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SHORT NOTE

The arbuscular mycorrhizal fungus *Rhizophagus irregularis* MUCL 43194 induces the gene expression of citrate synthase in the tricarboxylic acid cycle of the phosphate-solubilizing bacterium *Rahnella aquatilis* HX2

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Abstract

An increasing number of studies have demonstrated that arbuscular mycorrhizal fungi can cooperate with other soil microorganisms, e.g., bacteria, which develop near or on the surface of the extraradical hyphae where they perform multiple functions. However, the mechanisms involved in this privileged relationship are still poorly known. In the present study, we investigated how the arbuscular mycorrhizal fungus *Rhizophagus irregularis* MUCL 43194 influences the three pace-making enzymes (i.e., citrate synthase, isocitrate dehydrogenase, and α-oxoglutarate dehydrogenase) of the tricarboxylic acid (TCA) cycle in the phosphate-solubilizing bacterium *Rahnella aquatilis* HX2. The study was conducted under strict in vitro culture conditions and analysis made at the transcriptional level. Results showed that *R. irregularis* induced the expression of the gene-encoding citrate synthase (*gltA*), the pace-making enzyme involved in the first step of the TCA cycle, in *R. aquatilis* at all time points of observation (i.e., 1, 6, 12, 24, 48, and 72 h). The expression of the gene-encoding isocitrate dehydrogenase (*icd*) significantly decreased at 6, 12, 24, 48, and 72 h and the expression of the gene-encoding α-oxoglutarate dehydrogenase E1 component (*kgdhc*) significantly increased at 1, 6, and 48 h. The above results suggested that *R. irregularis* may influence the level of adenosine triphosphate production in *R. aquatilis* and thus the metabolism of the bacterium by stimulating the expression of *gltA* involved in the TCA cycle. Our results suggest a fine-tuned dialog between *R. irregularis* MUCL 43194 and *R. aquatilis* HX2 and emphasize the complexity of the interactions that might take place at the hyphal surface of arbuscular mycorrhizal fungi hosting communities of microbes.

Keywords Citrate synthase · Isocitrate dehydrogenase · α-Oxoglutarate dehydrogenase · In vitro culture · Tricarboxylic acid cycle

Introduction

The below-ground association between arbuscular mycorrhizal fungi (AMF) and plant roots is probably the most ancient symbiosis on earth (Redecker et al. 2000) that nowadays involves 80% of terrestrial plants (Smith and Read 2008). In this association, AMF receive their carbon (C) from the host plants, in return for which they compensate the plants by the provision of mineral nutrients (subsequently called 'nutrients'), in particular the poorly mobile phosphate (P) ions (Smith and Read 2008; Karasawa et al. 2012). Due to their strict dependence on plants for C acquisition, AMF have lost most of the gene-encoding proteins involved in saprotrophic function (Tisserant et al. 2013). Thus, they cannot access soil organic nutrients by themselves. However, other soil

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microorganisms, e.g., bacteria, can develop near or on the surface of extraradical hyphae where they perform multiple functions (Artursson et al. 2006; Agnolucci et al. 2015; Iffis et al. 2016). Under nutrient-limited conditions, these bacteria may compete for the break-down products solubilized by their exo-enzymes (Leigh et al. 2011; Zhang et al. 2014). Conversely, when the nutrient level is appropriate, these bacteria may cooperate with AMF and enhance the access of the fungi to the organic nutrient (Zhang et al. 2018). These bacteria receive C-rich compounds (e.g., sugars, carboxylates, amino acids, etc.) exuded by the hyphae (Kaiser et al. 2015; Wang et al. 2016), and some of them are able to mineralize soil organic N and P that become available to the AMF (Leigh et al. 2011; Nuccio et al. 2013; Zhang et al. 2016, 2018). In a previous study, Zhang et al. (2018) demonstrated cooperation between the arbuscular mycorrhizal fungus *Rhizophagus irregularis* MUCL 43194 and the phosphate-solubilizing bacterium (PSB) *Rahnella aquatilis* HX2. However, the mechanisms involved in this relationship remain poorly understood.

