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1 **Dual transcriptomics and proteomics analyses of the early stage of interaction between**
2 ***Caballeronia mineralivorans* PML1(12) and mineral**

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31 **ABSTRACT**

32 Minerals and rocks represent essential reservoirs of nutritive elements for the long-lasting
33 functioning of forest ecosystems developed on nutrient-poor soils. While the presence of
34 effective mineral weathering bacteria was evidenced in the rhizosphere of different plants, the
35 molecular mechanisms involved remain uncharacterized. To fill this gap, we combined
36 transcriptomic, proteomics, geo-chemical and physiological analyses to decipher the potential
37 molecular mechanisms explaining the mineral weathering effectiveness of strain PML1(12) of
38 *Caballeronia mineralivorans*. Considering the early-stage of the interaction between mineral and
39 bacteria, we identified the genes and proteins differentially expressed when: i) the environment is
40 depleted of certain essential nutrients (*i.e.*, Mg and Fe), ii) a mineral is added and iii) the carbon
41 source (*i.e.*, glucose vs. mannitol) differs. The integration of these data demonstrate that strain
42 PML1(12) is capable of i) mobilizing iron through the production of a NRPS-independent
43 siderophore, ii) inducing chemotaxis and motility in response to nutrient availability and iii)
44 strongly acidifying its environment in the presence of glucose using a suite of GMC
45 oxidoreductases to weather mineral. These results provide new insights into the molecular
46 mechanisms involved in mineral weathering and their regulation, and highlight the complex
47 sequence of events triggered by bacteria to weather minerals.

48

49 **Originality-Significance Statement**

50 The access and the recycling of nutrients are key processes in nutrient poor environments. In such
51 environments, plants have developed particular strategies to adapt, notably based on the selection
52 in their rhizosphere of functional communities effective at collecting the nutrients available in the
53 soil or at weathering minerals. Bacteria are considered as very effective at weathering, but our
54 understanding of the molecular mechanisms, the genes and potential regulations involved during
55 the mineral-bacteria interaction and the mineral weathering (MW) process remains limited. To
56 date, a comprehensive investigation of MW was performed on only a single model bacterial
57 species, *i.e.* strain CH34 of *Cupriavidus metallidurans*. In this context, we investigated the
58 molecular mechanism regulated in strain PML1(12) of *Caballeronia mineralivorans* (an effective
59 MW bacterial strain) depending on the level of nutrient availability, on the presence of a mineral

60 and on the carbon source. The novel results obtained through the combination of geo-chemical
61 analyses associated to genomic, transcriptomics and proteomics provided a detailed and
62 comprehensive analysis of the functions activated or repressed depending on the
63 presence/absence of the mineral. Our findings provide relevant information for different field of
64 research such as environmental genomics, geochemistry, mineralogy and soil sciences.

65

66 **Introduction**

67 Minerals and rocks represent an essential source of inorganic nutrients for the long lasting
68 functioning of nutrient-poor or/and low-input ecosystems (April and Newton, 1992). Indeed,
69 beside the nutrients originating from organic matter decomposition and atmospheric deposits,
70 they are the main reservoirs of nutritive elements such as magnesium, potassium, calcium and
71 iron. However, the nutritive content of these minerals remains entrapped into their crystal
72 structure and is not directly accessible to the biosphere. Beside their nutritive role, minerals and
73 rocks also serve as physical supports for the attachment and the development of complex
74 microbial communities (Hutchens *et al.*, 2009; Or *et al.*, 2007). They can consequently be
75 considered as reactive interfaces where inorganic nutrients are released and a microbial habitat
76 (*i.e.*, the mineralosphere) (Uroz *et al.*, 2015). The dissolution of these minerals (*i.e.*, mineral
77 weathering) is known to depend on the physico-chemical properties of these minerals (Brantley
78 *et al.*, 2008), the action of abiotic processes (protons, water circulation), and to be amplified
79 through the action of plants, fungi or bacteria (Drever, 1994; Landeweert *et al.*, 2001). However,
80 our understanding of the linkage between minerals, microorganisms and nutrient availability and
81 the impact of these factors on mineral weathering remains limited. From the few studies that have
82 analyzed the mineral-colonizing bacterial communities into the soil, it seems that this
83 colonization depends on the weatherability of the minerals, their nutritive content but also the
84 local nutrient availability (Colin *et al.*, 2017; Hutchens *et al.*, 2009; Vieira *et al.*, 2020).

85 Resource availability is known to affect the structure of the bacterial communities. While for
86 certain categories of substrates (*e.g.*, carbon and nitrogen related), the presence of the substrate
87 allows for the enrichment of substrate-degraders, for other substrates (*e.g.*, iron) the low
88 concentrations, or depletion, of the substrates allow for the selection of oligotrophic
89 microorganisms capable of recovering this substrate from its insoluble forms or of using
90 alternative substrates. It seems to be the case for mineral weathering bacteria (Huang *et al.*, 2014;
91 Wang *et al.*, 2014; Uroz *et al.*, 2009). Indeed, it has been shown through direct observation or
92 after fertilizations that this functional ability is enriched in nutrient depleted conditions. Such
93 relation was reported for grassland or forest ecosystems, where an enrichment of effective
94 mineral weathering bacteria was observed in soils presenting the lower availability of P and/or
95 the lower nutrient availability compared to nutrient-rich soils (Mander *et al.*, 2016; Nicolitch *et*
96 *al.*, 2016). Similarly, several studies analyzing different horizons of a soil profile revealed an

97 enrichment of mineral weathering bacteria in the deeper and nutrient-poorer horizons (Huang *et*
98 *al.*, 2014; Wang *et al.*, 2014). Fertilization with calcium, potassium and/or magnesium was also
99 shown to affect the structure of the mineral weathering bacterial communities (Uroz *et al.*, 2011;
100 Lepleux *et al.*, 2012; Nicolitch *et al.*, 2019). In this case, the amended conditions presented a
101 lower frequency of effective mineral weathering bacteria compared to the non-amended
102 treatment. These findings were confirmed through short-term microcosm experiments, where a
103 single input of Mg or K in a nutrient-poor soil was shown to quickly (*i.e.*, 15 days) modify the
104 frequency and effectiveness of mineral weathering bacteria (Nicolitch *et al.*, 2019). Together,
105 these studies suggest that the mineral weathering bacterial communities are reactive to variations
106 of nutrient availability, making them especially competitive in nutrient-poor conditions.

107 At the cell level, nutrient limitation is known to induce important physiological regulations in
108 bacteria, notably increasing the production of secondary metabolites, the expression of uptake
109 systems and in certain cases motility and chemotaxis (Andrew *et al.*, 2003; Buch *et al.*, 2008;
110 Childers *et al.*, 2002 ; Ishige *et al.*, 2003 ; Zheng *et al.*, 2017). These modifications evidence the
111 capability of bacteria to perceive environmental changes and to adapt to them. One of the best
112 documented example is when iron is depleted in their environment (Cai *et al.*, 2018; Lim *et al.*,
113 2012; Sasnow *et al.*, 2016). In such conditions, bacteria activate the production of iron chelating
114 molecules (*i.e.*, siderophores, haem) and enzymes (*i.e.*, deferriochelatase, haem oxygenase)
115 involved in the access to iron as well as the expression of dedicated transport systems (*i.e.*, TonB
116 dependent or ABC transporters/permeases) (Andrew *et al.*, 2003; Lim *et al.*, 2012). Due to their
117 high affinity with ferric iron and the presence of this microelement in several types of minerals,
118 the production of siderophores has been proposed as one of the main molecular mechanism used
119 by bacteria to weather minerals (Ahmed *et al.*, 2014 ; Liermann *et al.*, 2000). Indeed, these
120 molecules may directly recover the iron entrapped into the minerals or indirectly by changing the
121 equilibrium of the solution chemistry through the chelation of the available iron. Phosphorous
122 represents another important nutrient, which is frequently found limiting in soils. In P depleted
123 conditions bacteria have been shown to regulate a wide array of genes (Ishige *et al.*, 2003; Zeng
124 *et al.*, 2016, 2017). Glucose metabolism, ABC transporters and Pho system are generally down-
125 regulated under P depleted conditions (Zheng *et al.*, 2016; 2017). Notably, the expression of a
126 pyrroloquinoline quinone (PQQ)-glucose dehydrogenase gene, an enzyme involved in the
127 solubilization of inorganic phosphorous (Goldstein, 1995), was shown to decrease in

128 *Pseudomonas* when the concentration of soluble phosphate increased in solution (Zheng *et al.*,
129 2016). The important changes occurring in depleted conditions and the modulation of the
130 production of chelating and/or acidifying molecules suggest that bacteria may have developed
131 molecular mechanisms suitable to interact and to recover the nutrients entrapped into the
132 minerals, but experimental demonstrations are missing.

133 Comparatively to the studies deciphering the physiological responses of aerobic bacteria to
134 depleted vs repleted conditions, their physiological responses to the presence of a mineral
135 remains under-investigated. To our knowledge, the sole work addressing this question and
136 providing a comprehensive view was done on strain CH34 of *Cupriavidus metallidurans*, an
137 organism isolated from Icelandic basalt (Olsson-Francis *et al.*, 2010; Bryce *et al.*, 2016). Using
138 whole-genome microarray and proteomics, the authors revealed that this bacterial strain up-
139 regulated the expression of genes, or produced higher levels of proteins, involved in phosphate
140 uptake, nutrient transport, metal and redox homeostasis and motility in presence of basalt, while
141 TonB-dependent transporters and siderophore production appeared down-regulated (Olsson-
142 Francis *et al.*, 2010; Bryce *et al.*, 2016). In addition, Xia *et al.* (2012) evidenced the up-regulated
143 production of some proteins (assigned as glycine hydroxyl-methyltransferase, phosphoserine
144 aminotransferase, and ketol-acid reducto-isomerase) in a strain of *Bacillus mucilaginosus* in
145 presence of a K- bearing mineral (*i.e.*, feldspar). Those first results suggest that complex
146 mechanisms are activated or repressed during the mineral-bacteria interaction, but our knowledge
147 remains limited to few bacterial strains and experimental conditions.

