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# Rhizobacterial Plant Drought Stress Tolerance Enhancement: Towards Sustainable Water Resource Management and Food Security

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**Abstract** Global climate change is one of the most serious challenges facing us today. As agricultural activities expand to less fertile areas to satisfy growing demands for food, the scenarios of global environmental change suggest future increases in aridity in many areas on the earth making drought stress an important issue worldwide. Accordingly, novel solutions for plant survival and growth under restricted water availability are of central significance in contemporary plant science. Rhizobacterial ability to increase plant growth and provide protection to various pathogens has been frequently reported and applied in agricultural systems. Relatively few reports have been published on the bacterial ability to induce drought stress tolerance. Application of the isolates together with novel technologies for their monitoring and risk evaluations can contribute to solving food security issues in the changing climates. Commercial applications of the rhizobacterial isolates need complex approaches, both in the technology in the field, and in the commercial financing and ownership of the patent rights.

***Keywords:*** *abiotic stress, climate change, plant growth promoting rhizobacteria, ACC deaminase, bacterial biofilms*

## 1. Global Water Shortage is the Key Challenge for Food Security

The world population is predicted to increase beyond 8 billion by 2030, implying major challenges for the agricultural sector to secure food availability [1]. A key challenge for plant growth is global water shortage which limits crop yields already today. As the drought limitations gain in importance in the near future the agricultural activities must expand to less fertile areas to satisfy growing demands for food [2]. Agricultural practices determine the level of food production and, to a great extent, the state of the global environment. Agriculturalists are the chief managers of terrestrial ‘useable’ lands, which we broadly define as all land that is not desert, tundra, rock or boreal. Sustainability implies both high yields that can be maintained, even in the face of climate change, and agricultural practices that have acceptable environmental impacts.

It is predicted that during the next years, many countries will experience water problems—shortages, poor water quality, or floods—that will increase regional tensions. By 2030, available fresh water will not keep up with the demand, and without more efficient management of our water resources, the problems we now encounter will hinder food production in key countries. This is a global problem for food markets and the associated economic growth. Due to developing economic pressures, North Africa, the Middle East and South Asia will face dramatic challenges in dealing with water problems. Because of depleted and contaminated surface water supplies, many of these countries have resulted in over pumping the groundwater to satisfy growing food demands, which has resulted in a serious threat to food security, thereby risking social turmoil i.e. loss in agricultural jobs, significantly stressing the economy. A strong correlation exists between water supplies for agriculture and GDP. Approximately 70% of the global fresh water supply is used in agriculture, creating a great opportunity and potential for technology to provide solutions in order to effectively use the available water [3].

## 2. Rhizobacteria Can Alleviate Plant Drought Stress

Several breeding and genetic engineering strategies have been proposed to increase the ability of plants to tolerate stress in order to find practical solutions. Past efforts to improve plant tolerance have has been slower than expected owing to the genetic complexity of stress responses. Given the large number of different crops with huge variety of cultivars, and the plethora of genes, expression of which need to be altered or novel genes engineered into plants, it is currently unclear whether the engineering technology will develop fast enough to cope with rapidly increasing food demands. At the same time the resources already present in natural, complex self-regulating systems i.e. the underground resources of the plant rhizosphere offer new opportunities for agricultural biotechnology [4,5].