At the cellular level, the activity of bacteria depends on the high-energy phosphate compound adenosine triphosphate (ATP), which is primarily produced in the tricarboxylic acid (TCA) cycle (Tortora et al. 2016). This cycle comprises eight steps which are catalyzed by nine enzymes (or enzyme complexes). The first step, which is the most limiting step in the cycle, is the synthesis of citric acid catalyzed by citrate synthase (Krebs 1970). Isocitrate dehydrogenase and α-oxoglutarate dehydrogenase are the other pace-making enzymes involved in the cycle (Tortora et al. 2016). Any increase in the amounts or activity of these enzymes will enhance the production of ATP and thus the activity of bacteria that live in close vicinity to AMF hyphae.

In the present study, we investigated at the transcriptional level, how the arbuscular mycorrhizal fungus *R. irregularis* MUCL 43194 influences the three pace-making enzymes of the TCA cycle in the PSB *R. aquatilis* HX2. We postulated that the presence of *R. irregularis* hyphae would increase the expression of genes encoding the three pace-making enzymes in *R. aquatilis* because hyphal exudates provide C-rich compounds (e.g., sugars, carboxylates, amino acids, etc.) to the bacterium which can be transformed into substrates for the TCA cycle.

Materials and methods

The arbuscular mycorrhizal fungus strain used was *R. irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler as "irregulare" MUCL 43194. This strain was provided by the Glomeromycota in vitro collection (GINCO—http://www.mycorrhiza.be/ginco-bel) associated with Ri T-DNA transformed carrot (*Daucus carota* L.) roots clone DC2 on Petri plates (90 × 15 mm) containing modified Strullu-Romand (MSR) medium (Declerck et al. 1998) solidified with 3 g L^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA). The bacterial strain used was *R. aquatilis* HX2, isolated from a vineyard soil in Beijing, China (Guo et al. 2012). This strain has the ability to mineralize organic P, solubilize mineral P, fix nitrogen, and produce pyrroloquinoline quinine, indole-3-acetic acid, and antibacterial substances (Chen et al. 2007; Guo et al. 2009). It colonizes the root of maize and the hyphae of AMF (Chen et al. 2007; Zhang et al. 2016). This strain has been whole-genome sequenced (Guo et al. 2012) and was shown effective in the mineralization and utilization of phytate (Zhang et al. 2016, 2018).

The whole genome sequence of *R. aquatilis* is available at the National Center for Biotechnology Information (NCBI) (GenBank assembly accession: GCA_000255535). We investigated three genes, i.e., citrate synthase (*gltA*), isocitrate dehydrogenase (*icd*), and α-oxoglutarate dehydrogenase E1 component (*kgdhc*), encoding three pace-making enzymes in the TCA cycle of *R. aquatilis*. The primers for the genes were designed with the software Primer 5. The sequences of primers and other information related to the genes are described in Supplementary Materials Table S1.

Experimental set-up

Bi-compartmented Petri plates (90 × 15 mm) were used to grow the roots and AMF as detailed in Declerck et al. (2003). Briefly, in one compartment (i.e., the root compartment—RC), an excised transformed root of carrot (*Daucus carota* L.) clone DC2 was associated with the arbuscular mycorrhizal fungus on 25-ml MSR medium solidified with 3 g L^{-1} Phytagel. After 4 weeks, the extraradical mycelium (ERM) developed profusely in the RC and started to cross the plastic barrier separating the RC from the other compartment (i.e., the hyphal compartment—HC) in which only the ERM was allowed to develop. In the HC, 4-ml MSR medium solidified with 3 g L^{-1} Phytagel but without sucrose and vitamins was added in a slope from the top of the plastic barrier to the bottom of the HC (Toljander et al. 2007). The bi-compartmented Petri plates were incubated in a growth chamber at 27 °C in the dark. The cultures were checked on a regular basis, and any roots in the RC close to the plastic barrier were removed before they started to grow into the HC. Two additional control treatments were included under strictly identical conditions. The first control consisted of Petri plates without roots and AMF in the RC. The second control comprised carrot roots associated with the arbuscular mycorrhizal fungus in the RC without proliferation of ERM in the HC (the MSR medium was cut 1 cm away from the plastic barrier in the RC to prevent the hyphae from crossing the partition wall separating the RC from the HC). Three treatments were thus considered: absence of mycorrhizal roots in

the RC and *R. irregularis* in the HC (RC^{-MR}/HC^{-RI}); presence of mycorrhizal roots in the RC but without proliferation of *R. irregularis* in the HC (RC^{+MR}/HC^{-RI}); presence of mycorrhizal roots in the RC; and proliferation of *R. irregularis* in the HC (RC^{+MR}/HC^{+RI}) (see Fig. 1). Each treatment consisted of four replicates.

