



## Quantitative PCR reveals the frequency and distribution of 3 indigenous yeast species across a range of specialty cheeses

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### ABSTRACT

Indigenous microorganisms are important components of the complex ecosystem of many dairy foods including cheeses, and they are potential contributors to the development of a specific cheese's sensory properties. Among these indigenous microorganisms are the yeasts *Cyberlindnera jadinii*, *Pichia kudriavzevii*, and *Kazachstania servazzii*, which were previously detected using traditional microbiological methods in both raw milk and some artisanal specialty cheeses produced in the province of Québec, Canada. However, their levels across different cheese varieties are unknown. A highly specific and sensitive real-time quantitative PCR assay was developed to quantitate these yeast species in a variety of specialty cheeses (bloomy-rind, washed-rind, and natural-rind cheeses from raw, thermized, and pasteurized milks). The specificity of the quantitative PCR assay was validated, and it showed no cross-amplification with 11 other fungal microorganisms usually found in bloomy-rind and washed-rind cheeses. *Cyberlindnera jadinii* and *P. kudriavzevii* were found in the majority of the cheeses analyzed (25 of 29 and 24 of 29 cheeses, respectively) in concentrations up to  $10^4$  to  $10^8$  gene copies/g in the cheese cores, which are considered oxygen-poor environments, and  $10^1$  to  $10^4$  gene copies/cm<sup>2</sup> in the rind. However, their high abundance was not observed in the same samples. Whereas *C. jadinii* was present and dominant in all core and rind samples, *P. kudriavzevii* was mostly present in cheese cores. In contrast, *K. servazzii* was present in the rinds of only 2 cheeses, in concentrations ranging from  $10^1$  to  $10^3$  gene copies/cm<sup>2</sup>, and in 1 cheese core at  $10^5$  gene copies/g. Thus, in the ecosystems of specialty cheeses, indigenous yeasts are highly frequent but variable, with certain species selectively present in specific varieties. These

results shed light on some indigenous yeasts that establish during the ripening of specialty cheeses.

**Key words:** cheese ecosystem, indigenous yeast, *Cyberlindnera jadinii*, *Pichia kudriavzevii*, *Kazachstania servazzii*

### INTRODUCTION

A consistent cheese quality and flavor can be difficult to achieve from batch to batch, potentially due to the complex microbial ecosystem and the presence of indigenous microorganisms (Beresford and Williams, 2004). Recent studies have demonstrated that the cheese ecosystem is composed of hundreds of adventitious microbial species that emerge during the ripening process (Bokulich and Mills, 2013; Wolfe et al., 2014). They may be introduced into milk at the farm or the dairy plant (Bokulich and Mills, 2013).

Yeasts are natural members of the cheese ecosystem because of their capacity to grow in wide ranges of pH and temperature and their tolerance to high salt concentrations and low water activity (Fleet, 2011). Because of their common presence, multiple techniques are used to detect, identify, and quantify yeasts in cheese, including traditional microbiology techniques, quantitative (q)PCR, and omics methods (Lessard et al., 2014; Wolfe et al., 2014; Dugat-Bony et al., 2016). To date, few studies have addressed the frequency and distribution of indigenous yeasts in cheeses, despite their potential effects on the physicochemical composition of the cheeses and their microbial ecosystem (Wyder and Puhán, 1999; Chen et al., 2012).

The presence of indigenous yeasts *Cyberlindnera jadinii* (synonyms: *Pichia jadinii*, *Saccharomyces jadinii*; anamorph: *Candida utilis*), *Pichia kudriavzevii* (synonym: *Issatchenkia orientalis*; anamorph: *Candida krusei*), and *Kazachstania servazzii* (synonym: *Saccharomyces servazzii*) has been previously established in various fermented foods. *Kazachstania servazzii* has been found in pickles and kefir (Tominaga, 2004; Büchl and Seiler, 2011), *C. jadinii* has been isolated from

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**Table 1.** Yeast species and isolates used to develop and test the quantitative PCR method

Species	LMA <sup>1</sup> strain number (isolated from)	Reference
<i>Yarrowia lipolytica</i>	LMA-97 (cheese)	
<i>Cyberlindnera jadinii</i>	LMA-361 (milk), LMA-839 (cheese)	Lavoie et al., 2012
<i>Kluyveromyces lactis</i>	LMA-437	Lessard et al., 2012
<i>Candida parapsilosis</i>	LMA-463 (cheese)	Lavoie et al., 2012
<i>Pichia fermentans</i>	LMA-623 (cheese)	Lavoie et al., 2012
<i>Kazachstania servazzii</i>	LMA-647, LMA-687 (milk)	Lavoie et al., 2012
<i>Pichia kudriavzevii</i>	LMA-503, LMA-584, LMA-615, LMA-652 (cheese), LMA-660, LMA-666, LMA-726, LMA-845, LMA-933 (milk)	Lavoie et al., 2012
<i>Cryptococcus curvatus</i>	LMA-784 (milk)	Lavoie et al., 2012
<i>Rhodotorula mucilaginosa</i>	LMA-808 (cheese)	Lavoie et al., 2012
<i>Debaryomyces hansenii</i>	LMA-1019 (commercial cheese starter)	Lessard et al., 2012
<i>Geotrichum candidum</i>	LMA-1028 (commercial cheese starter)	Lessard et al., 2014

