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MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY BASED IDENTIFICATION OF THE FISH GUT MICROBIOTA

Miroslava Kačániová[1,2], Margarita Terentjeva[3], Alīna Klūga[3], Monika Březinová[4], Miriam Kádasi Horáková[5]*

Address(es): prof. Ing. Miroslava Kačániová, PhD.
[1]*Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, SK-94976 Nitra, Slovakia,*
[2]*Department of Bioenergy and Food Technology, Faculty of Biology and Agriculture, University of Rzeszow, Zelwerowicza St. 4, PL-35601 Rzeszow, Poland,*
[3]*Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture, K. Helmaņa iela 8, LV-3004, Jelgava, Latvia,*
[5]*Department of Landscape Management, Faculty of Agriculture, University of South Bohemia, Studentská 1668, CZ-37005 České Budějovice, Czech Republic,*
[5]*Department of Plant Pathology and Mycology, Institute of Forest Ecology, Slovak Academy of Sciences, Akademická 2, SK-94901 Nitra, Slovakia.*

*Corresponding author: miroslava.kacaniova@gmail.com

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ABSTRACT

The aim of the study was to investigate the composition of intestinal microflora of freshwater fish in Latvia. A total amount of 28 fish were collected from fishermen (n=20) and retail outlets (n=8), including roach *Rutilus rutilus*, n=15, crucian carp *Carassius carassius*, n=5, perch *Perca fluvialitis*, n=5, bream *Blicca bjoerkna*, n=3. Microbiological testing consisted of the detection of total viable count (TPC), *Enterobacteriaceae* and coliforms with subsequent identification with MALDI-TOF Biotyper. TPC, coliforms and *Enterobacteriaceae* counts ranged from 2.7±0.4 to 5.4±0.3, 2.4±1.5 to 3.7±0.7 and 2.00±1.2 to 3.7±2.5 log cfu/g in gut of wild crucian carp and retailed roach, wild perch and retailed roach, and crucian carp and bream, accordingly. The TPC, coliforms and *Enterobacteriaceae* counts were significantly higher in retailed fish than in wild fish gut samples (P≤0.05). Gut microbiota were represented by *Proteobacteria* (93.0%), *Firmicutes* (3.9%) and *Ascomycota* (3.1%). The most abundant families were *Enterobacteriacea* (50.8%) and *Pseudomonadaceae* (36.7%). *Rahnella aquatilis*, *Serratia fonticola* and *Pantoe aagglomerans* were the most abundant among *Enterobacteriaceae* while *Pseudomonas extremorientalis* and *P. fragi* among the *Pseudomonadaceae*. Results of the present study show that the gut of freshwater fish were mostly represented by *Enterobacteriaceae* and *Pseudomonadaceae* and the presence of fish bacterial pathogens must be considered.

Keywords: bream, crucian carp, MALDI-TOF MS Biotyper, *Enterobacteriaceae*, *Pseudomonadaceae*, roach

INTRODUCTION

The digestive tract of fish is a habitat of heterogeneous microflora and is colonized by a high variety and number of microorganisms (**Burr *et al.*, 2005**). The existing symbiosis between host and the intestinal microflora is of great significance for all live organisms, including fish (**Sugita *et al.*, 1997**). In fish, the gut microbiota is important for digestion of food, protection of fish against the bacterial pathogens and development of immunological response. Impairment in balance of gut microbiota affects the fish health, consequently, the stability and composition of gut microbiota are important (**Gómez and Balcázar, 2008**).
Composition of fish gut microbiota depends on various influencing factors, including fish species, age, nutritional, genetic factors and environmental conditions of habitat (**Gómez and Balcázar, 2008; Floris *et al.*, 2013**). Differences between the composition of intestinal microbiota of marine and freshwater fish were identified and the freshwater fish gut microorganisms were more diverse with the genera *Aeromonas*, *Flavobacterium*, *Pseudomonas* and the family *Enterobacteriaceae* were found to be dominant (**Skrodenyté-Arbačiauskiené, 2008**). Composition of gut microbiota may differ between the individual fish and fish species (**González *et al.*, 1999**).
Since the gut microbiota consist of various groups of microorganisms of different functional significance, an establishment of balanced gut microflora is essential. The normal indigenous microbiota act competitively and prevent the colonization of gut by pathogens, however, the different other groups of pathogenic microorganisms of fish, animal and human health significance can be found in gut (**Austin, 2006**). Thus, the identification and analysis of fish gut microbiota helps not only recognize the composition of fish microflora in different environments, but also to tackle potentially pathogenic microorganisms affecting the fish health. Fish is an important food source and the studies on fish gut microbiota are important for an assessment of fish health and safety of fish for consumption as well (**Holben et al., 2002**).