148 In this context, we investigated the molecular mechanisms used by strain PML1(12) of
149 *Caballeronia mineralivorans* to interact with and weather minerals. This strain was isolated from
150 a nutrient-poor forest soil and was previously characterized for its high mineral weathering
151 effectiveness (Uroz *et al.*, 2007) and its ability to promote tree growth (Calvaruso *et al.*, 2006,
152 2013 ; Koele *et al.*, 2009). In this study, we developed a microcosm approach to investigate i) the
153 genes and proteins regulated in condition of deficiency of certain nutritive elements, ii) the genes
154 and proteins regulated in presence of a mineral in such depleted conditions and iii) how the
155 carbon source (*i.e.* glucose vs. mannitol) impacts the response of strain PML1(12). To answer
156 these questions, we used a dual transcriptomics and proteomics approach combined with solution
157 and mineral chemistry to document the physiological processes activated or repressed at the early
158 stage of the interaction between mineral and bacteria.

159

160 **RESULTS**

161

162 *Chemical analyses of the solution*

163 The analyses done on the solution after 20h of incubation (*i.e.*, culture medium) revealed
164 significant differences between the different treatments (GB, Glucose with bacteria; GBwB,
165 glucose with bacteria and biotite; MBwB, mannitol with bacteria and biotite) (Table 1; Figure 1).
166 The measures done on the abiotic controls (C, Control without bacteria and biotite and CB,
167 Control with biotite without bacteria) confirmed that in the absence of biotite, the culture medium
168 was depleted in iron and magnesium ($p < 0.05$). The addition of biotite modified the solution
169 chemistry, provoking a significant increase in solution of the nutrients originating from biotite
170 dissolution. The spontaneous hydrolysis of minerals when introduced in aqueous solution is well-
171 described phenomenon, especially for mineral particles freshly prepared (Brantley, 2008). Due to
172 the spontaneous hydrolysis of biotite, the amount of iron, aluminium and magnesium were
173 increased ($p < 0.05$), making the medium amended with biotite capable of sustaining all the
174 nutritional needs of strain PML1(12).

175 In absence of biotite, the inoculation of PML1(12) (*i.e.*, GB treatment) lead to the same
176 solution chemistry as the non-inoculated treatment (*i.e.*, C treatment), except that the pH of the
177 solution decreased from pH 6.5 to 2.78. The comparison of the treatments amended with biotite
178 and containing glucose evidenced a significant increase of the concentration of iron, magnesium
179 and aluminium in the solution of the inoculated treatment, while the concentrations of other
180 nutrients (Na, P, Ca) decreased ($p < 0.05$). In presence of glucose (GB and GBwB), the pH of the
181 solution reached a value of 2.77 in presence or absence of biotite. When the microcosms were
182 amended with mannitol (MBwB) as carbon substrate only a significant increase of the
183 concentration of aluminium in the solution of the inoculated treatment and a decrease of Na and P
184 were observed, without variations of the concentrations of magnesium and iron in comparison to
185 the control. In presence of mannitol, the pH of the solution reached a value of 4.03. Last, the
186 comparison of the treatments amended with biotite and a carbon source (*i.e.*, glucose (GBwB) or
187 mannitol (MBwB)) evidenced a significant increase of the concentration of iron, magnesium and
188 aluminium in the solution of the glucose amended treatment (GBwB ; $p < 0.05$), and no

189 differences for the other nutrients (Na, P, Ca). The pH of the solution appeared significantly more
190 acidic in the treatment amended with glucose than with mannitol ($p < 0.05$).

191 In the inoculated treatments, the main organic acids detected were gluconate and pyruvate
192 (Figure 1). Very low concentrations of lactate, formate, oxalate and acetate were detected. In
193 presence of glucose, significantly more gluconate was produced in absence (GB, 874 mg L⁻¹)
194 than in presence of biotite (GBwB, 586 mg/L; $p < 0.05$). Gluconate concentration was
195 comparatively very low in presence of mannitol (3.65 mg L⁻¹). No organic acid was detected in
196 the abiotic treatments.

197

198 *Physiological properties*

199 When the growth assays were done in the presence of iron and magnesium, no difference was
200 observed in the first 20h of incubation between the glucose or mannitol conditions (Figure S1).
201 However, we observed that for longer incubation periods, a higher biomass was obtained with
202 mannitol (OD_{max}=0.8 after 46h ; ca. 8.10⁸ cell mL⁻¹) compared to glucose (OD_{max}=0.65 after 40h;
203 ca. 5.10⁷ cell mL⁻¹). In the absence of iron, we observed a delay in growth in comparison to the
204 nutrient rich (*i.e.*, with iron and magnesium) conditions, but no significant difference in the first
205 20h of growth between the glucose and mannitol conditions. In such environments, a higher
206 biomass was obtained with mannitol (OD_{max}=0.53 after 65h; ca. 3.10⁷ cell mL⁻¹) compared to
207 glucose (OD_{max}=0.47 after 55h; ca. 8.10⁶ cell mL⁻¹) (Figure S1). In absence of iron in the culture
208 medium, the chromazurol assay highlighted that strain PML1(12) produced a chelating agent in
209 both glucose and mannitol treatments (Figure S2). In presence of iron, no chelating agent was
210 detected using the CAS assay. Similarly, no chelating agent was detected in the culture
211 supernatant in presence of biotite.

212

213 *Transcriptome and proteome statistics and comparisons*

214 After 20h of incubation, the same samples were used to extract RNA and recover proteins. For
215 the RNAseq dataset, the number of reads retained after barcode removal and quality control,
216 varied from 35 to 45 million, giving a total of 138 Gb of 150bp paired-end sequence data.
217 Between 98.16 and 99.78% of reads mapped at least once on the genome of *C. mineralivorans*
218 PML1(12). After the removal unmapped sequences and the remaining rRNA sequences, between
219 80 to 91% of the total number of reads were retained. Without log₂Foldchange (log₂FC)

220 threshold, a total of 3,984 and 3,644 differentially expressed genes (DEG); corresponding to 43.5
221 and 39.8% of the genes of PML1(12), respectively) were detected in GBwB and MBwB
222 treatments with a significant pvalue (details in material and methods). When a threshold of 1/-1
223 (log₂FC) was applied, DEG numbers reached 1,153 and 1,241 (corresponding to 12.6 and 13.5%
224 of the genes of PML1(12), respectively) in the GB/GBwB and GBwB/MBwB treatment
225 comparisons respectively. The proteomics dataset consisted in a total of 576,330 MS/MS spectra
226 that were recorded at high resolution, among which 368,661 (61%) were assigned to peptide
227 sequences attributed to strain PML1(12). The number of peptide sequences per sample varied
228 from 25,000 to 37,000. Based on stringent parameters (at least two peptides per protein and FDR
229 below 1%), a total of 2,408 proteins were identified.

230 To obtain a global view of our dataset, multivariate analyses (*i.e.*, Partial Least Squares
231 regression (PLS)) was performed. Due to the high amount of genes and to permit a good and
232 interpretable visualization of the genes associated to each treatment a Sparse Partial Least
233 Squares regression (sPLS) was preferred to a PLS analysis. These analyses clearly permitted to
234 visualize the differentiation of the treatments (*i.e.*, GB vs GBwB vs MBwB). Both the RNA-
235 based, the protein-based and the combined (RNA+protein) analyses gave a good separation of the
236 different treatments considered (Figure 2). The list of the genes the most associated to each
237 treatment whatever the approach (*i.e.*, RNA or protein) is presented in Table S1. This global
238 analysis was then completed by a detailed analysis using DESeq2. For each treatment comparison
239 (*i.e.*, GB vs GBwB or MBwB vs GBwB), the transcriptome and proteome data were compared
240 according to the differential expression/abundance (*i.e.*, RNA and proteins). These analyses
241 revealed that though the proteomes were supported by a smaller number of proteins identified as
242 significantly differentially produced (DPP) compared the DEG of the transcriptomes, the
243 sequences encoding these proteins were found in the related transcriptome. In the comparison
244 GB/GBwB, 100% of the differentially produced proteins (*i.e.*, 41 in total) were recovered in the
245 1153 DEG of the related transcriptome (Figure 3A). In the comparison GBwB/MBwB, 82% of
246 the DPP (*i.e.*, 198 in total) were recovered in the 1241 DEG of the related transcriptome (Figure
247 3B). The comparison of the DEG and DPP obtained through the transcriptomic and proteomics
248 approaches revealed significant correlations according to linear regression analyses ($p < 0.0001$;
249 R^2 : 0.78 for GB/GBwB; R^2 : 0.58 for GBwB/MBwB), showing that the common down- and up-
250 regulated genes followed the same trend in the transcriptome and proteome datasets. The genes

251 differentially regulated ($|\log_2FC| \geq 1$) and common to the RNAseq and proteomics dataset are
252 presented in Table 2.

253

254 *Overview of the variations in the Clusters of Orthologous Groups (COG)*

255 Although the depth of analysis was very different between the two methods (i.e., proteomics
256 and transcriptomics), a functional analysis was performed to assign the differentially regulated
257 genes of *C. mineralivorans* PML1(12) into the COG categories both at the RNA and protein
258 levels (Figure 4). In the glucose treatments (i.e., GB vs GBwB), the higher percentages of DEG
259 and DPP were observed for the amino acid transport and metabolism (RNA: 11.2% vs Protein: 17
260 %), cell wall/membrane/envelope biogenesis (RNA: 8.2% vs Protein: 19.5 %), carbohydrate
261 transport (RNA: 8.2% vs Protein: 19.5 %) and inorganic ion transport and metabolism (RNA:
262 7.45% vs Protein: 19.5 %). The main differences between these two treatments in the
263 transcriptome were observed for cell motility (6% in GBwB vs 0.13% in GB), cell
264 wall/membrane/envelope biogenesis (6% in GBwB vs 2.2% in GB) and in carbohydrate
265 metabolism and signal transduction (Figure 4AB). A focus on the GB treatment revealed only a
266 higher proportion of DEG in the categories S (poorly characterized functions, GB:3.85 % vs
267 GBwB:1.72%), H (coenzyme transport and metabolism, GB:1.40 % vs GBwB:0.66%) and K
268 (Transcription, GB:3.36 % vs GBwB:2.78%) detected by the transcriptome. At the proteome
269 level, several COG categories were not detected in the GB treatment among the DPP (i.e.,
270 C,D,F,G,H,K,L,N,S). Cell wall/membrane/envelope biogenesis and amino acids were more
271 represented in the GBwB treatment, while only the category P (Inorganic ion transport and
272 metabolism) appeared higher in the GB treatment (GB: 12.20% vs GBwB:4.88%).

