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Background:

Denitrification is a microbial respiratory process contributing to the emission of greenhouse gas. The study of denitrifying bacteria, like that of others, is hindered by characteristics that can prevent up to 99% of soil bacteria from being cultivated *in vitro*. New approaches based on the direct extraction of DNA from the natural environment and PCR amplifications can overcome limitations due to bacterial unculturability, but until now their application to denitrification genes has led only to the recovery of partial sequences for some of these genes.

Objectives:

Our goals in this study were to apply a metagenomic approach characterized by cloning of DNA extracted from soil and screening of metagenomic DNA library clones in order to identify and characterize gene clusters involved in the denitrification process.

Methods:

A technique was developed in which the 77 000 clones of a metagenomic library were spotted on high-density membranes and hybridized with a probe solution consisting of a mixture of oligonucleotides complementary to 14 different genes. The pool of targeted genes included those associated with functions as wide as denitrification, antibiotic resistance, and dehalogenation.

Results:

After hybridization, 134 positive clones were detected out of the 77 000 tested, thus providing a drastic selection process. Positive clone DNA was pooled and pyrosequenced, and sequences compared (BLAST) to those obtained by 454FLX pyrosequencing of the original extracted metagenomic DNA. In the case of the denitrification genes, contig assembly with bioinformatics tools produced 5 contigs containing *nirS*, 4 contigs containing *nirK*, 2 contigs containing *nosZ* and 1 contig containing both *nirK* and *nosZ*.

Conclusions:

This study demonstrates the potential of metagenomic approaches to characterize functional genes present in small populations (<5%) of the soil microbial community.