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Cadaverine, putrescine, and histamine formation of *Morganella morganii* in raclette-type cheese

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ABSTRACT

The influence of *Morganella morganii* isolated from cheese on the formation of biogenic amines was studied in raclette-type cheeses. Three variants were produced. One variant containing a cadaverine- and histamine-forming strain, one variant with a putrescine- and histamine-forming strain, and a variant without *M. morganii*. After 130 d of ripening, live *M. morganii* was found in the outer layers but no longer inside the cheese. The cheeses with the cadaverine-forming strain exhibited a decreasing cadaverine gradient from the outside (on average 310 mg kg^{-1}) to the inside (160 mg kg^{-1}). Putrescine was present in the cheeses with the putrescine-forming strain. Its concentration averaged 59 mg kg^{-1} in all layers. All cheeses with *M. morganii* contained also histamine with concentration less than 50 mg kg^{-1}. The results reveal new information on the survival of *M. morganii* as well as its ability to form biogenic amines in cheese.

1. Introduction

Morganella morganii is a Gram-negative bacterium that is found in humans and animals (Liu, Zhu, Hu, & Rao, 2016; Manos & Belas, 2006) and in food such as fish (Kim, Field, Chang, Wei, & An, 2001) and cheese (Coton et al., 2012; Ryser, Arias-Roth, Perreten, Irmler, & Bruggmann, 2021). The species is considered to be one of the main causes for histamine formation in fish (Kim et al., 2001, 2002). The formation of histamine and other biogenic amines in food is undesired since its consumption can cause intoxications (Ruiz-Capillas & Herrero, 2019; Schirone, Visciano, Tofalo, & Suzzi, 2016; Suzzi, Perpetuini, & Tofalo, 2022).

An important step to prevent biogenic amine formation in food is to identify the causative microorganisms. While some potent microbial histamine and tyramine producers such as *Lentilactobacillus parabuchneri, Latilactobacillus curvatus*, and enterococci have been identified in cheese (Berthoud et al., 2017; Bunkova et al., 2010; Ladero, Fernández, Cuesta, & Alvarez, 2010), to our knowledge, few bacterial species have been studied for putrescine and cadaverine formation in this matrix. Marino, Maifreni, Moret, and Rondinini (2000) found a positive correlation between the presence of enterobacteria and cadaverine concentrations in cheese. However, no cheese trials were conducted. In another study, the formation of biogenic amines by enterobacteria isolated from cheese products was investigated in small model cheeses (Delbes-Paus et al., 2012). In this study, *Hafnia alvei* B16, *Halomonas venusta* 4C1A, *M. morganii* 3A2A, and *Klebsiella oxytoca* 927 produced cadaverine in the experimental cheeses. Of these, only *H. alvei* formed putrescine. In another study, potential amine-forming bacteria were isolated from Edam-cheese samples that contained biogenic amines (Bunkova et al., 2010). The authors were able to identify *L. curvatus* as putrescine producer. They also isolated two strains of *Lacticaseibacillus paracasei* and one *L. curvatus* strain that exhibited very weak cadaverine formation in vitro.

We recently characterised several *M. morganii* strains isolated from cheese (Ryser et al., 2021). We found that all isolates were able to produce histamine in broth. With regard to cadaverine and putrescine formation, we observed strain-specific differences. We linked the cadaverine formation to a lysine decarboxylase-encoding gene. The formation of putrescine results from the activity of two different ornithine decarboxylases. These are encoded by the genes *speF* and *speC* (de las Rivas, González, Landete, &

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Munoz, 2008; de las Rivas, Marcobal, & Munoz, 2007). We found that the interruption of the *speF* gene by an insertion sequence prevents strong putrescine formation in vitro (Ryser et al., 2021). However, the presence of bacteria encoding for amino acid decarboxylases is not the only factor influencing the biogenic amine levels in food. Interactions between microorganisms, pH, ripening and storage temperatures, water activity, proteolysis that provides the precursor amino acids, and salt concentration also affect the biogenic amine concentrations (Benkerroum, 2016; Suzzi et al., 2022).

The principal objective of this study is to describe the development of cadaverine, putrescine, and histamine in raclette-type cheese inoculated with two different *M. morganii* strains, which showed a distinct biogenic amine formation profile in vitro. We monitored *M. morganii* growth dynamics using culture-independent and culture-dependent methods in three different layers of the cheeses during a 130-d ripening period. In addition, we determined the formation of biogenic amines in the cheese layers.

2. Materials and methods

2.1. M. morganii *inoculum preparation*

We used two *M. morganii* strains that had different properties with regard to the formation of biogenic amines. The strain FAM24206 was able to produce cadaverine (CAD-positive), whereas strain FAM24676 was able to produce putrescine (PUT-positive) under laboratory conditions (Ryser et al., 2021). Both strains were able to produce histamine in broth.

