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Genetic Identification and Symbiotic Efficiency of an Indigenous *Sinorhizobium meliloti* Field Population

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Summary

Soil bacteria *Sinorhizobium meliloti* are of enormous agricultural value, because of their ability to fix atmospheric nitrogen in symbiosis with an important forage crop legume – alfalfa. The main aim of this study was (i) to isolate indigenous *S. meliloti* strains from different field sites in Croatia, (ii) to assess genetic diversity and genetic relationships amongst strains of natural populations and (iii) to provide information about nodulation and symbiotic efficiency of indigenous *S. meliloti* strains. The nine strains isolated from alfalfa nodules collected from different field sites and three reference strains were analysed. Genetic characterisation by PCR-RFLP of the 16S rDNA, rep-PCR and RAPD-PCR was applied to study the status of *Sinorhizobium meliloti* populations inhabiting nodules of alfalfa. The results of PCR-RFLP of the 16S rDNA revealed that all isolates belong to the *S. meliloti* species. Cluster analysis of rep-PCR and RAPD-PCR profiles showed significant differences among *S. meliloti* isolates. Both methods resulted in identical grouping of strains. Among indigenous strains two divergent groups could be determined. The biggest differences were detected among two reference strains and all field isolates. Greenhouse studies were performed for evaluation of symbiotic efficiency and compatibility of *S. meliloti* strains with two alfalfa cultivars. Quantitative expression of symbiotic efficiency was evaluated by measurement of nodule dry weight, content of proteins and total nitrogen in plants, dry matter and green mass yield of plants. All strains nodulated both alfalfa cultivars but with different efficiency. Significant differences in dry matter and green mass yield of alfalfa as well as protein content were determined depending on the strain used. The results indicate that three indigenous *S. meliloti* strains can be characterised as the most efficient of all strains used in this study.

Key words: nitrogen fixation, alfalfa, *Sinorhizobium meliloti*, PCR-RFLP, rep-PCR, RAPD, symbiotic efficiency

Introduction

Biological fixation of molecular nitrogen from the atmosphere is one of the main sources of nitrogen pool enhancement in agricultural soils. Rhizobia are a group

of bacteria, normally found in soil, which induce nitrogen-fixing nodules on leguminous plants. Alfalfa (*Medicago sativa*) is a perennial forage legume and it is one of

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the most important protein sources for nutrition of livestock. As a legume, alfalfa forms a symbiotic relation with nitrogen fixing bacteria belonging to *Sinorhizobium meliloti* (1). Symbiotic efficiency has been shown to vary among different *S. meliloti* strains. The efficiency of sinorhizobial nodulation and symbiotic nitrogen fixation is determined by their individual genotype. In order to identify genotypes displaying, for instance, superior nitrogen fixing capabilities, it is essential to characterise the natural population of competitors that are well adapted to a certain environment. Rapid and reliable molecular methods have been used for differentiation of rhizobial strains (2–8). A large number of molecular methods based on polymerase chain reaction have been proposed to characterise *Sinorhizobium* strains and to provide a high degree of differentiation among closely related bacterial strains. Restriction fragment length polymorphism (RFLP) analysis of PCR-amplified 16S rRNA genes is especially applicable for determination of inter- and intra-generic relationship among rhizobial species. This method is also suitable for grouping strains at the species level or higher, and it can be used to detect potential new taxa or phylogenetically classify a vast number of rhizobia (9). However, 16S rDNA analysis is based on the features of only one gene, whereas rep-PCR and RAPD generates fingerprints of the entire genome. The random amplified polymorphic DNA (RAPD) fingerprinting method is based on the use of short primers which hybridise with sufficient affinity to chromosomal DNA sequences at low annealing temperatures in a way that they can be used to initiate amplification of regions of the bacterial genome (10). The number and location of these random sites vary for different strains of a bacterial species. In many studies, RAPD-PCR have been proposed for identification and phylogenetic grouping of *Sinorhizobium* isolates (11,12). DNA fingerprints can also be generated by using pairs of primers derived from repetitive extragenic palindromic (REP) and enterobacterial repetitive intergenic consensus (ERIC) sequences and the BOX element (13). It has been shown that REP- and ERIC-like sequences are present in rhizobia and in other Gram-negative soil bacteria and that they can be used for bacterial taxonomy (4,14–18). De Bruijn demonstrated the usefulness of DNA fingerprinting by PCR using REP and ERIC primers for identification and classification of the members of several *Sinorhizobium* species (19). The fingerprints obtained by rep-PCR and RAPD are specific and reproducible and allow the distinction of bacteria at the (sub)species and strain level (17).

