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Analysis of cultured aerobic and capnophilic bacteria of the digestive tract of common garden snail *Cornu aspersum aspersum* (Müller, 1774) by MALDI-TOF-MS

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Summary

This study investigated the gastrointestinal bacteria of the snail *Cornu aspersum aspersum* under capnophilic and aerobic conditions using MALDI-TOF-MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry). Under capnophilic conditions, 13 different bacterial species were identified: *Acinetobacter pittii*, *Aeromonas eucrenophila*, *Aeromonas hydrophila*, *Buttiauxella agrestis*, *Buttiauxella gaviniae*, *Enterococcus caseliflavus*, *Klebsiella oxytoca*, *Lactococcus lactis*, *Leclercia adecarboxylata*, *Pantoea agglomerans*, *Raoultellaplanticola*, and *Raoultella terrigena*. In contrast, bacterial identification under aerobic conditions revealed 12 species: *Aeromonas hydrophila* (n = 4), *Aeromonas sobria* (n = 2), *Buttiauxella agrestis*, *Buttiauxella izardii*, *Buttiauxella warmboldiae* (n = 3), *Citrobacter freundii*, *Escherichia vulneris*, *Klebsiella oxytoca*, *Morganella morganii* (n = 2), *Myroides odoratimimus*, *Proteus vulgaris* (n = 4), and *Providencia rettgeri*. Although several species (e.g., *Aeromonas hydrophila*, *Buttiauxella agrestis*, *Klebsiella oxytoca*) were identified under both conditions, some species, such as *Acinetobacter pittii*, *Aeromonas eucrenophila*, *Buttiauxella gaviniae*, *Enterococcus caseliflavus*, *Lactococcus lactis*, *Leclercia adecarboxylata*, *Pantoea agglomerans*, *Raoultella planticola*, and *Raoultella terrigena*, were found exclusively under capnophilic conditions. The results of the study suggest that aerobic and capnophilic bacteria of the gastrointestinal tract of *C. aspersum* may play an important epidemiological role as a potential source or reservoir of pathogens harmful to humans and animals.

Keywords: *Cornu aspersum*, snails, digestive tract

The European brown garden snail *Cornu aspersum* (Müller, 1774), formerly classified as *Helix aspersa*, is a terrestrial snail species of significant economic importance, commonly bred in various regions of Europe and beyond, primarily for culinary purposes (46, 47, 63). In recent years, *C. aspersum* has also gained popularity as a subject of scientific research in fields such as ecology, immunology, and microbiology (17, 69-71). Particular attention has been directed toward its gastrointestinal tract, which constitutes a complex and dynamic microbiological ecosystem inhabited by diverse microorganisms that play a key role in digestion, metabolism, and the organism's defense mechanisms.

The gut microbiota of *C. aspersum* includes both aerobic and anaerobic bacteria, and its composition and activity are closely linked to the environmental conditions prevailing in different sections of the gastrointestinal tract (7, 42). The structure of this microbiota can be shaped by physicochemical gradients, such as varying oxygen concentrations, leading to the formation of specialized ecological niches for different bacterial groups. Previous studies have

revealed the presence of bacteria from the phyla Pseudomonadota (formerly Proteobacteria), Bacillota (formerly Firmicutes), Actinomycetota (formerly Actinobacteria), Chloroflexota (formerly Chloroflexi), and Bacteroidota (formerly Bacteroidetes) in the gastrointestinal tracts of various terrestrial and aquatic snail species (11, 18, 23, 35).

According to the holobiont concept (5, 37), the gut microbiota and the host form an integrated ecological and evolutionary unit, playing a significant role not only in environmental adaptation but also in maintaining the organism's health and homeostasis (56). In healthy *Cornu aspersum* snails, the dominant intestinal bacteria have been shown to belong to the genera *Lactobacillus*, *Lactococcus*, and *Pediococcus* (8). In enteropathological cases, the microbial structure was dominated by potentially pathogenic genera such as *Klebsiella*, *Pantoea*, *Citrobacter*, and *Enterobacter*, which may suggest a role of the risk of zoonoses (6, 30, 46, 47). Lactic acid bacteria (LAB), through the production of lactic acid, bacteriocins, and fermentative enzymes, support the digestion of plant-derived sugars and may act antagonistically against potential pathogens (11). Moreover, some bacterial species found in the snail gut have previously been associated with cases of sepsis, urinary tract infections, and wound infections (3, 28, 33).