<sup>1</sup>LMA = Laboratoire de Mycologie Alimentaire (Université Laval, Québec, Canada).

cucumber brine and coffee cherries (Silva et al., 2000; Tornai-Lehoczki, 2003), and *P. kudriavzevii* has been found in wine (Del Mónaco et al., 2016), sorghum beer (Lyumugabe et al., 2014), cocoa (Koné et al., 2016), Italian sourdoughs (Succi et al., 2003), and green olive brine (Romero-Gil et al., 2016). In dairy foods, *K. servazzii* is found less frequently than *C. jadinii* and *P. kudriavzevii*, and fewer studies have been conducted to evaluate its role in the dairy environment (Mei et al., 2014). In cheese slurries, *C. jadinii*, present in Münster cheese, imparts a distinct odor in addition to an elevated pH, and *P. kudriavzevii* influences the chemical environment of cheese by increasing pH and proteolysis (Wyder and Puhán, 1999; Chen et al., 2012).

In raw milk or artisanal specialty cheeses from the province of Québec, *P. kudriavzevii* was isolated and identified, using ITS1–5.8S–ITS2 rDNA sequencing, in numerous cheese samples, whereas *C. jadinii* and *K. servazzii* were found less frequently (Lavoie et al., 2012). Because the approach was not quantitative, the abundance of these yeasts across different cheeses and their ability to influence the cheese ripening process remain unclear.

The aim of this study was to develop a qPCR method to determine the concentrations of *C. jadinii*, *P. kudriavzevii*, and *K. servazzii* in different areas (core and rind) of several types of commercial specialty cheeses.

## MATERIALS AND METHODS

### Biological Material and Genomic DNA Extraction

Reference fungal strains from the Laboratoire de Mycologie Alimentaire (LMA, Université Laval, Québec, Canada) collection were used to develop a qPCR assay (Table 1). Strains were cultured from culture stock stored at  $-80^{\circ}\text{C}$  in YEG medium (10 g/L yeast extract, Bio Basic; 10 g/L glucose, EMD Chemicals) containing 15% glycerol, and were plated on YEG agar (15 g/L

bacto agar; BD Diagnostics). Individual colonies were cultured in YEG liquid medium and incubated at  $25^{\circ}\text{C}$  for 48 h. Yeasts were pelleted by centrifugation (5 min at  $20,000 \times g$  at room temperature), which were used for genomic DNA extraction using a phenol-chloroform extraction protocol (Al-Samarrai and Schmid, 2000). Yield and quality of DNA were determined using a Nanodrop spectrophotometer (ND-1000, ThermoFisher Scientific).

### Cheese Samples and Total DNA Extraction

Triplicates of each of 29 ready-to-eat specialty cheese varieties from 22 cheese plants (for a total of 87 individual cheeses sampled) from the province of Québec, Canada, were sampled. They were classified according to their variety: washed rind ( $n = 15$ ), bloomy rind ( $n = 12$ ) or natural rind ( $n = 2$ ), and by heat treatment of the milk: raw ( $n = 6$ ), thermized ( $n = 14$ ), or pasteurized ( $n = 9$ ), and were coded for confidentiality (Table 2). Thermization was conducted by each cheesemaker according to the regulation of the province of Québec; that is, between 15 and 20 s at  $57$  to  $68^{\circ}\text{C}$ . The cheeses were stored at  $4^{\circ}\text{C}$  for 3 d. A total of  $50 \text{ cm}^2$  was sampled and weighed for each cheese rind; in brief,  $25 \text{ cm}^2$  was scraped from each flat surface to recover the microbiota and a minimum of the cheese matrix underneath ( $<1 \text{ mm}$ ). In addition, 10 g of each cheese variety's core was sampled for 1 of 3 specimens for each of the 29 cheeses. All rind and core samples were stored at  $-80^{\circ}\text{C}$  before grinding using a liquid nitrogen-cooled CryoMill (Retsch). Grinding consisted of a precooling cycle at 5 Hz, followed by a 4-min grinding step at 25 Hz.

Cheese total DNA extraction was performed in triplicate for each cheese sample using a Purelink viral RNA/DNA kit (Invitrogen/Life Technologies) with the following modifications: a total of 30 to 40 mg of each ground cheese sample was homogenized in  $200 \mu\text{L}$  of

0.9% NaCl, before the addition of proteinase K and lysis buffer from the kit. After DNA elution, RNA was removed using a 0.5 mg/mL RNase A treatment for 1 h at 37°C. Yield and quality of the extracted DNA were measured using a Synergy HI microplate reader and Gen5 software (BioTek).

