RESEARCH LETTER

Carnobacterium divergens – a dominating bacterium of pork meat juice

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Abstract

Nonspoiled food that nevertheless contains bacterial pathogens constitutes a much more serious health problem than spoiled food, as the consumer is not warned beforehand. However, data on the diversity of bacterial species in meat juice are rare. To study the bacterial load of fresh pork from ten different distributors, we applied a combination of the conventional culture-based and molecular methods for detecting and quantifying the microbial spectrum of fresh pork meat juice samples. Altogether, we identified 23 bacterial species of ten different families analyzed by 16S rRNA gene sequencing. The majority of isolates were belonging to the typical spoilage bacterial population of lactic acid bacteria (LAB), *Enterococcaceae*, and *Pseudomonadaceae*. Several additional isolates were identified as *Staphylococcus* spp. and *Bacillus* spp. originating from human and animal skin and other environmental niches including plants, soil, and water. *Carnobacterium divergens*, a LAB contributing to the spoilage of raw meat even at refrigeration temperature, was the most frequently isolated species in our study (5/10) with a bacterial load of 10^3–10^7 CFU mL^{-1}. In several of the analyzed pork meat juice samples, two bacterial faecal indicators, *Serratia grimesii* and *Serratia proteamaculans*, were identified together with another opportunistic food-borne pathogen, *Staphylococcus equorum*. Our data reveal a high bacterial load of fresh pork meat supporting the potential health risk of meat juice for the end consumer even under refrigerated conditions.

Introduction

Raw meat is a 'land of plenty' for most of the bacteria species transferred to this ecological niche – it is an aquatic environment rich in nutrients. Therefore, it is one of the most perishable foods that potentially contain animal-derived pathogenic bacteria (zoonotic agents); thus, it constitutes a potential risk factor for spreading pathogens in its environment. During the last two decades, several studies investigated the spoilage microbiota of refrigerated fresh and vacuum-packaged (VP) meat under diverse modified atmosphere conditions (MAP) to determine appropriate preservation methods (Shaw & Harding, 1984; McMullen & Stiles, 1993; Borch *et al.*, 1996; Sakala *et al.*, 2002; Holley *et al.*, 2004; Ercolini *et al.*, 2006, 2011; Nychas *et al.*, 2008; Schirmer *et al.*, 2009; Doulgeraki *et al.*, 2010; Jiang *et al.*, 2010; Pennacchia *et al.*, 2011). The main focus was set on the improvement of the shelf life of food products by trying to establish other bacterial genus such as lactic acid bacteria (LAB) to compete and displace contaminations by food-borne pathogens and spoilage microflora such as *Enterobacteriaceae* and *Pseudomonadaceae* (Yildirim & Johnson, 1998; Metaxopoulos *et al.*, 2002; Budde *et al.*, 2003; Jacobsen *et al.*, 2003), whereas species of the latter family, which are strict aerobic bacteria, showed a delay of growth under MAP conditions (Jimenez *et al.*, 1997; Viana *et al.*, 2005; Alp & Aksu, 2010). In contrast, most species belonging to the LAB group multiply even under VP conditions but do not initially damage the quality of the meat product as recently affirmed by studies with *Carnobacterium maltaromaticum* (Jones, 2004; Casaburi *et al.*, 2011; Pennacchia *et al.*, 2011). *Pseudomonas* spp. and *Serratia* spp. are metabolizing the abundant nutrient sources, for example, carbohydrates, amino acids, and lipids to end products that spoil the food product; thus, it becomes sensory undesirable for the customer to purchase because of color change, off-odors, and also slime production – a definite

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Table 1. Properties of the ten meat samples

Sample no.	Location of purchase	Designation	Packaging	Volume of meat juice (mL)	Total colony count (CFU mL^{-1})
I	Supermarket	Pork fillet	In air	0.3	5×10^6
II	Butcher shop	Pork fillet	In air	12	1.9×10^8
III	Butcher shop	Pork fillet	In air	20	7.7×10^5
IV	Supermarket	Pork fillet	In air	1	1.2×10^6
V	Supermarket	Pork fillet	In air	0.5	8.2×10^4
VI	Supermarket	Pork fillet	Vacuum	20	5×10^7
VII	Butcher shop	Pork fillet	In air	0.2	2×10^7
VIII	Supermarket	Pork fillet	Vacuum	15	1.5×10^4
IX	Supermarket	Pork loin	In air	0.1	3.4×10^4
X	Supermarket	Pork loin	In air	0.05	3.5×10^4

impairment of the meat quality (Labadie, 1999; Gram *et al.*, 2002; Jay *et al.*, 2003; Koutsoumanis *et al.*, 2006).