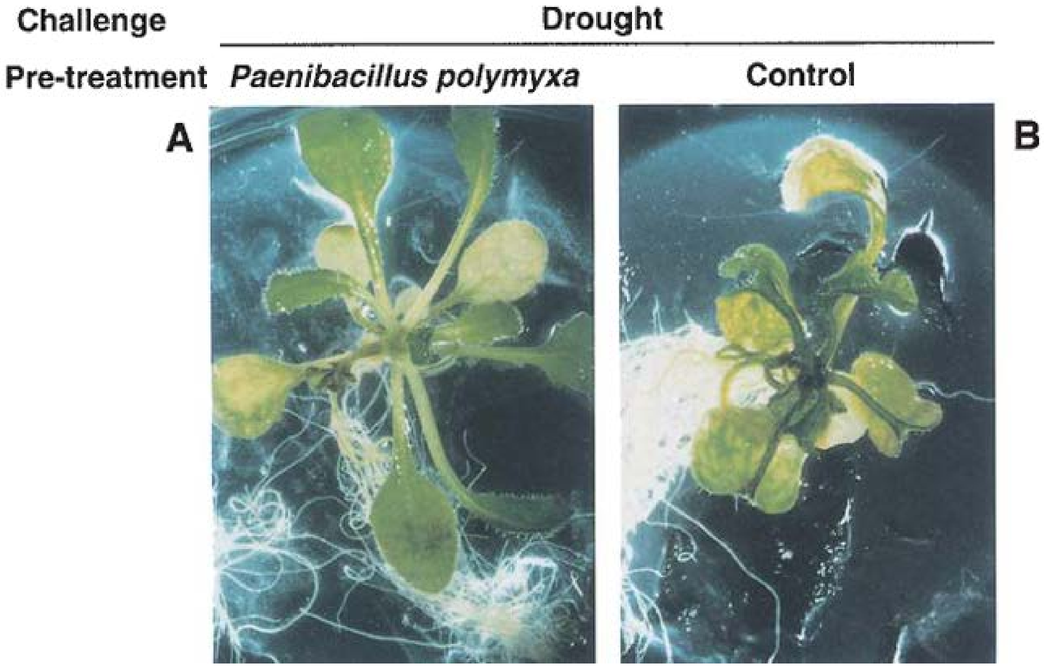


**Figure 1.** Enhancement of plant drought tolerance by *Paenibacillus polymyxa*

*P. polymyxa B2* inoculated plants (A) survived drought stress two weeks longer than untreated control plants (B). Image after three day drought exposure is shown. Plants were grown and inoculated and subsequently exposed to drought stress as described by Timmusk and Wagner (1999).

The very first report on plant drought tolerance enhancement by plant growth promoting rhizobacteria (PGPR) was published in Uppsala, Sweden [6] Figure 1. It was shown that *Arabidopsis thaliana* inoculated with PGPR *Paenibacillus polymyxa* B2 could survive drought stress remarkably longer compared to the untreated control plant. The report was followed by Prof. Glick group in Canada [7]. How do plants cope with drought stress? Survival mechanisms at dry sites involve drought escape, dehydration avoidance and dehydration tolerance. Drought escape is an important strategy for Mediterranean and monsoon climates there drought periods mostly occur at a predictable times. It could be temporal when whole life cycle or physiologically active phase is shifted to periods without stress e.g. winter wheat, winter barley – well suited for their place of origin such as Iraq, Iran; Israel. It also could be spatial involving development of water-storing belowground organs e.g. geophytes. Drought i.e. dehydration avoidance occurs when tissues which are sensitive to dehydration maintain high water potentials as long as possible. Generally there are two groups of drought avoiders: i) Water savers which conserve water and ii) Water spenders which absorb water fast enough to meet transpirational losses. Anatomical and morphological traits help the plant to increase water uptake and reduce water spending. Water uptake could be improved through extensive root system with large active surface area and then shoot/root ratio shifted in favor of the roots. Water loss is reduced through reduced transpiration i.e. timely stomatal closure. It could also happen via smaller but more densely distributed stomata, thick cuticle, epicuticular waxes, leaf colour (yellow, glaucous), white hairs on leaves, leaf angle, leaf rolling, plant senescence, leaf senescence or leaf shedding. Drought tolerance occurs through species-specific capacity of protoplasma to tolerate severe water loss. Physiological processes proceed even at high dehydration levels. Tolerance mechanisms such as osmoprotection, detoxification, ion transport or chaperone functions take over when tissues are no longer protected by avoidance mechanisms (*8*). The type of drought tolerance is known in xerophytes. Any of the above mentioned mechanisms for plant drought tolerance but also some avoidance mechanisms could involve rhizobacteria.

