1 # Luteibacter rhizovicinus MIMR1 promotes root development in
2 # barley (*Hordeum vulgare* L) under laboratory conditions

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Abstract

In order to preserve environmental quality, alternative strategies to chemical-intensive agriculture are strongly needed. In this study, we characterized *in vitro* the potential plant growth promoting (PGP) properties of a gamma-proteobacterium, named MIMR1, originally isolated from apple shoots in micropropagation. The analysis of the 16S rRNA gene sequence allowed the taxonomic identification of MIMR1 as *Luteibacter rhizovicinus*. The PGP properties of MIMR1 were compared to *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM 19603^T, which was selected as a reference PGP bacterium. By means of *in vitro* experiments, we showed that *L. rhizovicinus* MIMR1 and *P. chlororaphis* DSM 19603^T have the ability to produce molecules able to chelate ferric ions and solubilize monocalcium phosphate. On the contrary, both strains were apparently unable to solubilize tricalcium phosphate. Furthermore, the ability to produce 3-indol acetic acid by MIMR1 was approximately three times higher than that of DSM 19603^T. By using fluorescent recombinants of strains MIMR1 and DSM 19603^T, we also demonstrated that both bacteria are able to abundantly proliferate and colonize the barley rhizosphere, preferentially localizing on root tips and in the rhizoplane. Finally, we observed a negative effect of DSM 19603^T on barley seed germination and plant growth, whereas MIMR1, compared to the control, determined a significant increase of the weight of aerial part (+ 22 %), and the weight and length of roots (+ 53 % and + 32 %, respectively). The results obtained in this work make *Luteibacter rhizovicinus* MIMR1 a good candidate for possible use in the formulation of bio-fertilizers.

Key words: *Luteibacter rhizovicinus*, plant growth-promoting bacteria, auxins, *Hordeum vulgare* L, root development

39 **Introduction**

40 The massive increase in the use of nitrogen and phosphorus fertilizers during last decades by intensive
41 agricultural practices has significantly contributed to severe environmental pollution (Vance 2001).
42 Particularly, nitrogen is accumulating in the environment globally (Walvoord et al. 2003), leading to
43 eutrophication, hypoxia, loss of biodiversity, and habitat degradation (Galloway et al. 2003). In order to
44 preserve environmental quality, alternative strategies to chemical-intensive agriculture are strongly
45 needed. Such environmental-friendly approaches are generally indicated as sustainable agriculture, which
46 Golley et al. (1992) defined as agriculture "managed toward greater resource efficiency and conservation
47 while maintaining an environment favorable for the evolution of all species". A possible agricultural
48 sustainable strategy consists in the use of biofertilizers, *i.e.* "a substance which contains living
49 microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the
50 interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to
51 the host plant" (Vessey 2003). The microorganisms most commonly included in biofertilizers are
52 rhizosphere-competent bacteria, which are able to benefit plants and consequently to improve crop
53 production. For this reason, they are generally called "plant growth promoting rhizobacteria" (PGPR).
54 PGPR can benefit plants development through multiple mechanisms, including antagonism to pathogenic
55 fungi, siderophore production, nitrogen fixation, phosphate solubilization, the production of organic
56 acids, indole acetic acid (IAA), NH_3 and HCN, the release of enzymes (soil dehydrogenase, phosphatase,
57 nitrogenase, etc.), and the induction of systemic disease resistance (Babalola 2010). The research
58 throughout last 20 years identified PGPR strains in many different bacterial genera, belonging to the taxa
59 α-proteobacteria (genera *Acetobacter*, *Azospirillum*, *Beijerinckia*, *Gluconacetobacter*, *Ochrobactrum*), β-
60 proteobacteria (*Alcaligenes*, *Azoarcus*, *Zoogloea*, *Burkholderia*, *Derxia*, *Herbaspirillum*), γ-proteobacteria
61 (*Enterobacter*, *Klebsiella*, *Pantoae*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, *Acinetobacter*,
62 *Azotobacter*), Actinobacteria (*Rhodococcus*, *Arthrobacter*), and Firmicutes (*Bacillus*) (Babalola 2010).
63 In this study, we investigated the PGP abilities of the γ-proteobacterium *Luteibacter rhizovicinus* MIMR1,
64 a microbial strain isolated from apple shoots (*Malus domestica* L. cultivar Golden Delicious) in

65 micropropagation (Piagnani et al. 2007). The results collected during this study showed that *L.*
66 *rhizovicinus* MIMR1can colonize the rhizosphere of barley *in vitro*, promoting root development and
67 plant growth. This is the first time that a member of the genus *Luteibacter* is proposed as PGPR.
68

