Original Article

Global transcriptional response to salt shock of the plant microsymbiont *Mesorhizobium loti* MAFF303099

Marta Laranjo [a], Ana Alexandre [a], Solange Oliveira [a,b,∗]

[a] *Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Instituto de Investigação e Formação Avançada (IIFA), Universidade de Évora, Ap. 94, 7002-554 Évora, Portugal*
[b] *Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Polo da Mitra, Ap. 94, 7002-554 Évora, Portugal*

Abstract

Soil salinity affects rhizobia both as free-living bacteria and in symbiosis with the host. The aim of this study was to examine the transcriptional response of the *Lotus* microsymbiont *Mesorhizobium loti* MAFF303099 to salt shock. Changes in the transcriptome of bacterial cells subjected to a salt shock of 10% NaCl for 30 min were analyzed. From a total of 7231 protein-coding genes, 385 were found to be differentially expressed upon salt shock, among which 272 were overexpressed. Although a large number of overexpressed genes encode hypothetical proteins, the two most frequently represented COG categories are "defense mechanisms" and "nucleotide transport and metabolism". A significant number of transcriptional regulators and ABC transporters genes were upregulated. Chemotaxis and motility genes were not differentially expressed. Moreover, most genes previously reported to be involved in salt tolerance were not differentially expressed. The transcriptional response to salt shock of a rhizobium with low ability to grow under salinity conditions, but enduring a salinity shock, may enlighten us concerning salinity stress response mechanisms.
© 2016 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Rhizobia; Microarray; Transcriptome; Osmotic stress; Salt

1. Introduction

A worldwide increasing area of about 800 Mha of arable lands is affected by soil salinity [27], which is a crucial factor in legume crop yield. Salt stress affects both symbiotic bacteria and plants in two ways: it induces ionic stress due to the high concentration of ions and it also induces osmotic stress through the change in the solute concentration around the cells, leading to water shortage and desiccation. Generally, legumes are more sensitive to salinity than their rhizobial counterparts, and consequently symbiosis is more sensitive to salt stress than free-living rhizobia [26]. Salt stress may inhibit the initial steps in symbiosis (root infection, nodule initiation and development), but it also has a depressive effect on nitrogen fixation [42,18].

Rhizobia subjected to salt stress may undergo changes in cell size and morphology or in the profile of extracellular polysaccharides (EPSs) [37,23] and lipopolysaccharides (LPSs) [35,38]. The latter responses may have an impact on the symbiotic interaction, because EPS and LPS are necessary to establish symbiosis and for the development of root nodules.

Many bacteria, including rhizobia, use distinct mechanisms for osmotic adaptation upon salt stress [42]. In general, the metabolism of *Rhizobium* is slowed down under osmotic stress due to repression of genes implicated in the tricarboxylic acid cycle, in the uptake of carbon supply, in glycogen metabolism, in the respiratory chains, and in ribosome composition [11]. Furthermore, changes in the expression of genes encoding chaperones and elongation factors as well as genes involved in

* Corresponding author. Universidade de Évora, Departamento de Biologia, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.
E-mail address: ismo@uevora.pt (S. Oliveira).

cell division were observed in rhizobia during salt stress [41,25].

Accumulation of intracellular low-molecular-weight osmolytes, including sugars, amino acids and polyamines, or accumulation of ions, to equilibrate internal and external osmotic concentrations have been observed in rhizobia subjected to salt stress [14,30,32,36]. Some compatible solutes can be used as either nitrogen or carbon sources for growth, suggesting that their catabolism may be regulated to prevent degradation during salt stress. For example, *Ensifer meliloti* 1021 overcomes salt-stress-induced growth inhibition by accumulating compatible solutes, glutamate and proline [11,32].

The transport systems of ions may also be involved in the rhizobia response to salt stress due to the intracellular accumulation of potassium and some polyamines [40,42]. Nogales et al. [28] identified a *kup* gene encoding an inner membrane protein involved in potassium uptake, which confers salt tolerance in *Rhizobium tropici* CIAT 899. Another identified transporter involved in the early response to osmotic stress was BetS (betaine/proline transporter) in *E. meliloti* [3]. Several genes such as *ntrY* (nitrogen regulator), *noeJ* (mannose-1-phosphate guanylytransferase), *alaS* (alanyl-tRNA synthase), *dnaJ* (heat-shock chaperone), *greA* (transcription elongation factor), *omp*10 (outer membrane lipoprotein), *relA* ((p)ppGpp synthethase) and *nuoL* (NADH dehydronase I chain L protein) were identified by mutant analysis as being involved in the salt stress response in *E. meliloti* and *R. tropici* [28,41]. Despite the fact that several genes have been identified in rhizobial response to salinity, the mechanisms for overcoming salt stress remain unknown in rhizobia, mainly due to the fact that response and adaptation to salinity stress are complex phenomena involving many physiological and biochemical processes that involve differential expression of a high number of genes.