## 3. Mechanisms Behind the Bacterial Action should be Studied to Ensure Their Reproducible Application

Research is being conducted to understand the mode of action of the bacteria to find possibilities for economically efficient applications for plant drought tolerance enhancement. It has been shown that certain PGPR enhance plant stress tolerance through 1-aminocyclopropane-1-carboxylate deaminase (ACCd) and provide significant protection to a wide range of plant species from the damage caused by various abiotic stress conditions [9]. ACC breakdown and ethylene synthesis inhibition by ACCd decreases the damage of various stress situations by enhancing homeostasis in and around the plant root, especially at early stages of stress exposure. Hence ACCd can be considered one of the signalling compounds mediating plant basic stress tolerance. Our results indicate that the ACCd containing bacteria which show the best drought tolerance enhancing ability excrete the 'matrix' to provide a buffer against the and hold themselves in place [4,10] (Figure 2, Figure 3). The dense biofilm matrix limits diffusion of bioactive substances and nutritional elements secreted by root bacteria and these are therefore concentrated at the root surface where they may affect plant growth [4,11]. What is inside a matrix? An extracellular matrix can provide an almost infinite range of macromolecules. It was suggested that in the model bacterium *Bacillus subtlis* polysaccharides and a protein Tas A are the major components of its biofilm. Mutations that eliminate Tas A and extracellular polysaccharides (EPS) production have a severe effect on biofilm production [12,13]. The sugars in biofilms can be divided into simple sugars (monosaccarides, oligosaccharides, polysaccharides), and complex sugars: all of which can play various roles in host microbe interactions [14,15]. Water retention varies with the type of polysaccharides but EPS water retention capacity may exceed 70g water per g polysaccharide [15,16,17,18]. Our experiments in Israel 'Evolution Canyon' show that bacteria can engineer their own microenvironment in a form of porous extracellular matrix mixed soil particles [4,19]. The environment immediately interacts with plant root providing buffered and predictable hydration and transport properties [19] Figure 3. The extracellular matrix producing *Paenibacillus* sp. strains significantly increased soil aggregation in comparison to the strains with limited matrix production (Timmusk manuscript). The biofilm enhanced soil aggregation, improves water stability and enhances microbial biomass which in turn stimulates root exudation under stress 8. Hence, there is a strong selective advantage for the production of a slimy layer of extracellular matrix in the rhizosphere, especially under stressful conditions. The matrix may also contribute to mechanical stability of the biofilm and interact with other macromolecules and low molecular mass solutes, providing a multitude of microenvironments within the biofilm [11].

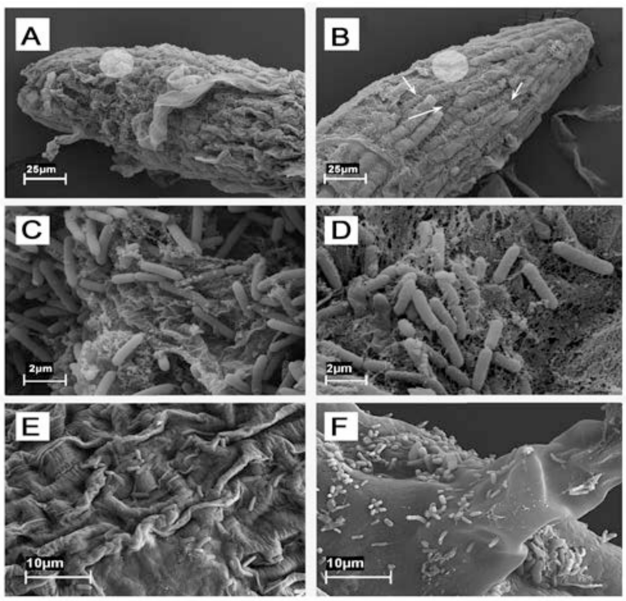


**Figure 2.** Typical pattern of *P. polymyxa* colonization and biofilm formation on plant roots Scanning electron microscopy (SEM) micrographs of colonized roots

Studies in the controlled system after two hours of colonization (A, C, E) and in non-sterile soil assays after one week of colonization (B, D, F). Plants were grown and inoculated as described by Timmusk et al. 2005. Images were taken from the root tips (A, B, C and D) and from tip-distal regions (E and F) Timmusk et al.2005.

