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COMPARATIVE GENOMICS OF SINORHI*ZOBIUM MELILOTI* ISOLATES DIFFERED FROM SALT TOLERANCE

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Sinorhizobium meliloti are able to survive in two different niches –in a soil as a saprophyte bacteria and in root threads developing into a nitrogen-fixing root nodule on alfalfa (*Medicago sativa*).

Genome of type strain *Rm*1021 comprises one circle chromosome (3.65 Mb) and two megaplasmids: *pSymA* (1.35 Mb) and *pSymB* (1.68 Mb). Recently it was sequenced [1] and the first full-genome biochip was constructed [2].

Comparative genomic hybridization (CGH) relies on microarray genome DNA comparison is a powerful modern approach to explore genomic variations in native bacteria isolates in order to learn more about evolutionary dynamics of all three *S. meliloti* / *S. medicae* replicons and to relate genotypic features to ecological adaptation.

Main objective: to estimate genetic variation at the genomic level in *S. meliloti / S. medicae* isolates native to geographically distinct origins of legumes: Central Asian, Siberian and Aral Sea region by applying CGH approach with the sequenced strain *Rm*1021 on a full-genome *S. meliloti* microarray and by producing PCR products of the most polymorph regions at the chromosome.

Three nodule isolates of *S. meliloti* native to salinised Aral Sea region were evaluated by genomic comparative analysis with Sm6kPCR containing 6046 internal open reading frame (ORF)-specific DNA fragments of 80 to 350 bp and 161 70-mer oligonucleotide [2].

Two isolates from our collection were previously analyzed by microarray technique [3]. No remarkable differences could be stressed out when these two isolates were compared with the type strain *Rm*1021. While that was not the case when three salt sensitive isolates from the same region were studied for the genomic diversity in all replicons across both *Rm*1021 and two mentioned above isolates with moderate salt tolerance. Chromosome and megaplasmid *pSymB* of salt sensitive isolates have lost or contained much more divergent regions than the strains with moderate salt tolerance. The symbiotic megaplasmid *pSymA* in all three tested salt sensitive isolates showed great diversity in comparison with moderately salt tolerant *Rm*1021 and native isolates.

Chromosomal divergent sequences polymorphism in genomes of *S. meliloti / S. medicae* isolates native to different origins.

Genomic diversity is high among rhizobia and results from the acquisition of foreign DNA and from frequent recombination events in the genome. Foreign DNA have been acquired by horizontal transfer and are characterized by one or more of the following criteria: (1) are absent from the identical location in closely related strains or species; (2) contain an altered G/C content as compared to the rest of the genome; (3) associated with mobile DNA sequences (4) contain blocks of genes that work together for a specific function such as molecular transport, specialized metabolism or host cell interactions [4].

According to the sequence data [1] at least six regions with the low G+C content (54-58%) could be identified through the chromosome. Two short- and one large-sequence polymorph regions on the chromosome of *Rm*1021 according to the sequence data [5] were evaluated for divergence across 36 *S. meliloti* and 5 *S. medicae* isolates differed in geographical origin by PCR with specially designed primers. Each of three "islands" were bounded by tRNA genes and enriched in IS. Remarkable, that all but one *S. medicae* isolates including CC169 had shown no differences in chromosomal structure organization with thus of *Rm*1021.

One short "island" predominantly harboring genes related to defense function was divergent (or absent) in 69% of *S. meliloti* isolates and another short "island" was divergent among 53% of *S. meliloti* isolates native to evaluated gene centers. The large "island" harboring genes in all major functional categories was found to be partially or completely deleted among 11% of isolates. While significant difference between groups of isolates native to different gene centers of alfalfa was observed towards polymorph regions

Using DNA microarray technology, we assessed genetic variation of all three replicons of three salt sensitive isolates of *S. meliloti*. We found out that:

- all three replicons of salt sensitive isolates are accumulated much more genetic differences (lost or divergent), than that of *Rm*1021 and isolates with moderate salt tolerance;
- *pSymA* of tested isolates is the major replicon for differentiation towards environment within *S. meliloti* species;
- resemblance in chromosome organization of *S. meliloti* and *S. medicae* isolates native to origin of host plants could vote for their common predecessor.

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