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The abattoir source of culturable psychrophilic *Clostridium* spp. causing 'blown pack' spoilage of vacuum-packed chilled venison

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Aims: To identify the abattoir source(s) of culturable psychrophilic clostridia causing 'blown pack' spoilage of vacuum-packed chilled meats.

Methods and Results: Psychrophilic and psychrotolerant clostridia were isolated from hides, faeces and tonsils of deer slaughter stock, and from a meat plant environment. The isolates were differentiated using restriction fragment length polymorphism analysis of the 16S rDNA gene (PCR–RFLP) and 16S-23S rDNA internal transcribed spacer (ITS) analysis. PCR–RFLP group I clostridia were found to have restriction patterns indistinguishable from the patterns of 'blown pack'-causing *Clostridium gasigenes* DB1A^T and R26. Gas production in packs inoculated with vegetative cells of PCR–RFLP group I clostridia was first evident after 14 days at 2 °C. The prevalence of these clostridia was similar in hide and faecal samples from slaughter animals, but these micro-organisms were absent from tonsils and the meat plant environment. Banding patterns of PCR–RFLP group II clostridia showed some cross-similarity with patterns of the 'blown pack'-causing micro-organism *Cl. estertheticum* DSM 8809^T and *Cl. estertheticum*-like meat strains. The majority of clostridia in PCR–RFLP group II were found in the faeces of slaughter animals. Isolates representing PCR–RFLP group II did not, however, produce gas in vacuum packs stored at 2 °C for 84 days.

Conclusions: The data suggest that soil particles attached to hide or present in faeces are the most probable primary reservoir from which 'blown pack' clostridia are introduced onto carcasses. Therefore, dressing procedure hygiene remains paramount in order to control the spread of psychrophilic *Clostridium* spp. in a meat plant.

Significance and Impact of the Study: The paper provides information critical for controlling 'blown pack' spoilage in meat processing plants. It reports on the use of molecular techniques for determination of abattoir sources of 'blown pack'-causing clostridia.

INTRODUCTION

Psychrophilic clostridia have been recognized as causative agents of 'blown pack' spoilage of vacuum-packed chilled meats (Dainty *et al*. 1989; Kalchayanand *et al*. 1989; Broda *et al*. 2000b). To eliminate this type of spoilage, the source of the causative micro-organisms in the meat plant must be identified and the transfer of these micro-organisms from that source onto meat cuts prevented.

Little is known, however, of the probable source(s) of carcass contamination with psychrophilic clostridia. The majority of spoilage bacteria associated with vacuum-packed meat cuts are believed to originate from the exogenous environment of slaughter stock (Gill 1979; Nottingham 1982). In the meat plant, soil and animal faeces attached to pelts of slaughter stock are considered to be the primary reservoir for direct carcass contamination with these spoilage bacteria (Bell 1997; McEvoy *et al*. 2000). With 'blown pack'

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spoilage, however, the vacuum pack distension during chilled storage was reported to be associated with abattoir foci of contamination, such as the conveyers carrying unwrapped meat prior to vacuum packaging or boning room air vents (Kalchayanand *et al*. 1991).

Psychrotolerant micro-organisms have been isolated previously from permanently cold Arctic or Antarctic environments (Jordan and McNicol 1979; Mountfort *et al*. 1997) and, because of their ability to tolerate higher temperatures, from seasonally cold environments (Kotsyurbenko *et al*. 1995). Although psychrophilic micro-organisms are frequently unable to proliferate at temperatures above 22 °C, their spores survive exposure to sub-optimal environmental conditions, including elevated temperatures. Therefore, such spores entering the abattoir in soil particles attached to an animal's hide and/or in animal faeces, as well as those resident within the abattoir environment, can be regarded as in-plant potential source(s) for psychrophilic clostridial contamination of carcasses.

The aim of this study was to identify the abattoir source(s) of culturable psychrophilic clostridia causing 'blown pack' spoilage of vacuum-packed chilled meats. Faeces were examined to determine whether the gastrointestinal tract could be a source of psychrophilic *Clostridium* spp. contamination of carcass meat. Similarly, hide samples were examined to determine whether hides, or soil or faecal matter attached to them, could be the source of contamination.

