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Diversity of Nitrogen-Fixing Bacteria Associated with Yams (*Dioscorea* spp.)

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Abstract

Yams are important tuber crops in the tropical zones. Nitrogen is one of the limiting nutrients in yam production. In Africa, where the highest production is held, yam tuber yields are very low due to loss of soil fertility, and to unaffordable and expansive mineral fertilisers which are also not eco-friendly. In this context, identifying yam accessions that can rely on biological nitrogen fixation is necessary for achieving efficient and sustainable yam production. The aim of this study was to isolate and identify endophytic diazotrophic bacteria of yam. Endophytic bacteria were isolated from surface-sterilized roots, stems and leaves of yam accessions grown in nutrient-poor subsoil 160 days after planting. The nitrogen-free MR medium was used for culturing and purifying the endophytic bacteria. Sequencing of the 16S rRNA genes was performed for the fast-growing pure colonies. In total, 41 endophytes were found, belonging to 18 bacterial genera including *Bacillus, Rhizobium, Burkholderia, Curtobacterium, Devosia, Mesorhizobium, Paenibacillus, Sphingomonas, Achromobacter, Bradyrhizobium, Ensifer, Enterobacter, Kocuria, Mycobacterium, Neorhizobium, Pseudaminobacter* and *Pseudoxanthomonas*. Strains of Proteobacteria accounted for 56.10 % of total isolates, followed by Firmicutes (34.15 %) and Actinobacteria (9.75 %). Most endophytes were isolated from stems (21) and roots (14), while only six were from the leaves. This is, to our knowledge, the first report where bacterial strains related to *Curtobacterium* sp., *Ensifer* sp., *Kocuria* sp., *Mesorhizobium camelthorni*, *Mycobacterium cosmeticum*, *Pseudoxanthomonas indica*, *Paenibacillus panacisoli* and *Neorhizobium huautlense* were fast grown on nitrogen-free medium, and their nitrogenase activities assessed through acetylene reduction assay. In *D. esculenta*, the highest nitrogenase activity (57.4 nmol $C_2H_4\,tube^{-1}day^{-1}$.) was recorded in the isolate E1T0R_Y6, belonging to the genus *Bradyrhizobium* sp., while it was 164 nmol $C_2H_4\,tube^{-1}day^{-1}$ in *D. alata*, strain A23T1R_Y40, a strain related to the bacterial genus *Ensifer* sp. The plant growth promotion activities (PGP) of these isolates are to be investigated for selecting most promising nitrogen-fixing bacteria of yams.

Accession numbers of the bacterial 16S rRNA sequences obtained in this were submitted to the DNA Data Bank of Japan (DDBJ).

Keywords: Diazotrophes, *Dioscorea*, endophytes, yams

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