of metal resistant, potentially plant growth-promoting bacteria.
9 401 *FEMS Microbiol Ecol* 52: 153–162
10
11
12
13 402
14
15 403 Dworkin, M., and Foster, J., 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *J.*
16 404 *Bacteriol.* 75, 592–601.
17
18
19 405
20
21 406 Forchetti, G. Masciarelli, O., Alemano, S., Alvarez D., Abdala, G., 2007. Endophytic bacteria in sunflower
22 407 (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture
23 408 medium. *Appl. Microbiol. Biotechnol.* 76, 1145–1152.
24
25
26
27 409
28
29 410 Glick, B.R., Penrose, D.M., Li J.P., 1998. A model for the lowering of plant ethylene concentrations by plant
30 411 growth promoting bacteria. *J. Theor. Biol.* 190, 63–68.
31
32
33 412
34
35 413 Glick, B.R., 2003. Phytoremediation: Synergistic use of plants and bacteria to clean up the environment.
36 414 *Biotechnol. Advan.* 21, 383–393.
37
38
39 415
40
41 416 Glickmann, E., Dessaux, Y., 1995. A critical examination of the specificity of the Salkowski reagent for indolic
42 417 compounds produced by phytopathogenic bacteria. *Appl. Environ. Microbiol.* 61, 793–796.
43
44
45
46 418
47
48 419 Goldstein, A.H., Liu, S.T., 1987. Molecular cloning and regulation of a mineral phosphate solubilizing gene
49 420 from *Erwinia herbicola*. *Nature Biotechnol.* 5, 72–74.
50
51
52 421
53
54 422 Grichko, V.P., Filby, B., Glickm B.R., 2000. Increased ability of transgenic plants expressing the bacterial
55 423 enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb and Zn. *J. Biotechnol.* 81, 45–53.
56
57
58 424
59
60
61
62
63
64
65

425 Huang, A., Teplitski, M., Rathinasabapathi, B., Ma, L., 2010. Characterization of arsenic-resistant bacteria from
1 426 the rhizosphere of arsenic hyperaccumulator *Pteris vittata*. *Can. J. Microbiol.* 56, 236–246.
2
3 427
4
5
6 428 Hu, M., Li, F., Liu, C., Wu, W., 2015. The diversity and abundance of As(III) oxidizers on root iron plaque is
7 429 critical for arsenic bioavailability to rice. *Sci. Rep.* 5, 13611.
8
9
10 430
11
12 431 Kim, Y.T., Yoon, C., Woo, N.C., 2007. An assessment of sampling preservation and analytical procedures for
13 432 arsenic speciation in potentially contaminated waters. *Environ. Geochem. Health* 29, 337–346.
14
15
16 433
17
18 434 Kunz, D.A., Chapman, P.J., 1981. Catabolism of pseudocumene and 3-ethyltoluene by *Pseudomonas putida*
19 435 (*arvilla*) mt-2: evidence for new functions of the TOL (pWW0) plasmid. *J. Bacteriol.* 146, 179–191.
20
21
22 436
23
24
25 437 Liu, X., Zhang, S., Shan, X., Zhu, Y., 2005. Toxicity of arsenate and arsenite on germination, seedling growth
26 438 and amylolytic activity of wheat. *Chemosphere* 61, 293–301.
27
28
29 439
30
31 440 Madhaiyan, M., Poonguzhali, S., Senthilkumar, M., Seshadri, S., Chung, H., Yang, J., Sundaram, S., Sa, T.,
32 441 2004. Growth promotion and induction of systemic resistance in rice cultivar Co-47 (*Oryza sativa* L.) by
33 442 *Methylobacterium* spp.. *Bot. Bull. Acad. Sin* 45, 315–324.
34
35
36 443
37
38
39 444 Mandal, B.K., Suzuki, K.T., 2002. Arsenic around the world: a review. *Talanta* 58, 201–235.
40
41
42 445
43
44 446 Meharg, A.A., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant
45 447 plant species. *New Phytol.* 154, 29–43.
46
47
48 448
49
50 449 Meharg, A.A., Williams, P.N., Adomako, E., Lawgali, Y.Y., Deacon, C., Villada, A., Cambell, R.C.J, Sun, G.,
51 450 Zhu, Y-G., Feldmann, J., Raab, A., Zhao, F-J., Islam, R., Hossain, S., Yanai, J., 2009. Geographical variation in
52 451 total and inorganic arsenic content in polished (white) rice *Environ. Sci. Technol.* 43, 1612–1617
53
54
55 452
56
57
58 453 Mendoza-Hernández, J.C., Perea-Vélez, Y.S., Arriola-Morales, J., Martínez-Simón, S.M., Pérez-Osorio, G.,
59 454 2016. Assessing the effects of heavy metals in ACC deaminase and IAA production on plant growth-promoting
60 455 bacteria. *Microbiol Res.* 188-189, 53-61.
61
62
63
64
65