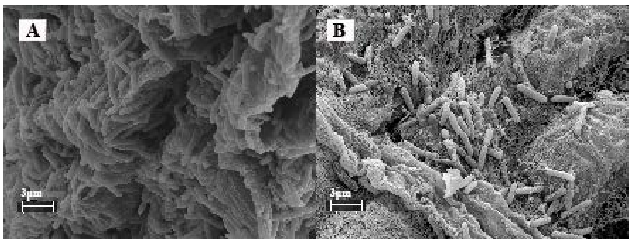


**Figure 3.** Typical pattern of wild barley *Hordeum spontaneum* roots colonized by biofilm forming bacteria

Scanning electron microscopy micrographs of bacterial biofilm formation on wild barley root tips under drought stressed (A) and non-stressed (B) conditions. Wild barley plants were sampled, prepared and analyzed as described in Timmusk et al 2011. Note that wild barley root tips under drought stress (A) are well colonized with *Bacillus* type biofilm forming bacteria compared to less stressful conditions (B).

The bioactive compounds inside the matrix could include: (1) plant hormones like abscisic acid, gibberellic acid, cytokinins, and auxin, i.e IAA; enzymes for (2) nitrogen fixation; (3) nutrient e.g. phosphorus mineralization; (4) production of siderophores; [5,20,21,22]. At the same time it is clear that any of the compounds produced can't be singularly considered responsible for the observed drought stress tolerance enhancement. Rather it is likely that different mechanisms are used at the different growth stadiums of plant drought tolerance enhancement. As our understanding of biology at the level of DNA, RNA, and proteins has increased, it has become clear that biological processes occur not in isolation but rather within the context of complex systems of components, regulated by intricate networks of feedback loops. These systems operate on a variety of levels: from that of RNA polymerase interacting with a DNA strand to start the process of DNA transcription, to a signal-transduction pathway within a cell, to complex interactions between systems of organisms. While our appreciation of the complexity of interactions within and between these systems has grown, there has been a corresponding recognition that the traditional, reductionistic, scientific approach severely limits our ability to understand biological phenomena and interactions within and between cells. Investigators have increasingly embraced systems approaches in their efforts to understand biological interactions, taking advantage of the power of mathematical and computer modelling to examine the interactions between components of a biological system. Hence to understand the behaviour of complex biological organization and processes of PGPR and plant interaction high-throughput, genome-wide research involving molecular networks coupled to high resolution microscopy should be performed.

## 4. Commercial Applications Need Complex Approaches, Both in the Technology in the Field, and in the Commercial Financing and Ownership of the Patent Rights

It is obvious that the underground resources of the plant rhizosphere could provide advantages associated with global water shortage and climate change. Yet a lot has to be done before the bacterial inoculants could be massively used in agricultural practice. The adoption of the bacterial inoculation technology comes with a variety of constrictions for formulation and delivery of the inoculates which should be solved individually in each case. As the inoculates are composed of living organisms there is often specific host range where the effect is more pronounced as well as certain environmental factors for development such as adequate temperature, moisture, UV radiation, which again could be specific for every inoculate. Gram positive bacteria in general are preferred as microbial inoculates because they are able to resist pesticides and form endospores that can survive under various stress conditions in the field, providing more reproducible results under natural conditions. With the help of proper monitoring systems, the limitations of applying microbial inoculation on fields can be remarkably reduced. Hence a large number of field trials have to be performed, coupled with advanced molecular and biochemical monitoring systems, for the development of management strategies to foster indigenous knowledge system.

In order to interest commercial investors, the novel bacterial application technologies should be patented. This means that researcher should not publish the discoveries before the patent application is accepted. Yet the research communities are dependent on publishing as usually their finances are based on the frequency and quality of publications. The European Commission's increasing concern to reduce chemicals in the food and food chain, and facilitate ecological production (Directive 2009/128/EC) has widened the market for microbial inoculates in EU countries. Yet the present restrictions -- the paper work, including efficacy, safety, composition, toxicity, and degradation -- the risk assessment and such warrant that each country must isolate and develop its own microbial inoculates because current regulations do not encourage transfer of these inoculates between countries. Hence it is necessary that the researcher's investors/granting organizations both from agricultural production and environmental side, and legislative people should sit together and discuss the work organization, financing and law enforcement for the common benefit.

The underground resources of rhizobacteria have the potential for solving many future food security issues; however, this potential has been neglected to a large extent. Hopefully with the help of new and more comprehensive approaches we will be able to understand and employ the natural potential of biofilms for our agro-ecosystems soon.

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