ACTA VET. BRNO 2013, 82: 237–241; doi:10.2754/avb201382030237

Weissella viridescens in meat products – a review

Marta Dušková[1], Josef Kameník[2], Renáta Karpíšková[1,3]

[1]University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Milk Hygiene and Technology, [2]Department of Meat Hygiene and Technology, Brno, Czech Republic
[3]Veterinary Research Institute, Brno, Czech Republic

Received December 19, 2012
Accepted May 29, 2012

Abstract

Although ubiquitous, bacteria of the *Weissella* genus are not given sufficient attention. Many members of the genus were originally classified as *Leuconostoc* or *Lactobacillus*. With the development of molecular methods, these phylogenetically closely related bacteria formed a separate group, the *Weissella* genus. Due to its heterofermentative metabolism, *Weissella* spp. may cause considerable damage particularly in the meat industry. Slime formation and greening of meat products are sensory defects for which the technologically important species *Weissella viridescens* is responsible. This article summarizes basic information about the influence of *Weissella viridescens* on meat processing.

Lactic acid bacteria, Weissella spp., spoilage, greening, thermal resistance

The negative effects of heterofermentative lactobacilli in cooked meat products were described almost 60 years ago by Niven et al. (1954). The researchers' attention was drawn to the causative agents of greening, both on the surface and in the core of products. While colour changes of the surface were caused by bacteria contaminating the product only after cooking, the greening of the product core was due to the activity of bacteria that survived elevated temperatures. In their experiment, they paid particular attention to the testing of thermal resistance of the isolated lactobacilli. While strains collected from surface colorations did not survive exposure to the temperature of 65.56 °C (150 F) for only 10–12 min, lactobacilli in meat product cores survived that temperature for 120 min. Under laboratory conditions, it was possible to even increase thermotolerance of certain species by successive heating.

Lörincz and Incze (1961) mentioned these heterofermentative lactic acid bacteria under the name of *Lactobacillus viridescens*.

Collins et al. (1993) began to be interested in a group of unknown bacteria that very closely resembled leuconostocs (the so-called *Leuconostoc*-like microorganisms) isolated from fermented Greek salami. Based on the results of sequential analysis of the genes 16S rRNA and other previous phylogenetic studies, a new genus *Weissella* was proposed. The new genus was named after the German microbiologist Norbert Weiss, whose scientific interests focused mainly on lactic acid bacteria, which also include weissellas (Collins et al. 1993). At present, we recognize 15 species of weissellas: *W. beninensis*, *W. ceti*, *W. cibaria*, *W. confusa*, *W. fabaria*, *W. ghanensis*, *W. halotolerans*, *W. hellenica*, *W. kandleri*, *W. kimchii* (*W. cibaria*), *W. koreensis*, *W. minor*, *W. paramesenteroides*, *W. soli*, *W. thailandensis* and *W. viridescens*. Weissellas belong to the family *Leuconostocaceae*, order *Lactobacillales*, class *Bacilli*, phylum *Firmicutes* and domain *Bacteria* (Collins et al. 1993; Tanasupawat et al. 2000; Björkroth et al. 2002; Choi et al. 2002; Lee et al. 2002; Magnusson et al. 2002; De Bruyne et al. 2008; De Bruyne et al. 2010; Padonou et al. 2010; Vela et al. 2011).

The aim of this paper was to summarize the data about the influence of *Weissella viridescens* on meat and meat products.

Address for correspondence:
MVDr. Josef Kameník, CSc., MBA
Department of Meat Hygiene and Technology
Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences Brno
Palackého tř. 1/3, CZ-61242 Brno, Czech Republi

Phone: +420 604 220 851
E-mail: kamenikj@vfu.cz
http://actavet.vfu.cz/

Characteristic and occurrence of *Weissella* spp.