The strains were stored at −80 °C in Trypticase Soy Broth (TSB) (BD, Dr. Grogg Chemie AG, Stettlen-Deisswil, Switzerland) that contained 30% (v/v) glycerol. To prepare the inoculum, the strains were reactivated without shaking in TSB at 37 °C for 20 h. One day before the cheesemaking, 45 mL sterile reconstituted 10% (w/v) skim milk were inoculated with 450 μL of the *M. morganii* culture. The sample was incubated without shaking at 37 °C for 20 h, and then used as inoculum for the cheese experiment.

To enumerate *M. morganii* in the inoculum for the time being, we carried out three independent incubations at the end of which a bacterial count was performed using the TEMPO® Enterobacteriaceae enumeration kit (bioMérieux Industry, Geneva, Switzerland) according to the manufacturers' instructions. Thus, we determined that the cell count normally reached between 7 and 8 log cfu mL^{-1}. The cell counts of the inoculi used for the cheese experiment, which could only be determined after the cheesemaking, were 7.6 (±0.3) log cfu mL^{-1} for FAM24206 and 7.9 (±0.2) log cfu mL^{-1} for FAM24676.

2.2. Cheesemaking

A total of nine raclette-type cheeses were made on three different production days. On each day, 35-L milk vats were used to produce three cheese variants: a control cheese without an adjunct culture and two cheeses inoculated with one of the two *M. morganii* strains. To produce three cheese loaves weighing approximately 7 kg, the curds from two vats were mixed together.

The starter culture FD-DVS-CHN19 (Chr. Hansen Holding A/S, Hørsholm, Denmark), which contains a mixture of *Lactococcus lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *lactis* biovar *diacetylactis and Leuconostoc*, was used to initiate acidification. Therefore, 7.56 g of the lyophilised starter culture was first suspended in 600 mL of milk of which 100 mL was then added to 35 L of pasteurised full-fat (33 g kg^{-1}) cows' milk. Then, 4.7 mL of $CaCl_2$ 52% (w/v, aqueous solution) and 3.5 mL of *M. morganii* adjunct culture was added to the vat milk. After an incubation of 30 min, 8.5 mL of rennet extract Winkler GR orange (Winkler AG, Konolfingen, Switzerland) was added to the vat and the incubation continued for another 30 min at 32 °C. The coagulum was cut into pieces (4–8 mm) using a cheese harp for 12 min. While stirring, the temperature was increased to 38 °C within 13 min. To lower the lactose concentration, 5 L of whey was replaced with 5 L of water (38 °C). After a final stirring for 30 min, the curds of the two vats per cheese variant were mixed together by transferring the grains/whey mixture into perforated moulds. The curd was pressed first with 1.5 bar for 15 min followed with 3 bar for 30 min. When the cheeses reached a pH of 5.25, they were immersed in 12–13.5 °C cold, sterile brine solution 20% (NaCl, w/w) for 17 h. Afterward the cheeses were ripened for 130 d at 10 °C and 96% relative humidity. The individual steps of the cheesemaking are shown graphically in Fig. 1.

The cheeses were smeared daily during the first 5 d of ripening using OMK 702 (Liebefeld Kulturen AG, Bern, Switzerland), which contains a mixture of *Brevibacterium linens*, *Glutamicibacter arilaitensis*, and *Debaryomyces hansenii*. Afterward, the smear culture was applied once a week.

2.3. Cheese sampling

Cheese samples were collected after 1, 21, 42, 70, 90, and 130 d of ripening with a cheese trier (diameter of 5 cm). The opening around the plug was sealed with a sterile plastic wax to avoid water evaporation and contamination of the cheese core. The samples of day 1 were directly processed for microbiological analysis and stored at −20 °C for chemical analysis. Due to closed labs during the COVID-19 crisis, the samples of days 21, 42, 70, 90, and 130 were frozen at −20 °C and analysed at the end of ripening. The cheese samples were divided aseptically into three layers, namely rind (0.0–0.5 cm), under-the-rind (0.5–2 cm), and core (remaining sample material; Fig. 2). Both the rind and the under-the-rind samples from each side of the cheese were mixed together, respectively.

2.4. Quantitative polymerase chain reaction

Quantitative polymerase chain reaction (qPCR) assays were performed to enumerate *M. morganii* in the cheese samples. Therefore, 5 g of the cheese was added to 45 mL of potassium buffer (3 g L^{-1} potassium dihydrogen phosphate, 17 g L^{-1} dipotassium hydrogen phosphate), incubated at 40 °C for 10 min and then homogenised for 4 min in a stomacher (Masticator, IUL instruments GmbH, Königswinter, Germany). Ten mL of the homogenate was centrifuged at room temperature at 4000×*g* for 30 min. The DNA of the pellet was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Lucerna Chem AG, Lucerne, Switzerland) according to the manufacturer's instructions.

The *ybgC* gene was found to be a species-specific target for polymerase chain reaction (PCR) assays using the SpeciesPrimer tool (v1.0; options: target: *M. morganii*, qc_gene: [tuf, recA, dnaK], assemblylevel: all, mfold: −3.0; mpprimer: −3.0; January 2019; Dreier, Berthoud, Shani, Wechsler, and Junier (2020)). The primers and the hydrolysis probe were designed with the Primer Express software (v3.0.1, standard parameters, Thermo Fisher Scientific).