It is also important to evaluate the most effective *S. meliloti* strains for alfalfa inoculation in agricultural production. Dry matter analyses of plants provide a useful measurement of the efficiency of symbioses formed between the crop and the rhizobia (20). The term »symbiotic efficiency« is used to describe the ability of nodulated plants to fix nitrogen, and a quantitative expression is dependent on comparisons of dry weights (20). Besides the measurement of dry weight, quantitative expression of symbiotic efficiency could be evaluated by the measurement of content of proteins, content of total nitrogen in plants and green mass yield of plants.

The main objective of this study was (i) to isolate indigenous *S. meliloti* strains from different field sites in Croatia, (ii) to assess genetic diversity and genetic relationship amongst strains of natural populations and (iii) to provide information about nodulation and symbiotic efficiency of indigenous *S. meliloti* strains.

Materials and Methods

Bacterial strains

Forty-one *S. meliloti* isolates were obtained from alfalfa nodules collected from different soil types (Table 2). Strains were isolated from surface sterilised nodules following a standard protocol (21). Isolates were grown in YMA (yeast mannitol agar) medium with the addition of Congo red. The symbiotic ability of isolates was checked by nodulation tests (21). Nine isolates were used for further characterisation. Three *S. meliloti* strains, obtained from the Culture Collection of Rhizobium – Praha (strain D 469 and D 528) and Institute of Grassland and Environmental Research in Dyfed (strain 2011), were also included in these investigations as reference strains.

DNA isolation

Total genomic DNAs from nine field isolates and three reference strains were isolated using standard phenol-chloroform-isoamyl extraction. The concentration and the purity of DNA were estimated spectrophotometrically at 260 and 280 nm.

PCR-RFLP analysis of 16S rDNA

PCR amplification reactions were performed in a 25 µL volume, containing: 20 mmol/L Tris-HCl (pH=8.4), 50 mmol/L KCl, 2.5 mmol/L MgCl₂, 200 mmol/L each of dNTPs, 1 µmol/L primer, 30 ng of genomic DNA and 1.5 U taq DNA polymerase (Life Technologies). Primers fd1 and rd1 described by Weisburg *et al.* (22) were used for PCR amplification of 16S rDNA. Amplification was performed in PCR thermocycler Crocodile II – version 1.2 (Appligen Inc., Pleasanton, CA). The following temperature profiles were used for PCR amplification: 95 °C for 3 min for an initial denaturation; 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C; and a final extension at 72 °C for 3 min. Amplified PCR products were digested with restriction endonucleases *Hae*III, *Hinf*I, *Msp*I and *Mbo*I (La Roche, Germany) in separate reactions according to the manufacturer's recommendations. Digested DNAs were analysed by submerged gel electrophoresis on precast 6 % poly(NAT) gels run in SEA 2000 apparatus (Elchrom Scientific AG, Cham, Switzerland). A molecular size marker, a 1 kb DNA ladder (Life Technologies), was run in all gels. Electrophoresis was carried out at 7 V/cm and 20 °C during 2 h and 45 min. The restriction patterns were visualised under UV illumination and photographed with Polaroid type 667 positive film.