MATERIALS AND METHODS

Bacteria

Stringent anaerobic procedures and pre-reduced media (Holdeman *et al*. 1977) were used for culturing all *Clostridium* strains.

The reference strain of *Clostridium estertheticum* DSM 8809^T (T = type strain) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. This strain was revived in sterile anaerobic milk and was subcultured onto Columbia Blood Agar (CBA, Oxoid) containing 5% v/v sheep's blood. The strain was grown at 10 °C for 7 days. The reference strain of *Clostridium gasigenes* $DB1A^T$ (DSM 12272^T) was the original New Zealand strain obtained from an incident of 'blown pack' spoilage of chilled vacuum-packed lamb (Broda *et al*. 2000b). This strain was maintained as described previously (Broda *et al*. 2000a).

Psychrophilic clostridia of meat origin, *Cl. gasigenes* R26, and *Cl. estertheticum*-like strains K21 and K24, were isolated using methods described previously (Broda *et al*. 1996). These strains were obtained between 1993 and 1997 from blown packs of chilled vacuum-packed lamb (strain R26) and venison (strains K21 and K24). With meat strains, the ability to cause pack 'blowing' was checked under laboratory conditions. At 2 °C, all three strains produced the first signs of gas production in vacuum packs within 11 to 14 days of storage. The meat strains, maintained as freeze-dried cultures, were revived in Peptone Yeast Extract Glucose Starch (PYGS) (Lund *et al*. 1990) broth, plated onto CBA supplemented with 5% v/v sheep's blood and incubated at 20 °C (strain R26) or 10 °C (strains K21 and K24) for 48–96 h, respectively.

A total of 359 strains of psychrophilic and psychrotolerant clostridia were isolated from hide swabs, faeces and tonsils of 100 slaughter animals, and from 33 environmental samples collected at various points on a venison processing chain. A multi-step isolation procedure was followed using media and techniques described previously (Broda *et al*. 1998). Vegetative cells and spores were recovered on Shahidi Ferguson Perfringens (SFP, Oxoid) agar with 5% egg-yolk. Spores were recovered after ethanol treatment (the sample was mixed with an equal volume of absolute ethanol and incubated at 15 °C for 60 min) on Peptone Yeast Extract Glucose agar (Lund *et al*. 1990) with 625 U ml^{-1} lysozyme, and after heat treatment (80 °C for 10 min) on Glucose Starch agar (Nakamura *et al*. 1985). All inoculated plates were incubated at 15 °C for 14 days.

Isolates representing every colony morphotype (one per type) on each primary isolation plate were streaked onto CBA agar and incubated anaerobically at 15 °C for 7–10 days to confirm purity. Isolates that were obligately anaerobic, catalase-negative, oxidase-negative, Gram-positive (sometimes Gram-variable) large rods with terminal or subterminal spores, and able to grow at 4 °C, were considered to be psychrophilic or psychrotolerant clostridia. Of these isolates, those that were unable to grow at 25 °C and above were considered to be psychrophilic (Morita 1975). Following their original isolation, the isolates, referred to as industry strains, were stored anaerobically on CBA agar at 10 °C for 3 months without further transfers. Before use in molecular typing, these strains were subcultured onto CBA plates and incubated at 15 °C for 7–10 days to obtain individual colonies.

DNA isolation

To isolate DNA, cells from exponentially-growing reference, meat and industry strains of psychrophilic and psychrotolerant clostridia were harvested from the surface of CBA plates. Cells were suspended in 1 ml sterile TE buffer and the optical density of the cell suspension adjusted to approximately 1·0 O.D. (equivalent to 10^9 cells ml^{-1}). Genomic DNA was isolated using a HighPure DNA preparation kit (Boehringer Mannheim GmbH, Mannheim, Germany). The manufacturer's recommended protocol for the isolation of nucleic acids from bacteria and yeast was

followed, with the exception that, to improve lysis, cells were resuspended in 200 μl of 50 mg ml^{-1} lysozyme and incubated at 37 °C for 60 min. From this step, the recommended protocol, incorporating Proteinase K lysis, guanidine–HCl binding and subsequent isopropanol precipitation and ethanol washes, was followed. Eluted DNA was stored at − 20 °C pending PCR amplification.