The qPCR assay was performed in a final volume of 12 μL containing 2 μL DNA extract, 300 nM ybgC_Mmorg_F (5′-GAC ATT TAC GCA GAC ACT TAT GCA T-3′), 300 nM ybgC_Mmorg_R (5′-CCG GCT TCA TTA AAG AAA TAT TCA C-3′), 100 nM hydrolysis probe (5'- (FAM)

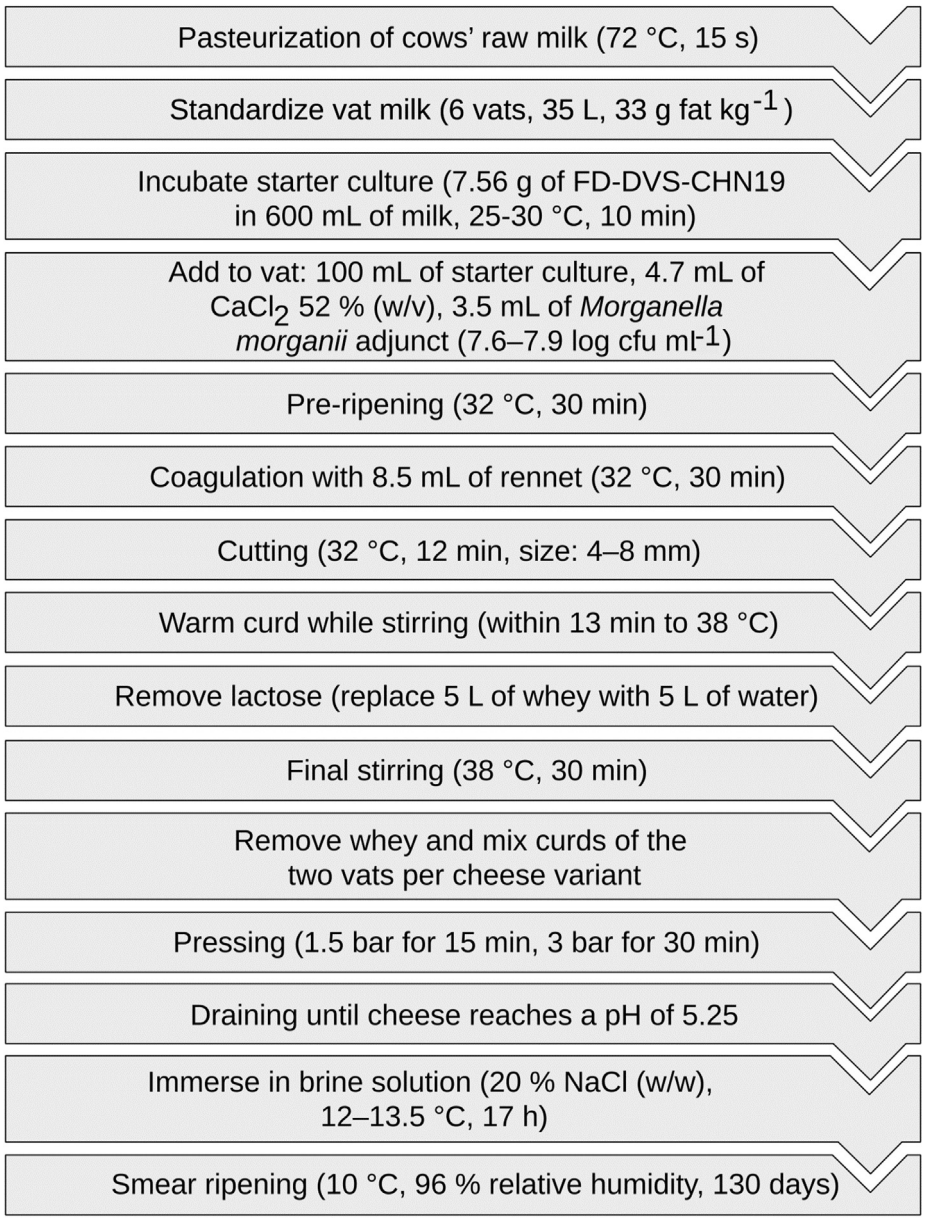

Fig. 1. Flow diagram for the manufacture of the raclette-type cheeses.

TCG CGC AGA GGT ACT GGT CGC C(BHQ-1)-3′), and 6 μL Takyon™ No Rox Probe MasterMix UNG (Eurogentec, Seraing, Belgium). The PCR reactions were carried out on a Rotor-Gene 6000 device (Corbett Life Science, Sydney, Australia) under the following conditions: 50 °C for 2 min, 95 °C for 3 min, and finally 40 cycles of 95 °C for 3 s and 60 °C for 20 s. The fluorescence emission of the FAM dye was detected at 510 nm. The measurements were analysed using the Rotor Gene Q Series software (v.2.3.1) with a Cq threshold at 0.03. All reactions were carried out in duplicates per run and each run was repeated once.