Advances in microbial identification methods have revolutionized environmental microbiology. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) now represents a rapid, precise, and cost-effective tool enabling bacterial identification at the species level (44). This technique is increasingly used in both ecological microbiology and clinical diagnostics, allowing for the identification of cultivable bacteria.

The aim of this study was to characterize the composition of the gut microbiome of *C. aspersum aspersum* cultured under aerobic and capnophilic conditions using MALDI-TOF-MS technology. It was hypothesized that the applied microbial culture conditions influence the identified microbiome composition, and that introducing capnophilic conditions may enable the detection of species not observable under standard aerobic conditions. The obtained results expand the knowledge on the potential metabolic and ecological functions of the gut bacteria of this species and provide information relevant to snail health as well as the assessment of possible zoonotic risks.

Material and methods

C. aspersum aspersum snails from a laboratory breeding colony maintained at the Department of Epizootiology and the Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin were used for the study. The animals were kept under laboratory conditions (temperature 20-22°C, humidity 70-80%), fed with a standard snail feed (Patent no. 237356) and fresh plants. Prior to sampling, the snails (n = 5) were subjected to a 24-hour fasting period to clear the intestines of any transient flora that could be cultured.

After externally cleaning the shell surfaces with 70% ethanol, the snails were euthanized using 0.1 mM succinyl chloride in a 2% $MgCl_2$ solution (12), and their gastrointestinal tracts were dissected. After sectioning the intestines, their contents were flushed with PBS buffer, and a scraping from each intestine was combined into a single pooled sample. The obtained sample was inoculated onto MRS medium (De Man, Rogosa, Sharpe) and incubated at 30°C for 48 hours under both aerobic and capnophilic conditions (5% CO_2). Selected colonies that exhibited distinct morphologies were re-streaked to obtain pure cultures.

Bacterial identification. Pure isolates were identified using MALDI-TOF-MS mass spectrometry (Microflex Biotyper, Bruker Daltonics, USA), following the method described by Pastuszka et al. (45). Mass spectra were recorded using the Flex Control software ver. 3.4, Build 135.10. Spectral analysis was performed according to the procedures recommended by the manufacturer and subsequently compared with the reference Bruker database for species-level assignment. The results were compared with the manufacturer's database, according to the interpretative criteria: a score ≥ 2.300 indicates a high probability of species-level identification; 2.000-2.299 indicates a probable species-level identification and a secure genus-level identification; 1.700-1.999 indicates a probable genus-level identification; and a score < 1.700 indicates an unreliable identification.

The taxonomic classification of the strains was conducted using the NCBI Taxonomy Browser database ([https://www.ncbi.nlm.nih.gov/taxonomy] (https://www.ncbi.nlm.nih.gov/taxonomy)), as of April 18, 2025.

Results and discussion

As a result of the analysis of the gastrointestinal microbiota of *Cornu aspersum aspersum* using MALDI-TOF-MS technology, a total of 22 bacterial species were identified, with 13 species detected under capnophilic conditions and 12 species under aerobic conditions (Tab. 1 and 2, Fig. 1-3).

Under capnophilic conditions, 13 bacterial species were detected, with a predominance of *Lactococcus lactis* (n = 26) and *Acinetobacter pittii* (n = 5), and *Buttiauxella gaviniae* (n = 4). Other identified species included: *Aeromonas eucrenophila*, *Aeromonas hydrophila*, *Buttiauxella agrestis*, *Enterococcus casseliflavus*, *Klebsiella oxytoca*, *Kluyvera ascorbata*, *Leclercia adecarboxylata*, *Pantoea agglomerans*, *Raoultella planticola*, and *Raoultella terrigena* (Tab. 1 and 3, Fig. 1).

Under aerobic conditions, 12 species were identified, including: *Aeromonas hydrophila* (n = 4), *Proteus vulgaris* (n = 4), *Buttiauxella warmboldiae* (n = 3), *Aeromonas sobria* (n = 2), *Morganella morganii* (n = 2), *Buttiauxella agrestis*, *Buttiauxella izardii*, *Citrobacter freundii*, *Escherichia vulneris*, *Klebsiella oxytoca*, *Myroides odoratimimus*, and *Providencia rettgeri* (Tab. 2 and 3, Fig. 2).

