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Research Paper

Methylobacterium-plant interaction genes regulated by plant exudate and quorum sensing molecules

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Abstract

Bacteria from the genus *Methylobacterium* interact symbiotically (endophytically and epiphytically) with different plant species. These interactions can promote plant growth or induce systemic resistance, increasing plant fitness. The plant colonization is guided by molecular communication between bacteria-bacteria and bacteria-plants, where the bacteria recognize specific exuded compounds by other bacteria (*e.g.* homoserine molecules) and/or by the plant roots (*e.g.* flavonoids, ethanol and methanol), respectively. In this context, the aim of this study was to evaluate the effect of quorum sensing molecules (N-acyl-homoserine lactones) and plant exudates (including ethanol) in the expression of a series of bacterial genes involved in *Methylobacterium*-plant interaction. The selected genes are related to bacterial metabolism (*mxa*F), adaptation to stressful environment (*crt*I, *pho*U and *sss*), to interactions with plant metabolism compounds (*acd*S) and pathogenicity (*patatin* and *pho*U). Under in vitro conditions, our results showed the differential expression of some important genes related to metabolism, stress and pathogenesis, thereby AHL molecules up-regulate all tested genes, except *pho*U, while plant exudates induce only *mxa*F gene expression. In the presence of plant exudates there is a lower bacterial density (due the endophytic and epiphytic colonization), which produce less AHL, leading to down regulation of genes when compared to the control. Therefore, bacterial density, more than plant exudate, influences the expression of genes related to plant-bacteria interaction.

Key words: endophyte, *Methylobacterium*, quantitative PCR (qPCR), plant-bacteria Interaction, homoserine.

Introduction

Endophytes colonize the plant inner tissues, commonly coming from the soil and entering the plants by intercellular spaces or little root fissure. The genus *Methylobacterium* is constituted by methylotrophic bacteria, which are able to interact symbiotically with different plant species, where they occupy mainly the inner tissues of the host, as endophytes. In agriculture, it has been described that *M. nodulans* is involved with nitrogen fixation and nodulation of plants (Sy *et al.*, 2003), while other species can promote plant growth or induce systemic resistance (Araújo *et al.*, 2002; Lacava *et al.*, 2004; Madahaiyan *et al.*, 2006). In citrus, it has been shown that endophytic

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Methylobacterium can interact with *Xylella fastidiosa*, the causal agent of citrus variegate clorose (CVC) (Araújo *et al.*, 2002; Lacava *et al.*, 2004). Gai *et al.* (2009) manipulated *M. mesophilicum* SR1.6/6 to express the green fluorescence protein (GFP) in *C. roseus*, and observed that the colonization and transmission of *M. mesophilicum* by *Bucephalogonia xanthophis*, the *X. fastidiosa* insect-vector. Their results proved that *M. mesophilicum* is really transmitted by the insect to host plant living in the same niche of *X. fastidiosa* inside of *C. roseus*. Thus, the authors proposed the *M. mesophilicum* as a candidate to symbiotic control of *X. fastidiosa* due to this intimate association of SR1.6/6 with the plant, it can be suggested that this bacterium can act in plant, influencing the microbial balance in the plant host and participating on the plant development.

The occurrence of plant colonization by pathogenic or non-pathogenic bacteria is dependent of a communication between plants and microorganisms (Rosenblueth and Martinez-Romero, 2006; Hardoim *et al.*, 2008; Wang *et al.*, 2010), which is believed to be made by plant exudation of 17 compounds, such as flavonoids (Hardoim *et al.*, 2008), ethanol (Williams and Yavitt, 2009) and methanol (Sudtachat *et al.*, 2009; Sy *et al.*, 2005; Jourand *et al.*, 2005) that attracts microorganisms. Bacterial communication during the plant-interaction can be coordinated physiological and adaptive changes in their population (Cha *et al.*, 1998), favoring its adaptation to specific environments (Bauer and Mathesius, 2004; Joint, 2006; Soto *et al.*, 2006; Sanches-Contreras *et al.*, 2007). Some quorum sense (QS) systems use N-acyl-homoserine lactones (AHLs) as signaling molecules, commonly found in Gram-negative bacteria that living in association with plants (Cha *et al.*, 1998; Loh *et al.*, 2002; Camilli and Bassler, 2006; Sanches-Contreras *et al.*, 2007; Barnard *et al.*, 2007; White and Winans, 2007). QS system allows bacteria to function as multicellular organisms, because the extracellular concentration of autoinducer increases with bacteria population growth, after reaches a determinate number, this molecules diffuse back into the bacteria and regulate the transcription of different genes that may be related with the secretion of virulence factors, biofilm formation, sporulation, exchange of DNA and others (Zhu and Sun, 2008).