After an additional 2 weeks, the ERM of the RC^{+MR}/HC^{+RI} treatment developed extensively on the slope. Ten milliliters of liquid MSR medium without $Ca(NO_3)_2{\cdot}4H_2O$, sucrose, and vitamins, but containing 280 μM phytate (Na-phytate, Sigma-Aldrich, Saint Louis, USA), was added to the HC. Phytate was added as unique source of P to investigate the cooperation between *R. irregularis* and *R. aquatilis*. The ERM extended from the slope into the whole HC. There were two reasons to remove $Ca(NO_3)_2{\cdot}4H_2O$ from the liquid MSR medium. First, the uptake of NO_3^- by the ERM in the HC of the RC^{+MR}/HC^{+Ri} treatment would decrease its concentration as compared with the two other treatments, and at the same time would increase the pH of the MSR medium in this compartment which could then decrease the solubility of phytate. Second, Ca^{2+} could precipitate the added phytate and decrease its concentration in the MSR medium.

After another 4 weeks, the surface of the HC was covered by actively growing hyphae. The remaining liquid MSR medium (approximately 9 ml) in the HC was transferred to a 15-ml tube with a micropipette and adjusted to 10 ml using the same liquid MSR medium as above but without phytate. Then, 2 ml of bacterial supernatant with a concentration of approximately 10^8 CFUs ml^{-1} was added and mixed uniformly. The 12-ml MSR medium containing the bacteria was added back to the HC of each experimental system with a micropipette. The liquid medium was removed carefully and the Petri plates refilled carefully as well. The medium was removed along the border of the Petri plate, where no hyphae were present (at the furthest distance from the hyphae). So, there was no physical contact between the micropipette and the hyphae. Therefore, although we cannot entirely exclude that some hyphae may have been injured, the risk of that was extremely low. The *R. aquatilis* inoculum was prepared as follows: bacteria were cultured in liquid Luria–Bertani medium with shaking at 180 rpm at 28 °C until OD_{600} reached 0.4–0.6 (logarithmic phase) and then centrifuged at 5878*g* for 6 min. The supernatant was discarded, and the pellet was resuspended and washed with sterilized 0.85% (*w*/*v*) NaCl solution three times. The supernatant was then adjusted to $OD_{600} = 0.8$ with the autoclaved 0.85% NaCl solution and stored for 4 h before use.

Harvest and gene expression analysis

The liquid MSR medium containing *R. aquatilis* was added to the HC of each Petri plate. At 1, 6, 12, 24, 48, and 72 h, 0.5-ml medium was sampled with a micropipette and added to 1-ml RNAprotect® Bacteria Reagent (Qiagen) according to the handbook, to stabilize the bacterial RNA. The samples were then stored at − 80 °C until RNA extraction.

Total RNA was extracted from the frozen bacteria using the RNeasy® Mini Kit (Qiagen), according to the manufacturer's instructions and treated with a TURBO DNA-*free*™ Kit (Ambion) to remove possible DNA contamination. For single-strand cDNA synthesis, 300 ng of total RNA was reverse-transcribed at 65 °C for 10 min, 55 °C for 20 min, and 85 °C for 5 min in a final volume of 20 μl containing 1 μl random primer using Transcriptor High Fidelity cDNA Synthesis Kit (Roche). The products were then diluted to 100 μl.

Quantitative RT-PCR (qRT-PCR) was performed using a LightCycler® 96 Real-Time PCR System (Roche). Each PCR reaction was carried out in a total volume of 10 μl containing 2.5 μl cDNA, 5 μl 2 × FastStart Essential DNA Green Master (Roche), and 0.5 μl of each primer (5 μM). The following PCR program was used: 95 °C for 600 s, 45 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s. A melting curve was recorded at the end of each run to exclude that the primers had generated non-specific PCR products (Ririe et al. 1997). All reactions were performed on three technical replicates and their mean was used. Baseline range and threshold cycle (Ct) values were automatically calculated using LightCycler® 96 software. The ΔCt was calculated by subtracting the Ct value of a reference gene from the Ct value of each target gene.