Tab. 1. Identification results of 48 isolates obtained under capnophilic conditions from the intestines of *C. aspersum aspersum* snails using the Bruker Biotyper MALDI database

Identified species	Strains	Organism (best match)	Score
Acinetobacter pittii n = 5	X1	*Acinetobacter pittii* Serovar 26 DSM 9321 DSM	2.225
	X4	*Acinetobacter pittii* Serovar 26 DSM 9321 DSM	2.103
	X5	*Acinetobacter pittii* Serovar 26 DSM 9321 DSM	2.124
	X7	*Acinetobacter pittii* Serovar 21 DSM 9342 DSM	2.061
	X8	*Acinetobacter pittii* 60 HCB	1.922
Aeromonas eucrenophila	Mu7	*Aeromonas eucrenophila* CECT 4224T DSM	2.013
Aeromonas hydrophila	Mu1	*Aeromonas hydrophila* ssp. *hydrophila* DSM 30187T DSM	2.247
Buttiauxella agrestis	Mu8	*Buttiauxella agrestis* DSM 4586T HAM	1.975
Buttiauxella gaviniae n = 4	Mu11	*Buttiauxella gaviniae* DSM 9393T HAM	2.119
	Mu4	*Buttiauxella gaviniae* DSM 9393T HAM	2.105
	Mu5	*Buttiauxella gaviniae* DSM 9393T HAM	2.027
	Mu2	*Buttiauxella gaviniae* DSM 9393T HAM	2.096
Enterococcus casseliflavus	3H	*Enterococcus casseliflavus* DSM 7370 DSM	1.949
Klebsiella oxytoca	Mu10	*Klebsiella oxytoca* VA31877_09 ERL	2.200
Kluyvera ascorbata n = 4	X10	*Kluyvera ascorbata* DSM 4611T HAM	1.845
	X2	*Kluyvera ascorbata* ST280_10 ERL	2.056
	X3	*Kluyvera ascorbata* ST280_10 ERL	2.099
	X9	*Kluyvera ascorbata* ST280_10 ERL	2.216
Lactococcus lactis n = 26	4HI	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.231
	X12	*Lactococcus lactis* IBS_MS_6 IBS	2.087
	X13	*Lactococcus lactis* IBS_MS_6 IBS	2.188
	Mu1	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	1.976
	X18	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.036
	X19	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	1.998
	Mu2	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.268
	X21	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.002
	X22	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.202
	X23	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.132
	X24	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.222
	X25	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	1.988
	X26	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.142
	X27	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.219
	X29	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	1.718
	Mu3	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.283
	Mu37	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	1.967
	Mu38	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.030
	Mu39	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.289
	Mu4	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.149
	Mu40	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.184
	Mu5	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.214
	Mu6	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.047
	Mu7	*Lactococcus lactis* ssp. *cremoris* DSM 20388 DSM	2.183
	Mu8	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.263
	Mu9	*Lactococcus lactis* ssp. *lactis* DSM 20661 DSM	2.033
Leclercia adecarboxylata	Mu9	*Leclercia adecarboxylata* CCM 4443 CCM	1.834
Pantoea agglomerans	X6	*Pantoea agglomerans* CCM 2406 CCM	2.236
Raoultella planticola	X14	*Raoultella planticola* DSM 4617 DSM	2.249
Raoultella terrigena	Mu3	*Raoultella terrigena* DSM 7333 DSM	2.358

Some species, such as *A. hydrophila*, *B. agrestis*, and *K. oxytoca*, were found in both types of incubation environments. The remaining species (n = 11), including *A. pittii*, *A. eucrenophila*, *B. agrestis*, *B. gaviniae*, *L. lactis*, *E. casseliflavus*, *K. ascorbata*, *P. agglomerans*, *L. adecarboxylata*, and representatives of the genus *Raoultella*, were isolated exclusively from capnophilic cultures. In contrast, *A. sobria*, *B. izardii*, *B. warmboldiae*, *C. freundii*, *E. vulneris*, *M. morganii*, *M. odoratimimus*, *P. vulgaris*, and *P. rettgeri* appeared only under aerobic conditions (Tab. 3).