Although several studies demonstrate the importance of the association between *Methylobacterium*-plants (Pirttila *et al.*, 2000; Sy *et al.*, 2001, Lee *et al.*, 2006; Madhaiyan *et al.*, 2009a; 2009b; Dourado *et al.*, 2012a,b) and that members of the *Methylobacterium* genus produces AHL (Penãlver *et al.*, 2006; Pomini *et al.*, 2009; Poonguzhali *et al.*, 2007), little is known about the role of plant exudates and AHL on the expression of bacterial genes that are involved in bacterium plant-interaction. Studies reported that biofilm production seems to be dependent of the production of AHL (Penãlver *et al.*, 2006), and that biofilm formation on the plant may be the first step towards endophytic colonization by *Methylobacterium* (Andreote *et al.*, 2006; Rossetto *et al.*, 2011). Despite of the difficulty to understand the complexity of mechanisms involved in plant-microbe interactions, some bacterial genes responsible for metabolism, stress defence and pathogenicity that present an important role on plant bacterial interaction are described. The *mxa*F gene, responsible for the methylotrofic metabolism, encodes a subunit of the dehydrogenase methanol enzyme (MDH) (Zhang and Lindstrom, 2003) its expression confers adaptive advantages in competitive conditions on the plant surface (Willians and Yavitt, 2009), increasing methylotrophic activity during symbioses (Sy *et al.*, 2005).

The genes *pho*U and *sss* are transport genes, where *pho*U gene is responsible mainly for the phosphate homeostasis regulation, but also controls and interferes on stress, antibiotic production (Li and Zhang, 2007; Gristwood *et al.*, 2009) and virulence gene expression (Cheng *et al.*, 2009) and the *sss* (sodium solute symporter) gene is responsible for the symport transport of solute with the sodium (Scier, 1998). Genes *crt*I and *acd*S genes are associated to the stress response (Sandmann, 2009) and to plant metabolism (Hardoim *et al.*, 2008). Phytoene dehydrogenase gene (*crt*I) codifies an enzyme that catalyses the denaturation reaction resulting on the lycopene synthesis that protects the cell against oxidative damages, different types of radiations and dissections (Xu *et al.*, 2007) and the *acd*S gene codifies the enzyme carboxylic acid aminocyclopropane deaminase that degrades ACC (1-carboxilate amineciclopropane), forerunner of plant ethylene (Glick *et al.*, 2007; Hardoim *et al.*, 2008).

The *patatin* gene is associated to the pathogenic bacteria and is activated during the pathogeneses process, the *patatin* family genes encode phospholypases enzymes that hydrolyze phospholipids, frequently used by pathogenic bacteria on the effective host colonization hydrolyzing the membrane phospholipids, resulting in a membrane damage and cytotoxicity (Camera *et al.*, 2009), the *pho*U gene is also involved in the bacteria pathogeneses process (Cheng *et al.*, 2009).

Thus, the aim of the present study was to evaluate the expression of several genes previously related to plant-bacteria and bacteria-bacteria interaction (*mxa*F, *pho*U, *crt*I, *acd*S, *sss* and *patatin*) using as model an endophytic bacterium *M. mesophilicum* SR1.6/6, quorum sense molecules (AHL (S)-N-dodecanoyl HSL-extracted from this isolate) and rice and eucalyptus plants as well ethanol as rooting exudate carbon source.