### Target Gene Sequencing and Design of Real-Time qPCR Primers and Probes

Specific gene sequences for the targeted yeast species were selected based on their availability in GenBank. The genes coding for malic enzyme (*CME1*; accession number DQ173437.1), glutathione S-transferase Y-1 (*GSTY-1*; accession number AB021655.1), and centromere H3 (*CenH3*; accession number DQ826421.1) were selected as qPCR gene targets and sequenced for *C. jadinii* (forward primer 5'-CTGAAGGTGACGCCATTGCT-3' and reverse primer 5'-GTGGACGACCAGTGTGACA-3'), *P. kudriavzevii* (forward primer 5'-CTGAAGGTGACGCCATTGCT-3' and reverse primer 5'-AACAAATGTGCTGTAAGGTTACGTA TAG G-3'), and *K. servazzii* (forward primer 5'-TTCCCCCAGTGACCTTGATG-3' and reverse primer 5'-TTCGCATGTTCCAGTAAACCAA-3'), respectively. Based on the target gene sequences obtained, qPCR-specific primers and TaqMan probes were designed using Primer Express v2.0 (ThermoFisher Scientific) and analyzed with OligoAnalyzer v3.1 (Integrated DNA Technologies; Table 3). Primer specificity was verified by PCR on several strains of the targeted yeast species (Table 1), and resulting PCR amplicons were sequenced and verified using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Each qPCR design was assessed for cross-amplification against 10 other fungal species commonly found in dairy products (Table 1).

**Table 2.** Distribution of specialty cheeses (C1 to C29) selected from the province of Québec

Type of rind	Heat treatment of milk		
	Raw	Thermized <sup>1</sup>	Pasteurized
Washed	3 (C1 to C3)	5 (C4 to C8)	7 (C9 to C15)
Bloomy	3 (C16 to C18)	2 (C19, C20)	7 (C21 to C27)
Natural <sup>2</sup>	0	2 (C28, C29)	0

<sup>1</sup>Thermization was conducted by the cheesemaker according to the regulation of the province of Québec; that is, between 15 and 20 s at 57 to 68°C.

<sup>2</sup>No raw or pasteurized natural-rind cheeses were available at the time of sampling.

### qPCR Absolute Quantification Using Standard Curve Assays

The qPCR target genes (*CEM1*, *GSTY-1*, and *CenH3*) were individually PCR amplified, cloned in the pCR-II TOPO vector, and transformed in *Escherichia coli* TOP10 using a TOPO TA Cloning kit (Invitrogen/Life Technologies). Recombinant plasmid DNA was purified with a Plasmid DNA Maxiprep kit (Qiagen), and then sequenced. To use the recombinant plasmid DNA as a quantification standard, the concentration was measured with a Synergy HI microplate reader and Gen5 software (BioTek). The number of gene copies was calculated using the following formula:

No. of gene copies =

$$\frac{\text{Amt. of plasmid DNA (ng)} \times 6.0221 \times 10^{23} \text{ molecules/mol}}{[\text{Length of plasmid DNA (bp)} \times 660 \text{ g/mol}] \times 1 \times 10^9 \text{ ng/g}}$$

where Amt. = amount, and 660 g/mol is the average mass of 1 bp of double-stranded DNA.

**Table 3.** Primers and TaqMan probes used for quantitative (q)PCR quantification of indigenous yeasts and efficiency of each qPCR assay

Yeast species and targeted gene	Primers and TaqMan probes (5' → 3') <sup>1</sup>	Dynamic range (gene copy/reaction)	qPCR efficiency, range (%)	R <sup>2</sup>
<i>Cyberlindnera jadinii</i> Malic enzyme ( <i>CME1</i> )	CME1-F: GGTGTTGGTGGTGTCCGTATT CME1-R: GGATACCACCACAAAGGGTCAT CME1-Prb: 5'FAM-CCATCTCTAAGCTTGCC-TAMRA3'	3 × 10 <sup>0</sup> to 3 × 10 <sup>8</sup>	90.4 to 102.4	≥0.99
<i>Kazachstania servazzii</i> Centromere H3 ( <i>CenH3</i> )	CenH3-F: GCAGTAAGAATTACAGACGCAGAGA CenH3-R: GTTGCACTTTTATTTGCTTTTTTCT CenH3-Prb: 5'FAM-TCTAGGTGGTGAGATTAG-TAMRA3'	3 × 10 <sup>0</sup> to 3 × 10 <sup>9</sup>	92.6 to 100.7	>0.99
<i>Pichia kudriavzevii</i> Glutathione S-transferase Y-1 ( <i>GSTY-1</i> )	GSTY1-F: TCCTTTGCCAACTCGGATTT GSTY1-R: TCGTCTTTGCAGAACCACACA GSTY1-Prb: 5'FAM-TGGCGCAATGGTT-TAMRA3'	3 × 10 <sup>0</sup> to 3 × 10 <sup>9</sup>	91.5 to 105.3	≥0.99

<sup>1</sup>F= forward primer; R = reverse primer; Prb = probe; FAM = 6-carboxyfluorescein; TAMRA = 6-carboxytetramethylrhodamine.

When used as a quantification standard during qPCR assays, the recombinant plasmid DNA was diluted 6- to 10-fold and deposited in triplicate in 96-well plates. These serial dilutions allowed us to determine a limit of detection of 3 gene copies per PCR reaction. Standard curves were generated to determine the qPCR efficiency and dynamic range of each reaction (dynamic range for target gene *CME1*: 3 to  $3 \times 10^8$  gene copies per reaction; for *GSTY-1* and *CenH3*: 3 to  $3 \times 10^9$  gene copies per reaction).