The taxonomic classification of all isolated strains was performed using the NCBI Taxonomy Browser database, assigning them to appropriate systematic levels – from kingdom to species (Tab. 3).

In this study, using the MALDI-TOF-MS technique, 22 bacterial species belonging to 16 genera were identified, representing three main phyla: Pseudomonadota, Bacteroidota, and Bacillota. Analysis of the results obtained under aerobic and capnophilic conditions showed that most (n = 19) of the isolated bacterial species belonged to the phylum Pseudomonadota. Two species were assigned to Bacillota and one to Bacteroidota (Tab. 3). The dominance of bacteria from the phylum Pseudomonadota, to which the majority of the identified strains belonged, is consistent with previous reports on the invertebrate microbiome, including terrestrial herbivorous snails (7, 42). It is important to emphasize the significance of these microorganisms – both in the context of their role in metabolic processes and host–environment interactions, as well as their potential as vectors of zoonotic pathogens.

In the context of the obtained results, it should be noted that the actual diversity of the snail microbiome may be much greater than indicated by the data obtained with the MALDI-TOF-MS method. The fact that 22 bacterial species were identified, with a dominance of Pseudomonadota, reflects only a part of the actual spectrum of microorganisms colonizing the digestive tract. The MALDI-TOF-MS method is currently considered the gold standard for the rapid and precise identification of numerous bacterial species; however, its application is associated with significant limitations. The most important of these is the fact that analysis is only possible for microorganisms capable of growing on culture media, generating sufficient biomass required to obtain high-quality mass spectra (13, 66). Consequently, non-culturable bacteria or those entering the VBNC (viable but non-culturable) state remain beyond the reach of this technology (62). Another limitation is the presence of mixed cultures,

Tab. 2. Identification results of 22 isolates obtained under aerobic conditions from the intestines of *C. aspersum aspersum* snails using the Bruker Biotyper MALDI database

Species	Strains	Matched pattern	Score
Aeromonas hydrophila n = 4	T15.2	*Aeromonas hydrophila* CECT 839T DSM	2.032
	T33	*Aeromonas hydrophila* CECT 839T DSM	2.123
	T34	*Aeromonas hydrophila* CECT 839T DSM	2.217
	T43	*Aeromonas hydrophila* ssp. *hydrophila* DSM 30187T DSM	2.002
Aeromonas sobria n = 2	T37	*Aeromonas sobria* CECT 4245T DSM	2.102
	T54	*Aeromonas sobria* LMG 3783T HAM	2.223
Buttiauxella agrestis	T6.1	*Buttiauxella agrestis* DSM 4586T HAM	1.804
Buttiauxella izardii	T3.2	*Buttiauxella izardii* DSM 9397T HAM	1.844
Buttiauxella warmboldiae n = 3	T1.3	*Buttiauxella warmboldiae* DSM 9404T HAM	1.812
	T2.2	*Buttiauxella warmboldiae* DSM 9404T HAM	1.728
	T8.3	*Buttiauxella warmboldiae* DSM 9404T HAM	1.751
Citrobacter freundii	T19.3	*Citrobacter freundii* DSM 30039T HAM	2.209
Escherichia vulneris	T7.1	*Escherichia vulneris* DSM 4564T DSM	1.828
Klebsiella oxytoca	T5.2	*Klebsiella oxytoca* VA31877_09 ERL	2.139
Morganella morganii n = 2	T11.3	*Morganella morganii* ssp. *sibonii* Mb19277_2 CHB	2.242
	T13.2	*Morganella morganii* ssp. *sibonii* Mb19277_2 CHB	1.742
Myroides odoratimimus	T17.2	*Myroides odoratimimus* LMG 4029T HAM	1.711
Proteus vulgaris n = 4	T9.3	*Proteus vulgaris* LMG 5586 LMG	1.890
	T10.3	*Proteus vulgaris* (PX) 22086129 MLD	1.969
	T12.2	*Proteus vulgaris* LMG 5586 LMG	2.188
	T14.3	*Proteus vulgaris* DSM 30119 DSM	1.978
Providencia rettgeri	T18.3	*Providencia rettgeri* CCM 5617 CCM	1.718

in which MALDI-TOF-MS most often identifies only the dominant species, overlooking less abundant microorganisms (58). An additional barrier is the incompleteness of reference databases – the absence of spectra for rare or environmental species results in identification only at the genus level or leads to misclassification (57). Even for well-characterized species, factors such as growth phase, type of culture medium, or incubation conditions may affect the quality of the spectrum and the reliability of identification (4). In light of these limitations, the MALDI-TOF-MS technique, despite its high usefulness in routine clinical diagnostics, does not replace molecular and sequencing methods in the identification of bacteria requiring specific growth conditions, rare species, or non-culturable microorganisms.