Restriction fragment length polymorphism (RFLP) analysis of 16S rDNA gene (PCR–RFLP)

For amplification of the 16S rDNA gene, genomic DNA was used as the PCR template. PCR was performed with universal (eu)bacterial primers complementary to conserved regions of the 5′ and 3′ ends of the 16S rRNA gene. The primer sequences were: pA (forward) 5′-AGA GTT TGA TCC TGG CTC AG-3′ and pH* (reverse) 5′-AAG GAG GTG ATC CAG CCG CA-3′ (Hutson *et al*. 1993).

The PCR mix was prepared according to the manufacturer's (Boehringer Mannheim) recommendation and contained: PCR buffer 10× (10 μl), 0·2 mmol l^{-1} of each deoxynucleoside triphosphate, 0·5 μmol l^{-1} of each primer, 2·5 U of Taq polymerase and 10 μl of template DNA (100 ng) in a total volume of 100 μl. Amplifications were performed in a heated lid thermal cycler (Techne Genius, Princeton, NJ, USA). After initial denaturation for 3 min at 93 °C, target DNA was amplified in 30 cycles. Each cycle consisted of denaturation for 1 min at 92 °C, annealing for 1 min at 55 °C and extension for 2 min at 72 °C. The final extension was for 3 min at 72 °C. The PCR reaction tubes, each containing an amplified 16S rDNA fragment, were held at 4 °C until further analysis.

PCR-amplified 16S rDNA genes of reference, meat and industry strains were digested with *Alu*I endonuclease (Boehringer Mannheim). Restriction digests containing 10 μl of the PCR product, 2 μl of the appropriate buffer and 10 U of enzyme in a total volume of 20 μl were prepared and incubated according to the manufacturer's recommendations. Digestion products were separated by gel electrophoresis in 2·0% (w/v) agarose (Seakem) gels at 90 V for 1·5 h. The DNA molecular weight marker VI (Boehringer Mannheim) was used as a size marker. Banding patterns were visualized with ethidium bromide by u.v. transillumination.

Isolates were grouped on the basis of banding pattern similarity as assessed by comparison of resolved fragments larger than 154 bp. Isolates whose PCR-amplified 16S rDNA genes digested with *Alu*I yielded indistinguishable banding patterns were considered members of the same PCR–RFLP type (Broda *et al*. 2000a). PCR products from the isolates that had *Alu*I restriction patterns similar to patterns of the known 'blowers' *Cl. estertheticum* DSM 8809T, *Cl. gasigenes* DB1AT and meat strains R26, K21 and K24, were then digested with three additional restriction endonucleases, *Hae*III, *Taq*I and *Cfo*I (Boehringer Mannheim). With these three enzymes, digestion and restriction fragment separation was conducted as with *Alu*I.

16S–23S rDNA internal transcribed spacer (ITS) length polymorphism analysis

Spacers from reference, meat and industry strains that had *Alu*I restriction patterns similar to the known 'blowers' were PCR amplified with universal (eu)bacterial primers ISRA (forward) 5′-AAG TCG TAA CAA GGT ARC-3′ and ISRC (reverse) 5′-GGG TTB CCC CAT TCR G-3′ (Lane 1991). These primers are complementary to conserved regions of the 3′ end of 16S rRNA and the 5′ end of 23S rRNA genes directly flanking the spacer. The PCR mix was prepared and ITS amplifications were performed as described for the RFLP analysis of 16S rDNA genes.

A 5 μl aliquot of the PCR reaction was electrophoresed on a 1·5% (w/v) agarose gel at 90 V for 1·5 h. The DNA molecular weight marker VI (Boehringer Mannheim) was used as a size marker. Banding patterns were visualized with ethidium bromide by u.v. transillumination.

