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## Metagenomic analysis of bacteria associated with soybean nodules in continuous cropping field

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The continuous cropping often causes soybean yield decline, even when no disease and pest damages are observed. To investigate whether nodule-associated bacteria are involved in this yield decline, we analyzed a metagenome of the bacteria associated with nodules of soybean cultivated in the continuous cropping field. Soybean nodules were sampled from three or six years continuous cropping fields at Sakata city in Yamagata prefecture. These fields were converted from paddy fields. The fields received a basal fertilizer (6 kg N/10a) on May 28, 2013. Soybean (cultivar 'Enrei') was sowed on June 7. Nodule numbers and the relative ureide content of xylem sap were measured on August 21 (R3; beginning pod) and September 12 (R6; full seed). The nodules on R3 stage were used for the metagenomic analysis. The metagenomic DNA of bacteria associated with these nodules were extracted without the cultivation by bacterial enrichment method (Ikeda et al. 2009, *Microb. Ecol.* 58: 703-714). The metagenomic DNAs were paired-end sequenced (2 × 250 bp) by a MiSeq sequencer. To estimate the relative abundances of *Bradyrhizobium diazoefficiens*, *B. japonicum* and *B. elkanii*, the reads were mapped to the concatenated reference sequence that was consisted of three genomes of *B. diazoefficiens* USDA110<sup>T</sup>, *B. japonicum* USDA6<sup>T</sup> and *B. elkanii* USDA76<sup>T</sup> by using the CLC Workbench software. Taxonomic assignment analysis of these reads was performed by using the MG-RAST service (Meyer et al. 2008, *BMC bioinformatics.* 9:386). Yields in 3 and 6 years continuous cropping fields were 335 g/m<sup>2</sup> and 303 g/m<sup>2</sup>, respectively. In addition, nodule numbers and the relative ureide content at R6 stage in the 6 years field were lower than that in the 3 years field. The metagenome analysis successfully showed the fluctuations of species composition of soybean *Bradyrhizobia* and nodule bacterial endophytes along with the continuous cropping, which will be discussed in our presentation.

keywords:Continuous cropping ,Metagenome,Nodule,Soybean,Symbiosis