Traditional analyses of the bacterial flora of meat and meat products in the past have primarily concentrated on cultivation on selective plates for LAB, *Pseudomonas* spp., and *Enterobacteriaceae* (Blixt & Borch, 2002; Jiang *et al.*, 2010; Pennacchia *et al.*, 2011). The isolation and phenotypic identification of the bacterial species are time-consuming and can be restricted by limiting biochemical differentiation options. Recently, molecular techniques such as PCR-based rapid species identification have been established using genus or species-specific DNA probes or primers for studying food spoilage processes (Muyzer *et al.*, 1993; Macian *et al.*, 2004; Rachman *et al.*, 2004; Fontana *et al.*, 2006; Liu *et al.*, 2010; Ercolini *et al.*, 2011). Ercolini *et al.* stated that the use of both culture-based and molecular methods has been shown to enhance the detection of microbial diversity in foods (Ercolini, 2004; Pennacchia *et al.*, 2011).

In general, bacteria prefer to adhere to surface structures, colonizing the meat surface, because an attachment by glycocalix formation could be shown (Ercolini *et al.*, 2006). Nevertheless, some of the bacteria are planktonic and grow in the meat juice, which is an exudate of the stored meat. Especially, the bacterial load of meat juices is harboring a potential safety hazard for the consumer when handling meat juice in an unhygienic manner, for example, in the consumer's home where, in the refrigerator or on a cutting board, meat juice spillage does not become noticeable and, therefore, harbors a considerable health risk by cross-contamination (de Jong *et al.*, 2008). However, a reliable and comprehensive study of bacterial contamination of pork meat juice is still pending. Our study could have industrial implications, exploring a method to grade the bacterial contamination of the meat by a package integrated sensor which is only in contact with the meat juice. To determine the range of bacterial species and the bacterial load common in the juice of refrigerated pork meat, we applied the combination of both the conventional cultivation as well as a molecular technique.

Material and methods

Meat samples

From different supermarkets or butcher shops, a total of ten portions of fresh pork meat fillet or loin (about 500 g each) were purchased by local distributors at the same day. Most of the samples were from an open counter, only two were vacuum wrapped. The open meat samples were transferred to a sterile plastic bag and together with the vacuum wrapped ones immediately stored in a fridge at +4 °C. After 6 h, the accumulated meat juices were collected into a sterile tube (Table 1).

Bacterial growth conditions and colony counts

Of each meat juice, a sterile 1 : 10 dilution series with PBS solution (0.8% NaCl, 0.144% Na_2HPO_4, 0.024% KH_2PO_4, 0.02% KCl, pH 7.4) were prepared and 100 μL of the appropriate dilutions spread on GCF agar plates (GC agar base; Remel, Wien, Austria) containing 5% fetal calf serum (FCS) in three replicates. After 72 h of incubation at 37 °C, the obtained colonies were counted and used for isolating different bacterial species. The colony-forming units (CFU) per mL were calculated as mean value of triplicates.

Isolation and differentiation of bacterial colonies

Of each countable (25–250 colonies) plate, up to seven single macroscopically different bacterial colonies were purified by subcultivation on GCF agar plates. To minimize repeated sequencing of the same strain macroscopically, similar colonies were screened by Gram staining, cell morphology, and quick enzyme tests such as catalase (4% H_2O_2), coagulase (Staphaurex-Plus; Remel, Dartford, UK), oxidase (BBL-Oxidase-DrySlide, Becton Dickinson), and urease reaction (urea broth; Oxoid, Wesel, Germany). Gram staining was performed using a single colony of a

purified bacterial culture on GCF agar plates applying AxonGram solutions (Axonlab, Austria) according to the manufacturer's instructions. The stained slides were analyzed with a Leica Microscope at 1000× magnification. Pure bacterial clones were stored at −80 °C.