Fingerprinting of genomic DNA by rep-PCR and RAPD-PCR

Primers REP 1R and REP 2I, ERIC 1R and ERIC 2 and BOX A1R were used for rep-PCR fingerprinting analysis described by de Bruijn (19). Five arbitrarily chosen primers used for RAPD fingerprinting were 10 nucleotides in length. Primer sequences were as follows: 5' – GATCGGACCG – 3' (P1), 5' – TCGCCAGCCA – 3' (P16), 5' – GATCCTGCCG – 3' (P5), 5' – GATCGGACGG – 3' (P2), 5' – GATCCCAGCG – 3' (P4). All the primers used in this investigation were obtained from Microsynth (Balgach, Switzerland). Reaction mixture composition was performed as described above for PCR-RFLP analysis of 16S rDNA. The cycling conditions for RAPD and rep-PCR differ in annealing time and temperature. The reaction mixtures were overlaid with two drops of mineral oil, incubated at 95 °C for 5 min for initial denaturation, 35 cycles in intervals of 30 sec at 94 °C, 30 sec at 36 °C (RAPD) or 1 min at 40 °C (REP-PCR) or 30 sec at 50 °C and 1 min at 52 °C (ERIC-PCR) or 1 min at 53 °C (BOX-PCR), and 1 min at 72 °C followed by 7 min of incubation at 72 °C. PCR-amplified DNA fragments were separated by horizontal gel electrophoresis on precast 6 % poly(NAT) gels and visualised as described for PCR-RFLP analysis of 16S rDNA

DNA fingerprinting analysis

All RAPD and rep-PCR fingerprints and restriction patterns were converted into a two-dimensional binary matrix (1, presence of a band; 0, absence of a band) and analysed by using the NTSYS-pc package (version 1.8; Exeter Software, Setauket, N.Y.). For each pair of strains, a simple matching (Sm) coefficient was calculated, and a UPGMA algorithm was used to perform hierarchical cluster analysis and to construct a dendrogram.

Plant assays

Evaluation of symbiotic efficiency and compatibility of *S. meliloti* strains with alfalfa cultivars was performed in a greenhouse experiment. Alfalfa seeds of two cultivars Mirna and Posavina were surface sterilised using standard protocols (21) and separately inoculated with nine *S. meliloti* isolates and three reference strains including uninoculated seed of both cultivars. Plastic pots (20 cm in diameter) were sterilised in 0.1 % sodium hypochlorite, rinsed in sterile water and filled with 6 kg of sterilised soil (gamma radiation with 5.0 Mrads). Experiment was established on the eutric brown soil. The physical and chemical properties of the soil were as follows: clay 8.8 %; silt 22.2 %; sand 20.4 %; pH = 5.6 (measured in KCl); humus 1.1 %, total nitrogen 0.07 %, physiologically active K and P (12.2 mg K₂O/ 100 g soil; 16.71 mg P₂O₅/100 g soil). Approximately 30 seeds of alfalfa cultivars were sown in each pot. Plants were grown in the greenhouse with natural sunlight and temperatures ranging from 8–24 °C and watered with non-nitrogen solution (21) during the vegetation period. The experimental design was a randomised complete block with three replicates of each inoculant and cultivar combination as well as uninoculated controls. In full bloom stage of alfalfa, 10 plants were taken from

each pot. Nodule dry weight, protein content in plants, dry matter and green mass yield of the plants were measured. All the obtained data were processed by analysis of variance using the MSTAT-C statistical program. Mean separation was calculated using the LSD values if the F-test was significant at P=0.05.

Results

PCR-RFLP analysis of 16S rDNA

A pair of primers fD1 and rD1 were used to amplify the 16S rDNA of the isolates and reference strains. The PCR amplification with all samples produced a single fragment, size of about 1.5 kb. This size corresponds to the expected size of the 16S rRNA genes among bacteria. Digestion with different enzymes (*Hae*III, *Hinf*I, *Msp*I and *Mbo*I) revealed differences between field isolates and reference strains. The combined *Hae*III, *Hinf*I, *Msp*I and *Mbo*I restriction patterns of the amplified 16S rDNA were converted into a two-dimensional binary matrix and analysed using NTSYS-pc biostatistics program. Dendrogram derived from RFLP patterns showed the grouping of the strains into two divergent groups (Fig. 1). Field isolates from the Slavonian region (OS 2, OS 3, OS 4, OS 5, S 26, OS 6, V 11) had identical RFLP patterns, closely related to *Sinorhizobium meliloti* reference strain 2011. Indigenous strain Z 21 was also included in this first major group of strains. The second group consisted of two reference strains, 528 and 469, with identical RFLP pattern and indigenous strain C 16 which differed from the two reference strains at similarity level of 0.63.