Both macroscopic and microscopic morphology of weissellas is easily interchangeable with that of other representatives of lactic acid bacteria, in particular with the leuconostocs and lactobacilli. Weissellas are Gram-positive irregular short rods with rounded ends or coccoid rods, occurring singly, in pairs or in short chains (Plate I, Fig. 1). On de Man, Rogosa and Sharpe (MRS) agar, weissellas form small transparent colonies, circular in shape of a slightly elevated profile, older cultures form a concentric structure (Plates I, II, Figs 2 and 3). These microorganisms are optionally anaerobic, and grow rapidly under microaerophilic incubation. They multiply at 15 °C but not at 45 °C, with the exception of certain *W. confusa* strains. They are non-sporulating and mostly non-motile, and both oxidase and catalase negative. The *W. paramesenteroides* species is able to produce catalase at low glucose content. Under aerobic conditions, weissellas are capable of accumulating hydrogen peroxide, are unable to reduce nitrates and do not hydrolize gelatin (Niven and Evans 1957; Collins et al. 1993).

Bacteria of the genus *Weissella* can exist in very diverse environments. They are frequently isolated from plant material, e.g. fresh vegetables, cassava and silage (Wang and Nishinno 2008), cacao beans (De Bruyne et al. 2008), as well as from meat and meat products (Milbourne 1983; Collins et al. 1993; Han et al. 2010; Patterson et al. 2010; Han et al. 2011; Comi and Iacumin 2012; Doulgeraki et al. 2012; Kesmen et al. 2012), fish (Tanasupawat et al. 2000; Liu et al. 2009), kimchi (Choi et al. 2002; Jang et al. 2002; Lee et al. 2002), specialties from Malaysia (Björkroth et al. 2002), soil (Magnusson et al. 2002) and, in isolated cases, they can also occur in clinical material of human or animal origin. In such cases, they are of course associated with other bacteria. Weissellas are generally considered as non-pathogenic (Walter et al. 2001; Björkroth et al. 2002; Liu et al. 2009).

Methods of *Weissella* spp. identification

Representatives of the *Weissella* genus are not easy to identify by means of phenotypic methods. It is particularly problematic to differentiate between weissellas and heterofermentative lactobacilli and leuconostocs. Weissellas can be distinguished from homofermentative lactobacilli, pediococci, enterococci, lactococci and streptococci by gas production during saccharide fermentation (Collins et al. 1993). Molecular biology techniques, such as polymerase chain reaction (PCR) with genus-specific primers Weissgrp and LWrev (Schillinger et al. 2008) during which an amplicon of 1200 bp is produced at weissellas (Plate II, Fig. 4) are more useful. To facilitate rapid identification of *Weissella* species, Jang et al. (2002) developed an amplified rDNA restriction analysis (ARDRA). They used restriction endonucleases *Mnl*I, *Mse*I and *Bce*AI to cleave *Weissella* genus-specific product of 725 bp. For the identification of microbial diversity in Turkish dry fermented salami of the sucuk (sudžuk, soudjouk) type, Kesmen et al. (2012) used polymerase chain reaction with denaturing gradient gel electrophoresis (PCR-DGGE) for V1 and V3 regions of genes 16S rDNA, and the interrepetitive PCR (rep-PCR) and the sequencing of genes 16S rDNA and 16S-23S rDNA of intergenic regions.

Weissella viridescens in meat products

Because of their heterofermentative metabolism, weissellas may also be involved in food spoilage. They ferment glucose to DL lactic acid, with the exception of *W. paramesenteroides* and *W. hellenica* that produce D(–)-lactic acid (Collins et al. 1993).

The most important species in the meat processing industry is *W. viridescens* (formerly *Lactobacillus viridescens*), which may be involved in meat product spoilage. *W. viridescens* ferments glucose, mannose, fructose, maltose, sometimes saccharose, and rarely also mannitol (Niven and Evans 1957).

W. viridescens may be the cause of slime formation or the greening of meat. Slime formation begins with individual colonies appearing on wet surface, to form a continuous layer of greenish slime later on. The underlying cause of the greening of meat products is the formation of hydrogen peroxide, which oxidizes nitrosomyochromogen, the colour pigment of meat products. Green discolouration of packaged meat products (sausages, smoked meat, vacuum-packaged meat) develops when the product is exposed to oxygen. *Weissella* is known to have been the cause of core greening of smoked pork loin and sausages with a lower redox potential which allowed for H_2O_2 accumulation (Marsden et al. 2009).