Material and Methods

Microorganism and plant material

The selected bacterial strain SR1.6/6, *M. mesophilicum*, was previously isolated from *Citrus sinensis*

(Araújo *et al.*, 2002). The SR1.6/6 was routinely cultivated at 28 °C in CHOI3 medium (Toyama *et al.*, 1998). The bacterium is stored in dilute liquid CHOI3 supplemented with glycerol at -80 °C. Fresh cultures were started from glycerol stocks for each experiment by plating portions onto CHOI3. The plant hosts used in this study were rice (*Oryza sativa*) and eucalyptus (*Eucalyptus citriodora*) seedlings. The axenic seedlings of rice and eucalyptus were obtained from seeds previously disinfected, washing in 70% ethanol for 2 min, sodium hypochlorite solution (2% available Cl^-) for 6 min, and again 70% ethanol for 2 min for rice seeds and washed for 1 min in 70% ethanol, 2 min in sodium hypochlorite solution and 1 min in 70% ethanol for eucalyptus seeds, both seeds followed by two rinses in sterile distilled water. The seeds were germinated on the MS medium (Ramakers *et al.*, 2003) in a wet chamber at 28 °C under 16 hours of photoperiod.

AHL (S)-N-dodecanoyl-HSL assay

The AHL (S)-N-dodecanoyl-HSL was purified from the same bacterial strain (SR1.6/6 - *M. mesophilicum*) and synthesized in Chemistry Institute of Unicamp University (Pomini *et al.*, 2009). To minimize the levels of endogenous AHL in *M. mesophilicum* SR1.6/6, the bacteria was initially cultivated in 25 mL of CHOI3 medium (Toyama *et al.*, 1998) at 28 °C to $O.D._{600}$ of 0.5. Then the cells were pelletized, washed in PBS buffer (10 mM Na_2HPO_4; 2 mM KH_2PO_4; 3 mM KCl; 140 mM NaCl; pH 7.4), inoculated into 50 mL of CHOI3 medium and grown at 28 °C to $O.D._{600}$ of 0.5. Proceeding as before, the culture was centrifuged; the pellet cells were washed in PBS buffer and transferred to 100 mL of CHOI3 medium supplemented with the amount of AHL naturally produced by this isolated (0.1 g/mL) (Pomini *et al.*, 2009). The treated cultures were incubated at 28 °C to transition to stationary phase (18 hours; $O.D._{600}$ -1.7). The experiment was performed in three biological replicates. Triplicate control cultures no added AHL were also grown under the same conditions to RNA extraction.

Root exudates role in the gene expression

After seven and twenty days of rice and eucalyptus respectively disinfected seeds germination, the seedlings were removed from pots and inoculated with *M. mesophilicum* SR1.6/6 cell by adding 5 mL of bacterial suspension (10^8 UFC mL^{-1}) on CHOI3 medium (Toyama *et al.*, 1998). Two assays were developed, to observe the biotic factors, all the inoculums were done in liquid MS medium (Murashige and Skoog, 1962), in the first assay SR1.6/6 was inoculated with rice or eucalyptus seedlings and to accesses the effects of carbon source SR1.6/6 in CHOI3, using methanol or ethanol on this medium. All treatments were constituted by three biological repetitions and the incubation period before RNA extraction was 48 hours.