Under salt stress, an osmoadaptive response is induced in *E. meliloti*, which is mainly characterized by repression of flagella and pilin biosynthesis genes (*fla*A, *fla*B, *fla*C, *fla*D, *pil*A), as well as chemotaxis genes (*che*Y1, *che*W3, *mcp*X, *mcp*Z), and by induction of genes involved in surface polysaccharide biosynthesis (*exo*N, *exo*Y, *exs*I) [32].

Transcriptome profiling using DNA microarrays has been used for the study of the response to osmoadaptation in *E. meliloti* [11]. Both NaCl and sucrose stresses resulted in induction of a large number of genes with unknown function and in repression of genes coding for proteins involved mainly in iron uptake or uptake and metabolism of mannitol. The *glgA2*, *glgB2*, and *glgX* genes involved in glycogen metabolism are expressed at higher levels during exposure to salt stress, indicating that glycogen accumulates during salt stress [11].

Other authors have analyzed the transcriptome of *Mesorhizobium alhagi* CCNWXJ12-2, a relatively high salt-resistant strain [21], using RNA-Seq in order to elucidate the salt resistance mechanism [22]. Approximately the same number of genes were found to be up- and downregulated. Upon salt stress, several genes already known to be involved in salt tolerance were found to be upregulated. In addition, the transcription levels of some heat shock proteins as well as of most protein secretion systems were found to be reduced.

Mesorhizobia are known for their metabolic diversity and versatility [20] and their tolerance to different environmental conditions has been investigated [2,4−6]. Regarding salt stress, some *Mesorhizobium* species can tolerate up to 2% NaCl [9]. *Mesorhizobium loti* MAFF303099 is salt-sensitive (28% growth with 1.5% NaCl), contrary to other species, such as *E. meliloti* 1021, which prefers 1.5% NaCl (109% growth) and can grow in 3% NaCl (48% growth) [21].

The aim of the present study was to analyze the transcriptional response of *M. loti* MAFF303099 following salt shock, as this strain is able to endure salinity shock despite its slow growth rate under continuous salt conditions.

2. Materials and methods

2.1. Bacterial growth conditions and RNA isolation

The bacterial strain used in this study was *M. loti* MAFF303099 [16]. For gene expression profiling, three independent cultures were grown overnight at 28 °C in YMB [39] medium to an optical density of 0.3 (540 nm). A 10 ml volume of cells was used for each treatment: cells transiently subjected to a NaCl concentration of 10% (1.71 M) for 30 min and cells exposed to no change in the NaCl concentration (control). NaCl was chosen for this study, since its effect seems to be an overall good indicator of the response of rhizobia to salinity conditions (Abdelmoumen et al., 1999). The growth of *M. loti* MAFF303099 after salt shock was evaluated. This strain rapidly recovers from salt shock with growth rates similar to those of the control conditions (data not shown). Cells were harvested and total RNA was extracted using the RNeasy Mini Kit (Qiagen) with DNase (Roche Applied Sciences) treatment following the manufacturer's instructions. Once the absence of residual DNA was confirmed, concentration and purity were determined using a Nanodrop ND-1000 UV−visible spectrophotometer (Thermo Scientific). RNA integrity was checked with an Agilent 2100 Bioanalyser using an RNA Nano assay (Agilent Technologies).

2.2. Microarray experiments and analysis

Microarray experiments were performed as a service at the BIOCANT-Genomics Unit (Portugal). Three biological replicates were used for each microarray experiment. mRNA microarray analyses were carried out as described by the manufacturer using the 40K array for *M. loti* MAFF303099 (MYcroarray). The slide images were acquired using the DNA Microarray B scanner (Agilent Technologies) and scanned with an intensity of 100% PTM in the green channel. Data were extracted using QuantArray software (Packard BioScience). The arrays were analyzed using BRB data analysis tools for Excel [34], and normalized by the median. These data were then used to identify genes with differential expression using the MeV4.0 software package [33]. A statistical Student t-Test with a P-value threshold of 0.01 was applied. The data were