273 In the comparison glucose (GBwB)/mannitol(MBwB) treatments, the higher proportions of
274 DEG/DPP were observed for amino acid transport and metabolism (RNA: 21.5% vs Protein: 16.7
275 %), cell wall biogenesis (RNA: 8% vs Protein: 10 %), carbohydrate transport (RNA: 8.6% vs
276 Protein: 13.6 %), signal transduction (RNA: 7.2% vs Protein: 11 %) and inorganic ion transport
277 and metabolism (RNA: 7.5% vs Protein: 3.5 %)(Figure 4CD). Most of the categories were
278 mainly found in the MBwB treatment, except the categories C, D, H and O. The same
279 distribution was observed in the proteome.

280

281 *Functional changes induced by growth in nutrient-deprived conditions*

282 The comparison done between the GB and GBwB treatments permitted to identify the genes
283 and/or proteins significantly expressed in the condition deprived in iron and magnesium. On the
284 1,153 DEG and the 41 DPP identified in the comparison of the GB/GBwB treatments, a total of
285 621 and 23 were significantly higher in the GB treatment in the transcriptome and proteome,
286 respectively.

287 Among the most up-regulated genes or the proteins presenting an increased abundance in the
288 GB treatment (Table S2A and B), several appeared involved in ion transport. Indeed, higher
289 signal were observed for ion porin (KLU25682.1; log₂FC:5.5) or transporter (KLU24497.1), ion
290 permease (KLU25664.1; Log₂FC:5.5). All these genes are homologous to genes coding for
291 proteins predicted to allow the incorporation of manganese, magnesium, potassium or
292 phosphorous. Interestingly, the molecular markers supporting these functions were members of
293 the top 5 of the up-regulated genes in the DEG dataset and the most produced in the DPP dataset.
294 A total of 33 genes involved in iron access and storage appeared up-regulated in the nutrient-
295 deprived condition (*i.e.*, GB). Several iron transporter/receptors (TonB and ABC types) were in
296 the top 20 of the most expressed genes (Table S2A and B). Indeed, 5 of the 20 putative TonB
297 receptors detected in the genome of PML1(12) were significantly up-regulated. In addition, a
298 cluster of 9 successive genes (KLU26370.1 to KLU26361.1) encoding a putative siderophore was
299 detected using antiSMASH based on the presence of the gene KLU26365.1 (log₂FC:4.4)
300 presenting high homology to the *IucA/IucC* system, know in *Escherichia coli* to code for the
301 production of aerobactin. In addition, two regions involved in the mobilization of iron from the
302 cell reserve appeared significantly more expressed. The first region is composed of a gene
303 encoding a putative deferrochelatase (KLU25830.1, log₂FC:4.5) and several neighbouring genes
304 (KLU25828.1-KLU25831.1) appeared up-regulated. The second region (KLU20274.1-
305 KLU20238.1) is characterized by the presence of a bacterioferritin-associated ferredoxin, iron
306 transporters and a permease, all up-regulated. Several of the proteins specified by these genes
307 were also recovered in the proteins presenting an increased abundance in the comparative
308 proteome analysis as for example the deferrochelatase (KLU25830.1), iron ABC transporter
309 (KLU25829.1, KLU25827.1) (Table S2A and B).

310 Different genes related to glycogen, glucose and by-product metabolisms appeared up-
311 regulated according to the transcriptome data. Notably, several genes encoding enzymes involved
312 in the synthesis of glycogen and trehalose were detected (Glycogen operon protein homologous

313 to GlgX (KLU21704.1, log2FC:2.6) ;Glycogen debranching enzyme (KLU25338.1, log2FC:1.5);
314 Glycogen synthase (KLU25341.1, log2FC:2.1) ; trehalose-6-phosphate synthase (KLU22111.1;
315 log2FC:1.8); Malto-oligosyltrehalose synthase (KLU25336.1, log2FC:2.4) ; Trehalose synthase
316 (KLU25392.1, log2FC:2). Among the genes related to glucose metabolism, two regions involved
317 in the production of gluconic acid were detected among the up-regulated genes. The first region
318 corresponds to the locus encoding a flavin adenine dinucleotide (FAD)-dependent enzyme
319 presenting high sequence similarity with a Glucose/methanol/choline (GMC) oxidoreductase.
320 This enzyme (termed GMC1) is composed of three subunits, a large unit (KLU25169.1,
321 log2FC:1.1), a small unit (KLU25168.1, log2FC:1) and a cytochrome unit (KLU25978.1,
322 log2FC:1.1), all the corresponding cistrons being significantly up-regulated. The second region
323 encompasses genes coding for proteins involved in the synthesis of pyrroloquinoline quinone
324 (pqq), which is known to be the cofactor of pqq-dependent enzymes such as the glucose
325 dehydrogenase. Based on sequence similarity, genes *pqqA, B, C, D, E* as well as several pqq-
326 dependent enzymes were detected in the genome of the strain PML1(12). Three of the pqq-
327 dependent enzymes have as potential substrates methanol (*i.e.*, pqq-dependent methanol DH;
328 KLU21389.1 and KLU25669.1) and glucose (pqq-dependent glucose DH; KLU26637.1), while
329 the substrate for the last one remains undefined (pqq-dependent alcohol DH ; KLU25853.1).
330 Among the cofactor genes, *pqqB,C,D* and *E* ($1.2 < \log_2FC < 1.6$) were up-regulated in the GB
331 treatment, with a low expression level. For the pqq-dependent enzymes, all were expressed, but
332 only genes KLU25853.1 (Log2FC:1.3), KLU25669.1 (Log2FC:0.9) and KLU21389.1
333 (Log2FC:0.6) were slightly up-regulated, but poorly expressed. In addition, gene KLU20907.1
334 encoding a 2,5-diketo-D-gluconic acid reductase (*i.e.*, the enzyme which catalyzes the reduction
335 of 2,5-diketo-D-gluconic acid to 2-keto-L-gulononic acid) appeared significantly up-regulated
336 (Log2FC:2.2) in the nutrient limited treatment.

337

338 *Changes induced by growth in nutrient-deprived condition amended with biotite*

339 The comparison done between the GBwB and GB treatments permitted to identify the genes
340 and/or proteins significantly expressed in presence of biotite. However, biotite spontaneously
341 releases nutrients in the medium as observed by comparing the CB and GBwB treatments at the
342 chemical level (Table 1). Consequently, part of the transcriptomics and proteomics changes
343 observed correspond to a sum of mechanisms involved in the perception and adaptation to the

344 presence of the mineral, but also to the different nutrients released in the solution from the biotite
345 (part from the spontaneous dissolution and part related to the mineral weathering ability of strain
346 PML1(12)). Among the DEG and DPP identified molecular players, 532 and 18 were
347 significantly higher in the GBwB treatment (compared to the GB treatment) in the transcriptome
348 and proteome, respectively (Table S3A and B).

349 A total of 43 genes involved in motility presented a higher expression in presence of the
350 mineral, although no surface adhesion was observed. Notably, 34 genes of a cluster of 38
351 successive genes (KLU23267.1 to KLU27815.1) are involved in flagella construction and
352 functioning (Table S3A and B). Some of these genes as well as from other genomic regions were
353 present in the top 10 of the most up-regulated genes such as FliS (KLU23268.1, log₂FC:4.4),
354 FliT (KLU23267.1, log₂FC:4.1), flagellin synthesis (KLU23267.1, log₂FC:4.1). Notably, the
355 flagellin synthesis (KLU23267.1) was also found in the proteins presenting an increased
356 abundance (log₂FC:2). In addition to the motility, several genes related to chemotaxis or linking
357 chemotaxis/motility (n=24) were up-regulated. It was notably the case of several genes annotated
358 as *cheB,D,E,Y* homologs and grouped in the same genomic region (KLU22901.1 to
359 KLU22934.1). Beside the Che related genes, several genes encoding methyl-accepting
360 chemotaxis sensory transducers (n=16) were also up-regulated.

361 Different genes related to monosaccharides and byproduct metabolisms appeared up-regulated
362 in presence of the mineral according to the transcriptome data, confirming the analyses done at
363 the COG level. Several genes encoding glycosyl transferases such as (e.g., KLU27900.1,
364 KLU27927.1, KLU27959.1, KLU27926.1, KLU27889.1, KLU20777.1, KLU20776.1,
365 KLU20778.1, KLU26149.1, KLU26333.1, KLU26150.1). Among these genes related to glucose
366 metabolism, a region potentially involved in the production of gluconic acid was detected. It
367 corresponded to a flavin adenine dinucleotide (FAD)-dependent enzyme presenting high
368 sequence similarity with a second GMC oxidoreductase. This enzyme (termed GMC2) is
369 composed of three subunits encoding a large unit (KLU25979.1), a small unit (KLU25980.1) and
370 a cytochrome unit (KLU25978.1), all significantly up-regulated (log₂FC:1.1 to 1.9). These genes
371 appeared up-regulated, but expressed at a relatively low level. The GMC2 enzyme presented
372 sequence similarity (21%) with the D-gluconate 2-dehydrogenase large subunit of *Gluconobacter*
373 *frateurii* (BAH80545.1).

374 Among the up-regulated genes or proteins, several corresponded to potential reductases
375 involved in the reduction of ammonium (KLU22414.1, log₂FC:2.4), sulfate (KLU22416.1,
376 log₂FC:2.9) and hydroperoxide (KLU26653.1, log₂FC:1.2) and to specific transporters such as
377 gene KLU22692.1 (ammonium transporter, log₂FC:3.5).

378
379 *Change induced by growth in nutrient-deprived condition amended with biotite and according to*
380 *the carbon substrate*

381 Previous experiments revealed that strain PML1(12) was very effective at weathering biotite
382 in presence of glucose, but poorly effective with mannitol (Uroz et al., 2007). The mineral
383 weathering data presented above confirmed these observations. Both carbon substrates are
384 preferential substrates used by the strain PML1(12) for its growth, with a tendency to grow better
385 with mannitol. To avoid a growth effect and to test the impact of these substrates, a short-term
386 incubation time (20h) was done using mannitol or glucose. The comparison done between the
387 GBwB and MBwB treatments (i.e., presence of biotite but two C substrates) permitted to identify
388 the genes and/or proteins significantly expressed in the condition deprived in iron and magnesium
389 in relation to the carbon substrate available for the growth of strain PML1(12) (Table S4A and
390 B).

