

Environmental Stress Response and Adaptation Mechanisms in Rhizobia

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Summary

Rhizobia are bacteria that can fixate atmospheric nitrogen in association within the root or the stem nodules of legume plants and transform atmospheric nitrogen to ammonia. Soil environmental conditions are critical factors for the persistence and survival of rhizobia in the soil. The changes in the rhizosphere environment can affect both growth and saprophytic competence, which will influence competitiveness and persistence. Environmental stress imposes a major threat to symbiotic nitrogen fixation and agriculture that can be limited by soil and climatic factors such as salinity, drought, temperature, acidity/alkalinity and heavy metals. In this review we present several different mechanisms in rhizobia adaptation under stress factors.

Key words

Rhizobium, environmental stress, adaptation mechanism, nitrogen fixation

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Introduction

Rhizobia is the common name given to a group of small, rod-shaped, Gram-negative bacteria that collectively have the ability to produce nodules on the roots of leguminous plants and belong to the family *Rhizobiaceae*, which are part of the α -proteobacteria. They form white pigmented, circular, convex, semi-translucent raised mucilaginous colonies. The genus *Rhizobium* was first described in 1889 (Frank, 1889). The metabolic diversity found within rhizobia might be one explanation for their large and complex genome. In fact, the size of the rhizobial plasmids, which may carry functional genes, has raised some questions on the bacterial genome organization and on the variability between plasmids and chromosomes (Downie & Young, 2001). Until the end of 2009, 16 complete rhizobial genomes have been sequenced, including two rhizobia species from the Betaproteobacteria class. The size of those genomes ranges from 5.4 to 9.1 Mb and plasmid number varies between 0 to 7. Symbiosis genes are often plasmid encoded, but this strain displays a chromosomal symbiosis island, which means that nitrogen fixation and nodulation genes are clustered in a 610 bp DNA fragment in the chromosome. The size and diversity of indigenous rhizobia populations in soil are influenced by the host presence. In order to survive, in the changing environment, every bacterium has its own optimum conditions that make this process easier. This adaptation is a stress response. There are two types of stress responses that operate in microorganisms. First being the general stress response and second that is specific stress response. The general stress response is normally controlled by a single or a few master regulators (Bremer & Krämer, 2001) and provides cross-protection against a wide variety of environmental cues, regardless of the initial stimulant (Hecker & Völker, 1998). This response is effective in a way that it allows the cell to survive, but it may not be enough to let the cell grow under the stressful conditions (Bremer & Krämer, 2001). There is also a complex relationship between cellular response systems and global regulators, adding another level of control to the cell's emergency stress response and long term survival reactions (Hengge-Aronis, 1999). Environmental constraints, such as soil and water salinity, water deficit, phosphorus deficiency and soil pH, recorded in many regions of world, particularly in arid and semi-arid areas, are still the main limiting factors for the productivity of leguminous plants and their symbiotic nitrogen fixation (Bargaz et al., 2013; Farissi et al., 2013). Soil environmental conditions are critical factors for the persistence and survival of rhizobia in the soil. The changes in the rhizosphere environment can affect both growth and saprophytic competence, which will influence competitiveness and persistence (Abd-Alla, 2013). In the last few years, the most important strategies employed to reduce the adverse effects of unfavorable environmental conditions on legume production have been focused on the selection of host genotypes adapted to drastic conditions (Farissi et al., 2011; Bargaz et al., 2013).

Examples of stress response in *Rhizobium*

Osmotic stress

Water is one of the most vital environmental factors to affect the growth and survival of microorganisms (Potts, 1994). Water deficit is an abiotic factor that affects the agricultural production

with high frequency and intensity, influencing aspects related to plant development, such as decrease in photosynthesis rate and reduction in leaf area (Kramer, 1994). When water deficiency occurs, crops normally present various alterations, such as lower growth and development with progressive reduction in leaf dry matter and consequent repercussion on production parameters, e.g. a number of grains and pods per plant (Costa 2011). Bacteria may detect a change in osmotic pressure by many different ways, including: a change in cell turgor, deformation of cell membrane and changes in the hydration state of membrane proteins, but the key signal is believed to be a change in intercellular ionic solutes (Poolman et al., 2002). Two distinct mechanisms are responsible for initializing the movement of water across a cell membrane under osmotic stress. Simple diffusion is usually adequate in balancing solute levels under low osmotic conditions. However, a much faster transfer of water is achieved through water specific channels (Bremer & Krämer, 2001). A more flexible and versatile osmotic stress response is used by bacteria that generally inhabit environments of varying salinity or water activity (Bremer & Krämer, 2001).

The temperature stress

In arid and semiarid regions of the tropics the soil temperatures near the surface can be very high. In Egyptian sandy soils, the temperature near the soil surface may reach 59°C when the air temperature is 39°C. The soil temperature decreases rapidly with depth, reaching moderate 35°C, at 15 cm. It appears that rhizobia are more resistant to high temperatures in soil than in laboratory conditions. The temperature plays a critical role in the exchange of molecular signals between rhizobia and their host, thus reducing nodulation. Low temperature inhibits inter-organismal signaling between the two symbiotic partners. It has been shown that low temperature inhibits the biosynthesis and rhizosecretion of plant to bacterial signal molecules (Abd-Alla, 2001).

