



Rhizobacteria for reduced fertiliser inputs in wheat (RHIBAC)

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RHIBAC introduction

High inputs of agricultural fertilisers, particularly containing nitrogen and phosphorus compounds, have increased crop yields dramatically over the past fifty years. However, it has been clear for a number of years that intensive arable cultivation of this kind is no longer sustainable. Firstly, the nitrogen compounds in fertilisers contribute to a number of environmental problems. Run-off from agricultural land causes pollution of rivers and lakes, and nitrate levels must be carefully monitored to ensure the quality of drinking water. Nitrogen oxides which are emitted during the application of nitrogen fertilisers are implicated in poor air quality at ground level and also nitrous oxide is a highly potent greenhouse gas. Furthermore, the production of chemical fertiliser depends on natural gas or oil as a primary feedstock, and supplies of these commodities will become increasingly more expensive in the coming decades.

The European Union is aiming through its Framework 6 programme to support research projects which pave the way for reductions in inputs of chemical fertiliser. One approach, to be investigated in RHIBAC, is to introduce plant growth promoting rhizobacteria (PGPR) to the root systems of crops. Unlike symbiotic Rhizobia which invade the plant tissues of legumes, these rhizobacteria have a more associative interaction with the plant. The best known example is *Azospirillum brasilense*, which has been isolated from various plants, including wheat. Like Rhizobia, *Azospirillum* and other PGPR fix nitrogen from the air, but the plant growth promotion effect is thought to be due mainly to other factors, including excretion of plant hormones which increase the efficiency of crop root systems, and increased mobilisation of soil nutrients.

For over 30 years, researchers have been investigating the application of PGPR such as *Azospirillum* in inocula to promote the growth of key arable crops such as wheat and maize. Results of greenhouse and field trials have been variable, and much remains to understand concerning the plant-microbe interactions.

The RHIBAC project aims to develop PGPR inocula for plant growth promotion of wheat. It aims for a better understanding of PGPR-plant interactions through studies of the molecular biology of PGPR, and how their interaction with plants is related to the crop variety. The project builds on previous research, particularly working with new PGPR strains previously isolated by research projects in the EU (FP5 project MicroNfix), Brazil, Chile, and Turkey.



AGRON

Field trials in Israel



RHIBAC workplan

RHIBAC includes the following activities to understand the plant-PGPR interaction and develop PGPR inocula for application in wheat cultivation:

- Study of molecular pathways in PGPR involved in plant growth promotion
- Examination the interaction of PGPR and specific crop genotypes
- Observation of root colonisation by PGPR
- Development of new inocula formulations, including encapsulation and seed coating
- Small-scale field trial to assess efficacy and effect on soil nutrients of PGPR inoculation
- Assessment of compatibility of PGPR with other common biological agents used in agriculture
- If effective PGPR inocula are developed, larger scale demonstration field trials

Progress in 2007

Several of the RHIBAC partners have previously isolated PGPR that have potential for growth promotion in wheat. These include strains isolated from the natural environment in Turkey, Chile and Brazil, as well as two strains identified from strain collections in Europe during the course of the FP5 MicroNfix project.

The model PGPR strain has been selected as *Azospirillum brasilense* Sp245, since this is one of the most studied PGPR strains and has been demonstrated to give growth promotion under certain conditions in greenhouse and field trials. However, this organism is not very hardy and it is important to find alternative PGPR especially for use in temperate zones.

During the first year of the project, the partners have exchanged PGPR strains and have commenced testing these in greenhouse and small-scale field trials. Field trials are necessary at this stage of the project, since greenhouse trials in pots do not always replicate well the actual conditions of plants in the field. Plant growth promotion can often be observed in field trials where it may not be observed in a greenhouse experiment.

Partners in Brazil, Chile and Turkey have their own facilities for fermentation and preparation of PGPR inocula. For other partners, the consortium is using the services of a fermentation company in Germany.

Field trials have so far been conducted in Turkey, Brazil and Chile, and Dalgety Arable Research completed a field trial commenced at the end of the MicroNfix project. Further field trials were commenced in late 2007 in the UK and Israel.

The results of the field trials conducted so far are still being analysed, but some of the trials have given extremely interesting results, where plots treated with various PGPR have given similar biomass and grain yields with plots fertilised with chemical nitrogen fertilisers at the same sites. Field trials have also indicated some differences in the plant growth promotion effect according to the wheat variety.

The field trials have been accompanied by greenhouse trials in Belgium, Chile, Turkey and Brazil. Again, some of these trials have shown promising results. A comparison of different *A. brasilense* strains by K.U.Leuven has confirmed the previously known optimal concentration of 10^7 cells of PGPR inocula per seed, and that PGPR appear to have the most beneficial effect under conditions of moderate N fertilisation. Embrapa obtained a positive PGPR effect under some conditions for *A. brasilense* Sp245 and some of the new PGPR strains.