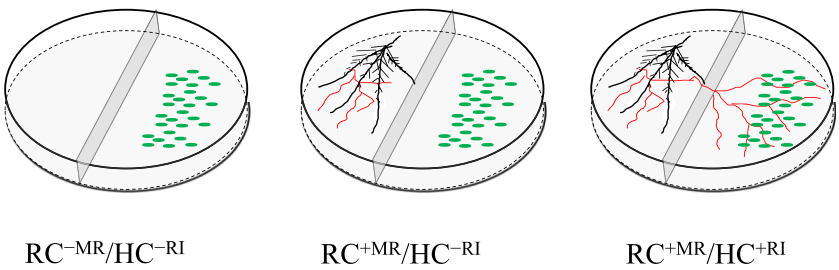

Fig. 1 Schematic representation of the experimental set-up. Red lines, extraradical hyphae of *R. irregularis*; green dots, bacterial cells of *R. aquatilis*. Treatment codes are as follows: RC^{-MR}/HC^{-RI}, absence of mycorrhizal roots in the RC and *R. irregularis* in the HC; RC^{+MR}/HC^{-RI}, presence of mycorrhizal roots in the RC but without proliferation of *R. irregularis* in the HC; RC^{+MR}/HC^{+RI}, presence of mycorrhizal roots in the RC and proliferation of *R. irregularis* in the HC. RC, root compartment; HC, hyphal compartment; RI, *R. irregularis*

Relative fold-change of each target gene was normalized by the $2^{-\Delta\Delta Ct}$ method, with reference to the ΔCt value in the RC^{-MR}/HC^{-RI} treatment harvested at the 1-h sampling time. The constitutively expressed RNA polymerase sigma factor *RpoD* was used as the house-keeping gene.

Data analysis

Statistical analyses were performed using SPSS v. 16.0 (SPSS Inc., Chicago, IL, USA). One-way repeated measures ANOVAs were performed to compare the between-subject effect of arbuscular mycorrhizal fungus, the within-subject effects of harvest time, and the time × arbuscular mycorrhizal fungus interaction on expression of three key genes, i.e., *gltA*, *icd*, and *kgdhc* in TCA cycle. Significant differences among the arbuscular mycorrhizal fungus treatments at a specific time and among the harvest times in a specific arbuscular mycorrhizal fungus treatment were evaluated by a Tukey's honest significant difference (HSD) test at $P \leq 0.05$.

Results

The one-way repeated measures ANOVA analysis showed that the between-subject effect of arbuscular mycorrhizal fungus and the within-subject effect of harvest time significantly affected the expression of all three genes (i.e., *gltA*, *icd*, and *kgdhc*), while the within-subject effect of time × arbuscular mycorrhizal fungus interaction significantly affected the expression of *gltA* and *icd* but not *kgdhc* (see Supplementary Materials Table S2).

No significant difference was found in the expression of *gltA* between the two control treatments (i.e., RC^{-MR}/HC^{-RI} and RC^{+MR}/HC^{-RI}) at any time, except 1 h. In the RC^{-MR}/HC^{-RI} treatment, the expression of *gltA* did not significantly differ among 1, 6, 12, and 24 h, but was significantly greater at 48 and 72 h than that at the other times. In the RC^{-MR}/HC^{+RI} and RC^{+MR}/HC^{+RI} treatments, the expression of *gltA* remained stable from 1 to 72 h. In the presence of the extraradical hyphae in the HC (i.e., the RC^{+MR}/HC^{+RI} treatment), the expression of *gltA* was significantly greater than either control treatment whatever the harvest time point (Fig. 2a).

No significant difference was noticed in the expression of *icd* between the two control treatments at any time point of harvest, except 6 h. In the RC^{-MR}/HC^{-RI} treatment, the expression of *icd* was the greatest at 6 h, was significantly smaller at 12 h and the following times than that at 6 h, and did not differ significantly between 48 and 72 h. In the RC^{+MR}/HC^{-RI} treatment, the expression of *icd* increased from 6 to 12 h and was the greatest at 12 h. It was significantly smaller at 24 h and the following harvest times than that at 12 h and did not differ significantly between 48 and 72 h. In the RC^{+MR}/HC^{+RI} treatment, the expression of *icd* was significantly greater at 1 and 6 h than that at 12, 24, 48, and 72 h. In the presence of the extraradical hyphae in the HC, the expression of *icd* was significantly smaller than either control treatment at all time points of harvest, except 1 h (Fig. 2b).