Confirmation of 'blowing' ability

The ability to cause 'blown pack' spoilage in a laboratory meat model was determined for six isolates representing each of the two PCR–RFLP *Alu*I groups that showed pattern similarity to known 'blowers'. In addition, two isolates representing each of the remaining PCR–RFLP *Alu*I groups were also tested. Reference strains *Cl. estertheticum* DSM 8809^T and *Cl. gasigenes* DB1A^T were used as positive controls. Each strain was grown in 10 ml PYGS broth until a heavy suspension (approximately 10^8 ml^{-1}) was obtained. Cells of each strain were harvested by centrifugation at 6000 *g* for 15 min and washed five times with ice-cold saline (0·85% w/v NaCl). These washed suspensions were used as inocula.

Chilled boneless lamb chumps were obtained one day after boning at a local meat plant and placed individually into barrier bags (Cryovac BB4L; Cryovac Sealed Air, Porirua, New Zealand) pending inoculation. Duplicate packs were prepared for each strain tested. The packs were inoculated with 0·5 ml of a cell suspension of each strain to obtain a concentration of approximately 10^3 cfu cm^{-2}. Inoculated packs and two uninoculated controls were immediately vacuum-packed using a controlled atmosphere packaging machine (Securepack 10; Securefresh Pacific Ltd, Auckland, New Zealand) and were stored at 2 °C for up to 84 days (the maximum shelf-life expected for vacuum-packed chilled lamb). Packs were examined regularly for the presence of gas bubbles in the meat drip, followed by loss of vacuum and, subsequently, by substantial pack distension.

RESULTS

RFLP analysis of 16S rDNA gene (PCR–RFLP)

On the basis of RFLP analysis of PCR-amplified 16S rDNAs, the majority of the industry strains were classified into six distinct groups. Each of these groups contained multiple isolates that had banding patterns similar to each other, but that were readily differentiated from other PCR–RFLP groups. Only two PCR–RFLP groups (named groups I and II) showed *Alu*I restriction pattern similarity with the known 'blowers' *Cl. estertheticum* DSMZ 8809^T, *Cl. gasigenes* and *Cl. estertheticum*-like strains K21 and K24. Isolates in both groups were unable to grow at or above 25 °C. An additional 12 isolates had banding patterns that differed from those of the six major groups, the reference or meat strains. These isolates, representing eight unique banding patterns, were for convenience designated as miscellaneous.

The results of endonuclease digestion of 16S rDNA genes of *Cl. gasigenes*, and PCR–RFLP group I isolates V3, V4 and V5 (representatives of all PCR–RFLP group I industry strains), are shown in Fig. 1. PCR–RFLP group I contained 11 isolates that had *Alu*I banding patterns indistinguishable from those of *Cl. gasigenes* strains. Subsequent digestion of 16S rDNA genes of these industry isolates with *Taq*I and *Cfo*I yielded, with each endonuclease, restriction patterns indistinguishable from those of *Cl. gasigenes* strains DB1A^T and R26 (results not shown). However, *Hae*III digestion of 16S rDNA genes demonstrated restriction pattern polymorphism within PCR–RFLP group I. Within this group, five isolates had *Hae*III restriction patterns indistinguishable from the pattern of *Cl. gasigenes* DB1A^T, whereas the other six isolates had *Hae*III restriction patterns similar to the pattern of strain R26.

The results of endonuclease digestion of 16S rDNA genes of *Cl. estertheticum*, strains K21 and K24, and PCR–RFLP group II isolate V13 (representative of all PCR–RFLP group II industry strains) are shown in Fig. 2. PCR–RFLP group II contained 17 isolates that, with *Alu*I, *Hae*III, *Taq*I or *Cfo*I, had restriction patterns indistinguishable from each other. These isolates shared some *Alu*I and *Hae*III pattern similarity with the reference strain *Cl. estertheticum* DSM 8809^T and *Cl. estertheticum*-like meat strains K21 and K24. With *Alu*I, however, the restriction patterns of industry isolates lacked one band at approximately 453 bp, while an additional band below 154 bp was present in restriction patterns of PCR–RFLP group II isolates but not in patterns of the reference or meat strains. With *Hae*III, a change in position of one band was observed between patterns of the industry isolates and strains K21, K24 and *Cl. estertheticum* DSMZ 8809^T. On subsequent digestion with *Taq*I the restriction patterns of the industry isolates were readily differentiated from the reference strain *Cl. estertheticum* and meat strains K21 and K24. With the same enzyme, restriction pattern of *Cl. estertheticum* DSMZ 8809^T was distinctly different from patterns of strains K21 and K24 (Fig. 2).