Quantitative PCR was performed on an ABI7500 Fast system apparatus (Applied Biosystems/ThermoFisher Scientific). The qPCR plates were set up using an epMotion 5075 VAC automated pipetting system (Eppendorf). Optimal dilutions of DNA samples were chosen to fit the dynamic range of the standard curves and to ensure the absence of PCR inhibitors. The qPCR reagents were used as described by Lessard et al. (2012). The qPCR program began with an initial denaturation step at 95°C for 20 s, followed by 40 cycles of denaturation 95°C for 3 s and annealing/extension at 60°C for 30 s. The qPCR data were analyzed using 7500 software v2.0.6 (ThermoFisher Scientific).

### Statistical Analysis

A one-way ANOVA was used to analyze the effect of the type of cheese on the detection of each indigenous yeast being tested. Because of the non-normality of the data, *P*-values were adjusted using a permutation distribution. Variety of cheese was considered a fixed factor, and comparisons were made between specific types of cheese (rind type and heat treatment). SAS/STAT software (2015; SAS Institute Inc.) was used for analysis; specifically, the GLM procedure was followed by the MULTTEST procedure.

## RESULTS

### qPCR Assay Efficiency

Highly specific and sensitive qPCR assays were developed for the quantification of 3 indigenous yeast species (*C. jadinii*, *P. kudriavzevii*, and *K. servazzii*) in samples originating from both the rind and core of specialty cheeses. The specificity of qPCR amplification was confirmed by sequencing the obtained qPCR amplicons and by negative qPCR detection using 10 other fungal species isolated from dairy products. BLAST searches of the *CME1*, *GSTY-1*, and *CenH3* target genes revealed that these genes were present in only one copy in the genomes of *C. jadinii* [whole-genome sequencing (WGS) project BAEL01], *P. kudriavzevii* (WGS project

JQFK01), and *K. servazzii* (unpublished WGS project LMA-647, Laboratoire de Mycologie Alimentaire), respectively. Resulting average qPCR efficiencies were 98.4, 97.8, and 96.6% for *C. jadinii*, *P. kudriavzevii*, and *K. servazzii*, respectively ( $R^2$  values  $\geq 0.990$ ; Table 3).

### Distribution of *C. jadinii* in Quebec's Specialty Cheeses

*Cyberlindnera jadinii* was detected in 25 of 29 cheese cores at concentrations of  $10^5$  to  $10^8$  gene copies/g (Table 4), and was detected in the rinds of 24 of 29 cheeses at  $10^0$  to  $10^4$  gene copies/cm<sup>2</sup> in all cheese varieties. It was absent in both the core and rind of only one cheese, C1, a washed-rind cheese made with raw milk. The population of *C. jadinii* in the cheese core was not dependent on the cheese variety ( $P > 0.05$ ) but was correlated with the milk heat treatment applied ( $P < 0.01$ , Figure 1), especially in bloomy-rind cheeses made from raw and thermized milk ( $P < 0.001$ ). At the cheese surface, high *C. jadinii* counts were obtained in bloomy-rind cheeses (12/29 cheeses) compared with washed-rind cheeses (15/29 cheeses;  $P \leq 0.001$ , Table 4), although this difference was not observed in core samples taken from the same cheese varieties. Cheeses made from raw and thermized milk had more *C. jadinii* on their surface than pasteurized milk cheeses ( $P < 0.001$ ), but we detected no differences between raw and thermized milk cheeses (Figure 2).

The presence of *C. jadinii* in multiple cheeses manufactured in the same cheese plant was verified by analyzing 2 different cheese varieties from each of 8 artisanal cheese plants (plant A: C1 and C4; plant B: C3 and C17; plant C: C5 and C19; plant D: C6 and C8; plant E: C9 and C10; plant F: C12 and C27; plant G: C16 and C18; plant H: C20 and C29, Table 4). Counts of *C. jadinii* on the surface of a bloomy-rind and a washed-rind cheese (C17 and C3) produced at the same cheese plant were significantly different ( $10^4$  gene copies/cm<sup>2</sup> and 1 gene copy/cm<sup>2</sup>, respectively;  $P < 0.001$ ). The same significant difference in *C. jadinii* counts was observed in the cores of these cheeses: *C. jadinii* was more abundant in bloomy-rind cheeses than in washed-rind cheeses ( $P < 0.001$ ; Table 4).

### Distribution of *P. kudriavzevii* in Quebec's Specialty Cheeses

*Pichia kudriavzevii* was detected on the surface of 6 varieties of cheese at  $0.9 \pm 1.5$  to  $1.2 \pm 2.1 \times 10^2$  gene copies/cm<sup>2</sup>, and in the core of 24 cheeses from  $2.4 \pm 4.2 \times 10^5$  to  $4.15 \pm 1.03 \times 10^7$  gene copies/g (Table 5). It was not detected in the rind or core of 4 cheeses