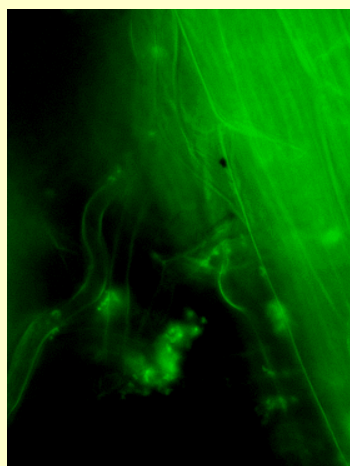
YUDGB

Field trials in Turkey

In the laboratory, the RHIBAC partners have been working to understand better the mechanisms involved in plant growth promotion, and the variations in interaction found between PGPR and different varieties of crops. ARC are working on the genetic sequences of the *nifH* genes which are responsible for nitrogen fixation, in two of their PGPR strains. Once these are identified, it will be possible to label them with a fluorescent marker, in order to observe activation of nitrogen fixation as the PGPR interact with plant roots. Also, a novel PCR approach is being used by ARC to obtain strain-specific PCR primers. In the meantime, the partners are also investigating the possibility of using antibiotic resistance with antibiotics not commonly used in human medicine. YUDGB is also using Raman spectroscopy and FAME analysis for characterisation and rapid identification of PGPR strains.

K.U.Leuven, ARC and University of Hohenheim have also worked on modified PGPR strains, where genes thought to be important for plant growth promotion have been knocked out or upregulated. Several genetically modified strains of *A.brasilense* are already available from K.U.Leuven and are being compared in pot trials for differences in plant growth promotion.

The partners are also working on labelling of PGPR strains with fluorescent markers to aid observation of the rhizobacteria on plant roots. The challenge here is that plant roots are also show fluorescence and it is not always easy to obtain a good contrast between the PGPR cells and the background fluorescence.



K.U.Leuven

Image of PGPR labelled with green fluorescent protein, colonising plant roots

Work on understanding the interaction between PGPR effects and the genetic diversity of crop varieties is being led by University of Hohenheim. The work is focusing on the differences in chemical composition of root and seed exudates, as the chemicals released by the plant are thought to attract PGPR under certain conditions. Barley is being used as a model crop in addition to wheat, since this plant is much simpler to investigate in terms of genetics, while having many similarities with wheat.

The University of Hohenheim group have gathered a collection of representative barley and wheat crop varieties, and these are being grown hydroponically to collect seed and root exudates. The exudates are being analysed by high pressure liquid chromatography and thin layer chromatography to identify changes in chemical composition under conditions of nitrogen fertilisation, nitrogen deficiency.

The group is also studying the direct effect of PGPR on root system development. Previously in MicroNfix, the University of Hohenheim showed that inoculation one of the MicroNfix PGPR strains had a significant effect on the growth of lateral roots in *Arabidopsis*, a model plant. In RHIBAC, these experiments are continuing with additional studies on the effect of different types of nitrogen source, and also conducting the same experiment with barley. For these experiments a new system of software has been set up for quantitative measurements of root systems. Promising results have so far been obtained identifying nutrient factors influencing the root growth promotion effect, and investigations of the related plant biochemistry are planned.

ENITIAA has worked on further improvements to the PGPR encapsulation technique developed in the previous MicroNfix project. PGPR survival is not at acceptable levels and ENITIAA are examining each step of the process to determine suitable changes to increase cell viability. ENITIAA are also studying the adsorption of PGPR onto starch granules and the release of PGPR into the soil from the capsules.

A RHIBAC website has been developed at <http://www.rhibac.org> and this will feature regular updates on the project. The project was also presented at the RHIZOSPHERE2 conference held in Montpellier, France in August 2007.

RHIBAC partners



The RHIBAC consortium is international, with a strong contribution from two agricultural companies.

Institute of Plant Nutrition, University of Hohenheim, Germany

Austrian Research Centers, Austria

Ecole Nationale d'Ingénieurs des Techniques des Industries Agricoles et Alimentaires, France

AGRON, Agrochemicals Development and Marketing Ltd, Israel

Dalgety Arable Research, part of Masstock Arable (UK) Ltd, UK

Centre of Microbial and Plant Genetics, Catholic University of Leuven, Belgium

Universidad Austral de Chile, Chile

Fundação Educacional Charles Darwin, Brazil

Embrapa Agrobiology, Brazil

Department of Genetics and Bioengineering, Yeditepe University, Turkey

The partners have links with agricultural companies, farming organisations and related research projects through the RHIBAC observer group.

If you are interested to find out more about RHIBAC, please consult the project website at www.rhibac.org or contact us below:

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