Total RNA isolation and cDNA synthesis

Bacteria cells were collected from all treatment describe above by centrifugation (15 min at 6000 g), and the RNA was isolated from the bacterial pellet according to the Invitrogen protocol (TRIzol, Invitrogen). The RNA was ressuspended in 30 μL of dietyl-pirocarbonate (DEPC) treated water and stored at -80 °C. The integrity and amount of extracted RNA was verified in a denaturant agarose gel 1.2% in FA 1X (MOPS 200 mM, sodium acetate 50 mM and EDTA 10 mM), containing formaldehyde (0.7%) and ethidium bromide (0.3 $g.mL^{-1}$). The RNA was quantified by $O.D._{260}$, measured in spectrophotometer NanoDrop ND-1000 (Thermo Scientific, USA). All material used obtain and treat the RNA was sterilized and/or treated with DEPC to eliminated RNAse. Total RNA (1-10 μg) was reverse-transcribed into cDNA using random hexamer primers (Invitrogen) and 200 U SuperscriptII RNAse H- reverse transcriptase (Invitrogen) according to the procedure supplied with the enzyme. For each RNA sample, a negative RT (no addition of reverse transcriptase) was performed and used as a negative control in subsequent PCRs.

Primers designing and validation

The construction of the primers to amplify the target genes in this study were designed based on the six genomes sequences of *Methylobacterium* genus (*M. extorquens* DM4, *M. extorquens* AM1, *M. chloromethanicum* CM4, *M. populi* BJ001, *M. nodulans* ORS 2060, *M. radiotolerans* JCM 2831) available at GenBank database (National Center for Biotechnology Information). Sequences from all genes were aligned, and primers were designed, with the software Primer 3 program (v. 0.4.0) (http://frodo.wi.mit.edu/), to anneal in conserved regions of the gene (Table 1). Firstly, the conventional PCR was used to validate the design primers. PCRs were performed in 25 μL reaction containing 1 X enzyme buffer, 3.75 mM of $MgCl_2$, 0.2 mM of each dNTPs, 0.2 μM of each primer and 0.1 U/μL of Taq DNA Polymerase (Invitrogen, Brazil). An initial denaturation was carried out at 94 °C for 5 min, followed by 35 thermal cycles of 30 s at 95 °C, 1 min at 59 °C and 1 min at 72 °C, with a final extension performed at 72 °C for 5 min. All PCR amplificons were checked by electrophoresis on agarose gel (1.5% w/v agarose) and UV visualization of the ethidium bromide stained gels.

The specificity of each primer pair was tested in 17 bacterial strains of *Methylobacterium* genus, and also in strains affiliated to *Rhizobium*, *Sinorizobium* and *Bacillus* genera (Table 2). All amplification products of the seven genes (*mxa*F, *acd*S, *crt*I, *patatin*, *pho*U, *sss* and *rec*A) were purified, sequenced and compared to the GenBank data by BLASTn (http://blast.ncbi.nlm.nih.gov/) confirming the identity of the amplified fragment.

Table 1 - Sequencing of designed primers to evaluate the gene expression of the endophytic bacterium *M. mesophilicum* SR1.6/6 in plants.

Primer	Target gene	Sequencing (5'-3')	Fragment length	Reference
MxaFqPCRAF	*mxaF*	CGTCAACGTCATGATGCT(C/G)T	250 pb	This study
MxaFqPCRAR		GATGTCCTTGGCGAG(A/G)TG		This study
ACC Met1 f	*acdS*	GACCGGGTCGGCAACATC	200 pb	This study
ACC Met2 r		AGCCCGCCGTACTTGTGC		This study
*Patatin*F	*Patatin*	CTTCAACGCCAACCTGATG	250 pb	This study
*Patatin*R		CCGATCCGCTCGTAGTTCT		This study
PhyF	*crtI*	AATACTTCAAGCCGGTGCTG	186 pb	This study
PhyR		GACATGCCGAGGTACTTGGT		This study
*sss*F	*sss*	ATCGACGCCCTGTACAATTC	221 pb	This study
*sss*R		ACCGTCGCGTAGTTCGAC		This study
*phoU*F	*phoU*	TTCGACGGGCTGATCTACTC	189 pb	This study
*phoU*R		GATCAGGTAGAAGGCCACCA		This study
recAF	*recA*	CGAACTGCATGGTC(G)ATCTTC	232 pb	This study
recAR		ATGTCGAACTCGACCTGCTT '		This study
zwfrF	*zwf*	AGCAGCTGGAACATGTGGTT	231 pb	This study
zwfrR		CGACGAGAGCCAGTTCTACC		This study
rpoDrF	*rpoD*	ACGACCTCGAGAACAACGTC	229 pb	This study
rpoDrR		ACGACCTCGAGAACAACGTC		This study
proCF	proC	CCAGCAGGAAGACGTAGGC	282 pb	This study
proCR		ACACGCTCCTCGTCTCGAT		This study
MMC1	16S *rRNA*	TACGTGGAGAGATTCACGGTC	390 pb	Lacava *et al.* (2006)
MMC2		GTACAAGGCCCGGGAACGTAC		Lacava *et al.* (2006)