Freshwater fish is a significant part of fish fauna in Latvia and usually the most accessible for fishermen. Of total more than 40 freshwater species represented, roach *Rutilus rutilus*, perch *Perca fluviatilis* and bream *Blicca bjoerkna* were found to be the main species caught by fishermen in inland waters (**Birzaks, 2008**).
Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged in the recent years as a reliable and rapid method for identification and characterization of microorganisms. The MALDI-TOF MS has several advantages in comparison with microbiological method in terms of sensitivity and economical aspects, including processing, application and staff costs. The applications of the MALDI-TOF for identification of microorganisms, detection of food-borne pathogens, strain typing, characterization of antibiotic resistance, epidemiological studies etc. were described (**Singhal *et al.*, 2015**). The studies on the characterization of freshwater fish microbiota with MALFI-TOF MS are very limited.
The aim of the present study was to identify and analyse the freshwater fish gut microbiota with MALDI-TOF MS Biotyper.

MATERIAL AND METHODS

Sampling

Altogether, an amount of 28 freshwater fish were collected in Latvia. Samples included roach (*Rutilus rutilus* n=15), crucian carp (*Carassius carassius*, n=5), perch (*Perca fluviatilis*, n=5) and bream (*Blicca bjoerkna*, n=3). Samples were obtained from fishermen immediately after angling (n=20) and purchased at retail market (n=8). Fish from retail market were purchased as a whole, ungutted fish. Sampled fish were placed on ice and transported to the laboratory for microbiological testing. Examination of fish was initiated within 2 h after sampling and the gut was aseptically removed from surrounding tissues and used for further investigations.

Microbiological testing of gut content

For microbiological testing, the total plate count (TPC), *Enterobacteriaceae* and total coliforms were detected. An amount of 1 g of the gut content was transferred to 9 mL of peptone buffered water (Oxoid, Basingstoke, *United Kingdom)* and mixed to obtain the initial dilution. The initial dilution was used for the preparation of further serial dilutions. Then, a quantity of 1 mL of the initial and serial dilutions was transferred in sterile Petri dishes and covered with 15 mL of molten Plate Count Agar (PCA) for TPC, Violet Red Bile Glucose Agar (VRBGA) for *Enterobacteriaceae* and Violet Red Bile Lactose Agar (VRBLA) for total coliforms *(Oxoid)*. After inoculation, the PCA plates were incubated at 30°C for 72h, but VRBGA and VRBLA at 37°C for 24h with evaluation of bacterial growth after incubation. All colonies were counted on PCA, while the typical *Enterobacteriaceae* and coliform colonies were enumerated on VRBGA and VRBLA. Altogether, one to twenty five colonies were selected from the each plate for further confirmation with MALDI-TOF.

Identification of bacteria with MALDI-TOF MS Biotyper

Altogether, 128 microbial colonies were used for confirmation and the colonies were picked up from agar, suspended in 300 µl of sterile distilled water and mixed thoroughly. Then, an amount of 900 µL of absolute ethanol (99%, Sigma-Aldrich, USA) was added. The mixture was centrifuged at 13 000 x g for 2 min. Later, the supernatant was removed and the pellet was centrifuged. Residual ethanol was completely removed by pipetting and the pellet was allowed to dry at a room temperature. Subsequently, an amount of 10 µL of formic acid (70%, Sigma-Aldrich, USA) was added to the pellet and mixed with a sterile toothpick. Next, a 10 µL of acetonitrile (100%, Sigma-Aldrich, USA) was added and mixed thoroughly. The solution was centrifuged at maximum speed for 2 min and 1 µL of the supernatant was spotted on a polished MALDI target plate (Bruker Daltonics, Germany). Immediately after drying a 1 µL of the matrix solution was added to each spot and allowed to air dry. The matrix used was a saturated solution of HCCA: α-cyano-4-hydroxycinnamic acid (Bruker Daltonics, Germany) dissolved in 50 % acetonitrile with 0.025 % trifluoroacetic acid (TFA) (100%, Sigma-Aldrich, USA). The matrix solution preparation (2.5 mg of HCCA) contains 500 µL of acetonitrile, 475 µL of ultrapure water and 25 µL of trifluoroacetic acid. An amount of 250 µL of the solution was added to the 2.5 mg of HCCA. Samples were processed in the MALDI-TOF MS (Microflex LT/SH, Bruker Daltonics, Germany) with flex Control software and results were obtained with Realtime Classification software (RTC) (Bruker Daltonics, Germany) (**Kačániová *et al.*, 2019**).