69 Material and methods

70 *Bacterial strains, culture conditions and plant seeds*
71 *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM 19603^T (purchased from Deutsche Sammlung von
72 Mikroorganismen und Zellkulturen GmbH, DSMZ, Braunschweig, Germany) and *Luteibacter* sp.
73 MIMR1 were routinely grown overnight at 28 °C in Luria Bertani broth under constant agitation (from
74 100 to 250 rpm). In this study, we used seeds of *Hordeum vulgare* L. variety "Cometa" (Apsovsementi
75 S.p.A., Voghera, Italy).
76

77 *Taxonomic identification and phylogenesis of* Luteibacter *sp. MIMR1*
78 The bacterial isolate *Luteibacter* sp. MIMR1 was taxonomically identified by means of 16S rRNA gene
79 sequence analysis as previously described (Guglielmetti et al. 2010). The BLAST programs
80 (http://www.ncbi.nlm.nih.gov/blast/) were used to conduct similarity searches against GenBank and
81 EMBL sequence databases, with subsequent alignment and neighbour-joining phylogenetic analysis of
82 16S rRNA gene sequences with bootstrap values (1000 replicates) using ClustalW and Treecon software.
83

84 In vitro *screening of bacterial strains for their plant growth promoting (PGP) activities*
85 Siderophore production. Bacterial strains were assayed for siderophores production on the Chrome azurol
86 S agar medium (Sigma-Aldrich, Steinheim, Germany) according to Milagres et al. (1999). In brief, we
87 prepared King's B agar plates, removed half of the solid medium with a sterile scalpel, and poured
88 Chrome azurol S agar. Test organisms were inoculated with a loop on King's B medium and plates were
89 incubated at 28 °C for 48–72 h. Development of yellow–orange halo on Chrome azurol S agar was
90 considered as positive for siderophore production.

91 Inorganic phosphate solubilization. The qualitative analysis of solubilization of calcium hydrogen
92 phosphate ($CaHPO_4$) and tricalcium phosphate ($Ca_3(PO_4)_2$) was made on agar plates containing T1 (10 g/l
93 glucose, 2 g/l $CaHPO_4$, 10 ml/l Alazarin Red 1 %, 5 g/l tryptone) or T2 (20 g/l glucose, 5 g/l $Ca_3(PO_4)_2$,
94 10 g/l $MgCl_2$, 0.25 g/l $MgSO_4$, 0.20 g/l KCl, 0.10 g/l $(NH_4)_2SO_4$) agar medium, respectively. After the
95 inoculation, plates were incubated at 28 °C. The formation of a clarification area around bacterial growth
96 was considered a positive indication of the ability to solubilize phosphates.
97 Indoleacetic acid (IAA) production. Quantitative analysis of IAA was performed in King's B broth
98 supplemented with 500 µg/ml of tryptophan according to Glickmann and Dessaux (1995). Bacterial
99 cultures were incubated for 5 days at 28 °C; broth cultures were then centrifuged and 0.4 ml of the
100 supernatant was mixed with 1.6 ml of Salkowski reagent (60 % H_2SO_4; 3 % of a 0.5 M $FeCl_3$ solution).
101 After 30 min of incubation at room temperature in dark, the optical density was measured at 530 nm.
102 Concentration of IAA produced by cultures was measured with the help of standard graph of IAA
103 obtained in the range of 4–500 µg/ml.
104
105 *Bacterial colonization of the rhizosphere of barley (*Hordeum vulgare *L.)*
106 Tagging of bacterial strains with Gfp. GFP-tagged bacteria were generated by transferring the
107 plasmid pPnptII:gfp (Stiner and Halverson 2002) into *Luteibacter* sp. MIMR1 by electroporation and the
108 plasmid pUTgfp2x (Tombolini et al. 1997) into *P. chlororaphis* DSM 19603^T by conjugation.
109 Transformation of strain MIMR1 was carried out according to a method conventionally employed for the
110 electro-transformation of *Escherichia coli*. Conjugation experiments were carried out according to Unge
111 et al. (1997). In brief, strain DSM 19603^T was co-incubated with *Escherichia coli* SM10/λ pir, which is
112 the donor of vector pUTgfp2x. After 18 hours of growth in LB medium at 28 °C under agitation (100
113 rpm), 0.1 ml aliquots were spread on LB agar plates containing 25 µg/ml kanamycin (selection for
114 plasmid pUTgfp2x) and 10 µg/ml chloramphenicol (selection for DSM 19603^T). Mutant strains, named
115 MIMR1^{Gfp} and DSM 19603^{Gfp}, were maintained in LB medium supplemented with 25 µg/ml kanamycin.
116 Both recombinant strains were highly stable and could be maintained for more than 5 days of culture
117 without antibiotic selection.