DNA isolation

Bacterial genome DNA was isolated by applying DNA Mini and Blood Mini Kit from Qiagen (Hilden, Germany). Freshly subcultured single colonies were harvested with sterile wooden stick cotton swaps and resuspended in PBS. After centrifugation, the pellet was lysed in lysis buffer containing proteinase K provided by the manufacturer. In case of Gram-positive bacteria, lysozyme (20 mg mL^{-1}) was added as recommended by the manufacturer. In brief, the bacterial DNA was isolated by adhering to silicate in mini columns and eluted with water after washing with an ethanol-containing solution. The DNA concentration was measured with a Nanodrop photometric apparatus (Peqlab, Erlangen, Germany).

PCR amplification and product purification

Purified bacterial genomic DNA was used to amplify a fragment of 1500 bp of the 16S rRNA gene by polymerase chain reaction (PCR) with the forward primer 8F 5′-AGAGTTTGATCCTGGCTCAG-3′ (Galkiewicz & Kellogg, 2008) and reverse primer DG74 5′-AGGAGGTGATCC AACCGCA-3′ (Greisen *et al.*, 1994) (Eurofins, Ebersberg, Germany). The PCR (25 μL) contained 1 U Dream Taq DNA Polymerase (Fermentas, St. Leon-Roth, Germany), 1× Dream Taq Buffer, 0.5 mM dNTPs, 0.15 μM forward and reverse primer, and 30–50 ng genomic DNA. The PCR mixture was subjected to an initial denaturation step of 5 min at 95 °C, followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 52 °C, and extension of 2 min at 72 °C, and a final extension of 10 min at 72 °C in a Peltier Thermal Cycler PTC-200 (BioRad, Vienna, Austria). The amplification product was visualized by agarose gel electrophoresis (1% agarose in 1× TAE-buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 8.0)). Midori green (Fermentas) stained DNA bands (1.5 kb) were excised under a 360-nm UV light box and purified with the NucleoSpin Extract II Kit (Macherey-Nagel, Dueren, Germany).

Sequencing and identification of the species

The sequencing of both strands of the amplified 16S rRNA gene was run by Eurofins sending 150 ng of the purified PCR product. The quality of the obtained sequence was checked by screening the chromatogram of each read. The complete sequence was then compared to the DNA databases using the program BLAST (http://www.ncbi.nlm.nih.gov). Sequence alignments with the highest score were investigated for identifying the bacterial strain by specific 16S rRNA gene sequence.

Results

Total bacterial count of meat juice samples

The total bacterial count of each pork meat juice sample is summarized in Table 1 ranging from 10^4 to 10^8 CFU mL^{-1} after 6 h storage at 4 °C. Only 30% of the analyzed samples reached a bacterial load between 10^7 and 10^8 CFU mL^{-1}. The results did not reveal any differences between the bacterial count of juice from VP pork meat and in air stored ones. In general, up to seven macroscopically different colonies were observed on the agar plates. These single colonies were differentiated by size, color, and shape as well as by rapid enzymatic assays such as oxidase, catalase, coagulase, and urease and Gram staining. Following this procedure, out of each meat juice sample between three and seven different bacterial colonies could be purified for further analyses.

