The occurrence and characterisation of lactobacilli in meat products

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Abstract

The aim of this study was to monitor the frequency of lactobacilli in ready-to-eat meat products and to determine the species and the antimicrobial susceptibility. Identification of suspect isolates was investigated in this work by polymerase chain reaction. A total of 148 samples of meat products were examined, and lactobacilli were detected in 107 samples (72%). Four lactobacilli species were identified (*L. sakei*, *L. plantarum*, *L. brevis* and *L. curvatus*). Resistance to antimicrobials was screened by the broth microdilution method and minimum inhibitory concentrations were determined. Nine strains (60%) were resistant to at least one antimicrobial and one strain was resistant to three groups of antimicrobial agents.

Lactobacillus spp., species identification, polymerase chain reaction, antibiotic resistance, minimum inhibitory concentration (MIC), broth microdilution method

Introduction

The genus *Lactobacillus* is one of the most abundant groups of bacteria and is of great importance to the food industry. More than 150 species of lactobacilli are currently known, and many find applications as starting cultures in the meat and dairy industries, in which a number of lactobacilli contribute, thanks to their metabolic abilities, to the creation of aromatically active substances and influence the texture of fermented products (Casaburi et al. 2008; Kant et al. 2011). Certain strains have probiotic properties or preservative properties thanks to the creation of bacteriocins, though others may play a part in the deterioration of foodstuffs and the formation of biogenic amines (Bonomo et al. 2008; Marsden et al. 2009; Pircher et al. 2007). The species *L. sakei* and *L. curvatus* often play a part in the deterioration of meat products (Drosinos and Paramithiotis 2009) and, along with *L. plantarum*, make up the largest proportion of lactic acid bacteria in fermented meat products (Cocolin et al. 2009).

One undesirable property of lactobacilli is their resistance to antimicrobial substances and the potential risk of this resistance spreading to other bacteria through mobile genetic elements (Mathur and Singh 2005). The absence of obtained antimicrobial resistance should be an important criterion in assessing the safety of lactobacilli used as starting cultures or probiotics (Mayrhofer et al. 2008). The European Food Safety Authority has issued microbiological breakpoints (EFSA 2008) for the purpose of easier differentiation of strains carrying obtained or natural antibacterial resistance from strains sensitive to these substances.

The aim of this study was to monitor the occurrence of lactobacilli in meat products, their species representation and their sensitivity to antimicrobial substances.

Materials and Methods

A total of 148 samples of meat products, taken from the retail network in the Czech Republic, were tested. These were dry salamis (fermented and cooked), cooked salamis, cooked meat products and small meat products (Table 1).

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Table 1. An overview of tested samples and isolates of lactobacilli obtained

Commodity	Number of samples	Number of positive samples	Number of suspect isolates	Number of positive isolates
Dry fermented salami	25	23 (92%)	56	49 (88%)
Dry cooked salami	25	15 (60%)	38	21 (55%)
Cooked salami	25	22 (88%)	41	27 (66%)
Liver salami	7	4 (57%)	12	8 (67%)
Pork ham	16	11 (69%)	26	15 (58%)
Pork presswurst	8	5 (63%)	16	8 (50%)
White pudding	8	5 (63%)	13	6 (46%)
Black pudding	12	7 (58%)	23	14 (61%)
Sausage	9	7 (78%)	13	10 (77%)
Frankfurter	13	8 (62%)	18	11 (61%)
Total	**148**	**107 (72%)**	**256**	**169 (66%)**

Isolates were cultivated on MRS agar (Oxoid, GB) at 30 °C for 48–72 hours in a microaerophilic conditions. Cell morphology in suspect colonies was studied microscopically (Gram staining), while the cultures were also tested for the presence of catalase and oxidase (JK Trading, CZ). Genotype confirmation of lactobacilli isolates was performed with the use of a polymerase chain reaction (PCR) with genus-specific primers LbLMA 1-rev and R16-1 (Dubernet et al. 2002). Amplification took place in a PTC-200 thermocycler (MJ Research, USA). A two-step multiplex PCR method was used for species identification in selected isolates and subsequently, following the classification of lactobacilli into groups, PCR with species-specific primers was also used (Table 2) based on the detection of nucleotide sequences of the 16S-23S rRNA intergenic spacer region and adjacent 23S rRNA gene differing for individual species of lactobacilli (Berthier and Ehrlich 1998; Song et al. 2000; Walter et al. 2000; Guarneri et al. 2001). Amplicons were detected by agarose gel electrophoresis, stained with ethidium bromide, and visualised using a UV transilluminator (λ = 305 nm).

Sensitivity to antimicrobial substances was tested on 15 identified lactobacilli isolates. The minimum inhibitory concentrations for this collection of strains were determined by the broth microdilution method (Trios spol. s r. o., CZ). The following concentrations were tested for individual antibiotics: ampicillin (0.015–2 mg·l^{-1}), gentamicin (1–128 mg·l^{-1}), chloramphenicol (0.5–64 mg·l^{-1}), streptomycin (2–256 mg·l^{-1}), tetracycline (2–256 mg·l^{-1}), erythromycin (0.031–4 mg·l^{-1}) and clindamycin (0.031–4 mg·l^{-1}). Plates with an inoculated 24-hour culture (turbidity 0.5 °McF) were incubated microaerophilically at 30 °C. Minimum inhibitory concentrations (MIC) were read after 24 hours and the sensitivity or resistance of individual strains determined according to EFSA microbiological breakpoints (2008).