Many studies investigated the capability of weissellas to survive heat treatment, and the possible increase in their thermal resistance after repeated exposure to thermal shock. *W. viridescens* has been isolated from products after heating at 65 °C for 30 min, or at 50 °C for 175 min. It has also been detected in brine at a 10–14% concentration (Lörincz and Incze 1961). Thermoresistance of *W. viridescens* was studied also by Vrchlabský and Leistner (1971). They quote a literary reference on the strain's ability to survive in liver pâté at 95 °C for 10 min. Under laboratory conditions, they demonstrated the protective effect of NaCl at concentrations in the 1.4–3.4% range on *W. viridescens* survival during cooking. Products with a_w in the 0.985–0.975 range thus offer better conditions for the survival of these bacteria at elevated temperatures.

D_{60} values of a majority of lactic acid bacteria are around 0.25–0.66 min (Franz and von Holy 1996). When Milbourne (1983) tested *W. viridescens* for survival in heated MRS broth, the D values that he obtained were 23.5 min (D_{65}), 12 min (D_{75}) and 9.5 min (D_{80}). However, after the weissellas had been exposed to a succession of thermal shocks, they were able to withstand the temperature of 65 °C for 140 min. If the *Weissella* can adapt itself to withstand high temperatures in the laboratory, it can do the same in processing facilities, which poses an increased risk of meat product spoilage by thermoresistant *Weissella* strains. D_{65} values obtained from survival tests of these strains in sliced ham were higher than those obtained from tests with cultivation media. Peirson et al. (2003) also obtained higher D values from cooked sausage test (D_{60} = 14.7 min), weissellas in ATP broth were sensitive to heat.

Comi and Iacumin (2012) investigated spoilage in cooked hams where cavities in the muscle of hams were caused by the growth of *Weissella viridescens* and the CO_2 produced by the fermentation of carbohydrates added to the brine which was used for the production of the hams. Spoiled hams had a slight vinegar off-flavour because weissellas produce acetic acid during fermentation. The same authors also investigated physiological properties of *W. viridescens* isolates and studied weissellas growth in 6 and 8% NaCl. The 6% NaCl environment had no effect on the growth of weissellas, and 80% of strains grew even in 8% NaCl. Comi and Iacumin (2012) also found that *Weissella viridescens* was able to grow at refrigeration temperatures with generation times at 8 °C, 6 °C and 4 °C being 5, 12 and 20 h, respectively.

Among microbial cultures involved in ham spoilage, *Weissella viridescens* is the most resistant against high-pressure processing of hams. It can withstand pressures of 400 to 600 MPa at 22 °C for 10 min. *W. minor* was also able to survive under those conditions, whereas *W. paramesenteroides* was not (Han et al. 2010; Han et al. 2011).

Antimicrobial activity of *W. viridescens*

Patterson et al. (2010) demonstrated antimicrobial activity in *W. viridescens* strains against a broad range of microorganisms. This property could in the future be used to enhance microbiological safety of foods. The problem is, however, that not all of the tested strains demonstrated the inhibitory action against *Listeria monocytogenes*, *Clostridium botulinum*, *E. coli* and *Salmonella* spp.

In conclusion, although weissellas may cause significant financial losses particularly in the meat processing industry, they do not belong among lactic acid bacteria that get the attention of either processors or service organisations. This opens up the area for new investigation that could help to prevent sensory defects caused by the bacterial genus of *Weissella*.

References

Björkroth KJ, Schillinger U, Geisen R, Weiss N, Hoste B, Holzapfel WH, Korkeala HJ, Vandamme P 2002: Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. Int J Syst Evol Micr **52**: 141-148

Choi HJ, Cheigh CI, Kim SB, Lee JC, Lee DW, Choi SW, Park JM, Puyn YR 2002: *Weissella kimchi* sp. nov., a novel lactic acid bacterium from kimchi. Int J Syst Evol Micr **52**: 507-511

Collins MD, Samelis J, Metaxopoulos J, Wallbanks S 1993: Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. J Appl Microbiol **75**: 595-603

