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Isolation and characterization of yeasts associated with plants growing in heavy metal- and arsenic-contaminated soils

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Manuscripts

1 **Research Article**

2 **Isolation and characterization of yeasts associated with plants growing in heavy**
3 **metal- and arsenic-contaminated soils**

4

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37 **Short title**: Heavy metals, arsenic-resistance yeasts.

38

39 **Abstract**

40 Yeasts were quantified and isolated from the rhizospheres of five plant species grown at
41 two sites of a Mexican region contaminated with arsenic, lead, and other heavy metals.
42 Yeast abundance was about 10^2 CFU g^{-1} soil and 31 isolates were obtained. Based on the
43 phylogenetic analysis of *26S rRNA* and ITS fragment, six species within five genera were
44 identified, including *Cryptococcus* (80.64%), *Rhodotorula* (6.45%), *Exophiala* (6.45%),
45 *Trichosporon* (3.22%), and *Cystobasidium* (3.22%). *Cryptococcus* spp. was the
46 predominant group. Pectinases (51.6%), proteases (51.6%), and xylanases (41.9%) were
47 the enzymes most common, while poor siderophores (16.1%) and Indole Acetic Acid
48 (IAA) (9.67%) production was detected. Isolates of *Rhodotorula mucilaginosa* and
49 *Cystobasidium sloffiae* could promote plant growth and seed germination in bioassay
50 with *Brassica juncea*. Resistance of isolates by As and heavy metals was as follows: As^{3+}
51 ≥100 mM; As^{5+} ≥30 mM; Zn^{2+} ≥2 mM; Pb^{2+} ≥1.2 mM, and Cu^{2+} ≥0.5 mM. Strains of
52 *Cryptococcus albidus* were able to reduce Arsenate (As^{5+}) into Arsenite (As^{3+}), but no
53 isolate was capable of oxidizing arsenite. This is the first study on the abundance and
54 identification of rhizosphere yeasts and in a HM and arsenic-contaminated soil and in
55 arsenate reduction by the species *C. albidus*.

56

57 **Keywords:** Yeast, Rhizosphere, Reduction, Arsenic, Speciation.

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60 **Introduction**

61 Arsenic (As) is a common toxic contaminant produced by industry extraction or
62 anthropogenic input in nature, but it is also released as a consequence of rock weathering
63 or volcanic explosions (Donahoe et al. 2004; Wang and Mulligan 2006). In different
64 environments, As is present as inorganic (As^{5+} and As^{3+}) or organic [Monomethylarsonic
65 Acid (MMA), DimethylArsonic Acid (DMA), and TrimethylArsine Oxide (TMAO)]
66 forms, which can be transformed by microbial activities, such as oxidation-reduction and
67 methylation-demethylation (Turpeinen et al. 2002).

68 Many bacteria, archaea, and fungi have developed mechanisms for avoiding arsenic
69 toxicity, including the following: (i) reduction of Arsenate (As^{5+}) to Arsenite (As^{3+}) and
70 expelling the latter outside of the cells (Čerňanský et al. 2009); (ii) arsenic complexation
71 with other molecules inside the cells, such as glutathione and vacuolar sequestration
72 (Rosen 2002); (iii) methylation of arsenic to the less toxic organic forms (Qin et al.
73 2006); (iv) oxidation of arsenite to the less toxic arsenate (Gihring and Banfield 2001),
74 and (v) metalloid immobilization by absorption and accumulation in biomass (Wang and
75 Xhao 2009).

76 Similar to other microorganisms in the soil and rhizosphere, yeasts play an important
77 ecological role, such as recycling nutriments, aggregating soil particles, assimilating
78 secondary products from bacteria and other fungi, exhibiting different interactions
79 (amensalism, predation, and competition) with other microorganisms and plants, and
80 possessing versatile metabolisms for utilizing and transforming nitrogen compounds

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81 (Botha 2011). Some yeasts, such as *Trichosporon* spp., *Rhodotorula* spp., and *Candida*
82 *tropicalis,* are known for their capability to resist and accumulate arsenic and heavy
83 metals (Olasupo et al. 1993; Ilyas et al. 2014; Ilyas et al. 2015). The biotransformation of
84 arsenic is well studied in the yeast *Saccharomyces cerevisiae*, and its related pathways
85 and the genes and mutants involved have been characterized (Menezes et al. 2004; Shah
86 et al. 2010; Todorova et al. 2010). Other yeasts, such as *Schizosaccharomyces pombe*,
87 *Trichosporon* spp., *Rhodotorula* spp., and *Cryptococcus humicolus* have been studied in
88 terms of the accumulation, biosorption, and quantification of the oxidation and reduction
89 process (Button et al. 1973; Salgado et al. 2012; Ilyas 2014). However, no information
90 exists about the yeast population associated with HM- and arsenic-resistant plants or their
91 characterization.

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92 The aim of this work was to evaluate the diversity of rhizospheric yeasts associated with
93 HM- and arsenic-resistant plants and to evaluate their ability to biotransform As. In order
94 to accomplish this, we isolated and characterized the rhizosphere yeasts associated with
95 five plant species grown at two sites in a small town, namely Villa de la Paz, in the State
96 of San Luis Potosí, Mexico, a traditional mining region with mine tailings, soils, and
97 plants contaminated with high concentrations of arsenic and HM (Franco-Hernández
98 2010). To date, no study on microbial diversity and the HM and arsenic detoxification
99 potential in this region has been reported.

100 **Materials and Methods**

101 *Sampling sites*

102 The sampling sites are located in Villa de la Paz in the State of San Luis Potosí (23.7 N,
103 178.7 W), a zone with a mean annual temperature of 18°C and an average annual
104 precipitation of 486 mm. The two sampling sites comprise a mine tailing with an altitude
105 of 1,557 m, and a natural hill with an altitude of 1,830 m, with a distance of about 5 km
106 between them. In a previous study, it was reported that this soil is contaminated with risk
107 elements (Franco-Hernández et al. 2010). Soil analysis revealed the following: Arsenic
108 (As) >3,574 $mg.kg^{-1}$, lead (Pb) >555.45 $mg.kg^{-1}$, Copper (Cu) >2,012 $mg.kg^{-1}$, and Zinc
109 (Zn) >1,337 $mg.kg^{-1}$ have been detected in the mine tailing; at the hill site, the data were
110 the following: As >1,071.50 $mg.kg^{-1}$; Pb >5,478.60 $mg.kg^{-1}$; Cu >749.30 $mg.kg^{-1}$, and Zn,
111 >1,672.85 $mg.kg^{-1}$ (data shown in Supplementary Material). In this work, the
112 physicochemical parameters of the mine and hill site were analyzed using the methods
113 described by Vásquez-Murrieta et al. (2006).

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114 *Plant identification and soil sampling characterization*

115 At both sites, four individual plants of the most abundant plant species were sampled
116 randomly by extracting plants with their roots together with their soils. The samples were
117 maintained in plastic bags, transported immediately to the laboratory, and stored
118 thereafter at 4°C. All of the sampled plants were mature and appeared healthy without the
119 presence of parasites. The plants were identified by Botanists in the *Plant Ecology*
120 *Laboratory* of the National School of Biological Science (ENCB) of the National
121 Polytechnic Institute (IPN) in Mexico City, based on typical morphology and taxonomic
122 features depending on the plant species (Calderón de Rzedowski and Rzedowski 2001;
123 McVaugh 1984; McVaugh 1987). Rhizosphere soils were prepared by brushing off the

124 soils adhering to the root surface of the plants, as previously described (Trujillo-Cabrera
125 et al. 2013), and these were used for yeast isolation. Soils sampled together with the
126 plants of each species at the same site were compiled in the same volume, which was then
127 employed for analysis of the contents of HM and arsenic and nutrients, as well as the
128 physicochemical features, as reported previously (Franco-Hernández et al. 2010;
129 Vásquez-Murrieta et al. 2006).

130 *Yeast isolation*

131 For isolation of yeasts, 0.5 g of rhizospheric soil from each plant species was suspended
132 in 4.5 mL of sterile saline solution (NaCl 0.85%). Serial dilutions were prepared up to 10^{-}
133 ^{3}, and an aliquot of 0.1 mL from each dilution was spread on the following media in Petri
134 dishes: Yeast-Peptone-Dextrose (YPD) agar (Yeast extract, 10 g; Peptone, 20 g;
135 Dextrose, 20 g; Agar, 15 g; Distilled water, 1 L, added with Streptomycin, 20 mg) and
136 Rose-Bengal Agar (DIFCO) (Chloramphenicol, 0.1 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; KH_2PO_4, 1.0
137 g; Dextrose, 10 g; Rose-Bengal, 0.05 g; Soy peptone, 5.0 g; agar 15 g, and Distilled
138 water, 1 L). The inoculated Petri dishes were incubated at 28°C for 7 days. Single
139 colonies were selected according to their morphology and were purified by repeated
140 cross-striking on YPD plates of until all of the colonies in the same isolate presented
141 similar morphology (Al-gabr et al. 2014). Purity of the isolates was verified
142 microscopically, and the pure isolates were conserved at –70°C in microtubes (1.5 mL)
143 half filled with YPD broth supplied with glycerol (50%, w/v).