Identification of the bacterial spectrum in pork meat juice

All together, 52 colonies were successfully purified, genomic DNA isolated, and applied for 16S rRNA gene amplification by PCR. Both strands of the eluted PCR product were sequenced and the obtained consensus sequence addressed to a BLAST search. In a preliminary experiment, 12 macroscopically different colonies were isolated and purified in duplicates. The BLAST results of these sequenced 16S rRNA genes revealed in 75% an identical identification of the species. In three cases, further exclusion criteria had to be applied such as Gram staining, bacterial shape, and rapid enzymatic tests to determine the bacterial species (data not shown). In one case of such a duplicate isolation, the sequence of two different *Serratia* spp. (*S. grimesii* and *S. liquefaciens*) was listed with an equal BLAST score value. Further, differentiation of these two Gram-negative bacteria species was not addressed because the usual applied exclusion criteria were not sufficient. Altogether, 23 different bacterial species were identified in juice samples of fresh pork meat (Table 2). The distribution of Gram-positive and Gram-negative species was more or less equal, whereas approximately 50% of these species did not belong to the taxonomy families of *Enterobacteriaceae*, *Pseudomonadaceae*, and LAB. Most of the other families belong to the Gram-positive species of skin flora and environmental

Table 2. List of identified bacterial species of all pork meat juice samples sorted by its taxonomic family and typical ecological niche named

Taxonomy (family)	Isolated species	Ecological niche	Accession no.	% Identity [query coverage/ max. identity]	References
Bacillaceae	*Bacillus amyloliquefaciens*	Plants	GU125630.1	100/99	Arguelles-Arias *et al.* (2009)
	Bacillus subtilis	Soil	GU994112.1	100/99	Ali *et al.* (2012)
Carnobacteriaceae	*Carnobacterium divergens*	Skin, mucosa	HQ259724.1	99/100	Leisner *et al.* (2007)
	Carnobacterium gallinarum	Fish, meat	AJ387905.1	100/99	Leisner *et al.* (2007)
Enterobacteriaceae	*Pectobacterium carotovorum (Erwinia carotovorum)*	Plant pathogen	FJ527481.1	100/100	Terta *et al.* (2010)
	Serratia grimesii	Faecal pollution	DQ991163.1	100/100	Ashelford *et al.* (2002)
	Serratia liquefaciens	Soil, plants, water, nosocomial infection	HQ335000.1	99/99	Ashelford *et al.* (2002)
	Serratia proteamaculans	Faecal pollution	AJ233435.1	100/100	Ashelford *et al.* (2002)
	Yersinia ruckeri	Fish (pathogen)	HQ222844.1	100/99	Wortberg *et al.* (2012)
Flavobacteriaceae	*Kaistella flava*	Soil	AM421015.1	100/98	Gargiulo *et al.* (2008)
Lactobacilliaceae	*Lactobacillus sakei*	Skin, mucosa	HQ992696.1	100/99	Chiaramonte *et al.* (2010)
Micrococcaceae	*Kocuria rhizophila*	Soil, surface water	FR682683.1	100/99	Lecomte *et al.* (2011)
	Rothia amarae	Soil and water, sludge of foul water	FR682692.1	100/98	Fan *et al.* (2002)
Moraxellaceae	*Enhydrobacter* sp.	Water	FN377702.1	99/99	Srinivas *et al.* (2009)
Pseudomonadaceae	*Pseudomonas fluorescens*	Soil, water	EU434349.1	99/99	Bodilis *et al.* (2006)
	Pseudomonas synxantha	Soil, rhizosphere	GQ900609.1	100/99	Wechter *et al.* (2002)
Shewanellaceae	*Shewanella putrefaciens*	Marine fish	AB057660.1	99/99	Tryfinopoulou *et al.* (2007)
Staphylococcaceae	*Macrococcus caseolyticus*	Animal skin	AP009484.1	100/99	Tsubakishita *et al.* (2010)
	Staphylococcus epidermidis	Skin of human and animals	HM209751.1	100/99	Nagase *et al.* (2002)
	Staphylococcus equorum	Skin of human and animals	FR691468.1	99/99	Novakova *et al.* (2006)
	Staphylococcus pasteuri	Skin of human and animals, food	AB269765.1	100/99	Chesneau *et al.* (1993)
	Staphylococcus succinus	Skin of human and animals, cheese	HQ018602.1	99/100	Novakova *et al.* (2006)
	Staphylococcus xylosus	Skin of human and animals	GQ222240.1	99/100	Dordet-Frisoni *et al.* (2007)

bacteria such as *Staphylococcaceae* and *Bacillaceae*, respectively.