The results were quantified using the standard curve method. Therefore, a PCR amplicon (1478 bp) containing the target sequence of the qPCR assay was amplified from genomic DNA of the *M. morganii* strain FAM24091 (Agroscope Culture Collection, Liebefeld, Switzerland) with the primer pair Std_Mmorg_F (5′- CCG GTG CCA CCA TCT GTA -3′) and Std_Mmorg_R (5′- TCA GCC TGA CAG AAA ACA CCA TA -3′). The PCR was performed in a final volume of 50 μL containing Mix Platinum™ SuperFi™ PCR Master Mix (Thermo Fisher Scientific), 500 nM each primer, and 1 ng genomic DNA under the following conditions: 98 °C for 30 s, 35 cycles of [98 °C for 10 s, 58 °C for 10 s, 72 °C for 60 s], and a final elongation at 72 °C for 5 min. The PCR product was purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and quantified using a NanoDrop instrument (Thermo Fisher Scientific). The copy number was calculated using the following formula:

$$copy\ number = \frac{\left(amount\ of\ DNA\ (ng) * 6.022 * 10^{23}\right)}{\left(length\ of\ DNA\ template\ (bp) * 1 * 10^{9} * 660\right)}$$

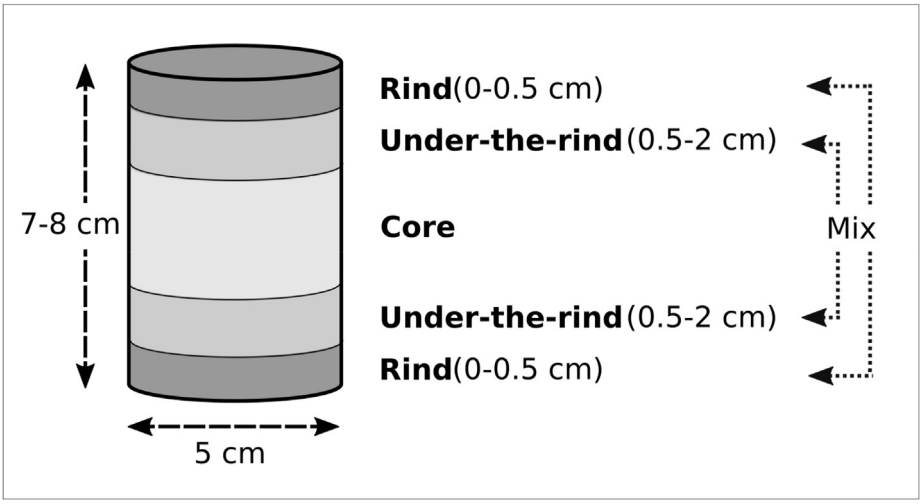

Fig. 2. Section scheme of individual layers of a cheese sample. The upper and lower layer of rind and under-the-rind, respectively, were mixed for the various analyses.

2.5. Plate counting and species identification with MALDI-TOF-MS

To detect live *M. morganii*, 1 mL of serial 10-fold dilutions of the above homogenates were plated into Violet Red Bile Glucose (VRBG) agar. After an aerobic incubation at 37 °C overnight, dark-purple colonies with a 1–2 mm diameter were counted.

To identify the bacteria with matrix-assisted laser desorption ionisation-time of flight-mass spectrometry (MALDI-TOF-MS), the colonies were transferred from within the agar to the surface of a new VRBG agar plate, which was then incubated at 37 °C overnight. The species identity of the colony was determined using a MALDI Biotyper system (Bruker Daltonics GmbH, Bremen, Germany) according to the manufacturer's instruction.

2.6. Chemical analysis

Dry matter was determined by measuring the weight of the cheese samples before and after drying at 102 °C (IDF, 2004).

The pH of the cheese during cheesemaking and at 130 d of ripening was determined with a 926 VTV pH-meter with an Ingold electrode 406 MX (Mettler Toledo S.A., Viroflay, France). The content of biogenic amines and free amino acids was determined using high-pressure liquid chromatography as described previously (Ascone et al., 2017; Wenzel et al., 2018).

2.7. Statistical analysis

The experimental design includes the three factors *cheese variant, layer*, and *ripening time*, which were treated as categorical variables. Statistical analyses were performed with R (v3.6.3, http://www.R-project.org) for an $\alpha = 5\%$. The cheese samples were taken over a time period (longitudinal data) and the group differences were therefore assessed using the R library nparLD (Noguchi, Gel, Brunner, & Konietschke, 2012). Statistical analysis of the free amino-acid concentrations at 130 d of ripening was carried using the nonparametric Aligned Rank Transform test (ARTool, v 0.11.0) (Wobbrock, Findlater, Gergle, & Higgins, 2011). The global tests were performed for each biogenic amine and free amino acid separately. The nonparametric Conover's test (PMCMR, v4.3, https://cran.r-project.org/web/packages/PMCMR/index.html) was used for the pairwise comparisons of the sample groups at 130 d of ripening. The pairwise comparisons were not corrected for multiple testing, because the hypotheses were constructed before performing the statistical tests.