Table 2 - Isolates of *Methylobacterium* spp. and other genera from different plant hosts, identified by partial 16S rRNA gene sequencing, and used to evaluate primers specificity.

Isolate	Host plant	Genes						
		mxaF	*acdS*	*Patatin*	*crtI*	*phoU*	*sss*	*recA*
Methylobacterium sp. TC3-6	Coffee	+	-	+	-	+	+	+
Methylobacterium sp. TC3-7	Coffee	+	-	+	-	+	+	+
M. fujisawaense F5	Sugarcane	+	-	+	-	+	+	+
Methylobacterium sp. F7	Sugarcane	+	-	+	+	+	+	+
Methylobacterium sp. F8	Sugarcane	+	-	-	-	+	+	+
Methylobacterium sp. F9	Sugarcane	+	+	+	-	+	+	+
M. mesophilicum SR1.6/6	Citrus	+	+	+	+	+	+	+
Methylobacterium sp. SR3/27	Citrus	+	-	-	-	+	+	+
M. mesophilicum PR1/3	Citrus	+	+	-	-	+	+	-
Methylobacterium sp. PR3/11	Citrus	+	+	+	+	+	+	-
Methylotrophyc bacteria TP7	Sweet peper	+	+	+	+	+	+	+
M. hispanicum TP8	Sweet peper	+	-	+	-	+	+	+
Methylobacterium spp. R3E	*Eucalyptus* spp.	+	-	-	-	-	+	+
Methylobacterium spp. MA2.9	*Laguncularia racemosa*	+	+	+	-	+	+	+
Methylobacterium spp. MA3.1	*Aviscenia shaueriana*	+	+	-	+	+	+	+
Methylobacterium spp. MB1.1	*Rhizophora mangle*	+	-	+	-	+	-	+
Methylobacterium spp. MB1.3	*Rhizophora mangle*	+	-	-	-	-	-	-
Rhizobium SP	Laboratory collection	-	+	-	-	-	-	-
Sinorhizobium SP	Laboratory collection	-	-	-	-	-	-	-
Bacillus cereus	Laboratory collection	-	-	-	-	-	-	-
Total of 20		17	8	11	5	15	15	14

RT qPCR analysis

All the amplification reactions by qPCR used the thermocycler iQTM5 (Bio-Rad) programmed to an initial denaturation of 5 min at 94 °C, followed by 40 cycles of 15 seconds at 94 °C and 1 min at 62 °C. The specificity of qPCR primer sets were evaluated by the melting curve with gradient from 60 to 96 °C ranging 1 °C each 30 s. Each amplification reaction it was used 2 μL of cDNA (100 ng), 10 μM of each primer and the Platinum SYBR Green qPCR SuperMix-UDG kit (Invitrogen). The primer-efficiencies were calculated through LinRegPCR software program (Ramakers *et al.*, 2003).

The selection of endogenous gene, to be used as a normalizer was made by testing the Ct values for five different genes (16S rRNA, *rec*A, *zwf*, *rpo*D e *pro*C) (Stevenson and Weimer, 2005). Thereby, the *rec*A presented the lowest deltaCt and the higher amplification efficiency, resulting in its selection as the normalizer for gene expression analysis.

The Pfaffl method (Pfaffl, 2001) was used to determine a relative quantification of the target genes in comparison to the reference gene. The DNA recombination and repair protein - *rec*A gene was used as a reference gene.

