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Proteomic analysis of *Rhizobium freirei* PRF 81^T reveals the key role of central metabolic pathways in acid tolerance



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ARTICLE INFO

Keywords: Oxidative stress Abiotic stresses Biological nitrogen fixation pH homeostasis Acid consuming Two-dimensional electrophoresis

ABSTRACT

Soil acidity strongly affects microbial diversity and represents a constraint to legume production. *Rhizobium freirei* is a common bean symbiont recognized by its tolerance to environmental stresses, including low pH. The protein expression profiles of *R. freirei* PRF 81^T grown in pH 6.8 and 4.8 were analyzed to clarify the mechanisms responsible for acid tolerance in this species. Bacteria were grown up to exponential phase in tryptone-yeast (TY) medium at pH 6.8 and 4.8. Whole-cell protein extracts were separated by two-dimensional electrophoresis (2-DE), and spots that showed statistical difference in their relative volumes (%vol) between the treatments were selected and excised for identification by MALDI-TOF mass spectrometry. Data showed that protein synthesis was increased at pH 4.8, which consequently raises energy demand. Differential expression of membrane-associated proteins suggested an increased proton extrusion and decreased influx, while central metabolism seemed to be through inducing acid-consuming catabolic pathways and preventing fatty acid biosynthesis. Consequently, the respiratory chain was stimulated along with the production of reactive oxygen species (ROS). The broad range of metabolic pathways modulated by acidified pH endorses the adaptive response to environmental stresses as a multigenic character in *R. freirei* PRF 81. Moreover, our data emphasize the key role of central metabolism in acid stress tolerance.

1. Introduction

Soil acidity represents a current constraint to legume production (Graham and Vance, 2003). The legume-rhizobium interaction is fundamental to ecology and agriculture and can help crop species to cope with stressful environmental conditions (Buckley and Schmidt, 2002). Soil acidity is also described as the main factor affecting microbial diversity and structure (Fierer and Jackson, 2006), besides interfering with the nodulation and symbiosis with legume host, and nitrogen fixation capacity (Hungria and Vargas, 2000). The *Rhizobium freirei* strain PRF 81 shows many interesting features, mainly its high tolerance to environmental stresses and high efficiency in fixing N₂ in association with the common bean (*Phaseolus vulgaris*), that led to its use in commercial inoculants for this legume in Brazil (Hungria et al., 2000).

Despite the economic and environmental importance of rhizobia, a

limited number of studies attempted to elucidate the mechanisms of tolerance to abiotic stresses employed by these bacteria (Gomes et al., 2012a,b), and most of the studies are related to clinical and industrial microorganisms such as *Escherichia coli, Lactobacillus* spp., and *Streptococcus mutans* (Lund et al., 2014). To better understand the biological processes involved in stress response, differential proteomics has been successfully employed (Gomes et al., 2012b; Batista and Hungria, 2012; Luche et al., 2016). Here differential proteomics was employed to investigate the acid tolerance responses of *R. freirei* PRF 81.

2. Materials and methods

Rhizobium freirei PRF 81^T, isolated from common bean nodules in Brazil, is deposited at "Diazotrophic and Plant Growth Promoting Bacteria Culture Collection of Embrapa Soja" (WFCC Collection # 1213, WDCM Collection # 1054). Further information about the strain is

https://doi.org/10.1016/j.apsoil.2018.11.014

Received 20 May 2018; Received in revised form 26 November 2018; Accepted 26 November 2018 Available online 02 December 2018 0929-1393/ © 2018 Elsevier B.V. All rights reserved.

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available elsewhere (Hungria et al., 2000; Ormeño-Orrillo et al., 2012; Dall'Agnol et al., 2013; Gomes et al., 2015). The whole protein extract was achieved after growing the bacterium in TY under two conditions: control (pH 6.8) and with acid stress (pH 4.8). The procedures of total protein extraction (I), 2-DE electrophoresis and gel analysis (II), mass spectrometry (III), and *in silico* identification of proteins (IV), were made as described by Gomes et al. (2012b); except for using: the automatic data acquisition mode on the mass spectrometer (III), and the newer Mascot software version 2.4 adopting peptide tolerance of 100 or 150 ppm (IV). Microbes Online (Alm et al., 2005) and the Integrated Microbial Genomes system (Markowitz et al., 2006) were also used for protein characterization.

3. Results and discussion

Although R. *freirei* PRF 81 grow at pH 4.0 (Hungria et al., 2000; Dall'Agnol et al., 2013), the pH 4.8 was chosen because the growth kinetics at this pH was not significantly different from the growth kinetics observed at pH 6.8 (data not shown). This choice is also supported by the similar growth kinetics results of *R. tropici* CIAT 899 Graham et al. (1994), which shares genotypic (Ormeño-Orrillo et al., 2012) and morphological similarities with *R. freirei* (Dall'Agnol et al., 2013). Also, a recent study evaluated the response of *R. freirei* CIAT 899 grown under acid conditions and the pH of choice was 4.5 (Guerrero-Castro et al., 2018).

Thirty-six proteins were successfully identified (Table 1 and Supplementary Material S1) and classified according to functional groups of Clusters of Orthologous Groups (COG) (Table 1) (Galperin et al., 2015). In relation to cell location, thirty-two proteins were classified as cytoplasmic, spots 23 and 35 as outer membrane and periplasmic proteins, respectively; location of spots 8 and 11 were not determined by the software used. Additional data are available in Supplementary Material S2.

3.1. Induction of protein synthesis

Bacterial exposure to acidic conditions can shift cytoplasmic pH to levels too low to be regulated by primary responses such as buffering or ionic flux. In these cases, inducible responses become important for the reestablishment of the normal pH (Lund et al., 2014). Such responses may include intensification of aerobic respiration due to the higher energy demand, as suggested by the differential expression of energy-related genes observed in *Sinorhizobium meliloti* (Draghi et al., 2016). In this study, the profile of protein spots was also modified in *R. freirei* PRF 81 grown at low pH, and transcriptional and translational processes were intensified in this condition (Table 1).

The 50S ribosomal protein L9, RpII, is responsible for maintaining translation fidelity, while the elongation factor P, EF-P, is involved in relieving stalled ribosomes (Keiler, 2015; Naganathan et al., 2015). Increased expression of RpII and EF-P were observed at pH 4.8 in *R. freirei* PRF 81 and in *S. meliloti* exposed to pH 6.1, in addition to other translation-related proteins (Draghi et al., 2016). The importance of these proteins for cell survival is reinforced by the observation that *E. coli* deficient for RpII and EF-P are usually non viable (Naganathan et al., 2015).

3.2. Glutathione involvement in acid pH responses

Glutathione (GSH) is essential for protecting rhizobia against environmental stresses (Riccillo et al., 2000; Sobrevals et al., 2006) and is involved in nodulation and nitrogen fixation (Harrison et al., 2005; Cheng et al., 2017). GSH synthetase (GshB) and serine hydroxymethyltransferase (SHMT) are enzymes involved in GSH biosynthesis (Sobrevals et al., 2006; Schirch et al., 1985). Previous reports showed that *R. tropici* CIAT 899 under acid shock increases *gshB* expression (Muglia et al., 2007) and is particularly sensitive to acidic conditions when *gshB* is mutated (Riccillo et al., 2000). In contrast, GshB and SHMT are down-regulated in *R. freirei* PRF 81 grown at low pH (Table 1).

R. freirei was grown in TY medium which contains yeast extract, rich in GSH (Sobrevals et al., 2006). Noteworthy, GSH itself, and not its anabolic pathway, is required for stress tolerance, and exogenously supplied GSH can modulate metabolic and symbiotic responses (Cheng et al., 2017) and lead to increased stress tolerance (Corticeiro et al., 2006). Therefore, the TY medium may be supplying the bacterial GSH demand, thus leading to decreased GSH synthesis and down-regulation of GshB and SHMT at pH 4.8.

3.3. Reduced cellular susceptibility to proton influx

RopB, an up-regulated outer membrane protein in acid pH (Table 1), have an important role in maintain membrane stability (Vanderlinde and Yost, 2012) and is a main structural component in some rhizobia (de Maagd et al., 1989). *Rhizobium leguminosarum* bv. *viciae ropB* mutants are more sensitive to low pH and other abiotic stresses (Foreman et al., 2010) and *Agrobacterium tumefaciens* growing under acidic conditions presented an increased expression of a homologous *ropB* gene (Li et al., 2002; Yuan et al., 2008).