456

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

457 Pandey, V.C., Singh, J.S., Singh, R.P., Singh, N., Yunus, M., 2011. Arsenic hazards in coal fly ash and its fate in
458 Indian scenario. *Resour. Conserv. Recy.* 55, 819–835.

459
460 Pandey, S., Ghosh, P.K., Ghosh, S., De, T.K., Maiti, T.K., 2013. Role of heavy metal resistant *Ochrobactrum* sp.
461 and *Bacillus* spp. strains in bioremediation of a rice cultivar and their PGPR like activities *J. Microbiol.* 51, 11–
462 17.

463
464 Quéméneur, M., Heinrich-Salmeron, A., Muller, D., Lièvreumont, D., Jauzein, M., Bertin, P.N., Garrido, F.,
465 Jouliau, C., 2008. Diversity surveys and evolutionary relationships of *aoxB* genes in aerobic arsenite-oxidizing
466 bacteria. *Appl. Environ. Microbiol.* 74, 4567–4573.

467
468 Rahman, M.A., Hasegawa, H., Rahman, M.M., Islam, M.N., Miah, M.A.M., Tasmien, A., 2007. Effect of arsenic
469 on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh.
470 *Chemosphere* 67, 1072-1079.

471
472 Sadouk, A., Mergeay, M., 1993. Chromosome mapping in *Alcaligenes eutrophus* CH34, *Mol. Gen. Gen.* 240,
473 181-187.

474
475 Sahoo, A., Agarwal, N., Kamra, D.N., Chudhary, L.C., Pathak, N.N., 1999. Influence of the level of molasses in
476 de-oiled rice bran-based concentrate mixture on rumen fermentation pattern in crossbred cattle calves. *Anim.*
477 *Feed Sci. Technol.* 80, 83–90.

478
479 Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores.
480 *Anal. Biochem.* 160, 47-56.

481
482 Shri, M., Kumar, S., Chakrabarty, D., Trivedi, P.K., Mallick, S., Misra, P., Shukla, D., Mishra, S., Srivastava, S.,
483 Tripathi, R.D., Tuli, R., 2009. Effect of arsenic on growth, oxidative stress, and antioxidant system in rice
484 seedlings. *Ecotoxicol Environ Saf* 72, 1102-1110.

485
486 Smibert, R.M., Krieg, N.R. 1994. Phenotypic characterization. In: *Method for general and molecular*
487 *bacteriology.* *Am. Soc. Microbiol.* 34, 607–654.

488

- 489 Sosa, L., Llanes, A., Reinoso, H., Reginato, M., Luna, V., 2005. Osmotic and specific ion effect on the
 1 490 germination of *Prosopis strombulifera*. *Ann. Bot.* 96, 261-267.
 2
 3
 4 491
 5
 6 492 Spanu, A., Daga, L., Orlandoni, A.M., Sanna, G., 2012. The role of irrigation techniques in arsenic
 7 493 bioaccumulation in rice (*Oryza sativa* L.). *Environ. Sci. Technol.* 46, 8333–8340.
 8
 9
 10 494
 11
 12 495 Tamura, K., Dudley, J., Nei, M., Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA)
 13 496 Software Version 4.0. *Mol. Biol. Evol.* 24, 1596–1599.
 14
 15
 16 497
 17
 18 498 Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA for phylogenetic study, *J.*
 19 499 *Bacteriol.* 173, 697-703.
 20
 21 500
 22
 23
 24 501 Williams, P.N., Price, A.H., Raab, A., Hossain, A.A., Feldmann, J., Meharg, A.A., 2005 Variation in arsenic
 25 502 speciation and concentration in paddy rice related to dietary exposure. *Environ. Sci. Technol.* 39, 5531-5540.
 26
 27
 28 503
 29
 30 504 Yang, G.D., Xie, W.Y., Zhu, X., Huang, Y., Yang, X.J., Qiu, Z.Q., Lv, Z.M., Wang, W.N., Lin, W.X., 2015.
 31 505 Effect of arsenite-oxidizing bacterium *B. laterosporus* on arsenite toxicity and arsenic translocation in rice
 32 506 seedlings. *Ecotoxicol. Environ. Saf.* 120, 7-12.
 33
 34
 35
 36 507
 37
 38 508 Zhang, Y., Zhao, L., Wang, Y., Yang, B., Chen, S., 2008 Enhancement of heavy metal accumulation by tissue
 39 509 specific co-expression of *iaaM* and ACC deaminase genes in plants. *Chemosphere* 72, 564-571.
 40
 41
 42 510
 43
 44 511 Zhao, F.-J., McGrath, S.P., Meharg, A.A., 2010. Arsenic as a food chain contaminant: mechanisms of plant
 45 512 uptake and metabolism and mitigation strategies. *Annu. Rev. Plant Biol.* 61, 535-559.
 46
 47
 48 513
 49
 50