Results and Discussion

The designed primers (Table 1) were specific for 17 *Methylobacterium* isolates exhibiting PCR products with expected size. However, these fragments were not observed from *Sinorhizobium* sp., *Rhizobium* sp. and *Bacillus cereus*, except for the primer that amplifies *acd*S gene, which amplified the target gene from *Rhizobium* sp. (Table 2). Therefore, these primers were used to evaluate the effects of bacteria gene expression under the following conditions: 1. AHL (S)-N-dodecanoyl-HSL; 2. Rice root exudates; 3. Eucalyptus root exudates and 4. Ethanol as carbon source.

No differences were observed between the bacterial growth with and without AHL (S)-N-dodecanoyl-HSL as well in the assay with ethanol as carbon source. However, there were less growth in the presence of the plant, due to bacteria colonization. During experimental period all plants exhibited normal growth and healthy aspects, with no lesions or chlorotic spots. In general, bacterial cell growth actively in culture medium with plant roots and after 48 hours some cells were observed attached on root surface. Taking in mind that one of our objective was to evaluate the differential gene expression in response to AHL, root exudates and carbon source, and that the planktonic cells should vary the gene expression response when attached to the root when plant-associated, in the present study only planktonic cells of *M. mesophilicum* SR1.6/6 under the mentioned conditions were accessed.

Looking specifically to each analyzed gene, the gene *mxa*F was over-expressed (more than 10 times) in the presence of AHL, and it was repressed only during the bacterial incubation in medium with ethanol as the carbon source (Figure 1A), the bacteria of eucalyptus treatments presented an increase in gene expression, but no significant differences occurred between the gene expression of *M. mesophilicum* in control and rice host.

The *mxa*F gene codifies an enzyme responsible for the transformation of methanol on formaldehyde (McDonald *et al.*, 2005; Zhang and Lindstrom, 2003), and it was over-expressed in the presence of AHL showing that this gene responds to quorum sensing therefore, in the presence of ethanol the expression of this gene was repressed.

On leaves, the production and liberation of methanol is readily metabolized by *M. mesophilicum*, fact that could confer competitive advantage to the bacterium over other bacteria that do not present such metabolic alternative (Sudtachat *et al.*, 2009; Sy *et al.*, 2005; Jourand *et al.*, 2005), different from rhizosphere, where (considering plant treatments) no significant differences occurred between the gene expression of *M. mesophilicum* in control and plant hosts, considering the conditions found in this study, where the bacterium was not under competition with other microorganisms, it is possible to generate the hypothesis that the expression of *mxa*F gene is stimulated mainly by quorum sensing molecules (produced by itself or other bacteria), and in a competing condition, produces *mxa*F gene as an advantage.

The expression of genes related to plant metabolism and bacterial stress (*acd*S and *crt*I genes, respectively) presented the same pattern. It was not affected by ethanol amendment, however it was super expressed in the presence of AHL and repressed in bacterial-rice and Eucalyptus interaction (Figure 1C and E). Considering the genes roles, it can be suggested that on the evaluated conditions, the bacteria did not feel under stressing conditions while in contact with plant roots and its exudates. It is believed that the product of *acd*S gene is responsible for increasing the plant capacity to support physic and biological stresses. Tittabutr *et al.* (2008) showed that the introduction of multiples copies of *acd*S gene increased the enzyme ACC deaminase activity in *Rhizobium* sp and enhances its symbiotic efficiency on plant host (*L. leucocephala*). Besides that, it was demonstrated that in some rhizobia strains the *acd*S gene is regulated by the promoter responsible for the transcription activation of *nif* genes, which encode for nitrogen fixation (Glick *et al.*, 2007). Therefore, with ACC deaminase enzyme activity there is an increase in plant growth (Cheng *et al.*, 2009).