**Table 4.** Quantitative PCR of *Cyberlindnera jadinii* in specialty cheeses

Type of rind and milk heat treatment	Cheese number (cheese plant) <sup>1</sup>	Rind (gene copies/cm <sup>2</sup> )	Core (gene copies/g)	
Washed Raw	C1 (A)	<LOD <sup>2</sup>	<LOD	
	C2	4.7 ± 1.9 × 10 <sup>1</sup>	1.5 ± 1.3 × 10 <sup>6</sup>	
Thermized	C3 (B)	3.6 ± 6.2	5.4 ± 9.4 × 10 <sup>5</sup>	
	C4 (A)	<LOD	1.6 ± 1.6 × 10 <sup>6</sup>	
	C5 (C)	8.2 ± 3.4 × 10 <sup>2</sup>	1.6 ± 0.2 × 10 <sup>8</sup>	
	C6 (D)	<LOD	1.6 ± 2.8 × 10 <sup>6</sup>	
	C7	0.6 ± 1.1 × 10 <sup>1</sup>	<LOD	
Pasteurized	C8 (D)	2.4 ± 4.2 × 10 <sup>1</sup>	1.3 ± 1.8 × 10 <sup>6</sup>	
	C9 (E)	0.6 ± 1.0 × 10 <sup>1</sup>	<LOD	
	C10 (E)	1.7 ± 0.8 × 10 <sup>2</sup>	3.6 ± 1.3 × 10 <sup>6</sup>	
	C11	1.0 ± 1.8 × 10 <sup>1</sup>	1.0 ± 0.1 × 10 <sup>7</sup>	
	C12 (F)	<LOD	1.9 ± 1.1 × 10 <sup>6</sup>	
	C13	<LOD	1.5 ± 2.1 × 10 <sup>6</sup>	
	C14	1.0 ± 4.0 × 10 <sup>1</sup>	1.5 ± 1.6 × 10 <sup>6</sup>	
Bloomy Raw	C15	4.1 ± 7.0	2.8 ± 4.9 × 10 <sup>6</sup>	
	C16 (G)	8.8 ± 3.0	5.6 ± 0.8 × 10 <sup>6</sup>	
	C17 (B)	1.6 ± 0.4 × 10 <sup>4</sup>	3.8 ± 1.2 × 10 <sup>7</sup>	
	C18 (G)	1.7 ± 2.9 × 10 <sup>1</sup>	1.4 ± 2.3 × 10 <sup>6</sup>	
	Thermized	C19 (C)	9.2 ± 2.3 × 10 <sup>3</sup>	1.0 ± 0.1 × 10 <sup>8</sup>
		C20 (H)	0.9 ± 1.6 × 10 <sup>1</sup>	<LOD
	Pasteurized	C21	9.4 ± 8.1	3.4 ± 0.7 × 10 <sup>6</sup>
		C22	1.0 ± 1.7 × 10 <sup>2</sup>	2.9 ± 2.0 × 10 <sup>6</sup>
		C23	6.6 ± 5.5 × 10 <sup>1</sup>	1.6 ± 0.4 × 10 <sup>6</sup>
		C24	0.9 ± 1.6 × 10 <sup>1</sup>	2.7 ± 0.4 × 10 <sup>6</sup>
		C25	3.6 ± 3.6 × 10 <sup>1</sup>	5.1 ± 2.6 × 10 <sup>6</sup>
C26		1.0 ± 1.7 × 10 <sup>1</sup>	2.8 ± 0.3 × 10 <sup>6</sup>	
C27 (F)		3.7 ± 2.1 × 10 <sup>1</sup>	2.4 ± 0.5 × 10 <sup>6</sup>	
Natural Thermized	C28	5.6 ± 7.5	2.7 ± 0.7 × 10 <sup>6</sup>	
	C29 (H)	4.9 ± 8.4	3.0 ± 5.2 × 10 <sup>6</sup>	

<sup>1</sup>Cheeses with the same letter (A to H) were produced by the same cheese plant. Cheeses that do not have a letter to identify the cheese plant do not share a production site with any other cheeses analyzed in this study. Means and SE were calculated from means of each of 3 biological replicates (3 technical replicates were performed per biological replicate) for the rinds of each cheese variety, and from the means of values obtained from 3 technical replicates for each core (1 biological replicate was sampled per cheese variety).

<sup>2</sup>LOD = limit of detection, determined using serial dilutions, and set to 3 gene copies per PCR reaction.