51 **Figure captions**

52
 53 515 Figure 1. Time course of bacterial growth of *Achromobacter* sp. strain N2 under different hyperosmotic stresses
 54 516 (-1.5 MPa) (mean±SD, n=3, where bar is absent, SD is within the point). ○, growth in LB; ■, growth in LB+400
 55 517 mmol L⁻¹ NaCl; ◆, growth in LB+175 mmol L⁻¹ As(V); ▲, growth in LB+26% (w/v) PEG6000.
 56
 57

58 518
 59 519 Figure 2 Phylogenetic relationships deduced amino acid sequence of arsenite oxidase gene *aioA* of
 60 520 *Achromobacter* sp. Strain N2 (in bold). The evolutionary history was inferred using the Neighbor-Joining
 61
 62
 63
 64
 65

521 method. The bar indicates 2% sequence difference. The sequence of *Hoeflea phototrophica* (ZP02167371) was
1 522 used as an outgroup.

2
3 523

4 524 Figure 3 Plant growth-promoting ability of the strain N2 in the absence (black bars) and in the presence of
5
6 525 As(III) 0.1 mM (grey bars) as measured in terms of germination percentage. Data reported in the bars under
7 526 different doses of As with same letter are not significantly different ($p < 0.05$) according to Bonferroni test.

8
9
10 527

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

528

529 Table 1 Primer pairs used to amplify As-transforming genes from *Achromobacter* sp. N2

Target	Primer	Sequence (5'-3')	Reference
<i>arsC</i>	P52f	AGCCAAATGGCAGAAGC-3	Bachate et al. (2009)
	P323r	GCTGGRTCRTCAAATCCCCA	
<i>arsB</i>	darsB1F	TGTGGAACATCGTCTGGAAYGCNAC	Achour et al. (2007)
	darsB1R	CAGGCCGTACACCACCAGRTACATNCC	
<i>aioA</i>	aoxBM1-2F	CCACTTCTGCATCGTGGGNTGYGGNTA	Quémèneur et al. (2008)
	aoxBM3-2R	TGTCGTTGCCCCAGATGADNCCYTTYTC	

530

531

532

533

534

Table 2 Plant growth promotion traits, biocontrol properties and metal resistance of *Achromobacter* sp. N2

Characteristic	N2
PGP traits	
Indole acetic acid production	+
Jasmonic acid production	+
Abscissic acid production	-
Growth on ACC	+
Siderophores production	+
Mineral-P solubilization	+
Proteolytic activity	+
Chitinase activity	-
Biocontrol activity	
% inhibition of <i>Botrytis cinerea</i> ss 177v	20%
% inhibition of <i>Botrytis cinerea</i> ss 140t	17%
As(III) oxidation (1 mmol L ⁻¹)	100%
Metal resistance (mmol L ⁻¹)	
As(V)	100
As(III)	5
SbIII	10
CuII	5
CrVI	1
NiII	0.1
CdII	0.1
ZnII	0.1

535

+, positive; -, negative.

536

537

538

539

540 Table 3 Response of rice seeds to different As(III) concentrations.

As(III) (mmol L ⁻¹)	Seeds germination (%)	Root length (mm)	Seedling height (mm)	Seedling dry weight (g)
0	75 b	50 c	36 b	0.511 b
0.05	32 a	27 b	24 a	0.179 a
0.1	23 a	10 a	27 a	0.114 a
0.5	0	-	-	-

541 Data followed by the same letter in a column for each treatment do not differ significantly at $p < 0.05$, as determined using Bonferroni test;
 542 n.d., not determinable.