118 Colonization of barley rizosphere by *gfp*-tagged bacteria. Healthy *Hordeum vulgare* seeds were
119 washed for 5 min with filter-sterilized 70 % ethanol and for 1 min with 3 % hydrogen peroxide, followed
120 by five washes with sterile distilled water. Seeds were incubated in the dark at room temperature for 2/3
121 days for germination on plates containing water agar (10 g/l agar in tap water). Seedlings with 1 cm long
122 radicles were sterilely transferred into 1 l Roux bottles (one plant per bottle) containing Fahreus mineral
123 agar medium (0.01 g/l $CaCl_2$; 0.12 g/l $MgSO_4$; 0.1 g/l KH_2PO_4; 0.15 g/l Na_2HPO_4; 1.650 g/l NH_4NO_3;
124 0.005 g/l ferric citrate; traces of Mn, Cu, Zn, B, Mo; 0.8 % agar). Afterwards, each plantlet was sprinkled
125 with 0.5 ml of the bacterial suspension, which contained 10^9 cells. Bacterial suspensions were prepared as
126 follows. Bacterial cells were grown over night in LB broth supplemented with 25 µg/ml kanamycin,
127 washed once with saline, counted by means of a Neubauer-improved counting chamber (Marienfeld
128 GmbH, Lauda-Königshofen, Germany), and resuspended in 10 mM $MgSO_4$ at a concentration of 2×10^9
129 cell/ml. After bacterial inoculation, Roux bottles were kept in a greenhouse programmed for 12h
130 photoperiod, temperature of 25 °C and 70 % relative humidity. Uninoculated seedlings served as control.
131 *Hordeum vulgare* plants were harvested 5 days after inoculation and the roots were gently removed. Root
132 samples were finally observed using fluorescence optical digital microscope Leica DM1000 (Leica
133 Microsystems, Wetzlar, Germany).
134
135 *Bacterial promotion of barley growth*
136 The first experiment was carried out as described above for the root colonization experiments. After 5-
137 days incubation in greenhouse, the following parameters were recorded: root length, root weight, leaf
138 (aerial part) length and aerial part weight.
139 In the second experiment, we incubated bacteria with barley seeds before germination. Specifically,
140 we prepared Petri plates (20 cm diameter) containing 40 ml of Fahreus agar medium and 10^7 bacterial
141 cell/ml (uninoculated plates served as control). Afterwards, 21 sterilized non-germinated barley seeds
142 were laid down on a single Petri plate and incubated as described above. After 5 days of incubation, the
143 following parameters were recorded: number of germinated seeds, root length, root weight, aerial part
144 length and aerial part weight.

145

146 **Results**

147 *Taxomonic identification of the bacterial isolate MIMR1*

148 In the present study, we obtained the nucleotidic sequence of about 1400 bp from the 16S rRNA gene of

149 MIMR1. Following GenBank database search by nBLAST and phylogenetic analysis, strain MIMR1 was

150 identified as *Luteibacter rhizovicinus* (99 % sequence similarity with the type strain *Luteibacter*

151 *rhizovicinus* LJ96^T, Fig. 1).

152

153 *Phenotypic characterization of strain MIMR1*

154 In order to understand the potential PGP properties of MIMR1, we performed *in vitro* assays aimed to

155 determinate the ability of the bacterial isolate under study to chelate iron, to produce indol acetic acid

156 (IAA) and to solubilize phosphates. We also included in the study strain DSM 19603^T, which belongs to

157 the taxon *Pseudomonas chlororaphis* subsp. *aurantiaca*, a subspecies known to display PGP properties

158 (Andrés et al. 2011) and for this reason often included in industrial bio-fertilizer products.

159 After four days of incubation at 28 °C, strain MIMR1 and, more prominently, strain DSM 19603^T

160 induced a change of the color from blue to orange in CAS agar (Supplementary information 1), indicating

161 the potential ability of both bacteria to produce molecules able to chelate Fe^{3+} (syderophores).

162 Furthermore, we observed the ability of *L. rhizovicinus* MIMR1 and *P. chlororaphis* DSM 19603^T to

163 solubilize $Ca(HPO_4)_2$. On the contrary, both strains were apparently unable to solubilize the inorganic

164 phosphate $Ca_3(PO_4)_2$ (Data not shown).

165 We also assessed spectrophotometrically the capacity of strains MIMR1 and DSM 19603^T to produce

166 3-indol acetic acid (IAA) in King's B broth supplemented with 500 µg/ml of L-tryptophan. After five

167 days of incubation, the cell production index (CPI, *i.e.* µg of IAA per billion of cells) of strain MIMR1

168 was approximately three times higher than the CPI of strain DSM 19603^T (Table 1).

169

170 *Colonization of barley (*Hordeum vulgare *L.) rhizosphere*

171 In order to assess the ability of the bacteria under investigation to colonize barley rhizoshere, 10^9 cells of
172 the recombinant strains *L. rhizovicinus* MIMR1^{Gfp} and *P. chlororaphis* DSM 19603^{Gfp}, expressing a green
173 fluorescent protein (Gfp), were inoculated on barley plantlets in Fahreus mineral agar medium. After one
174 week of incubation, fluorescence microscope observation of roots revealed that both bacteria were able to
175 abundantly proliferate and colonize the rhizosphere. Particularly, MIMR1^{Gfp} and DSM 19603^{Gfp} were
176 preferentially localized on root tips and in the rhizoplane (Fig. 2).