Although the F_1F_0 -ATPase channel has been reported as induced by acidic conditions (Len et al., 2004; Draghi et al., 2016), the subunit AtpD was down-regulated at pH 4.8 in *R. freirei*; in contrast, the NADH dehydrogenase subunit C of the Complex 1 (NuoC) was up-regulated. Up-regulation of *nuoC* was previously reported at the transcriptional level in *S. meliloti* at pH 6.1 (Draghi et al., 2016), and in *E. coli* exposed to acid shift from pH 7.6 to 5.5 (Kannan et al., 2008) or growing at pH 5.0 (Maurer et al., 2005).

In the *R. freirei* genome, both F_1F_0 -ATPase and the Complex 1 are composed of several subunits (Duary et al., 2010; Spero et al., 2015) encoded by the same operons (Ormeño-Orrillo et al., 2012). The downregulation of AtpD and the up-regulation of the NuoC suggest a mechanism that decreases proton intrusion and increases proton extrusion to control the internal pH (Fig. 1). In *Staphylococcus aureus*, decreased expression of the *atp* operon was observed 2 min after exposure to pH 4.5, and increased expression of *nuo* genes after 10 min (Bore et al., 2007). These opposing mechanisms are needed to raise the cytoplasmic proton content in *E. coli* exposed to alkaline pH (Maurer et al., 2005).

Although pH 6.0 limits the growth of *S. meliloti* (Draghi et al., 2016), *R. freirei* (Dall'Agnol et al., 2013) and *S. aureus* (Bore et al., 2007) can grow at pHs lower than 5.0. While *R. freirei* and *S. aureus* show down-regulation of ATPase and up-regulation of Complex 1 components under acidic pH, *S. meliloti* induces these two proteins (Draghi et al., 2016).

The up-regulation of the Complex 1 suggests that respiration was stepped up in *R. freirei* grown at pH 4.8, as previously reported in *E. coli* at pH 5.0 (Maurer et al., 2005). *S. meliloti* O₂ consumption is more than 5-fold higher at pH 6.1 in comparison with neutral pH (Draghi et al., 2016). In addition, the proton extrusion catalyzed by the Complex 1 is coupled to the conversion of NADH + H⁺ into NAD⁺ (Spero et al., 2015), essential for the maintenance of the central metabolism (Geddes and Oresnik, 2014).

Considering that increased protein synthesis raises energy demand (Draghi et al., 2016) and ATPase was down-regulated in *R. freirei*, besides its growth kinetics was not significantly affected at pH 4.8, it is likely that the ATP supply was being provided by other alternative sources such as catabolic reactions. These reactions consume NAD⁺, thus explaining the induction of the Complex 1. These results are in accordance with the observations in *E. coli* showing the induction of *nuo* genes after acid shift is delayed (Kannan et al., 2008), suggesting that Complex 1 activation is a consequence of de-energization following acid stress, as a secondary response of acid-associated metabolism.

Table 1

Proteins of *Rhizobium freirei* PRF 81 whole-cell extract differentially expressed in low pH conditions, arranged according to COG functional categories. Spots on Table 1, Supplementary Material S1 and Supplementary Material S2 are marked with the same number.

