

# The detection of psychrotrophic lactobacilli and the occurrence of *Lactobacillus curvatus* and *Lactobacillus sakei* in samples of pork meat and ham

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## Abstract

The aim of this study was to detect psychrotrophic lactobacilli, to monitor the occurrence of *Lactobacillus curvatus* and *Lactobacillus sakei* in raw pork meat and pork ham, and to compare the results of the identification of bacteria by MALDI-TOF mass spectrometry and genus- and species-specific polymerase chain reaction (PCR). The samples (n = 23) came from one producer in the Czech Republic. Catalase and oxidase negative isolates cultivated on MRS agar were identified by two methods – MALDI-TOF MS and genus- and species-specific PCR. Lactobacilli were detected in all analysed samples. Forty strains (33%) of 120 suspect isolates were confirmed as *Lactobacillus* spp. by both methods. The results of species identification were the same for both methods. *Lactobacillus curvatus* was detected in one sample of raw pork meat (4%) and six samples of pork ham (26%). *Lactobacillus sakei* was detected in six samples of raw pork meat (26%) and thirteen samples of pork ham (57%).

*Lactobacillus* spp., MALDI-TOF MS, meat products, PCR

## Introduction

Lactic acid bacteria (LAB) are an ubiquitous microbial group comprised of more than twenty genera whose main product of glucose fermentation is lactic acid. *Lactobacillus* is an extremely important genus of LAB made up of more than 150 species (Limsowtin et al. 2002; Salmien et al. 2004 and Kant et al. 2011). The genus *Lactobacillus* is a heterogeneous group of Gram-positive, non-spore-forming, catalase and oxidase negative rods. The significance of many species of lactobacilli lies in the use of their fermentation ability (they make up part of starter cultures in dairy and meat products) and their ability to conserve foodstuffs and animal feeds. Lactobacilli, however, can also contribute to the spoilage of meat and meat products, can form biogenic amines, and can carry genes of antimicrobial resistance (Cury and Crow 2002; Giraffa et al. 2010).

*Lactobacillus sakei* and *Lactobacillus curvatus* are two related species belonging to the *Lactobacillus casei* group (Pot and Tsakalidou 2009). Both species contribute to the fermentation of meat products either as starter cultures or by finding their way into such products by means of secondary contamination (Rimaux et al. 2011). Their metabolic products affect the sensory properties of meat products. They prevent the growth of pathogenic microorganisms such as *Escherichia coli*, *Listeria monocytogenes* etc. by producing bacteriocins and reducing the pH of foodstuffs (Lücke 2000; Martinović and Vesковиć-Moračanin 2006 and Todorov et al. 2013).

Meat is a commodity of a high nutritional value. It contains a relatively large proportion of water, proteins and other substances soluble in water, which makes it a suitable environment for the growth of microorganisms, including bacteria with undesirable properties that cause spoilage (Fernandes 2009).

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The aim of this study was to detect psychrotrophic strains of lactobacilli and to monitor the occurrence of *Lactobacillus curvatus* and *Lactobacillus sakei* in raw pork and ham. It also aimed to compare polymerase chain reaction and MALDI-TOF mass spectrometry in species identification.

### Materials and Methods

Twenty-three samples (6 samples of raw pork muscle and 17 slices of pork ham) obtained from one producer in the Czech Republic were analysed. The samples of ham were stored at 4 °C and analysed over the course of a period of four weeks.

The samples were analysed according to the standards ISO 7218 and ISO 6887-1. Twenty-five grams were taken from the samples of pork ham and homogenised in 225 ml of sterile MRS (De Man-Rogosa-Sharpe) broth (Oxoid, UK). Swabs were taken from the samples of raw pork with a sterile swab from an area of 100 cm<sup>2</sup> and shaken into MRS broth. An inoculum of the appropriate dilution was applied to Petri dishes with MRS agar (Oxoid, UK) and cultivated at 15 °C for six days. Suspect colonies were tested for evidence of the presence of catalase and oxidase (JK Trading, CR). Confirmation of the genus *Lactobacillus* and species identification of *L. sakei* and *L. curvatus* by the polymerase chain reaction (PCR) and MALDI-TOF mass spectrometry methods were performed on isolates with negative oxidase and catalase reactions.

