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IDENTIFICATION OF WILD *BRADYRHIZOBIUM* STRAINS AND THEIR PLANT GROWTH-PROMOTING EFFECT ON SOYBEAN IN FIELD CONDITIONS

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Abstract. Efficient nodulation by nitrogen-fixing rhizobia underlies sustainable soybean production and reduces the need for mineral fertilizers. Wild *Bradyrhizobium* strains were isolated from surface-sterilized root nodules of soybean cv. ES Mentor and identified by whole-genome and 16S rRNA gene sequencing, and annotated with Average Nucleotide Identity (ANI) analysis in DFAST. Two isolates, 31 and 82, were identified as *B. japonicum* and *B. diazoefficiens*, respectively, both showing 100 % identity to their closest reference type strains. A consortium of the two strains, used for seed pre-sowing inoculation, significantly increased root length (by 57.9 %) and root diameter (by 16.4 %) in a field experiment, while stimulating nitrogen-fixing and phosphate-mobilizing rhizosphere microbiota and lowering the nitrate nitrogen content in soil. The results confirm the plant growth-promoting potential of the isolated *Bradyrhizobium* strains for soybean cultivation.
Keywords: *Bradyrhizobium*, soybean, symbiotic nitrogen fixation, plant growth-promoting bacteria, rhizosphere microbiota.

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important protein- and oil-yielding crops, and increasing its productivity while reducing dependence on chemical fertilizers is central to sustainable agriculture. This goal is closely linked to the efficiency of symbiotic nitrogen fixation carried out by root-nodule bacteria (rhizobia), which engage in a finely regulated molecular dialogue with the host legume during nodule formation and functioning (Yeremko et al., 2025). Screening, isolation, and identification of highly effective indigenous rhizobial strains from local agrocenoses is therefore a key step toward developing novel microbial consortia for seed inoculation and for enhancing legume-rhizobium symbiosis. The aim of this study was to isolate and identify wild strains of *Bradyrhizobium* spp. from nodules by whole-genome sequencing and 16S rRNA gene analysis, and to evaluate their phytostimulatory effect on soybean cv. ES Mentor plants under field conditions.

Microorganisms were isolated from surface-sterilized soybean root nodules by plating on YEME (Yeast Extract Malt Extract) agar. Total DNA was extracted from the resulting pure cultures. Taxonomic identification was based on PCR amplification and whole-genome sequencing (Illumina NovaSeq, 2×150 bp), together with 16S rRNA gene sequencing by the Sanger method. Average Nucleotide Identity (ANI) was calculated using the FastANI algorithm implemented in DFAST 1.3.9 (DDBJ Fast Annotation and Submission Tool) (Tanizawa et al., 2018). For the nitrogen-fixing consortium, strains were cultivated in liquid YEM medium for 4 days at 28 °C on a shaker-incubator to a titre of $1 \cdot 10^6$ CFU/mL, combined, and used for pre-sowing treatment of soybean (*G. max* L.) seeds. Morphometric parameters of the plants were measured at the pod-formation stage, together with microbiological and agrochemical analyses of the rhizosphere soil. Root length, root diameter, stem length, and leaf number were analyzed by one-way ANOVA and Welch's t-test with Bonferroni correction.

Two strains, *Bradyrhizobium* sp. 31 and 82, were isolated from soybean nodules. Strain 31 was phylogenetically related to *B. japonicum* DSM 3014 and *B. japonicum* ACCC 15027 (100 % identity for both), whereas strain 82 was related to *B. diazoefficiens* KG2-6 and *B. diazoefficiens* FK2-4 (100 % identity for both). Whole-genome sequencing identified strain 31 as *B. japonicum* and strain 82 as *B. diazoefficiens* (Delamuta et al., 2013), both recognised as effective soybean microsymbionts. Sequencing yielded a genome of 9.2 Mb in size; its annotation revealed 8,443 protein-coding regions. A check of the genome's completeness indicates that the sequence obtained is 99.24 % complete and contains 0.15 % contamination. The successful identification of slow-growing bradyrhizobia confirmed the targeted isolation of active nitrogen fixers with considerable biotechnological potential.

In the field experiment, seed inoculation with the two-strain consortium significantly increased root length by 57.9 % (179.2 mm in the control vs. 282.9 mm with the inoculant; $p = 0.0015$ after Bonferroni correction; Cohen's $d = 3.08$) and root diameter by 16.4 %, while stem length increased by 2.9 % and leaf number by 3.5 %, the latter two changes being statistically non-significant. This agrees with reports that *B. inoculation* improves nodulation and vegetative growth parameters of soybean under field conditions (Jarecki et al., 2024). Agrochemical analysis of the rhizosphere soil showed a decrease in nitrate nitrogen content from 3.8 to 2.0 mg/kg (–47.4 %), attributable to more intensive uptake by the better-developed root system, while organic matter content increased by 6.1 %. Potassium, sulfur, zinc, and iron contents were slightly lower in the inoculated variant (by 4.6–16.7 %), whereas calcium, magnesium, copper, manganese, boron, cation exchange capacity, and base saturation differed only minimally (within ±3 %). Pre-sowing inoculation increased the abundance of nitrogen-fixing, oligoazotrophic, and phosphate-mobilising microorganisms in the soil and decreased the abundance of oligotrophic microorganisms.

Thus, the identified strains *Bradyrhizobium japonicum* 31 and *B. diazoefficiens* 82 proved promising for forming an effective plant-microorganism symbiosis and showed a phytostimulatory effect on soybean in a field experiment, while positively influencing the microbial and agrochemical dynamics of the rhizosphere soil.

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