Applied and Environmental Microbiology, Oct. 1997, p. 3992–3994 Vol. 63, No. 10
0099-2240/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Ribotyping for Strain Characterization of *Clostridium perfringens* Isolates from Food Poisoning Cases and Outbreaks

B. Schalch,[1] J. Björkroth,[2] H. Eisgruber,[1] H. Korkeala,[2] and A. Stolle[1]*

Institute for Hygiene and Technology of Foods of Animal Origin, Veterinary Faculty, Ludwig-Maximilians-University, 80539 Munich, Germany,[1] and Department of Food and Environmental Hygiene, Helsinki University, Helsinki, Finland[2]

Received 19 February 1997/Accepted 14 July 1997

Ribotyping was used to characterize 34 *Clostridium perfringens* strains isolated from 10 food poisoning cases and outbreaks over a 7-year period. Twelve different ribopatterns were generated by *Eco*RI digestion. In eight food poisoning cases and outbreaks, all of the ribotypes of each food and stool isolate were found to be identical. Two *C. perfringens* isolates showed unique patterns. Ribotyping was found to be a useful tool for determining the genetic relationship of *C. perfringens* isolates in the context of foodborne poisoning cases.

Clostridium perfringens is a gram-positive, spore-forming, anaerobic rod. This bacterium can be responsible for food spoilage and can produce an enterotoxin (CPE) which is released upon lysis of the vegetative cell during sporulation in the intestinal tract. CPE causes food poisoning in humans and some animals (2, 8, 10). *C. perfringens* is also a part of the normal intestinal flora. Therefore, typing of *C. perfringens* is of great importance for investigating food poisoning sources and for studying the epidemiology of this microorganism.

Phenotypical differentiation procedures as well as DNA-based typing methods have been used successfully for strain differentiation of *C. perfringens*. Among them were plasmid isolation (3, 7), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6), and ribotyping of patients' and hospital environment isolates (4). This report describes the examination of 34 food poisoning-related *C. perfringens* isolates by ribotyping (5).

MATERIALS AND METHODS

Strains. Table 1 shows the 34 *C. perfringens* isolates investigated from 10 foodborne outbreaks and cases which occurred between 1984 and 1991 in Eastern Germany. Two to six isolates from foods and patient stool samples were available for each outbreak and case. Isolates were cultured, purified, identified, and kept in a Microbank storage system (Mast Diagnostica, Reinfeld, Germany) at −18°C as described by Eisgruber et al. (3). Before the ribotyping, the purity of the isolates was reensured by culture of the isolates twice on Columbia sheep blood agar (Unipath, Ltd., Basingstoke, Hampshire, United Kingdom).

Ribotyping. Ribotyping was carried out according to the method described by Grimont and Grimont (5). Clostridia were grown anaerobically overnight in brain heart infusion broth (Unipath Ltd.). An aliquot of 1.5 ml of that suspension was centrifuged (13,000 × *g*, 10 min). DNA was isolated by the guanidium thiocyanate method of Pitcher et al. (9) with the modifications described by Björkroth and Korkeala (1). Five micrograms of DNA was cleaved with *Eco*RI (Boehringer Mannheim GmbH, Mannheim, Germany) according to the manufacturer's instructions. The DNA concentrations were determined with a UV spectrophotometer (UV/VIS spectrometer Lambda 2; Perkin-Elmer, Norwalk, Conn.). DNA fragments were separated in 0.8% agarose gels (16 h, 25 V). Digoxigenin (DIG)-labeled phage lambda DNA (Boehringer Mannheim GmbH) was used as a molecular size marker. After gel electrophoresis, DNA fragments were transferred by the method of Southern (11) to a nylon membrane (Sigma-Aldrich, Deisenhofen, Germany). The membrane was air dried first, followed by DNA fixation at 180°C for 0.5 h. The membrane was sealed in a plastic bag and prehybridized for 2 to 4 h in a 58°C water bath. Hybridization with the DIG-labeled probe was carried out overnight at 58°C. The solutions used for hybridization, washes, and detection of the DIG label were the ones described for the DIG DNA labeling and detection kit (Boehringer Mannheim GmbH).

DIG-labeled probe. The DNA probe was prepared from *Escherichia coli* 16S and 23S rRNA (Boehringer Mannheim GmbH) by reverse transcription and labeled by incorporation of DIG-dUTP with avian myeloblastosis virus reverse transcriptase according to the manufacturer's instructions.

Evaluation of ribotyping results. The pattern was read visually. Patterns of the isolates were compared within each case and outbreak, respectively, and with all other isolates. All 34 *C. perfringens* isolates were analyzed repeatedly to test the reproducibility of the results. The DNA of each strain was prepared three times, and the samples were electrophoresed on different gels.