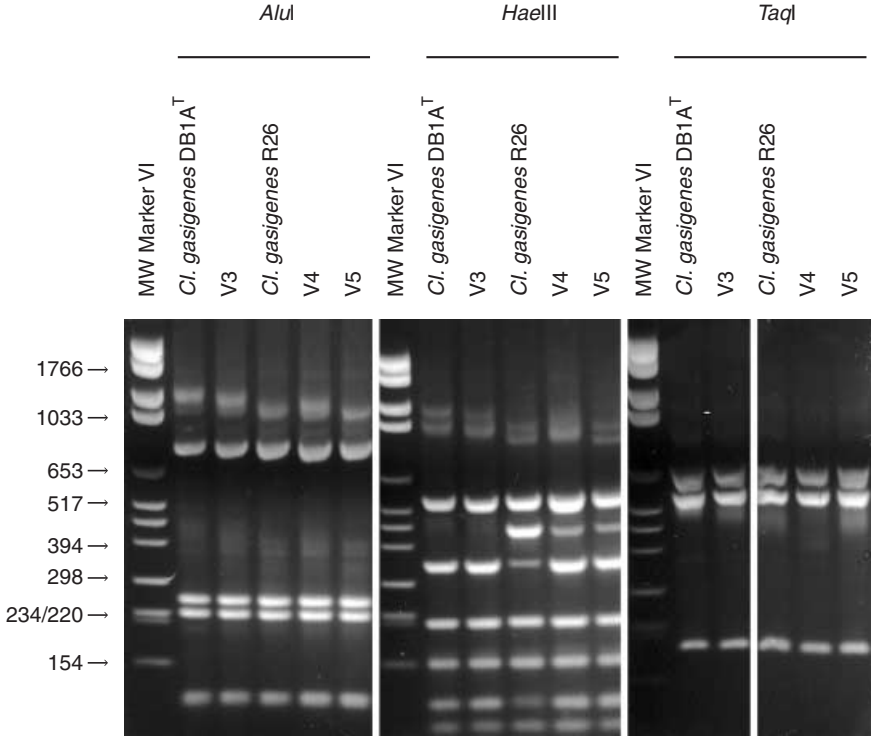

Fig. 1 Restriction patterns of digested 16S rDNA genes of *Clostridium gasigenes* DB1AT and R26, and three industry isolates representing PCR–RFLP group I. The remaining eight isolates in this group had restriction patterns indistinguishable from the pattern of either industry isolate V3 or V4 and V5.