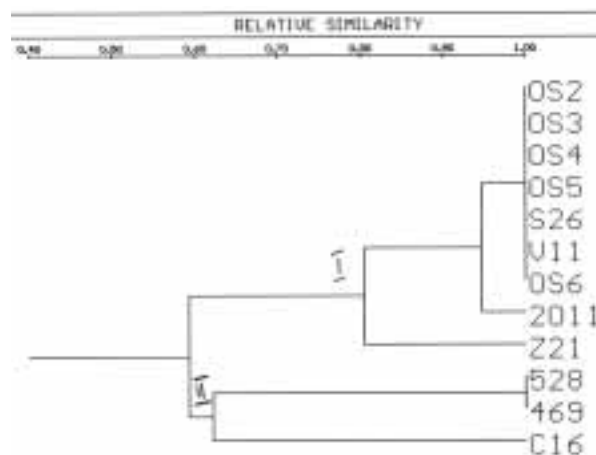


Fig. 1. Dendrogram of *Sinorhizobium meliloti* strains derived from RFLP analysis of PCR amplified 16S rDNA

DNA fingerprinting by rep-PCR and RAPD

Total genomic DNAs from all strains examined were used as templates in amplification reactions with REP (REP 1R and REP 2I), ERIC (ERIC 1R and ERIC 2) and BOX (BOX A1R) sets of primers. The PCR products from a representative group of strains were separated by horizontal gel electrophoresis on precast 6 % poly(NAT) gels. The primers yielded multiple DNA

products ranging in size from 0.1 to 4.0 kb with REP and ERIC primers and 0.2–3.0 kb with BOX primer. The number of amplified fragments ranged from 6 to 23 per strain, depending on the primer used in the amplification reaction. The dendrogram obtained by numerical analysis of gel data (Fig. 2) shows that all tested strains could be divided into two major clusters (similarity level of 0.59). Most of *S. meliloti* field isolates were grouped within the first major cluster, together with reference strain 2011. The second major cluster comprised only four strains divided into two distinct subgroups (similarity level of 0.6). Subgroup IIa included the very similar reference strains 528 and 469 from the Culture Collection of Rhizobium – Praha and subgroup IIb included two field isolates, C 16 and Z 21, which considerably diverged from all other strains.

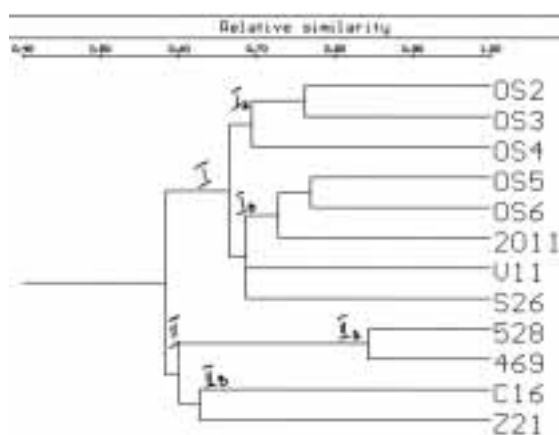


Fig. 2. Dendrogram of *Sinorhizobium meliloti* strains derived from rep-PCR fingerprints generated using REP, ERIC and BOX of primers

The diversity of three reference strains and nine field isolates was also estimated by RAPD-PCR fingerprinting. The amplification reaction with five arbitrarily chosen primers (P 1, P 16, P 5, P 2, P 4) resulted in specific and different fingerprints for all strains tested. The fragment sizes ranged from less than 0.3 to approximately 5.0 kb. The dendrogram derived from combined RAPD profiles showed that all strains could be divided into two major clusters which diverged at the similarity level of 0.61 (Fig. 3). The majority of the isolates were found to belong to the first cluster together with reference strain 2011. The second cluster consisted of only three strains and they represented a separate group of sinorhizobia. Reference strains 469 and 528 formed one subgroup, while indigenous strain Z 21 diverged from them at a similarity level of 0.65. Indigenous strain C 16 was not included in any group of strains and the greatest differences were determined between that isolate and all other strains tested.

