the risk of transmission to wildlife. Until the 10 days have passed, MRDT104 bacteria are present, and as such constitute a potential hazard. However, as it is not allowed to spread slurry from MRDT104 infected herds on pasture or ready-to-eat vegetables, there is no direct exposure to grazing stock or humans.

Risk analysis of *Bacillus* spp. isolated from cured pork sausages

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Summary: This study was undertaken to acquire information about the toxigenic potential of *Bacillus* strains isolated from eight cured pork sausages obtained from traditional or industrial processings. The application of RAPD-PCR protocols made it possible to identify 52 different biotypes among 220 heat-resistant Gram-positive endospore-forming colonies. The sequence analysis of the 5’ region of 16S rDNA revealed that 36 strains belonged to *B. subtilis* and 16 to *B. pumilus* species. No strains belonging to *B. cereus* species were isolated from the cured sausages analysed. The toxigenic potential of these strains was assayed by PCR analysis and physiological tests to identify the most important *B. cereus* toxins and virulence factors. No specific PCR fragment was obtained from any of the strains; however, some of them were found positive for hemolytic and lecithinase activity. These preliminary results reassure about the microbiological risk related to the presence of pathogenic *Bacillus* strains in cured pork sausages analysed even though the hemolytic and lecithinase activities found in some strains suggest that more in-depth analyses need to be carried out.

Keywords: PCR, toxins, virulence factors, *B. cereus*, cured meat products

Introduction: A wide variety of microorganisms such as, lactic acid bacteria (LAB), *Staphylococcus*, *Kocuria* and *Bacillus* are involved in meat fermentation. Most cases of food poisoning attributed to *Bacillus* species are associated with *B. cereus*; this bacterium is known to cause a variety of foodborne disorders characterized by either diarrhea or emesis. Lately, other *Bacillus* species have been gaining recognition as organisms relevant in causing food poisoning, with recent epidemiological evidence linking *B. subtilis*, *B. pumilus*, *B. licheniformis*, and *B. thuringensis* with incidents of foodborne illness. Evaluation of toxin gene presence and expression in *Bacillus* spp. other than *B. cereus* has not been thoroughly investigated. The survival of *Bacillus* strains through meat processing leads to suppose that potentially pathogenic ones could be present in cured sausages. For these reasons we analyzed *Bacillus* strains isolated from industrial and traditional cured pork sausages to gain insight into their potential role in foodborne infections.

Material and Methods: Eight sausage samples were collected from the local market; the sausage casing was removed aseptically and 20 g sample from the central portion of each sausage was homogenized (10 % w/w) in a saline solution. Five milliliters of cell suspension were pasteurized at 80 °C for 10 min and then cooled to room temperature. Serial decimal dilutions in 0.1 % peptone water were poured onto non-selective tryptose soy agar plates (Oxoid, Basingstoke, UK). Aerobic mesophilic counts were determined after incubation at 30 °C for 72 h. Thirty colonies from each sample were collected and analyzed for Gram stain, cell morphology, presence of endospores and catalase reaction. The genomic DNA of each isolate was extracted with DNA Purification Kit (Promega, UK). The isolates were biotyped and taxonomically identified by using a two-step RAPD-PCR protocol and 16S rDNA sequencing (Baruzzi et al., 2000). By means of PCR assays, the strains were analysed for most
important B. cereus virulence factors: enterotoxins FM/S (entFM), T (bceT), and NHE (nheB), HBL, a three-component hemolysin with hemolytic and dermonecrotic activities (hbl-D), sphingomyelinase (sph), and phosphatidylinositol specific phospholipases (pipIc) (Ghelardi et al., 2002).

All the strains were tested for hemolytic activity after growth on blood agar plates (Merck, Darmstadt, Germany) containing 5 % sheep blood. Lecithinase-positive strains produced an halo around the colonies grown onto nutrient agar supplemented with 8 % egg yolk emulsion (Oxoid).

Results: RAPD-PCR analysis showed 52 different fingerprints from 220 colonies. The 52 biotypes identified by means 16S rDNA sequencing belonged to B. subtilis and B. pumilus species with 36 and 16 strains, respectively. No B. cereus strain was isolated from cured pork sausages analyzed. The distribution of total viable cell counts of B. subtilis and B. pumilus strains from each sample is shown in Figure 1.

No PCR fragment related to B. cereus virulence factors was obtained from B. subtilis and B. pumilus strains from cured sausages. The sequence analyses of DNA fragments amplified from positive control strains (B. cereus type strains DSM4312 and DSM4313 and B. cereus BAC1 dairy isolate) showed that they exhibited a high degree of identity (97-100 %) with the B. cereus virulence genes.

The results relative to hemolytic activity showed that 15 out of 16 B. pumilus strains were positive whereas only two B. subtilis strains produced a weak halo of hemolysis after 48 h growth. Lecithinase activity was developed by 72 % B. subtilis and one B. pumilus strains.

Discussion and Conclusions: This is a report of the preliminary results relative to the risk assessment of Bacillus spp. in cured pork sausages. The low levels of viable endospore counts in sausages (the minimal infective dose for the diarrhoeal type is assumed to be 100,000 B. cereus cells), the absence of B. cereus isolates and the absence of B. cereus virulence factors in B. subtilis and B. pumilus strains from traditional and industrial sausages seem to indicate that the samples analyzed do not pose any risk to consumer. Although the hemolytic and lecithinase activity could be considered normal metabolisms in cells from food matrix, more in-depth studies should be carried out to understand if new potential virulence factors are expressed in B. pumilus and B. subtilis.

References: