fied as *Salmonella enterica* subspecies *enterica* (9, 10).

Ribosomal DNA sequence-based methods are currently widely accepted as rapid and accurate techniques for determining the taxonomic position of unknown bacteria. However, the interspecies distinction in a genus is almost impossible because of their high sequence similarities. For example, we found that all established species in the genus *Klebsiella* have more than 98% similarity in their 16S rDNAs.

We previously reported a highly quantitative fluorometric microtiter plate hybridization method that uses chromosomal DNA-DNA hybridization to differentiate species such as these that share almost identical 16S rDNA sequences (8). Our current microarray technique is an even more promising method because much less DNA is required. This new microarray technique utilizes thousands of individual DNAs printed in a high-density array on a glass slide. Using this microarray technique, we were able to hybridize the unknown labeled DNAs to DNAs from all 5,000 established bacterial species at once.

Generally, chromosomal hybridization has been used only in some taxonomy laboratories to determine bacterial species because highly purified DNA has been necessary (1, 2, 5, 6, 16, 28, 29). Modern taxonomic guidelines suggest that DNA-DNA similarity values among strains within an established species should be more than 70% under optimal conditions (12, 31). This indicates species are considered new when the strains share less than 70% similarity with established species.

Based on the G+C content of the DNAs from the *Enterobacteriaceae* family, the optimal temperature for DNA-DNA hybridization should be approximately 36 C in the 50% formamide-containing solution. However, we used 45 to 50 C as the hybridization temperature to increase the stringency of the experiment and to minimize cross-hybridization. By employing this stringent condition, most established species among *Enterobacteriaceae* were found to share less than 30% similarity on the microarray. Our final conditions for hybridizing to DNA from members of *Enterobacteriaceae* on the microarray included 0.5 mg/ml DNA concentration for spotting and hybridization for 120 min at 45 C in 2× SSC containing 50% formamide.

Values of chromosomal DNA similarity reported by multiple laboratories have differed even when the same strains have been used because each laboratory has used their own method for determining chromosome similarity. Thus, the Ad Hoc Committee of International Systematic Bacteriology had released recommendation that the DNA sequence for at least five housekeeping genes be used for taxonomy as alternative to using chromosomal DNA-DNA similarity (28). *rpoB*, *gyrB*, ITS, and *groEL* are candidate because they have larger sequence variation than the 16S rDNA sequence (17). The *rpoB* sequence of members of the *Enterobacteriaceae* family has been determined in detail and has proven to be promising for differentiating closely related species within this family *Enterobacteriaceae* (20). We have recently reported the variation in the *dnaJ* sequence among the genus *Legionella* is another candidate for classification (19). Our ongoing sequencing project for the members of family *Enterobacteriaceae* has, in fact, shown that the *dnaJ* sequence is much more variable than *rpoB* (our unpublished observations). However, we still do not have enough data to definitively establish a species using this gene. Until enough sequence data for these multilocus genes is accumulated and is used for setting new criteria for species, the chromosomal DNA-DNA similarity value will remain important for the identification and classification of bacteria.

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