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Community analysis of ciliates in soil based on 18S rDNA

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The interaction between protozoa and bacteria in the soil is very important in agricultural systems. The beneficial effects of protozoa on plant growth have been attributed to nutrients released from the consumed bacterial biomass. However, there are few reports analyzing protozoan diversity in soils. Although culturing techniques and microscopy are commonly used, procedures for isolation of protozoa from soil have not been fully exploited. Molecular techniques offer a powerful tool, which is rapid and high throughput, for analysing the diversity of the microbial community. We designed a ciliate-specific PCR primer, and performed PCR amplification of 18S rDNA of five ciliates, three flagellates, two amoebae, four nematodes, three fungi and one yeast species. Eukaryote 18S rDNAs of all organisms were amplified using the universal primer set EU347F & EU929R (ca 582 bp in length). The PCR bands for ciliates were observed using the ciliate-specific primer set CS322F & EU929R. Unexpectedly, 18S rDNA of one of the tested ciliates (*Dileptus anser*) was not amplified, while that of *Aphelencus avenae* (a nematode) was amplified. The DNA sequence of *A. avenae* is identical to that of the CS322F primer except for one position. The designed ciliate-specific PCR primer is therefore not completely specific for ciliates, but may nevertheless be useful to study the diversity of ciliates. To analyze the communities and diversity of ciliates, and their interaction with bacteria in the soil, we used the ciliate-specific primer and PCR-denaturing gradient gel electrophoresis (DGGE) analysis based on 18S rDNA.