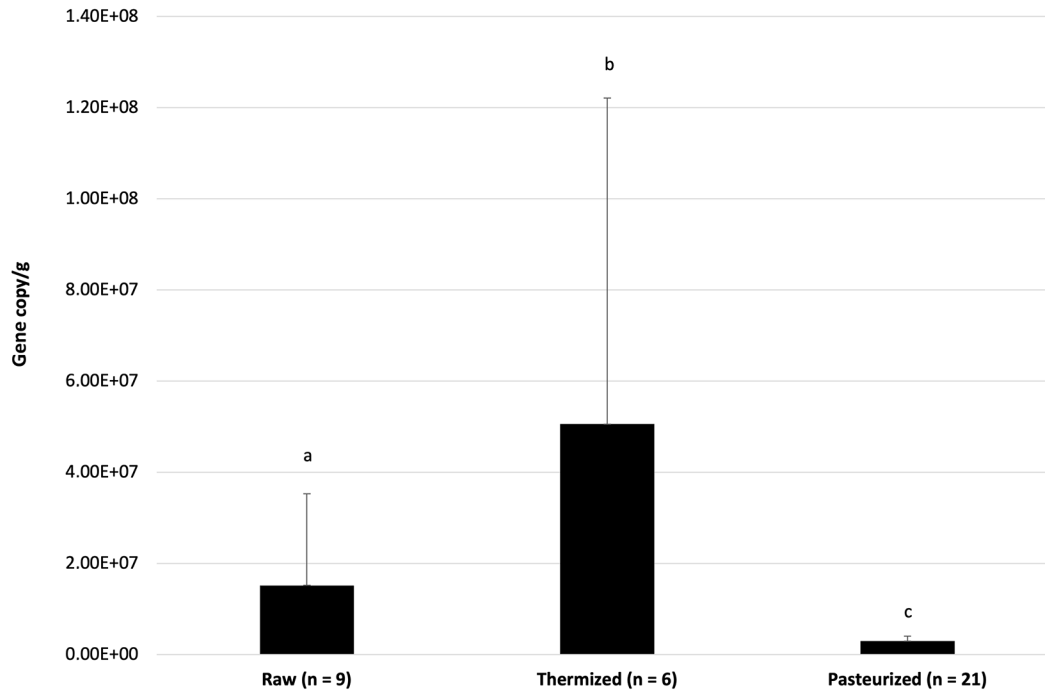
(C1, C12, C15, and C20). The type of heat treatment applied to the milk used for each cheese did not affect the presence or levels of *P. kudriavzevii* in the cores of washed-rind cheeses. In the bloomy-rind cheeses, however, a significantly higher count of *P. kudriavzevii* was detected in raw milk cheeses than in thermized and pasteurized milk cheeses ( $P < 0.05$ ; Figure 3). This indigenous yeast was detected in the core of 11 of 12 bloomy-rind cheeses in amounts ranging from 10<sup>5</sup> to 10<sup>7</sup> gene copies/g. Finally, different cheeses produced in the same plant showed different levels of *P. kudriavzevii*. For example, a difference was observed between washed-rind and bloomy-rind cheeses C3 and C17 from cheese plant B (10<sup>5</sup> and 10<sup>7</sup> gene copies/g, respectively). Only 1 of the 2 cheese varieties from cheese plant H, which produced the bloomy-rind cheese C20 (< limit of detection) and the natural-rind cheese C29 (10<sup>7</sup> gene copies/g), contained *P. kudriavzevii*.

### Distribution of *K. servazzii* in Quebec's Specialty Cheeses

*Kazachstania servazzii* was detected in the rind of only 2 cheeses: natural-rind cheeses C28 and C29 (both made from thermized milk), at 1.89 ± 0.51 × 10<sup>3</sup> gene copies/cm<sup>2</sup> and 0.6 ± 1.0 × 10<sup>1</sup> gene copies/cm<sup>2</sup>, respectively (Table 6). *Kazachstania servazzii* was detected in only 1 cheese core: cheese C12 at 0.9 ± 1.6 × 10<sup>5</sup> gene copies/g.

## DISCUSSION

This study aimed to establish the frequency and distribution of the 3 indigenous yeast species *C. jadinii*, *P. kudriavzevii*, and *K. servazzii* in specialty cheeses. These yeasts have been previously detected in cheeses from Québec (Lavoie et al., 2012) and in other cheese



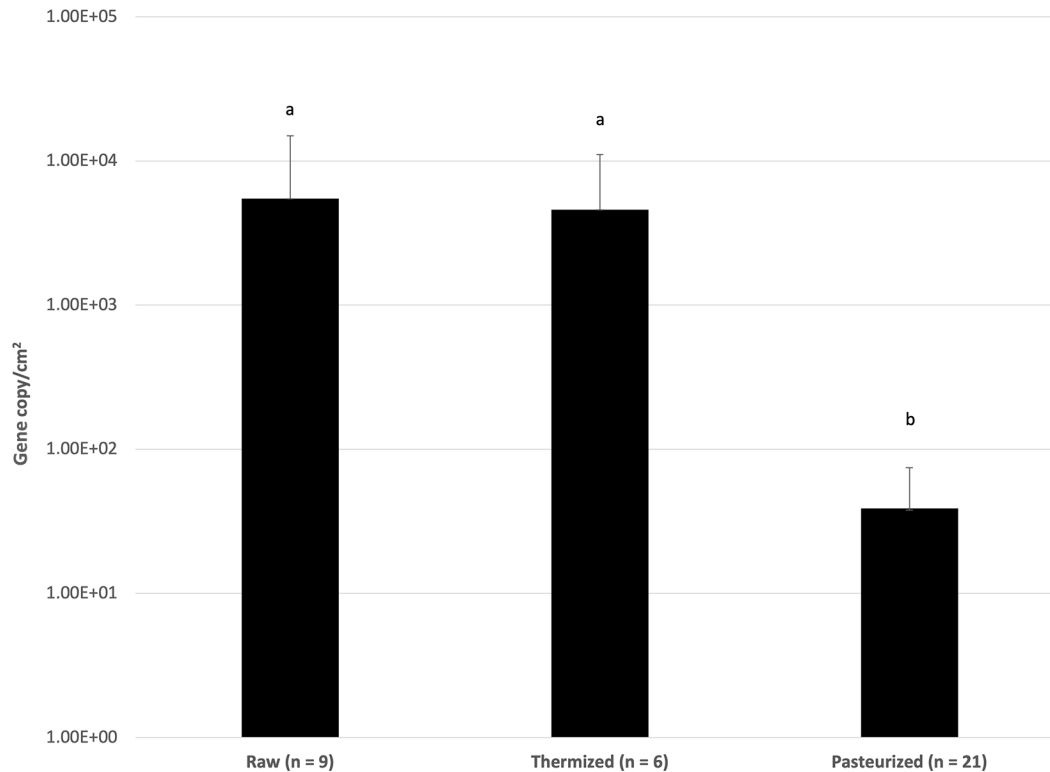
**Figure 1.** Quantitative PCR enumeration (gene copies/g) of *Cyberlindnera jadinii* in the cores of bloomy-rind specialty cheeses. Different letters (a–c) indicate significant differences ( $P < 0.01$ ) among core samples. Error bars represent SE.