144 *Amplification and sequencing of yeasts* 26S rRNA *gene and ITS fragment*

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145 Genomic DNA extracted from each yeast isolate using the protocols of Allers and
146 Lichten (2000) was used as template to amplify the *26S rRNA and* ITS fragments by PCR
147 with a thermocycler (MaxyGene Thermal Cycler THERM 1061; AxyGen Scientific),
148 following the subsequent protocol: an initial 10-min denaturing step at 94°C, followed by
149 30, 1-min cycles at 94°C, 1 min of annealing at 54°C, 1 min extension at 72°C, and a
150 final 8-min polymerization step at 72°C. The PCR mixture (50 µL) contained 50–100 ng
151 of DNA template, 3 mM $MgCl_2$, 2.5 U *Taq* DNA polymerase (Invitrogen, USA), 1X
152 PCR buffer, 20 pmol of primers for *26S rRNA*: NL1 (5′-
153 GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-
154 GGTCCGTGTTTCAAGACGG-3′) (Redecker 2000), and the following for the ITS
155 fragment: ITS1 (5′TCC CGT AGG TGA CCT GCG G 3′) and ITS4 (5′TCC TCC GCT
156 TAT TGA TAT GC 3′) under the same thermal condition (Gardes and Bruns 1993) and
157 200 µM of each dNTP. Amplification products were visualized after electrophoresis in
158 agarose gel (1%, w/v) in 0.5X TAE buffer by staining with an aqueous solution of
159 ethidium bromide (0.5 $\mu g.mL^{-1}$). A PureLink™ 310002 commercial kit (Invitrogen) was
160 utilized for purifying the PCR products, which were then sequenced under Big Dye™
161 terminator cycling conditions with the same primers using the Automatic Sequencer
162 3730XL in Macrogen (Korea).

163 *Phylogenetic analysis*

164 The D1/D2 regions of the *26S rRNA* sequences obtained and the ITS fragment were
165 employed to estimate the taxonomic affiliation and genotype designations of the yeast
166 isolates. BLASTn searches for *26S rRNA* and ITS sequence data in the National Center

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167 for Biotechnology Information (NCBI) GenBank database
168 (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to extract the sequences for related
169 taxa and were utilized as references for the subsequent phylogenetic analysis. All of the
170 sequences obtained in this study were manually edited with the reference alignment
171 editor BIOEDIT and aligned together with the reference sequences using CLUSTAL X
172 ver. 1.7 software (Thompson 1997). The phylogenetic tree was constructed by the
173 Maximum Likelihood method (Guindon and Gascuel 2003) using the best models:
174 (Kimura 2 Parameter + I + G) for *26S* rRNA and Tamura 3 parameter + I + G for ITS
175 fragments selected by using jModelTest ver 3.06 software, based on the Akaike
176 Information Criterion (AIC) (Posada 2008). Statistical validation at each node was
177 determined by 1,000 bootstrap replicates. A zygomycete, *Rhizopus microsporum,* was
178 employed as out-group for tree rooting.

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179 *Determination of enzymatic activities*

180 The productions of the following enzymes were analyzed: amylase; pectinase; xylanase;
181 cellulase; protease, and pectinase. Enzymatic activities were performed by initially
182 growing the isolates in YPD broth for 24 h at 28ºC. Afterward, 100 µL aliquots were
183 inoculated on the specific culture media for the investigation of each enzyme. The plates
184 were incubated at 28ºC during 72 h. Screening for amylases and pectinases was
185 performed in Castañeda medium (Castañeda-Agullo 1956), into which 2% (w/v) of starch
186 or 1% (w/v) of pectin was added, correspondingly. For xylanase and cellulase screening,
187 the Congo red medium was produced (Hankin and Anagnostakis 1977; Suto et al. 2002).
188 For protease detection, the skim-milk medium was utilized (Atlas 1997). Positive

189 amylase activity was revealed by the presence of a clear zone around a colony after
190 immersing the yeasts colonies in Lugol solution. Positive pectinase activity was
191 recognized by the presence of a clear ring around a colony following the immersion in
192 Cethyl Trimetyl Ammonium Bromide (CTAB) solution (CTAB 5%, w/v). Positive
193 degradation of cellulose, xylan, or casein was identified in the corresponding medium by
194 the presence of a clear ring around a colony. The Enzymatic Index (EI) was determined
195 within 72 h of incubation, which was expressed as the relationship between the average
196 diameter of the transparent (degradation) ring and the average diameter of the colony
197 (Hankin and Anagnostakis 1975).

198 *Determination of the potential of plant growth- promoting traits for isolates*

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199 Phosphate solubilization capacity was evaluated with Pikovskaya medium (Paul and
200 Sundara Rao 1971) and was considered positive if a clear ring appeared around the
201 colony. Polyamine production was detected using the Long Asthon Decarboxylase (LAD)
202 medium (Arena and Manca de Nadra 2001), with the color change of the medium from
203 yellow to red considered as a positive test. Siderophore production was evaluated
204 according to Schwyn and Neilands (1987) using Cromo-Azurol S (CAS) medium, and the
205 color change of the medium to yellow, orange, or purple around the colony was
206 considered as a positive test. IAA production was evaluated in YPD broth according to
207 Nassar et al. (2005) modified by Limtong and Koowadjanakul (2012); the appearance of
208 a pink color within 30 min after treatment with Salkowski reagent was recorded as a
209 positive test.

210

211 Quantification of IAA

212 Quantification of the IAA produced by the yeast isolates was carried out using the
213 Salkowski method (Glickmann and Dessaux, 1994). One pre-inoculum of each yeast was
214 prepared in YPD at 28°C for 24 h. One mL of this culture was employed to inoculate a
215 second flask with YPD medium and was incubated at 28ºC at 150 rpm, until reaching
216 about 10^8 cell/mL^{-1}, at which time tryptophan 1% (Sigma™) was added. One- mL aliquots
217 were taken every 24 h; these were placed in an Eppendorf tube and centrifuged at 1,500
218 rpm/5 min. The supernatant was transferred into a new tube, and the same amount of
219 Salkowski reagent was added to this (12 g.L^{-1} $FeCl_3$, 7.9 M H_2SO_4), the reaction was left
220 for 45 min at 28°C under conditions of darkness, and the reaction was read in a
221 spectrophotometer at 540 nm. The quantity of IAA was determined using a standard
222 curve with a range of concentrations of 1, 2, 3, 5, 6, 8, 9, 10, 15, and 20 µg.mL^{-1}. The
223 standard curve was prepared using a stock solution of IAA (JT Baker®) with a final
224 concentration of 1 mg.mL^{-1}, effecting proper dilutions in YPD medium. Each
225 determination was carried out in triplicate. Statistical analysis by means of two-way
226 Analysis of variance (ANOVA) was conducted using the method of Duncan with $p < 0.05$
227 to determine significant differences.

228 Effect of IAA produced by rhizospheric yeast on *Brassica juncea* seed germination

229 Five hundred µL of sterile, filtered supernatants from the cultures described previously
230 and containing 4 µg of IAA were allowed to be absorbed onto Petri dishes containing
231 water agar. Ten seeds of *Brassica juncea* disinfected with 2% sodium hypochlorite were
232 placed in each Petri dish. The negative control had 10 seeds per Petri dishes without IAA.

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233 All Petri dishes were incubated at 28°C and germination percentage was determined at 24
234 and 48 h. All assays were performed in triplicate. Kruskal-Wallis statistical analysis was
235 performed to evaluate the germination percentage and the one-way ANOVA were
236 conducted to evaluate plant growth. The means of each experiment were compared using
237 the Duncan multiple range test. All analyses were carried out using SigmaPlot ver. 12.0
238 statistical software (Copyright© 2006; Systat Software, Inc.), and we considered p <0.05
239 acceptable for statistical significance.

240 *Resistance of the isolates to HM and arsenic*

241 Minimum Inhibitory Concentration (MIC) was evaluated using resistance to HM and
242 arsenic in MES-Buffered Minimal Medium (MBMM) proposed by Rathnayake et al.
243 (2013). HM and As were supplemented at concentrations of 0.05, 0.1, 2.0, 4.0, 10, 20, 30,
244 40, 50, 60, 70, 80, 90, and 100 mM) in the forms of $CuSO_4$, $ZnSO_4$, $Pb(NO_3)_2$,
245 NaH_2AsO_4, and $NaAsO_2$. Inoculation and incubation conditions were the same as those
246 for resistance to salinity.

247 *Oxidation and reduction of arsenic compounds by the isolates*

248 The ability to oxidize the arsenite or reduce the arsenate of the yeast isolates was
249 estimated by utilizing the $KMnO_4$ qualitative test (Salmassi et al. 2002). The yeasts were
250 cultured in liquid Chemically Defined Medium (CDM) (Weeger et al. 1999) medium
251 containing NaH_2AsO_4 or $NaAsO_2$ at a final concentration of 100 $mg.L^{-1}$ with an initial
252 Optical Density $(OD)_{600}$ of 0.4. The culture was incubated for 5 days with shaking (120
253 rpm). Then, 20 µL of $KMnO_4$ solution (0.01 $mol.L^{-1}$) was added to 1 mL of the culture.

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254 The presence of pink color in the broth indicates positivity for arsenite oxidation and
255 generation of yellow color indicated arsenate reduction. The isolates positive in the
256 qualitative test were evaluated with the quantitative method of Molybdenum blue (Hu et
257 al. 2012).

258 **Results**

259 *Plant Identification and Soil Characterization*

260 A total of five plant species were collected in the region of Villa de La Paz. At the mine
261 tailing site, the plants *Sphaeralcea angustifolia*, *Prosopis* spp., *Flaveria angustifolia*, and
262 *Thitonia diversifolia* were the most abundant, while *Bahia absinthifolia, Sphaeralcea*
263 *angustifolia*, and *Prosopis* spp. comprised the main plants on the hill. Soils at both
264 sampling sites were seriously contaminated by multiple HM and As, especially As, Cd,
265 Cu, Mn, Pb, and Zn, which presented at concentrations (mg.kg^{-1}) of 3,574, 129, 2,012,
266 1,382, 555, and 1,337 in the mine tailing, and of 1,071, 62, 749, 3,054, 5,488, and 1,672
267 on the hill, respectively (detailed information available in Supplementary Table S1). In
268 addition, greater values related with nutrient features were observed in the hill soil, such
269 as Cation Exchange Capacity (CEC), Organic Carbon (OC), Total Nitrogen (TN), and
270 Total Phosphorous (TP), were 77 cmolc.kg^{-1}, 7.7%, 0.32 mg.kg^{-1}, and 69 mg.kg^{-1},
271 respectively, while corresponding values were 38, 1.9, 0.09, and 33 at the mine tailing.