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

De Bruyne K, Camu N, De Vuyst L, Vandamme P 2010: *Weissella fabaria* sp. nov., from a Ghanaian cocoa fermentation. Int J Syst Evol Micr **60**: 1999-2005

De Bruyne K, Camu N, Lefebre K, De Vuyst L, Vandamme P 2008: *Weissella ghanensis* sp. nov., isolated from a Ghanaian cocoa fermentation. Int J Syst Evol Micr **58**: 2721-2725

Doulgeraki AI, Ercolini D, Villani F, Nychas GJE 2012: Spoilage microbiota associated to the storage of raw meat in different conditions. Meat Sci **157**: 130-141

Franz CMAP, Von Holy A 1996: Thermotolerance of meat spoilage lactic acid bacteria and their inactivation in vacuum-packaged vienna sausages. Int J Food Microbiol **29**: 59-73

Han Y, Jiang Y, Xu X, Sun X, Xu B, Zhou G 2011: Effect of high pressure treatment on microbial populations of sliced vacuum-packed cooked ham. Meat Sci **88**: 682-688

Han Y, Xu X, Jiang Y, Zhou G, Sun X, Xu B 2010: Inactivation of food spoilage bacteria by high pressure processing: Evaluation with conventional media and PCR-DGGE analysis. Food Res Int **43**: 1719-1724

Jang J, Kim B, Lee J, Kim J, Jeong G, Han HG 2002: Identification of *Weissella* species by the genus-specific amplified ribosomal DNA restriction analysis. FEMS Microbiol Lett **212**: 29-34

Kesmen Z, Yetiman AE, Gulluce A, Kacmaz N, Sagdic O, Cetin B, Adiguzel A, Sahin F, Yetim H 2012: Combination of culture-dependent and culture-independent molecular methods for the determination of lactic microbiota in sucuk. Int J Food Microbiol **153**: 428-435

Lee JS, Lee KC, Ahn JS, Mheen TI, Pyun YR, Park YH 2002: *Weissella koreensis* sp. nov., isolated from kimchi. Int J Syst Evol Micr **52**: 1257-1261

Liu JY, Li AH, Ji C, Yang WM 2009: First description of novel *Weissella* species as an opportunistic pathogen for rainbow trout *Oncorhynchus mykiss* (Walbaum) in China. Vet Microbiol **136**: 314-320

Lörincz F, Incze K 1961: Angaben über die Grünverfärbung von Fleisch und Fleischerzeugnissen hervorrufenden Laktobazillen. Fleischwirtschaft **13**: 406-408

Magnusson J, Jonsson H, Schnürer J, Roos S 2002: *Weissella soli* sp. nov., a lactic acid bacterium isolated from soil. Int J Syst Evol Micr **52**: 831-834

Marsden J, Ahmed-Kotrola N, Saini J, Fung DYC, Phebus R 2009: Cooked meats, poultry and their products. In: Fernandes R (Ed.): Meat products. Leatherhead Food International Ltd, UK, pp. 53-82

Milbourne K 1983: Thermal tolerance of *Lactobacillus viridescens* in ham. Meat Sci **9**: 113-119

Niven CF Jr, Buettner LG, Evans JB 1954: Thermal tolerance studies on the heterofermentative lactobacilli that cause greening of cured meat products. Appl Microbiol **2**: 26-29

Niven CF Jr, Evans JB 1957: *Lactobacillus viridescens* nov. spec., a heterofermentative species that produces a green discoloration of cured meat pigments. J Bacteriol **73**: 758-759

Padonou SW, Schillinger U, Nielsen DS, Franz CMAP, Hansen M, Hounhouigan JD, Nago MC, Jakobsen M 2010: *Weissella beninensis* sp. nov., a motile lactic acid bacterium from submerged cassava fermentations, and emended description of the genus *Weissella*. Int J Syst Evol Micr **60**: 2193-2121

Patterson MF, McKay AM, Connolly M, Linton M 2010: Effect of high pressure on the microbiological quality of cooked chicken during storage at normal and abuse refrigeration temperatures. Food Microbiol **27**: 226-273

Peirson MD, Guan TY, Holley RA 2003: Thermal resistances and lactate and diacetate sensitivities of bacteria causing bologna discolouration. Int J Food Microbiol **86**: 223-230