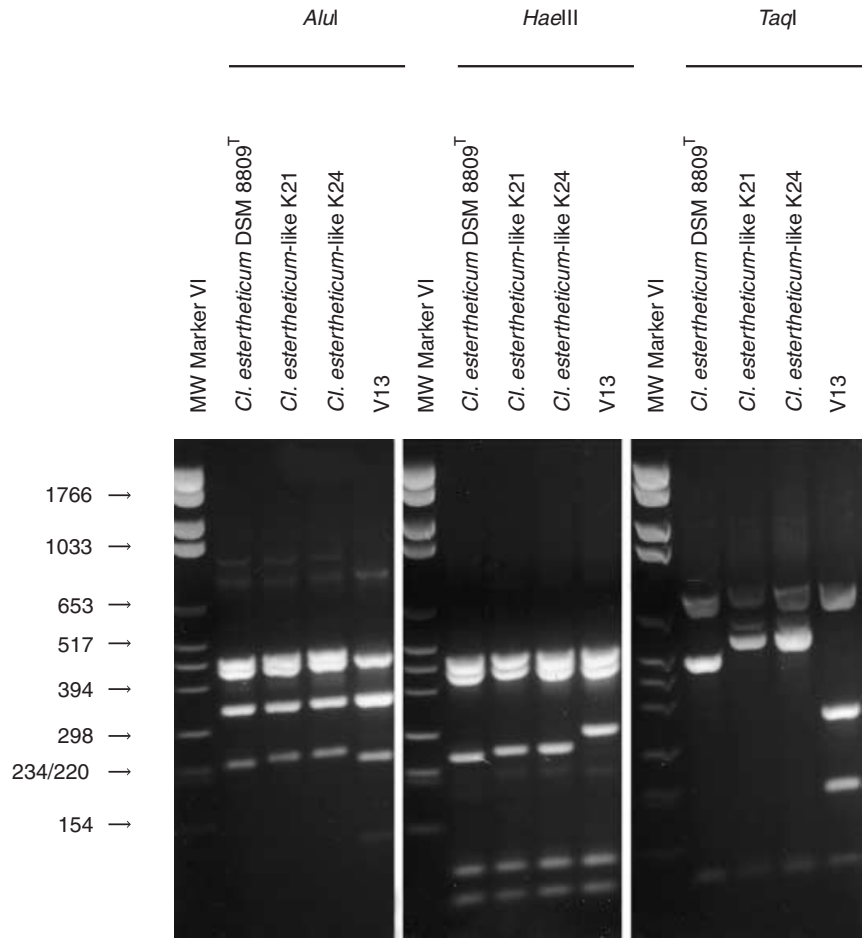

Fig. 2 Restriction patterns of digested 16S rDNA genes of reference strain *Clostridium estertheticum* DSM 8809T, *Cl. estertheticum*-like meat strains K21 and K24, and industry isolate V13 representing PCR–RFLP group II. The remaining 16 isolates in this group had PCR–RFLP restriction patterns indistinguishable from the pattern of isolate V13.

Internal transcribed spacer (ITS) polymorphism analysis

The ITS banding patterns of PCR–RFLP group I industry strains (represented on Fig. 3 by isolates V3, V4 and V5) consisted of one main band at approximately 346 bp (primary ITS product) and multiple minor bands (secondary ITS products). All PCR–RFLP group I isolates, and *Cl. gasigenes* DB1A^T and R26, had indistinguishable overall banding patterns and a similar-sized main spacer PCR amplification product visualized on the gel (Fig. 3).

The ITS banding patterns of PCR–RFLP group II industry strains (represented on Fig. 3 by isolate V13) consisted of two main bands at approximately 490 and 410 bp, and multiple minor bands. Within PCR–RFLP group II, all the industry isolates had indistinguishable ITS banding patterns and primary ITS products of similar size. The industry isolates differed, however, from *Cl. estertheticum* DSMZ 8809^T in the overall ITS banding pattern and in the size of the two primary ITS products (Fig. 3). These isolates also differed from meat strains K21 and K24 in the overall ITS banding pattern. In contrast to the industry isolates whose patterns consisted of two main bands, the banding patterns of strains K21 and K24 had only one main band at approximately 410 bp.

Confirmation of 'blowing' ability

In packs inoculated with vegetative cells of PCR–RFLP group I isolates, small gas bubbles were first present in the meat drip after 14 days of storage at 2 °C. In comparison, gas production in packs inoculated with vegetative cells of the reference strain of *Cl. estertheticum* DSM 8809^T and *Cl. gasigenes* DB1A^T was first evident at 11 and 15 days of storage, respectively. While packs inoculated with *Cl. estertheticum* were grossly distended after 35 days of storage, a smaller volume of gas was produced in packs inoculated with PCR–RFLP group I isolates. These packs did not reach the grossly distended state within 84 days of storage. No gas was present in uninoculated control packs stored at 2 °C for 84 days.