Plant assay

Symbiotic efficiency of *Sinorhizobium meliloti* isolates and reference strains was evaluated in a greenhouse experiment. All strains nodulated alfalfa cultivars Mirna and Posavina. Statistical analysis data revealed that inoculation significantly affected nodule dry weight, pro-

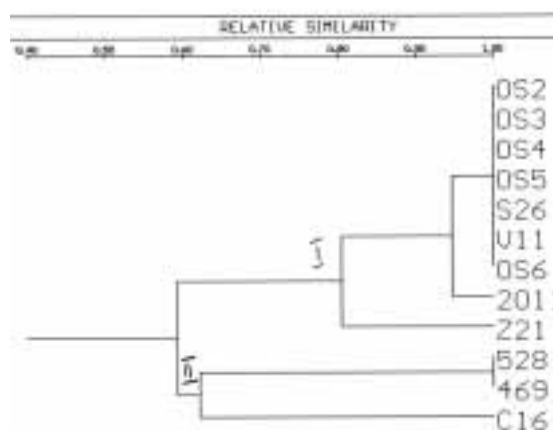


Fig. 3. Dendrogram of *Sinorhizobium meliloti* strains derived from RAPD fingerprints generated using five different primers (P1, P16, P5, P2, P4)

tein content, green mass and dry matter yield of plants (Table 1). Effect of strains on cultivars (S x C) was determined by protein content and dry matter yield. A positive effect of inoculation with all strains on dry the matter and green mass yield of alfalfa as well as protein content in plant with respect to all uninoculated vari-

Table 1. Combined analysis of variance for nodule dry weight, green mass yield, content of proteins and dry matter yield

Source of variation	df	Nodule dry weight (g/plant)	Green mass yield (g/10 plants)	Content of proteins (g/10 plants)	Dry matter yield (g/10 plants)
Strains (S)	12	**	**	**	**
Cultivar (C)	1	**	**	ns	ns
S x C	12	ns	ns	**	*

* significant at the 0.05 level

** significant at the 0.01 level

df degrees of freedom

ns not significant

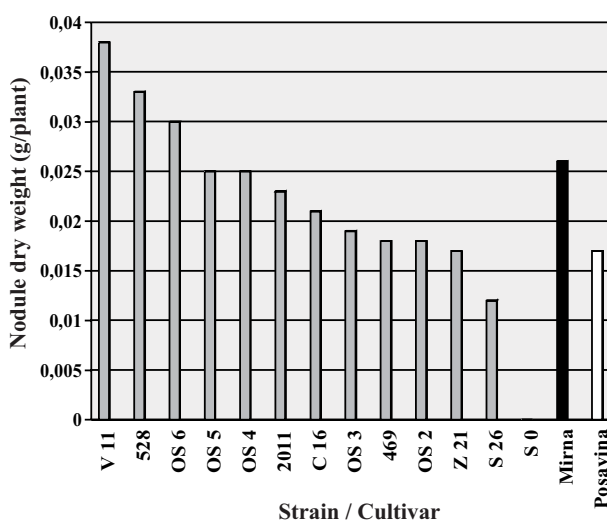


Fig. 4. Effect of inoculation with different *S. meliloti* strains on nodule dry weight; ■ cultivar Mirna; □ cultivar Posavina

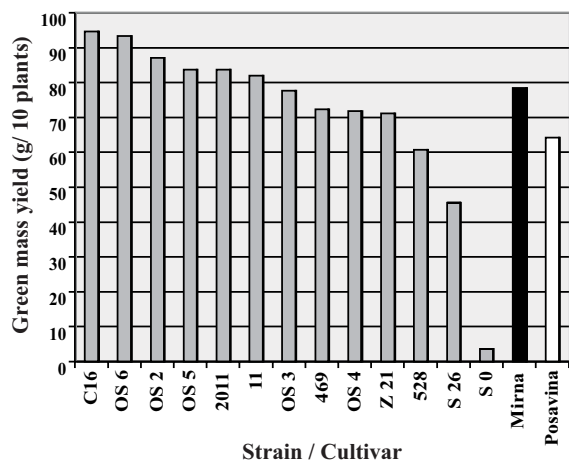


Fig. 5. Symbiotic efficiency of indigenous and reference *S. meliloti* strains determined by green mass yield of alfalfa cultivars