272 *Yeast Isolation and Identification*

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273 During the isolations, we found that the abundance of yeasts in rhizosphere soils was
274 around 200 $CFU.g^{-1}$ of soil. In total, 31 rhizospheric yeasts representing different colony
275 morphology were isolated from the sampled plants (Table 1). The D1/D2 fragment of the
276 *26S rRNA* gene was amplified from all of the isolates, with the expected size of 600 pb.
277 Additionally, the ITS fragment was amplified with the same expected size. The
278 nucleotide sequences acquired in this study have been deposited in GenBank under
279 accession numbers KP455389-KP455419 for *26S rRNA* and KT716400-KT716430 for
280 the ITS fragment.

281 Phylogenetic analyses of the D1/D2 sequences grouped the isolates within the phyla
282 *Basidiomycota* (29 isolates) and *Ascomycota* (two isolates), with 25 isolates belonging to
283 the genus *Cryptococcus* (80.64%), two to *Rhodotorula* (6.45%), two to *Exophiala*
284 (6.45%), one to *Trichosporon* (3.22%), and one to *Cystobasidium* (3.22%) (Fig. 1A). The
285 majority of the isolates exhibited high sequence coverage and identity (100 and 99–
286 100%) with the defined species (data available as Supplementary Table S2). Only two
287 isolates, YR30 and YR31, presented 100% coverage and 97% identity with *Exophiala*
288 *pisciphila*. Twenty four isolates were identified as *Cryptococcus* spp. related with the
289 species *Cryptococcus albidus/Cryptococcus kuetzingii/Cryptococcus adeliensis*; YR14
290 was designed as *Cryptococcus uzbekistanensis*, isolate YR11 was identified as
291 *Cystobasidium slooffiae*, and finally, isolate YR20 was designated as *Trichosporon* spp.
292 and was related with the group *Trichosporon japonicum/Trichosporon*
293 *coremiiforme/Trichosporon insectorum.*

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294 The ITS sequences demonstrated the following information: The *Cryptococcus* genus
295 was the most common and was represented mainly by the species *C. albidus* and *C.*
296 *uzbekistanensis* (25 isolates exhibited an identity range of 99–100%). The genus
297 *Rhodotorula mucilaginosa* was represented by three isolates (YR24, YR29, and YR11).
298 The *Exophiala* genus was represented by isolates belonging to the species *Exophiala*
299 *capensis* with low identity of 87%. Finally, isolate YR20 was again designated as
300 *Trichosporon*, related with the group *T. japonicum*, *T. insectorum*, *Trichosporon faecale*,
301 and *Trichosporon asteroides,* as depicted in Fig. 1B.

302 *Enzymatic Activity and Potential Plant Growth- promoting Features of Rhizospheric*
303 *Yeast Isolates*

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304 Results of the enzymatic characterization demonstrated that 51.6% (16/31), 51.6%
305 (16/31), 41.9% (13/31), 12.9% (4/31), and 0% of the rhizospheric yeasts presented
306 pectinase, protease, xylanase, cellulase, and amylase activities, respectively (Table 1).
307 For the plant growth promoting features, only siderophore and IAA productions were
308 detected among some isolates at the proportions of 16.1% (5/31) and 9.67% (3/31),
309 respectively. The species *R. mucilaginosa* (two isolates) and *C. sloffiae* (one isolate) were
310 positive for AIA production, and both genera were selected for the IAA quantification.
311 Polyamine production and phosphate solubilization were absent in all isolates.

312 *IAA Quantification of the Two Isolated Yeasts*

313 Three yeasts are capable of producing IAA, two of them belonging to *R. mucilaginosa*
314 (YR29 and YR24,*)* with auxin produced concentrations of 9.61 and 9.02 $mg.mL^{-1}$ in 7

315 days respectively, while *C. slooffiae* produced only 6.8 $mg.mL^{-1}$, with a significant
316 difference in IAA production between strains and between incubation times ($p < 0.05$). In
317 addition, the concentration of IAA increased as time passed from incubation (Fig. 2).

318 Germination Promotion of *Brassica juncea* Seed by IAA Secreted by Rhizospheric
319 Yeasts

320 A significant increase was observed in seed germination of *B. juncea* with treatments
321 containing filtrates of rhizospheric yeasts (YR24, YR11, and YR29) compared with the
322 control. Seed germination at 48 h of incubation increased by >70% in all treatments
323 including the control. Only the treatment with the YR24 strain filtrate demonstrated a
324 difference significant difference ($p < 0.05$) from the rest of these, with 96.6% germination
325 (Fig. 4). In the case of seedling growth, in all treatments supplemented with filtrate yeast,
326 seedling growth exhibited a significant increase compared with the control ($p < 0.05$)
327 (Figs. 3 and 4). These results suggest that the IAA produced by the rhizospheric yeasts
328 promotes germination and plant growth under the conditions tested.

329 *Heavy-metal Resistance and Arsenic Oxidation/Reduction*

330 The isolated yeasts demonstrated high resistance to Arsenic (As^{3+} and As^{5+}), but were
331 sensitive to the remaining HM, especially to Cu. The MIC of the 31 isolates in MBMM
332 was 0–5 mM for $ZnSO_4$, 0–1.2 mM for $Pb(NO_3)_2$, 0–0.5 mM for $CuSO_4$, 0–30 mM for
333 NaH_2AsO_4, and 0–100 mM for $NaAsO_2$ (Table 2). The order of toxicity of RE to the
334 isolates was $Cu>Zn>Pb>As^{5+}>As^{3+}$. Higher resistance was observed among yeasts
335 isolated from the mining tailing. Arsenic oxidation/reduction assays showed that 51.6%

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336 (16/31) of the rhizospheric yeasts can reduce arsenate, but none of these oxidized
337 arsenite; all positive isolates belonged to the species *C. albidus* and exhibited a reduction
338 percentage within the range of 10–40% in samples with 0.15 mM of As^{V} (Table 2),

339 **Discussion**

340 In the present study, the rhizospheric yeasts were isolated and characterized, to our
341 knowledge for the first time, from the plants grown in a region that were seriously
342 contaminated with multiple HM and arsenic. Of the two sampling sites, both the hill and
343 the mining tailing could be classified as extreme environments based on their low
344 nutrients and high As, Pb, Zn, Mn, Cd, and Cu (see data in Supplementary Table S1). The
345 mine tailing possesses high concentration of HM and arsenic (As, Pb, Zn, Mn, Cd, and
346 Cu), poor organic matter, low nitrogen, and is slightly salty and slightly acidic to neutral
347 pH, while the hill also possesses high concentrations of HM and arsenic; however, the
348 concentration of nitrogen and organic matter presented a normal-to-high range and was
349 slightly acid to neutral pH. The parameters of these soils are important because they
350 could affect the bioavailability of HM and arsenic and their toxicity for plants and
351 microorganisms (Jasim et al. 2014).

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352 Yeasts have been isolated from different environments, including extreme environments
353 and from the rhizosphere of some plants (Hong et al. 2002; Hong et al. 2006), and are
354 employed as biocontrol agents or plant growth promoters (El-Mehalawy 2004; Mestre et
355 al. 2011). However, little information is available on yeasts associated with HM- and
356 arsenic-resistant plants. Deng and contributors (Deng et al. 2012) characterized an HM-
357 resistant endophytic yeast, *Cryptococcus* spp. CBSB78, which was resistant to 20 mM

358 Cd^{2+}, 20 mM Pb^{2+}, 10 mM Zn^{2+}, and 7 mM Cu^{2+}, and could improve the growth and
359 heavy-metal extraction of inoculated *Brassica* plants. Castro-Silva et al. (2003) isolated
360 and analyzed 12 HM-resistant yeasts from water, sediment/soil, and plant resources,
361 which exhibited HM resistance to 1 mM $CdCl_2$ and/or 1–20 mM $ZnCl_2$, but the authors
362 did not identify the isolates.

363 Currently, molecular identification employing the large-subunit rDNA D1/D2 domain
364 and ITS fragment sequence analysis has been utilized to estimate the taxonomic
365 affiliation of the yeasts (Kurtzman and Robnett 1998; Linton et al. 2007; Gardes and
366 Bruns 1993). In the present study, these techniques could successfully identify the
367 isolates into five genera (Figs. 1A and 1B) (Table 1). However, species affiliations were
368 uncertain in the following one case: the isolate YR20 possessed a sequence nearly
369 identical to those of *T. japonicum*, *T. insectorum*, and *T. coremiiforme* on *26S rRNA*
370 analysis and was related with the group including *T. japonicum, T. insectorum, T. faecale*,
371 and *T. asteroides* on ITS. These cases indicated the inability of two molecular markers
372 for differentiating closely related species; therefore, the exact species definition of
373 *Trichosporon* spp. isolates requires additional molecular analysis, such as multi-locus
374 sequencing (Bovers et al. 2008; Bernhardt et al. 2014; Linton et al. 2007).

375 Both the abundance and the taxon number detected in this study were rather low, which
376 might be related with the extreme environmental factors, especially the high
377 concentrations of HM and arsenic. Botha (2006) revealed that the pH and Cu
378 concentration in soil were the principal factors for decreasing yeast number. In our study,
379 Cu concentration reached 2,012 and 749 $mg.kg^{-1}$ soil at the mining tailing and at the hill

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380 soil, respectively, much greater than their MIC values (0–0.5 mM ≈0–32.8 $mg.kg^{-1}$) for
381 the isolates (Table 2). These results suggest a difference in the rhizospheric Cu
382 concentration and in the soil surrounding the plants, suggesting positive interaction
383 between yeast and plants. These possible interactions require further investigation.