3. Results and discussion

3.1. Chemical analysis

External factors such as temperature, pH, and water content can influence the metabolic performance of bacteria. Since all cheeses were stored in the same ripening cellar, we excluded the strong influence of abiotic factors during ripening. However, varying temperature during production can affect the water content. The dry matter content of the core ranged between 58.5 and 58.6% after 130 d of ripening (Table 1). As there were no significant differences, we concluded that the production conditions of the three production days were comparable.

Metabolic activities of the microorganisms present in the cheeses could directly influence the pH. With regard to the pH, we found that the pH value was higher in the rind (6.43–6.54) than in the core (5.73–5.80) (Table 1). The increase of the pH in the rind can be explained by the metabolism of lactate by the surface smear culture (McSweeney & Fox, 2004).

Furthermore, we found a trend that the pH of the cheese inoculated with the cadaverine-positive *M. morganii* strain FAM24206 was 0.1 units lower than of the other cheeses. Since we kept all external factors as constant as possible, we attribute this difference to metabolic activities of the added *M. morganii* strain. As we will see later, this strain produced the highest amounts of biogenic amines. This is remarkable, as it is generally assumed that amine formation is associated with a pH increase.

3.2. Detection and enumeration of the M. morganii strains

The knowledge whether *M. morganii* can survive in cheese is limited. Delbes-Paus et al. (2012) showed that *M. morganii* 3A2A

Table 1
Dry matter and pH of the cheese variants after 130 d of ripening.[a]

Parameter	Control	FAM24206	FAM24676
Dry matter (%)	58.5 ± 0.6	58.6 ± 0.6	58.5 ± 0.6
pH, rind	6.54 ± 0.16^{L}	6.43 ± 0.09^{L}	6.53 ± 0.12^{L}
pH, core	5.80 ± 0.01^{L}	$5.73 \pm 0.03^{L,p}$	$5.86 \pm 0.03^{L,c}$

[a] Results represent the mean (±SEM) of the three cheesemaking experiments. Statistically significant differences assessed using *t*-test ($P < 0.05$) as follows:[c] different from FAM24206;[p] different from FAM24676;[L] different from the other layer.

could grow in model cheeses during the first days of ripening. As there is no selective medium for *M. morganii* available, we decided to use a species-specific qPCR to enumerate the *M. morganii* strains. The PrimerSpecies tool (Dreier et al., 2020) proposed the *ybgC* gene, which encodes a putative 4-hydroxybenzoyl-CoA thioesterase, as a species-specific target (data not shown). Based on this result, a qPCR assay was developed that amplifies a 95 bp long fragment of the *ybgC* gene. Primer-BLAST searches confirmed the specificity of the primer pair (data not shown).

DNA extracts from the control cheese samples did not yield a positive qPCR signal, whereas a signal was obtained from the cheeses inoculated with *M. morganii* (Fig. 3). This confirmed that the qPCR assay was specific for the *M. morganii* strains used. Moreover, we were confident that no cross-contamination between the cheese groups had occurred.

All the layers from the cheeses inoculated with FAM24206 and FAM24676 contained between 5.6 and 6.4 log copy number g^{-1} after 1 d of ripening. We did not observe any strain-specific differences. The population density remained constant in the under-the-rind zone and in the core during ripening until day 130. In contrast, we observed a drop to approximately 4 log copy number g^{-1} in the rind after 42 d of ripening. Thereafter, an increase of *M. morganii* up to 5 log copy number g^{-1} took place until 90 d. Finally, the density of *M. morganii* decreased to 3.1 (FAM24206) and 1.2 (FAM24676) log copy number g^{-1} in the rind at 130 d of ripening.

Since a qPCR test does not discriminate between dead and living cells, we additionally used a culture-based enumeration method.

3.3. Changes in bacterial counts during ripening

We observed that various strains of *M. morganii* grew in and on VRBG agar (data not shown). Kim et al. (2001) also used VRBG agar to isolate *M. morganii* from fish. Therefore, we think that this medium is suitable to detect live *M. morganii* cells in food. However, VRBG is not selective for *M. morganii* and allows the growth of other enteric bacteria. Thus, MALDI-TOF-MS analyses were performed to determine the species of randomly selected colonies.

The bacterial count in control cheeses at day 1 ranged between 1.6 and 2.8 log cfu g^{-1}. In contrast, the population density in the 1-d cheeses with *M. morganii* ranged between 5.4 and 6.4 log cfu g^{-1} (Fig. 4). None of the eight tested colonies from the control cheese were identified as *M. morganii* (Table 2). This result was in line with the qPCR result, in which no *M. morganii* was found in the control cheeses either.