Bacterial DNA for PCR was isolated with a 20% solution of Chelex 100 (Bio-Rad Laboratories, USA). Genotype confirmation of genus was performed according to Dubernet et al. (2002) and species identification according to Berthier and Ehrlich (1998).

MALDI-TOF MS analysis was performed according to a standard protocol (Freiwald and Sauer 2009; Dušková et al. 2012).

### Results and Discussion

Six samples of raw pork muscle and 17 samples of sliced pork ham were analysed. One hundred and twenty suspect isolates of lactobacilli (34 from meat surface, 86 from hams) with a negative oxidase and catalase test were obtained. This genus was confirmed in 40 strains (33%) by PCR and MALDI-TOF MS. The presence of bacteria of the genus *Lactobacillus* was confirmed in all the analysed samples (n = 23) (Table 1).

Species identification by MALDI-TOF MS (Fig. 1) showed *L. curvatus* and *L. sakei* to be the commonest species of lactobacilli in the analysed samples. All the isolates had a Biotyper log (score) higher than 2.0. Twelve strains (32%) had a Biotyper log (score) of between 2.0 and 2.3 (indicating a high probability of genus identification and probable identification at the species level), while 26 strains (68%) had a Biotyper log (score) higher than 2.3 (expressing a high probability at the species level). *L. oligofermentans*, *L. coryniformis* and *L. fructivorans* were other species of lactobacilli detected.

The results of the two methods, MALDI-TOF MS and PCR, show that the species *L. sakei* was detected in 19 analysed samples (83%), while *L. curvatus* was detected in only 7 samples (30%). The conformity of identification of the species *L. sakei* and *L. curvatus* by the PCR and MALDI-TOF MS methods was 100%. The conformity achieved by the MALDI-TOF MS and PCR methods has also been demonstrated during the identification of lactobacilli, including the species *L. sakei* and *L. curvatus* (Dušková et al. 2012).

Matrix-assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF) Mass Spectrometry is a chemotaxonomic method that is currently finding continually increasing application not merely in the identification of pathogens in clinical microbiology, but also in the identification of bacteria isolated from foodstuffs. Lactic acid bacteria and lactobacilli in particular, are a large and problematic group for identification by phenotype methods. A polymerase chain reaction is suitable for their identification, though primers must be selected correctly and it is not always suitable for routine use as it is expensive, time-consuming and laborious. Identification by the MALDI-TOF MS method is an ideal tool for this large and heterogeneous group. The inadequate number of reference strains in the database may prove to be a limitation of this method (Šedo et al. 2011; Doan et al. 2012; Dušková et al. 2012 and Callaway et al. 2013).

Table 1. The origin and frequency of occurrence of lactobacilli isolates (including *L. curvatus* and *L. sakei*) in samples of pork meat and sliced pork ham

Commodity	Number of positive samples ( <i>Lactobacillus</i> spp.)	Number of isolates of lactobacilli	<i>Lactobacillus curvatus</i>	<i>Lactobacillus sakei</i>
			PCR / MALDI-TOF MS identification	
Raw pork	6 (100%)	11 (32%)	1/1	9/9
Sliced pork ham	17 (100%)	29 (34%)	7/7	21/21
Total	23 (100%)	40 (33%)	8 (20%)	30 (75%)

*L. sakei* is a common species that can be isolated from heat-treated meat products such as frankfurters and steamed hams (Audenaert et al. 2010; Comi and Iacumin 2012). Iacumin et al. (2014) detected a predominance of homofermentative lactobacilli (*Lb. sakei*), comprising as much as 70% of the population of LAB, in frankfurters of Italian origin without sensory deviations.

*L. sakei* is also the dominant species in dry fermented sausages. It accounts for as much as 89% (Chorizo) or 76% (Fuet) of the population in Spanish products, and as much as 100% in French products at the end of ripening (Kamenik 2011). *L. sakei* and *L. curvatus* comprise more than 90% of all identified LAB in traditional Italian dry fermented sausages from the southern and central part of the country (Silvestri et al. 2007; Bonomo et al. 2008 and Cocolin et al. 2009). The species *L. sakei*, *L. curvatus* and *L. plantarum* were also the most frequently isolated species in salamis from Sardinia (Greco et al. 2005).

