CNR- Short Term Mobility Program 2011

Report of the scientific activity of Dr Vera Safronova

at the CNR-ISPAAM u.o.s. Sassari

Introduction

The visit of dr Vera Safronova at the laboratories of the CNR-ISPAAM u.o.s. Sassari was aimed at developing scientific collaborations with CNR scientists. During the time spent at the ISPAAM in Sassari, Dr Safronova experience with rhizobia and rhizobacteria isolation and characterization, was used to support a project currently running, about the study of interactions between plant species and rhizospheric microorganisms in coastal sandy habitats of the island and gulf of Asinara. Dr Safronova support has provided some useful information for the characterization of microbial and plant resources, which may have potential in the development of ecological restoration activities on coastal sandy areas suffering from heavy anthropic impact. During her stay at the Institute Laboratories, Dr Safronova has been working at the isolation of several strains of rhizobia from *Lotus cytisoides* root nodules and of ACC utilising rhizobacteria from the rhizosphere of *L. cytisoides* and *Matthiola tricuspidata*. The recovery of the Institute microbial collection was also performed through the multiplication of the isolates brought back from ARRIAM. Apart from the laboratory activity, Dr Safronova was also involved in the training of laboratory personnel in microbiological techniques, in round table discussions to illustrate her scientific activity, in the establishing of new scientific collaborations and in the planning of a scientific publication in cooperation with the CNR colleagues. All such activities are better specified in the following paragraphs.

Activities performed in the period June 6th- June 18th

1) Collection of root samples and isolation of ACC utilizing rhizobacteria.

Several local expeditions in the regions of Castelsardo, Stintino and Platamona (north Sardinia) were organised to collect root samples of plants inhabiting coastal environments. The roots of the perennial legume species *Lotus cytisoides* and the annual non-legume species *Matthiola tricuspidata* were collected for further inspection of the presence and isolation of rhizobacteria, containing ACC deaminase. *L. cytisoides* is widespread either in coastal sandy areas and on rocky

areas, is adapted to drought stress, very poor soils and can be successfully used in stressful environments for soil protection on slopes of Mediterranean areas (Meloni et al., 2000). *M. tricuspidata* is typical of sand dunes, is considered tolerant to drought and suitable for xeriscaping.

They are also interesting species for studies about interactions between legume and non-legume species and rhizospheric microorganisms on sandy and rocky coastal environments, useful to better exploit the potential of the species in recovery actions on coastal areas degraded by human frequentation and activities.

The collected root samples were used for isolation of bacteria containing ACC deaminase. This kind of beneficial bacteria can utilize ACC, the immediate precursor in biosynthesis of phytohormone ethylene, as a nutrient source (Glick et al., 1997; Belimov et al., 2005). Bacteria that have ACC deaminase are capable of stimulating plant growth and the mechanism of bacterial effect is as follows (Belimov et al., 2009). Some of the plant ACC is exuded from roots or seeds, taken up by the bacteria, and then cleaved by ACC deaminase to ammonium and α-ketobutyrate (Fig. 1). The reduction of ACC levels within the plant results in a decrease in the ethylene content and leads to elimination of plant growth inhibition caused by this hormone (Glick et al. 1998; Belimov et al., 2001). Since biosynthesis of ethylene increases under various unfavourable environment conditions, including heavy metal toxicity, drought and salinity, the growth-promoting effect of ACC-utilizing rhizobacteria may be of particular importance in the presence of stresses.

Application of selective nutrient medium containing ACC as a sole source of nitrogen (Belimov et al., 2001, 2005) showed that such bacteria are present in all root samples collected. During the isolation procedures six bacterial strains have been isolated. The ability of these isolates to utilize ACC was checked by repeated cultivation on selective medium. At the next step the purification of obtained ACC deaminase containing strains, was carried out by subsequent cloning. The isolation technique can be easily adapted and used for the study of beneficial microorganisms inhabiting the rhizosphere of various plant species and soils.

2) Determination of root elongation in seedlings inoculated with rhizobacterial isolates.

The plant root elongation promoting (PREP) activity of the isolated bacteria was determined using the modified root elongation assay of Belimov et al. (2001). Bacteria were grown on solid medium containing ACC for 48 h at 28^{o}C and resuspended to about 10^7 cells ml^{-1} in sterile tap water. Three ml of the bacterial suspensions or sterile water (uninoculated control) were added to plastic Petri dishes with filter paper. The seeds of *Lotus cytisoides* and *Matthiola tricuspidata* were

surface-sterilized with sulphuric acid (for 10 min) and hypochlorite (for 15 min) respectively, washed with sterile water and placed on wetted filter paper. Root length of seedlings was measured after incubation of closed Petri dishes for 6 d at 28^{o}C in the dark. The assay was performed with three dishes (with 20 seeds per dish) for each treatment.

