

Microbial identification by mass cataloging.

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BACKGROUND: The public availability of over 180,000 bacterial 16S ribosomal RNA (rRNA) sequences has facilitated microbial identification and classification using hybridization and other molecular approaches. In their usual format, such assays are based on the presence of unique subsequences in the target RNA and require a prior knowledge of what organisms are likely to be in a sample. They are thus limited in generality when analyzing an unknown sample. Herein, we demonstrate the utility of catalogs of masses to characterize the bacterial 16S rRNA(s) in any sample. Sample nucleic acids are digested with a nuclease of known specificity and the products characterized using mass spectrometry. The resulting catalogs of masses can subsequently be compared to the masses known to occur in previously-sequenced 16S rRNAs allowing organism identification. Alternatively, if the organism is not in the existing database, it will still be possible to determine its genetic affinity relative to the known organisms.

RESULTS: Ribonuclease T1 and ribonuclease A digestion patterns were calculated for 1,921 complete 16S rRNAs. Oligoribonucleotides generated by RNase T1 of length 9 and longer produce sufficient diversity of masses to be informative. In addition, individual fragments or combinations thereof can be used to recognize the presence of specific organisms in a complex sample. In this regard, 140 strains out of 1,921 organisms (7.3%) could be identified by the presence of a unique RNase T1-generated oligoribonucleotide mass.

Combinations of just two and three oligoribonucleotide masses allowed 54% and 72% of the specific strains to be identified, respectively. An initial algorithm for recovering likely organisms present in complex samples is also described.

CONCLUSION: The use of catalogs of compositions (masses) of characteristic oligoribonucleotides for microbial identification appears extremely promising. RNase T1 is more useful than ribonuclease A in generating characteristic masses, though RNase A produces oligomers which are more readily distinguished due to the large mass difference between A and G. Identification of multiple species in mixtures is also feasible. Practical applicability of the method depends on high performance mass spectrometric determination, and/or use of methods that increase the one dalton (Da) mass difference between uracil and cytosine.

PMID: 16524471 [PubMed - indexed for MEDLINE]