The analysis of the 21-d cheeses showed a strong decrease in bacterial cell numbers (Fig. 4). This decrease was most pronounced in the under-the-rind and core zones. A decrease in *M. morganii* cell count was also observed in model cheese after 18 d of ripening by Delbes-Paus et al. (2012). In our study, the bacterial count in the core tended toward zero, but a steady increase was observed from day 42 to day 130 for the other layers. At the end of ripening, most of the tested colonies from the rind and under-the-rind layers were assigned to *M. morganii* (Table 2).

M. morganii is taxonomically closely related with the *Enterobacteriaceae*. The levels of the latter are regularly monitored in raw milk cheeses, as they are regarded as hygiene indicator. Metz, Sheehan, and Feng (2020) performed an in-depth literature search to gather knowledge on the distribution and growth of these indicator bacteria. The authors could derive the general picture that during the first days of ripening, there is an increase of number of coliforms and/or *Escherichia coli* followed by a decline throughout the ripening.

In the rind, we did not observe the same pattern in our study since the bacterial count increased after an initial decrease in the cheeses inoculated with *M. morganii*. The identification of several colonies as *M. morganii* at the end of ripening showed that the strains survived within the rind microbiota. The higher counts in the rind and under-the-rind layers compared with the core are probably related to the higher pH of approximately 6.4, which is close to a neutral pH.

Remarkably, the bacterial cell counts, especially in the core, are not consistent with the qPCR results. We have two hypothesis that can explain this matter. On the one hand, many dead *M. morganii* cells were present in the cheese samples whose DNA was not degraded. On the other hand, *M. morganii* was able to enter the viable but nonculturable (VBNC) state, which is a survival strategy of many bacteria (Pinto, Santos, & Chambel, 2015). To our knowledge, nucleotide metabolism in cheese has been rarely studied. In this context, it has been reported that various lactic acid bacteria can secrete nucleases (Péant & LaPointe, 2004) that would degrade free DNA in the cheese matrix. Since we did not detect any considerable decrease or increase in *M. morganii* with the qPCR assay (Fig. 3), there is much to suggest that the species has reached a viable but nonculturable state. As VBNC has been detected in various enterobacteria (Pinto et al., 2015), we think that the hypothesis that *M. morganii* can enter a VBNC state is not far-fetched, but definitely needs further investigation.

3.4. Biogenic amine formation

An essential question of this study was whether there is a link between *M. morganii* and the formation of biogenic amines. When we determined the biogenic amines' levels over the ripening period, strain-specific differences emerged.

The cheeses produced with the CAD-positive *M. morganii* FAM24206 contained cadaverine, which was not detected in the other cheese variants (Fig. 5). We determined concentrations of 310 (±59) mg kg^{-1} in rind, 298 (±32) mg kg^{-1} under-the-rind, and 162 (±11) mg kg^{-1} in the core after 130 d of ripening. Thus, there was a gradient, with the highest values in the rind. The gradient correlated with the cell counts, according to which living *M. morganii* cells were present in the rind but not in the core. We concluded

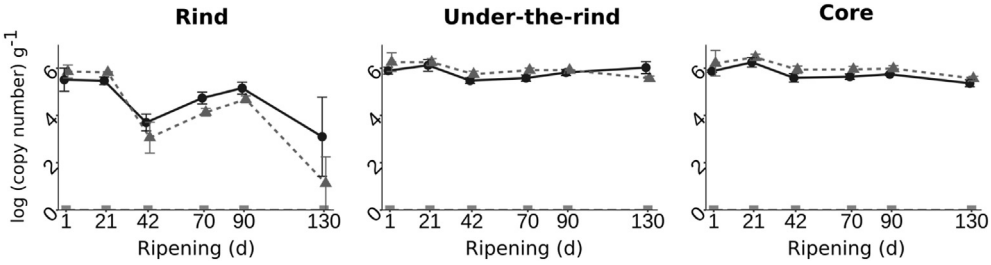

Fig. 3. Enumeration of *Morganella morganii* in the experimental cheese variants using a species-specific, quantitative PCR assay: —●—, FAM24206; ···▲···, FAM24676; – ■ –, control. The values represent the mean of the three cheese-making experiments; error bars show the standard error of the mean.

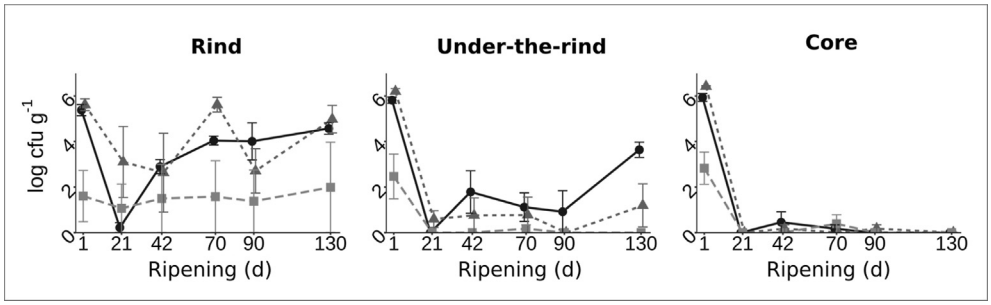

Fig. 4. Enumeration of *Morganella morganii* (and enterobacteria) in the experimental cheese variants using VRBG agar: —●—, FAM24206; ···▲···, FAM24676; – ■ –, control. The values represent the mean of the three cheesemaking experiments. Error bars show the standard error of the mean. (cfu: colony-forming units).