According to the holobiont theory, the diversity of bacterial species in the animal gastrointestinal tract, by influencing environmental adaptation, is of key importance for understanding the ecology and evolution of a given species (5). Many authors emphasize that representatives of bacterial phyla commonly found in the gut of invertebrates participate in digestive processes, breaking down complex organic compounds and supplying the host with essential metabolites (9, 20, 43, 50). Bordenstein and Theis (5) argue that the gut microbiome is an integral part of host physiology and influences its environmental adaptation. In our study, we confirmed the presence of bacteria such as *K. oxytoca*, *C. freundii*, *A. hydrophila*, and *P. agglomerans*, whose ability to degrade cellulose and other complex plant polysaccharides has been demonstrated by other authors in snails such as *Achatina fulica*, *Pila globosa*, and *H. aspersa* (7, 15-17, 25, 29, 49-51). Their enzymatic activity (including cellulases, hemicellulases, and proteases) promotes efficient digestion and nutrient absorption.

The presence of LAB with potential probiotic properties has been demonstrated in the gastrointestinal tract of snails (18). LAB play an important role in maintaining microbial homeostasis and host health. They are capable of adhering to the host's intestinal epithelium and stimulating the immune system by enhancing chemotaxis and phagocytosis of amebocytes, increasing expression of TLR2, TLR4, and TLR6 in the intestinal mucosa, and boosting the bactericidal activity of the hemolymph (18). In our study, we isolated 27 LAB strains: 26 *L. lactis* and 1 *E. casseliflavus*. The presence of *L. lactis* has previously been documented in the microbiome of *Helix pomatia* (11), while *E. casseliflavus* has been isolated from the gastrointestinal tract of *C. aspersum* (11, 31). These bacteria are known for producing lactic acid and bacteriocins, which inhibit the growth of pathogens and help protect the host from infections. They also exhibit probiotic properties and may participate in the fermentation of plant-derived carbohydrates, facilitating their digestion and improving nutrient availability. The study by Koleva et al. (31) on the gut microbiota of *C. aspersum* showed the presence of LAB belonging to the genera *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Lactobacillus*, and *Weissella*. The main LAB species included *L. lactis*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus graminis*, and *Lactobacillus curvatus* (31). Their potential probiotic and immunomodulatory roles in invertebrates deserve further research, particularly in the context of ecologically dynamic environments.

Additionally, in this study, diazotrophic bacteria such as *K. oxytoca* and *P. agglomerans* were identified. This indicates a potential role of the microbiota in supporting the host by compensating for nitrogen deficiencies resulting from a low-protein diet. The process of biological nitrogen fixation, well-documented in other invertebrates (54), may represent an important mechanism supporting the growth and development of snails in environments low in available mineral nitrogen. *K. oxytoca* is commonly present in the natural environment but has also been isolated as a commensal bacterium from the human gastrointestinal tract (65).

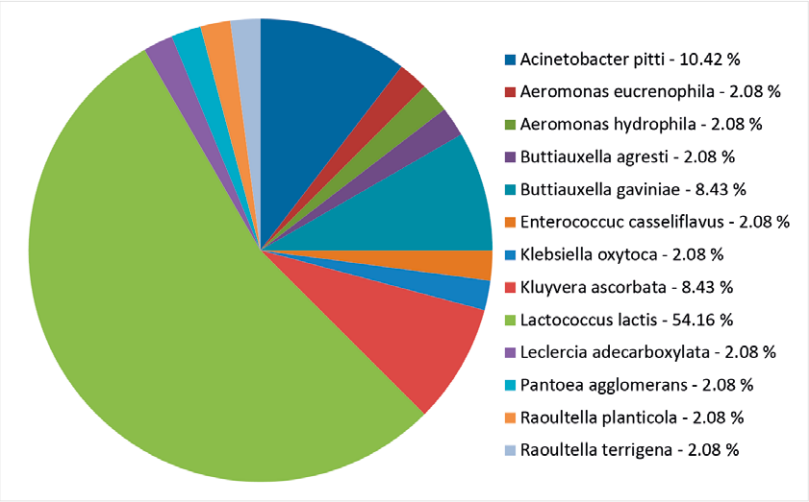

Fig. 1. Percentage share of bacterial species identified by MALDI-TOF-MS in the gastrointestinal tract of *C. aspersum aspersum* under capnophilic conditions (n = 48)