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9 Apartate semialariyate derivariogenaie (40%) ↑ 0.8641 ± 0.102 1.11806 ± 0.041 10 Histadiariyate derivariogenaie (40%) ↑ 0.8674 ± 0.012 0.3368 ± 0.016 11 Amino acid ABC transporter subtrates binding (24%) ↑ 0.8764 ± 0.012 0.3765 ± 0.021 0 Bartates emiladiorizations (65%) ↑ 0.8764 ± 0.048 1.1220 ± 0.095 0 Galcosco-ophosphate 1-delvárogenase (17%) ↑ 0.8764 ± 0.048 1.1220 ± 0.096 13 Galcosco-ophosphate 1-delvárogenase (17%) ↑ 0.8764 ± 0.048 1.1220 ± 0.096 14 KHO-KOPC binnectional adolase (28%) ↓ 1.3196 ± 0.067 0.86804 ± 0.078 1- Lipid transport and metabolim 1.30584 ± 0.051 0.69416 ± 0.122 14 Acetyl-CoA carboxylase biotin carboxylase subunit (56%) ↓ 1.3141 ± 0.043 0.86586 ± 0.036 18 Porpholingen synthase (26%) ↓ 1.3141 ± 0.043 0.86586 ± 0.036 10 Galcaschine carboxylase biotin carboxylase subunit (36%) ↓ 1.3141 ± 0.043 0.86586 ± 0.036 10 Galcaschine carboxylase divide (26%) ↓ 1.3146 ± 0.0273 0.68394 ± 0.010 10 Galcaschine carboxididereducase (26%) ↓	8	Spermidine/putrescine ABC transporter (2/%)	1.13657 ± 0.105	0.86342 ± 0.013	
10 Initiation operating and call ABC transport substrate below (1 = 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,	9	Aspartate-semialdehyde dehydrogenase (40%) ↑	0.88641 ± 0.102	1.11360 ± 0.041	
11 Amino acid Anc, transporter subtrate-animaly (2*%) (0.050 1 ± 0.173 1.2499 ± 0.007 12 Amino acid Aniontranserse (14%) (0.87784 ± 0.050 1.12405 ± 0.007 13 Glucose-Sphosphate 1.delydrogenase (17%) (0.87594 ± 0.048 1.1206 ± 0.096 14 KHG-KDPC birturcional adolase (28%) (1.13196 ± 0.067 0.86804 ± 0.078 1- Lipid transport and metabolism - - - - 16 Acetyl-CoA carboxylase biotin carboxylase subunit (56%) 1 1.13414 ± 0.043 0.86586 ± 0.036 0.0351 ± 0.052 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0352 ± 0.061 0.0352 ± 0.061 0.0352 ± 0.061 0.0352 ± 0.061 0.0352 ± 0.061 0.0352 ± 0.061 0.0352 ± 0.061 0.0352 ± 0.061 0.0352 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0351 ± 0.039 0.0352 ± 0.036 0.0466 0.0362 ± 0.035 0.0466 0.0466 0.0466 0.0466 0.0466 0.0466 0.0466 0.0466 0.0466 0.0466 0.0466 <td< td=""><td>10</td><td>Australianoi-phosphate aminotransferase (46%)</td><td>1.56135 ± 0.221</td><td>0.43865 ± 0.016</td></td<>	10	Australianoi-phosphate aminotransferase (46%)	1.56135 ± 0.221	0.43865 ± 0.016	
12 Amino and animotranserias (41%) [0.87 A4 ± 0.050 1.1220 ± 0.096 13 Glacose-5-phosphate 1-dehydrogenase (17%) [0.87594 ± 0.048 1.1240 ± 0.096 14 KHG-KOPC b/interclone aldolase (28%) [0.7640 ± 0.018 1.1240 ± 0.096 15 Fractose-bisphosphate 1-dehydrogenase (17%) [0.7640 ± 0.027 0.86604 ± 0.078 1 Lipid transport and metabolism 1.13196 ± 0.051 0.69416 ± 0.122 16 Acetyl-CoA carboxylase blotin carboxylase subunit (56%) [1.03058 ± 0.051 0.69416 ± 0.122 17 Hypothetical protein RHSP_82341 (22%) [1.13414 ± 0.043 0.86586 ± 0.036 18 Porphohilinoeg mynthase (26%) [1.31606 ± 0.273 0.66394 ± 0.010 20 Flavin-dependent oxidoreductase (20%) [1.31606 ± 0.273 0.66394 ± 0.010 21 Aromatic ring-hydroxylating dioxygenase subunit alpha (30%) [1.21173 ± 0.118 0.78827 ± 0.087 24 VelC/PmpR family DA-binding transcriptional regulator (37%) [0.80793 ± 0.038 1.12619 ± 0.045 25 Liongation factor P (24%) [0.78821 ± 0.0168 1.12179 ± 0.045 1.12179 ± 0.045 24 VelC/PmpR family DA-binding transcriptional regulator (37%) [0.78531 ±	11	Amino acid ABC transporter substrate-binding (24%)	0.76501 ± 0.175	1.23499 ± 0.077	
G - Carbolydraic transport and metabolism 1.2406 transport 0.87594 ± 0.048 1.12406 ± 0.096 13 Gloces 6-phosphate 1-dehydrogenase (17%) ↑ 0.87594 ± 0.048 0.27951 ± 0.230 15 Fructose-bisphosphate aldolase (28%) ↑ 1.3196 ± 0.067 0.8684 ± 0.078 <i>I - Lipid transport and metabolism</i> 1 1.3196 ± 0.067 0.8694 ± 0.120 H - Corenzyme transport and metabolism 1 1.3414 ± 0.043 0.86586 ± 0.036 16 Acetyl-CoA carboxylase (28%) ↓ 1.04948 ± 0.029 0.9612 ± 0.039 17 Hypothetical protein RHSP_82341 (22%) ↓ 1.04948 ± 0.023 0.86588 ± 0.061 18 Porphoshilongen synthesise (28%) ↓ 1.04948 ± 0.023 0.86589 ± 0.061 20 Flavin-dependent oxidoreductase (20%) ↓ 1.3166 ± 0.273 0.68394 ± 0.081 21 Aromatic ring hydroxylating dioxygenase subunit alpha (30%) ↓ 1.21173 ± 0.118 0.78827 ± 0.081 23 Outer membrane protein Rop8 (23%) ↑ 0.81340 ± 0.033 1.18661 ± 0.096 23 Outer membrane protein Rop8 (23%) ↑ 0.81340 ± 0.033 1.18661 ± 0.096 24 V Pc/CPmpR family DNA-binding transcriptional regulator (37%) ↑ 0.83793 ± 0.066 1.16109 ± 0.032	12	Amino acid aminotransferase (41%) †	0.87784 ± 0.050	1.12217 ± 0.095	
13 Glucose-b-phosphate 1-dehydrogenase (1/%) † 0.87/94 ± 0.048 1.12408 ± 0.039 14 KHC-KOPC bifunctional aldolase (08%) † 0.13196 ± 0.067 0.86694 ± 0.078 1- Lipid transport and metabolism 1.13196 ± 0.067 0.86694 ± 0.078 1- Lipid transport and metabolism 1.03054 ± 0.051 0.669116 ± 0.122 17 Hardbolism 0.00548 ± 0.051 0.66916 ± 0.022 18 Porphobilinogen synthase (26%) ↓ 1.0344 ± 0.043 0.86586 ± 0.036 18 Porphobilinogen synthase (26%) ↓ 1.03468 ± 0.029 0.90512 ± 0.039 19 Glucatione synthesize (20%) ↓ 1.31606 ± 0.273 0.66394 ± 0.010 10 Ravin-dependent xidoreductase (20%) ↓ 1.31666 ± 0.027 0.66394 ± 0.011 10 Aromatic ring-bydroxylating dioxygenase subunit alpha (30%) ↓ 1.21173 ± 0.118 0.78827 ± 0.087 4 VebC/PmpR family DNA-binding transcriptional regulator (37%) † 0.8134 ± 0.073 0.89616 ± 0.046 24 VebC/PmpR family DNA-binding transcriptional regulator (37%) † 0.81393 ± 0.066 1.16109 ± 0.134 27 Pepide chain release factor 2 (14%) ↓ 1.21705 ± 0.125 0.78205 ± 0.076 28 Glorgation factor P (24%) † <td>G – Carbohydrate transport</td> <td>and metabolism</td> <td></td> <td></td>	G – Carbohydrate transport	and metabolism			
14 KHC+KDPL bitunctional alcolase (69%) 1 0.70449 \pm 0.210 1.23951 \pm 0.230 15 Furctose-hisphophate alcolases (28%) 1 1.30584 \pm 0.051 0.69416 \pm 0.072 16 Acceyl-CoA carboxylase biotin carboxylase subunit (56%) 1 1.30584 \pm 0.051 0.69416 \pm 0.122 16 Acceyl-CoA carboxylase biotin carboxylase subunit (56%) 1 1.30584 \pm 0.051 0.69416 \pm 0.122 17 Hypothetical protein RHSP 23241 (22%) 1 1.13414 \pm 0.043 0.65586 \pm 0.036 18 Porpholinogen synthesse (26%) 1 1.04684 \pm 0.027 0.68394 \pm 0.010 20 Flavin-dependent oxidoreductase (20%) 1 1.31606 \pm 0.273 0.68394 \pm 0.010 21 Aromatic ring-hydroxylating dioxygenase subunit alpha (30%) 1 1.21175 \pm 0.118 0.7827 \pm 0.087 4 Cell wall/membershow biogenesis 22 UTP-glucose-1-phophate uridylyltransferase (60%) 1 1.10184 \pm 0.077 0.89216 \pm 0.086 23 UTP-glucose-1-phophate uridylyltransferase (60%) 1 1.0184 \pm 0.039 1.9207 \pm 0.011 3 Forestription 0.83941 \pm 0.045 0.039 1.9207 \pm 0.012 3 Torstation, ribosonal arotein R058 (23%) 1 0.80793 \pm 0.039 1.9207 \pm 0.016 0.7217 \pm 0	13	Glucose-6-phosphate 1-dehydrogenase (17%) ↑	0.87594 ± 0.048	1.12406 ± 0.096	
15 Pructose-hsphosphate alcolase (28%) j 1.1319 ± 0.067 0.08604 ± 0.078 1 - Lipli transport and metabolism 1 1.30584 ± 0.051 0.69416 ± 0.122 H - Coensyme transport and metabolism 1 1.13414 ± 0.043 0.86586 ± 0.036 18 Porphobilingen synthase (34%) j 1.13414 ± 0.043 0.86586 ± 0.036 19 Glutathone syntheses (34%) j 1.04488 ± 0.029 0.90512 ± 0.039 20 Flavin-dependent oxidoreductase (20%) j 1.31606 ± 0.273 0.68394 ± 0.010 P - Inorganic in transport and metabolism 1.21173 ± 0.118 0.78827 ± 0.087 21 Aromatic ring-hydroxylating dioxygenase subunit alpha (30%) j 1.21173 ± 0.118 0.78827 ± 0.087 M - Cell wall/membrane/metable biogenesis 1 1.0184 ± 0.077 0.89816 ± 0.049 K - Transcription 2 UTP-glucose-1-phosphate uridylytransferase (60%) j 1.0184 ± 0.077 0.89816 ± 0.049 K - Transcription 2 UTP-glucose-1-phosphate gravity in anscriptional regulator (37%) † 0.80793 ± 0.033 1.13601 ± 0.049 J - Translation, rindosomal structure and biogenesis 2 1.21075 ± 0.125 0.78595 ± 0.076 24 YebC/PmpR family DNA-binding transcriptional regulator (37%)	14	KHG-KDPG bifunctional aldolase (68%) ↑	0.70449 ± 0.210	1.29551 ± 0.230	
I - Ligid transport and metabolism 1.30584 ± 0.051 0.69416 ± 0.122 H - Coenzyme transport and metabolism 1.30544 ± 0.043 0.86586 ± 0.036 Norphobiling on synthase (26%) ↓ 1.31414 ± 0.043 0.86586 ± 0.036 18 Porphobiling on synthase (26%) ↓ 1.31416 ± 0.058 0.85239 ± 0.061 20 Glutathione synthase (26%) ↓ 1.31606 ± 0.273 0.68394 ± 0.101 20 Flavin dependent oxidoreductase (20%) ↓ 1.21173 ± 0.118 0.78827 ± 0.087 P - Inorganic int transp-ty and metabolism 1.20173 ± 0.118 0.78827 ± 0.087 21 M romatic ring-hydroxylating dioxygenase subunit alpha (30%) ↓ 1.21173 ± 0.118 0.78827 ± 0.087 22 UT P-glucose-1-phosphate uridylyltransferase (60%) ↓ 1.10184 ± 0.077 0.89816 ± 0.048 23 Outer membrane protein RopB (23%) ↑ 0.81340 ± 0.038 1.19207 ± 0.019 Chrascription 1.10184 ± 0.077 0.89816 ± 0.048 1.19207 ± 0.019 24 V F>Qlucose-1-phosphate uridylyltransferase (60%) ↓ 1.10184 ± 0.077 0.89816 ± 0.049 25 Elongation factor P (24%) ↑ 0.80793 ± 0.039 1.19207 ± 0.019 26 <td>15</td> <td>Fructose-bisphosphate aldolase (28%) 1</td> <td>1.13196 ± 0.067</td> <td>0.86804 ± 0.078</td>	15	Fructose-bisphosphate aldolase (28%) 1	1.13196 ± 0.067	0.86804 ± 0.078	
16 Actyl-CoA carboxylase siobin carboxylase subunit (56%) ↓ 1.0958 ± 0.051 0.05416 ± 0.122 H - Coanzyme transport and metabolism 1.13414 ± 0.043 0.86586 ± 0.036 17 Hypothetical protein RHSP_82341 (22%) ↓ 1.04948 ± 0.029 0.90512 ± 0.039 18 Porphobilinogen synthase (26%) ↓ 1.0476 ± 0.058 0.8539 ± 0.010 20 Flavin-dependent oxidoreductase (20%) ↓ 1.31606 ± 0.273 0.68394 ± 0.101 P - Inorganic in transport and metabolism	I – Lipid transport and meta	ibolism			