177

178 *Impact of bacteria on barley vegetal growth*

179 Two different experiments were carried out in order to assess the effect of *Luteibacter rhizovicinus*
180 MIMR1 and *Pseudomonas chlororaphis* DSM 19603^T on barley plant development. In the first
181 experiments, 3-days old barley plants germinated in water agar were transferred to agarized Fahreus
182 mineral solution and inoculated with 10^9 bacterial cells. After 5 days of incubation in greenhouse, plant
183 growth parameters were measured. Concerning the aerial parts, the only significant differences observed
184 between samples consisted in a reduction of length (- 11 %) and weight (- 12 %) induced by strain DSM
185 19603^T compared to MIMR1 (Table 2; Data not shown 2, only for referees). Also root weight was
186 decreased by the incubation with DSM 19603^T (- 33 %) compared to strain MIMR1 and the control (no
187 inoculated bacterial cells). More interestingly, plants incubated with strain MIMR1 had significantly
188 longer roots compared to the control (+ 20 %) and strain DSM 19603^T (+ 76 %) (Table 2; Supplementary
189 information 2).

190 In the following experiment, 10^7 bacterial cells per ml were inoculated directly in agarized Fahreus
191 medium before sawing not-yet-germinated barley seeds. After 5 days of incubation, we counted the
192 number or germinated seeds and measured plant growth parameters. First, we observed a drastic negative
193 effect of *P. chlororaphis* DSM 19603^T on all considered plant parameters, germination rate included (Fig.
194 3). On the contrary, *L. rhizovicinus* MIMR1, compared to the control, determined a significant increase of
195 the weight of aerial part (+ 22 %), and the weight and length of roots (+ 53 % and + 32 %, respectively)
196 (Fig. 3). Germination rate was substantially unaffected by the presence of *L. rhizovicinus* MIMR1.

197

198 **Discussion**

199 The need to integrate traditional farming practices with more environmentally friendly approaches
200 stimulated the interest towards plant growth promoting rhizobacteria (PGPR) since the early 80's. Over
201 the past 20 years, research and industry developed bio-fertilizer products containing PGPR micro-
202 organisms, which have been specifically selected to increase the bioavailability of the primary plant
203 nutrients in the soil and acting as bio-control agents against plant pathogens (Vessey 2003).

204 In this study, we characterized *in vitro* the potential PGP properties of two bacterial strains:
205 *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM 19603^{T} and *Luteibacter rhizovicinus* MIMR1.
206 Members of the bacterial taxon *Pseudomonas chlororaphis* subsp. *aurantiaca* have been already proposed
207 as bio-control agents towards fungal pathogens (Rosas et al. 2001). Furthermore, recent studies have also
208 demonstrated the ability of these bacteria to promote plant growth through mechanisms independent from
209 the antagonism against plant pathogens (Carlier et al. 2008; Rosas et al. 2009; Andrés et al. 2011). For
210 these reasons, we selected *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM 19603^{T} as a PGPR
211 reference strain to compare with MIMR1, a bacterial strain that has been isolated from shoots of the apple
212 cultivar “Golden Delicious” in micropropagation (Piagnani et al. 2007). The isolate MIMR1 did not affect
213 apple shoot proliferation and growth, but was associated to a sensible loss of leaf organogenic ability and
214 to a more abundant callus production (Piagnani et al. 2007). We therefore supposed that strain MIMR1
215 could deliver growth regulators to the plant cells.

216 The genus *Luteibacter* belongs to γ-proteobacteria, a class of microorganisms frequently proposed
217 and even commercially employed as PGPR, such as, for instance, *Azotobacter chroococcum* (Kumar and
218 Narula 1999), *Pseudomonas chlororaphis* and *Pseudomonas putida* (Cattelan et al. 1999), *Xanthomonas*
219 *maltophilia* (de Freitas et al. 1997). Members of the species *Luteibacter rhizovicinus* were described for
220 the first time as yellow-pigmented bacteria isolated from the rhizosphere of barley (*Hordeum vulgare* L.;
221 Johansen et al. 2005). According to the above mentioned observations, we decided to evaluate whether
222 strain MIMR1 could affect the growth of barley plants.

223 Initially, the ability of *L. rhizovicinus* MIMR1 and *P. chlororaphis* subsp. *aurantiaca* DSM 19603^{T}
224 to produce siderophores, solubilize inorganic phosphates and synthetize phytohormonal compounds was

225 tested *in vitro*. These features are considered common ways through which PGPR promote the
226 development of the host plant (Glick 1995). The experiments performed in this study showed that both
227 DSM 19603^T and MIMR1 can produce agar-diffusible molecules capable of chelating trivalent iron ions,
228 thus suggesting the hypothesis of siderophore production by these bacteria. The siderophores are
229 compounds belonging to different classes of molecules, which possess the property of chelating Fe^{3+}, thus
230 favoring the bioavailability of this micronutrient. It was reported the ability of numerous members of the
231 genus *Pseudomonas*, and more generally of the γ-proteobacteria, to produce a great variety of soluble
232 siderophores, which reflects the wide capacity of these microorganisms to colonize numerous diverse
233 ecological niches (Cornelis and Matthijs 2002).