384 Previously, *Cryptococcus*, *Rhodotorula*, and *Trichosporon* have been isolated from
385 phylloplane and rhizosphere of sugar cane (Biswas et al. 2001; Insuellas de Azeredo et al.
386 1998) and from bulk and rhizosphere soils in forest region (Hong et al. 2002; Mestre et
387 al. 2011). The isolation of these in the present study further confirmed their ubiquitous
388 distribution.

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389 Identification of *Cryptococcus* as the main group in our study was similar to previous
390 reports that *Cryptococcus* comprised the cosmopolitan yeasts in different soils (Slavikova
391 and Vadkertiova 2003; Slavikova et al. 2007; Vishniac 1995) and in
392 rhizosphere/endophytic environments (Deng et al. 2012; Insuellas de Azeredo et al. 1998;
393 Mestre et al. 2011). The *C. albidus* clade, which covered the majority of the isolates
394 obtained in the present study, is the most important organism in sandy and desert soils,
395 described as dominant in Mexican soils (Vishniac 2006). However, all of the three
396 species in this clade that is, *C. albidus*, *C. adeliensis*, and *C. kuetzingii* (synonym for
397 *Cryptococcus albidus* var. *kuetzingii*) have rarely been isolated as pathogens (Liu et al.
398 2013; Tintelnot and Losert 2005).

399 In the past decade, *Rhodotorula* species, including *R. mucilaginosa*, which covered two
400 isolates in this study, are found to be ubiquitous in soil and rhizosphere (Hong et al.

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401 2002). Similar to *Cryptococcus, Rhodotorula* species belonged to the capsulated yeast,
402 but presented an orange or red color. Both capsule and pigments were able to protect
403 these from different stress conditions, such as radiation (Molinè et al. 2010).

404 The genus *Trichosporon* contained species that are widely distributed in different
405 environments. It has rarely infected immunocompromised persons as a pathogen
406 (Colombo et al. 2011). Some of the saprophytic species possessed capacities for
407 bioremediation and biotechnology (Santos et al. 2001). *T. insectorum* is a killer yeast
408 isolated from the gut of insects and artisanal cheese (Fuentefria et al. 2008), while *T.*
409 *coremiiforme* could degrade corncob acid and produce diverse lipids (Huang et al. 2013).

410 Previously, little information on *Exophiala* and *Cystobasidium* from the environments
411 was available. In the present study, two isolates were identified as members of *Exophiala*,
412 classified as a Dark Septate Endophyte (DSE); there are some reports concerning the
413 extreme Cadmium (Cd) tolerance of *Exophiala pisciphila*, including metal ion binding
414 and transportation, and organic acid metabolism (Zhao et al. 2015). Because DSE are
415 typical root endophytes, their potential effects on host plants should also be considered.
416 Under heavy-metal stress, these fungi can affect heavy metal uptake and the tolerance of
417 the host plants, e.g., *Zea mays* L. (Li et al. 2011). *Cystobasidium* is a genus described as
418 living in different associations with other fungi, lichens, and woods; in addition, some
419 isolates have clinical relevance (Yurkov et al. 2015). To our knowledge, this is the first
420 time that *Cystobasidium* yeast has been isolated from rhizosphere of HM- and arsenic-
421 resistant plants. Detection of *Cystobasidium* and *Exophiala* in the present study enlarged

422 the diversity of rhizosphere yeasts, especially in HM-contaminated regions, and implied
423 that they may play some ecological role in HM-contaminated soils.

424 In tests for enzymatic and potential plant growth- promoting activities (Table 1),
425 considerable proportions of the isolates presented pectinases (51.6%), proteases (51.6%),
426 and xylanases (42.9%), but only 12.9% of the isolates presented cellulases. These
427 enzymes are involved in the degradation of the plant cell wall (Riou et al. 1991; Schulz
428 and Boyle 2005). Therefore, the presence of these enzymes may help these rhizospheric
429 yeasts to colonize the rhizosphere.

430 Previously, it was reported that some fungi, including the *Rhodotorula* yeast, could
431 produce siderophore (such as rhodotorulic acid) under iron-deficient conditions, which
432 exerts an antagonic effect against other filamentous fungi (Calvente et al. 2001).
433 However, the soils involved in the present study are iron-rich (about 35 g Fe kg^{-1} soil);
434 thus, siderophore production should not be related with iron deficiency, but rather linked
435 with other functions. It has been reported that the siderophore could increase producer-
436 resistance to HM or metalloids (Schalk et al. 2011), increase As (Shah et al. 2010),
437 improve soil fertility, and inhibit fungal pathogens (Ali and Vidhale 2013; Matthijs et al.
438 2007; Shah et al. 1992). The six siderophore-producing yeasts were defined as members
439 of four genera and were isolated from rhizosphere of three plant species grown at both
440 sites: *Cryptococcus* spp. YR6, YR12, and *Trichosporon* spp. YR20 from *Flaveria*
441 *angustifolia, Sphaeralcea angustifolia*, and *Prosopis* plants, respectively, grown at the
442 mine tailing, *Rhodotorula* spp. YR24 and *Exophiala* spp. YR30 and YR31 from *Prosopis*
443 plants grown at the hill. However, the low proportion (16.1% of the isolates) and the low

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444 index implied that siderophore production might not comprise determinants for the
445 colonization and survival of yeasts in the rhizosphere of plants grown in the tested
446 environments.

447 IAA production was detected in three isolates, associated with three plant species grown
448 at the mine tailing or at the hill. These were members of *Cystobasidium* spp. YR11 (from
449 *F. angustifolia*, mine tailing) *Rhodotorula* spp. YR24 (from *S. angustifolia* at the hill),
450 YR29 (from *Prosopis* spp. at the hill). The genera *Rhodotorula* and *Cystobasidium*
451 produced IAA in amounts of 6.8–9.61 μg/mL, and these concentrations are considered
452 low in comparison with other genera in *Ascomycota*, such as *Candida maltosa*, *Candida*
453 *glabrata*, and *Candida tropicalis* (Limtong and Koowadjanakul 2012). Their low
454 proportion in this study demonstrated that this feature was not selected by the rhizosphere
455 in the soils tested. However, IAA production was sufficient for promoting seed
456 germination and plant growth. Polyamines and inorganic phosphate solubilization were
457 not detected among the yeasts isolated in this study, perhaps based upon the same reason
458 estimated for IAA production.

459 HM and As resistance has been reported for some yeasts (Olasupo et al. 1993,
460 Vadkertiová and Sláviková 2006). In our work, the yeast isolates demonstrated poor-to-
461 intermediate resistance to HM and As, compared with the results of other authors
462 (Vadkertiová and Sláviková 2006). However, *C. albidus*-related yeasts exhibited high
463 resistance to arsenic: up to 30 mM for arsenate and 100 mM for arsenite. In general,
464 arsenate is less toxic than arsenite, and greater resistance to arsenate than arsenite has
465 been reported in some bacteria and fungi. Thus, it could be estimated that these 13 yeast

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466 isolates may possess a different mechanism for arsenite resistance. A mechanism related
467 with this estimation has been well studied in *Saccharomyces cerevisiae*, in which the
468 arsenate was reduced to arsenite and was expelled from the cell (Čerňanský et al. 2009).
469 In nature, arsenite can be also detoxified by methylation, which transforms the arsenite
470 into a volatile form (Su et al. 2011). Further study is required on our isolates in order to
471 ascertain whether they possess the methylation function or another. To date, scarce
472 information is available on As transformation by yeasts, and there is no report on As
473 transformation by *Cryptococcus*. Therefore, our study was, to our knowledge, the first
474 report on arsenate reduction by this yeast.

475 In conclusion, yeasts associated with the rhizosphere of *S. angustifolia*, *Prosopis* spp.,
476 *Thitonia diversifolia*, *F. angustifolia*, and *Bahia absinthifolia* plants grown in soils
477 seriously contaminated with arsenic and other HM presented low abundance (10^2 CFU g^{-1}
478 of soil) and low diversity, covering only five species in five genera. The *Cryptococcus*
479 group comprised the major yeast found in all of the five plants grown at both sampling
480 sites. Many yeast isolates produce pectinases, proteases, and cellulases and have a low
481 production of siderophore and IAA. The majority of the yeasts demonstrated resistance to
482 salinity, alkaline conditions, and also resistance to multiple HM and arsenic at high
483 concentrations. Some isolates presented higher resistance to arsenite than to arsenate and
484 they reduced arsenate to arsenite. Future study to evaluate the capacity for transforming
485 As^{3+} into methyl-compounds and the potential for bioremediation is needed.

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495 **References**

496 Al-gabr, H.M., Zheng, T., Yu, X. 2014. Occurrence and quantification of fungi and
497 detection of mycotoxigenic fungi in drinking water in Xiamen City, China [online]. Sci.
498 Total. Environ. 466–467:11031111. doi: 10.1016/j.scitotenv.2012.12.060

499 Ali, S.S., and Vidhale, N.N. 2013. Bacterial siderophore and their application: a review.
500 Int. J. Curr. Microbiol. Appl. Sci. 2:303–331.

501 Allers, T., and Lichten, M. 2000. A method for preparing genomic DNA that restrains
502 branch migration of Hollyday junctions. Nucleic Acids Res. 15: 28(2):e6.