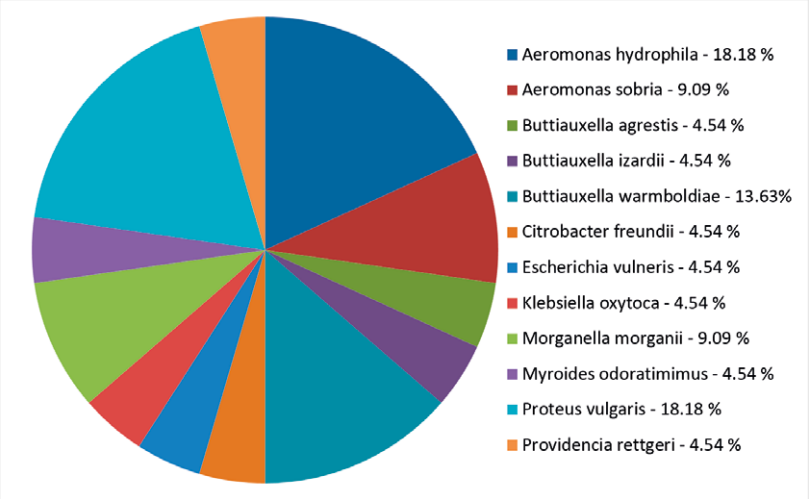

Fig. 2. Percentage share of bacterial species identified by MALDI-TOF-MS in the gastrointestinal tract of *C. aspersum aspersum* under aerobic conditions (n = 22)

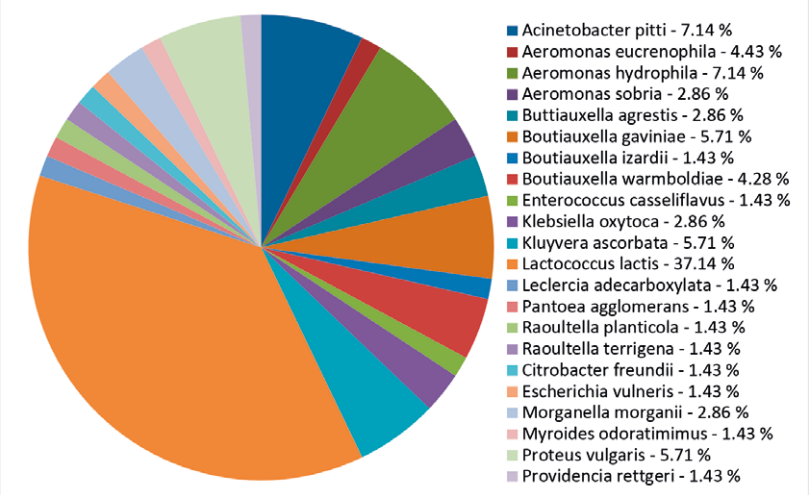

Fig. 3. Percentage share of bacterial species identified by MALDI-TOF-MS in the gastrointestinal tract of *C. aspersum aspersum* under capnophilic and aerobic conditions (combined, n = 70)

Tab. 3. Classification of bacteria isolated from the gut of *C. aspersum aspersum*