H - Coarsyme transport and metabolism 17 Hypothetical protein RHSP.82341 (22%) ↓ 1.13414 ± 0.043 0.86586 ± 0.039 18 Porphobilinogen synthase (35%) ↓ 1.04968 ± 0.029 0.96512 ± 0.039 19 Glutathione synthetae (35%) ↓ 1.31606 ± 0.058 0.86538 ± 0.010 20 Rain-dependent oxidoreductase (20%) ↓ 1.3160 ± 0.058 0.8533 ± 0.010 21 Aromatic ring-hydroxylating dioxygenase subunit alpha (30%) ↓ 1.21173 ± 0.118 0.78827 ± 0.086 22 UTP-glucose-1-phosphate uridylyltransferase (60%) ↓ 1.21173 ± 0.118 0.78827 ± 0.086 23 Outer membrane protein RopB (23%) ↑ 0.80793 ± 0.039 1.19207 ± 0.011 24 VebC/PmpR family DNA-binding transcriptional regulator (37%) ↑ 0.80793 ± 0.039 1.19207 ± 0.011 25 Elongation factor P (24%) ↑ 0.78581 ± 0.108 1.21419 ± 0.045 26 S05 thosomal protein 19 (30%) ↑ 0.78581 ± 0.108 1.21419 ± 0.045 27 Pepide chain release factor 2 (14%) ↓ 0.7550 ± 0.078 1.24470 ± 0.170 28 Glutathione S-transferase (25%) ↓ 0.78581 ± 0.108 0.83397 ± 0.015 29 ATP-dependent Cly protease proteolyti subunit (25%) ↓ 0.78	16	Acetyl-CoA carboxylase biotin carboxylase subunit (56%)	1.30584 ± 0.051	0.69416 ± 0.122	
17 Hypothetical protein RISP \$2341 (22%) j. 1.13414 ± 0.043 0.86566 ± 0.036 18 Porphoblingors purthase (26%) j. 1.09484 ± 0.029 0.9051 ± 0.039 19 Glurathione synthase (20%) j. 1.31606 ± 0.273 0.85239 ± 0.061 20 Flavin-dependent oxidoreductase (20%) j. 1.31606 ± 0.273 0.85239 ± 0.061 20 Flavin-dependent oxidoreductase (20%) j. 1.21173 ± 0.118 0.75827 ± 0.087 P - lorgenic ion transport Aromatic ring-hydroxylating dioxygenase subunit alpha (30%) j. 1.21173 ± 0.118 0.75827 ± 0.087 M - Cell wall/membrane/~vicepte biogenesis UTP-glucose-1-phosphate uridylytransferase (60%) j. 1.1018 ± 0.077 0.89816 ± 0.068 23 Outer membrane protein Ropb (23%) † 0.81340 ± 0.038 1.19207 ± 0.011 J - Transcription I Interferation (137%) † 0.8073 ± 0.039 1.19207 ± 0.015 J - Transcription I Interferation (137%) † 0.75581 ± 0.108 1.21419 ± 0.045 26 S05 robosmal protein Is (05%) † 0.75581 ± 0.108 1.21419 ± 0.045 27 Piptide chain relase factor 2 (14%) j. 1.21705 ± 0.125 0.78295 ± 0.076 28 Glutathione S-transferase (25%) †	H – Coenzyme transport an	d metabolism			
18 Porphobilinogen synthase (24%) ↓ 1.09488 ± 0.029 0.90512 ± 0.039 19 Glutathone synthase (24%) ↓ 1.31606 ± 0.273 0.68394 ± 0.010 20 Flavin-dependent oxidoreductase (20%) ↓ 1.31606 ± 0.273 0.68394 ± 0.010 P - Inorganic ion transport ord metabolim 1 1.2173 ± 0.118 0.78827 ± 0.087 21 Aromatic ring-hydroxylating dioxygenase subunit alpha (30%) ↓ 1.21173 ± 0.118 0.78827 ± 0.087 M - Cell wall/membrane/=vlope biogenesis 1 1.0184 ± 0.077 0.89816 ± 0.086 23 Outer membrane protein RopB (23%) ↑ 0.81340 ± 0.038 1.18661 ± 0.049 K - Transcription 2 4 YebC/PmpR family DNA-binding transcriptional regulator (37%) ↑ 0.80793 ± 0.039 1.19207 ± 0.011 J - Translation, ribosomal structure and biogenesis 2 1.21419 ± 0.045 1.6505 1.6505 1.6506 ↑ 1.16109 ± 0.134 26 505 ribosomal protein J9 (36%) ↑ 0.78581 ± 0.108 1.21419 ± 0.045 1.6938 ± 0.040 0.93062 ± 0.076 27 Peptide chain release factor 2 (14%) ↓ 0.78503 ± 0.078 1.24470 ± 0.176 0.78503 ± 0.078 1.24470 ± 0.176 0.78503 ± 0.078 1.24470 ± 0.076 0.78529 ± 0.076 <	17	Hypothetical protein RHSP_82341 (22%)↓	1.13414 ± 0.043	0.86586 ± 0.036	
19 Glutathine synthetase (31%)↓ 1.14761 ± 0.058 0.65239 ± 0.061 20 Falvin-dependent oxidoreductase (20%) ↓ 1.31606 ± 0.273 0.68394 ± 0.101 P - Inorganic ion transport and metabolis 1.21173 ± 0.118 0.7827 ± 0.087 M - Cell wall/membrane/emvelore 1.21173 ± 0.118 0.7827 ± 0.087 M - Cell wall/membrane/emvelore 0.0000 0.81340 ± 0.038 1.8661 ± 0.049 Z2 UTP-glucose-1-phosphate uridylyltransferase (60%) ↓ 1.01184 ± 0.077 0.89816 ± 0.049 K - Transcription 1.20173 0.80793 ± 0.039 1.19207 ± 0.011 J - Translation, ribosomal structure and biogenesis 1.21705 ± 0.125 0.78581 ± 0.108 1.21419 ± 0.045 Z6 S06 ribosomal protein 19 (36%) ↑ 0.83891 ± 0.066 1.16109 ± 0.034 Z7 Peptide chain release factor 2 (14%) ↓ 1.21705 ± 0.125 0.78295 ± 0.076 Z8 Glutathine stransferase (25%) ↑ 0.75530 ± 0.078 1.24470 ± 0.170 Z9 Altr-dependent Clp protease proteolytic subunit (25%) ↓ 0.65331 ± 0.074 1.36869 ± 0.144 R - General function protein 28%) ↑ 0.41470 ± 0.029 0.80290 ± 0.166 0.14470 R - General function protein SU 0.79	18	Porphobilinogen synthase (26%) ↓	1.09488 ± 0.029	0.90512 ± 0.039	
20Flavin-dependent oxidoreductase (20%) ↓1.31606 ± 0.2730.68394 ± 0.101P - Inorganic ion transport und metabolismNormatic ring-hydroxylating dioxygenase subunit alpha (30%) ↓1.21173 ± 0.1180.78827 ± 0.087M - Cell wall/membrane/etw-lope biogenesis1.0184 ± 0.0770.89816 ± 0.0860.81340 ± 0.0381.18661 ± 0.049Z2UTP-glucose-1-phosphate uridylyltransferase (60%) ↓1.10184 ± 0.0770.89816 ± 0.0860.089Z3Outer membrane protein RopB (23%) ↑0.81340 ± 0.0381.18661 ± 0.049K - TranscriptionT1.10184 ± 0.0770.89816 ± 0.086J - Translation, ribosomal structure and biogenesisTTZ5Elongation factor P (24%) ↑0.78581 ± 0.1081.21419 ± 0.014Z6S0S ribosomal protein 19 (36%) ↑0.83891 ± 0.0661.16109 ± 0.134Z7Peptide chain release factor 2 (14%) ↓0.75530 ± 0.0781.24470 ± 0.170Z9ATP-dependent Clp protease proteolytic subunit (25%) ↓0.65331 ± 0.0581.04470 ± 0.170Z9ATP-dependent Clp protease proteolytic subunit (25%) ↓1.14602 ± 0.0320.85397 ± 0.014Z9Marioxidant protein (28%) ↑0.63131 ± 0.0741.36669 ± 0.144Z9Calcender function prediction1.19710 ± 0.0290.80290 ± 0.166Z9Calcender function protein CobW (21%) ↓1.21741 ± 0.0740.78259 ± 0.056S - Function Uhknown1.21741 ± 0.0740.78259 ± 0.056Z9Calcender function protein RoHSP_28418 (46%) ↓1.38591 ± 0.1360.61409 ± 0.055S - Function Uhknow	19	Glutathione synthetase (31%)↓	1.14761 ± 0.058	0.85239 ± 0.061	
P - Inorganic ion transport and metabolism 1.21173 ± 0.118 0.78827 ± 0.078 21 Aromatic ring-hydroxylating dioxygenase subunit alph (30%) ↓ 1.21173 ± 0.118 0.78827 ± 0.078 21 UPP-glucose-1-phosphate uridylyltransferase (60%) ↓ 1.21173 ± 0.118 0.78827 ± 0.087 22 UPP-glucose-1-phosphate uridylyltransferase (60%) ↓ 1.10184 ± 0.077 0.89816 ± 0.086 23 Outer membrane protein RopB (23%) ↑ 0.80793 ± 0.038 1.18661 ± 0.049 K - Transcription K K K K 24 YebC/Pmg family DNA-binding transcriptional regulator (37%) ↑ 0.80793 ± 0.038 1.19207 ± 0.015 25 Elongation factor P (24%) ↑ 0.78581 ± 0.108 1.21419 ± 0.045 26 S05 ribosomal protein 10 (36%) ↑ 0.83891 ± 0.066 1.16109 ± 0.134 27 Peptide chain release factor 2 (14%) ↓ 0.75530 ± 0.078 1.24470 ± 0.170 28 Glutathione S-transferase (25%) ↑ 0.75530 ± 0.078 1.24470 ± 0.170 29 ATP-dependent Clp protease proteolytic subunit (25%) ↓ 1.14602 ± 0.032 0.85397 ± 0.015 V - Defense mechanisms 1.9710 ± 0.029 0.85297 ± 0.056 0.85291 ± 0.056 2 <	20	Flavin-dependent oxidoreductase (20%)	1.31606 ± 0.273	0.68394 ± 0.101	
21Aromatic ring-hydroxylating dioxygenase subunit alpha (30%) ↓1.21173 ± 0.1180.78827 ± 0.087 $M - Cell wall/membra/emvelope biogenesisUT - glucosc-hydosphate uridylyltransferase (60%) ↓1.10184 ± 0.0770.89916 ± 0.08623Outer membrane protein RopB (23%) ↑0.81340 ± 0.0381.18661 ± 0.049K - TranscriptionUT - glucosc-hydosphate uridylyltransferase (60%) ↑0.80793 ± 0.0391.19207 ± 0.011J - Translation, ribosomal structure and biogenesisUT - GSSB1 \pm 0.1081.21419 ± 0.04525Elongation factor P (24%) ↑0.78581 ± 0.1081.21419 ± 0.04526508 ribosomal protein L9 (36%) ↑0.78531 ± 0.1081.21419 ± 0.04527Peptide chain release factor 2 (14%) ↓0.75530 ± 0.0781.24470 ± 0.17028Glutathione S-transferase (25%) ↑0.75530 ± 0.0781.24470 ± 0.17029ATP-dependent Clp protease proteolytic subunit (25%) ↓1.06938 ± 0.0400.93062 ± 0.0737 - Signal transductionT.114602 ± 0.0320.85397 ± 0.015V - Defense mechanisms11.0741.36869 ± 0.14431Anti-oxidant protein (28%) ↑1.01700.63131 ± 0.0741.36869 ± 0.144R - General function prediteix1.2174 ± 0.0740.78259 ± 0.05632GTP-binding protein CobW (21%) ↓1.21741 ± 0.0740.78259 ± 0.05634Hypothetical protein RHSP_28418 (46%) ↓1.38591 ± 0.1360.61409 ± 0.05035Putative outer membrane protein (36%) ↓1.4387 ± 0.1490.85131 ± 0.05836Glucation protein C$	P – Inorganic ion transport and metabolism				
M - Cell wall/membrane/====biogenesis 1.10184 ± 0.077 0.89816 ± 0.089 23 0uter membrane protein RopB (23%) ↑ 0.81340 ± 0.038 1.19661 ± 0.049 K - Transcription 24 VebC/PmpR family DNA-binding transcriptional regulator (37%) ↑ 0.80793 ± 0.039 1.29207 ± 0.011 J - Translation, ribosomal structure and biogenesis 25 Elongation factor P (24%) ↑ 0.78581 ± 0.108 1.21419 ± 0.045 26 50 sribosomal protein 1.9 (36%) ↑ 0.78581 ± 0.108 1.21419 ± 0.014 27 Peptide chain release factor 2 (14%) ↓ 1.21705 ± 0.125 0.78295 ± 0.076 28 Glutathione S-transferase (25%) ↑ 0.75530 ± 0.078 1.24470 ± 0.170 29 ATP-dependent Clp protease proteolytic subunit (25%) ↓ 1.1602 ± 0.032 0.85397 ± 0.015 30 Mypothetical protein RHSP_58990 (58%) ↓ 1.14602 ± 0.032 0.85397 ± 0.015 7 - Signal transduction Natio ant protein (28%) ↑ 0.311 ± 0.074 0.8569 ± 0.146 31 Anti-oxidant protein (28%) ↑ 1.19710 ± 0.029 0.80290 ± 0.166 33 GTP-binding protein CoW (21%) ↓ 1.21741 ± 0.074 0.78259 ± 0.056 S - Function UMknown	21	Aromatic ring-hydroxylating dioxygenase subunit alpha (30%) \downarrow	1.21173 ± 0.118	0.78827 ± 0.087	
22 23UTP-glucose-1-phosphate uridylyltransferase (60%) ↓1.10184 ± 0.0770.89816 ± 0.08623Outer membrane protein RopB (23%) ↑0.81340 ± 0.0381.18661 ± 0.049K - TranscriptionVebC/PmpR family DNA-binding transcriptional regulator (37%) ↑0.80793 ± 0.0391.19207 ± 0.011J - Translation, ribosomal structure and biogenesis0.81340 ± 0.0381.21419 ± 0.04525Elongation factor P (24%) ↑0.78581 ± 0.1081.21419 ± 0.0452650S ribosomal protein 1056%) ↑0.83891 ± 0.0661.16109 ± 0.13427Peptide chain release factor 2 (14%) ↓1.21705 ± 0.1250.78295 ± 0.07628Glutathione S-transferase (25%) ↑0.75530 ± 0.0781.24470 ± 0.17029ATP-dependent Clp protease proteolytic subunit (25%) ↓1.06938 ± 0.0400.93062 ± 0.0737 - Signal transduction1.14602 ± 0.0320.85397 ± 0.0157 - Defense mechanisms1.14602 ± 0.0320.85397 ± 0.01530Hypothetical protein (28%) ↑0.63131 ± 0.0741.36869 ± 0.14631Anti-oxidant protein (28%) ↑1.21711 ± 0.0740.78259 ± 0.05633GTP-binding protein CobW (21%) ↓1.21741 ± 0.0740.78259 ± 0.05635Putative outer membrane protein (36%) ↓1.38591 ± 0.1360.61409 ± 0.05035Putative outer membrane protein (36%) ↓1.38591 ± 0.1360.61409 ± 0.05036Grup and	M – Cell wall/membrane/envelope biogenesis				
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	30	Gyciase (21%) ↓	1.28959 ± 0.163	0.71041 ± 0.060	