234 Phosphorus is an important micronutrient for plants and represents about 0.2 % of their dry weight.
235 Although the total amount of phosphorus in the soil is generally high, it is often present in non-
236 bioavailable forms. The ability to solubilize the complexed forms of phosphorus thus plays a very
237 important role in improving the nutritional status of crop plants. Both microorganisms under study
238 displayed phosphate-lytic activity towards the monocalcium phosphate. This activity was particularly
239 accentuated for strain DSM 19603^T. On the contrary, tricalcium phosphate was apparently not solubilized
240 by the bacteria. Since the modalities through which the PGPR solubilize inorganic phosphates are linked
241 to the synthesis of specific enzymes (phosphatases) or the activity of acidification through the secretion of
242 organic acids (Kim et al. 1998), further investigations should be carried out to better understand the
243 mechanism underlying this capacity.

244 In the next step, the ability of MIMR1 and DSM 19603^T to produce compounds with auxinic
245 activity, such as 3-indole-acetic acid (IAA), was investigated. IAA is the most active phytohormone
246 within the class of auxins and the major player in the stimulation of the processes of rooting and cell
247 distension (Salisbury 1994). The root exudates of various plants contain rich supplies of tryptophan,
248 which are used by the microorganisms for synthesis and release of auxins as secondary metabolites in the
249 rhizosphere (Kravchenko 2004). In the experimental conditions adopted in this study, the *in vitro*
250 production of IAA by MIMR1 was found to be significantly greater than that of strain DSM 19603^T. This

251 result suggests the potential ability of MIMR1 to affect plant rooting and growth. This hypothesis has
252 been tested in the following experiments.

253 Irrespective of the mode of action, efficient colonization of root surfaces is a key feature of all
254 plant-beneficial bacteria (Whipps 2001). Therefore, we studied the rhizosphere competence of strains
255 MIMR1 and DSM 19603^T by using fluorescent recombinants. In our experimental conditions, when
256 barley shoots were incubated with bacteria for five days, we observed directly (*i.e.* microscopically) the
257 marked ability of fluorescent MIMR1^{Gfp} and DSM 19603^{Gfp} recombinants to colonize homogeneously the
258 rhizoplane, locating on the whole radical surface. The use of confocal microscopy could demonstrate
259 whether, besides rhizosphere competence, the bacteria under investigation could also colonize plant
260 tissues in endophytic manner. This feature, in fact, has already been reported for *P. chlororaphis* subsp.
261 *aurantiaca* (Rosas et al. 2005).

262 In the last part of this research, potential ability of the bacteria to stimulate plant growth was tested.
263 This analysis was carried out by evaluating various parameters such as the weight and length of the roots,
264 and the weight and the height of the aerial part of barley plants. The results showed that *Luteibacter*
265 *rhizovicinus* MIMR1 has the potential to increase the length and weight of the roots. The microorganisms
266 of the species *Luteibacter rhizovicinus* were originally isolated from the rhizosphere of barley; it,
267 therefore, seems plausible that these bacteria may have physiological characteristics that allow a
268 symbiotic interaction with plants of barley, as confirmed by the results collected in this study. Our results
269 could be partly explained by the ability of MIMR1 to efficiently produce auxins, which are
270 phytohormones able to induce a variety of effects on plants, including cell proliferation and elongation,
271 and the formation of new roots.

272 On the contrary, in the same experiments *P. chlororaphis* DSM 19603^T showed negative effects on
273 barley growth, both on the aerial part and roots. This bacterium displayed a very marked ability to
274 colonize the rhizosphere of barley. It is therefore possible that the negative effects observed might be due
275 to an excessive proliferation of the bacterium, facilitated by the conditions of sterility in which the tests
276 were conducted, which are characterized by the absence of microbial competitors. Unexpectedly, a

277 dramatic inhibitory activity of DSM 19603^T on barley seed germination was also observed. This result,
278 which appears in contrast with previous studies (Cattelan et al. 1999), could be due to the use of a too
279 high bacterial cell concentration in contact with the seeds, which may have determined the colonization of
280 internal seed tissues, limiting their germination. On the contrary, plant tolerance toward MIMR1 cells
281 appeared to be higher, suggesting a potential evolutive mutual adaptation between barley and *Luteibacter*
282 *rizhovicinus*.

