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The report of the study visit

Short Term Scientific Mission, COST Action FA1103
Host: Silke Ruppel, Leibniz-Institute of Vegetable- and Ornamental Crops Großbeeren
Period: from 02/10/2013 to 30/11/2013
Place: Großbeeren (Germany)
Reference code: COST-STSM-ECOST-STSM-FA1103-020913-031009

Title of STSM project:

"Molecular diversity of microbial structures of bacterial endophytes in the roots of *Salicornia europaea* (*S.herbacea*) and *Aster tripolium* under salinity stress and potential of selected strains for N_2 fixation".

<u>Most important results received during the visit:</u>

1. Density and diversity of bacterial diazotrophs isolated from the leaves, stems and roots of two halophytic plants (*S. europaea* and *A. tripolium*)

In total 9 variants of experiment were analysed:

- plants: *S. europea* (test site I – anthropogenic salinity), *S. europea* (test site II – natural salinity), *A. tripolium* (test site I – anthropogenic salinity),
- source of isolation: leaves, shoots, roots.

Bacterial endophytes were isolated from the surface-sterilized leaves, shoots and roots of halophytic plants on the selective medium CC medium (Rennie et al. 1981) to isolate diazotrophic, that means atmospheric nitrogen fixing, bacteria. Statistical analysis revealed significantly higher density of culturable diazotrophic bacterial endophytes in the shoots compared to the leaves and roots of examined plants. From each variant of experiment (9 in total) 24 bacterial strains were isolated, purified and selected. A bacterial culture collection was set up and these bacterial strains were identified using 16S rDNA sequencing analysis (216 diazotrophic bacterial endophytes in total). Among the identified strains dominated bacteria belonging to the class of Gammaproteobacteria (~50%), e.g. *Pseudomonas* sp. The highest diversity of identified strains was characteristic for shoots and the lowest for leaves. Data analysis is still in progress.

2. Quantification of the 16S rDNA and *nifH* gene pool in leaves, shoots and roots of halophytes – quantitative real-time PCR analysis.

Total DNA was isolated from leaves, shoots and roots of analysed plants in three replicates (27 samples in total). In experiment 2 different primer pairs were used to analyse: (1) 16S rDNA-gene copy numbers of total bacterial cells and (2) copy numbers of the *nifH* gene – the marker gene for biological nitrogen fixation of diazotrophic bacteria. To measure PCR products the nonspecific intercalating dye SYBR Green was used. The highest level of 16S rDNA-gene and *nifH*-gene (quantified per μg of DNA) was observed in the leaves of analysed plants. Abundance of *nifH*-gene in plant tissues was only slightly lower compare to the level of 16S rDNA-gene. The ratio of *nifH*-gene to 16S rDNA-gene was higher at naturally saline test site (0.6) compare to the test site with anthropogenic salinity (0.4).

3. DGGE

PCR-DGGE and band pattern multivariate comparison analysis were proceeded for total bacterial and diazotrophic bacterial communities extracted from *Salicornia europaea* plant material. The data analysis is still in progress.

Other experiences which occurred during the stay:

1. From 8 selected bacterial strains total proteins were extracted. Level of proteins was measured with the use of Bradford method and separated in the SDS-page analysis. In the last step of experiment extracted proteins were immunoblotted with *nifH* specific antibody. For two analysed strains two bands were detected after analysis. We expect to get different forms of *nifH* proteins produced by various diazotrophic endophytes. Further detailed studies using extracted proteins are in progress in near collaboration with the Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V..

The results received during the project greatly expanded the knowledge about diazotrophic bacteria associated with the leaves, shoots an roots of halophytes growing in soils with high concentration of NaCl. Predominant environmentally adapted diazotrophic selected bacterial strains will be investigated and can probably be used in practice to improve plant growth in salt affected agricultural areas in future time.

After completion of the analysis we intend to publish the results in two scientific papers with the following tentative titles:

1. K. Hrynkiewicz, S. Ruppel*: Quantification and identification of culturable and non-culturable endophytic diazotrophic bacteria in plant tissues of *Salicornia europea* (in preparation).

2. K. Hrynkiewicz, K.Witzel, A.-C. Scherwinski, S. Ruppel*: Genomic and proteomic diversity of the nitrogenase operon of endophytic diazotrophic bacterial strains originated from halophytic plants (in preparation).

Prospect of future co-operation with German institution

Continuation of the cooperation in the investigation of *nifH* gene and proteins:

- Quantification of *nifH* gene in the bacterial cultures growing under salt stress – qPCR analysis.
- Analysis of nifH proteins – analysis of selected bacterial strains and plant tissues inoculated with endophytes.

Joint application for a project will allow to finance above experiments.