RESULTS

Altogether, 12 distinct ribotype patterns were found among the 34 *C. perfringens* isolates analyzed. Those 12 patterns were clearly reproducible in three different runs. Patterns differing by one or more bands were considered different. An example of 14 ribotyping patterns derived from three outbreaks (I, III, and IX) is given in Fig. 1. The results of ribotyping are listed in Table 1, together with the results obtained by plasmid profiling, as previously described by Eisgruber et al. (3).

In 8 of 10 outbreaks and cases, identical ribotype patterns for all isolates were detected. Two unique ribotypes were found in outbreaks III and IX. Outbreak III showed five identical ribotypes among six isolates derived from foods and feces. One feces isolate (954/85) revealed a different pattern.

In outbreak IX, all four fecal isolates showed identical ribotypes. However, the *C. perfringens* strain isolated from the presumptive outbreak related to chicken fricassee (174/90) had a different and unique pattern.

All results were clearly reproducible. The strains gave identical ribotype patterns in three runs on different membranes.

DISCUSSION

DNA-based typing of isolates is of great importance for tracking epidemiological and causal relationships within given food poisoning outbreaks. In this study, the use of ribotyping for the differentiation of *C. perfringens* was investigated by using food and stool isolates from 10 clinical food poisoning outbreaks and cases.

The *C. perfringens* strains of all 10 outbreaks and cases showed different ribotype patterns. Within eight outbreaks and cases (I, II, IV to VIII, and X), all isolates had identical ribotypes. These results are consistent with the findings of a previous study (3), in which identical or very similar plasmid

* Corresponding author. Mailing address: Institute for Hygiene and Technology of Foods of Animal Origin, Veterinary Faculty, Ludwig-Maximilians-University Munich, Veterinärstr. 13, 80539 Munich, Germany. Phone: 49-89-2180-2522. Fax: 49-89-2180-3872. E-mail: lmhyg@lrz.uni-muenchen.de.

Downloaded from http://aem.asm.org/ on October 29, 2015 by guest

TABLE 1. *C. perfringens* isolates from food poisoning outbreaks

Outbreak or case	Strain	Origin of strain	Ribotype	Plasmid(s) (mol mass [MDa])
I	721/84	Rabbit meat	I	7.1
	731/84	Feces	I	7.1
	732/84	Feces	I	7.1
II	310/85	Rabbit meat	II	28.5
	313/85	Feces	II	28.5
	314/85	Feces	II	28.5
III	945/85	Heart goulash	III	25.5, 22.5
	948/85	Cauliflower salad	III	27.0
	949/85	Cauliflower salad	III	27.0
	953/85	Feces	III	25.5, 22.5
	954/85	Feces	XI	21.0
	955/85	Feces	III	22.5
IV	10/86	Pork	IV	28.5, 8.4, 6.6
	18/86	Feces	IV	8.4, 6.6
	26/86	Feces	IV	28.5, 8.4, 6.6
V	349/86	Beef	V	30.5
	344/86	Feces	V	30.5
	345/86	Feces	V	30.5
VI	834/87	Pea mash	VI	5.6
	836/87	Feces	VI	29.5, 5.6
VII	216/88	Poultry meat	VII	0
	227/88	Feces	VII	0
	231/88	Feces	VII	0
	234/88	Feces	VII	0
VIII	1291/88	Chicken fricassee	VIII	29.5, 8.7, 2.4
	1295/88	Feces	VIII	29.5, 8.7, 2.4, 2.3
IX	174/90	Poultry fricassee	XII	28.0, 3.0
	175/90	Feces	IX	28.0
	176/90	Feces	IX	0
	192/90	Feces	IX	0
	195/90	Feces	IX	0
X	344/91	Beef	X	0
	346/91	Feces	X	8.8
	347/91	Feces	X	0

profiles were reported. The ribotyping results give a strong indication that the isolates within each of these eight outbreaks and cases are genetically very closely related. Since the food isolates tested were epidemiologically implicated as sources of the outbreak or case (3), it can be assumed that the *C. perfringens* strains found in food and stool caused the disease. Therefore, the respective foods have been confirmed by ribotyping as the source of infection in 8 of 10 outbreaks or cases.

Two food poisoning outbreaks (III and IX) showed different ribotypes within each. Their different plasmid profiles have been reported (3). Among the six isolates of outbreak III, one unique ribotype was detected (954/85 [feces isolate]). The other five isolates from foods and stool were identical. In outbreak IX, the ribopattern of the food isolate (174/90) differed from that of the four feces isolates with identical patterns. The following possibilities have to be considered concerning outbreaks III and IX. On the one hand, two genetically different *C. perfringens* strains could have caused the disease, but randomly only one of them was detected from food. This

