

# DuPont Qualicon RiboPrinter® System

## APPLICATION PROFILE

## Brewer Discovers Two Novel Beer Spoilage Species and the Best Way to Profile Their Microbial Environment

With the help of accurate and in-depth microbial information, a whole new world of organisms is beginning to be uncovered. Which technology best provides this kind of information?

To find out, researchers at Asahi Brewing in Japan recently conducted a survival study of environmental organisms using several molecular methodologies including the RiboPrinter® Microbial Characterization System. Their findings include not only two previously unknown species of beer-spoilage *Lactobacilli*, but also some startling comparisons with other methods of microbial analysis.

For the study, Asahi spiked their beer with 170 strains of *Lactobacillus*. Of these 170 strains, 55 were able to survive. The survivors included 45 strains of *L. brevis*, three strains of *L. lindneri*, one strain of *L. casei*, and six unknown strains that were not identified by either PCR or a fluorescent staining assay. The RiboPrint® patterns of these same six did not match any of the other *Lactobacillus* RiboGroups. Instead, they formed two new RiboGroups consisting of three strains each, which Asahi called LA-2 and LA-6 respectively.

RiboGroups are created by the system when related patterns fall within specifically defined similarity threshold limits. Any pattern that does not meet an established RiboGroup's limit triggers a new RiboGroup.

### New levels of information

Researchers also examined these isolates using additional techniques. 16S rDNA sequencing indicated that LA-2 has a >99.5% similarity to *L. collinoides*. Although these results from the 16S rDNA sequencing indicated a high level of relatedness, the RiboPrint® patterns for LA-2 were distinctively different from the database pattern for *L. collinoides*. (See Figure 1). This showed the Asahi researchers the much finer distinction available with the below-species level information that would allow tracking of organisms specific to their environment.

In the case of LA-6, 16S rDNA sequencing could not match the isolates to any organism and therefore offered no information on the isolates.

Asahi Brewing's Quality Assurance could now monitor these two environmental spoilage organisms by routine use of the RiboPrinter® system.



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## Brewer endorses system

At a recent gathering of brewing chemists, Asahi presented this survival study and other work that showed the patterns of five different *Lactobacillus* species plus two species of *Pectinatus*, a genus of Gram-negative bacteria that has been isolated from spoiled beers. This second study compared PFGE, plasmid profiling, arbitrary primer PCR and automated ribotyping using the RiboPrinter® System.

Of these various assays, Asahi enthusiastically endorsed the RiboPrinter® system based on its ease of use, time to results, efficiency and reproducibility, in addition to the advantages of being able to automatically compare data from previously run samples.

Asahi's work also reaffirmed the utility of the RiboPrinter® system's restriction enzyme flexibility. They were able to show increased differentiation of strains with *Hind* II and *Bam*HI restriction endonucleases, which would further extend the system's discrimination capability for *Lactobacilli*, which are of particular interest to the brewing industry.

## Conclusion

Since its commercialization two years ago, the RiboPrinter® Microbial Characterization System has been adopted by leading brewers around the world. The system's flexibility, accuracy, speed and ability to share information have made it a popular choice for creating a microbial profile of the brewing environment.

The results of the Ashai study, presented at the 1997 ASBC annual meeting, concluded that the RiboPrinter® Microbial Characterization System is a powerful tool for analyzing microorganisms found in brewing. The instrument is of special use in identifying beer spoilage bacteria, contamination sources and in building a custom database of organisms to determine whether particular bacteria have been encountered in the past or not.

Faced with the need to control spoilage organisms and pathogens in their production processes, brewers are using the information from the RiboPrinter® system to control costs, target decontamination and manage their inventory.

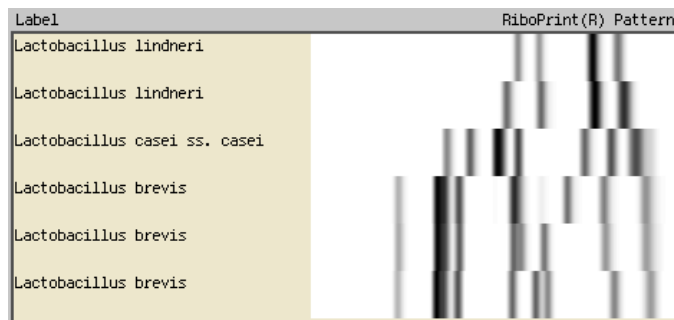


Figure 1. In addition to the six standard *Lactobacillus* strains represented above, researchers at Asahi Breweries, Ltd. isolated six additional beer-spoilage *Lactobacilli* that are considered to belong to novel species. The brewer's scientists subsequently developed primer sets for the detection of these strains.

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