543

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

544

1
2 545 Table 4 Response of rice seeds to ACCD-induced *Achromobacter* sp. N2 cells (time of incubation 10 days).
3

4	Condition	Seeds germination (%)	Root length (mm)	Seedling height (mm)	Seedling dry weight (g)
7	ACCD-induced cells	73 ns	58 c	41 b	0.364 ns
8	Uninduced cells	55	40 a	33 a	0.275
9	Not inoculated seeds	75	50 b	36 ab	0.511

10 546 Data followed by the same letter in a column for each treatment do not differ significantly at $p < 0.05$, as determined using Bonferroni test.

11 547

12 548

13 549

14 550

16 551 Table 5 Evaluation of rice growth-promotion responses to inoculation of *Achromobacter* sp. N2 in the presence
17 552 of 0.1 mmol L⁻¹ As(III).
18

19	As(III) (mmol L ⁻¹)	Condition	Seeds germination (%)	Root length (mm)	Seedling height (mm)	Seedling dry weight (g)	Specific As content in seedling (mmol kg ⁻¹ d.w.)
26	0.1	ACCD-induced cells	30 b	16 b	27 b	0.060 a	1.695 d
27		ACCD-uninduced cells	20 a	18 b	24 a	0.106 a	1.141 c
28		Not inoculated seeds	23 a	10 a	25 ab	0.114 a	0.698 b
30	0	Not inoculated seeds	75 c	50 c	36 c	0.511 a	0.009 a

31 553 Data followed by the same letter in a column for each treatment do not differ significantly at $p < 0.05$, as determined using Bonferroni test.