Whatever the harvest time, no significant difference was noticed in the expression of *kgdhc* between the two control treatments. In the RC^{-MR}/HC^{-RI} and RC^{+MR}/HC^{-RI} treatments, the expression of *kgdhc* remained stable from 1 to

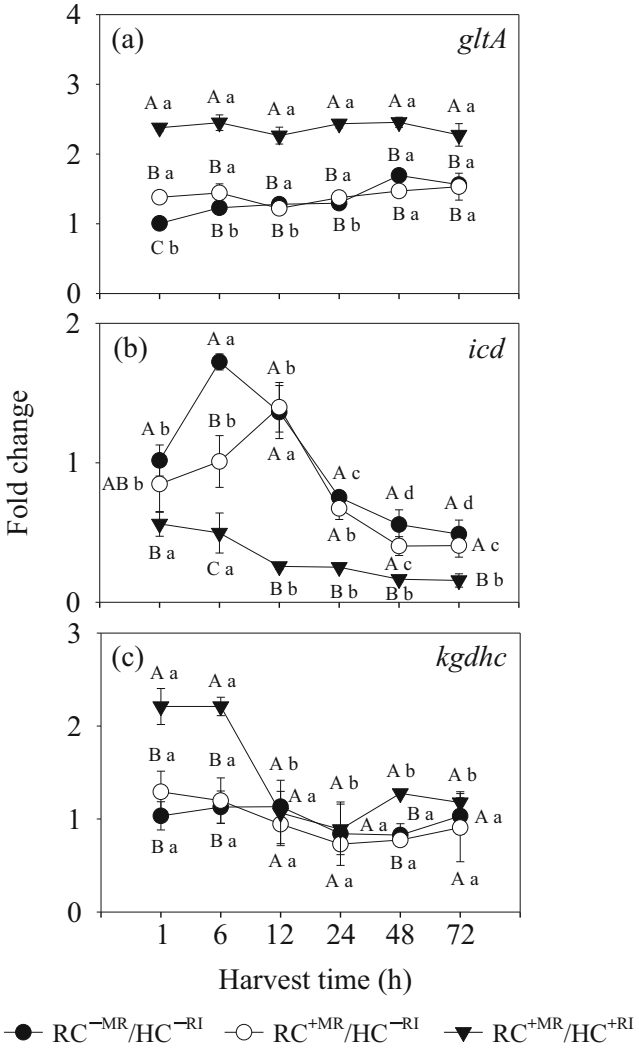

Fig. 2 Expression of **a**–**c** key genes (*gltA*, *icd*, and *kgdhc*) in the TCA cycle of *R. aquatilis* harvested from the HC of the Petri plates at different times. RC^{-MR}/HC^{-RI}, absence of mycorrhizal roots in the RC and *R. irregularis* in the HC; RC^{+MR}/HC^{-RI}, presence of mycorrhizal roots in the RC but without proliferation of *R. irregularis* in the HC; RC^{+MR}/HC^{+RI}, presence of mycorrhizal roots in the RC and proliferation of *R. irregularis* in the HC. RC, root compartment; HC, hyphal compartment; RI, *Rhizophagus irregularis*. The error bars represent the SE. The same capital letter means there is no significant difference among the arbuscular mycorrhizal fungus treatments at specific time points at $P \leq 0.05$ by Tukey's HSD test. The same lowercase letter means that there is no significant difference among the harvest times in specific arbuscular mycorrhizal fungus treatments at $P \leq 0.05$ by Tukey's HSD test

72 h. In the RC^{+MR}/HC^{+RI} treatment, the expression of *kgdhc* was significantly greater at 1 and 6 h than that at 12, 24, 48, and 72 h. In the presence of the extraradical hyphae in the HC, the expression of *kgdhc* was significantly greater than either control treatment at 1, 6, and 48 h (Fig. 2c).

Discussion

The surface of extraradical hyphae is the place of important exchanges between AMF, exuding C and other compounds, and bacteria, involved in organic N and P mobilization (Leigh et al. 2011; Zhang et al. 2014). Recently, Zhang et al. (2018) demonstrated that fructose exuded by the arbuscular mycorrhizal fungus *R. irregularis* MUCL 43194 induces the expression of phosphatase genes of the PSB *R. aquatilis* HX2, thus supporting cooperation between the two microorganisms. The stimulation of bacterial metabolism, via the provision of sugars or other compounds by the fungus, most probably depends on the cellular ATP level, which is primarily controlled by the TCA cycle (Tortora et al. 2016). In the present study, we demonstrated that the three pace-making genes were influenced differently by the AMF: *gltA* and *kgdhc* were increased and *icd* was decreased, making it difficult to determine the overall effect of the fungus on the TCA rate. We anticipated that the overall effect was positive because the expression of *gltA*, which has the greatest influence on the TCA rate, was increased, even though this has to be demonstrated conclusively.