391 The GBwB and MBwB treatment comparison evidenced significant metabolic changes. On
392 the 1241 DEG and the 198 DPP identified in the comparison of the GBwB/MBwB treatments, a
393 total of 370 and 58 were significantly higher in the GBwB treatment in the transcriptome and
394 proteome, respectively. Several genes related to the pentose phosphate, glycolysis, glyoxylate or
395 Entner-Doudoroff pathways appeared significantly more expressed in the GBwB treatment.
396 Among the most up-regulated genes or in the proteins presenting an increased abundance those
397 involved in gluconate and 2-ketogluconate metabolism and transport are noteworthy
398 (KLU20897.1 to KLU20902.1) (Table S4A and B). The genes KLU20902.1 (log₂FC:4.8)
399 encoding a putative 2-dehydro-3-deoxygluconokinase and KLU20900.1 (log₂FC:4.11) encoding
400 a gluconate-2-dehydrogenase (GADH) were the second and the sixth genes the most up-regulated
401 in the GBwB treatment compared to MBwB, respectively. In addition, several genes encoding
402 GMC oxidoreductases were up-regulated in the transcriptome (GMC1: small (KLU25168.1) /
403 large (KLU25169.1) / cytochrome (KLU25978.1), log₂FC:1.87 for each subunit ; GMC3:
404 cytochrome (KLU25394.1) / large (KLU25343.1) / small (KLU25394.1), log₂FC=1.15 for each

405 subunit), but only the GMC1 (KLU25169.1) presented a significantly increased abundance in the
406 proteome (log₂FC:5.49). Several other genes related to cytochrome oxidases involved in the
407 proton motive force (PMF) and in the electron transport/respiratory chain appeared also up-
408 regulated (Cytochrome d ubiquinol oxidase subunit II, KLU26331.1; Cytochrome b561,
409 KLU25473.1; Cytochrome c-type biogenesis DsbD type, 1.18 RNA and 1.8 protein). Porin and
410 transporters, and efflux systems were also up-regulated (effluxtransporters (KLU24564.1),
411 log₂FC:3.54; porin (KLU23737.1), log₂FC:3.84). Some genes especially related to the transport
412 and mobilization of iron appeared also up-regulated like some iron permeases (KLU20236.1,
413 log₂FC:1.34; KLU25828.1, log₂FC:1.08), bacterioferritin (KLU20274.1, log₂FC:1.13) and an
414 enzyme involved in the mobilization of iron from the cell (*i.e.*, deferrochelatase/peroxidase EfeN
415 (KLU25830.1, log₂FC:1.69).

416 Comparatively to the GBwB treatment, a higher number of genes and proteins appeared up-
417 regulated in the MBwB treatment (Table S5A and B). Most of these changes were related to
418 mannitol metabolism, which differs from glucose metabolism. Notably several genes encoding
419 mannitol ABC transporter permeases (KLU22511.1, log₂FC:5.39; KLU22510.1, log₂FC:5.24;
420 KLU22513.1, log₂FC:5.01) appeared highly expressed. The gene and protein encoding a
421 mannitol-2-dehydrogenase were significantly up-regulated (KLU22486.1; log₂FC:4 with RNA
422 and 3.8 with protein). Notably, the different genes encoding the subunits (KLU25980.1,
423 KLU25979.1, KLU25978.1) of the GMC oxidoreductase (GMC2) appeared up-regulated in the
424 transcriptome in MBwB treatment, but with low expression level and log₂FC value of 0.70.

425

426 **DISCUSSION**

427 To decipher the molecular mechanisms involved in the early stage of the interaction between
428 mineral and bacteria and in the weathering of biotite, we used a combination of transcriptomics,
429 proteomics and (geo)chemistry. The single time-point considered in our study (*i.e.*, 20h) was
430 determined based on preliminary data to be in the early stage of interaction
431 bacteria/mineral/solution at both biological (almost no growth) and mineralogical levels (almost
432 no alteration). Our goal was to obtain a view of the mechanisms activated or repressed without
433 much cell replication and as early as we could quantify biotite dissolution. Indeed, after a longer
434 incubation period, cells have the time to replicate differentially between the carbon sources and
435 the system (mineral/solution/bacteria) is no longer deprived in nutrients. The transcriptomics and

436 proteomics approaches are complementary and provide a more integrative view of the regulated
437 pathways, while the concomitant (geo)chemical analyses tell us about the processes themselves.
438 The RNA-seq approach permitted to identify massively the transcriptional regulatory effects of
439 the different treatments, while proteomics gave access to the most abundant functions activated
440 according to the treatments considered. Although the depth of analysis was very different
441 between the two methods due to the methodologies, it is noteworthy that both provided a
442 congruent view of the molecular basis of mineral weathering. Indeed, we found significant
443 correlations between the proteins differentially produced (PDP) and the genes differentially
444 expressed (DEG) both using DESeq2 and Mixomics analyses. This good overlap is probably
445 linked to the fact that we considered the early stage of interaction between mineral/bacteria (*i.e.*,
446 a short-term event). Our results revealed the pathways activated or repressed in the
447 presence/absence of mineral and according to the available carbon source. Significant changes in
448 the solution chemistry as well as in the transcriptome and proteome of strain PML1(12) were
449 observed, highlighting that as expected strain PML1(12) was reactive to the presence/absence of
450 mineral in its environment and potentially induced molecular mechanisms involved in the
451 mineral weathering process.

452 The analyses performed on the solution chemistry revealed a stronger acidification in presence
453 of glucose (pH 2.7) than in presence of mannitol (pH 4.03), confirming previous observations on
454 this strain but obtained after a longer incubation time (*i.e.*, 48h; Uroz *et al.*, 2007). In
455 presence/absence of biotite, strain PML1(12) induced the same acidification. In addition, strain
456 PML1(12) was shown to produce a siderophore independently of the carbon source, but
457 dependent on Fe-depletion. The production of siderophore was not detected in presence of biotite,
458 suggesting that the amount of Fe released spontaneously was sufficient to repress its synthesis.
459 The growth assays revealed no difference in biomass development in the first 20h of
460 bacteria/mineral interaction whatever the treatment, evidencing that the differences measured in
461 our setup were not attributable to putative differences in cell growth. After longer incubation
462 times, a growth lag was observed, evidencing the long-term impact of iron and magnesium
463 depletion on the physiology of the bacterial strain. Together with this stronger acidification, we
464 measured a significantly higher dissolution of biotite in the glucose (GBwB) than in the mannitol
465 (MBwB) treatment as revealed by the quantities of iron and magnesium released in the solution.
466 Those results further confirm the strong connection between bacterial metabolism and mineral

467 weathering (Mapelli *et al.*, 2012; Uroz *et al.*, 2009). Gluconic acid appeared as the main organic
468 acid detected in presence of glucose as carbon source, while it was almost absent in the mannitol
469 treatment. Notably, significantly higher concentrations of gluconic acid was measured in the
470 treatment without mineral (*i.e.*, GB; Fe and Mg depleted) than with mineral (*i.e.*, GBwB; Fe and
471 Mg released from biotite). Those results suggest that Mg- and Fe-dependent metabolic
472 regulations occur in strain PML1(12). This is congruent with the results of Sasnow *et al.* (2016)
473 showing that when a *Pseudomonas* strain was grown in Fe-repleted conditions, glucose was
474 mostly converted into biomass and CO₂ (96%), while in Fe-depleted conditions it was mostly
475 converted into gluconate (40%), siderophore (10%), CO₂ (30%) and marginally into biomass
476 (10%). Together our results show that the presence of biotite in the culture medium leads to
477 quantifiable chemical and physiological changes as revealed by the differences in the solution
478 chemistry and the differential production of siderophore and organic acids.

479 Due to the depletion of the culture medium in iron and magnesium, the up-regulation of
480 molecular mechanisms involved in the mobilization of iron was expected. While the ability to
481 weather mineral is well known for strain PML1(12), its ability to produce chelating agents was
482 never investigated at both chemical and genomic levels. Interestingly, the transcriptome and
483 proteome analyses revealed that several genes/proteins involved in iron mobilization and
484 transport were up-regulated in the GB treatment. Beside the different transporters and permeases,
485 two main pathways were identified. The first one corresponded to the mechanisms involved in
486 the access to extra-cellular iron. A cluster of genes encoding a putative siderophore appeared
487 among the most up-regulated genes in the Fe-depleted treatment (GB). The siderophore
488 characteristics of this cluster were confirmed by an antiSMASH analysis on the genome, which
489 revealed the presence of *iucA/iucC* homologs. The *iucA/iucC* genes encode a non-ribosomal
490 peptide synthetase (NRPS)-independent siderophore (NIS) synthetase, which is responsible of the
491 condensation of citrate, or a derivative molecule, with an amine or alcohol group, and last of the
492 displacement of a citryl intermediate (Carroll and Moore, 2018). In PML1(12) the gene found
493 presented high sequence similarity (26.5%) with the N(2)-citryl-N(6)-acetyl-N(6)-hydroxylysine
494 synthase (*iucA*) and the aerobactin synthase (*iucC*) of *E. coli*. NIS synthetases produce non-
495 peptidic siderophores such as aerobactin, rhizobactin or woodybactins (Challis, 2005; Carmichael
496 *et al.*, 2019; Lynch *et al.*, 2001; de Lorenzo *et al.*, 1986; Carroll and Moore, 2018). A similar
497 system was also reported as up-regulated by Olsson-Francis *et al.* (2010) in *Cupriavidus*

498 *metallidurans* strain CH34 growing in Fe-depleted environment compared to the same condition
499 amended with obsidian. Notably, the exact same gene organization found in PML1(12) was
500 observed in the genome of several strains of *Pseudomonas*, *Massilia*, *Rasltonia* or *Sinorhizobium*
501 and in some *Burkholderia sensu largo* (comprising *Burkholderia*, *Caballeronia* and
502 *Paraburkholderia*). The antiSMASH analysis did not identify other siderophore systems such as
503 non-ribosomal peptide synthetases (NRPS) in the genome of strain PML1(12), suggesting that
504 this gene cluster is the only one involved in the production of a siderophore. The second pathway
505 up-regulated in strain PML1(12) corresponded to mechanisms involved in the re-mobilization of
506 intra-cellular iron (Andrews *et al.*, 2003). Indeed, several enzymes involved in the mobilization
507 of iron from the cell reserves such as bacterioferritin-associated thioredoxin and deferriochelatase
508 appeared up-regulated in both transcriptome and proteome datasets. Notably, these enzymes have
509 been described to allow for the storage of this element in bacterioferritin in excess of iron to
510 protect the cell against iron toxicity, and to perform the reverse reaction in nutrient-poor
511 conditions (Andrews *et al.*, 2003). Together, these data show that under Fe-depletion, strain
512 PML1(12) activates different molecular mechanisms which may participate in its ability to live in
513 nutrient limited conditions and are potentially involved in the first step of the mineral weathering
514 process.