Table 2
Identification of *Morganella morganii* colonies grown on VRBG agar.

Cheese variant	Layer	Day	Number of analysed colonies	Number of *Morganella morganii* identifications
Control	Rind	1	8	0
	Rind	130	14	0
FAM24676	Rind	130	32	28
	Under-the-rind	130	11	9
FAM24206	Rind	130	25	23
	Under-the-rind	130	26	26

from these two observations that cadaverine was mainly formed on the cheese surface and diffused into the core during ripening.

The cheese variants containing the PUT-positive *M. morganii* FAM24676 developed putrescine, which was not found in the other cheese variants (Fig. 5). In contrast to cadaverine, no gradient from the outside to the inside was observed. We determined 59 (±12) mg kg^{-1} in the rind, 65 (±19) mg kg^{-1} in under-the-rind, and 54 (±16) mg kg^{-1} in the core after 130 d of ripening.

Histamine was only detected in the cheese variants inoculated with *M. morganii*. There were no strain-specific differences in regard to histamine concentrations. In the cheeses with

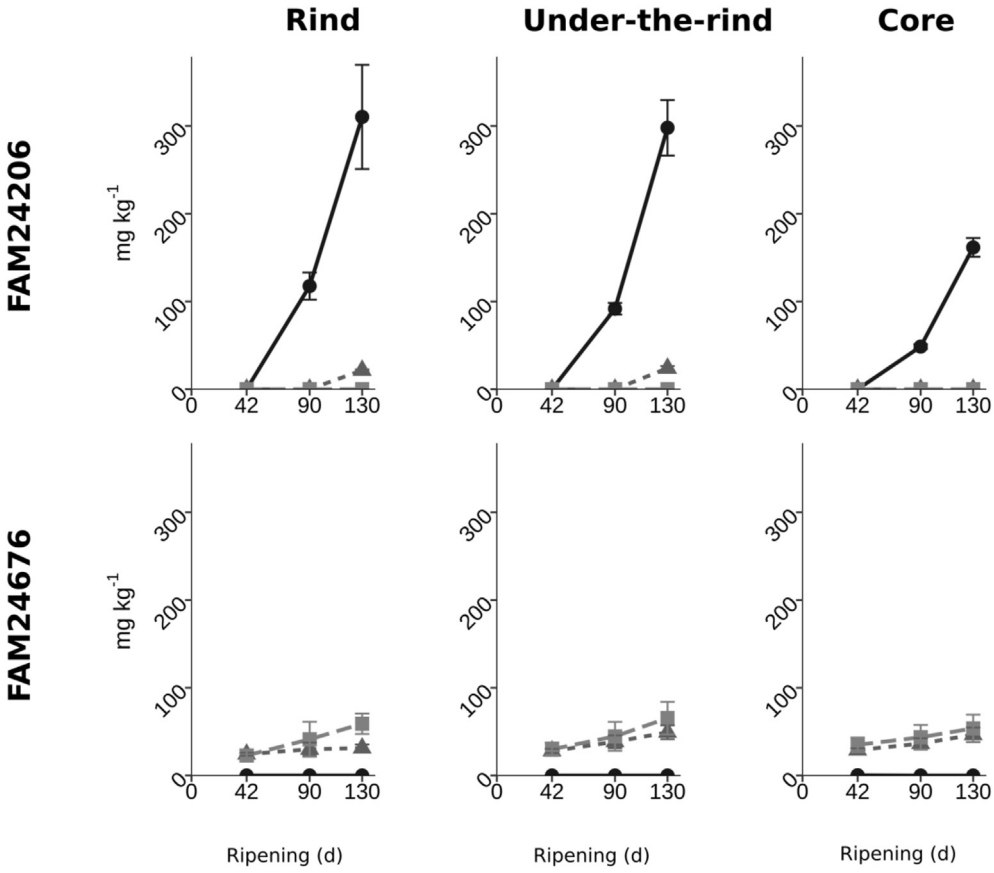

Fig. 5. Determination of cadaverine (—●—), putrescine (– ■ –), and histamine (···▲···) in the experimental cheese variants. The three biogenic amines were not detected in the control cheeses. The values indicate the mean of three cheesemaking experiments; error bars show the standard error of the mean.

FAM24676, histamine was already detected after 21 d of ripening, whereas in the cheeses with FAM24206, histamine was only detected after 130 d of ripening (Fig. 5). The histamine concentrations were below 50 mg kg^{-1} after 130 d in all cheese samples that contained histamine.

