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Prevalence of *Clostridium estertheticum* and *Clostridium gasigenes* in cattle at a slaughterhouse in Belgium.

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INTRODUCTION

Globalization of meat exchanges led to a generalized application of conservation techniques assuring a longer shelf-life, such as chilling and vacuum-packaging. However, a type of spoilage of chilled vacuum-packaged meat is becoming a major problem for the meat industry. This spoilage phenomenon referred to as "Blown Pack Spoilage" can be caused by different species of psychrophilic and psychrotrophic clostridia such as *Clostridium estertheticum* and *Clostridium gasigenes*.

OBJECTIVES

The first part of our study was to determine whether these bacteria are present in cattle brought to Belgian slaughterhouses and if so, to determine their prevalence. The second part of our study focuses on the genotypic characterization of these strains

MATERIAL AND METHODS

Bovine faecal samples were collected at a Belgian slaughterhouse, from the slaughter line, directly from the large intestine in the viscera processing area. Cattle intestinal samples were recovered from animals with ages ranging between 11 and 52 months coming from different herds. Samples were kept in individual sterile 50 ml tubes and were brought the same day to the lab and kept refrigerated until analysis. A total of 175 samples was analysed as follows: 1 gram of each faecal sample was added to a sterile tube containing 9 ml of broth. The culture broth was glucose and sodium bicarbonate supplemented Reinforced Clostridial Medium (Oxoid N.V., Aalst, BE). The suspension was kept in a water bath at 70°C for 15 minutes to select spores of the psychrophilic/trophic species and induce germination. The heat-shock was immediately stopped by means of an ice-bath. An enrichment step at 8°C for 3 weeks in anaerobiosis was then carried out. The enriched bacterial suspension was then plated onto Columbia agar supplemented with 5% of sheep blood (Biomérieux, Craponne, FR) and incubated anaerobically at 8°C for another 2 weeks, until colonies would appear. Once the psychrophilic/trophic *Clostridium* colonies were selected, a species-specific PCR for *Cl. gasigenes* and *Cl. estertheticum* was conducted for identification (Broda *et al*., 2003). Isolates identified as *Cl. estertheticum* by PCR were then characterized genotypically by means of 16S rDNA Sanger sequencing (GIGA, Liège, BE). Raw sequences were assembled using Geneious 6.1 (Biomatters Ltd., Auckland, NZ) and compared thereafter against the open nucleotide database using BLAST® and against each other with a Clustal Omega multiple alignment (EMBL-EBI, Cambridgeshire , UK) .

RESULTS

A total of 40 isolates, characterized as psychrophilic/trophic, spore-forming, Gram-positive rods, were identified by PCR as belonging to *Cl. estertheticum* (17,1%) or *Cl. gasigenes* (5,7%). Other psychrophilic/trophic clostridia were isolated from the faecal samples but were PCR negative and further tests for identification shall be conducted.

Regarding the 16S rDNA sequences of the isolates identified by PCR as *Cl. estertheticum*, Table 1 shows the species identification obtained using BLAST® and Figure 1 represents the phylogenetic distance between these isolates based exclusively on the 16S rDNA sequences.

Strain	HQ% of assembled sequence	BLAST results (%ID)
2903/3	90	*Clostridium frigoriphilum* (100%)
2903/4	79	*Clostridium bowmanii* (99%)
2903/7	76	*Clostridium bowmanii* (99%)
2903/8	85	*Cl. bowmanii/estertheticum* (99%)
2903/11	78	*Cl. bowmanii/estertheticum* (99%)
1205/1	88	*Cl. bowmanii/estertheticum* (99%)
1205/2	91	*Cl. bowmanii/estertheticum* (99%)
1205/5	54	*Cl. bowmanii* (98%)
1205/6	79	*Cl. bowmanii/estertheticum* (99%)
1205/18	33	*Cl. bowmanii* (98%)
1205/20	84	*Cl. bowmanii/estertheticum* (99%)
1205/23	68	*Cl. bowmanii* (99%)
0411/1	85	*Cl. bowmanii/estertheticum* (99%)
0411/3	80	*Cl. bowmanii/estertheticum* (99%)
0411/4	91	*Clostridium tagluense* (99%)
0411/6	86	*Cl. bowmanii/estertheticum* (99%)
0411/7	86	*Cl. bowmanii* (99%)
0411/8	5	*Clostridium algidicarnis* (95%)
0411/11	94	*Cl. tagluense* (99%)
2211/1	84	*Cl. bowmanii/estertheticum* (99%)
2211/4	63	*Cl. bowmanii/estertheticum* (98%)
2211/12	59	*Cl. bowmanii* (98%)
2211/13	87	*Clostridium frigoriphilum* (100%)
2211/19	90	*Cl. bowmanii/estertheticum* (98%)
2911/1	47	*Cl. bowmanii* (97%)

High trace score for the two strands
Medium trace score for one of the two strands
Medium trace score for the two strands

Table 1 - Species identification based on 16S rDNA sequence alignment against the nucleotide database (BLAST®).

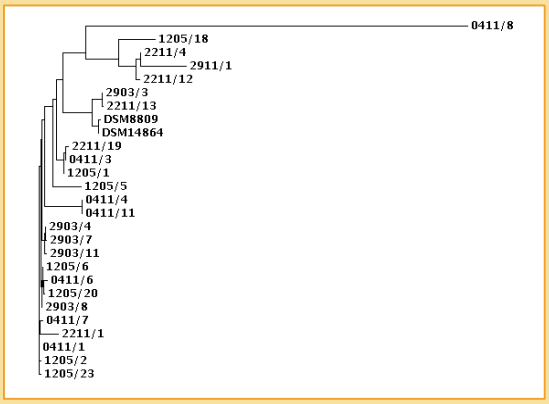

Figure 1 - Phylogram tree (neighbour-joining clustering method) showing the distance between isolates based on a multiple alignment of the 16S rDNA sequences (ClustalOmega and ClustalW2 Phylogenetic).

CONCLUSIONS

- Psychrophilic/trophic clostridia responsible for meat spoilage are carried by cattle brought to slaughterhouses in Belgium.
- In Belgium, *Cl. estertheticum* seems to be more prevalent in cattle than *Cl. gasigenes*.
- 16S rDNA sequencing results show that other species close to *Cl. estertheticum* can be detected with the PCR described by Broda.
- The intestinal content of cattle shows a significant diversity of psychrophilic/trophic clostridia that can be associated with meat spoilage.

BIBLIOGRAPHIC REFERENCES

Broda D.M., Boerema J.A., Bell R.G., 2003, "PCR detection of psychrophilic *Clostridium* spp. causing 'blown pack' spoilage of vacuum-packed chilled meats", *J Appl Microbiol*, **94 (3),** 515-22.