283 This study is a preliminary work, which has the aim to propose *Luteibacter rhizovicinus* as a
284 potential new PGP bacterium. Since it is preliminary, this study has several limitations. Firstly, the
285 bacteria under examination were investigated in the absence of a complex microbial community
286 associated to plants. In field conditions, live roots and root exudates provide a diverse range of resources
287 to soil organisms, the vast majority of which are bacteria (with densities as high as 10^9 cells per gram of
288 soil) that compete with each other for these carbon resources (Hol et al. 2013). At this stage, it is
289 questionable if strain MIMR1 can efficiently compete with other soil bacteria when exogenously added to
290 barley rhizosphere in field. Nonetheless, the root colonization ability displayed by this bacterium in
291 greenhouse trials is noticeable and encourages the achievement of field experiments involving strain
292 MIMR1.

293

294 In conclusion, the results obtained in this work highlighted the potential PGP capabilities of
295 *Luteibacter rhizovicinus* MIMR1, which makes this bacterium a good candidate for a possible use in the
296 formulation of bio-fertilizers. In perspective, open field and greenhouse trials will be carried out in order
297 to assess the ability of this bacterium to promote plant growth in relation to physical and nutritional
298 stressors.

299

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304

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366 **Figure legends**

367 **Fig. 1.** *Neighbour Joining* dendrogram obtained through clustalW alignment of 1384 bp of the 16S rRNA

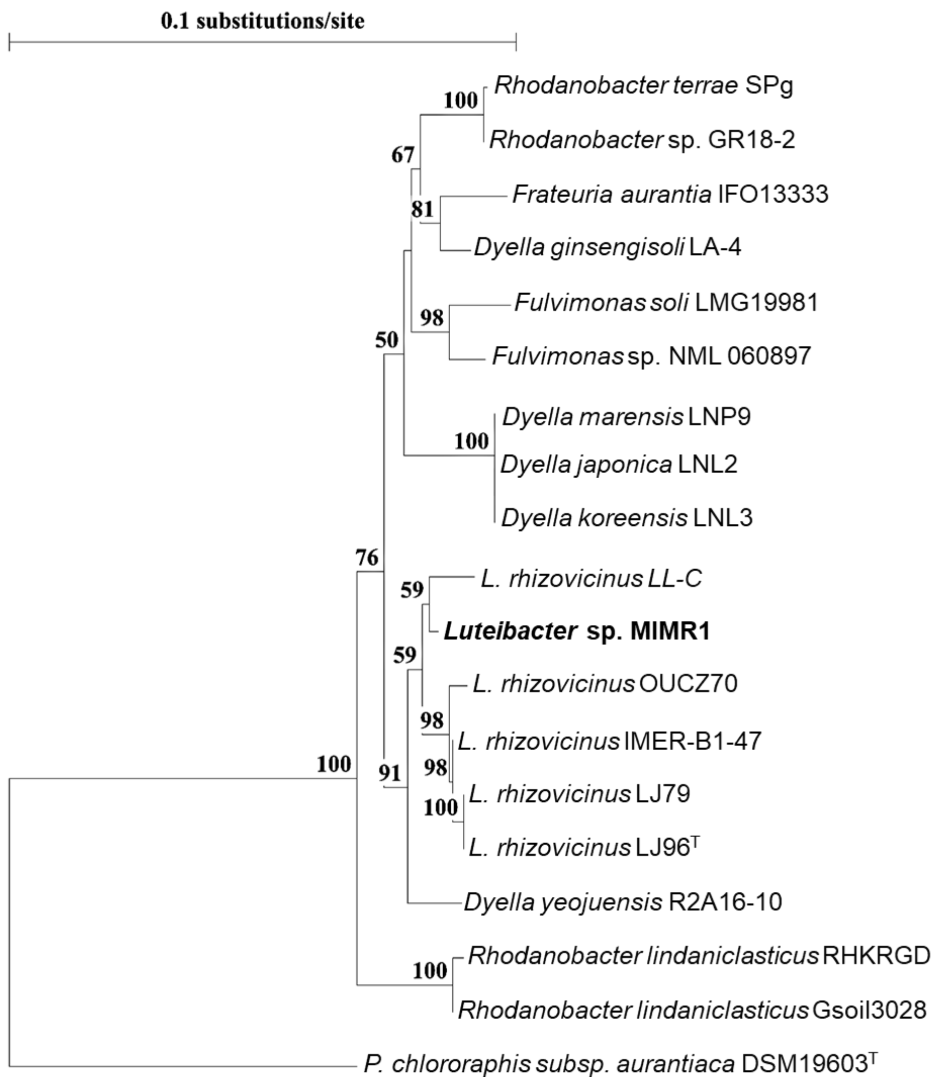
368 gene of *Luteibacter* sp. MIMR1 and the corresponding region of the phylogenetically most closely related

369 microbial strains available in GenBank, according to a nBLAST search. *L.* = *Luteibacter*; *P.* =

370 *Pseudomonas*. Outgroup: *P. chlororaphis* subsp. *aurantiaca* DSM19603^{T}. Percentual bootstraps higher

371 than 50 % are shown. Total bootstrap: 1000.