515 As the production of gluconic acid appeared significantly more important in absence of biotite
516 in the culture medium, a specific focus was made on the genes and proteins related to glucose
517 metabolism which regulation was increased compared to the GBwB treatment. This survey
518 evidenced two important modifications in the cell functioning. The first was related to the up-
519 regulation of genes involved in carbon storage. Indeed, several genes involved in glycogen and
520 trehalose production were up-regulated in the GB treatment. These two carbohydrates are
521 polymers of glucose produced by bacteria as storage compounds. Glycogen and trehalose are
522 usually produced when carbon is abundant but another nutritive element required for growth is
523 limiting (Argüelles, 2000; Preiss and Romeo, 1994). The up-regulation of these genes in our
524 experiments clearly evidences the physiological changes induced by Fe and Mg depletion. The
525 second modification is linked to the production of gluconic acid. From the literature, gluconic
526 acid is usually produced by a glucose deshydrogenase (GDH), which releases protons jointly with
527 the production of the gluconic acid. Glucose is usually oxidized in the periplasm to gluconate and
528 partially further oxidized to 2-ketogluconate (Hanke *et al.*, 2013). The PQQ-dependent GDHs are

529 suspected to be key enzymes involved in mineral weathering (Babu-Khan *et al.*, 1955; Golstein
530 *et al.*, 1995; Wang *et al.*, 2020). The genome of strain PML1(12) contains several enzymes
531 related to GDH, some pyrroloquinoline quinone (PQQ)-dependent and some others non-PQQ
532 dependent. Concerning the PQQ-dependent enzymes, this strain possesses three identified
533 enzymes as well as a suite of *pqq* genes (A to E) encoding the PQQ cofactor. All these genes
534 (except *pqqA*) showed a significant up-regulation in GB than in GBwB, but supported by a low
535 expression level. The potential regulation of the PQQ system was reported for some bacterial
536 strains (An and Moe, 2016; Wang *et al.*, 2020), but other studies suggested that it may be a
537 constitutive mechanism (Zheng *et al.*, 2017). Concerning the PQQ-independent enzymes, a total
538 of 4 GMC oxidoreductases were detected in this study in the genome of strain PML1(12). These
539 enzymes depend on FAD as co-factor and allow the production of gluconic acid from glucose. In
540 the depleted treatment (GB), only the GMC1 (small subunit (KLU25168.1); large subunit
541 (KLU25169.1) and cytochrome subunit (KLU25978.1)) appeared significantly up-regulated,
542 supported by a high expression level. In addition, a 2,5-diketo-D-gluconic acid reductase was
543 also significantly up-regulated in the depleted treatment (GB>GBwB). This enzyme is involved
544 in the transformation of 2,5 ketogluconic acid into 2 ketogluconic acid. The ionic
545 chromatographic analyses done in our study did not allow to distinguish between the different
546 forms of gluconic acid produced (*i.e.*, gluconic acid, 2 ketogluconic acid, 5 ketogluconic acid, or
547 2,5 ketogluconic acid) by strain PML1(12). However, we showed that strain PML1(12) is capable
548 of using gluconic acid, 2-ketogluconic acid and 5-ketogluconic acid as carbon sources (2,5
549 ketogluconic acid was not tested), suggesting that this strain produces the different forms and is
550 capable of metabolizing them. Our genomic analyses suggest that strain PML1(12) is capable of
551 forming gluconic acid through two types of GDH depending on two types of co-factors: PQQ or
552 FAD. However, the GMC oxidoreductase showed a far higher expression level than the PQQ-
553 dependent GDH and differential expression in the depleted condition (*i.e.*, GB). The down-
554 regulation of a GMC oxidoreductase was also reported for another strain of *Burkholderia* (*B.*
555 *multivorans* WS-FJ9) when phosphate concentration increased in the culture medium, but its
556 function was not investigated (Zheng *et al.*, 2017). Together, our data suggest that this pathway
557 may be the main one involved in the production of gluconic acid in strain PML1(12) in our
558 experimental conditions.

559 The comparison made between the GB and GBwB treatments highlighted the potential
560 molecular mechanisms activated in presence of biotite both using specific (i.e., RNA or Protein)
561 or combined (i.e., RNA + Protein) analyses on our omic data. The transcriptome analysis of these
562 treatments revealed that 12.6% of the genes were differentially expressed, and half of them (532
563 genes; 5.8%) were up-regulated in presence of the mineral. At the COG level, the stronger
564 differences between the two treatments (GB vs GBwB) were related to cell motility, cell
565 wall/membrane/envelope biogenesis and carbohydrate metabolism and signal transduction,
566 suggesting important physiological changes in strain PML1(12) due to the presence of the
567 mineral. The detailed analyses of the DEG confirmed these observations, with the notable
568 increase of the expression of genes involved in flagellum construction and functioning, but also
569 of the PML1(12) homologous genes of the chemotaxis Che system (*cheA,B,R,W,Z*). This system
570 allows bacteria to perceive chemical gradients through methyl-accepting chemotaxis proteins,
571 which then regulate the functioning of the flagellum (Wadhams and Armitage, 2004).
572 Interestingly, a similar increase of the motility and chemotactism gene expression was also
573 reported in the few studies dealing with bacteria (i.e., *Geobacter metallireducens*, *Cupriavidus*
574 *metallidurans*) growing in presence of complex minerals or Fe/Mn oxides (Childers *et al.*, 2002;
575 Olsson-Francis *et al.*, 2010; Bryce *et al.*, 2016). The combined activation of the chemotaxis and
576 motility suggests that PML1(12) can perceive the presence of the mineral and/or of the elements
577 released from its structure and migrates probably to the most nutrient-rich zone (i.e.,
578 mineral/solution interface). The question was to determine whether, they form a biofilm on
579 biotite. Cell attachment is known to be determined by several factors such as solution chemistry,
580 pH and quorum sensing communications (Banfield and Nealson, 1997; Yee *et al.*, 2000).
581 However, while the cells are able to migrate to the mineral, our microscopic observations have
582 not evidenced the formation of a PML1(12) biofilm on the biotite particles. In addition, in
583 previous experiments we always observed only a few cells on the mineral surfaces after
584 paraformaldehyde or alcohol fixation, suggesting that this strain does not form biofilms and is
585 only poorly adherent on the biotite particles under our experimental conditions. Consequently,
586 our results suggest that biotite dissolution induced by strain PML1(12) occurs without stable
587 colonization of the mineral surfaces and potentially through covalent interactions or distant
588 mechanisms.

589 The specific focus on the treatments with biotite (GBwB and MBwB) allowed us to uncover
590 the physiological changes induced or repressed as a function of the presence of the mineral with
591 two types of carbon source, and to quantify biotite dissolution. From these analyses, it appears
592 clearly that the acidification due to the production of gluconic acid is one of the main
593 mechanisms used by strain PML1(12) to access the nutrients trapped in the mineral. Indeed,
594 when the acidification is limited as in the MBwB treatment, the quantity of gluconic acid is
595 negligible and the dissolution of biotite very low. The genome analysis of strain PML1(12)
596 revealed the presence of a PQQ system and PQQ-dependent dehydrogenases. Indeed, strain
597 PML1(12) harbours the genes *pqqA,B,C,D* and *E* (KLU25599.1, KLU25560.1-KLU25563.1) and
598 some pqq-dependent DH (KLU25669.1:putative methanol DH; KLU21389.1:glucose DH;
599 KLU25669:methanol/ethanol DH). In addition, 4 potential GMC oxidoreductase genes are
600 distributed in the genome: the GMC1 (KLU25168.1), the GMC2 (KLU25978.1), the GMC3
601 (KLU25394.1) and the GMC4 (KLU26513.1). Three of these GMC oxidoreductases (GMC1-3)
602 are composed of 3 subunits (small, large and cytochrome) and correspond to the classical
603 architecture of such enzyme type (Cavener, 1992; Yamaoka *et al.*, 2008). The last GMC (GMC4)
604 detected in the genome does not present such organization and did not present significant up- or
605 down-regulation in the transcriptome or proteome. The GMC superfamily encompasses a wide
606 variety of FAD-dependent oxidoreductases such as gluconate dehydrogenase (GADH) glucose
607 dehydrogenase (GDH), fructose dehydrogenase (FDH), sorbitol dehydrogenase (SDH) (Sützl *et*
608 *al.*, 2019). The sequence comparison of each subunit with one another in our study revealed that
609 the GMC1 and GMC2 presented a high sequence similarity (65%; positive on 75%), while the
610 GMC3 (KLU25394.1) and GMC4 (KLU26513.1) presented a lower similarity (22% and 26%,
611 respectively only on a fraction of the protein sequence). Although their substrate remains
612 undetermined, part of these GMC enzymes may encode FAD-dependent GDH involved in the
613 production of gluconic acid. In this context, we made a correlation analysis for each FAD- or
614 PQQ-dependent enzyme using the expression data and the chemical analyses done for each
615 replicate and each treatment (Figure 5). These analyses revealed no correlation between the
616 quantity of gluconic acid and the expression level of the *pqq* genes (*pqqB,C,D*) and the related
617 PQQ-dependent DH. Although the GMC2 (gene KLU25978.1 and related subunits) was poorly
618 expressed compared to the other GMC1 and 3, we observed negative correlations with the
619 quantity of gluconic acid. These negative correlations and the fact that the GMC2 was up-

620 regulated in the treatments with low gluconic acid concentrations suggests that it does not encode
621 for a GDH. In this sense, the GMC2 large subunit has a strong sequence similarity with the D-
622 gluconate 2-dehydrogenase large subunit of *Gluconobacter frateurii*. Its up-regulation would
623 explain the low concentrations of gluconic acid detected in the supernatant in presence of biotite.
624 In contrast, significant positive correlations were observed between the quantity of gluconic acid
625 and the expression of the GMC1 (genes KLU25168.1 and related subunits) and the GMC3 (genes
626 KLU25394.1 and related subunits), which were the GMCs up-regulated in the GB and GBwB
627 treatments, respectively. Thus, our results clearly show that the production of gluconic acid in
628 strain PML1(12) is probably not due to the pqq system, but to the GMC1 and 3 oxidoreductases
629 and that the presence of glucose and the nutrient availability (*i.e.*, Fe, Mg) and/or the presence of
630 the biotite, induce a fine tuning of these two GMC enzymes in strain PML1(12). Interestingly, the
631 implication of such enzyme type (*i.e.*, FAD-dependent GDH) in mineral weathering was recently
632 evidenced for collimonads, another representative of the Betaproteobacteria (Picard *et al.*,
633 submitted). This study revealed that though collimonads did not contain pqq related genes in their
634 genome, they produce high amounts of gluconic acid and protons through the action of a GMC
635 oxidoreductase capable of oxidizing glucose to gluconic acid.