503 Alloway, B.J. 1995. Heavy Metals in Soils. 2nd. ed. London, U.K.: Blackie Academic &
504 Professional.

505 Arena, M.E., Manca De Nadra, M.C. 2001. Biogenic amine production by *Lactobacillus*
506 [online]. J Appl. Microbiol. 90: 158–162. doi: 10.1046/j.1365-2672.2001.01223.x

Draft

507 Atlas, R.M. 1997. Handbook of Microbiological Media. Boca Raton, FL, USA: CRC
508 Press.

509 Bernhardt, A., von Bomhard, W., Antweiler, E., et al. 2014. Molecular identification of
510 fungal pathogens in nodular skin lesions of cats [online]. Med. Mycol. 53:132–144. doi:
511 10.1093/mmy/myu082

512 Biswas, S.K., Yokoyama, K., Nishimura, K., et al. 2001. Molecular phylogenetics of the
513 genus *Rhodotorula* and related basidiomycetous yeasts inferred from the mitochondrial
514 cytochrome *b* gene [online]. Int. J. Syst. Evol. Microbiol. 51:1191–1199.
515 doi: 10.1099/00207713-51-3-1191

516 Botha, A. 2006. Yeast in soil. In: Rosa CA, Peter G (Editors). The Yeast Handbook.
517 Biodiversity and Ecophysiology of Yeast. Berlin, Germany. Springer-Verlag; pp. 1–100.

518 Botha, A. 2011. The importance and ecology of yeasts in soil [online]. Soil Biol Biochem
519 43:1–8. doi: 10.1016/j.soilbio.2010.10.001

520 Bovers, M., Hagen, F., Kuramae, E.E., et al. 2008. Six monophyletic lineages identified
521 within *Cryptococcus neoformans* and *Cryptococcus gattii* by multi-locus sequence typing
522 [online]. Fungal Genet. Biol. 45:400–421. doi: 10.1016/j.fgb.2007.12.004

523 Bowen, H.J.M. 1979. Environmental chemistry of the elements. London, U.K.: Academic
524 Press.

525 Button, D.K., Dunker S.S., and Morse, M.L. 1973. Continuous culture of *Rhodotorula*
526 *rubra* kinetics of phosphate-limited growth. J. Bacteriol. 113(2):599–611.

527 Calderón de Rzedowski, G. and Rzedowski J., et al. 2001. Flora fanerogámica del Valle
528 de México. 2a ed., Instituto de Ecología, A. C. y Comisión Nacional para el conocimiento
529 y uso de la biodiversidad, Pátzcuaro (Michoacán). p. 1406.

530 Calvente, V., Orellano De, M.E., Sansone, G., et al. 2001. Effect of nitrogen source and
531 pH on siderophore production by *Rhodotorula mucilaginosa* strains and their application
532 to biocontrol of phytopathogenic moulds [online]. J. Indust. Microbiol. Biotechnol.
533 2:226–229

534 Castañeda-Agullo, M. 1956. Studies on the biosynthesis of extracellular proteases by
535 bacteria. I. *Serratia marcescens*, synthetic and gelatin media [online]. J. Gen. Physiol.
536 39:369–375 doi: 10.1085/jgp.39.3.369

537 Castro-Silva, M.A., De Souza Lima, A.O., Gerchenski, A.V., et al. 2003. Heavy metal
538 resistance of microorganisms isolated from coal mining environments of Santa Catarina
539 [online]. Braz. J. Microbiol. 34:45–47, doi: 10.1590/S1517-83822003000500015

540 Čerňanský, S., Kolenčík, M., Ševc, J., et al. 2009. Fungal volatilization of trivalent and
541 pentavalent arsenic under laboratory conditions [online]. Biores. Technol. 100:1037–
542 1040. doi: 10.1016/j.biortech.2008.07.030

Draft

543 Colombo, A.L., Padovan, A.C., Chaves, G.M. 2011. Current knowledge of *Trichosporon*
544 spp. and Trichosporonosis [online]. Clin. Microbiol. Rev. 24:682–700 doi:
545 10.1128/CMR.00003-11

546 Deng, Z., Wang, W., Tan, H., et al. 2012. Characterization of heavy metal-resistant
547 endophytic yeast *Cryptococcus* spp. CBSB78 from rapes (*Brassica chinensis*) and its
548 potential in promoting the growth of *Brassica* spp. in metal-contaminated soils [online].
549 Water Air Soil Pollut. 223:5321–5329 doi: 10.1007/s11270-012-1282-6

550 Donahoe-Christiansen, J., D'Imperio, S., Jackson, C.R., et al. 2004. Arsenite-oxidizing
551 *Hydrogenobaculum* strain isolated from an acid-sulfate-chloride geothermal spring in
552 Yellowstone National Park [online]. Appl. Environ. Microbiol. 70:1865–1868. doi:
553 10.1128/AEM.70.3.1865-1868.2004

554 El-Mehalawy, A. 2004. The rhizosphere yeast fungi as biocontrol agents for wilt disease
555 of kidney bean caused by *Fusarium oxysporum* [online]. Int. J. Agri. Biol. 6:310–316 doi:
556 1560-8530/2004/06-2-310-316

557 Franco-Hernández M.O., Vásquez-Murrieta, M.S., Patiño-Siciliano, A., et al. 2010.
558 Heavy metals concentration in plants growing on mine tailings in Central Mexico
559 [online]. Bioresour. Technol. 101:3864–3869 doi: 10.1016/j.biortech.2010.01.013

560 Fuentefria, A.M., Suh, S.O., Landell, M.F. et al. 2008. *Trichosporon insectorum* spp.
561 nov., a new anamorphic basidiomycetous killer yeast [online]. Mycol. Res. 112:93–99
562 doi: 10.1016/j.mycres.2007.05.001

Draft

563 Gardes, M., Bruns, T.D. 1993. ITS primers with enhance specificity for *Basidiomycetes*-
564 application to the identification of mycorrhizae and roots. Mol. Ecol. 2:113-118.

565 Gihring, T.M., Banfield, J.F. 2001. Arsenite oxidation and arsenate respiration by a new
566 *Thermus* isolate. FEMS Microbiol. Lett. 204:335–340. doi: 10.1111/j.1574-
567 6968.2001.tb10907.x

568 Glickmann, E., Dessaux, Y. 1994. A critical examination of the specificity of the Salkoski
569 reagent for indolic compounds produced by phytopathogenic bacteria. Appl. Environ.
570 Microbiol. 61:793–796.

571 Guindon S and Gascuel O. 2003. A simple and accurate algorithm to estimate large
572 phylogenies by maximum likelihood. Syst Biol 52:696–704

573 Hankin, L., Anagnostakis, S.L. 1975. The use of solid media for detection of enzyme
574 production by fungi [online]. Mycologia. 67:597–607 doi: 10.2307/3758395

575 Hankin, L., Anagnostakis, S.L. 1977. Solid media containing carboxymethylcellulose to
576 detect CX cellulose activity of micro-organisms [online]. J. Gen. Microbiol. 98:109–115.
577 doi: 10.1099/00221287-98-1-109

578 Hind S.J., Mushrifah I., Aminah A., et al. 2012 Effects of physicochemical soil properties
579 on the heavy metal concentrations of diplaziumesculentum (medicinal plant) from the
580 UKM and tasikchini, Malaysia. Int. J. Chem. Tech. Res. 6(4):5519–5527.

Draft

581 Hong, S.G., Lee, K.H., Bae, K.S. 2002. Diversity of yeasts associated with natural
582 environments in Korea. J. Microbiol. 40:55–62

583 Hong, S.G., Lee, K.H., Kwak, J., et al. 2006. Diversity of yeasts associated with *Panax*
584 *ginseng*. J. Microbiol. 44:674–679.

585 Hu, S., Lu, J., and Jing, C. 2012. A novel colorimetric method for field arsenic speciation
586 analysis. J. Environ. Sci. 24:1341–1346.

587 Huang, C., Chen, X.F., Xiong, L., et al. 2013. Microbial oil production from corncob acid
588 hydrolysate by oleaginous yeast *Trichosporon coremiiforme* [online]. Biomass. Bioenerg.
589 49:273–278. doi: 10.1016/j.biombioe.2012.12.023

590 Ilyas, S., Rehman, A., Varela, A.C., et al. 2014. Redox proteomics changes in the fungal
591 pathogen *Trichosporon asahii* on arsenic exposure: identification of protein response to
592 metal-induced oxidative stress in an environmentally-sampled isolate [online]. PLOS.
593 ONE. 25:9(7):e102340. doi: 10.1371/journal.pone.0102340

594 Ilyas, S., Rehman, A. 2015. Oxidative stress, glutathione level and antioxidant response
595 to heavy metals in multi-resistant pathogen, *Candida tropicalis* [online]. Environ. Monit.
596 Assess. 187:4115. doi: 10.1007/s10661-014-4115-9

597 Insuellas de Azeredo, L.A., Gomes, E.A.T., Mendonça-Hagler, L.C., et al. 1998. Yeast
598 communities associated with sugarcane in Campos, Rio de Janeiro, Brazil. Int. Microbiol.
599 1:205–208.

600 Kurtzman, C.P., Robnett, C.J. 1998. Identification and phylogeny of ascomycetous yeasts
601 from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences [online].
602 Anton. Leeuw. Int. J. G. 73:331–371. doi: 10.1023/A:1001761008817

603 Li, T., Liu, M.J., Zhang, H.B., Sha, T., et al. 2011. Improved tolerance of maize (*Zea*
604 *mays* L.) to heavy metals by colonization of a dark septate endophyte (DSE) *Exophiala*
605 *pisciphila* [online]. Sci. Total. Environ. 409:1069–1074.