samples worldwide (Borelli et al., 2006; Lopandic et al., 2006; Larpin-Laborde et al., 2011; Mei et al., 2014; Chombo-Morales et al., 2016; Dugat-Bony et al., 2016). The use of qPCR in this study provided the first quantitative assessment of indigenous yeast presence in both the rind and the core of cheeses with different rind varieties (bloomy-, washed-, and natural-rind cheeses), and from milk undergoing different heat treatments (raw, thermized, and pasteurized).

Before this study, the presence of *C. jadinii* was rarely reported in cheese. It had been isolated from, but not quantified in, the rind of a Livarot cheese (a soft, red washed-rind cheese made from raw milk), during an early stage of ripening before salting (Larpin-Laborde et al., 2011). It was also reported in the core of a Portuguese Serpa cheese (Gonçalves Dos Santos et al., 2017). In an earlier study by our group, using traditional microbiological methods, *C. jadinii* was isolated in only a single raw milk sample and a single semi-hard washed-rind cheese core made with thermized milk (Lavoie et al., 2012). Interestingly, the qPCR method detected and quantified *C. jadinii* in 83% of surfaces and 86% of cores, a higher frequency than previously reported. The low detection rate in previous studies could be explained by the lack of specific detection methods (Cogan et al., 2014) or by the presence of *C. jadinii* as a subdominant

species of the cheese ecosystem because it is masked by other microorganisms. Given that *C. jadinii* was detected in relatively large quantities in several cheese varieties, the effect of *C. jadinii* on the physicochemical characteristics and microbial ecosystem of cheeses during ripening warrants further investigation.

*Kazachstania servazzii* was detected in only 3 varieties of specialty cheese analyzed in this study: on 2 natural-rind cheeses (C28 and C29) and in 1 washed-rind cheese core (C12). *Kazachstania servazzii* was found in the rind (surface) of all 3 biological and 27 technical replicates of C28. It was also isolated from the same cheese in 2012 (Lavoie et al., 2012), suggesting that this yeast is well established in the cheese plant where C28 is produced. *Kazachstania servazzii* is rarely reported in cheese, with the exception of a Camembert-type cheese produced using a Tibetan freeze-dried kefir coculture as a starter (Mei et al., 2014). In that particular case, this yeast was predominant during the beginning of the ripening process (d 0 to 15; Mei et al., 2014): 51 isolates of this species were found, compared with 15 isolates of *Debaryomyces hansenii* (Mei et al., 2014). This ecosystem clearly evolved during the ripening process, as *D. hansenii* was found in higher concentrations at the end of the ripening period (d 10 to 35) of the cheese. These results, taken in conjunction with the current report,



**Figure 2.** Quantitative PCR enumeration (gene copies/cm<sup>2</sup>) of *Cyberlindnera jadinii* on the surface of bloomy-rind specialty cheeses. Different letters (a, b) indicate significant differences ( $P < 0.001$ ) among surface samples. Error bars represent SE.

suggest that *K. servazzii* is not well adapted to survive in the cheese matrix or that it is displaced by other yeasts during ripening. This could be explained by the ability of *K. servazzii* to metabolize galactose but not lactate (Vaughan-Martini et al., 2011). Depending on techniques used during ripening (e.g., lactic vs. rennet coagulum, soft vs. hard cheese), residual galactose can be limited relative to lactate during the ripening process (Fox and McSweeney, 2004).

In previous work, *P. kudriavzevii* was found in raw milk from 8 farms and was the most frequently isolated yeast species from ripened cheese: 7 isolates came from cheese rinds and 12 from cheese cores (Lavoie et al., 2012). *Pichia kudriavzevii* is naturally present in milk, can survive the cheesemaking process, and can be found in cheese. In this study, *P. kudriavzevii* was found in 26 of 29 cheeses analyzed (6 surface and 24 cores), in all cheese rind varieties (washed, bloomy, and natural rind) and in cheese from milk submitted to all types of heat treatment (raw, thermized, and pasteurized). Our results confirm that *P. kudriavzevii* is frequently found in cheeses; it has been reported in Austrian fresh and sour curd cheeses (Lopandic et al., 2006), Graukäse cheese (Prillinger et al., 1999), and in

multiple raw milk cheeses (Borelli et al., 2006; Pangallo et al., 2014; Chombo-Morales et al., 2016). *Pichia kudriavzevii* was found to be one of the dominant species on the surface of Cotija raw milk cheese, as identified by PCR-denaturing gradient gel electrophoresis (PCR-DGGE; Chombo-Morales et al., 2016). This evidence, in conjunction with our results, suggests that the presence of *P. kudriavzevii* in cheese might have been underestimated in previous studies using traditional microbiological methods (Valdés-Stauber et al., 1997; Cogan et al., 2014).