Analyses of the genetic makeup of the species *L. sakei* have recently revealed this microbe's ability to adapt to the meat environment. Meat is a rich source of amino acids. *L. sakei* does not possess metabolic pathways that would allow it to synthesise amino acids

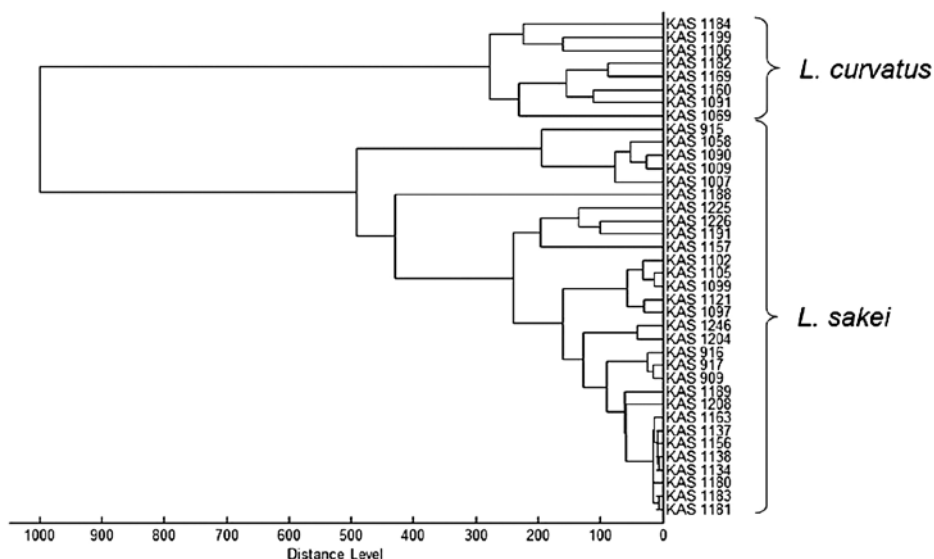


Fig. 1. Dendrogram created on the basis of the results of identification of *L. curvatus* and *L. sakei* isolates by MALDI-TOF MS

with the exception of glutamic acid and aspartic acid. *L. sakei* has also psychrotrophic and osmotolerant properties, what means it can grow in an environment with a low temperature and in the presence of as much as 10% of NaCl. It has obtained these properties thanks to genes that code proteins assuring an appropriate response to stress (stress-response proteins) such as cold-shock proteins and osmotolerant proteins. Equipped with these properties, this species is considerably better adapted to the meat and meat product environment than other lactobacilli. This explains the numerical predominance of *L. sakei* within the LAB population in meat products. The ability of lactobacilli to survive less favourable environmental conditions is well described in the literature (Kameník 2011).

## Conclusions

The results of this study show that psychrotrophic lactobacilli, including *Lactobacillus curvatus* and *Lactobacillus sakei*, occur commonly in pork meat and slices of pork ham. MALDI-TOF MS and PCR are suitable methods for identifying these species.