Fig. 1



372

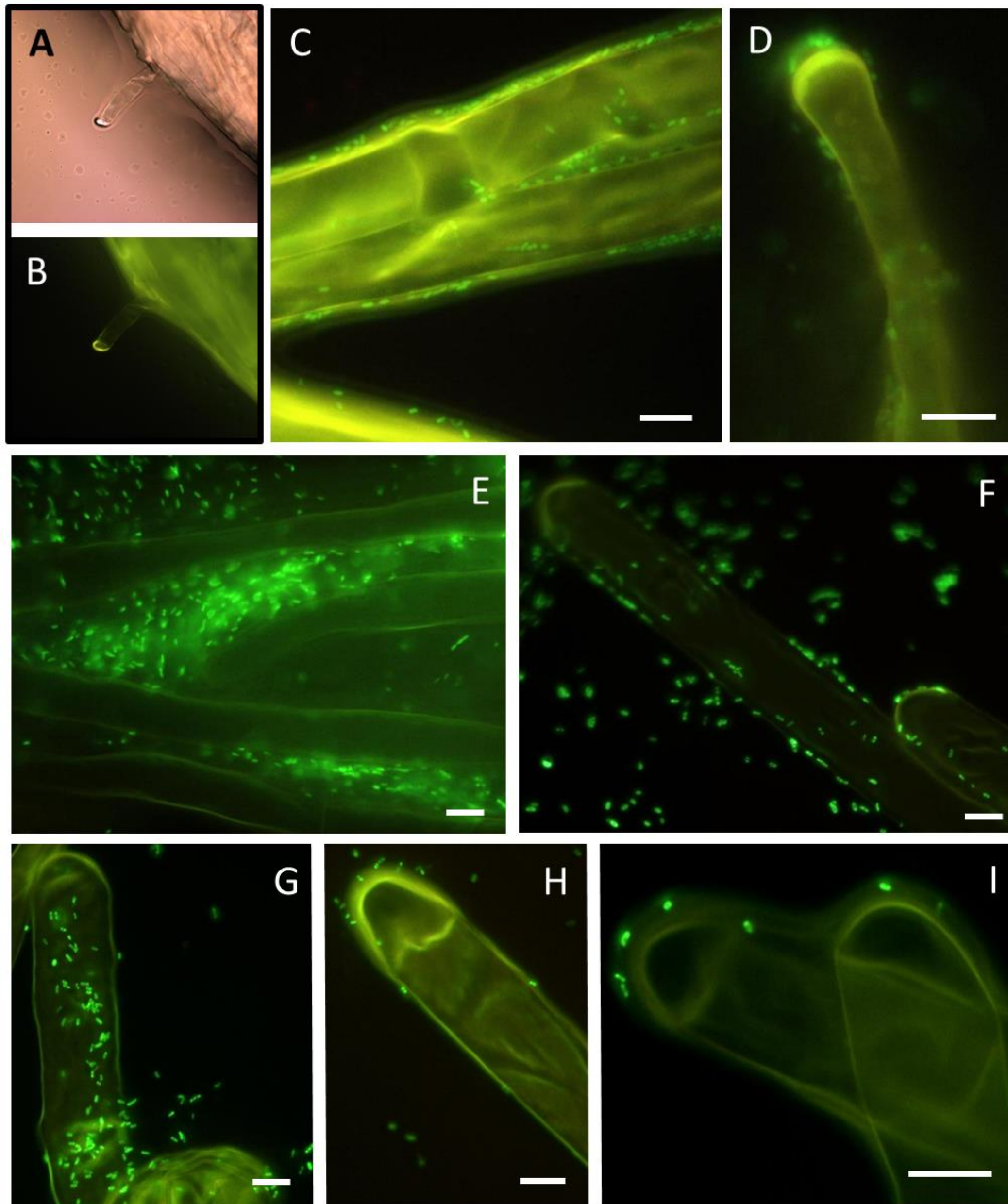
373 **Fig. 2.** Barley roots observed with an optical microscope. A, bright field. B, autofluorescence of plant

374 tissues observed with and epifluorescence microscope. C and D, green fluorescent *Pseudomonas*

375 *chlororaphis* subsp. *aurantiaca* DSM19603^T cells on root tips and rhizoplane. From image E to I, green

376 fluorescent *Luteibacter rhizovicinus* MIMR1 cells on root tips and rhizoplane. Magnification bar: 20 µm.