636 Based on the integration of our data and that of the literature (Olsson-Francis *et al.*, 2010;
637 Bryce *et al.*, 2016; Sasnow *et al.*, 2016; Zeng *et al.*, 2016), we progressed in our understanding of
638 the sequence of events involved in mineral/bacteria interaction and mineral weathering. Although
639 additional experiments are required to support the different steps (*i.e.*, other treatments and
640 kinetic analysis), we propose here a hypothetical functional model of the main mechanisms
641 activated by strain PML1(12) during the early stages of the interaction with the mineral that could
642 pave future experiments (Figure 6). i) The initial stage corresponds to the perception of a nutrient
643 depletion. In such condition, PML1(12) triggers the production of a siderophore, which chelates
644 the iron in solution and scavenges the Fe poorly associated to the mineral surfaces. The activation
645 of the production of siderophore in Fe-depleted conditions was expected, as it is a common
646 mechanism regulated in bacteria according to Fe-availability (Olsson-Francis *et al.*, 2010;
647 Sasnow *et al.*, 2016). At the same time and only in the presence of glucose, the bacterial strain
648 activates the pentose phosphate, glycolysis, glyoxylate and Entner-Doudoroff pathways resulting
649 in the production of gluconic acid and the acidification of the solution (Sasnow *et al.*, 2016; Zeng
650 *et al.*, 2016). Among the enzymes activated in strain PML1(12), we can point out the GMC1,

651 which activity is increased in Fe-depleted conditions. Such metabolic changes were recently
652 quantified using isotope-assisted metabolomics in *Pseudomonas putida* strain KT2440 (Sasnow
653 *et al.*, 2016). Comparing Fe-repleted and -depleted conditions, these authors showed a reduced
654 growth, a better glucose uptake and an important production of gluconic acid under Fe-
655 depletion. ; ii) when the acidification reaches a threshold in presence of mineral, siderophore
656 production is stopped as the protons released allow for a significant dissolution of the mineral and
657 for the release of sufficient levels of iron and other nutrients in the solution. Siderophore
658 production is known to be inhibited when Fe or a Fe-carrying mineral is added in a Fe-depleted
659 solution (Olsson-Francis *et al.*, 2010; Sasnow *et al.*, 2016). This raises questions about the role
660 played by the siderophore in the mineral weathering process. Indeed, as the release of the
661 nutrients entrapped into the mineral occurs spontaneously in aqueous solution and is amplified by
662 the activity of bacteria, we can wonder under which condition siderophores are produced. Indeed,
663 siderophore production is known to be repressed by minutes amount of iron (*e.g.*, 1mg/L or less)
664 (this study; Sasnow *et al.*, 2016); iii) due to the increase of nutrients in solution (*i.e.*, spontaneous
665 and microbial-increased dissolution), cells activate motility and chemotaxis and are attracted to
666 the mineral/solution interfaces. Interestingly, the *Cupriavidus metallidurans* strain CH34 was
667 also shown to activate motility and chemotaxis in presence of basalt (Olsson-Francis *et al.*, 2010),
668 suggesting that motility and chemotaxis may be common features used by bacteria to interact
669 with and weather minerals; iv) when nutrient availability increases in the solution, strain
670 PML1(12) increases the activity of a second GMC oxidoreductase (GMC3) identified as a FAD-
671 dependent GDH; and last, v) when the availability of glucose decreases, cells consume the
672 glucose derivatives (gluconic acids and derivatives; Sasnow *et al.*, 2016) and the production of
673 the GMC oxidoreductases is stopped.

674

675 **CONCLUSION**

676 Using a suite of omic approaches and (geo)chemical analyses, we were able to highlight the
677 significant physiological changes occurring in strain PML1(12) during the early stage of the
678 interaction with minerals. The suite of omics used in our study (*i.e.*, genome analysis,
679 transcriptome and proteome), their high complementarity and their integration provides a
680 comprehensive view of the molecular mechanisms activated or repressed by this bacterial strain
681 as a function of the presence or absence of the mineral. Notably, the genes evidenced in the

682 DESeq2 analyses of the transcriptomics or proteomics datasets were also found in the combined
683 RNA and proteins approach (*i.e.*, Mixomics), strengthening by this way our conclusions. Our
684 analyses showed that this bacterial strain is well equipped to survive in nutrient-poor
685 environments. Molecular mechanisms involved in iron chelation (NRPS-independent system) and
686 acidification (different types of GDH) appeared up-regulated, but in a different way depending on
687 the presence/absence of the mineral and the availability of Mg and Fe. Noticeably, our study
688 points out that GMC oxidoreductases play a stronger role in the mineral weathering process in the
689 strain PML1(12) than does the PQQ-dependent GDH. To elucidate the dynamics of the next steps
690 of the mineral weathering pathway proposed in our model will require further experimentation
691 and the verification of the role of the different candidate genes identified.

692

693 **MATERIAL AND METHODS**

694

695 *Bacterial strain and culture media*

696 Strain PML1(12) was used in our study as model organism due to its mineral weathering
697 effectiveness (Calvaruso *et al.*, 2006; Uroz *et al.*, 2007). Initially described as a *Burkholderia*,
698 genomic and physiological analyses allowed us to assign this strain to a new species of the genus
699 *Caballeronia* named *Caballeronia mineralivorans* (Uroz and Oger, 2015; 2017). In addition,
700 metagenomic analyses highlighted that culturable representatives of this genus are particularly
701 enriched in the rhizosphere of trees as well as on the surfaces of minerals (Lepleux *et al.*, 2012;
702 Colin *et al.*, 2017). Strain PML1(12) was grown at 25°C in solid and liquid Luria-Bertani (LB)
703 media as well as in 1/10 diluted Tryptic Soy Agar (TSA) medium (Tryptic Soy Broth from Difco,
704 final concentration : 3 g L⁻¹ and agar, 15 g L⁻¹). For the microcosm experiment described below, a
705 modified version of the Bushnell-Haas (BHm; devoid of iron and magnesium) medium was used
706 to create a nutrient deficiency complementary to the nutrients contained in the mineral used (*i.e.*,
707 the biotite; description below). The BHm composition was as follows (g L⁻¹): KCl, 0.020;
708 NaH₂PO₄·2H₂O, 0.080; Na₂HPO₄·2H₂O, 0.090; (NH₄)₂·SO₄, 0.065; KNO₃, 0.100; and CaCl₂,
709 0.020, buffered at pH 6.5 and supplemented with glucose or mannitol (2 g L⁻¹) as carbon source.
710 Glucose or mannitol were selected because they represent the dominant carbohydrate sources
711 found in forest soil (Jolivet *et al.*, 2006; Medeiros *et al.*, 2006) and also because strain

712 PML1(12) presented differential mineral weathering effectiveness in relation to these two
713 substrates (Uroz *et al.*, 2007).

714
715 *Mineral description and preparation*
716 The mineral chosen was a biotite from Bancroft (Canada). Biotite is a 2:1 phyllosilicate,
717 frequently present in acid soils, which weathers relatively quickly and holds K, Mg, and Fe
718 nutrient elements (which are indispensable for plants). Its chemical composition is 41.01% SiO₂,
719 10.9% Al₂O₃, 2.21% Fe₂O₃, 10.05% FeO, 0.27% MnO, 18.9% MgO, 0.41% Na₂O, 9.46%
720 K₂O, 2.28% TiO₂, 4.42% F, and 0.08% Zn (Calvaruso *et al.*, 2006). Its structural formula is
721 $(\text{Si}_3\text{Al}_1)(\text{Fe}^{3+}_{0.12}\text{Fe}^{2+}_{0.61}\text{Mg}_{2.06}\text{Mn}_{0.02}\text{Ti}_{0.13})$ and $\text{K}_{0.88}\text{Na}_{0.06}\text{O}_{10}(\text{OH}_{0.98}\text{F}_{1.02})$. This composition was
722 determined with inductively coupled plasma–atomic emission spectroscopy (ICP-AES), after
723 alkaline fusion with LiBO₂ and dissolution in HNO₃. The biotite crystals were ground, treated
724 ultrasonically (2 min, three times at 100V) to remove the fine particles that electrostatically
725 adhere to the particles, washed three times with distilled water, and then sieved (mesh size 200–
726 400 μm) to obtain size calibrated particles. The particles were then dried before any
727 manipulation. Freshly prepared minerals are known to release weak amount of nutrients when
728 introduced in aqueous solution (Brantley, 2008). This spontaneous hydrolysis is due to the
729 physical preparation of the size-calibrated particles.

730
731 *Bacterial inoculum preparation*
732 The bacterial strain PML1(12) was grown from a -80°C glycerol stock on 1/10 diluted TSA agar
733 medium at 25 °C for 48h. A single colony was used to inoculate 50 mL of LB medium and
734 incubated for 48h in at 25 °C. Ten milliliters of this culture were then collected (8,000 g 15 min
735 at 4°C), washed three times in sterile water and suspended in 5 mL sterile water to obtain a
736 calibrated suspension at OD_{595nm} (optical density) = 1 (ca. 10⁹ cell mL⁻¹).