Source: the author.

[†] Sequence coverage of identified protein on Mascot platform (Matrix Science).

^{*} ↑: higher expression in acid pH; ↓: lower expression in acid pH.

3.4. The central metabolic pathways and acid stress response

The up-regulation of Glucose-6-phosphate 1-dehydrogenase and KHG-KDPG bifunctional aldolase proteins at pH 4.8 (Table 1) can be associated with a responsive induction of central metabolic pathways. These enzymes catalyze irreversible reactions involved in the conversion of glucose into pyruvate through the Entner-Doudoroff (ED) pathway (Geddes and Oresnik, 2014) (Fig. 1), the preferential pathway for glucose metabolism in rhizobia (Fuhrer et al., 2005). An increased concentration of ED pathway metabolites when *S. meliloti* was cultured

at pH 6.1 was previously reported (Draghi et al., 2017).

The phosphoenolpyruvate (PEP) carboxykinase Pck, has a key role in catalyzing the reversible conversion of oxaloacetate into PEP (Fig. 1) and drives the metabolism towards catabolic tricarboxylic acid (TCA) cycle or anabolic gluconeogenesis (Klaffl et al., 2013). Although Pck regulation is not fully known, reducing conditions are known to favor anaplerotic reaction of Pck (Klaffl et al., 2013), as promoted by peroxidases and reductases, including Alkyl hydroperoxide reductase (Ahp) (Machová et al., 2014).