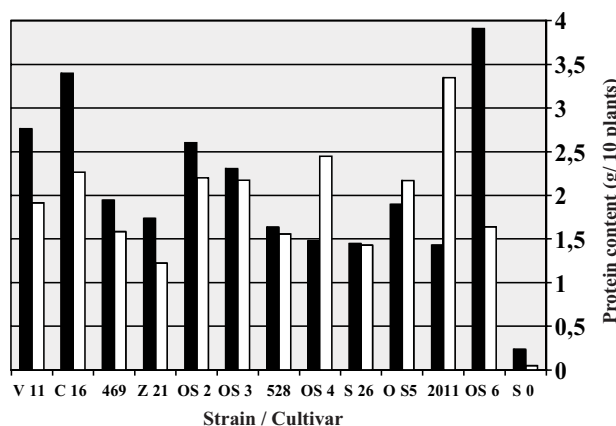


Fig. 6. Strains effect on cultivars Mirna and Posavina represented by protein content in alfalfa plants; ■ cultivar Mirna, □ cultivar Posavina

Table 2. Origin of *S. meliloti* isolates and effects of inoculation with 9 isolates and 3 reference strains on nodule dry weight, protein content, green mass and dry matter yield.

Strain designation	Location	Soil type	Nodule dry weight (g/plant)	Green mass yield (g) ^y	Content of proteins in plants (g) ^y	Dry matter yield (g) ^y
V 11	Grabovac	Luvisols on loess	0.038 ^a	81.963 ^{ab}	2.338 ^{ab}	25.077 ^{ab}
OS 2	Osijek	Eutric brown	0.018 ^{de}	87.090 ^{ab}	2.403 ^{ab}	21.328 ^{abc}
OS 3	Petrijevci	Luvisols pseudogleyic	0.019 ^{cde}	77.678 ^{ab}	2.241 ^{abc}	21.748 ^{abc}
OS 4	Valpovo	Luvisols pseudogleyic	0.025 ^{bcd}	71.812 ^{abc}	1.966 ^{bcd}	18.947 ^{bcd}
OS 5	Karanac	Chernozem	0.025 ^{bcd}	83.730 ^{ab}	2.031 ^{bcd}	23.018 ^{abc}
OS 6	Brestovac	Semigley	0.030 ^{abc}	93.285 ^a	2.777 ^a	25.987 ^a
S 26	Slavonski Brod	Hydromorphic gley	0.012 ^e	45.617 ^c	1.439 ^d	12.282 ^d
C 16	Čazma	Pseudogley	0.021 ^{bcd}	94.608 ^a	2.831 ^a	22.472 ^{abc}
Z 21	Zagreb	Eutric brown	0.017 ^{de}	71.157 ^{abc}	1.478 ^d	17.325 ^{cd}
S ₀ – non-inoculated (control)			0.000 ^f	3.705 ^d	0.141 ^e	1.010 ^e
469			0.018 ^{de}	72.297 ^{ab}	1.765 ^{bcd}	17.620 ^{cd}
528			0.033 ^{ab}	60.718 ^{bc}	1.596 ^{cd}	16.327 ^{cd}
2011			0.023 ^{bcd}	83.673 ^{ab}	2.389 ^{ab}	22.932 ^{abc}

Letters (a to f) indicate significant differences ($P < 0.05\%$) between values in each column. All values represent the mean of three replicates and when followed by the same letter in the same column, did not show statistical difference ($P < 0.05\%$).

ants was observed (Figs. 5–7). Differences in symbiotic efficiency among *S. meliloti* strains were also determined (Table 2). Alfalfa inoculation with indigenous strains V 11, OS 6 and reference strain 528 resulted in higher nodule dry weight in comparison with all other strains used in this study. The lowest nodule dry weight was determined on plants inoculated with indigenous strain S 26. Significantly higher nodule dry weight was determined on cultivar Mirna (Fig. 4). Yield of green mass was higher in plants inoculated with strains C 16, OS 6, OS 2 with respect to strains 528 ($P=5\%$) and S 26 ($P=1\%$) (Fig. 5). Data obtained for protein content in alfalfa plants revealed significant differences between strains and in strain-cultivar interaction (Table 1). According to interaction effect, the highest protein content was determined in cultivar Mirna inoculated with indigenous strains OS 6 and C 16, and in cultivar Posavina inoculated with strains 2011 and OS 4 (Fig. 6). The highest dry matter yield was determined in plants inoculated with strain OS 6 in respect to strains OS 4, 469, Z 21 ($P=5\%$) and 528, S 26 ($P=1\%$) (Table 2). A positive ef-

