A simple absolute quantification method for routine analysis of taxon-specific RT PCR data characterizing the fraction of archaeal DNA in soil samples

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Analysis of microbial communities of soils is necessary to study the relationship of their productivity with the composition of their microflora and to establish the main environmental roles, mainly performed by different taxa of microorganisms. Archaea make a significant share in soil microflora, participating in carbon and nitrogen cycles, however the specificity of their functions in soil microbial communities remains largely unclear. For screening of soil samples on the content of archaeal DNA need a convenient method to compare their number in the standard conditions of isolation of total DNA and of the analysis. Absolute quantitative data are preferable for studying archaea population dynamics and its comparison in different locations. To date, taxon-specific pairs of primers have been developed that recognize the genes of 16S rRNA of the Archaea domain. In this study, to calculate the absolute concentration of archaeal DNA, taking into account some important errors, we used the formula (fig. 1) based on data from a DNA sample with a known molar concentration. The equation is based on the equality of the fluorescence values of the standard and the studied samples at the intersection of the fluorescence threshold line. It takes into account the differences in the factors of amplification and amplicon lengths for standard and test samples. The method of determining the relative representation of the lower order taxon among the representatives of the higher-order taxon was taken as a basis. The proposed method does not claim to overcome all the systematic errors arising when they use RT-PCR in the analysis of microbial communities, but also does not contradict the basic principles adopted for the analysis of data obtained in the application of RT-PCR or various tasks. In the analysis of a black soil sample using standard lambda phage DNA, a value of 0.74 p.mol/mg for archaeal 16S rRNA genes in the soil was obtained.

Recent Publications

1. Bengtson P, Sterngren A E and Rousk J (2012) Archaeal abundance across a pH gradient in an arable soil and its relationship to bacterial and fungal growth rates. Applied and Environmental Microbiology, 78:5906–5911.
2. Bayer K, Kamke J and Hentschel U (2014) Quantification of bacterial and archaeal symbionts in high and low microbial abundance sponges using real-time PCR. FEMS Microbiology Ecology, 89: 679–690.
3. Bacchetti De Gregoris T B, Aldred N, Clare A S and Burgess J G (2011) Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa. Journal of Microbiological Methods, 86: 351–356.
4. Pfaffl M W (2004) Quantification strategies in real-time PCR. International University Line, La Jolla, USA 3:87-112.
5. Bustin S A, Benes V, Nolan T and Pfaffl M W (2005) Quantitative real-time RT-PCR – a perspective. Journal of Molecular Endocrinology 34:597–601.

Biography

Konstantin S Boyarshin has gained experience in Enzymological research, performing a PhD project on the study of mechanisms of products editing by aminoacyl-tRNA synthetases. Currently specializes in the study of complex microbial communities and the use of microbial preparations for the needs of agriculture and alternative energy. Develops principal approaches for the effective use of the results of fundamental research in applied science.

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