In this context, Madahaiyan *et al.* (2006) described that *Methyhlobacterium* is capable of inducing systemic resistance in rice and sugarcane. However, considering that in the presence of AHL increases *acd*S expression and in the presence of both plants reduces *acd*S gene expression, suggesting that this gene is regulated by quorum sense (AHL), and probably the treatment of planktonic bacteria with the plant presented lower bacteria cell density, conse-

quently less AHL, than planktonic bacterial cell without plant, despite of the same initial inoculums, in the presence of the plant, some bacterial colonizes plants (endophytically and epiphytically) (Andreote *et al.*, 2006; Rossetto *et al.*, 2011).

In this way, the results presented suggests that the expression of phytoene dehydrogenase gene (*crt*I), responsible for the lycopene synthesis that protects the cell against oxidative stress (Xu *et al.*, 2007), in the evaluated conditions were similar to *acd*S gene. It is associated to the fact that during the bacterium-plant interaction, the microbial defense system is probably not activated due the fact that the plant does not recognize the endophytic bacterium as a pathogen (Figure 1E).

In studied conditions, the expression of genes related to pathogenicity (*patatin* and *pho*U genes) are not differently expressed in the presence of plant exudates or ethanol, and it seems to be not regulated by plant interaction. But in the presence of AHL *patatin* gene is induced, but does not influence the *pho*U gene expression (Figure 1B and D).

The gene *pho*U is a transport gene, responsible for stress control (Gristwood *et al.*, 2009; Li and Zhang *et al.*, 2007) and bacteria pathogeneses process (Cheng *et al.*, 2009). While *patatin* gene is a phospholypases enzymes that hydrolyze phospholipids, activated during the patho-