737
738 *Experimental design*
739 The microcosms consisted of 100 mL capped Erlenmeyer flasks filled with 45 mL of sterile BHm
740 medium devoid of Fe and Mg, amended or not with 2 g of autoclaved biotite particles (size: 200–
741 400 μm). To avoid iron contamination, all glassware was washed with 3% HCl and rinsed with
742 deionised water. For each inoculated treatment, 5 mL of bacterial inoculum was added. As a

743 spontaneous hydrolysis of biotite is known to occur in aqueous solution, non-inoculated
744 microcosms containing culture medium with (control with mineral, CB) and without mineral
745 (control without mineral, C) particles were also included. In these treatments, a volume of 5 mL
746 of sterile water was added. For each condition treatment, four flasks were prepared. Our
747 experimental design was determined to allow a comparison between: i) the GB
748 (glucose+bacteria) treatment vs GBwB (glucose+bacteria+biotite) treatment, where GBwB is our
749 baseline (presence of the mineral and of all the nutrients required for growth); ii) the GBwB
750 (glucose+bacteria+biotite) treatment vs the MBwB (mannitol+bacteria+biotite) treatment, where
751 MBwB is our baseline (absence of mineral weathering). No treatment MB (mannitol+bacteria)
752 was considered as strain PML1(12) is not effective at weathering with mannitol (Uroz *et al.*,
753 2007). No ‘non nutrient-limited condition’ was included in our experimental design as we were
754 interested only in the direct and indirect effects (*i.e.*, the mineralosphere effect; Uroz *et al.*, 2015)
755 of the introduction of the mineral in our system. Microcosms were incubated at 22 °C with
756 agitation at 140 rpm in an orbital shaker (INFORS/Minitron). The duration of our experiment
757 (*i.e.*, 20h) was determined based on preliminary data to be in the early stage of the interaction
758 bacteria/mineral/solution at both biological (almost no growth) and mineralogical levels (almost
759 no alteration). Our goal was to obtain a view of the mechanisms activated or repressed without
760 much cell replication and as early as we could quantify biotite dissolution. Indeed, after a longer
761 period, cells have the time to replicate differentially between the different carbon sources and the
762 system (Mineral/solution/bacteria) is no longer deprived in nutrients. In addition, in our
763 experimental conditions, strain PML1(12) requires more than 4h to replicate and the acidification
764 of the solution in presence of glucose began evident only after more than 18h in our experimental
765 conditions and is maximum after 48h.

766

767 *Chemical analyses*

768 The supernatant was recovered by centrifugation (10,000 g for 15 min) and filtered at 0.22µm
769 (GHP Acrodisc 25 mm syringe filter ; PALL). A volume of 10 mL was used to determine the
770 concentrations of K, Na, P, Ca, Mg, Fe and Al in the different treatments. The analyses were
771 done by inductively coupled plasma-atomic emission spectrometry (700 Series ICP-OES, Agilent
772 Technologies). The pH was measured with a pH meter (DL70 ES, Mettler). Chemical analyses
773 were performed for all replicates. The culture supernatant was also analyzed to determine the

774 major organic acids released by the bacteria. These analyses were performed on an ion
775 chromatography with conductivity detection (ICS 3000, Dionex Corp.) associated to an analytical
776 column (IonPac® AS 11 HC, Dionex corp.) according to Balland *et al.* (2010). The supernatants
777 were eluted with KOH solutions of varying concentrations (0.9–60 mM) over time (step gradient)
778 with a flow rate of 1.3 mL/min. Synthetic organic acids were used as references (sodium formate,
779 D-gluconic acid, sodium butyrate, pyruvic acid sodium salt, sodium citrate tribasic, sodium
780 oxalate, sodium propionate, sodium acetate, succinic acid disodium salt, DL-malic acid disodium
781 salt, sodium-L-lactate and malonic acid disodium salt).

782

783 *Physiological assays*

784 The growth of strain PML1(12) was monitored in order to determine potential biases of biomass
785 development in presence of glucose or mannitol (2g/L) are sole carbon source. To do so, 10 µl of
786 bacterial inoculum was inoculated in 190 µl of medium (corresponding to 1.10^6 cell/mL) with or
787 without iron and magnesium incubated at 25°C. Iron was added as FeSO₄ (2.5 mg/L final
788 concentration) and magnesium as MgSO₄ (20 mg/L final concentration). The optical density (OD
789 600 nm) was measured during 4 days every 3h using a TECAN microplate reader to follow the
790 growth and determine its maximum (OD max). Similarly, we assayed the ability of PML1(12) to
791 grow on gluconic acid, 2 ketogluconic acid and 5 ketogluconic acid (2 g/L). To determine the
792 potential production of iron chelating agents by strain PML1(12), a volume of 100 µl of inoculum
793 was introduced in 10 mL of medium with glucose or mannitol as sole carbon source with or
794 without iron and magnesium. Incubation was done at 25°C during 3 days. After this incubation
795 period, the supernatant was recovered by centrifugation (8000g, 15 min) and filtrated at 0.22µm.
796 The presence of iron chelating agents was quantified using the liquid Chromazurol assay (CAS)
797 of Schwyn and Neilands (1987). To do it, a volume of 100 µl of the supernatant inoculated or not
798 was then mixed with the CAS solution and incubated 1h at ambient temperature before reading
799 the absorbance at 655 nm with a microplate reader. Measures of control solutions at different pH
800 (*i.e.*, culture medium with adjusted from 6.5 to 2.5) permitted to confirm that the results obtained
801 in the CAS assay were not simply due to acidification only, but to the production of a chelating
802 agent by the strain PML1(12).

803

804 *RNA extraction and sequencing*

805 After 20h of incubation, 10 mL of culture was recovered and centrifuged at 8000 g (15 min; 4°C)
806 to pellet the bacterial cells. Supernatants were removed and cells were frozen in liquid nitrogen
807 and stored at -80°C. Total RNA was purified using the RiboPure™ kit (Ambion, Austin, USA)
808 following the manufacturer's recommendations, then treated twice with DNase I (Ambion),
809 precipitated with ethanol and resuspended in RNase-free water. The concentration and quality of
810 the extracted RNA was determined by measuring its absorbance using a Nanodrop 2000
811 spectrophotometer (Thermo Scientific, Waltham, MA, USA), a Qubit® RNA Assay Kit in a
812 Qubit® 2.0 Fluorometer (Life Technologies, CA, United States) and TapeStation analyses. The
813 RNA integrity number value was 9.0 on average. The total RNA were sent to GENEWIZ
814 (location) which operated the rRNA depletion using the Ribo-Zero rRNA removal kit for
815 bacteria, performed the stranded RNA library preparation and sequenced the cDNA on their
816 Illumina HiSeq2500 platform using a 2x100bp paired-end (PE) configuration in High Output
817 mode (V4chemistry). This sequencing generated a total of more than 250 million reads per lane.

818

819 *Protein extraction and tandem mass spectrometry*

820 After 20h of incubation, 5 mL of culture were recovered and centrifuged to pellet the bacteria.
821 Supernatants were removed and cells were frozen in liquid nitrogen and stored at -80 °C. For
822 each sample, the cell pellet was homogenized in a volume (25 µL per mg of cellular pellet (wet
823 weight) of lithium dodecyl sulfate 1X lysis buffer (Invitrogen), supplemented with 200 mg of 0.1
824 mm silica beads (MP Biomedicals), and disrupted by bead-beating with a Precellys Evolution
825 instrument (Bertin Technologies) operated at 7,800 rpm for 3 cycles of 20 seconds, with 30
826 seconds of pause between each cycle. The samples were then centrifuged at 16,000 g for 1 min.
827 The resulting supernatants were heated at 99°C for 5 min. A volume of 25 µL of each sample was
828 loaded on a NuPAGE 4-12% Bis-Tris gel and subjected to electrophoresis for 5 min at 200 V.
829 After migration, the proteins were stained with Coomassie SimplyBlue SafeStain (Invitrogen).
830 In-gel proteolysis of the whole proteome sliced in a single polyacrylamide band was performed
831 with 0.2 µg of trypsin gold (Promega) supplemented with 50 µl of 0.01% ProteaseMAX
832 surfactant (Promega) as previously described (Hartmann *et al.*, 2014). After incubation à 37°C
833 for 4 h, the solution was acidified with 5% trifluoroacetic acid and the final volume adjusted to
834 50 µl of 0.1% trifluoroacetic acid if required. A volume of 5 µl of this suspension was then
835 injected to identify the resulting peptides by tandem mass spectrometry using a Q-Exactive HF

836 instrument (Thermo Scientific) coupled to an ultimate 300 nanoLC system (Thermo Scientific) in
837 the same conditions as those described by Hayoun (Hayoun *et al.*, 2019). Peptides were resolved
838 on an Acclaim PepMap100 C18 reversed-phase column (3 μm , 100 \AA , 75 μm id x 50 cm) over a
839 90 min gradient.

840

841 *Bioinformatic and statistical analyses*

842 The RNAseq reads were mapped to the coding sequences (CDS) of the genome of strain
843 PML1(12) through the Microscope (Vallenet *et al.*, 2019) annotation platform using the BWA
844 (version 0.7.4-r385; Li and Durbin, 2009). Genes differentially expressed were sorted according
845 to the genome sequence of strain PML1(12)(AEJF00000000) and raw data statistics are accessible
846 on the MICROSCOPE platform ([https://mage.genoscope.cns.fr/microscope/home/index.php?](https://mage.genoscope.cns.fr/microscope/home/index.php?act=logout)
847 [act=logout](https://mage.genoscope.cns.fr/microscope/home/index.php?act=logout); login: guest2020 password: Ic7Hf3). Raw data are under deposit on EMB-EBI
848 (<https://www.ebi.ac.uk/arrayexpress/>; E_N00000). For proteins, MS/MS spectra were queried
849 against a database with the theoretical coding sequences of *Caballeronia mineralivorans*
850 PML1(12) using the Mascot Daemon software version 2.6.1 (Matrix Science). The parameters
851 used for this search were: peptide charge of 2+ or 3+, 5 ppm peptide tolerance, 0.02 Da MS/MS
852 fragment tolerance, carbamidomethylation of cysteine as fixed modification, and oxidation of
853 methionine as variable modification. A protein was validated on the basis of two identified
854 peptide sequences and with a final discovery rate of less than 1% as estimated with a decoy
855 database search. Proteins differentially produced were also sorted according to the genome
856 sequence of strain PML1(12) and a matrix generated (Pride access n° PXD000000). The effect of
857 the different treatments (*i.e.*, GB, GBwB and MBwB) on both RNA and protein datasets was
858 determined using the Mixomics R package (González *et al.*, 2012; Rohart *et al.*, 2017). To do it,
859 the full raw dataset of each approach (*i.e.*, RNA or protein) was first normalized to conserve the
860 genes which abundance presented a minimum of 100 in the RNAseq dataset in one the treatments
861 considered and a minimum 30 in the proteome dataset. A partial Least Squares (PLS) regression
862 was first done to integrate the RNAseq and proteome matrix to generate a global view. Due to the
863 complexity of our dataset and to allow interpretability a Sparse Partial Least Squares regression
864 (sPLS) was then performed as it permits to perform simultaneous variable selection in the two
865 data sets. The dimensions selected were determined based on the higher variance explained and
866 the number of variables (*i.e.*, genes) considered in the sPLS were limited to 100 per component

867 axis and method (transcriptomics or proteomics) for legibility and interpretability reasons. The
868 distribution of the different genes considered was then visualized in a correlation circle plot
869 permitting to evidence treatment specific genes. The list of genes was then compared to the
870 outputs of the DESeq2 analysis. The DESeq2 (Anders and Huber, 2010; Love *et al.*, 2014) was
871 used to calculate differential gene expression and protein abundance between the different
872 treatments. A normalization of read numbers for replicates in each treatment was performed (by
873 estimates of size factors in DESeq2 and normalization), then differential expression values
874 (FoldChange) and statistical values (pVal and adjusted pVal through the Benjamini and
875 Hochberg's approaches) assessing the false discovery rate (FDR) were calculated. All genes
876 having an adjusted p-value inferior to 0.05 and a cut-off for absolute fold change > 1 were
877 considered as significantly differentially expressed. For the proteomics data, a cut-off for fold
878 change > 0.3 was considered.