Expression of both PckA and AhpC were induced in R. freirei PRF 81



Fig. 1. Schematic representation of the main molecular mechanisms of R. freirei PRF 81's adaptive response to acidified pH. Red and blue balloons correspond to up- and down-regulated proteins of the R. freirei proteome, respectively. Arrows correspond to enzymatic reactions. The central metabolism is represented by: the Entner-Doudoroff (ED) pathway (brown), fatty acid biosynthesis (orange), tricarboxylic acid (TCA) cycle (gray), and Gluconeogenesis (purple). Green arrows correspond to exopolysaccharide (EPS) biosynthesis pathway and black arrows represent antioxidant reactions. Metabolites are in the black boxes: glucose-6-phosphate (G6P), glucose-1-phosphate (G1P), UDP-glucose (UDP-G), 6-phosphogluconolactone (6PGL), 2-Keto-3-Deoxy 6-Phosphogluconate (KDPG), glyceraldehyde-3-phosphate (G3P), pyruvate (Pyr), phosphoenolpyruvate (PEP), acetyl-CoA (Ac-CoA), Malonyl-CoA (Ma-CoA), and oxaloacetate (OAA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

grown at acid pH (Table 1). In *A. tumefaciens* cultured under an acidified condition (pH 5.5), the *pckA* gene was also induced (Liu et al., 2005; Yuan et al., 2008). In contrast with the environmental bacteria *A. tumefaciens* and *R. freirei*, *E. coli* did not show differential expression of AhpC in a pH range from 4.9 to 9.1 (Stancik et al., 2002). Therefore, the reducing system may be driving Pck mainly towards the anaplerotic direction of catabolic TCA, thus increasing acid consuming. In addition, the down-regulation of fructose-bisphosphate aldolase Class I protein, which is involved in gluconeogenesis (Scamuffa and Caprioli, 1980), reinforces the hypothesis pro-catabolism rather than anabolism in *R. freirei* under acidic pH, although further research is needed to verify this hypothesis.

Produced by several *Rhizobium* species, succinoglycan is a type of exopolysaccharide (EPS) whose biosynthesis is dependent on the products of *exo* genes (Janczarek, 2011). In *S. meliloti*, the acid pH resulted in increased expression of *exo* genes (Hellweg et al., 2009; Lucena et al., 2010) and EPS production (Draghi et al., 2016). On the other hand, there is no evidence that EPS improves bacterial acid tolerance, as reported in a study with a *S. meliloti* mutant for *exoY* exposed to low pH (Dilworth et al., 1999). Moreover, a study with *Rhizobium ciceri* exposed to pH 5, 6, 7, 8, and 9 indicated that EPS yield is dramatically decreased under acidic pH (Küçük and Kivanç, 2009).

The ExoN protein was down-regulated in *R. freirei* PRF 81 grown at pH 4.8. Corroborating our result, ExoN2 protein expression is decreased in *S. meliloti* at pH 6.1 (Draghi et al., 2016), and *R. tropici* CIAT 899 strongly reduces EPS production under acidic pH 4.7 and 5.7 (Staudt et al., 2012). Therefore, the down-regulation of ExoN is in accordance with the inclination towards catabolism in low pH conditions.

The Acetyl-CoA carboxylase (Acc), a key enzymatic complex that catalyzes the first step in fatty acid biosynthesis (Rathnasingh et al., 2012), was down-regulated in *R. freirei* at pH 4.8 (Fig. 1). In *H. pylori*, a highly acid tolerant species, *accA* tended to be less expressed at pHs lower than 6.0 (Shao et al., 2008). Taken together, these data suggest that these bacteria bypass fatty acid biosynthesis in low pH conditions. To this end, the metabolism is probably driven to catabolism, catalyzing acid consuming rather than fatty acid biosynthesis, thus alleviating internal acidification.

3.5. Reactive oxygen species (ROS) formation and antioxidant defense

Although not fully elucidated, the correlation between acidity and oxidative stresses is often suggested (Bruno-Bárcena et al., 2010; Rangel, 2011; Ormeño-Orrillo et al., 2012). A large number of oxidative

stress genes show pH-dependent expression with most being induced in acid or inhibited in alkaline conditions (Shao et al., 2008; Draghi et al., 2016). Moreover, aerobic respiration steps up at pH 5 (Maurer et al., 2005). A proteomic analysis of *Yarrowia lipolytica* pointed out that one of the major cellular responses of this yeast species to low pH environment is the enhanced expression of TCA cycle enzymes and increased levels of ROS (Guo et al., 2016).

The respiratory chain is considered the major source of ROS (Pastor et al., 2009), and the Complex 1 is a known producer of endogenous superoxide (O_2^-) (Frick et al., 2015). Acid environmental conditions may lead to increased aerobic respiration and ROS formation, with consequent induction of oxidative stress.

ROS can be converted into hydrogen peroxide (H_2O_2) and Glutathione S-transferase (GST) belongs to a cluster of enzymes involved in some processes directly connected to the oxidative stress response, by efficiently reducing H_2O_2 (Imlay, 2013; Todorova et al., 2007). The up-regulation of GST at pH 4.8 suggests that this enzyme may be protecting the cell against oxidative damage.

Ahp is also involved in oxidative stress response; however, in contrast with catalases, Ahp degrades H_2O_2 into H_2O consuming NADH (Fig. 1) and does not lead to the formation of oxidizing species. Ahp is, therefore, the most effective scavenger during low-level H_2O_2 stress (Imlay, 2013). Nevertheless, Ahp depends on the NADH supply from metabolism and becomes saturated when intracellular H_2O_2 exceeds 20 μ M (Seaver and Imlay, 2001). Since Ahp was the most induced protein, it may be simultaneously detoxifying H_2O_2 and supplying metabolism with NAD⁺.

3.6. Overall response to acid pH

The wide range of *R. freirei* PRF 81 response to acid stress is summarized in the scheme depicted in Fig. 1. Several changes were revealed at translational level, including processes involved in protein biosynthesis, proton transport, proton motive force modulation, central carbon metabolism, ROS generation and antioxidative defense against ROS, among others.

Only few rhizobial species are able to grow in pH lower than 5.0 (Hungria and Vargas, 2000; Ribeiro et al., 2012; Dall'Agnol et al., 2013); this may be a key to symbiotic efficiency, since the pH in determinate nodules is slightly acid (approximately 6.4 in younger nodules and 5.5 in older or stressed nodules) (Becana and Klucas, 1992).

4. Conclusions

This study revealed that acidic growth conditions affect several *R*. *freirei* PRF 81's cellular processes and the response elicited by these bacteria to this stress. Our results emphasize the key role played by central metabolism in the acid tolerance response of *R*. *freirei* PRF 81 that may contribute with studies aiming to improve *R*. *freirei* PRF 81 symbiotic abilities under adverse soil conditions and to optimize the development and industrial production of inoculants.

Similar strategies seem to be employed by the related species *R. tropici* when exposed to pH 4.5, including changes on carbohydrate metabolism and energy generation processes, proton extrusion, reduced membrane permeability to protons, among others (Guerrero-Castro et al., 2018). In *S. meliloti* Rm 2011, a strain sensitive to low pH, it was observed the acid-induced expression of *fixN1* and *fixG* only at the transcriptional level (Draghi et al., 2016). Since both nitrogen-fixation genes are located in symbiotic plasmid pSymA, which is recognized to be involved in several stress responses, the authors indicate that the cellular role of *fix* genes may exceed their action in nitrogen fixation.

The molecular mechanisms discussed here suggest that the adaptive response of *R. freirei* PRF 81 to acid pH is a multigenic character, and acid tolerance responses seem to be orchestrated to alleviate internal acidification by inducing acid consuming, proton extrusion, reduction of proton influx, and oxidative stress defense, while decreasing fatty acid and EPS biosynthesis. The prevalence of differential cytoplasmic proteins highlights the crucial role of the central metabolism in the tolerance of *R. freirei* PRF 81 to acid stress.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Authors thanks Etto RM for helpful suggestions on the manuscript; Paulistch F, Klepa MS and Reichert PRS for help in setting up experiments.

Funding

Tullio LD received a scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Gomes DF from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Research was partially supported by CNPq (National Council for Scientific and Technological Development), Project Universal (449902/2014-5) and Science without Borders (400205/2012-5).

Authorship policy

Conceived or designed the study: Batista JSS, Gomes DF, Hungria M. Performed research: Batista JSS, Gomes DF, Silva LP, Tullio LD. Analyzed data: Batista JSS, Gomes DF, Silva LP, Tullio LD.

Contributed with new methods or models: Batista JSS, Gomes DF, Tullio LD.

Wrote the paper: Batista JSS, Hungria M, Tullio LD.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2018.11.014.