Quantification of isolated bacterial spectrum in pork meat juice

To quantify the bacterial species with the three highest bacterial loads of each meat juice sample, it was necessary to retrace the identified species to its macroscopic colony appearance on GCF agar plates. For that, all taken single colonies were initially numbered on the agar plate; thus, after identification of the bacterial species, a correlation to its colony appearance was possible. Because digital pictures were taken of all important agar plates, the bacterial load could be quantified by counting equally appearing colonies and calculating the approximate CFU mL^{-1}. In parallel, the total bacterial count of each meat juice sample was also determined (Table 3). The most frequently isolated species were as expected LAB (Leisner *et al.*, 2007), followed by species from the genera *Enterobacteriaceae*, *Pseudomonadaceae*, as well as some other environmental bacteria. In half of the sample of the LAB, *Carnobacterium divergens* revealed highest prevalence (Table 4). Other typical and most frequently isolated species from meat juice were *Serratia* spp., *Pseudomonas* spp., *Kocuria rhizophila*, *Staphylococcus equorum*, and *Lactobacillus sakei*. The logarithmic values of the highest frequent species in pork meat juice ranged from 10^3 up to 10^7 CFU mL^{-1}. These values were individually assessed in relation to the total bacterial amount. In general, the amount of the most frequent species isolated of each juice sample revealed a maximal difference of one logarithmic step regarding the total bacterial load of this sample (Table 3).

Table 3. Macroscopic characterization of isolated bacteria with the highest frequency (CFU mL^{-1}) within each of the 10 meat juice samples in relation to total CFU

No. of strain	Isolated species	Taxonomy (order – family)	Gram staining	Bacterial shape	Bacterial count of isolated species* (CFU mL^{-1})	Total bacterial count (CFU mL^{-1})
I-A	*Carnobacterium divergens*	*Lactobacillales – Carnobacteriaceae*	+	Coccoid rods	10^6	5×10^6
I-B	*Staphylococcus equorum*	*Bacillales – Staphylococcaceae*	+	Staphylococci	10^3	
I-G	*Pseudomonas fluorescens*	*Pseudomonadales – Pseudomonadaceae*	–	Rods	10^4	
II-D	*Kocuria rhizophila*	*Actinomycetales – Micrococcaceae*	+	Cocci	10^7	1.9×10^8
III-A	*Carnobacterium divergens*	*Lactobacillales – Carnobacteriaceae*	+	Coccoid rods	10^5	7.7×10^5
III-D	*Pseudomonas fluorescens*	*Pseudomonadales – Pseudomonadaceae*	–	Rods	10^4	
IV-A	*Carnobacterium divergens*	*Lactobacillales – Carnobacteriaceae*	+	Coccoid rods	10^5	1.2×10^6
V-A	*Kocuria rhizophila*	*Actinomycetales – Micrococcaceae*	+	Cocci (tetrade)	10^4	8.2×10^4
V-B	*Staphylococcus equorum*	*Bacillales – Staphylococcaceae*	+	Staphylococci	10^4	
V-C	*Serratia proteamaculans*	*Enterobacteriales – Enterobacteriaceae*	–	Rods	10^4	
VI-A	*Serratia proteamaculans*	*Enterobacteriales – Enterobacteriaceae*	–	Rods	10^6	5×10^7
VII-A	*Carnobacterium divergens*	*Lactobacillales – Carnobacteriaceae*	+	Coccoid rods	10^7	2×10^7
VIII-A	*Pectobacterium carotovorum*	*Enterobacteriales – Enterobacteriaceae*	–	Rods	10^3	1.5×10^4
VIII-B	*Pseudomonas synxantha*	*Pseudomonadales – Pseudomonadaceae*	–	Rods	10^3	
VIII-C	*Carnobacterium divergens*	*Lactobacillales – Carnobacteriaceae*	+	Coccoid rods	10^3	
IX-E	*Pseudomonas* sp.	*Pseudomonadales – Pseudomonadaceae*	–	Rods	10^4	3.4×10^4
X-A	*Kocuria* sp.	*Actinomycetales – Micrococcaceae*	+	Cocci (tetrade)	10^3	3.5×10^4

*These are approximate bacterial count values in CFU mL^{-1}, calculated by counting equally appearing colonies.

