Characterization of denitrification gene clusters of soil bacteria via a metagenomic approach

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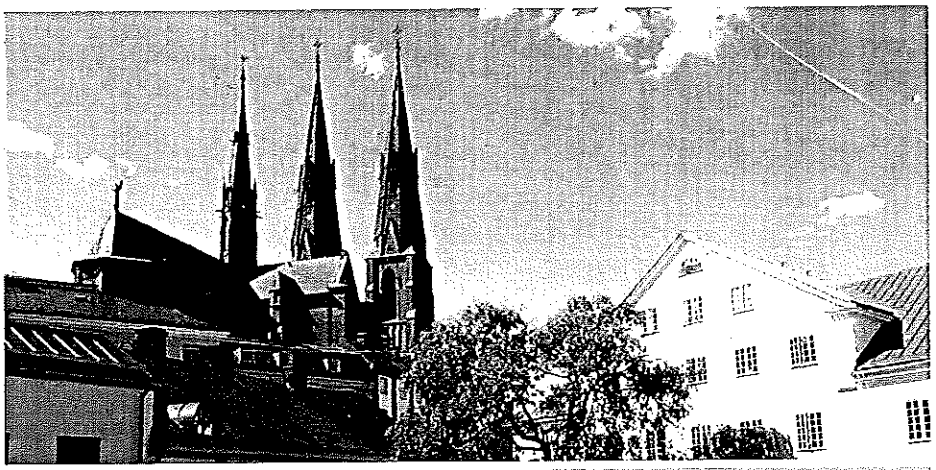


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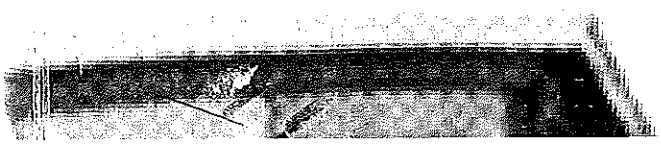

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CHARACTERIZATION OF DENITRIFICATION GENE CLUSTERS OF SOIL BACTERIA VIA A METAGENOMIC APPROACH

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Screening of metagenomic DNA libraries to detect clones whose inserts contain genes of interest is one of the technical challenges related to the development of the metagenomic approach.

A technique was developed in which the 77 000 clones of a metagenomic library are 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.

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