The optimum temperature for rhizobia growth is 25-30°C (Zhang et al., 1995). Most studies on rhizobia temperature stress tolerance focuses on soybean and common-bean microsymbionts. Soybean isolates grow weakly at 40°C and no isolate was able to grow at 45°C (Chen et al., 2002).

Temperature stress is generally divided into two classes: heat and cold shock. The heat shock response is very similar to the acid stress response when referred to synthesized proteins. The heat shock proteins (HSPs) contribute to heat tolerance by conferring heat protection on the bacteria but do not alter the internal temperature of the cell (Yura et al., 2000). Cold shock is essentially the opposite of heat shock. Instead of proteins misfolding and denaturing, cells undergoing cold shock have to contend with a loss of membrane and cytosol fluidity and with the stabilization of secondary structures of RNA/DNA (Phadtare et al., 2000).

The effects of temperature stress in nodulation and nitrogen fixation have been recognized for a long time, as the first studies addressing this subject can be traced back to the 1960's. Even before nodule formation, root zone temperature influences the rhizobia survival in soil, as well as the exchange of molecular signals between the two symbiotic partners (Sadowsky, 2005). High temperature was seen to have an inhibitory effect on adherence of bacteria to root hairs, root hair formation and infection thread formation (Hungria & Vargas, 2000). Nodule functioning

can also be influenced by high temperatures. Namely, the decrease in the rate of some essential reactions, as the synthesis of leghemoglobin or the nitrogenase activity. On the other hand, low temperatures also affect nodulation, as cold temperatures delay nodulation initiation or even completely inhibit the process and seem to influence nodule occupancy (Graham, 1992). A more recent study, with *B. japonicum*, emphasizes the fact that Nod factor production markedly decreases at 17°C or 15°C, despite the fact that its biological activity remains unaltered (Duzan et al., 2006). Bean and soybean have similar threshold, while lentil is more tolerant and nodulation is significantly delayed only at lower temperatures (10°C) (Lira et al., 2005).

pH stress

The optimum pH for rhizobia growth is considered to be between 6.0 and 7.0 (Hungria, 2000). The rhizobia strains vary widely in their acidity tolerance. Some mutants of *R. leguminosarum* have been reported to be able to grow at a pH as low as 4.5 (Chen et al., 1993), *S. meliloti* is viable only when the pH is down to 5.5 (Foster, 2000), *S. fredii* can grow well between pH 4 – 9.5, but *B. japonicum* cannot grow at the extremes of that range (Fujihara & Yoneyama, 1993). One of the most important factors that affect the efficiency of symbiosis between rhizobia and plants is the pH of the soil in which they interact (Glenn & Dilworth, 1994). The host plant seems to be a limiting factor for growth in extreme pH to any symbiotic *Rhizobium*, as most legumes require a neutral or slightly acidic soil for growth especially when they depend on symbiotic nitrogen fixation (Zahran, 1999). Alkaline or acidic agricultural soil has a great influence on the survival or multiplication of rhizobia and can affect both symbiosis partners. Many agricultural fields are alkaline with an average pH from 7.0 to 8.5. A reduction is a major problem in alkaline soils in nutrient availability. Alkalinity stress can also retard *Rhizobium* from growing and subsequent establishment of a viable nitrogen fixing symbiosis with a legume host. Therefore, it makes good agricultural sense, to select rhizobia isolates that are tolerant of alkaline conditions as well as their capability of nodulating on legumes (Farissi et al., 2014).

The more common, and characterized, pH stress found in soil is acidic one and although opposed to basic, the defense mechanisms of rhizobia are similar (Fujihara & Yoneyama, 1993). Some inducible systems raise the internal pH of the bacterium, in order to counter any intruding acidic molecules or protonated species. These systems employ ABC systems and other transport mechanisms to either move acidic molecules out of the cell, or import the more basic ones (Priefer et al., 2001). Another common response to acid shock from the bacteria is to produce acid shock proteins (ASPs). These contribute to acid tolerance by conferring acid protection on the bacteria but do not alter the internal pH of the cell (Foster, 1993). At least twenty genes have been identified in *R. leguminosarum* that are specific to acid stress response in rhizobia and are termed as the act genes (Kurchak et al., 2001). In order to bring an acid shock response the bacteria and/or root nodule must have some form of sensing mechanism (Glenn & Dilworth, 1994). Such systems for environmental sensing and response are generally made up of two components: the sensor and a regulator. One component like that has been found in *S. meliloti*, where the genes *actR* and *actS* encode for the regulator and sensor respectively (Tiwari et al., 1996).