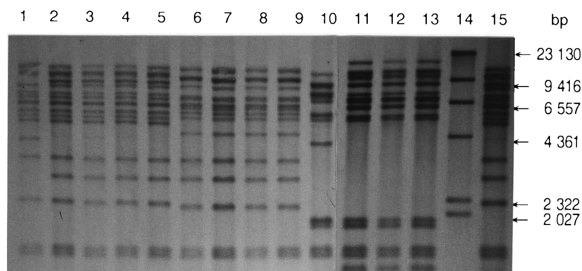

FIG. 1. Ribotyping patterns of 14 *C. perfringens* isolates obtained by *Eco*RI digestion. Outbreak III isolates (strain in parentheses) are shown by lanes 15 (955/85), 1 (954/85), 2 (953/85), 3 (949/85), 4 (948/85), and 5 (945/85); outbreak I isolates are shown by lanes 11 (732/84), 12 (731/84), and 13 (721/84); and outbreak IX isolates are shown by lanes 6 (195/90), 7 (192/90), 8 (176/90), 9 (175/90), and 10 (174/90). Lane 14, size markers.

could be suspected in outbreak IX, because only a single isolate from poultry fricassee was available for this examination, and it showed a pattern different from that of the four stool isolates which were identical. On the other hand, it must be considered that only one of the two ribotypes detected in outbreaks III and IX might have caused the disease; ribotyping results clearly show that, e.g., feces isolate 954/85 of outbreak III is genetically not related to any food isolate or any of the other patients' stool isolates. This is underlined by the result of plasmid profiling: 954/85 contains a plasmid with a unique molecular mass (21.0 MDa). It should also be considered that isolate 954/85 might belong to a *C. perfringens* population normally colonizing the intestinal tract of this patient and might not be involved at all in the outbreak.

According to the ribotyping results, food isolate 174/90 (outbreak IX) is genetically not related to any of the patients' stool isolates. This conclusion could not be drawn by plasmid profiling results. Here it must also be considered that the food isolate either might not be the only agent causing the disease or might not be involved in the outbreak at all. It is remarkable that in outbreak III, two *C. perfringens* isolates with identical ribotypes were isolated from two foods (heart goulash and cauliflower salad). This might be a hint of cross-contamination during preparation or storage of the foods.

These results show that ribotyping has a potential for classification of strains below the species level. Ribopatterns can be interpreted more easily than plasmid profiling results. Ribotyping was very useful for the epidemiologic investigation of foodborne disease outbreaks and cases caused by *C. perfringens*. Cleavage by *Eco*RI can be recommended as a standard method.

ACKNOWLEDGMENTS

We thank H.-P. Schau, Leipzig, for providing all of the *C. perfringens* strains investigated. The excellent technical support of Hanna Dietz is greatly acknowledged, and we thank Martin Wiedmann for help and criticism.

This work was supported by the DAAD and Academy of Finland.

REFERENCES

1. **Björkroth, J., and H. Korkeala.** 1996. rRNA gene restriction patterns as a characterization tool for *Lactobacillus sake* strains producing ropy slime. Int. J. Food Microbiol. **30:**293–302.
2. **Duncan, C. L., D. H. Strong, and M. Sebald.** 1972. Sporulation and enterotoxin production by mutants of *Clostridium perfringens*. J. Bacteriol. **110:**378–391.
3. **Eisgruber, H., M. Wiedmann, and A. Stolle.** 1995. Use of plasmid profiling as a typing method for epidemiologically related *Clostridium perfringens* isolates from food poisoning cases and outbreaks. Lett. Appl. Microbiol. **20:**290–294.

Downloaded from http://aem.asm.org/ on October 29, 2015 by guest

4. **Forsblom, B., A. Palmu, P. Hirvonen, and H. Jousimiessomer.** 1995. Ribotyping of *Clostridium perfringens*. Clin. Infect. Dis. **20**(Suppl. 2)**:**S323–S324.
5. **Grimont, F., and P. A. D. Grimont.** 1996. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann. Inst. Pasteur/Microbiol. (Paris) **137:**165–175.
6. **Klein, G., A. Pack, H. Eisgruber, and A. Stolle.** 1996. Mögliche Zusammenhänge zwischen *Clostridium perfringens*-Isolaten aus Lebensmitteln und *Clostridium perfringens* Erkrankungen, p. 49–54. *In* Proceedings of the 37th Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene, Garmisch-Partenkirchen Deutsche Veterinärmedizinische Gesellschaft, Giessen, Germany.
7. **Mahony, D. E., G. A. Clark, M. F. Stringer, M. C. MacDonald, D. R. Duchesne, and J. A. Mader.** 1986. Rapid extraction of plasmids from *Clostridium perfringens*. Appl. Environ. Microbiol. **51:**521–523.
8. **McDonel, J. L.** 1979. The molecular mode of action of *Clostridium perfringens* enterotoxin. Am. J. Clin. Nutr. **32:**210–218.
9. **Pitcher, D. G., N. A. Saunders, and R. J. Owen.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. **8:**151–156.
10. **Songer, J. G.** 1996. Clostridial enteric diseases of domestic animals. Clin. Microbiol. Rev. **9:**216–234.
11. **Southern E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.

Downloaded from http://aem.asm.org/ on October 29, 2015 by guest