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Visualization and quantification of archaeal and bacterial metabolically active cells in soil using fluorescence in situ hybridization method

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The method of in situ hybridization using fluorescent labeled 16S rRNA-targeted oligonucleotide probes (FISH - fluorescence in situ hybridization) combines identification and quantification of groups of microorganisms at different phylogenetic levels, from domain to species. The FISH method enables to study the soil microbial community in situ, avoiding plating on nutrient media, and allows to identify and quantify living, metabolically active cells of Bacteria and Archaea.

The full procedure consists of the following steps: desorption of the cells from the soil particles, fixation of cells, coating a fixed sample on the glass slide, hybridization with the specific probes and, finally, microscopic observation and cell counting. For the FISH analysis of Bacteria and Archaea, the paraformaldehyde-fixed samples were hybridized with Cy3-labeled Archaea-specific probe(Arc915) and 6-carboxyfluorescein (FAM)-labeled Bacteria-specific probe(EUB338). When a molecular probe is incorporated into a cell, it can hybridize solely with a complementary rRNA sequence. The hybridization can be visualized under the fluorescent microscope and counted.

The application of FISH will be demonstrated by the abundance of metabolically active cells of Archaea and Bacteria depending on soil properties, depth and land use. The research was carried out at field and natural ecosystems of European part of Russia. Samples were collected within the soil profiles (3-6 horizons) of Chernozem and Kastanozem with distinct land use.

Quantification of metabolically active cells in virgin and arable Chernozem revealed that the abundance of Archaea in topsoil of virgin Chernozem was doubled as compared with arable soil, but it leveled off in the deeper horizons. Plowing of Chernozem decreased an amount of archaeal and bacterial active cells simultaneously, however, Bacteria were more resistant to agrogenic impact than Archaea.

In Kastanozem, a significant change in the abundance of metabolically active cells due to plowing was detected only within 40 cm soil layer, and this effect disappeared in lower horizons. The abundance of Archaea was higher in the upper horizons of arable soil as compared to virgin. Conversely, the abundance of Bacteria in the upper layers of arable Kastanozem decreased versus virgin soil. A relationship between soil organic carbon and the amount of soil metabolically active Bacteria and Archaea cells revealed that distribution of both Bacteria and Archaea throughout the soil profile was governed mostly by the organic matter content. Thus, the organic matter was a main factor of declining the Bacteria:Archaea ratio with the soil depth (from 7.1 to 4.2 for virgin soil and from 5 to 3.9 for arable soil). As a result, Archaea out-compete Bacteria under conditions of reduced energy supply.

Thus, the FISH method combines classical microscopic and modern phylogenetic microbiological approaches and can be considered as an effective tool for ecological, diagnostic and environmental research in microbiology.