Statistical analyses

All bacterial counts were transferred into a decimal log. The One-Way ANOVA test was used for calculation of significance of differences between bacterial counts in different fish species.

RESULTS

Total plate count (TPC) ranged from 2.7±0.5 to 5.4±0.3 log cfu/g in the gut of wild crucian carp and retailed roach, respectively. Coliform counts were from 2.4±1.5 to 3.7±0.7 log cfu.g^{-1} in wild perch and retailed roach, but *Enterobacteriacea*e from 2.00±1.2 to 3.7±2.5 log cfu.g^{-1} in the gut of crucian carp and bream, accordingly (Table 1). The TPC, coliforms and *Enterobacteriaceae* counts were significantly higher in retailed fish gut than in wild fish gut samples (P≤0.05), while the significant differences between the bacterial counts of retailed fish were not identified (P≥0.05).

Table 1 Total bacterial count, *Enterobacteriaceae* and coliform counts in gut freshwater fish (in log cfu/g)

Species	Origin	No. of samples	TPC	Coliforms	*Enterobacteriaceae*
Roach	Wild	10	4.39±0.57[a]	2.96±0.46[b]	1.97±1.41
	Retailed	5	5.39±0.29[a]	3.69±0.69[b]	3.7±0.65
Crucian carp	Wild	5	2.72±0.46[a]	2.55±0.36[b]	1.95±1.15
Perch	Wild	5	4.15±0.53[a]	2.39±1.48[b]	2.74±0.61
Bream	Retailed	3	5.3±0.29[a]	3.62±2.35[b]	3.72±2.49

[a] differences between TPC in wild roach and crucian carp were significant (P<0.05), while there were no significant differences between TPC in retailed roach and bream gut (P>0.05)
[b] differences in coliform counts were not significant between wild (roach, crucian carp, perch) and retailed fish (roach, bream) (P>0.05)
[c] differences in *Enterobacteriaceae* counts between wild (roach, crucian carp, perch) and retailed fish (roach, bream) were significant (P<0.05)

The most abundant microbial phylum of fish gut was *Proteobacteria* (93.0%) followed by *Firmicutes* (3.9%) and *Ascomycota* (3.1%). The most abundant microbial families were *Enterobacteriaceae* (50.8%) and *Pseudomonadaceae* (36.7%) while the less abundant were *Bacillaceae*, *Lactobacillaceae*, *Peptostreptococcaceae*, *Sphingomonadaceae* and *Xanthobactereaceae* (0.8% each). *Enterobacteriaceae* was the predominant in gut of the wild roach (57.4%), crucian carp (50%), but there were no differences between the abundance of *Enterobacteriaceae* and *Pseudomonadaceae* in retailed roach and perch intestinal samples (P>0.05). The families *Aeromonadaceae* and *Clostridiaceae* were the most abundant in bream gut (33.3% each) (Table 2). The most diverse microbiota were recovered from the wild roach gut, but the less diverse from retailed roach gut with six and two phyla were identified, respectively.

Table 2 Abundance of microorganisms in freshwater fish gut

Family	Wild roach *Rutilus rutilus*	Roach retailed *Rutilus rutilus*	Crucuan carp *Carassius carassius*	Perch *Perca fluvialitis*	Bream *Blicca bjoerkna*
			No. of isolates (%)		
Aeromonadaceae	0 (0)	0 (0)	2 (5.9)	0 (0)	2 (33.3)
Bacillaceae	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
Clostridiaceae	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.3)
Enterobacteriaceae	35 (57.4)	7 (50.0)	17 (50.0)	5 (38.6)	1 (16.7)
Lactobacillaceae	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)
Peptostreptococcaceae	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomonadaceae	22 (36.2)	7 (50.0)	13 (38.2)	5 (38.6)	0 (0)
Sphingomonadaceae	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
Streptococcaceae	0 (0)	0 (0)	0 (0)	1 (7.6)	0 (0)
Xanthobactereaceae	0 (0)	0 (0)	0 (0)	1 (7.6)	0 (0)
Saccharomycetaceae	1.6 (0)	0 (0)	2 (5.9)	1 (7.6)	0 (0)
Total	61 (100)	14 (100)	34 (100)	13 (100)	6 (100)