High pH can also prevent *Rhizobium* from growing and undergoing nodulation, although *R. leguminosarum* bv. *trifolii* has been reported to colonize soil at a higher rate and produce nodules at a higher frequency in alkaline conditions (Lebrazi, 2014). The negative effect of alkaline soil's conditions is the unavailability of essential minerals such as iron for both rhizobia and host plant (Farissi et al., 2014).

Salt stress

Salinity is one of the major factors threatening agriculture in arid and semi-arid areas. Nearly 40% of the world's land surface can be categorized in having a potential salinity problem (Niste et al., 2013). The main cause of salinity is the nutrient imbalance in the soil that is considered as a constraint influencing the N₂ fixing symbiosis and the survival of both symbionts (Mohammadi et al., 2012). Biological N fixation by legumes may constitute a sustainable alternative to chemical fertilization in salinity-affected areas, when adapted cultivars and inoculants are available (El-Akhal MR et al., 2013). The legume-*Rhizobium* symbioses and nodule formation on legumes are more sensitive to salt or osmotic stress than the rhizobia (Zahran, 1991).

Salt stress inhibits the initial steps of *Rhizobium*-legume symbioses. The effects of salt stress on nodulation and nitrogen fixation of legumes have been examined previously by Delgado (1994). The reduction of N₂-fixing activity by salt stress is usually attributed to a reduction in respiration of the nodules (Walsh, 1995) and a reduction in cytosolic protein production, specifically leghemoglobin, by nodules (Delgado, 1994). The salt injury on the symbiotic interaction not only inhibits the formation of the nodules, but also thereby leads to the inhibition in nitrogenase activity and reduction of the growth of the host plant. Other effects of salinity on the nodulation include formation of non-functional nodules with abnormal structure, and degradation of peribacteroid membrane (Bolanos et al., 2003). Some strains of *Rhizobium* spp. are salt tolerant and under stress conditions root-associated beneficial bacteria can help to improve plant growth and nutrition of their host plants (Upadhyay et al., 2011). Reduced nodule conductance to O₂ diffusion is considered a major factor responsible for the inhibition of N₂ fixation by soil salinity. Salt tolerance was associated with higher stability of O₂ nodule conductance of the tolerant *Rhizobia* at increasing soil salinity (L'taief et al., 2007). Rhizobia strains differ in their ability to tolerate osmotic stress and can use different adaptation mechanisms such as intracellular accumulation of low molecular weight organic solutes (Zahran, 1999). Some authors have reported that tolerance to salinity may be due to a plasmid mediated resistance since salt resistance can be rapidly transferred from tolerant to sensitive bacteria (Pereira et al., 2008).

Metal stress

Heavy metals discharged from industrial operations and upon consequent accumulation in various ecological systems cause a massive threat to the varied agroecosystems (Cheung and Gu, 2007). When heavy metals accumulate into soil to an abnormal level, it causes dramatic changes in microbial composition and their activities (Khan et al., 2009; Krujatz et al.,

2011), leading consequently to losses in soil fertility. Many of the transitional elements function as essential cofactors in metabolic metal ions and can lead to harmful effects in bacteria,

including enzyme inhibition, biopolymer hydrolysis and uncontrolled redox reactions within the cell (Outten et al., 2000). Characterizing the minimum and maximum concentrations of each metal is imperative in determining the difference between a standard and a stress response. Stress response genes are induced as metal ion concentrations increase from starvation to toxic levels. It has been shown that there are genes that are expressed under general metal stress and genes expressed to a specific metal, such as nickel (Singh et al., 2001).

Responses to some of these metals have been well characterized. High intercellular carbohydrates and large cell inclusions increase the resistance of *R. leguminosarum* to cadmium, copper, nickel and zinc, whereas production of thiols has also been shown to counter heavy metal induced oxidation (Balestrasse et al., 2001). In *Rhizobium*-legume symbiosis, it is usually the plant that is the limiting factor regarding tolerance to metal toxicity. This has been reported with aluminum, copper, iron and cadmium (Balestrasse et al., 2001). Nodules can help plants survive because the bacteroids counter metal stress, further supporting the fact that symbiosis is mutually beneficial to legume and rhizobia (Balestrasse et al., 2001).

Conclusion

The impact of harsh environmental conditions plays an essential role in the control of legume-rhizobia interactions. They can arrest the growth, multiplication and survival of rhizobia in soil rhizosphere. The harsh environmental conditions may also have depressive effect on the steps involved in legume-*Rhizobium* symbiosis such as molecular signaling, infection process, nodule development and function, resulting in low nitrogen fixation and crop yield. Selection of hosts and their nitrogen-fixing endosymbionts that are tolerant to a broad range of environmental stresses is important for agriculture system. Environmental stress severely affects legumes on various metabolic activities including nod gene expression, photosynthesis, and synthesis of proteins, enzymes and carbohydrates.

Rhizobium-legume response to different environmental stress is complex phenomena that require the intervention of many genetic and biochemical adaptation mechanisms that should be included in future studies. Further investigation of the environmental stress is urgently required in order to understand the rhizobia-legume interactions for future growing of legumes under harsh environmental conditions.

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