To see if the precursor compounds were limiting the formation of biogenic amines, the content of free amino acids was determined in all cheese samples after 130 d of ripening. We found the amino acids lysine, ornithine, and histidine, which are the substrates for cadaverine, putrescine, and histamine, respectively, in all cheese samples (Table 3). We conclude that there was no substrate limitation for the amino acid decarboxylation.

Many members of the family *Enterobacteriaceae* found in food have decarboxylase activity and are capable of forming putrescine and cadaverine (Pircher, Bauer, & Paulsen, 2007). In cheese, they are considered to be mainly responsible for the formation of both substances as presence of putrescine and cadaverine is associated with the presence of *Enterobacteriacea* (Maifreni et al., 2013; Marino et al., 2000; Suzzi et al., 2022). *M. morganii* does not strictly belong to the *Enterobacteriaceae* but to the family *Morganellaceae.* This study shows that this species also can play a role in the formation of cadaverine and putrescine. In future, it would certainly be valuable to determine the identity of biogenic amine forming bacteria down to the species level to investigate not only correlation but also causality. Here, the MALDI-TOF-MS technique is very suitable as a rapid and simple identification method.

The low histamine concentrations at the end of ripening are remarkable, as *M. morganii* is a main cause of histamine formation in fish (Kim et al., 2001, 2002). It has been reported that Gram-negative bacteria could also play a role in the formation of histamine (Suzzi et al., 2022). We hypothesise that histamine formation in *M. morganii* is regulated by temperature, which is the reason for the low levels of histamine. For example, it was shown that the storage temperature is the most important factor for histamine formation by *M. morganii* in fish.

Taken together, the study shows that *M. morganii* is able to survive the ripening in the rind and has the ability to form biogenic amines in cheese. The phenotypes observed in broth can be used to predict biogenic amine formation in cheese. Further studies, for example, using different inoculation densities and storage temperatures, can clarify which factors are decisive and could be used to control the biogenic amine formation by *M. morganii* in cheese.

Table 3
Concentrations of free amino acids (mg kg^{-1}) in the raclette-type cheese variants.[a]

Amino acid	Layer	Control	FAM24206	FAM24676
Lysine	Rind	$2227 \pm 225^{y,z}$	$2136 \pm 658^{y,z}$	$3021 \pm 419^{y,z}$
	Under-the-rind	910 ± 176^{x}	$660 \pm 127^{p,x}$	$1163 \pm 133^{c,x,z}$
	Core	$735 \pm 75^{c,x}$	$546 \pm 19^{n,p,x}$	$848 \pm 46^{c,x,y}$
Arginine	Rind	$736 \pm 177^{y,z}$	$1207 \pm 501^{y,z}$	$1510 \pm 482^{y,z}$
	Under-the-rind	96 ± 11^{x}	142 ± 22^{x}	138 ± 30^{x}
	Core	110 ± 10^{x}	116 ± 23^{x}	81 ± 16^{x}
Citrulline	Rind	276 ± 67	246 ± 13^{z}	279 ± 49
	Under-the-rind	231 ± 12^{p}	249 ± 17^{z}	284 ± 14^{n}
	Core	193 ± 21	$193 \pm 13^{x,y}$	219 ± 10
Ornithine	Rind	351 ± 29^{p}	$369 \pm 40^{p,z}$	$247 \pm 13^{n,c,y,z}$
	Under-the-rind	452 ± 48^{p}	463 ± 26^{p}	$354 \pm 26^{n,c,x}$
	Core	456 ± 28	519 ± 51^{x}	397 ± 52^{x}
Histidine	Rind	$679 \pm 84^{y,z}$	$772 \pm 232^{y,z}$	$898 \pm 147^{y,z}$
	Under-the-rind	212 ± 30^{x}	220 ± 47^{x}	249 ± 45^{x}
	Core	150 ± 24^{x}	136 ± 22^{x}	140 ± 26^{x}

[a] Arginine and citrulline are listed as they are precursors for ornithine biosynthesis via the arginine deiminase pathway. Values (mg kg^{-1}) represent the mean (±SEM) of the three experimental cheeses variants. Statistically significant differences are as follows:[n] different from negative control;[c] different from FAM24206;[p] different from FAM24676;[x] different from rind;[y] different from under-the-rind;[z] different from core.

4. Conclusions

In the present study, it is demonstrated that *M. morganii* can grow in cheese. It survives in the edge of cheese and can produce the biogenic amines cadaverine and putrescine. Remarkably, histamine was not formed in significant quantities although this species possesses the genes for the formation of this compound. Moreover, *M. morganii* is known to carry antibiotic resistance genes. The species does not belong to the *Enterobacteriaceae* according to taxonomic criteria, but is also detected by the methods used for *Enterobacteriaceae*. This could mean that this species occurs more often in cheese than previously thought. Since biogenic amines as well as antibiotic resistance genes are undesirable in cheese, it is recommended that the occurrence and distribution of this species is analysed in more detail. Culture-based methods do not provide enough resolution to clearly identify and distinguish members of the *Enterobacteriaceae* and *Morganellaceae*. Here, the combination with MALDI-TOF-MS offers a possibility to compensate this shortcoming.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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