Among the *Enterobacteriaceae*, the most abundant bacterial species were *Rahnella aquatilis* (17 isolates), *Serratia fonticola* (9 isolates) and *Pantoea aagglomerans* (8 isolates). Beside the *Pseudomonadaceae*, *Pseudomonas extremorientalis* and *P. fragi* were the most abundant (6 isolates).
Rahnella aquatilis and *Providencia heimbachae* were the most abundant *Enterobacteriaceae* in wild (19.67%) and retail roach gut (21.43%), respectively. *Pantoea agglomerans* and *Rahnella aquatilis* were the most abundant in crucian carp gut (29.4%) but *Enterobacter cloacae* and *Enterobacter amnigenus* in perch and bream gut (40%) and one isolate (100%), respectively (Table 3).

Table 3 Microflora of gut of wild and retailed freshwater fish

Family	Identified species	Roach		Crucian carp	Perch	Bream
		Wild	Retailed	Wild	Wild	Retailed
		N=10	N=5	(n=5)	(n=5)	(n=3)
		No. of isolates identified (%)				
Aeromonadaceae	*A. bestiarum*	0 (0)	0 (0)	1 (2.9)	0 (0)	0 (0)
	Aeromonas eucrenophila	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)
	Aeromonas hydrophila	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
	Aeromonas veronii	0 (0)	0 (0)	1 (2.9)	0 (0)	0 (0)
Bacillaceae	*Bacillus megaterium*	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
Clostridiaceae	*Clostridium septicum*	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)
	Clostridium chauvoei	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)
Enterobacteriaceae	*Buttiauxella ferragutiae*	0 (0)	0(0)	3 (8.7)	0 (0)	0 (0)
	Citrobacter gillenii	2 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)
	Enterobacter amnigenus	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Enterobacter cloacae	2 (3.3)	0 (0)	0 (0)	2 (15.3)	0 (0)
	Ewingella americana	3 (4.9)	0 (0)	0 (0)	0 (0)	0 (0)
	Hafnia alvei	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Klebsiella oxytoca	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)
	Enterobacter amnigenus	1 (1.6)	0 (0)	0 (0)	0 (0)	1 (12.5)
	Moellerella wisconsensis	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Pantoea agglomerans	2 (3.3)	0 (0)	5 (14.8)	1 (7.7)	0 (0)
	Pluralibacter pyrinus	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Providencia heimbachae	0 (0)	3 (21.6)	0 (0)	0 (0)	0 (0)
	Rahnella aquatilis	12 (19.6)	2 (14.2)	5 (14.8)	0 (0)	1 (12.5)
	Raoultella ornithinolytica	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)
	Serratia entomophila	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Serratia fonticola	4 (7.3)	1 (7.1)	4 (11.6)	0 (0)	0 (0)
	Serratia liquefaciens	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Serratia plymuthica	2 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)
	Yersinia intermedia	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Yersinia ruckeri	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)
Lactobacillaceae	*Lactobacillus mucosae*	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)
Peptostreptococcaceae	*Filifactor villosus*	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomonadaceae	*Pseudomonas antarctica*	1 (1.6)	0 (0)	0 (0)	1 (20)	0 (0)
	Pseudomonas brenneri	0 (0)	0 (0)	1 (2.9)	0 (0)	0 (0)
	Pseudomonas extremorientalis	2 (3.3)	0 (0)	4 (11.6)	0 (0)	0 (0)
	Pseudomonas fluorescens	2 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)
	Pseudomonas frederiksbergensis	2 (3.3)	0 (0)	2 (6)	0 (0)	0 (0)
	Pseudomonas fragi	3(4.9)	3 (21.6)	0 (0)	0 (0)	1 (12.5)
	Pseudomonas fulva	0 (0)	0 (0)	1 (2.9)	0 (0)	0 (0)
	Pseudomonas gessardii	1 (1.6)	0 (0)	1 (2.9)	0 (0)	0 (0)
	Pseudomonas grimontii	1 (1.6)	0 (0)	0 (0)	1 (20.0)	0 (0)
	Pseudomonas koreensis	0 (0)	2 (14.2)	2 (6)	0 (0)	0 (0)
	Pseudomonas libanensis	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Pseudomonas lundensis	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Pseudomonas marginalis	1 (1.6)	0 (0)	2 (6)	1 (20.0)	0 (0)
	Pseudomonas orientalis	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Pseudomonas proteolytica	2 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)
	Pseudomonas putida	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Pseudomonas rhodesiae	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)
	Pseudomonas synxantha	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Pseudomonas thivervalensis	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)
	Pseudomonas tolaasii	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)
	Presudomonas trivialis	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)
	Pseudomonas veronii	2 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)
Sphingomonadaceae	*Sphingopyxis terrae*	1 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)
Streptococcaceae	*Streptococcus salivarius*	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)
Saccharomycetaceae	*Candida pelliculosa*	1 (1.6)	0 (0)	2 (6)	0 (0)	0 (0)
	Pichia fermentans	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)
Xanthobacteraceae	*Starkeya novella*	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)
Total		61 (100)	14 (100)	34 (100)	13 (100)	6 (100)