3) Multiplication and storage of the Sardinian collection of microorganisms brought back from ARRIAM.

Sixty strains of rhizobacteria and root nodule bacteria, previously isolated in different regions of Sardinia (Asinara Island and Iglesias area) were grown at 28^{o}C on solid Yeast-Mannitol (YM) medium during four days and resuspended in 5 ml of liquid YM medium supplemented with 15% of glycerol (concentration is about 10^{9} cells ml^{-1}). Per 1 ml of each suspension were dispensed into 3 cryovials and placed in the chest freezers at -20^{o}C and -80^{o}C for long-term storage.

4) Round table discussion and presentation of current research activities.

Dr Safronova also gave a presentation of her current research and illustrated the new system acquired by ARRIAM for automated storage of microorganisms at -80^{o}C. This system provides a stabile temperature of storage, allows organizing an authorized access to deposited strains and can be used for duplicate maintenance of the strains isolated during collaborative Russian-Italian projects.

5) Establishing of new collaborations with CNR-ISPAAM -u.o.s. of Sassari

During the discussions with ISPAAM scientists, some new ideas have been discussed on how to carry the current work forward. New experiments have been planned to confirm the results and to investigate further the findings so far acquired. A root nodulation trial and the taxonomic identification of isolates through DNA sequencing activities and comparison with gene bank data have been planned. A further opportunity for collaboration in a next project concerning with metagenomic investigations of rhizobacterial populations in different environmental conditions has been also discussed.

6) Scheduling of a new scientific paper

All data acquired and new coming data will be assembled to form a draft for a new paper dealing with the characterization of rhizobia and ACC utilizer rhizobacteria from the rhizosphere of the two examined species from coastal sandy environments.

References

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Fig. 1. Mechanism of interactions between plants and rhizobacteria containing ACC deaminase.

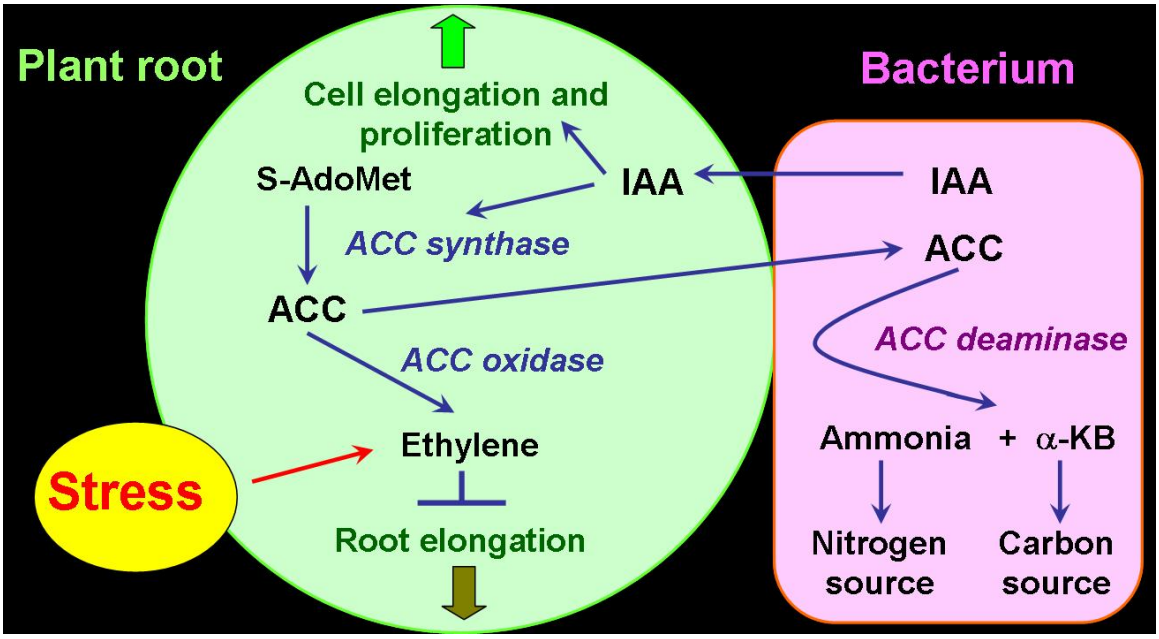

Fig. 2. *Lotus cytisoides* L.

Fig. 3. ***Matthiola tricuspidata*** **(L.) R. Br.**

Fig. 4. Bacterial growth on selective nutrient medium.