32 554

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

Figure 1

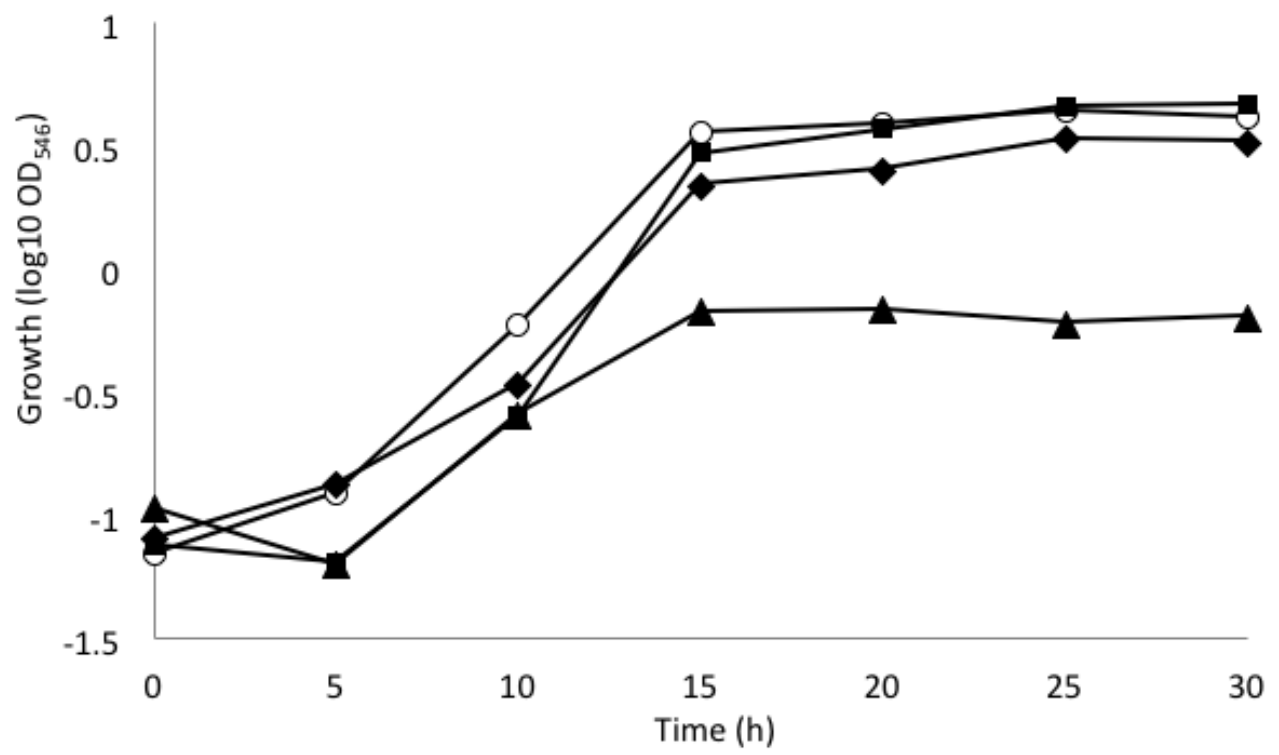


Figure 2

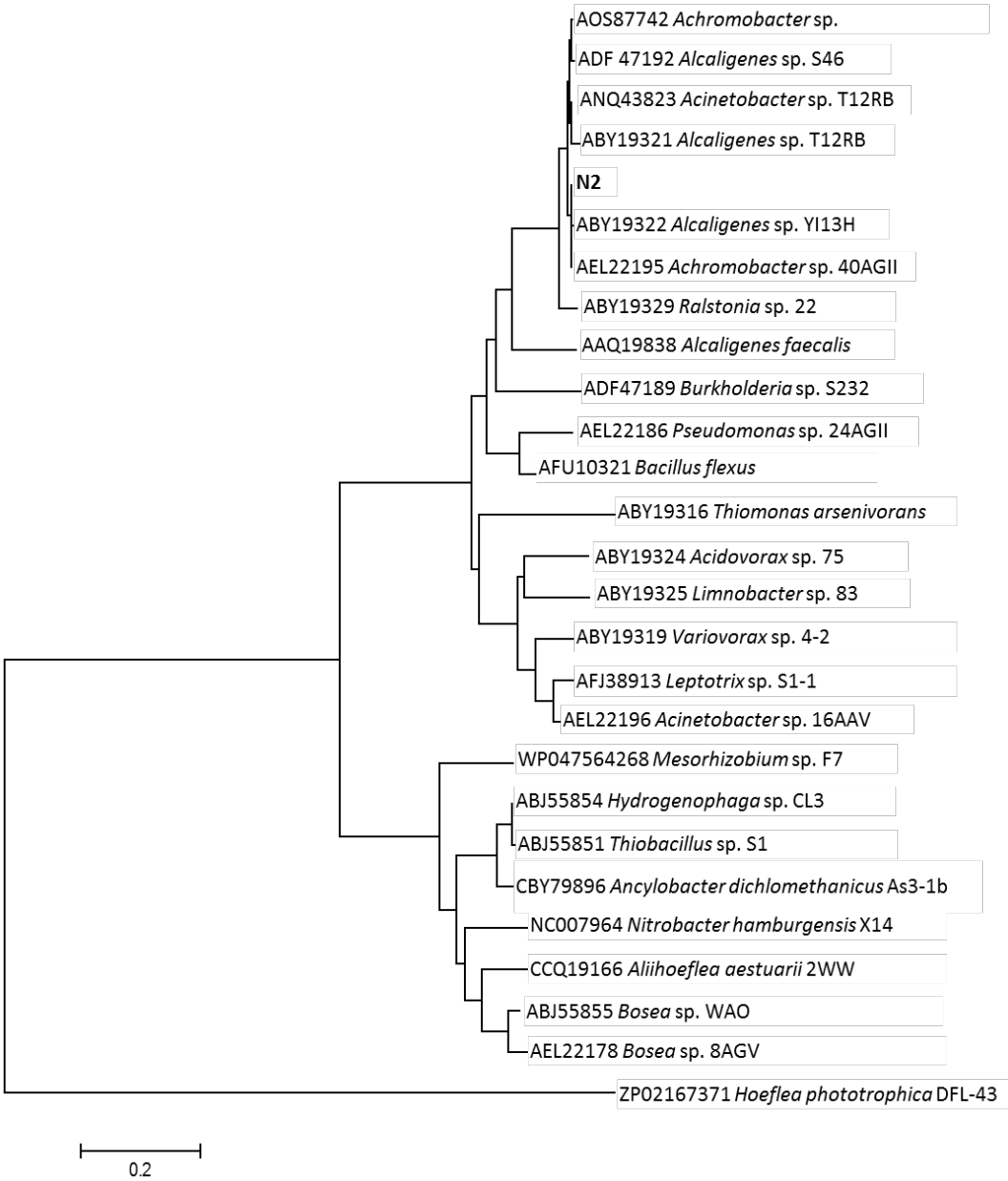


Figure 3