DISCUSSION

The TPC, *Enterobacteriaceae* and coliform counts identified in the present study were in agreement with the previously reported (**Navarrete *et al*., 2010; Wu *et al*., 2010; Floris, 2013; Kluga *et al*., 2017**). The bacterial counts in gut depend on environmental factors, water and feed quality and diet. Bacterial counts can vary between the individual fish but, in general, the gut is inhabited by a large number of microorganisms (**Spanggaard *et al*., 2000**). Bacterial counts in retailed fish were higher than in freshly caught fish that could be attributable to storage on ice before the sale, as it is known the bacteria may proliferate during the storage and impair quality of the fish product (**Al Bulushi *et al*., 2008**). Our results on the bacterial counts of freshly caught freshwater fish correspond to findings in fish gut originated from cold and relatively unpolluted waters (**González *et al*., 1999**).

A phylum *Proteobacteria* was found to be predominating in gut of all studies freshwater fish species. Bacteria representing the *Proteobacteria* and the *Firmicutes* phylum were frequently reported to be present in fish gut. *Proteobacteria* was the predominated phylum in the gut of yellow catfish (*Pelteobagrus fulvidraco*), gilthead sea bream (*Sparus aurata*) and crucian carp *Carassius gibelio* (**Wu *et al*., 2010; Floris, 2013; Kashinskaya *et al*., 2015**). The results of our study support the observation that *Proteobacteria* were prevalent in the gut of freshwater fish (**Kashinskaya *et al*., 2015**).

Enterobacteriaceae family was predominated in the gut of wild roach and crucian carp, while there were no differences between the abundance of *Enterobacteriaceae* and *Pseudomonas* in the gut of retailed roach, bream and perch. In general, our findings are in line with the previously reported and *Enterobacteriaceae* was found to be predominated in freshwater salmon (*Salmo salar*) and sea trout (*Salmo trutta trutta*) (**Skrodenyté-Arbačiauskienė *et al*.,**

2008). *Enterobacteriaceae* and *Pseudomonas* were found to be the most abundant in the gut of roach (*Rutilus rutilus*) (**Skrodenyté-Arbačiauskiené, 2007**). In contrast, **Floris *et al.* (2013)** reported that *Pseudomonas* spp. were predominant in gilthead sea bream (*Sparus aurata*) in two coastal lagoons of Sardinia. *Pseudomonas* are metabolically versatile microorganisms ubiquitous in the environment and frequently associated with fish and water as a habitat of fish (**Vaz-Moreira *et al.*, 2012**). Results of our study show that *Pseudomonas* spp. alongside with *Enterobacteriaceae* are the important representatives of the intestinal microbiota of wild freshwater fish. *Rahnella aquatilis*, *Serratia fonticola* and *Pantoea agglomerans* of the *Enterobacteriaceae* family were found be the most abundant that is in agreement with the previous studies on broad distribution of the bacteria in the environment, including the fish. The bacteria were identified in water, soil, plants, snails, slug, molluscs and the intestinal tract of fish (**Derlet and Carlson, 2004; Piotrowska-Seget *et al.*, 2005; Skrodenyté-Arbačiauskiené, 2007**).