Schillinger U, Boehringer B, Wallbaum S, Caroline L, Gonfa A, Huch M, Holzapfel WH, Franz CMAP 2008:

A genus-specific PCR method for differentiation between *Leuconostoc* and *Weissella* and its application in identification of heterofermentative lactic acid bacteria from coffee fermentation. FEMS Microbiol Lett **286**: 222-226

Tanasupawat S, Shida O, Okada S, Komagata K 2000: *Lactobacillus acidipiscis* sp. nov. and *Weissella thailandensis* sp. nov., isolated from fermented fish in Thailand. Int J Syst Evol Micr **50**: 1479-1485

Vela AI, Fernández A, De Quiros YB, Herraez P, Dominguez L, Fernandez-Garayzabal JF 2011: *Weissella ceti* sp. nov., isolated from besed whales (*Mesoplodon bidens*). Int J Syst Evol Micr **61**: 2758-2762

Vrchlabsky J, Lesitner L 1971: Thermoresistance of lactobacilli at various a_w-value (in German). Fleischwirtschaft **51**: 1368-1370

Walter J, Hertel C, Tannock GW, Lis CM, Munro K, Hammes WP 2001: Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella* species in human faeces by using group-specific PCR primers and denaturing gradient gel electrophoresis. Appl Environ Microb **671**: 2578-2585

Wang F, Nishinno N 2008: Ensiling of soybean curd residue and wet brewers grains with or without other feeds as a total mixed ration. J Dairy Sci **91**: 2380-2386

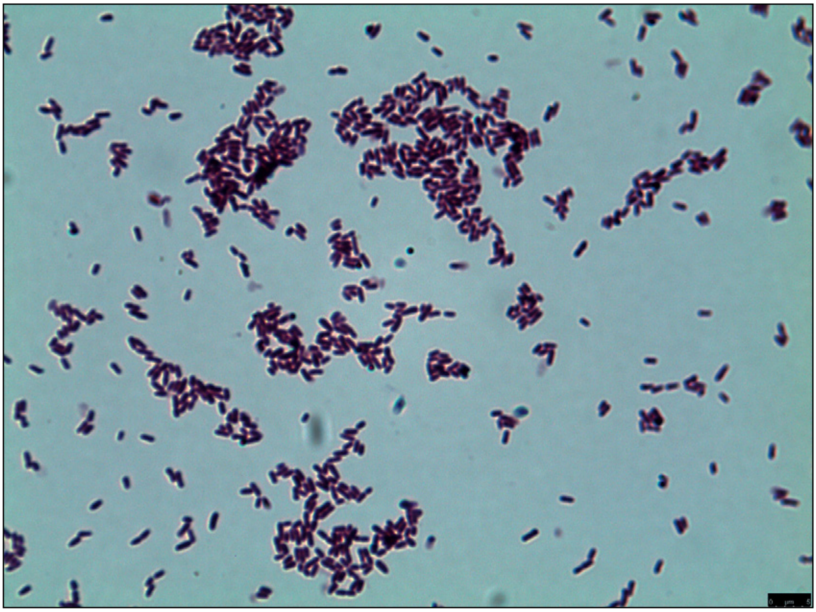

Fig. 1. Microscopy of *Weissella viridescens* cells stained with the Gram stain (×1000 magnification).