In the present study, we selected *rpoD* as the reference gene and used the SYBR Green method in quantitative gene expression analysis for the following reasons: first, *rpoD* was selected as the reference gene to calculate the relative expression of phosphatase genes of *R. aquatilis* HX2 in a previous study (Zhang et al. 2018). In the present study, four reference genes, i.e., 16S rDNA, *lpx*, *recA*, and *rpoD*, were compared and *rpoD* was again selected as the adequate reference gene. Secondly, SYBR green and TaqMan probes are two methods used in quantitative gene expression analysis. SYBR Green is relatively inexpensive and easy to use and technically based on binding the fluorescent dye to double-stranded deoxyribonucleic acid (dsDNA). Conversely, the TaqMan method is expensive and is based on dual-labeled oligonucleotide and exonuclease activity of Taq polymerase enzyme. Normally, one method is sufficient in qPCR. SYBR is popular, and by optimization of the SYBR Green method, its performance and quality can be comparable to the TaqMan method (Tajadini et al. 2014).

The pace of the TCA cycle is more determined by the citrate synthase reaction than by the other two reactions (i.e., isocitrate dehydrogenase and α-oxoglutarate dehydrogenase) (Krebs 1970). In the present study, the expression of *gltA* was significantly increased by the presence of the arbuscular mycorrhizal fungus at all observation times. This stimulation was most probably related to an increase in the concentration of substrates needed for the citrate synthase because the production of this enzyme can be induced by its substrates (Holmes et al. 2005). Basically, two substrates could be used by citrate synthase: oxaloacetate and acetyl-CoA. Oxaloacetate can be produced from pyruvate, malate, and aspartate (Utter and Keech 1960), while acetyl-CoA is mainly synthesized from pyruvate (Korkes et al. 1951). Interestingly, the hyphae exudates contain sugars and carboxylates, e.g., fructose, glucose, acetate (Zhang et al. 2018), which are involved in glycolysis and thus in the production of pyruvate. This may then lead to an increasing concentration of oxaloacetate and acetyl-CoA. For example, *gltA* transcripts in the environment are related to the availability of acetate (Holmes et al. 2005). Hyphal exudates also contain amino acids, e.g., aspartate (Bharadwaj et al. 2012), which may lead to an increasing concentration of oxaloacetate.

Curiously, the expression of *icd* in the bacteria was decreased at all times in the presence of the ERM. The expression of this gene is influenced by various environmental factors, e.g., the concentration of oxygen and nutrients (Chao et al. 1997; Domínguez-Martín et al. 2018). For instance, it has been shown that under anaerobic condition, the expression of *icd* in *Escherichia coli* was fivefold lower than under aerobic conditions (Chao et al. 1997). It is thus not excluded that the ERM of the arbuscular mycorrhizal fungus may compete with the bacterial cells for oxygen, decreasing its concentration near the bacteria (Storer et al. 2017). For instance, in soil conditions, previous studies have proposed that AMF may compete for oxygen with ammonia-oxidizing bacteria and decrease the conversion of NH_4^+ into NO_2^- and then NO_3^-, and ultimately to a decrease in N_2O emissions from soil (Bender et al. 2014; Storer et al. 2017). Therefore, it is not excluded that competition for oxygen within the liquid MSR medium may have influenced the expression of *icd* in *R. aquatilis*.

Several factors (e.g., Acyl-CoA, metallic ions, and α-oxoglutarate) have been reported to affect the activity of the α-oxoglutarate dehydrogenase protein (Webb 1964; Erfle and Sauer 1969; Peng et al. 2015), but how they influence the gene expression of the enzyme remains mostly unknown. Interestingly, we noticed an increase in expression of *kgdhc* at the early times (i.e., at 1 and 6 h) of contact with the ERM. The decrease of this gene at the later times seemed to be related to the depletion of some compounds in the hyphae exudates. In the same in vitro culture experiment (Zhang et al. 2018), we demonstrated that fructose depleted at 6 h. Interestingly, fructose-1-phosphate is the preferred effector of the catabolite repressor/activator, which can induce the expression of other genes in the soil bacterium *Pseudomonas putida* (Chavarría et al. 2014). However, if fructose-1-phosphate can regulate the expression of *kgdhc* needs further investigation.