Discussion

In this study, we investigated the microbiota and the bacterial load of pork meat juice. The pork fillet or loin was purchased by different distributors. In general, we were looking for refrigerated samples dated before expiration. The analysis was performed 6 h after the purchase, a time point mimicking the situation of a final customer buying a portion of pork meat for a meal at the same day. Meat juice handled in the kitchen might easily cross-contaminate other food items such as salad that is consumed raw. A transfer of bacteria via kitchen tools and especially cutting boards is easily imaginable. In such cases, the composition of the bacterial flora of the meat juice represents a potential hazard that could lead to food poisoning even under chilled conditions. Applying a combination of a culture-dependent analysis with a molecular method to characterize the microbial population present in meat juice, a broad range of bacteria could be identified. By means of 16S rRNA gene sequences, 23 different bacterial species of 10 different taxonomic families were depicted. The most frequently isolated bacteria species from pork meat juice were belonging to the families of *Enterobacteriaceae*, *Pseudomonadaceae*, and LAB. As demonstrated in several former studies, bacteria of these genera are assigned as typical spoilage flora (Borch *et al.*, 1996; Gill, 1996; Gram *et al.*, 2002; Jay *et al.*, 2003; Ercolini *et al.*, 2006; Koutsoumanis *et al.*, 2006) including *C. divergens*, *Pseudomonas* spp., and *Serratia* spp. The nonmotile, Gram-positive LAB, *C. divergens*, is a psychrotrophic and microaerophilic but oxygen-tolerating bacterium that is weakly acidotolerant (Leisner *et al.*, 2007), a predominant

bacterium in industrial foods, frequently associated with the spoilage of refrigerated meat and fish products (Borch *et al.*, 1996; Barakat *et al.*, 2000; Cailliez-Grimal *et al.*, 2005). However, it could be shown that *C. divergens* is only dominantly present in fresh meat products, but absence in spoiled products (Jones, 2004; Chenoll *et al.*, 2007). This contradiction is addressed in the literature (Laursen *et al.*, 2005; Leisner *et al.*, 2007); thus, *C. divergens* has not always been considered as important in terms of spoilage potential, indeed the potential of species belonging to the Carnobacterium genus as spoilage agents is not always clear-cut. There are studies that even propose *C. divergens* as biopreservative agent (Spanggaard *et al.*, 2001; Laursen *et al.*, 2005; Ringo *et al.*, 2007; Kim & Austin, 2008). Several studies were focusing on the shift of the microbiota during the process of meat deterioration (Borch *et al.*, 1996; Gram *et al.*, 2002; Ercolini *et al.*, 2006; Schirmer *et al.*, 2009). A shift from aerobic Gram-negative *Pseudomonas* spp. to Gram-positive LAB was observed during this process of pork meat spoilage (Schirmer *et al.*, 2009; Jiang *et al.*, 2010).

Other studies have revealed a LAB-dominating microbiota, including *Lactobacillus* spp. and *Leuconostoc* spp. in spoiled meat products (Borch *et al.*, 1996; Bjorkroth & Korkeala, 1997; Bjorkroth *et al.*, 2000; Santos *et al.*, 2005; Chenoll *et al.*, 2007), indicating an overgrowth of the fresh meat dominating *Carnobacterium* spp. by other LAB during storage (Jones, 2004; Chenoll *et al.*, 2007). But at the time of packaging, the concentration of these LAB were below the detection threshold of culturing methods of bacteria. This could be a plausible explanation why we did not dominantly isolate species of the genera *Lactobacillaceae*. In contrast to earlier observations, where *L. sakei* was mainly detected in psychrotrophic bacterial flora of vacuum-packed meat and meat products (Hugas, 1998; Jiang *et al.*, 2010), we have isolated *L. sakei* in our study out of in air-packaged fresh meat juice samples but not out of juice samples of VP meat. The literature is controversial about the benefit of LAB in raw meat. In one respect, these bacteria are discussed as causative agents of meat deterioration (Borch *et al.*, 1996; Labadie, 1999; Koutsoumanis *et al.*, 2006), and on the other hand, several studies have shown the importance of LAB in the microbiota of fresh meat (Hastings *et al.*, 1994; Gill, 1996). There it is supposed that LAB compete with other spoilage-related bacteria only in fresh meat under VP or MAP by releasing metabolites such as organic acids (e.g. lactate) and bacteriocins, thus preventing the growth of spoilage bacteria and, therefore, increasing the shelf life of the fresh meat and meat products.