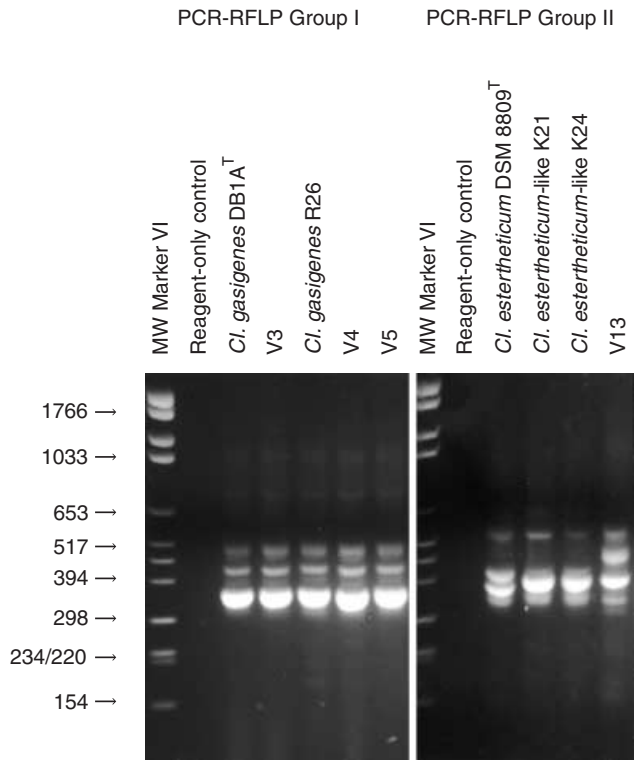

Fig. 3 Banding patterns of reference, meat and industry strains of psychrophilic clostridia representing PCR–RFLP groups I and II obtained in PCR amplification of the 16S–23S rDNA internal transcribed spacer. Industry isolates V3, V4 and V5 represent PCR–RFLP group I. The remaining eight isolates in this group had banding patterns indistinguishable from patterns of these isolates. Industry isolate V13 represents PCR–RFLP group II. The remaining 16 isolates in this group had banding patterns indistinguishable from the pattern of isolate V13.

No gas production or pack distension was observed in packs inoculated with PCR–RFLP group II isolates or those from any other PCR–RFLP group.

Source of psychrophilic clostridia

All PCR–RFLP group I isolates were obtained from either hide swabs or faecal samples. A similar proportion of hide and faecal samples (6% and 5% of the total number of samples, respectively) were positive for psychrophilic clostridia belonging to PCR–RFLP group I. None of the isolates belonging to PCR–RFLP group I were obtained from tonsil or environmental samples.

With PCR–RFLP group II, 15 out of 17 isolates were obtained from faecal samples. Of the two remaining isolates within this RFLP group, one was obtained from a hide swab and one from an environmental sample. The single environmental isolate was found in a swab sample taken from an inedible offal room. None of the isolates belonging to PCR–RFLP group II was obtained from tonsil samples.

DISCUSSION

PCR–RFLP group I psychrophilic clostridia caused gas production in vacuum-packed lamb stored at 2 °C for 14 days. With both RFLP analysis of 16S rDNA genes and ITS analysis, the banding patterns of these clostridia were found to be indistinguishable from the patterns of *Cl. gasigenes* DB1A^T or R26, which were previously confirmed as the causative organisms of 'blown pack' spoilage (Broda *et al.* 2000b). In this study, PCR–RFLP group I psychrophilic clostridia were obtained from either hides or faeces but not from the tonsils of slaughter animals. Since none of the isolates belonging to PCR–RFLP group I were obtained from an environmental sample, it appears that, with these micro-organisms, the primary sources of carcass contamination in the abattoir are the hides and faeces of the slaughter animals themselves. With clostridial 'blown pack' spoilage, this finding may explain the variable incidence and distribution of pack distension that is observed within processing batches or shipments (Dainty *et al.* 1989). Carcass contamination with PCR–RFLP group I clostridia appears most likely to result from contact either with spores that survive passage through the digestive system of slaughter animals, or with those from exogenous environmental sources present on the slaughter stock. Dressing procedure hygiene therefore remains paramount for controlling the spread of PCR–RFLP group I psychrophilic clostridia in a meat plant.

Although in the abattoir the immediate source of carcass contamination with psychrotolerant micro-organisms is usually the hides/fleeces of slaughter animals (Newton *et al.* 1978), a high proportion of PCR–RFLP group I, and the majority of PCR–RFLP group II psychrophilic clostridia were, in this study, found in faecal samples. The presence of clostridia from the gastrointestinal tract on hides can be anticipated as a consequence of faecal contamination occurring during transport and pre-slaughter holding of stock. Mesophilic clostridia, including those able to cause disease, are part of the normal flora of an animal's gastrointestinal tract (Cato *et al.* 1986; Roberts and Mead 1986). The results of the present study suggest that, in addition to mesophilic clostridia, the spores of psychrophilic clostridia may be carried in animals' intestines. Since the PCR–RFLP group I and group II isolates are unable to grow at temperatures above 25 °C, it is likely that, as with the majority of spoilage organisms in vacuum-packed meats, these micro-organisms originate from the exogenous environment of an animal (Gill 1979; Nottingham 1982). It is likely that, as with many other clostridial species, the primary sources of these micro-organisms in the farm environment are soil, animal feed or aerial plant surfaces (Lund 1986; Ercolani 1997).

