Letter to the Editor

“Differentiation between Types and Strains of Clostridium botulinum by Riboprinting,”

SEBASTIAN HELM, JOHANNA BJÖRKRÖTH, AND HANNU KORKEALA
Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, P.O. Box 57, FIN-00014 Helsinki University, Finland

We believe that some of the materials, and hence the results and conclusions, of this study are questionable, and we would like to point out and comment on those we regard as being of greatest significance. Also, the authors wrongfully belittle our earlier work on Clostridium botulinum PFGE typing (1) and ribotyping (2). This concerns us because we consider our studies to be far more extensive in both materials and methodological approach than this paper.

Identity of strains. With regard to ribotyping, the authors conclude that some proteolytic (group I) and nonproteolytic (group II) strains of C. botulinum belong to the same ribogroup. This finding, which we believe is likely to stem from a laboratory mix-up of the experimental strains, is taken as proof of the method’s inability for taxonomic deduction and its ability for strain characterization. The findings presented in this paper contradict all earlier works on clostridial ribotyping and phylogeny, which clearly state that C. botulinum groups I and II are two distinct species. Thus, they could hardly be placed in the same ribogroup. In ribotyping 73 (versus 31 in the discussed paper) C. botulinum strains (2), we used the same restriction enzyme as the Riboprinter does (EcoRI), as well as an additional one (HindIII), and found that the two genogroups were always easily distinguished. In EcoRI ribotyping, group II C. botulinum strains, regardless of toxin type, are easily recognized by one very heavily stained 3.6-kb fragment. Figure 1 in the paper shows this fragment in the profiles of ribogroups 121-4 and 152-2, whereas it is absent in the profiles of the supposed three group II strains contained in ribogroup 121-7. We have also genotyped strains 2-B and 17-B, which the authors claim are different, and we found them to be clonal not only by ribotyping but also by PFGE, Rep-PCR, and AP-PCR. The authors attribute their differing results to the differences in experimental conditions between manual and automated ribotyping; however, comparing the paper’s profiles to ours shows that this is not the case (2). In our experience with several bacterial species, the ribotyping profile of a single strain basically looks the same whether generated manually or with the Riboprinter.

Type E strain typeability. The authors state that the Riboprinter could type only 2 of 13 type E strains included (but not named) in the experiments, then they make some interesting parallels to our PFGE paper (1). They correctly state that we were able to type only 1 of 21 group II strains using a standard protocol, but they fail to mention that these DNase problems were overcome with a formaldehyde fixation step. In effect, we were able to type all but 1 strain, enabling us to effectively characterize 20 strains and to estimate the size of the group II genome. However, the DNase problem persists in some 10% of group II C. botulinum strains, which is why we proceeded to develop the ribotyping protocol for C. botulinum. Ribotyping (at least the manual type) was not hindered by DNA degradation because the C. botulinum genome fragments generated by a frequently cutting restriction enzyme such as EcoRI are far smaller than those resulting from DNase disruption. We did all of our ribotyping on formaldehyde-fixated DNA in agarose plugs used for PFGE typing and, regardless of DNase activity, we never encountered any typeability problems.

Other remarks. (i) The authors state that the ribotyping profile of Clostridium sporogenes resembled that of many type A strains. In fact, the profile was just as closely linked to three ribogroups of type B strains, including one containing nonproteolytic isolates (121-7), as seen in Figure 3. (ii) The authors idle at some length about the possible relation of six strains isolated from mushroom sources. Obviously, any relation found would be linked only to time and place because there is no active contamination of mushrooms on behalf of C. botulinum. (iii) According to the authors, the strain supposed to be Garlic-B presents a very distinct ribotype pattern. Once again we have to conclude that in our experience the profile presented in the paper (Fig. 1) does not belong to any strain of C. botulinum.

Summary. In light of so many conflicting results, we recommend that the authors verify the taxonomy of the experimental strains, using the mouse bioassay or BoNT-PCR for toxin type detection combined with plating on milk agar to detect possible proteolysis, before proceeding to incorporate these profiles into the Riboprinter database. We acknowledge that correct identification is a universal problem concerning the widely dispersed C. botulinum strain collections, but it is nevertheless a problem that could be alleviated by a collaborative research effort between laboratories capable of clostridial genotyping. However, the final attempt to justify the conflicting data by misquoting and disregarding other published scientific articles is simply unethical.

REFERENCES
Response

Guy E. Skinner, Steven M. Gendel, and Haim M. Solomon
Food Process Hazard Analysis Branch, Division of Food Processing and Packaging, Food and Drug Administration and National Center for Food Safety and Technology, 6502 South Archer Road, Summit-Argo, Illinois 60501, USA

We appreciate the comments of Hielm et al. and are pleased that they found the results of our manuscript (2) so interesting. As stated in our paper, the goal was to determine the efficacy of using the Qualicon Microbial Characterization System for strain differentiation with C. botulinum. As such, we were not attempting to duplicate or improve the isolation procedures described by Hielm et al., but to assess the results obtained with a standard procedure. We were as surprised as Hielm et al. at the pattern groupings obtained using this standard procedure and the Qualicon riboprinter. In fact, we cite their papers as the basis for our surprise. It should be noted that there are other reports showing differences in strain differentiation when using manual and automated ribotyping. For example, Dalsgaard et al. (1) showed that traditional ribotyping for Vibrio cholerae had greater discriminatory power than automated ribotyping with two different restriction enzymes. In addition, it is important to remember that relationships among fingerprint patterns (obtained with any technique) do not necessarily reflect the underlying genomic relationships and that relationships deduced from either sequencing or fingerprinting one small part of the genome (such as the ribosomal genes) do not necessarily reflect relationships for the rest of the genome.

Finally, we would like to assure Hielm et al. that, in addition to receiving freshly harvested spore crops for each of our inoculation studies, we use all appropriate microbiological procedures (including toxin typing by the mouse bioassay) to confirm and preserve strain identities. In fact, one of the potential applications we envisioned for automated ribotyping was strain identification within a culture collection and comparison between culture collections. We recognize this as an area of great concern, and Hielm et al. mention this as a universal problem in their comments as well.

REFERENCES
