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Sequence-Specific Cleavage of Small-Subunit (SSU) rRNA with Oligonucleotides and RNase H: a Rapid and Simple Approach to SSU rRNA-Based Quantitative Detection of Microorganisms

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A rapid and simple approach to the small-subunit (SSU) rRNA-based quantitative detection of a specific group of microorganisms in complex ecosystems has been developed. The method employs sequence-specific cleavage of rRNA molecules with oligonucleotides and RNase H. Defined mixtures of SSU rRNAs were mixed with an oligonucleotide (referred to as a "scissor probe") that was specifically designed to hybridize with a particular site of targeted rRNA and were subsequently digested with RNase H to proceed to sequence-dependent rRNA scission at the hybridization site. Under appropriate reaction conditions, the targeted rRNAs were correctly cut into two fragments, whereas nontargeted rRNAs remained intact under the same conditions. The specificity of the cleavage could be properly adjusted by controlling the hybridization stringency between the rRNA and the oligonucleotides, i.e., by controlling either the temperature of the reaction or the formamide concentration in the hybridization-digestion buffer used for the reaction. This enabled the reliable discrimination of completely matched rRNA sequences from single-base mismatched sequences. For the detection of targeted rRNAs, the resulting RNA fragment patterns were analyzed by gel electrophoresis with nucleotide-staining fluorescent dyes in order to separate cleaved and intact rRNA molecules. The relative abundance of the targeted SSU rRNA fragments in the total SSU rRNA could easily be calculated without the use of an external standard by determining the signal intensity of individual SSU rRNA bands in the electropherogram. This approach provides a fast and easy means of identification, detection, and quantification of a particular group of microbes in clinical and environmental specimens based on rRNA.

Microorganisms are an essential component of the earth's biota, playing integral roles in ecosystems in terms of function and sustainability. To unambiguously understand these roles, extensive studies of the microbial ecology of systems such as aquatic environments, soils, subsurfaces, and animals have been carried out. Due to such studies, there is now a much better understanding of microbial diversity as well as the functions of each microbial constituent and of the nature of the interactions among individual members (and environments or hosts) in various ecosystems. The recent accumulation of such knowledge in the field of microbial ecology can be attributed to a great extent to the development and application of molecular techniques in environmental microbiology, particularly those based on small-subunit (SSU) rRNA and the rRNA gene (6). Among rRNA-rRNA gene-based techniques developed to date, the use of group-specific DNA probes complementary to SSU rRNA provides the most powerful tool to precisely identify different populations in complex systems (3, 6). For example, whole-cell in situ hybridization based on SSU rRNA is now commonly used to detect specific groups of microbes and to quantify populations of interest in environments by direct counting (2). Another example of a method based on SSU rRNA is the quantitative membrane hybridization of labeled DNA probes to community rRNAs (43, 50). This method has also been applied to various environmental rRNAs for the quantitative detection of specific groups of microbes present in complex communities (21, 37, 38, 44, 45). More recently, an oligonucleotide-based DNA microchip format targeting multiple rRNA molecules is being developed, providing a powerful framework for the parallel hybridization of different rRNA fragments to a matrix array of DNA probes (15, 18, 30, 32, 52, 53). However, in spite of the potential advantages of these techniques, all of them are often laborious and require time-consuming procedures in general, particularly when they are employed for the quantitative detection of specific microbial groups in natural populations (5, 10, 55). To precisely and rapidly evaluate the abundance and activity of selected groups of microbes in complex ecosystems, more direct, rapid, simple, quantitative, and cost-effective tools which can be applied to various types of heterogeneous environments should be developed.

Here, we report the concept of sequence-specific cleavage of rRNA fragments using oligonucleotides and RNase H as a rapid and easy means of rRNA-based microbial identification, detection, and quantification (Fig. 1). RNase H is known to specifically degrade the RNA strand of RNA-DNA hybrid duplexes (9, 22, 24). By using this method, total RNAs from

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