879 Concerning the chemical analyses, the effect of the different treatments on the organic acid
880 production, the pH and inorganic nutrients released in solution was determined by analysis of
881 variance (one way ANOVA, $p < 0.05$, followed by a Tukey test).

882

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892

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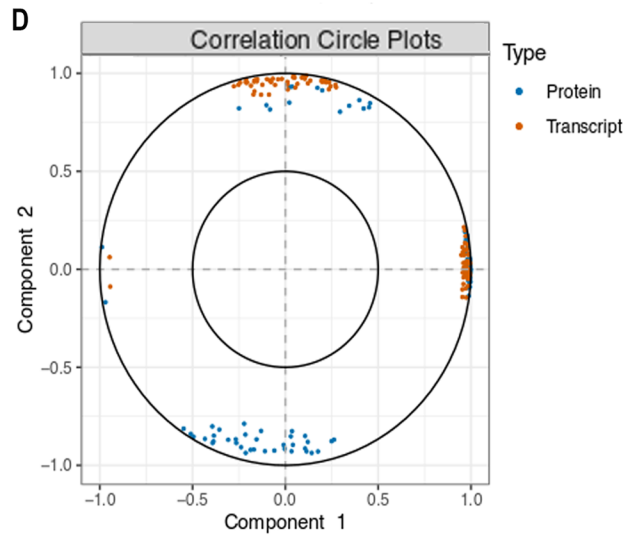
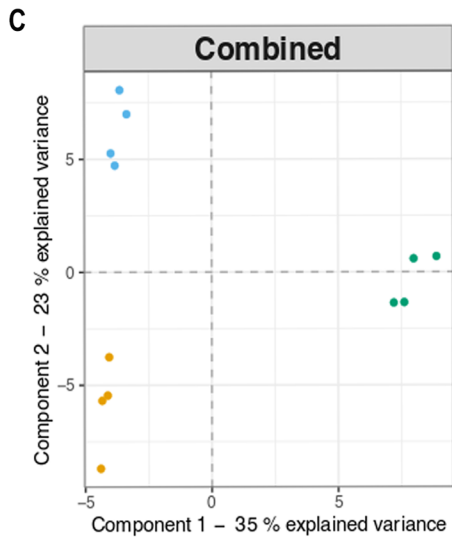
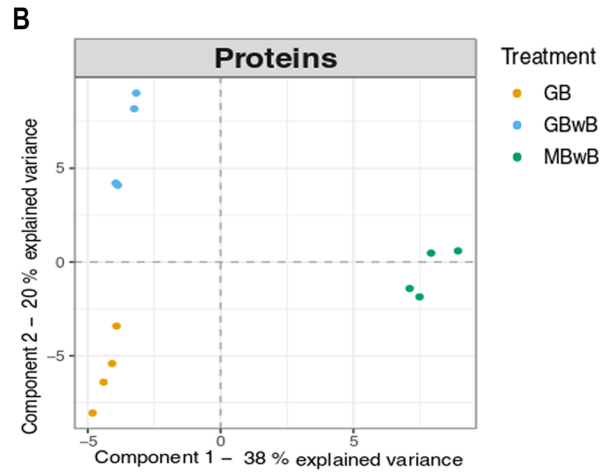
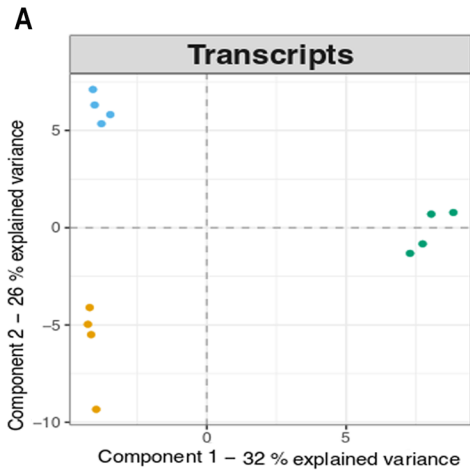
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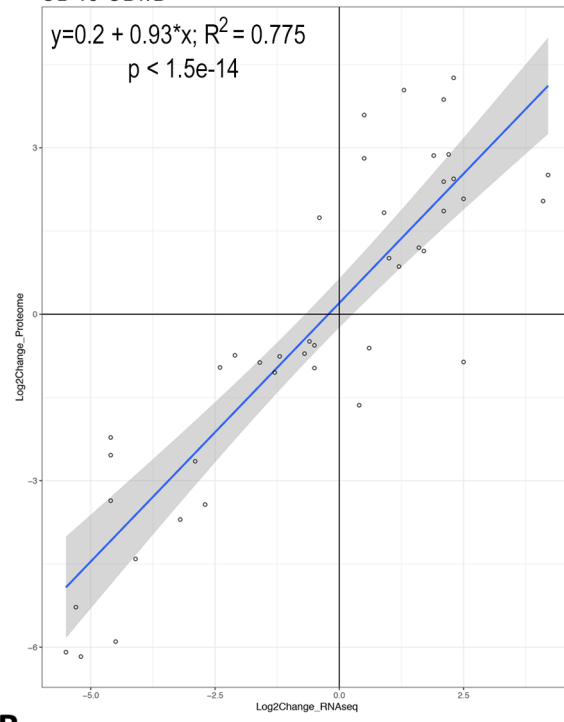
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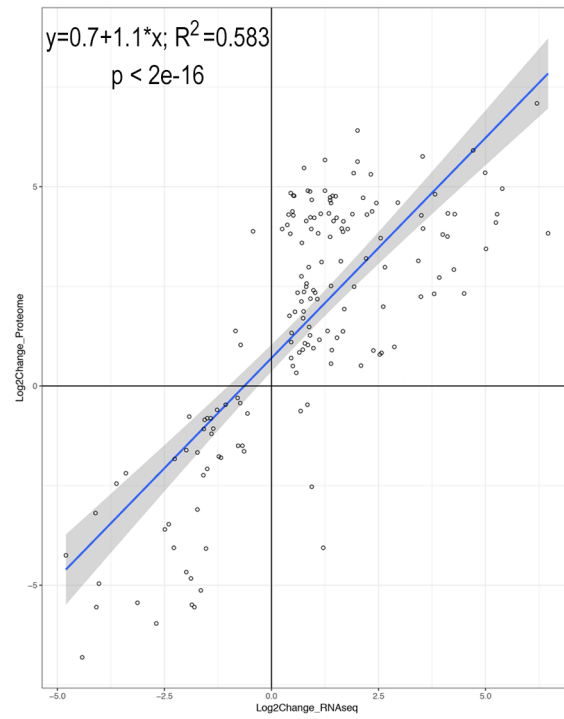
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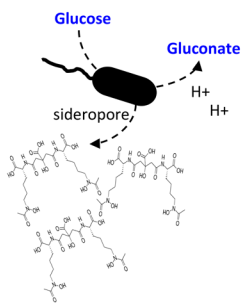
A GB vs GBwB



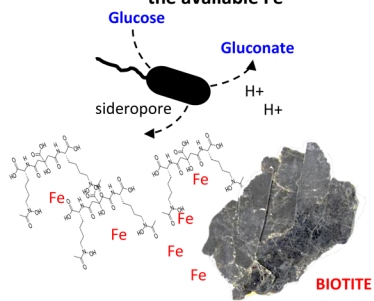
B GBwB vs MBwB



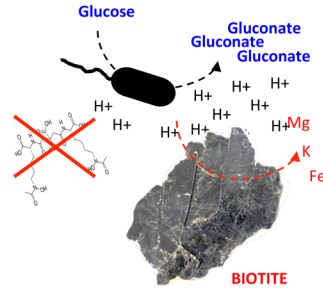
① **Nutrient-poor conditions**
(No mineral ; no Fe available)



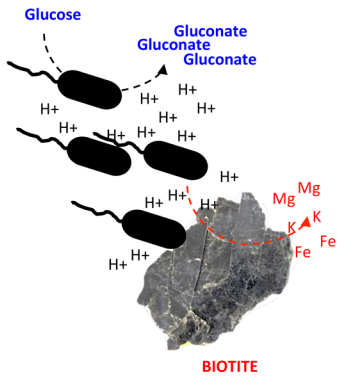
② **Nutrient-poor conditions + mineral** : Chelation of the available Fe



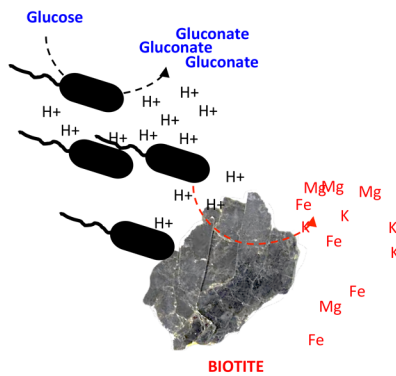
③ **Mineral weathering**
Increase of Fe available



④ **Mineral weathering**
Chemotaxis and motility



⑤ **Mineral weathering**
Regulation fn (nutrient availability)



⑥ **Mineral weathering stopped**
Glucose limiting concentration