From this study, we concluded that the ERM of *R. irregularis* induces the expression of the gene-encoding citrate synthase, the most pace-making enzyme in the TCA cycle, which may then increase the activity of *R. aquatilis*. This completed the recent study of Zhang et al. (2018) who demonstrated that the hyphae exudates of *R. irregularis* induce the expression of phosphatase genes in this bacterium, thus improving the cooperation between the two partners. The interactions between the arbuscular mycorrhizal fungus *R. irregularis* 43194 and the PSB *R. aquatilis* HX2 respond to a fine-tuned dialog favoring cooperation within a wider community of microorganisms in which cooperation but also antagonism with AMF may take place. These findings bring new insights on the impacts of AMF at the transcriptional level on the metabolism of phosphate-solubilizing bacteria (i.e., *R. aquatilis*) developing in the hyphosphere.

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References

Agnolucci M, Battini F, Cristani C, Giovannetti M (2015) Diverse bacterial communities are recruited on spores of different arbuscular mycorrhizal fungal isolates. Biol Fertil Soils 51:379–389

Artursson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8:1–10

Bender SF, Plantenga F, Neftel A, Jocher M, Oberholzer HR, Köhl L, Giles M, Daniell TJ, van der Heijden M (2014) Symbiotic relationships between soil fungi and plants reduce N_2O emissions from soil. ISME J 8:1336–1345

Bharadwaj DP, Alstrom S, Lundquist P-O (2012) Interactions among *Glomus irregulare*, arbuscular mycorrhizal spore-associated bacteria, and plant pathogens under *in vitro* conditions. Mycorrhiza 22: 437–447

Chao G, Shen J, Tseng CP, Park SJ, Gunsalus RP (1997) Aerobic regulation of isocitrate dehydrogenase gene (*icd*) expression in *Escherichia coli* by the *arcA* and *fnr* gene products. J Bacteriol 179:4299–4304

Chavarría M, Durante-Rodriguez G, Krell T, Santiago C, Brezovsky J, Damborsky J, de Lorenzo V (2014) Fructose 1-phosphate is the one and only physiological effector of the Cra (FruR) regulator of *Pseudomonas putida*. FEBS Open Bio 4:377–386

Chen F, Guo YB, Wang JH, Li JY, Wang HM (2007) Biological control of grape crown gall by *Rahnella aquatilis* HX2. Plant Dis 91:957–963

Declerck S, Strullu DG, Plenchette C (1998) Monoxenic culture of the intraradical forms of *Glomus* sp. isolated from a tropical ecosystem: a proposed methodology for germplasm collection. Mycologia 90: 579–585

Declerck S, Dupré de Boulois H, Bivort C, Delvaux B (2003) Extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus lamellosum* can take up, accumulate and translocate radiocaesium under root-organ culture conditions. Environ Microbiol 5:510–516

Domínguez-Martín MA, López-Lozano A, Rangel-Zúñiga OA, Díez J, García-Fernández JM (2018) Distinct features of C/N balance regulation in *Prochlorococcus* sp. strain MIT9313. FEMS Microbiol Lett 365:fnx278

Erfle JD, Sauer F (1969) The inhibitory effects of acyl-coenzyme a esters on the pyruvate and α-oxoglutarate dehydrogenase complexes. BBA-Enzymology 178:441–452

Guo YB, Li JY, Li L, Chen F, Wu WL, Wang JH, Wang HM (2009) Mutations that disrupt either the *pqq* or the *gdh* gene of *Rahnella aquatilis* abolish the production of an antibacterial substance and result in reduced biological control of grapevine crown gall. Appl Environ Microbiol 75:6792–6803

Guo Y, Jiao Z, Li L, Wu D, Crowley DE, Wang Y, Wu W (2012) Draft genome sequence of *Rahnella aquatilis* strain HX2, a plant growth-promoting rhizobacterium isolated from vineyard soil in Beijing, China. J Bacteriol 194:6646–6647