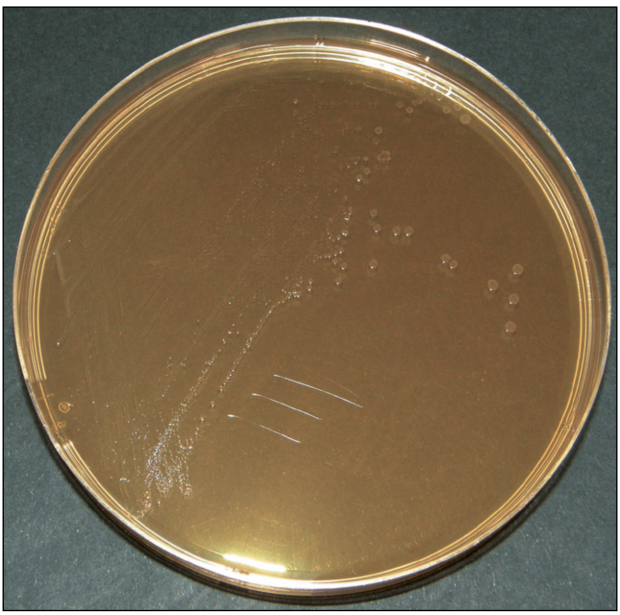

Fig. 2. Culture of *Weissella viridescens* isolated from dry heat-processed Vysočina salami on MRS agar (Oxoid, UK).

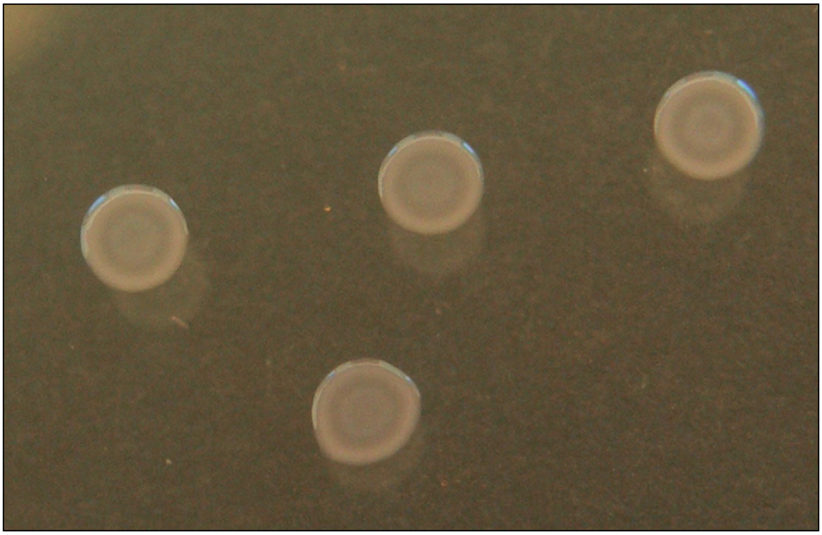

Fig. 3. A detailed view of colonies of *Weissella viridescens* bacteria with concentric structure on de Man, Rogosa and Sharpe agar.

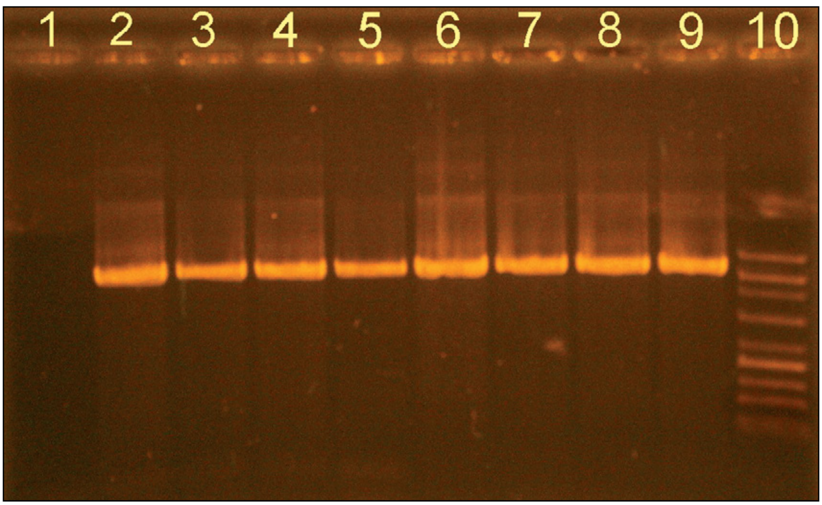

Fig. 4. Agarose gel electrophoresis of genus *Weissella*-specific PCR products (1200 bp). 1 - negative control (PCR H_2O without DNA), 2 - positive control (*Weissella viridescens* CCM 56^T), 3 - positive control (*Weissella confusa* CCM 7277), 4–9 - *Weissella* spp. (isolates from Vysočina salami), 10 - marker (200–1500 bp).