References

Alm, E.J., Huang, K.H., Price, M.N., Koche, R.P., Keller, K., Dubchak, I.L., Arkin, A.P., 2005. The Microbes Online Web site for comparative genomics. Genome Res. 15 (7), Applied Soil Ecology 135 (2019) 98-103

1015-1022.

- Batista, J.S.S., Hungria, M., 2012. Proteomics reveals differential expression of proteins related to a variety of metabolic pathways by genistein-induced *Bradyrhizobium japonicum* strains. J. Proteomics 75 (4), 1211–1219.
- Becana, M., Klucas, R.V., 1992. Oxidation and reduction of leghemoglobin in root nodules of leguminous plants. Plant physiol. 98 (4), 1217–1221.
- Bore, E., Langsrud, S., Langsrud, Ø., Rode, T.M., Holck, A., 2007. Acid-shock responses in *Staphylococcus aureus* investigated by global gene expression analysis. Microbiology 153 (7), 2289–2303.
- Bruno-Bárcena, J.M., Azcárate-Peril, M.A., Hassan, H.M., 2010. Role of antioxidant enzymes in bacterial resistance to organic acids. Appl. Environ. Microbiol. 76 (9), 2747–2753.
- Buckley, D.H., Schmidt, T.M., 2002. Exploring the biodiversity of soil—a microbial rain forest. Biodivers. Microb. Life 183–208.
- Cheng, G., Karunakaran, R., East, A.K., Munoz-Azcarate, O., Poole, P.S., 2017. Glutathione affects the transport activity of *Rhizobium leguminosarum* 3841 and is essential for efficient nodulation. FEMS Microbiol. Lett. 364 (8), fnx045.
- Corticeiro, S.C., Lima, A.I.G., Figueira, E.M.D.A.P., 2006. The importance of glutathione in oxidative status of *Rhizobium leguminosarum* biovar viciae under Cd exposure. Enzyme Microb. Technol. 40 (1), 132–137.
- Dall'Agnol, R.F., Ribeiro, R.A., Ormeño-Orrillo, E., Rogel, M.A., Delamuta, J.R.M., Andrade, D.S., Martínez-Romero, E., Hungria, M., 2013. *Rhizobium freirei* sp. nov., a symbiont of *Phaseolus vulgaris* that is very effective at fixing nitrogen. Int. J. Syst. Evol. Microbiol. 63 (11), 4167–4173.
- de Maagd, R., de Rijk, R.O.E.L., Mulders, I.H., Lugtenberg, B.J., 1989. Immunological characterization of *Rhizobium leguminosarum* outer membrane antigens by use of polyclonal and monoclonal antibodies. J. Bacteriol. 171 (2), 1136–1142.
- Dilworth, M.J., Rynne, F.G., Castelli, J.M., Vivas-Marfisi, A.I., Glenn, A.R., 1999. Survival and exopolysaccharide production in *Sinorhizobium meliloti* WSM419 are affected by calcium and low pH. Microbiology 145 (7), 1585–1593.
- Draghi, W.O., del Papa, M.F., Hellweg, C., Watt, S.A., Watt, T.F., Barsch, A., Lozano, M.J., Lagares Jr., A., Salas, M.E., López, J.L., Albicoro, F.J., Nilsson, J.F., Torres Tejerizo, G.A., Luna, M.F., Pistorio, M., Boiardi, J.L., Pühler, A., Weidner, S., Niehaus, K., Lagares, A., 2016. A consolidated analysis of the physiologic and molecular responses induced under acid stress in the legume-symbiont model-soil bacterium *Sinorhizobium meliloti*. Sci. Rep. 6.
- Draghi, W.O., del Papa, M.F., Barsch, A., Albicoro, F.J., Lozano, M.J., Pühler, A., Niehaus, K., Lagares, A., 2017. A metabolomic approach to characterize the acid-tolerance response in *Sinorhizobium meliloti*. Metabolomics 13 (6), 71.
- Duary, R.K., Batish, V.K., Grover, S., 2010. Expression of the *atpD* gene in probiotic *Lactobacillus plantarum* strains under in vitro acidic conditions using RT-qPCR. Res. Microbiol. 161 (5), 399–405.
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. U.S.A. 103 (3), 626–631.
- Foreman, D.L., Vanderlinde, E.M., Bay, D.C., Yost, C.K., 2010. Characterization of a gene family of outer membrane proteins (*ropB*) in *Rhizobium leguminosarum* bv. *viciae* VF39SM and the role of the sensor kinase ChvG in their regulation. J. Bacteriol. 192 (4), 975–983.
- Frick, K., Schulte, M., Friedrich, T., 2015. Reactive oxygen species production by *Escherichia coli* respiratory complex I. Biochemistry-US 54 (18), 2799–2801.
- Fuhrer, T., Fischer, E., Sauer, U., 2005. Experimental identification and quantification of glucose metabolism in seven bacterial species. J. Bacteriol. 187 (5), 1581–1590.
- Galperin, M.Y., Makarova, K.S., Wolf, Y.I., Koonin, E.V., 2015. Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucl. Acids Res. 43 (D1), D261–D269.
- Geddes, B.A., Oresnik, I.J., 2014. Physiology, genetics, and biochemistry of carbon metabolism in the alphaproteobacterium *Sinorhizobium meliloti*. Can. J. Microbiol. 60 (8), 491–507.
- Gomes, D.F., Batista, J.S.S., Torres, A.R., Andrade, D.S., Galli-Terasawa, L.V., Hungria, M., 2012a. Two-dimensional proteome reference map of *Rhizobium tropici* PRF 81 reveals several symbiotic determinants and strong resemblance with agrobacteria. Proteomics 12 (6), 859–863.
- Gomes, D.F., Batista, J.S.S., Schiavon, A.L., Andrade, D.S., Hungria, M., 2012b. Proteomic profiling of *Rhizobium tropici* PRF 81: identification of conserved and specific responses to heat stress. BMC Microbiol. 12 (1), 84.
- Gomes, D.F., Ormeño-Orrillo, E., Hungria, M., 2015. Biodiversity, symbiotic efficiency, and genomics of *Rhizobium tropici* and related species. Biol. Nitrogen Fixation 747–756.
- Graham, P.H., Draeger, K.J., Ferrey, M.L., Conroy, M.J., Hammer, B.E., Martinez, E., Aarons, S.R., Quinto, C., 1994. Acid pH tolerance in strains of *Rhizobium* and *Bradyrhizobium*, and initial studies on the basis for acid tolerance of *Rhizobium tropici* UMR1899. Can. J. Microbiol. 40 (3), 198–207.
- Graham, P.H., Vance, C.P., 2003. Legumes: importance and constraints to greater use. Plant Physiol. 131 (3), 872–877.
- Guerrero-Castro, J., Lozano, L., Sohlenkamp, C., 2018. Dissecting the acid stress response of *Rhizobium tropici* CIAT 899. Front. Microbiol. 9 (1), 1–14.
- Guo, H., Wan, H., Chen, H., Fang, F., Liu, S., Zhou, J., 2016. Proteomic analysis of the response of α-ketoglutarate-producer *Yarrowia lipolytica* WSH-Z06 to environmental pH stimuli. Appl. Microbiol. Biotechnol. 100 (20), 8829–8841.
- Harrison, J., Jamet, A., Muglia, C.I., van de Sype, G., Aguilar, O.M., Puppo, A., Frendo, P., 2005. Glutathione plays a fundamental role in growth and symbiotic capacity of *Sinorhizobium meliloti*. J. Bacteriol. 187 (1), 168–174.
- Hellweg, C., Pühler, A., Weidner, S., 2009. The time course of the transcriptomic response of *Sinorhizobium meliloti* 1021 following a shift to acidic pH. BMC Microbiol. 9 (1), 37.
- Hungria, M., Andrade, D.S., Chueire, L.M.O., Probanza, A., Guttierrez-Mañero, F.J., Megías, M., 2000. Isolation and characterization of new efficient and competitive

bean (Phaseolus vulgaris L.) rhizobia from Brazil. Soil Biol. Biochem. 32 (11), 1515–1528.