Results and Discussion

Two hundred and fifty-six suspect isolates were cultivated on MRS agar from the samples of meat products examined (n = 148), and these were subsequently identified by phenotype and genotype methods. A total of 169 isolates (66%) were gram-positive, catalase and oxidase negative, and characterised by the formation of a PCR product specific for the genus *Lactobacillus*. Table 1 gives an overview of the individual commodities for which the presence of lactobacilli was confirmed in the samples (72% of samples). Lactobacilli are frequently part of the starting cultures of dry fermented salamis, though an unexpected and relatively high occurrence of lactobacilli was also seen in unfermented meat products.

The species *L. sakei*, *L. plantarum*, *L. brevis* and *L. curvatus* were identified by polymerase chain reaction in selected isolates confirmed by the PCR method as *Lactobacillus* spp. These facultative and obligate heterofermentative species of lactobacilli are generally dominant in fermented uncooked meat products (Cocolin et al. 2009).

The values of minimum inhibitory concentrations for the tested lactobacilli were compared with the breakpoints given by the EFSA (2008). On the basis of these criteria, 9 isolates (60%) were resistant to at least one antibiotic and one strain of *L. plantarum* isolated from fermented salami was resistant to three groups of antimicrobial substances (Table 3). The most frequent resistance was to streptomycin (47% of tested isolates) and

Table 2. Oligonucleotide primers used for lactobacilli identification

PCR Type	Species	Primers	Sequence (5‘-3‘)	Annealing temperature (°C)	Reference
Genus -specific PCR	*Lactobacillus* spp.	LBLMA1-rev R16-1	CTCAAAACTAAACAAAGTTTC CTTGTACACACCGCCCGTCA	55	Dubernet et al. (2002)
Two-step multiplex PCR	Group I	Lac-2 Ldel-7	CCTCTTCGCTCGCCGCTACT ACAGATGGATGGAGAGCAGA	55	Song et al. (2000)
	Group II	Lac-2 LU-1‘	CCTCTTCGCTCGCCGCTACT ATTGTAGAGCGACCGAGAAG		Song et al. (2000)
	Group III	Lac-2 LU-3‘	CCTCTTCGCTCGCCGCTACT AAACCGAGAACACCGCGTT		Song et al. (2000)
	Group IV	Lac-2 LU-5	CCTCTTCGCTCGCCGCTACT CTAGCGGGTGCGACTTTGTT		Song et al. (2000)
Group IV	*L. fermentum*	Lfer-3 Lfer-4	ACTAACTTGACTGATCTACGA TTCACTGCTCAAGTAATCATC	55	Song et al. (2000)
	L. plantarum	Lpla-2 Lpla-3	CCTGAACTGAGAGAATTTGA ATTCATAGTCTAGTTGGAGGT		Song et al. (2000)
	L. reuteri	Lreu-1 Lreu-4	CAGACAATCTTTGATTGTTTAG GCTTGTTGGTTTGGGCTCTTC		Song et al. (2000)
	L. salivarius	Lsal-1 Lsal-2	AATCGCTAAACTCATAACCT CACTCTCTTTGGCTAATCTT		Song et al. (2000)
Species -specific PCR	*L. plantarum*	Lfpr Plan II	GCCGCCTAAGGTGGGACAGAT TTACCTAACGGTAAATGCGA	55	Walter et al. (2000)
	L. curvatus	16 Lc	GCTGGATCACCTCCTTTC TTGGTACTATTTAATTCTTAG	55	Berthier and Ehrlich (1998)
	L. sakei	16 Ls	GCTGGATCACCTCCTTTC ATGAAACTATTAAATTGGTAC	55	Berthier et al. (1998)
	L. brevis	BrevI BrevII	CTTGCACTGATTTTAACA GGGCGGTGTGTACAAGGC	40	Guarneri et al. (2001)

clindamycin (27% of tested isolates). An increased resistance of lactobacilli to clindamycin has also been stated by Klare et al. (2007) and to aminoglycosides by Katla et al. (2001). No resistance to chloramphenicol was discovered in our isolates. Although such resistance is not common, its testing could effectively cover the risk of obtained resistance to linezolid which is, like chloramphenicol, coded for the gene *cfr* (Arias et al. 2008; Toh et al. 2007; EFSA 2008).

Table 3. Resistant isolates according to EFSA breakpoints and resistance phenotypes

Strain	Species	Origin of isolate	Resistance phenotype*
B9	*L. brevis*	dry cooked salami	CLI
A54	*L. plantarum*	dry fermented salami	**GEN**, **STR**, TET, CLI
C16	*L. plantarum*	dry fermented salami	**GEN, STR**, CLI
C33	*L. plantarum*	soft salami	STR
C44	*L. plantarum*	dry fermented salami	STR, CLI
D42	*L. plantarum*	dry fermented salami	STR
D16	*L. sakei*	dry fermented salami	TET
M I 16	*L. sakei*	dry cooked salami	STR
M I 19	*L. sakei*	white pudding	STR

* in bold: antibiotitcs belonging to the same group (aminoglycosides)

For resistant isolates it is essential to focus on the molecular nature of the formation of resistance and to determine whether this involves resistance obtained by mutation or by the mobile genetic elements that represent the greatest risk the spreading of antibiotic resistance.

Conclusions

Representatives of the genus *Lactobacillus* are common, and not merely in fermented meat products intended for direct consumption. The occurrence of the species of lactobacilli that occur naturally in meat products and have no undesirable properties is seen as positive. The results of this study show that resistance to antimicrobial substances may occur among lactobacilli. Although lactobacilli are not pathogenic microorganisms, they occur frequently and in large numbers in foodstuffs, largely fermented foods. This fact may contribute negatively to the uncontrolled horizontal spreading of genes coding resistance to antimicrobial substances throughout the entire human food chain. Closer identification and characterisation of the individual species of lactobacilli isolated from meat products will enable a deeper understanding of their desirable and undesirable properties.

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