606 Limtong, S., Koowadjanakul, N. 2012. Yeasts from phylloplane and their capability to
607 produce indole-3-acetic acid [online]. World. J. Microbiol. Biotechnol. 28:3323–3335.
608 doi: 10.1007/s11274-012-1144-9

609 Linton, C.J., Borman, A.M., Cheung, G. et al. 2007. Molecular identification of unusual
610 pathogenic yeast isolates by large ribosomal subunit gene sequencing: 2 years of
611 experience at the United Kingdom Mycology Reference Laboratory [online]. J. Clin.
612 Microbiol. 45:1152–1158. doi: 10.1128/JCM.02061-06

613 Liu, Y., Ma, S., Wang, X., Xu, W., et al. 2013. *Cryptococcus albidus* encephalitis in
614 newly diagnosed HIV-patient and literature review [online]. Med. Mycol. Case. Rep.
615 3:8–10. doi: 10.1016/j.mmcr.2013.11.002

616 Matthijs, S., Tehrani, K.A., Laus, G. et al. 2007. Thioquinolobactin, a *Pseudomonas*
617 siderophore with antifungal and anti-*Pythium* activity [online]. Environ. Microbiol.
618 9:425–434. doi: 10.1111/j.1462-2920.2006.01154.x

Draft

619 McVaugh, R. 1984. A Descriptive Account of the Vascular Plants of Western Mexico.
620 Vol. 12, Compositae. USA: The University of Michigan; p. 1157.

621 McVaugh, R. 1987. A Descriptive Account of the Vascular Plants of Western Mexico.
622 Vol. 5, Leguminosae. USA: The University of Michigan; p. 786.

623 Menezes, R.A., Amaral, C., Delaunay, A., Toledano, M., et al. 2004. Yap8p activation in
624 *Saccharomyces cerevisiae* under arsenic conditions [online]. FEBS. Lett. 566:141–146.
625 doi: 10.1016/j.febslet.2004.04.019

626 Mestre, M.C., Rosa, C.A., Safar, S.V., et al. 2011. Yeast communities associated with the
627 bulk-soil, rhizosphere and ectomycorrhizosphere of a *Nothofagus pumilio* forest in
628 northwestern Patagonia, Argentina [online]. FEMS. Microbiol. Ecol. 78:531–541. doi:
629 10.1111/j.1574-6941.2011.01183.x

630 Moliné, M., Flores, M.R., Libkind, D., et al. 2010. Photoprotection by carotenoid
631 pigments in the yeast *Rhodotorula mucilaginosa*: the role of torularhodin [online].
632 Photochem. Photobiol. Sci. 9:1145–1151. doi: 10.1039/c0pp00009d

633 Nassar, A.H., El-Tarabily, K.A., Sivasithamparam, K. 2005. Promotion of plant growth
634 by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea*
635 *mays* L.) roots [online]. Biol. Fertil. Soils. 42:97–108. doi: 10.1007/s00374-005-0008-y

Draft

636 Olasupo, N.A., Scott-Emuakpor, M.B., Ogunshola, R.A. 1993. Resistance to heavy
637 metals by some Nigerian yeast strains [online]. Folia. Microbiol. (Praha). 38:285–287.
638 doi: 10.1007/BF02898595

639 Paul, N.B., Sundara Rao, W.V.B. 1971. Phosphate-dissolving bacteria in the rhizosphere
640 of some cultivated legumes. Plant. Soil. 35:127–132.

641 Posada, D. 2008. jModelTest: phylogenetic model averaging [online]. Mol. Biol. Evol.
642 25:1253–1256. doi: 10.1093/molbev/msn083

643 Qin, J., Rosen, B.P., Zhang, Y. et al. 2006. Arsenic detoxification and evolution of
644 trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase
645 [online]. Proc. Natl. Acad. Sci. U. S. A. 103:2075–2080. doi: 10.1073/pnas.0506836103

646 Rathnayake, I.V.N., Megharaj, M., Krishnamurti, G.S.R., et al. 2013. Heavy metal
647 toxicity to bacteria– Are the existing growth media accurate enough to determine heavy
648 metal toxicity? [online]. Chemosphere. 90:1195–1200. doi:
649 10.1016/j.chemosphere.2012.09.036

650 Redecker, D., 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi within
651 colonized roots. Mycorrhiza.10:73–80.

652 Riou, C., Freyssinet, G., Fevre, M. 1991. Production of cell wall-degrading enzymes by
653 the phytopathogenic fungus *Sclerotinia sclerotiorum*. Appl. Environ. Microbiol.
654 57:1478–1484.

Draft

655 Rosen, B.P. 2002. Biochemistry of arsenic detoxification [online]. FEBS. Lett.
656 529:86–92. doi: 10.1016/S0014-5793(02)03186-1

657 Salgado, A., López-Serrano, O.A., Matia-González AM, et al. (2012) Response to
658 arsenate treatment in *Schizosaccharomyces pombe* and the role of its arsenate
659 reductase activity. PLOS. ONE. 7(8):e43208. doi:10.1371/journal.pone.0043208

660 Salmassi, T.M., Venkateswaren, K., Satomi, M. et al. 2002. Oxidation of arsenite by
661 *Agrobacterium albertimagni*, AOL15, spp. nov., isolated from Hot Creek, California
662 [online]. Geomicrobiol. J. 19:53–66. doi: 10.1080/014904502317246165

663 Santos, V.L., Heilbuth, N.M., Linardi, V.R. 2001. Degradation of phenol by
664 *Trichosporon* spp. LE3 cells immobilized in alginate [online]. J. Basic. Microbiol.
665 41:171–178. doi: 10.1002/1521-4028(200107)41:3/43.0.CO;2-C

666 Schalk, I.J., Hannauer, M., Braud, A. 2011. New roles for bacterial siderophores in
667 metal transport and tolerance [online]. Environ. Microbiol. 13:2844–2854. doi:
668 10.1111/j.1462-2920.2011.02556.x

669 Schulz, B., and Boyle, C. (2005). The endophytic continuum. Mycol. Res. 109:661–
670 686.

671 Schwyn, B., and Neilands, J.B. 1987. Universal chemical assay for the detection and
672 determination of siderophores [online]. Anal. Biochem. 160:47–56. doi:
673 10.1016/0003-2697(87)90612-9

Draft

674 Shah, S., Karkhanis, V., Desai, A. 1992. Isolation and characterization of siderophore,
675 with antimicrobial activity, from *Azospirillum lipoferum* M. [online]. Curr. Microbiol.
676 25:347–351. doi: 10.1007/BF01577233

677 Shah, D., Shena, M.W.Y., Chen, W., et al. 2010. Enhanced arsenic accumulation in
678 *Saccharomyces cerevisiae* overexpressing transporters Fps1p or Hxt7p [online]. J.
679 Biotechnol. 150:101–107. doi: 10.1016/j.jbiotec.2010.07.012

680 Sláviková, E., and Vadkertiová, R. 2003. The diversity of yeasts in the agricultural
681 soil [online]. J Basic. Microbiol. 43:430–436. doi: 10.1002/jobm.200310277

682 Sláviková, E., Vadkertiová, R., Vránová, D. 2007. Yeasts colonizing the leaf surfaces
683 [online]. J. Basic. Microbiol. 47:344–350. doi: 10.1002/jobm.200710310

684 Su, S., Zeng, X., Bai, L. et al. 2011. Arsenic biotransformation by arsenic-resistant
685 fungi *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and
686 *Fusarium oxysporum* CZ-8F1 [online]. Sci. Total. Environ. 409:5057–5062. doi:
687 10.1016/j.scitotenv.2011.08.039

688 Suto, M., Takebayashi, M., Saito, K., et al. 2002, Endophytes as producers of
689 xylanase [online]. J. Biosci. Bioeng. 93:88–90. doi: 10.1016/S1389-1723(02)80059-7

690 Thompson, J.D., Gibson, T.J., Plewniak, F. et al. 1997. The CLUSTAL X windows
691 interface: flexible strategies for multiple sequence alignment aided by quality analysis
692 tools [online]. Nucleic. Acids. Res. 25:4876–4882. doi: 10.1093/nar/25.24.4876

Draft

693 Tintelnot, K., and Losert, H. 2005. Isolation of *Cryptococcus adeliensis* from clinical
694 samples and the environment in Germany [online]. J. Clin. Microbiol.
695 43:1007.doi: 10.1128/JCM.43.2.1007.2005

696 Todorova, T.T., Kujumdzieva, A.V., Vuilleumier, S. 2010. Non-enzymatic roles for
697 the URE2 glutathione S-transferase in the response of *Saccharomyces cerevisiae* to
698 arsenic [online]. Arch. Microbiol. 192:909–918. doi: 10.1007/s00203-010-0614-4

699 Trujillo-Cabrera, Y., Ponce-Mendoza, A., Vásquez-Murrieta, M.S., et al. 2013.
700 Diverse cellulolytic bacteria isolated from the high humus, alkaline-saline chinampa
701 soils [online]. Ann. Microbiol. 63:779–792. doi: 10.1007/s13213-012-0533-5

702 Turpeinen, R., Pantsar-Kallio, M., Kairesalo, T. 2002. Role of microbes in controlling
703 the speciation of arsenic and production of arsines in contaminated soils [online]. Sci.
704 Total. Environ. 285:133–145. doi: 10.1016/S0048-9697(01)00903-2

705 Vadkertiová, R., Sláviková, E. 2006. Metal tolerance of yeasts isolated from water,
706 soil and plant environments [online]. J. Basic. Microbiol. 46: 145–152. doi:
707 10.1002/jobm.200510609

708 Vásquez-Murrieta, M.S., Cruz-Mondragón, C., Trujillo-Tapia, N. et al. 2006. Nitrous
709 oxide production of heavy metal contaminated soil [online]. Soil. Biol. Biochem.
710 38:931–940. doi: 10.1016/j.soilbio.2005.08.007

Draft

711 Vishniac, H.S. 1995. Simulated *in situ* competitive ability and survival of a
712 representative soil yeast, *Cryptococcus albidus* [online]. Microb. Ecol. 30(3):309-20
713 doi: 10.1007/BF00171937

