

DuPont Qualicon RiboPrinter® System

APPLICATION PROFILE

Easy, Reliable and Fast Method Tracks Probiotic Strains of *Lactobacillus* with DNA Fingerprinting

Researchers who routinely study probiotics require dependable ways of identifying microorganisms below the species level. Until recently, these scientists have been limited by the instruments and tests available to them. These lab tools and techniques could not achieve the level and consistency of information that is now possible using genetics-based methods for recognizing bacteria.

However, DNA-based microbial analysis has been complex and slow. Its data was rarely reproducible from one lab to another even though the method was performed by highly trained and skilled microbiologists. These problems have now been overcome with the availability of an automated, tabletop instrument that is capable of fingerprinting virtually any bacterium, the RiboPrinter® Microbial Characterization System.

A recent study by a leading research institute, VTT Biotechnology and Food Research, Espoo, Finland along with Valio Ltd. and the Harjula Hospital also in Finland, demonstrated the RiboPrinter® system's ability to provide the needed sub-species information on an important probiotic organism. *Lactobacillus rhamnosus* GG (*L. rhamnosus* GG), one of the most often studied probiotic strains, was the focus of the work by the trio of Finnish labs.

Because the organism can survive and colonize the gastrointestinal tract in both adults and children, it is important in *L. rhamnosus* GG research — as well as in research on other probiotic organisms — to find methods capable of tracking the bacterium through the intestines. The often-weak reactions of many biochemical tests that depend on growth media and exact cultural conditions complicate any study of *L. rhamnosus* GG.

With few dependable ways to verify its presence, especially after several weeks in the human digestive system, identification of *Lactobacillus* species is usually difficult.

Finnish study structure

For the Finnish study, volunteer colonoscopy patients consumed a commercial product containing *L. rhamnosus* GG twice a day for 12 days. Test subjects were divided into three groups:

- Those subjects whose colonoscopy was performed immediately after consumption of the product;
- Subjects who had the procedure a week after consumption;
- And those who had a two-week break between administration of the organism and colonoscopy



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Fecal and biopsy samples were cultivated. Typical *L. rhamnosus* GG colonies were counted and a representative number were purified and verified using traditional assays based on the ability of the organism's ability to ferment lactose (*L. rhamnosus* GG is distinguished from most other of its species by its inability to ferment lactose.) Isolates were also tested using species-specific primers capable of discriminating between other lactobacilli and lactic acid bacteria by polymerase chain reaction (PCR). The capability of the ribotyping technique with the RiboPrinter® system to further distinguish *L. rhamnosus* GG from other strains of the same species was examined using pure cultures.

The results

Results showed that in all patients — even those who had consumed the bacteria two weeks before testing — *L. rhamnosus* GG was the dominant fecal lactic acid bacterium. The DNA fingerprints produced by the RiboPrinter® system were easily distinguishable from the fingerprints of other species. Not only did RiboPrint® patterns clearly distinguish these *Lactobacilli*, the consistently reproducible patterns were available within hours. In addition, the instrument could be operated by a technician, which freed highly skilled researchers for other work. The RiboPrinter® system also did not require the painstaking development of primers, which was necessary with the PCR assay.

The results of this study further showed that this organism was well able to adhere *in vivo* to the colon and remain there for a long time. Researchers in this study were pleased to find a dependable, automated, reproducible and easy way of confirming this important probiotic strain. Without this in-depth information from genetics-based testing, it would be difficult — perhaps impossible — to identify a probiotic strain.

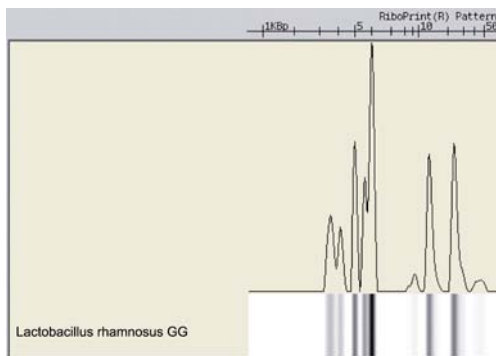


Figure 1. (above) In all patients — even those who had consumed the bacteria two weeks before testing — *L. rhamnosus* GG was the dominant fecal lactic acid bacterium. The DNA fingerprints produced by the RiboPrinter® system (above) were easily distinguishable from the fingerprints of other species.

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