With PCR–RFLP group I isolates, *Hae*III restriction pattern polymorphism of digested 16S rDNA genes was observed in this study. Despite this polymorphism, very little 16S–23S rDNA spacer length or overall ITS banding pattern difference existed between individual isolates in PCR–RFLP group I, or between PCR–RFLP group I strains and *Cl. gasigenes* strains DB1A^T and R26 that were originally obtained from spoiled product. The possibility exists that the lack of inter-strain spacer polymorphism observed with *Cl. gasigenes* is species-specific. Consequently, it appears that ITS analysis would not offer sufficient discriminatory power for tracing the *Cl. gasigenes* strains responsible for spoilage of vacuum-packed chilled meats back to their meat plant sources. The low inter-strain spacer heterogeneity of this species remains, however, to be confirmed with a larger number of isolates from different sources.

Despite some *Alu*I and *Hae*III restriction pattern cross similarity between PCR–RFLP group II isolates and known 'blowers' (*Cl. estertheticum*, and strains K21 and K24), these isolates did not cause pack blowing. Furthermore, the *Taq*I patterns of PCR–RFLP group II isolates were readily differentiated from patterns of the reference and meat strains. Distinct differences between restriction patterns of *Cl. estertheticum* DSMZ 8809^T and meat strains K21 and K24 were also observed after digestion of 16S rDNA genes with *Taq*I. Subsequent sequencing of an approximately 800 bp 16S rDNA gene fragment from PCR–RFLP group II isolate V13 (GenBank accession AF502398) showed that this isolate is more closely related to *Cl. subterminale* and thiosulphate-reducing clostridia (approximately 98% 16S rDNA sequence similarities within the 789 bp fragment) than to *Cl. estertheticum*. Similarly, ITS analysis demonstrated considerable polymorphism in spacer lengths of the reference strain *Cl. estertheticum*, meat strains K21 and K24, and PCR–RFLP group II psychrophilic clostridia. The results of the present study indicate that a considerable degree of 16S rDNA gene and internal transcribed spacer polymorphism exists between *Cl. estertheticum*, meat strains K21 and K24, and the RFLP group II isolates. The detailed taxonomic status of these meat and RFLP group II strains remains uncertain.

The possibility exists that micro-organisms causing 'blown pack' spoilage, *Cl. estertheticum*, *Cl. laramiense* or meat strains K21 and K24, were not present in the meat plant. It is more likely, however, that in this study, the failure to isolate clostridia that cause gross pack distension of vacuum-packed chilled meats reflects commonly encountered difficulties with the conventional isolation of these micro-organisms (Dainty *et al*. 1989; Kalchayanand *et al*. 1989; Broda *et al*. 1996). Isolation temperatures employed for culturing psychrophilic clostridia, even if carefully selected to fall within the growth range of a specific micro-organism, have been found to markedly influence the type of micro-organisms recovered, presumably due to the inability of these micro-organisms to compete successfully with the numerically dominant lactic acid bacteria present on vacuum-packed chilled meat (Bell *et al*. 1997). The development of direct molecular microbiological methods may therefore offer an attractive alternative to conventional methods for specific detection of 'blown pack'-causing clostridia in vacuum-packed meats and in the meat plant environment.

This study demonstrated that PCR–RFLP analysis of 16S rDNA genes assisted by ITS analysis could successfully determine the probable source of carcass contamination with psychrophilic clostridia. However, more discriminatory typing methods must be used to establish epidemiological relatedness of 'blown pack'-causing clostridial strains from spoiled packs to strains obtained *a priori*, in anticipation of spoilage, from slaughter animals. Only when such relatedness is established will the route of carcass contamination with psychrophilic clostridia be confirmed in a definitive manner.

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