- Hungria, M., Vargas, M.A., 2000. Environmental factors affecting N 2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crop Res. 65 (2), 151–164.
- Imlay, J.A., 2013. The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. Nat. Rev. Microbiol. 11 (7), 443–454. Janczarek, M., 2011. Environmental signals and regulatory pathways that influence
- exopolysaccharide production in rhizobia. Int. J. Mol. Sci. 12 (11), 7898–7933. Kannan, G., Wilks, J.C., Fitzgerald, D.M., Jones, B.D., BonDurant, S.S., Slonczewski, J.L., 2008. Rapid acid treatment of *Escherichia coli*: transcriptomic response and recovery. BMC Microbiol. 8 (1), 37.
- Keiler, K.C., 2015. Mechanisms of ribosome rescue in bacteria. Nat. Rev. Microbiol. 13 (5), 285–297.
- Klaffl, S., Brocker, M., Kalinowski, J., Eikmanns, B.J., Bott, M., 2013. Complex regulation of the phosphoenolpyruvate carboxykinase gene *pck* and characterization of its GntRtype regulator IoIR as a repressor of myo-inositol utilization genes in *Corynebacterium glutamicum*. J. Bacteriol. 195 (18), 4283–4296.
- Küçük, Ç., Kivanç, M., 2009. Extracellular polysaccharide production by *Rhizobium ciceri* from Turkey. Ann. Microbiol. 59 (1), 141–144.
- Len, A.C., Harty, D.W., Jacques, N.A., 2004. Proteome analysis of Streptococcus mutans metabolic phenotype during acid tolerance. Microbiology 150 (5), 1353–1366.
- Li, L., Jia, Y., Hou, Q., Charles, T.C., Nester, E.W., Pan, S.Q., 2002. A global pH sensor: *Agrobacterium* sensor protein ChvG regulates acid-inducible genes on its two chromosomes and Ti plasmid. Proc. Natl. Acad. Sci. U.S.A. 99 (19), 12369–12374.
- Liu, P., Wood, D., Nester, E.W., 2005. Phosphoenolpyruvate carboxykinase is an acidinduced, chromosomally encoded virulence factor in *Agrobacterium tumefaciens*. J. Bacteriol. 187 (17), 6039–6045.
- Lucena, D.K., Pühler, A., Weidner, S., 2010. The role of sigma factor RpoH1 in the pH stress response of *Sinorhizobium meliloti*. BMC Microbiol. 10 (1), 265.
- Luche, S., Eymard-Vernain, E., Diemer, H., van Dorsselaer, A., Rabilloud, T., Lelong, C., 2016. Zinc oxide induces the stringent response and major reorientations in the central metabolism of *Bacillus subtilis*. J. Proteomics 135, 170–180.
- Lund, P., Tramonti, A., de Biase, D., 2014. Coping with low pH: molecular strategies in neutralophilic bacteria. FEMS Microbiol. Rev. 38 (6), 1091–1125.
- Machová, I., Snášel, J., Zimmermann, M., Laubitz, D., Plocinski, P., Oehlmann, W., Singh, M., Dostál, J., Sauer, U., Pichová, I., 2014. *Mycobacterium tuberculosis* phosphoenolpyruvate carboxykinase is regulated by redox mechanisms and interaction with thioredoxin. J. Biol. Chem. 289 (19), 13066–13078.
- Markowitz, V.M., Korzeniewski, F., Palaniappan, K., Szeto, E., Werner, G., Padki, A., Zhao, X., Dubchak, I., Hugenholtz, P., Lykidis, A., Mavromatis, K., Ivanova, N., Kyrpides, N.C., 2006. The integrated microbial genomes (IMG) system. Nucl. Acids Res. 34 (suppl_1), D344–D348.
- Maurer, L.M., Yohannes, E., Bondurant, S.S., Radmacher, M., Slonczewski, J.L., 2005. pH regulates genes for flagellar motility, catabolism, and oxidative stress in *Escherichia coli* K-12. J. Bacteriol. 187 (1), 304–319.
- Muglia, C.I., Grasso, D.H., Aguilar, O.M., 2007. *Rhizobium tropici* response to acidity involves activation of glutathione synthesis. Microbiology 153 (4), 1286–1296.
- Naganathan, A., Wood, M.P., Moore, S.D., 2015. The large ribosomal subunit protein L9 enables the growth of EF-P deficient cells and enhances small subunit maturation. PLoS One 10 (4), e0120060.
- Ormeño-Orrillo, E., Menna, P., Almeida, L.G.P., Ollero, F.J., Nicolás, M.F., Rodrigues, E.P., Nakatani, A.S., Batista, J.S.S., Chueire, L.M.O., Souza, R.C., Vasconcelos, A.T.R.,

Megías, M., Hungria, M., Martínez-Romero, E., 2012. Genomic basis of broad host range and environmental adaptability of *Rhizobium tropici* CIAT 899 and *Rhizobium* sp. PRF 81 which are used in inoculants for common bean (*Phaseolus vulgaris* L.). BMC Genomics 13 (1), 735.

- Pastor, M.M., Proft, M., Pascual-Ahuir, A., 2009. Mitochondrial function is an inducible determinant of osmotic stress adaptation in yeast. J. Biol. Chem. 284 (44), 30307–30317.
- Rangel, D.E., 2011. Stress induced cross-protection against environmental challenges on prokaryotic and eukaryotic microbes. World J. Microbiol. Biotechnol. 27 (6), 1281–1296.
- Rathnasingh, C., Raj, S.M., Lee, Y., Catherine, C., Ashok, S., Park, S., 2012. Production of 3-hydroxypropionic acid via malonyl-CoA pathway using recombinant *Escherichia coli* strains. J. Biotechnol. 157 (4), 633–640.
- Ribeiro, R.A., Rogel, M.A., Lopez-Lopez, A., Ormeno-Orrillo, E., Barcellos, F.G., Martinez, J., Thompson, F.L., Martínez-Romero, E., Hungria, M., 2012. Reclassification of *Rhizobium tropici* type A strains as *Rhizobium leucaenae* sp. nov. Int. J. Syst. Evol. Microbiol. 62 (5), 1179–1184.
- Riccillo, P.M., Muglia, C.I., de Bruijn, F.J., Roe, A.J., Booth, I.R., Aguilar, O.M., 2000. Glutathione is involved in environmental stress responses in *Rhizobium tropici*, including acid tolerance. J. Bacteriol. 182 (6), 1748–1753.
- Scamuffa, M.D., Caprioli, R.M., 1980. Comparison of the mechanisms of two distinct aldolases from *Escherichia coli* grown on gluconeogenic substrates. BBA Enzymol. 614 (2), 583–590.
- Schirch, V., Hopkins, S., Villar, E., Angelaccio, S., 1985. Serine hydroxymethyltransferase from *Escherichia coli*: purification and properties. J. Bacteriol. 163 (1), 1–7.
- Seaver, L.C., Imlay, J.A., 2001. Alkyl hydroperoxide reductase is the primary scavenger of endogenous hydrogen peroxide in *Escherichia coli*. J. Bacteriol. 183 (24), 7173–7181.
- Shao, C., Zhang, Q., Tang, W., Qu, W., Zhou, Y., Sun, Y., Yu, H., Jia, J., 2008. The changes of proteomes components of *Helicobacter pylori* in response to acid stress without urea. J. Microbiol. 46 (3), 331–337.
- Sobrevals, L., Müller, P., Fabra, A., Castro, S., 2006. Role of glutathione in the growth of *Bradyrhizobium* sp. (peanut microsymbiont) under different environmental stresses and in symbiosis with the host plant. Can. J. Microbiol. 52 (7), 609–616.
- Spero, M.A., Aylward, F.O., Currie, C.R., Donohue, T.J., 2015. Phylogenomic analysis and predicted physiological role of the proton-translocating NADH: quinone oxidoreductase (complex I) across bacteria. MBio 6 (2), e00389–e415.
- Stancik, L.M., Stancik, D.M., Schmidt, B., Barnhart, D.M., Yoncheva, Y.N., Slonczewski, J.L., 2002. pH-dependent expression of periplasmic proteins and amino acid catabolism in *Escherichia coli*. J. Bacteriol. 184 (15), 4246–4258.
- Staudt, A.K., Wolfe, L.G., Shrout, J.D., 2012. Variations in exopolysaccharide production by *Rhizobium tropici*. Arch. Microbiol. 194 (3), 197–206.
- Todorova, T., Vuilleumier, S., Kujumdzieva, A., 2007. Role of glutathione s-transferases and glutathione in arsenic and peroxide resistance in *Saccharomyces cerevisiae*: a reverse genetic analysis approach. Biotechnol. Biotechnol. Eq. 21 (3), 348–352.
- Vanderlinde, E.M., Yost, C.K., 2012. Genetic analysis reveals links between lipid A structure and expression of the outer membrane protein gene, *ropB Rhizobium leguminosarum*. FEMS Microbiol. Lett. 335 (2), 130–139.
- Yuan, Z.C., Liu, P., Saenkham, P., Kerr, K., Nester, E.W., 2008. Transcriptome profiling and functional analysis of *Agrobacterium tumefaciens* reveals a general conserved response to acidic conditions (pH 5.5) and a complex acid-mediated signaling involved in *Agrobacterium*-plant interactions. J. Bacteriol. 190 (2), 494–507.