Our data reveal *C. divergens* as a dominating bacterium in fresh pork meat juice, whereas under continuous storage, Ercolini *et al.* demonstrated some species of the genus *Pseudomonas* as dominating active bacterial contributors to spoilage under aerobic conditions and even at refrigeration temperatures (Labadie, 1999; Ercolini *et al.*, 2006, 2011; Koutsoumanis *et al.*, 2006). In our study, *Pseudomonas fluorescens* were detected in 4/10 pork meat juice samples at moderate concentrations, supporting this observation. Besides other species, Pennacchia *et al.* (2011) detected in meat samples at time point zero following bacteria: *Pseudomonas* spp., *C. divergens*, and *Serratia* spp. For detecting these species, they and others (Ercolini *et al.*, 2006) were combining culture-based and molecular approaches such as PCR-denaturing gradient gel electrophoresis (DGGE) based on 16S rRNA gene amplification and pyrosequencing to enhance the understanding of the populations of spoilage bacteria. Because above-mentioned molecular methods are widely exploited for the characterization of fermented foods (Ercolini, 2004; Casaburi *et al.*, 2011), it is only in some cases optimized to monitor the microbiota and its changes during storage in meat (Ercolini *et al.*, 2006; Fontana *et al.*, 2006; Diez *et al.*, 2008). Therefore, we used the benefits of combining two methods, culturing and 16S rRNA gene sequencing of the isolated bacteria, to enhance the detection of microbial diversity in foods. Because fresh meat is easily contaminated by the slaughtering process, thus serving as substrate for different spoilage and pathogenic bacteria, it harbors a nonnegligible health risk for all end consumers. The question arose whether our identified meat juice microbiota of 23 bacterial species from ten different taxonomic families contains food poisoning-related bacteria and opportunistic bacterial pathogens. Typical food poisoning bacteria identified from meat products such as *Salmonella* spp., enteropathogenic *Escherichia coli*,

Table 4. Prevalence of most frequent isolated bacteria species in 10 pork meat juice samples

Isolated species	Taxonomy (order – family)	Colony count (CFU mL^{-1})	Prevalence in 10 samples
Carnobacterium divergens	*Lactobacillales – Carnobacteriaceae*	10^3–10^7	5
Pseudomonas fluorescens	*Pseudomonadales – Pseudomonadaceae*	10^3–10^4	4
Serratia proteamaculans	*Enterobacteriales – Enterobacteriaceae*	10^3–10^6	3
Kocuria rhizophila	*Actinomycetales – Micrococcaceae*	10^3–10^7	3
Lactobacillus sakei	*Lactobacillales – Lactobacillaceae*	10^5–10^6	2
Staphylococcus equorum	*Bacillales – Staphylococcaceae*	10^3–10^4	2

Shigella spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Kajikazawa, *et al.*, 2007) have not been detected in our samples, possibly because in fresh meat juice, these species, if any are present, might be in very low concentrations. Besides *S. grimesii* and *Serrtia proteamaculans* (Kajikazawa, *et al.*, 2007), a further opportunistic food-borne pathogen, *S. equorum*, residing the skin of human and animals, was detected in our meat juice samples. Depending on handling, these observations support the hazardous potential of meat juice for the end consumer. In general, the striking analogy of the microbiota of meat with meat juice offers useful opportunities for detecting the bacterial load and diversity by industrial implementation; for example, developing a package integrated sensor grading the bacterial contamination of meat juice.

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