Holmes DE, Nevin KP, O'Neil RA, Ward JE, Adams LA, Woodard TL, Vrionis HA, Lovley DR (2005) Potential for quantifying expression of the *Geobacteraceae* citrate synthase gene to assess the activity of *Geobacteraceae* in the subsurface and on current-harvesting electrodes. Appl Environ Microbiol 71:6870–6877

Iffis B, St-Arnaud M, Hijri M (2016) Petroleum hydrocarbon contamination, plant identity and arbuscular mycorrhizal fungal (AMF) community determine assemblages of the AMF spore-associated microbes. Environ Microbiol 18: 2689–2704

Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M, Cliff JB, Solaiman ZM, Murphy D (2015) Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. New Phytol 205:1537–1551

Karasawa T, Hodge A, Fitter AH (2012) Growth, respiration and nutrient acquisition by the arbuscular mycorrhizal fungus *Glomus mosseae* and its host plant *Plantago lanceolata* in cooled soil. Plant Cell Environ 35:819–828

Korkes S, Del Campillo A, Gunsalas IC, Ochoa S (1951) Enzymatic synthesis of citric acid. IV. Pyruvate as acetyl donor. J Biol Chem 193:721–735

Krebs HA (1970) Rate control of the tricarboxylic acid cycle. Adv Enzym Regul 8:335–353

Leigh J, Fitter AH, Hodge A (2011) Growth and symbiotic effectiveness of an arbuscular mycorrhizal fungus in organic matter in competition with soil bacteria. FEMS Microbiol Ecol 76:428–438

Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK (2013) An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environ Microbiol 15: 1870–1881

Peng B, Su Y, Li H, Han Y, Guo C, Tian Y, Peng X (2015) Exogenous alanine and/or glucose plus kanamycin kills antibiotic-resistant bacteria. Cell Metab 21:249–262

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science 289:1920–1921

Ririe KM, Rasmussen RP, Wittwer CT (1997) Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. Anal Biochem 245:154–160

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, New York

Storer K, Coggan A, Ineson P, Hodge A (2017) Arbuscular mycorrhizal fungi reduce nitrous oxide emissions from N_2O hotspots. New Phytol. https://doi.org/10.1111/nph.14931

Tajadini M, Panjehpour M, Javanmard SH (2014) Comparison of SYBR green and TaqMan methods in quantitative real-time polymerase chain reaction analysis of four adenosine receptor subtypes. Adv Biomed Res 3:85

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V, Gilbert LB, Handa Y, Herr JR, Hijri M, Koul R, Kawaguchi M, Krajinski F, Lammers PJ, Masclaux FG, Murat C, Morin E, Ndikumana S, Pagni M, Petitpierre D, Requena N, Rosikiewicz P, Riley R, Saito K, San Clemente H, Shapiro H, van Tuinen D, Becard G, Bonfante P, Paszkowski U, Shachar-Hill YY, Tuskan GA, Young JPW, Sanders IR, Henrissat B, Rensing SA, Grigoriev IV, Corradi N, Roux C, Martin F (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc Natl Acad Sci U S A 110:20117–20122

Toljander JF, Lindahl BD, Paul LR, Elfstrand M, Finlay RD (2007) Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. FEMS Microbiol Ecol 61: 295–304

Tortora GJ, Funke BR, Case CL (2016) Microbiology: an introduction, 12th edn. Pearson Education, USA

Utter MF, Keech DB (1960) Formation of oxaloacetate from pyruvate and CO_2. J Biol Chem 235:PC17–PC18

Wang F, Shi N, Jiang R, Zhang F, Feng G (2016) *In situ* stable isotope probing of phosphate-solubilizing bacteria in the hyphosphere. J Exp Bot 67:1689–1701

Webb M (1964) The biological action of cobalt and other metals: IV. Inhibition of α-oxoglutarate dehydrogenase. BBA – Specialized Section on Enzymological Subjects 89:431–446

Zhang L, Fan J, Ding X, He X, Zhang F, Feng G (2014) Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil. Soil Biol Biochem 74:177–183

Zhang L, Xu M, Liu Y, Zhang F, Hodge A, Feng G (2016) Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. New Phytol 210:1022–1032

Zhang L, Feng G, Declerck S (2018) Signal beyond nutrient, fructose, exuded by an arbuscular mycorrhizal fungus triggers phytate mineralization by a phosphate solubilizing bacterium. ISME J 12:2339–2351