Taxonomic classification of the identified bacterial species	
Kingdom: Bacteria	Species: *B. izardii*
Phylum: Bacillota	Species: *B. warmboldiae*
Class: Bacilli	Genus: *Citrobacter*
Order: Lactobacillales	Species: *C. freundi*
Family: Enterococcaceae	Genus: *Escherichia*
Genus: *Enterococcus*	Species: *E. vulneris*
Species: *E. casseliflavus*	Genus: *Klebsiella*
Family: Streptococcaceae	Species: *K. oxytoca*
Genus: *Lactococcus*	Genus: *Kluyvera*
Species: *L. lactis*	Species: *K. ascorbata*
Phylum: Bacteroidota	Genus: *Raoultella*
Class: Flavobacteria	Species: *R. planticola*
Order: Flavobacteriales	Species: *R. terrigena*
Family: Flavobacteriaceae	Genus: *Leclercia*
Genus: *Myroides*	Species: *L. adecarboxylata*
Species: *M. odoratimimus*	Family: Erviniaceae
Phylum: Pseudomonadota	Genus: *Pantoea*
Class: Gammaproteobacteria	Species: *P. agglomerans*
Order: Aeromonadales	Family: Morganellaceae
Family: Aeromonadaceae	Genus: *Morganella*
Genus: *Aeromonas*	Species: *M. morganii*
Species: *A. eucrenophila*	Genus: *Proteus*
Species: *A. hydrophila*	Species: *P. vulgaris*
Species: *A. sobria*	Genus: *Providencia*
Order: Enterobacterales	Species: *P. rettgeri*
Family: Enterobacteriaceae	Order: Moraxellales
Genus: *Buttiauxella*	Family: Moraxellaceae
Species: *B. agrestis*	Genus: *Acinetobacter*
Species: *B. gaviniae*	Species: *Acinetobacter pittii*

P. agglomerans is a bacterium that has been detected in insects, ticks, crabs, snails, fish, birds, and mammals (19).

One of the most concerning aspects of the obtained results is the isolation of numerous opportunistic pathogenic bacteria known to be involved in human infections. This group includes most of the bacterial species isolated in this study, such as *A. hydrophila*, *A. sobria*, *B. agrestis*, *B. gaviniae*, *C. freundii*, *E. vulneris*, *E. casseliflavus*, *K. oxytoca*, *K. ascorbata*, *L. lactis*, *M. morganii*, *M. odoratimimus*, *P. vulgaris*, *P. agglomerans*, *P. rettgeri*, and *R. planticola* (Tab. 3). To the authors' knowledge, human infections caused by *A. eucrenophila*, *B. izardii*, and *B. warmboldiae* have not yet been reported. *A. eucrenophila* has been isolated from water and ulcers of diseased fish (61), whereas *B. izardii* and *B. warmboldiae* are bacterial

species isolated from various environments, including soil, plants, and snails (40). Human infections caused by *A. pittii* (3), *A. hydrophila* (1, 21, 28, 32, 34), *A. sobria* (32), *B. agrestis* (48), *B. gaviniae* (2), *C. freundii* (26), *E. vulneris* (27), *E. casseliflavus* (67), *K. oxytoca* (52), *K. ascorbata* (55), *L. lactis* (22, 38), *L. adecarboxylata* (64), *M. morganii* (36), *M. odoratimimus* (10), *P. vulgaris* (33), *P. agglomerans* (14, 19), *P. rettgeri* (41), and *Raoultella spp*. (39, 53, 59, 60, 68) have been reported in the literature, leading to various diseases such as soft tissue infections, sepsis, osteomyelitis, and urinary tract infections. The fact that these bacteria were isolated from the gastrointestinal tract of *C. aspersum* suggests a potential for transmission between animals and humans. Their presence in the gut microbiome of *C. aspersum* may result from snail contact with soil and organic matter contaminated with animal feces, as well as from environments with a high microbial load. Considering that *C. aspersum* is an edible species used in gastronomy in many countries, these findings should prompt a reassessment of hygiene standards and snail processing procedures for food purposes.

Although the presence of these bacteria in the microbiota does not automatically imply pathogenicity, their detection should prompt further research into their role within the snail's ecosystem and the microbiological risks associated with their presence in the animal and human food chain.

The results of this study confirm that the gastrointestinal microbiota of *C. aspersum aspersum* is highly diverse and functionally specialized. Literature data indicate that the presence of bacteria involved in the digestion of plant fibers, nitrogen fixation, and stabilization of the gut microbial environment supports their importance in the host's adaptation to a plant-based diet. At the same time, the presence of numerous bacteria with potential zoonotic significance highlights the need for ongoing monitoring of the *C. aspersum* microbiome, particularly in the context of its consumption. It is recommended to expand research on the functional characteristics of the isolated strains, their enzymatic potential, and the microbiological risk associated with their presence in food.

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