Fig. 2



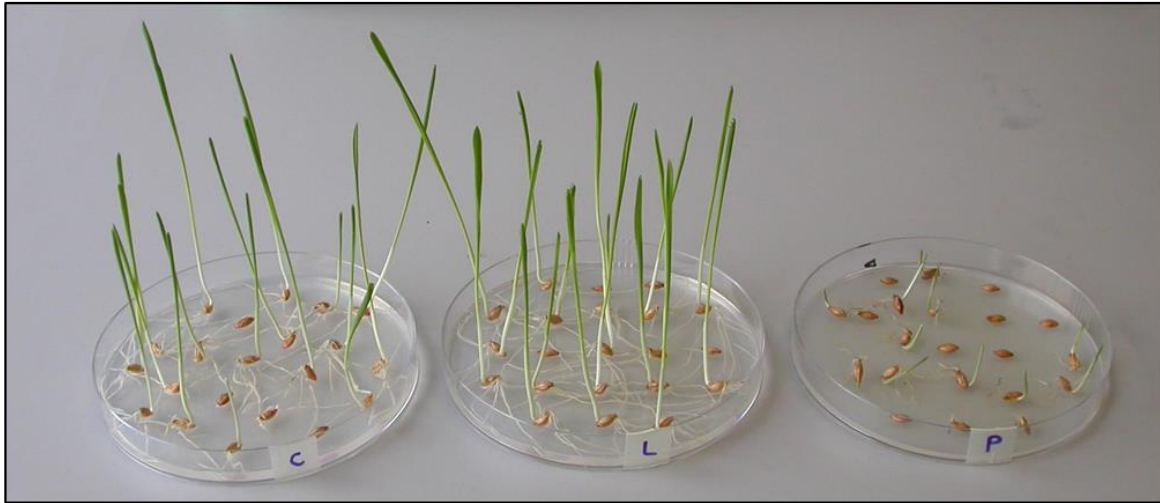
377

378 **Fig. 3.** Effect of *Luteibacter rhizovicinus* MIMR1 (B) and *Pseudomonas chlororaphis* DSM19603^{T} on

379 barley seed germination and plant growth on Fahreus agar mineral medium after 5 days of incubation at

380 25 °C. C, control (without bacterial cells).

Fig. 3



Control | ***Luteibater rhizovicinus*** **MIMR1** | ***Pseudomonas chlororaphis*** **DSM 19603^{T}**

381

382

383 **Tables**

384 **Table 1**. *In vitro* characterization of potential plant growth-promoting activities exerted by *Luteibacter*
385 *rhizovicinus* MIMR1 and *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM19603^T. IAA: indole acetic
386 acid (average of two experiments conducted in triplicate ± standard deviation). CPI: cell production index
387 (average of two experiments conducted in triplicate ± standard deviation).

Strain	Fe^{3+} chelation	$Ca(HPO_4)_2$ solubilization	$Ca_3(PO_4)_2$ solubilization	IAA production (mg/l)	CPI (µg/10^9 cells)
MIMR1	+	+	–	127.3 ± 8,8	14.1 ± 1,6
DSM19603^T	+	+	–	24.9 ± 1,6	4.8 ± 0.8

388 +: presence of activity
389 –: absence of activity

390

391 **Table 2**. Effect of bacterial strains on barley growth parameters. Germinated barley seeds were incubated
392 for 7 days in Roux bottles containing Fahreus agar medium in presence of 10^7 cell per ml of *Luteibacter*
393 *rhizovicinus* MIMR1, *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM19603^T or without bacteria
394 (control). Data are reported as the mean measures per plant calculated on two independent experiments (6
395 plants per tested condition per experiment) ± standard deviation.

	Aerial parts		Roots	
	length (cm)	weight (mg)	length (cm)	weight (mg)
Control	13.6 ± 2.2 **ab**	200 ± 20 **a**	17.9 ± 3.1 **a**	12 ± 3 **a**
MIMR1	14.1 ± 1.7 **a**	222 ± 35 **a**	21.5 ± 3.2 **b**	12 ± 3 **a**
DSM19603^T	12.6 ± 1.0 **b**	195 ± 28 **b**	12.2 ± 4.5 **c**	8 ± 2 **b**

396 Values with different suffix letters significantly differ at 0.05 level according to unpaired *t* Student's test.

397

398 **Table 3**. Effect of bacterial strains on barley seed germination and plant growth. Seeds were incubated in
399 Petri plates with Fahreus agar medium containing 10^7 cells per ml of *Luteibacter rhizovicinus* MIMR1,
400 *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM19603^T or without bacteria (control). Data are
401 reported as the mean measures per plant calculated on four independent experiments (21 plants per tested
402 condition per experiment) ± standard deviation.

	Aerial parts		Roots		Germinated seeds (%)
	length (cm)	weight (mg)	length (cm)	weight (mg)	
Control	9.3 ± 1.2 **a**	178 ± 11 **a**	13.7 ± 1.3 **a**	30 ± 3 **a**	75.0
MIMR1	10.3 ± 0.4 **a**	217 ± 18 **b**	18.0 ± 1.7 **b**	46 ± 7 **b**	73.8
DSM19603^T	2.1 ± 0.3 **b**	96 ± 5 **c**	5.1 ± 0.4 **c**	7 ± 1 **c**	53.6

403 Values with different suffix letters significantly differ at 0.05 level according to unpaired Student's t test.