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## References

- Audenaert K, D’Haene K, Messens K, Ruysen T, Vandamme P, Huys G 2010: Diversity of lactic acid bacteria from modified atmosphere packed sliced cooked meat products at sell-by date assessed by PCR-denaturing gradient gel electrophoresis. *Food Microbiol* **27**: 12-18
- Berthier F, Ehrlich SD 1998: Rapid species identification within two groups of closely related lactobacilli using PCR primers that target the 16S/23S rRNA spacer region. *FEMS Microbiol Lett* **161**: 97-106
- Bonomo MG, Ricciardi A, Zotta T, Parente E, Salzano G 2008: Molecular and technological characterization of lactic acid bacteria from traditional fermented sausages of Basilicata region (Southern Italy). *Meat Sci* **80**: 1238-1248
- Callaway A, Kostrzewa M, Willershausen B, Schmidt F, Thiede B, Kupper H, Kneist S 2013: Identification of Lactobacilli from deep carious lesions by means of species-specific PCR and MALDI-TOF Mass Spectrometry. *Clin Lab* **59**: 1373-1379
- Cocolin L, Dolci P, Rantsiou K, Urso R, Cantoni C, Comi G 2009: Lactic acid bacteria ecology of three traditional fermented sausages produced in the North of Italy as determined by molecular methods. *Meat Sci* **82**: 125-132
- Comi G, Iacumin L 2012: Identification and process origin of bacteria responsible for cavities and volatile off-flavour compounds in artisan cooked ham. *Int J Food Sci Tech* **47**: 114-121
- Curry B, Crow V 2002: *Lactobacillus* spp./General characteristics. In: Roginski H (Ed.): *Encyclopedia of Dairy Science*, Elsevier Science Ltd, 1479-1511
- Doan NTL, Van Hoorde K, Cnockaert M, De Brandt E, Aerts M, Le Thanh B, Vandamme P 2012: Validation of MALDI-TOF MS for rapid classification and identification of lactic acid bacteria, with a focus on isolates from traditional fermented foods in Northern Vietnam. *Lett Appl Microbiol* **55**: 265-273
- Dubernet S, Desmasures N, Gueguen M 2002: A PCR-based method for identification of lactobacilli at the genus level. *FEMS Microbiol Lett* **214**: 271-275
- Dušková M, Šedo O, Kšicová K, Zdráhal Z, Karpišková R 2012: Identification of lactobacilli isolated from food by genotypic methods and MALDI-TOF MS. *Int J Food Microbiol* **159**: 107-114
- Fernandes R 2009: *Microbiology handbook: Meat products*, ISBN 978-1-90522-466-1. Leatherhead Food International Ltd, 312 p
- Freiwald A, Sauer S 2009: Phylogenetic classification and identification of bacteria by mass spectrometry. *Nature Protocols* **4**: 732-742
- Giraffa G, Chanishvili N, Widyastuti Y 2010: Importance of lactobacilli in food and feed biotechnology. *Res Microbiol* **161**: 480-487
- Greco M, Mazzete R, DeSantis EPL, Corona A, Cosseddu AM 2005: Evolution and identification of lactic acid bacteria isolated during the ripening of Sardinian sausages. *Meat Sci* **69**: 733-739
- Iacumin L, Andyanto D, Manzano M, Comuzzo P 2014: A case of spoilage in wurstel sold in an Italian supermarket. *Food Cont* **43**: 245-250
- ISO6887-1 1999: Microbiology of food and animal feeding stuffs - Preparation of tests samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions

- ISO7218 2007: Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
- Kamenik J 2011: Durable meat products. University of Veterinary and Pharmaceutical Sciences Brno, 262 p
- Kant R, Blom J, Palva A, Siezen, RJ, De Vos WM 2011: Comparative genomics of *Lactobacillus*. *Microb Biotech* **4**: 323-332
- Limsowtin GKY, Broome MC, Powell IB 2002: Lactic acid bacteria, taxonomy. In: Roginski H (Ed.): Encyclopedia of Dairy Sciences, Elsevier Science Ltd, 1470-1478
- Lücke FK 2000: Utilization of microbes to process and preserve meat. *Meat Sci* **56**: 105-115
- Martinović A, Vesković-Moračanin S 2006: Primena starter kultura u industriji mesa. *Tehnologija mesa* **47**: 216-230 (In Croatian)
- Pot B, Tsakalidou E 2009: Taxonomy and Metabolism of *Lactobacillus*. In: Ljungh Å, Wadström T (Eds.): *Lactobacillus Molecular Biology*. Caister Academic Press, Norfolk, UK, 3-58
- Rimauxa, T, Vranckena G, Pothakos V, Maes D, De Vuyst L, Leroy F 2011: The kinetics of the arginine deaminase pathway in the meat starter culture *Lactobacillus sakei* CTC 494 are pH-dependent. *Food Microbiol* **28**: 597-604
- Salminen S, Von Wright A, Ouwehand A 2004: Lactic Acid Bacteria Microbiological and Functional Aspects. Marcel Dekker, Inc., 270 Madison Avenue, New York, 655 p
- Silvestri G, Santarelli S, Aquilanti L, Beccaceci A, Osimani A, Tonucci F, Clementi F 2007: Investigation of the microbial ecology of Ciauscolo, a traditional Italian salami, by culture-dependent techniques and PCR-DGGE. *Meat Sci* **77**: 413-423
- Šedo O, Sedláček I, Zdráhal Z 2011: Sample preparation methods for MALDI-MS profiling of bacteria. *Mass Spectrom Rev* **30**: 417-434
- Todorov SD, Vaz-Velho M, Gombossy De Melo Franco BD, Holzapfel WH 2013: Partial characterization of bacteriocins produced by three strains of *Lactobacillus sakei*, isolated from salpicão, a fermented meat product from North-West of Portugal. *Food Cont* **30**: 111-121