714 Vishniac, H.S. 2006. A multivariate analysis of soil yeasts isolated from a latitudinal
715 gradient [online]. Microb. Ecol. 52:90–103. doi: 10.1007/s00248-006-9066-4

716 Wang, S., Mulligan, C.N. 2006. Effect of natural organic matter on arsenic release
717 from soils and sediments into groundwater [online]. Environ. Geochem. Health.
718 28:197–214. doi: 10.1007/s10653-005-9032-y

719 Wang, S., Zhao, X. 2009. On the potential of biological treatment for arsenic
720 contaminated soils and groundwater [online]. J. Environ. Manage. 90:2367–2376.
721 doi: 10.1016/j.jenvman.2009.02.001

722 Weeger, W., Lièvremont, D., Perret, M. et al. 1999. Oxidation of arsenite to arsenate
723 by a bacterium isolated from an aquatic environment [online]. BioMetals. 12:141–
724 149. doi: 10.1023/A:1009255012328

725 Yurkov, A.M., Kachalkin, A.V., Daniel, H.M., et al. 2015. Two yeast species
726 *Cystobasidium psychroaquaticum* f.a. spp. nov. and *Cystobasidium rietchieii* f.a. spp.
727 nov. isolated from natural environments, and the transfer of *Rhodotorula minuta* clade
728 members to the genus *Cystobasidium* [online]. Anton. Leeuw. Int. J. G. 107:173–185.
729 doi: 10.1007/s10482-014-0315-0

Draft

730 Zhao, D., Li, T., Shen, M., Wang, J., et al. 2015. Diverse strategies conferring
731 extreme cadmium (Cd) tolerance in the Dark septate endophyte (DSE), *Exophiala*
732 *pisciphila*: evidence from RNA-seq data [online]. Microbiol. Res. 170:27–35. doi:
733 10.1016/j.micres.2014.09.005

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752 **Table and Supplementary Table Titles**

753

754 **Table 1.** Enzymatic activities and IAA production of the yeasts isolated of different plant
755 collected in the region of Villa de La Paz, San Luis Potosí, Mexico

756

757 **Table 2**. MIC of risk elements and capacity for oxidize or reducing arsenic of
758 rhizospheric yeasts

759

760 **Supplementary Table S1**. Physicochemical characteristic of the soil of the two sampling
761 sites in Villa de la Paz, San Luis Potosí, Mexico

762

763 **Supplementary Table S2.** Yeasts identified based upon *26S rRNA* and ITS sequence
764 analysis

765

766

767 **Figures Legends**

768

769 **Fig. 1A.** Phylogenetic tree showing the taxonomic affiliation of the rhizospheric yeasts
770 isolated from two soils seriously contaminated by HM and arsenic. The tree was
771 constructed by Maximum Likelihood by Kimura 2 parameter + I model with the
772 sequences of *26S rRNA* D1/D2 domain. Bootstrap values >50% are presented alongside
773 the branch. Isolate numbers are presented in boldface and sequence accession numbers
774 are indicated in parentheses.

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775 **Fig. 1B.** Phylogenetic tree showing the taxonomic affiliation of the rhizospheric yeasts
776 isolated from two soils seriously contaminated by HM and arsenic. The tree was
777 constructed by Maximum Likelihood by the Tamura 3 parameter + I + G model with
778 sequences of ITS fragment. Bootstrap values >50% are presented alongside the branch.
779 Isolate numbers are presented in boldface and sequence accession numbers are indicated
780 in parentheses.

781 **Fig. 2.** Kinetics of IAA production by yeasts *C. sloffiae* (YR11) and *R. mucilaginosa* (YR
782 24 and YR29). A statistical analysis of two-way ANOVA using the method of Duncan
783 with a $p < 0.05$ to determine significant differences.

784 **Fig. 3.** Percentage of seed germination at 24–48 h of incubation in water-agar
785 supplemented with yeast filtrates. YR24 = *R. mucilaginosa*, YR11 = *C. slooffiae*, and
786 YR29 = *R. mucilaginosa. P* <0.05. Bar = one times the Standard Error of the Mean
787 (SEM). Different letters indicate statistical significance.

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788 **Fig. 4.** Plant growth (cm) in water-agar supplemented with yeast filtrates. YR24 = *R.*
789 *mucilaginosa*, YR11 = *C. slooffiae*, and YR29 = *R. mucilaginosa. P* <0.05. Bar = one
790 times the Standard Error of the Mean (SEM). Different letters indicate statistical
791 significance.

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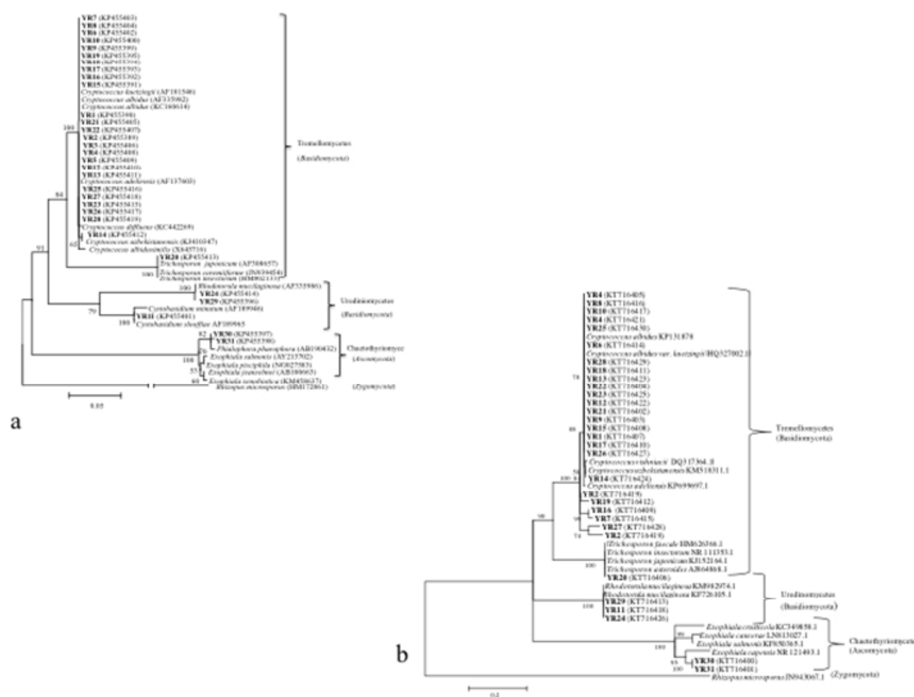

Fig. 1A. Phylogenetic tree showing the taxonomic affiliation of the rhizospheric yeasts isolated from two soils seriously contaminated by HM and arsenic. The tree was constructed by Maximum Likelihood by Kimura 2 parameter + I model with the sequences of 26S rRNA D1/D2 domain. Bootstrap values >50% are presented alongside the branch. Isolate numbers are presented in boldface and sequence accession numbers are indicated in parentheses.
Fig. 1B. Phylogenetic tree showing the taxonomic affiliation of the rhizospheric yeasts isolated from two soils seriously contaminated by HM and arsenic. The tree was constructed by Maximum Likelihood by the Tamura 3 parameter + I + G model with sequences of ITS fragment. Bootstrap values >50% are presented alongside the branch. Isolate numbers are presented in boldface and sequence accession numbers are indicated in parentheses.

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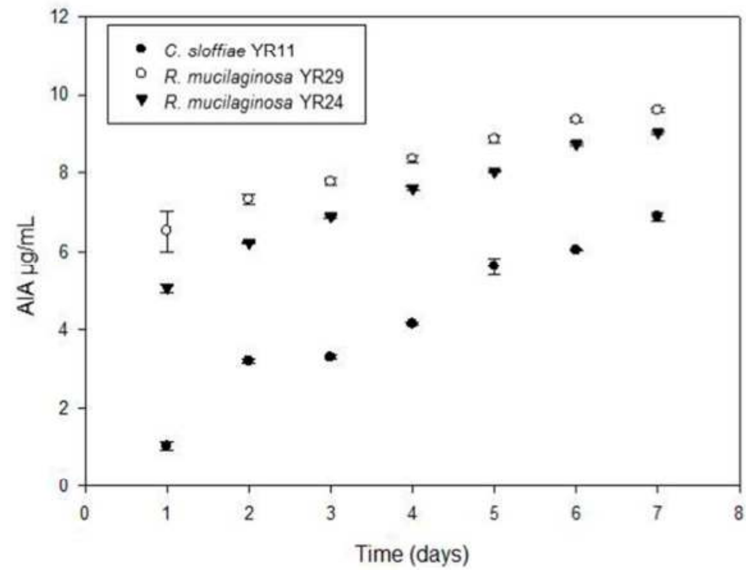

Kinetics of IAA production by yeasts C. sloffiae (YR11) and R. mucilaginosa (YR 24 and YR29). A statistical analysis of two-way ANOVA using the method of Duncan with a $p < 0.05$ to determine significant differences.

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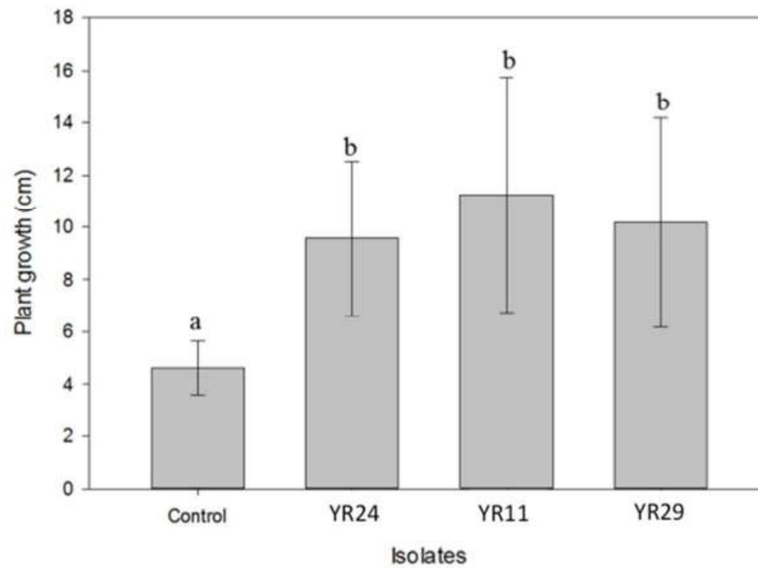

Percentage of seed germination at 24–48 h of incubation in water-agar supplemented with yeast filtrates. YR24 = R. mucilaginosa, YR11 = C. slooffiae, and YR29 = R. mucilaginosa. $P < 0.05$. Bar = one times the Standard Error of the Mean (SEM). Different letters indicate statistical significance
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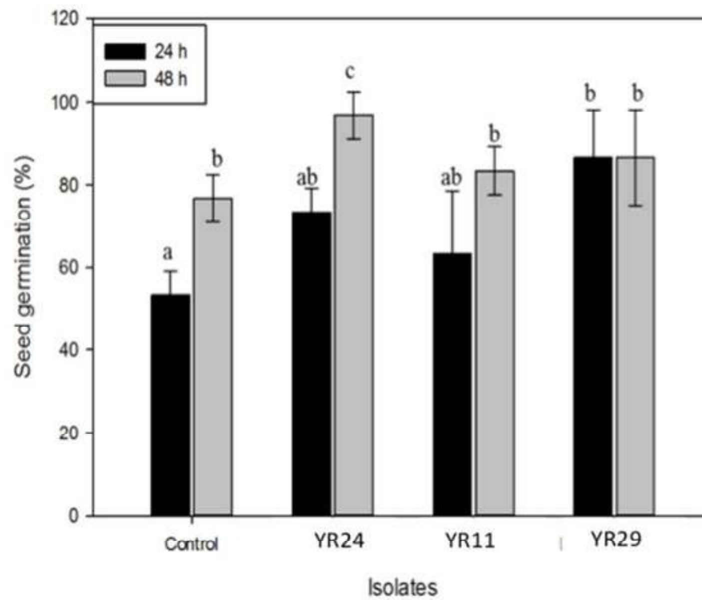

Plant growth (cm) in water-agar supplemented with yeast filtrates. YR24 = R. mucilaginosa, YR11 = C. slooffiae, and YR29 = R. mucilaginosa. P <0.05. Bar = one times the Standard Error of the Mean (SEM). Different letters indicate statistical significance.

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Table 1. Yeast isolates with their relevant information and enzymatic/plant growth promoting activities

Strain No.	Taxonomic affiliation	Index of activity for*				Production	
		Cellulases	Xylanases	Pectinases	Proteases	Siderophore#	IAA ug/ml
From *Thitonia diversifolia* in mine tailing							
YR1	*Cryptococcus albidus*	ND	ND	ND	ND	ND	-
YR2	*Cryptococcus albidus*	ND	ND	2	1	ND	-
YR3	*Cryptococcus albidus*	ND	ND	ND	1.33	ND	-
YR4	*Cryptococcus albidus*	ND	ND	ND	2	ND	-
YR5	*Cryptococcus albidus*	ND	ND	1	ND	ND	-
From *Flaveria angustifolia* in mine tailing							
YR6	*Cryptococcus albidus*	ND	ND	1.4	1.38	ND	-
YR7	*Cryptococcus albidus*	ND	ND	ND	ND	1.83	-
YR8	*Cryptococcus albidus*	ND	1.5	3.91	1.43	ND	-
YR9	*Cryptococcus albidus*	ND	1.5	2.48	1.52	ND	-
YR10	*Cryptococcus albidus*	ND	1.18	3.18	1.38	ND	-
YR11	*Cystobasidium sloffiae*	ND	1.2	ND	ND	ND	**6.8**
From *Spharealcea angustifolia* in mine tailing							
YR12	*Cryptococcus albidus*	ND	ND	ND	ND	1	-
YR13	*Cryptococcus albidus*	ND	ND	1.66	1.77	ND	-
YR14	*Cryptococcus uzbekistanensis*	ND	ND	1.29	ND	ND	-
From *Prosopis* sp in mine tailing							
YR15	*Cryptococcus albidus*	ND	ND	ND	ND	ND	-
YR16	*Cryptococcus albidus*	ND	ND	ND	ND	ND	-
YR17	*Cryptococcus albidus*	ND	ND	ND	ND	ND	-
YR18	*Cryptococcus albidus*	ND	ND	ND	1.94	ND	-
YR19	*Cryptococcus albidus*	ND	ND	ND	1.52	ND	-
YR20	*Trichosporon* sp	ND	1.52	ND	ND	1.34	-
From *Bahia absinthifolia* in hill							
YR21	*Cryptococcus albidus*	ND	1.5	2.16	1.88	ND	-
YR22	*Cryptococcus albidus*	ND	2	1.66	1.33	ND	-
From *Spharealcea angustifolia* in hill							
YR23	*Cryptococcus albidus*	ND	ND	ND	ND	ND	-
YR24	*Rhodotorula mucilaginosa*	1.5	2.11	3.75	ND	2.53	**9.02**
YR25	*Cryptococcus albidus*	ND	1.4	3.16	1.63	ND	-
YR26	*Cryptococcus albidus*	ND	1.63	2.33	2.16	ND	-
YR27	*Cryptococcus albidus*	ND	1.5	4.66	1	ND	-
YR28	*Cryptococcus albidus*	ND	ND	2.16	2.22	ND	-
From *Prosopis* sp in hill							
YR29	*Rhodotorula mucilaginosa*	1.75	ND	3.2	ND	1	**9.61**
YR30	*Exophiala pisciphila*	1.97	3.13	ND	ND	1	-
YR31	*Exophiala pisciphila*	2.16	2.38	ND	ND	ND	-
Proportion (%) of positive isolates		12.9	41.9	51.6	51.6	16.1	
Number of positive isolates		4	13	16	16	5	3

*. Index of rhizospheric yeast

ND- Not Detected

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Table 2. MICs of risk elements and capacity for oxidize or reducing arsenic of rhizospheric yeasts

Strain	Taxonomic affiliation	MIC (mM)					Arsenate Reduction Percentage (%)
		Zn^{+2}	Pb^{+2}	Cu^{+2}	As^{+5}	As^{+3}	
From *Thitonia diversifolia* in mine tailing							
YR1	*Cryptococcus albidus*	0.5	0.6	NG	2	30	10.24
YR2	*Cryptococcus albidus*	0.5	1.2	NG	1	30	10.24
YR3	*Cryptococcus albidus*	0.5	1.2	NG	NG	NG	–
YR4	*Cryptococcus albidus*	NG	1.2	NG	0.5	1	–
YR5	*Cryptococcus albidus*	1	0.6	NG	2	50	23.21
From *Flaveria angustifolia* in mine tailing							
YR6	*Cryptococcus albidus*	1	1.2	NG	2	100	30
YR7	*Cryptococcus albidus*	1	0.6	NG	1	20	–
YR8	*Cryptococcus albidus*	NG	1.2	NG	NG	30	15
YR9	*Cryptococcus albidus*	NG	1.2	NG	0.5	1	–
YR10	*Cryptococcus albidus*	1	1.2	0.5	NG	20	20
YR11	*Cystobasidium sloffiae*	2	NG	NG	2	10	–
From *Spharealcea angustifolia* in mine tailing							
YR12	*Cryptococcus albidus*	1	1.2	NG	2	30	20
YR13	*Cryptococcus albidus*	1	1.2	NG	30	100	40
YR14	*Cryptococcus uzbekistanensis*	2	1.2	0.5	NG	NG	–
From *Prosopis* sp. in mine tailing							
YR15	*Cryptococcus albidus*	2	0.6	0.5	10	30	16
YR16	*Cryptococcus albidus*	1	1.2	NG	30	30	15
YR17	*Cryptococcus albidus*	1	1.2	NG	4	60	15
YR18	*Cryptococcus albidus*	0.5	1.2	NG	20	90	30
YR19	*Cryptococcus albidus*	0.5	1.2	0.5	4	100	20

YR20	*Trichosporon japonicum*	0.5	NG	NG	NG	2	−
From *Bahia absinthifolia* in hill							
YR21	*Cryptococcus albidus*	1	1.2	NG	0.5	30	20
YR22	*Cryptococcus albidus*	NG	1.2	NG	0.5	4	−
From *Spharealcea angustifolia* in hill							
YR23	*Cryptococcus albidus*	1	1.2	NG	0.5	30	10
YR24	*Rhodotorula mucilaginosa*	0.5	NG	NG	NG	NG	−
YR25	*Cryptococcus albidus*	1	1.2	NG	0.5	30	−
YR26	*Cryptococcus albidus*	2	1.2	NG	0.5	30	−
YR27	*Cryptococcus albidus*	1	1.2	NG	0.5	20	10
YR28	*Cryptococcus albidus*	2	1.2	NG	NG	20	−
From *Prosopis* sp in hill							
YR29	*Rhodotorula mucilaginosa*	1	1.2	NG	NG	1	−
YR30	*Exophiala pisciphila*	5	0.3	NG	NG	1	−
YR31	*Exophiala pisciphila*	5	1.2	NG	NG	1	−
	Range or percentage	0.5-2	0.6-1.2	0-0.5	0.5-4	1-100	51.6%

*. Oxidation of arsenite was negative for all the isolates.
NG- Not growth