Background. The 16S rRNA gene is commonly used to identify Mycobacterium spp. in the clinical laboratory, but sequencing multiple DNA targets can provide better species resolution. RipSeq Dual Loci [Sintino, Bergen, Norway] is a software program that decodes mixed electropherograms enabling a laboratory to perform multi-locus sequencing in a single tube. We evaluated the ability of RipSeq to simultaneously analyze 16S rRNA and rpoB gene sequences for mycobacterial identification. Methods. Isolates identified as Mycobacterium spp. by partial 16S rRNA sequencing at ARUP Laboratories were obtained. Amplification was performed on lysates with primers targeting 500bp of 16S rRNA and 700bp of rpoB genes. Sequencing was performed with forward and reverse primers for each target. Electropherograms were analyzed with RipSeq. Final identification was determined by separate phylogenetic analyses of individual 16S rRNA and rpoB sequences. 149 Mycobacterium spp. were determined by lack of heterogeneity in the 16S rRNA gene. Many studies have shown that alternative targets such as the rpoB gene, may be more useful because they move at a faster evolutionary rate. Routine implementation of alternative targets is limited by reference databases and an uncertainty about the expected intra-species variability for each DNA target. Optimally both the 16S rRNA gene and an alternative target could be utilized to identify mycobacteria, but increased workflow and cost prevents this laboratory practice. In this study we explored a single reaction approach that allows for simultaneous amplification and sequence of the 16S rRNA and the rpoB genes, using a novel piece of software called RipSeq that can interpret mixed electropherograms generated by dual-locus sequencing.

RESULTS

Simultaneous Sequence Analysis of Two Loci to Identify Mycobacterium spp.

Keith E. Simmon1, Ø. Kommedal2, Ø. Saebø2, B. Karløsen2, and C. A. Petti1,3

1Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology, Salt Lake City, UT, 2iSentio, Bergen, Norway, and 3Departments of Medicine and Pathology, University of Utah School of Medicine, Salt Lake City, UT

CONCLUSIONS

• RipSeq Dual Loci was an effective tool for simultaneous analysis of dual loci sequences of 16S rRNA and rpoB genes.
  • This application provided greater resolution to species for 59 (42%) isolates, eliminating the need for reflex testing to discriminate between closely related species. Most notably RIPSeq identified all members of M. chelonae / abscessus complex, and distinguish between M. macrogenicum and M. phocaicum.
  • The 16S rRNA gene was required to identify 14 (10%) isolates because of insufficient rpoB data available.
  • Incorporation of dual loci sequencing has relatively low impact on current sequencing workflow and requires no additional cost in reagents and consumables, with the exception of primers.
  • Limitations included:
    - Database has to be manually updated and is not tied directly to Genbank.
    - Consensus sequence cannot be constructed.
    - Differences in signal strength between the two gene targets in RipSeq often resulted in lower identity scores for one of the targets, but identification was not compromised. We recommend optimizing the amplification and sequencing procedures to obtain good signal for both targets.
  • RipSeq provides a powerful tool to incorporate new DNA Targets with better resolution for identification without the need to extensively curate a database or sacrifice the experience and knowledge gained from using the 16S rRNA gene for identification.