

# Session 6 - Functional and structural diversity - bacteria

#### Page Lactic acid bacteria in organic agricultural soils

Somers Ellen', Adams Amke', Anja Croonenborghs', Van Overbeek Leo2, Vanderleyden Jos'

- 1 K.U.Leuven Microbial and Molecular Systems Kasteelpar Arenberg 20 3001 Heverlee, Belgium
- <sup>2</sup> Plant Research International, Wageningen, the Netherlands

According to a concept formulated by H.P. Rusch (1906-1977), soil fertility of organic agricultural soils can be related to the presence of lactic acid bacteria. However, there is little or no evidence in literature to support this concept.

We have sampled at different time points over a 2 year period (2005-2006) 15 organic farms in Flanders, Belgium. Soils used for the organic production of cabbage and leak were sampled. Direct plating of soil dilutions on either MRS (with glucose as carbon source) or M17 (with lactose as carbon), commonly used growth media for lactic acid bacteria, revealed no significant numbers of lactic acid bacteria. The same results were obtained with the culture independent DGGE analysis, using nested PCR as described by Heilig et al. (2002). Identification of colonies obtained by direct plating using Lactobacillus group-specific primers for 16S rDNA sequences indicated mainly Bacillus, Paenibacillus and Staphylococcus species.

In a second round, an enrichment step for lactic acid bacteria was included, by incubating diluted soil samples anaerobically in MRS or M17 medium, prior to plating or DGGE analysis, as described by Chen et al (2005). This revealed high numbers and diversity of lactic acid bacteria. Sequencing the 16S rDNA revealed the following lactic acid bacteria: Lactococcus garvieae, Lactococcus lactis, Leuconostoc mesenteroides, Weissella, Lactobacillus and Enterococcus. Some of these isolates were tested and found positive for biocontrol activity and/or direct plant growth promotion. Two Lactococcus lactis isolates showed significant plant growth promoting activity in greenhouse trials with cabbage.

It can be concluded that lactic acid bacteria, although present, are not dominant in organic farming soils in Flanders. Nevertheless, if they can be enriched through agricultural practices, some of these show potential for biocontrol and/or plant growth promotion.

Heilig et al (2002) Appl. Environm. Microbiol. 68, 114-123.

Chen et al (2005) Lett. Appl. Microbiol. 40, 195-200.

Rusch H.P. (1964) Bodenfruchbarkeit, Karl F. Hauh Verlag, ed. (Heidelberg).

## P.905 Symbiotic and phylogenetic diversity of rhizobia associated with native and introduced Acacias in Algeria

Boukhatem Zineb Faiza, Domergue Odile, Galiana Antoine, De Lajudie Philippe, Laguerre Giselle, Bekki Abdelkader, Galiana Antoine

Laboratoire des Symbioses Tropicales et Méditerranéennes, UMR 113 CIRAD / IRRA / SupAgro / UMII, 34398 Montpellier Cedex 5, France

In the arid zones where crop production is reduced due to drought combined to low soil fertility, the use of symbiotic native legume trees adapted to such harsh conditions could enhance the productivity of agroforestry systems. In North Africa, Acacia species are good candidates for this purpose since they can grow on N-deficient soils and improve their N balance due to their symbiotic association with rhizobia, nitrogen-fixing soil bacteria.

In order to identify and select efficient acacia-rhizobia symbiotic associations, soil samples and root nodules from seven Acacia species, five native (A. ehrenbergiana, A. laeta, A. nilotica, A. seyal, A. tortilis) and two introduced (A. karroo, A. saligna), were collected in arid and semi-arid regions of Algeria from Oran to Tamanrasset. A collection of 22 bacterial strains was obtained after trapping on Acacia seedlings inoculated with soil samples originating from nine geographic sites. 16S rDNA sequencing revealed that the new strains represented different species in Sinorhizobium and Rhizobium. This genetic diversity was confirmed by phenotypic characterisation through biochemical assays and host spectrum. Some strains were particularly tolerant to low pH and high NaCl concentrations, temperature and osmotic stress in in vitro conditions. In a next step, selection of efficient rhizobia displaying a high nitrogen-fixing potential under stress conditions will be carried out in controlled conditions before testing them in field conditions.

### 116

### Genetic diversity of rhizobia that nodulate Prosopis juliflora in the arid eastern area of Morocco

Benata Hanane, Ourarhi Mohammed, Berrichi Abdelbasset, Abdelmoumen Hanaa, Boukhatem Nourreddine, Missbah El Idrissi Mustapha
Université Mohamed Premier, Faculté des sciences, Oujda Biologie Laboratoire de Biologie des Plantes et des Microorganismes, Faculté des sciences, Oujda 60.000 Oujda Morocco

Symbiotic nitrogen fixation in the arid regions depends on the two partners of the symbiosis: legume - rhizobium and their aptitude to adapt to prevailing climatic and soil constraints. We showed that *Prosopis juliflora* is a salt tolerant legume species, since it is not affected by the salt concentrations as high as seawater levels. This plant represents a very good candidate to combat desertification.

We thus isolated 274 bacterial strains from the root nodules of *Prosopis juliflora*, cultivated in different soils of the Eastern area of Morocco. We found that 24 strains tolerated NaCl concentrations higher than 30 g/l (w/v). All the strains re-nodulated their originals host. They all possessed the nodC gene. This was checked by amplifying the nodC gene which codes for a NodC nodulation factor by PCR specific primers. The assessment of metabolic and physiologic properties of these isolate showed a high phenotypic diversity. This is an advantage for the plant because it can be always nodulated by different types of rhizobia even if the soil or climate conditions have to change.

The RFLP of the 16S rDNA using Mspl, HaelII and CfoI enzymes confirmed the diversity of the strains that nodulate this species in the same soil. We thus obtained three different profiles. The diversity of the strains was also assessed by the exploration of the bacterial chromosome carried out by rep PCR using BOXA1R, Rep (I and II) and ERIC (I and II) primers. The Results obtained showed a high diversity of the strains.

Some rhizobial strains kindly provided by colleagues of the "Laboratoire Commun de Microbiologie of the IRD/ISRA/UCAD" in Dakar, Senegal were used as references.