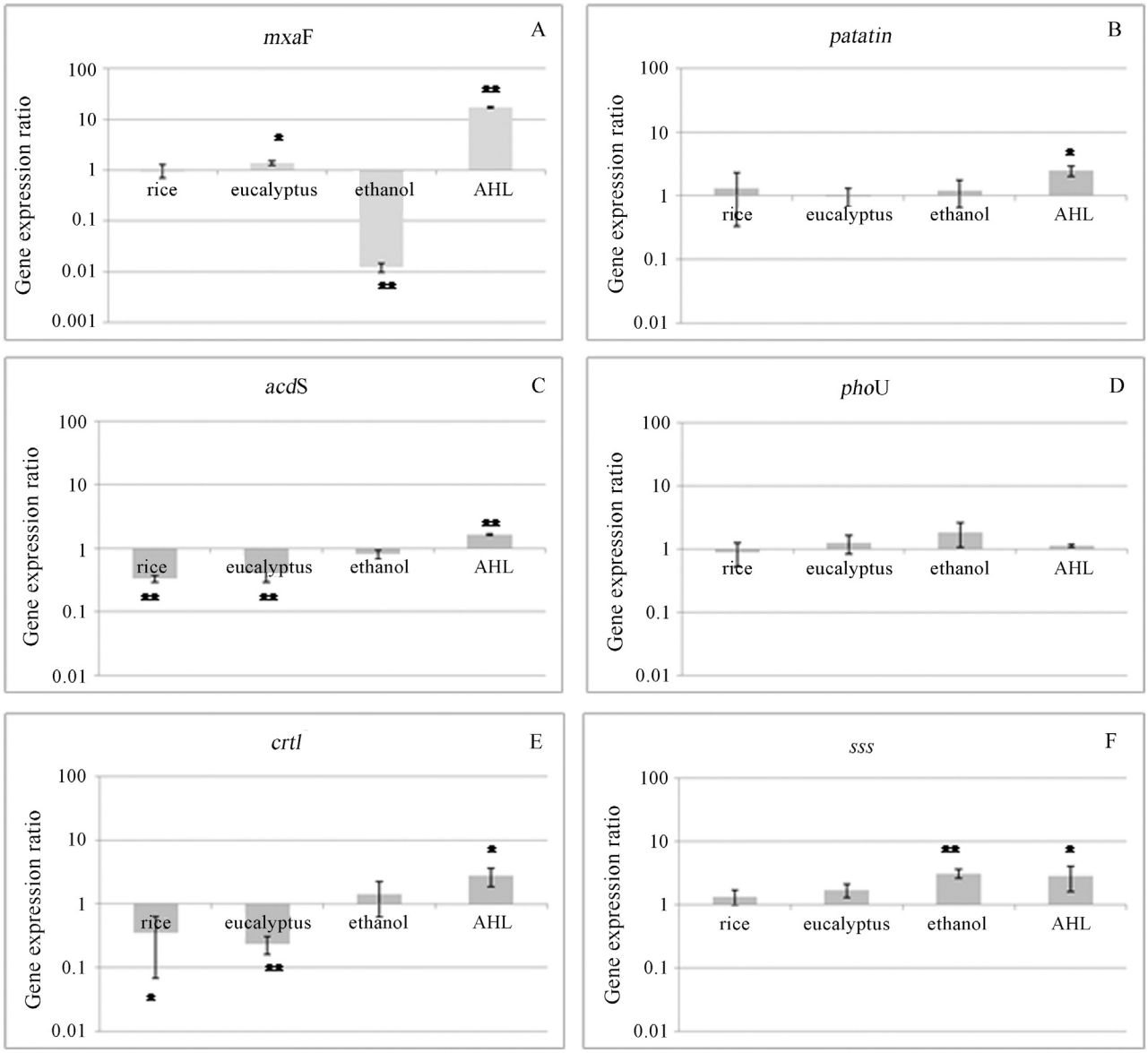

Figure 1 - Relative effects of ethanol, plant exudates and AHL on SR1.6/6 transcription of plant-interaction genes: *mxa*F (A), *patatin*(B), *acd*S (C), *pho*U (D), *crt*I (E) and *sss* (F). The gene expression ration were determined by the method of Pfaffl. The *rec*A as a reference gene. The results are means +/-standard errors of three replicates. Values of asterisks (* or **) differ statistically of control treatment (a = 0.05 and 0.01 respectively) according to t test of Student.

geneses process to hydrolyze the membrane phospholipids (Camera *et al.*, 2009). The expression of *patatin* and *pho*U genes, both related to bacterial pathogenicity, do not varies in the presence of plant exudates (Figure 1B and D), corroborating with previous studies that state *M. mesophilicum* as an endophyte able to colonize plants without cause damages to the host (Barnerji *et al.*, 2008). Also, the absence on the expression variation of these two genes in the different treatments shows that although present, the regulation of such genes in this endophytic bacterium does not function as their homologous found in pathogenic bacteria. However, these pathogenicity genes respond differently to AHL. It induces only *patatin* expression and does not influence *pho*U gene.

The *sss* (sodium solute symporter) gene responsible for the symport transport of solute with the sodium, in studied conditions, seems to be not regulated by plant interaction (Figure 1F). However, the *sss* gene expression was induced in medium containing ethanol and in the presence of AHL (Figure 1F).

This study allowed a better understanding of the endophytic gene expression during plant recognition and planktonic bacterial cell interaction with rice and eucalyptus, the effect of ethanol (root exudates) and the effect of quorum sense molecule (AHL (S)-N-dodecanoyl-HSL). It was observed that homoserine lactone induces all analyzed genes *mxa*F, *acd*S, *crt*I, *sss* and *patatin*, expect for *pho*U gene (it does not influence its expression). It was observed that the *mxa*F gene was not induced in rice, only in eucalyptus, possibly due to the differences on plant-bacteria interaction and on using different metabolic routes, also suggesting that the plant can induce the expression of this gene and indirectly increase the bacteria fitness during the plant host interaction.

In addition, the plant-related environment was stated as a free-of-stress niche for bacterial colonization, based on the analysis of the genes *crt*I, *acd*S and *pho*U expression. Hence, such symbiotic interaction was confirmed by the absence of induction for gene related to pathogenesis characteristics (*patatin* and *pho*U). Future studies might reveal differences in specific genes, modulated by the plant genotype, what will add information about the differential behavior of bacteria, according to the host plant. Concluding, we remark that more than plant exudates, bacterial density influences the expression of genes related to metabolism, stress and pathogenesis.

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