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. 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.

**Fig. 1 Bacillus subtilis and B. pumilus viable cell counts (black and white columns, respectively) from industrially (1-4) and traditionally (5-8) cured sausages**

**Discussion and Conclusions:** This is a report of the preliminary results relative to the risk assessment of *Bacillus* sp. 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:**