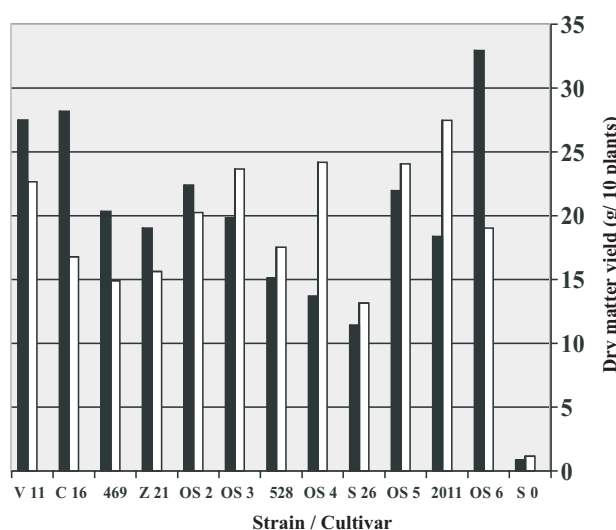


Fig. 7. Cultivars response on inoculation with different *S. meliloti* strains represented by measurement of dry matter yield; ■ cultivar Mirna □ cultivar Posavina

fect of inoculation with strains OS 6, C 16, V 11 resulted in the highest value for dry matter yield of cultivar Mirna in respect to strains OS 4 and S 26 ($P=1\%$). Inoculation with strain 2011 resulted in higher dry matter yield of cultivar Posavina in respect to strains 528 and C 16 ($P=5\%$) and strains Z 21, 469 and S 26 ($P=1\%$) (Fig. 7).

Discussion

The genetic diversity and symbiotic efficiency among *S. meliloti* isolates, obtained from different field sites in Croatia, were investigated in this study. To analyse the diversity among the isolates, 16S rDNA PCR-RFLP, rep-PCR and RAPD genomic fingerprints were generated. Characterisation based on 16S rDNA PCR-RFLP with four restriction enzymes revealed that it is possible to distinguish two well-defined groups of sinorhizobia used in this study, indicating the existence of intragenetic diversity. These results showed that most isolates (77 %) have identical 16S rDNA PCR-RFLP genotypes closely related to reference strain 2011. Two reference strains 469 and 528 and indigenous strain C 16 differ from other strains and form a separate group. In this work, distinction of isolates at the strain level was determined using the RAPD PCR and rep-PCR methods (16). All strains isolated from different field sites in Croatia had specific RAPD and rep-PCR patterns significantly different from the three reference strains. The results presented here showed that both methods grouped strains almost identically, which demonstrated that both PCR fingerprinting methods were equally suited for the characterisation of *S. meliloti* field populations. These results are in agreement with the results obtained by other authors (17). Strains isolated from East Slavonia (OS 2, OS 3, OS 4, OS 5, OS 6), Virovitica and Slavonski Brod were grouped within the first major cluster and closely related to reference strain 2011. Reference strains from the Culture Collection of Rhizobium – Praha significantly diverged from all isolates and were separated in subgroup IIa by both methods. The most different strain from all strains used in this study was indigenous strain C 16. With one exception, strain differentiation on the basis of their RAPD and rep-PCR fingerprints resulted in similar groupings as in characterisation based on 16S rDNA PCR-RFLP. 16S rDNA PCR-RFLP, RAPD and rep-PCR data revealed genetic diversity among alfalfa isolates and indicate the presence of indigenous field populations of *S. meliloti* in different field sites in Croatia.

Nodulation, symbiotic efficiency as well as compatibility of isolates and reference strains with domestic cultivars of alfalfa were also evaluated in this investigation. For this purpose, a greenhouse experiment was performed. Although strains nodulated alfalfa cultivars Mirna and Posavina, significant differences between *S. meliloti* strains were determined. Measurement of dry matter and green mass yield of plants, content of proteins and total nitrogen in plants provide useful information of the symbiotic effectiveness formed between alfalfa cultivars and *S. meliloti* strains. Our results indicate that indigenous strains C 16, OS 6 and V 11 can be characterised as the most efficient strains. However, effi-

cient strains V 11 and OS 6 produced higher nodule dry weight, which means that they induced better nodulation on alfalfa roots. Reference strain 528 produced higher nodule dry weight but inoculation of alfalfa cultivars with this strain resulted in significantly lower content of protein and nitrogen as well as dry matter and green mass yield of plants compared to efficient strains. These results suggest that the efficiency of the strain 528 was low, but with higher nodulation effect.

In order to determine the correlation between distribution of indigenous strains and ecological conditions of different field sites, from which the strains had been isolated, pedological analysis was performed (data not shown). The results indicated that indigenous *S. meliloti* strains were present in different ecological conditions such as acid soil reaction, different humus content, *etc.* Also, there seems to be no clear correlation between the isolation site, genomic fingerprint patterns and symbiotic efficiency of isolated strains.

Conclusion

The presence of indigenous *S. meliloti* strains was determined in all analysed soil types. Results from 16S rDNA PCR-RFLP method revealed that all isolates belong to the *S. meliloti* species. Results from RAPD and rep-PCR showed identical grouping of strains. Among indigenous strains two divergent groups can be determined. Indigenous strains C 16 and Z 21 significantly differed from all other isolates and reference strains. Compatibility between two alfalfa cultivars and all tested strains was determined in greenhouse studies. All strains nodulated both alfalfa cultivars with different symbiotic efficiency. The results of plant assay indicated that indigenous strains C 16, OS 6 and V 11 could be characterised as the most efficient of all strains examined and that, locally adapted, indigenous population of *S. meliloti* strains is capable of forming effective symbioses with two alfalfa cultivars. This preliminary results of symbiotic efficiency of indigenous strains provided in the greenhouse should be tested in field trials.

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Genetička identifikacija i simbiotna učinkovitost autohtone populacije *Sinorhizobium meliloti*

Sažetak

Bakterija *Sinorhizobium meliloti* ima važno mjesto u poljoprivrednoj proizvodnji zbog sposobnosti vezanja atmosferskog dušika u simbiozi s najvažnijom krmnom leguminozom – lucernom. Glavni je cilj ovih istraživanja (i) izolacija autohtonih sojeva *Sinorhizobium meliloti* s različitih agroekoloških područja Republike Hrvatske, (ii) identifikacija izoliranih sojeva i utvrđivanje genetičke raznolikosti unutar autohtone populacije *Sinorhizobium meliloti* te (iii) provjera simbiotne učinkovitosti izoliranih sojeva. Istraživanjem su obuhvaćena tri referentna soja *Sinorhizobium meliloti* i devet autohtonih sojeva izoliranih iz kvržica sakupljenih sa različitih lokaliteta i tipova tla. Genetička identifikacija autohtonih sojeva obavljena je primjenom molekularnih metoda PCR-RFLP 16S rDNA fragmenata, rep-PCR i RAPD-PCR. Rezultati restrikcijske analize amplificiranih 16S rDNA fragmenata upućuju na zaključak da svi izolati pripadaju vrsti *Sinorhizobium meliloti*. Primjenom RAPD i rep-PCR metode dobiveni su karakteristični DNA profili za sve analizirane sojeve, što znači da su obje metode iznimno osjetljive i precizne te se mogu uspješno primjenjivati za utvrđivanje genetičke raznolikosti u prirodnoj populaciji sojeva *S. meliloti*. Objе metode rezultirale su identičnim grupiranjem sojeva. Na osnovi RAPD i rep-PCR profila utvrđeno je da se svi ispitivani sojevi mogu podijeliti u dvije glavne skupine te da se svi autohtoni sojevi bitno razlikuju od dva referentna soja. Vegetacijski pokus u stakleniku postavljen je radi procjene simbiotne učinkovitosti sojeva *S. meliloti* i njihove kompatibilnosti s dvije sorte lucerne. Simbiotna učinkovitost sojeva utvrđena je mjerenjem udjela ukupnog dušika i proteina u biljci, mase suhe tvari kvržica po biljci, prinosa zelene mase i suhe tvari u biljci. Svi autohtoni i referentni sojevi su nodulirali upotrijebljene sorte lucerne i uspostavili s njima simbiotni odnos, ali s različitim učinkom. Signifikantne razlike između ispitivanih sojeva utvrđene su na osnovi prinosa zelene mase i suhe tvari lucerne te udjela proteina u biljci. Prema rezultatima dodivenim u vegetacijskom pokusu utvrđena je visoka simbiotna učinkovitost tri autohtona soja u usporedbi s ostalim ispitivanim sojevima.