Other microorganisms as *Bacillus*, *Buttiauxella*, *Ewingella*, *Serratia*, *Providencia*, *Raoultella*, *Sphingomonas*, *Candida*, *Pichia* and *Starkeya* were associated with water, soil, vegetables, foods, insects, plants and trees (**White *et al.*, 1996; Kelly *et al.*, 2000; Hurst and Jackson, 2002; Barchiesi *et al.*, 2005; Aravind *et al.*, 2009; Vadkertiová *et al.*, 2012**). Additionally, *C. gillenii* and *M. wisconsensis* were recognized as the members of fish microflora in previous studies (**Skrodenyté-Arbačiauskiené *et al.*, 2008; Lü *et al.*, 2011**). Due to wide distribution of the microorganisms in the environment, they could enter the intestinal tract of fish. Our findings revealed that the gut of freshwater fish may be a habitat of those microorganisms.

Pseudomonas spp. were frequently isolated from fish and are recognized to be the specific spoilage microorganism involved in the deterioration of the quality of freshly chilled fish (**Gram and Dalgaard, 2002**). Since the *Pseudomonas* spp. may develop the rapid growth in favourable condition, the predominance of *Pseudomonas* spp. in fish is undesirable and lead to the fish spoilage. *P. fragi*, *P. lundensis* and *P. fluorescens* were found to be the predominated in the fish at the end of shelf-life and contributed the spoilage (**Tryfinopoulou *et al.*, 2002**). Thus, the abundance of *Pseudomonas* in the gut may results in additional contamination of fish fillet during the gutting and predisposes the bacterial spoilage processes (**MacMillan and Santucci, 1990**).

Pseudomonas were associated with fish diseases and fish pathogenic *Pseudomonas* spp. identified in the present study. *P. fluorescens* was the causative agent of bacterial haemorrhagic septicaemia in rainbow trout, carp and chronic disease in catfish (**Shahi and Mallik, 2014**). *P. koreensis* caused the eye infection in golden mahseer in India (**Shahi and Mallik, 2014**), but *P. putida* ulcers in rainbow trout (**Altinok *et al.*, 2006**). Alongside with the *Pseudomonas* spp., *Hafnia alvei*, *Enterobacter cloaceae*, *Yersinia intermedia* and *Y. ruckeri* were reported to be the fish pathogenic (**Acosta *et al.*, 2002; Toback *et al.*, 2007; Sekar *et al.*, 2008**). *Aeromonas* are present primary in aquatic environments and *A. hydrophila* was found to inhabit normally the intestinal tract of fish (**Carvalho *et al.*, 2012**). However, the bacteria may become the opportunistic fish pathogen in a variety of farmed fish during stressful growth conditions (**Li *et al.*, 2013**). *A. bestiarum* and *A. veronii* are expected to be pathogenic for fish and were isolated from common carp and trout (**Kozińska, 2007**). Abovementioned bacteria and particularly *Y. ruckeri* may result in fish diseases with high mortality and attributed significant economic losses, therefore, the presence of those pathogens must be taken into consideration. The pathogenic microorganisms were isolated both from retail and wild fish, indicating the circulation of the pathogenic microorganisms in the environment and aquaculture.

Fish may carry the microorganisms which are opportunistic pathogens or pathogenic to consumers. Consumption of fish contaminated with *A. hydrophila*, *A. caviae* and *A. veronii* bv. *sobria* may cause the foodborne gastroenteritis. *Aeromonas* were linked to wound and respiratory infections, septicaemia, liver abscesses, urinary tract and eye infections. *K. oxytoca*, *E. cloaceae*, *P. agglomerans*, *R. aquatilis* and *R. ornitholytica* are nosocomial pathogens responsible for different clinical manifestations, including the urinary tract, respiratory tract, wound, skin and soft tissues infections and bacteremia. *Clostridium* spp. may result in gas gangrene with human and the animal patient became affected (**Tash, 2005; Cruz *et al.*, 2007; Gorkiewicz, 2009**). Our findings indicate that fish may serve as a source of the microorganisms of fish and public health significance. The present results reveal the potential risks of bacterial contamination of fishes from Latvia. Periodic monitoring of microorganisms pathogenic for fish and consumers, is important to identify any potential treat (**Alikunhi *et al.*, 2016**).

CONCLUSION

In conclusion, the present study confirms the predominance of *Enterobacteriaceae* and *Pseudomonadaceae* in of the gut freshwater fish. The composition of microbiota may alter the fish health alongside with the quality and safety of fish meat and fish products. The fish intestinal tract may serve as a habitat for microorganisms with fish and public health significance, therefore the results of present study indicate that fish may be an important vector for transmission of potentially pathogenic microorganisms for fish and fish consumers.

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