Although *P. kudriavzevii* appears to be common in cheese microbial ecosystems, its contribution to the cheese ripening process remains unclear. It is generally accepted that yeasts are found in 10- to 1,000-fold-higher concentrations on the surface of cheese than in the core because of their oxygen and nutritional requirements (Schmidt et al., 1980; Beresford and Williams, 2004; Chamba and Irlinger, 2004). However, our qPCR quantification method showed that the indigenous yeasts *C. jadinii* and *P. kudriavzevii* can be found in high concentrations (up to  $10^4$ – $10^8$  gene copies/g) in the cheese core compared with the surface ( $10^1$ – $10^4$  gene copies/cm<sup>2</sup>, equivalent to  $10^2$ – $10^5$  gene copies/g).

**Table 5.** Quantitative PCR of *Pichia kudriavzevii* in specialty cheeses

Type of rind and milk heat treatment	Cheese number (cheese plant) <sup>1</sup>	Rind (gene copies/cm <sup>2</sup> )	Core, (gene copies/g)	
Washed	Raw			
	C2	$3.9 \pm 6.7 \times 10^1$	<LOD <sup>2</sup>	
Thermized	C3 (B)	<LOD	$2.4 \pm 4.2 \times 10^5$	
	C4 (A)	<LOD	$1.08 \pm 0.20 \times 10^7$	
	C5 (C)	<LOD	$1.3 \pm 1.8 \times 10^7$	
	C6 (D)	<LOD	$4.3 \pm 7.4 \times 10^5$	
	C7	<LOD	$4.27 \pm 7.40 \times 10^6$	
Pasteurized	C8 (D)	<LOD	$0.6 \pm 1.1 \times 10^4$	
	C9 (E)	$1.4 \pm 2.4 \times 10^1$	$9.6 \pm 15.3 \times 10^6$	
	C10 (E)	<LOD	$3.71 \pm 0.54 \times 10^7$	
	C11	<LOD	$2.26 \pm 1.63 \times 10^7$	
	C13	<LOD	$1.6 \pm 2.6 \times 10^7$	
	C14	<LOD	$5.2 \pm 8.9 \times 10^5$	
Bloomy	Raw			
	C16 (G)	$0.9 \pm 1.5$	$1.72 \pm 2.09 \times 10^7$	
Thermized	C17 (B)	<LOD	$4.15 \pm 1.03 \times 10^7$	
	C18 (G)	$5.1 \pm 8.9$	$1.33 \pm 1.15 \times 10^7$	
	C19 (C)	<LOD	$8.76 \pm 1.29 \times 10^6$	
	Pasteurized	C21	<LOD	$6.28 \pm 4.72 \times 10^6$
		C22	<LOD	$7.7 \pm 7.3 \times 10^6$
	C23	$1.2 \pm 2.1 \times 10^2$	$3.3 \pm 4.7 \times 10^5$	
	C24	<LOD	$1.59 \pm 1.19 \times 10^7$	
	C25	<LOD	$3.33 \pm 3.25 \times 10^6$	
	C26	<LOD	$1.4 \pm 1.4 \times 10^7$	
	C27 (F)	<LOD	$1.3 \pm 2.0 \times 10^7$	
Natural	Thermized			
	C28	<LOD	$1.08 \pm 0.18 \times 10^7$	
	C29 (H)	$7.1 \pm 6.2 \times 10^1$	$2.41 \pm 1.25 \times 10^7$	

<sup>1</sup>Cheese samples in which *Pichia kudriavzevii* was not detected (in both the rind and core) are not presented. Cheeses with the same letter (A to H) were produced by the same cheese plant. (Cheeses with letters not repeated in this table indicate that another cheese produced by this cheese plant was analyzed but the microorganism was absent.) Means and SE were calculated from means of each of 3 biological replicates (3 technical replicates were performed per biological replicate) for the rinds of each cheese variety, and from the means of values obtained from 3 technical replicates for each core (1 biological replicate was sampled per cheese variety).

<sup>2</sup>LOD = limit of detection, determined using serial dilutions, and set to 3 gene copies per PCR reaction.

As the presence and metabolism of indigenous yeasts in the core and surface could affect the flavor development of the cheese (Beresford and Williams, 2004), further investigations of the role of *C. jadinii* and *P. kudriavzevii* in cheese ripening are warranted. Furthermore, the presence of these particular yeasts may reflect intrinsic properties, such as the ability to grow under low oxy-

gen conditions or in the presence of lactate and citrate (Kurtzman, 2011a,b).

Previous studies have shown that the type of heat treatment applied to milk affects the biodiversity of microorganisms found in cheese made from that milk (Delcenserie et al., 2014; Wolfe et al., 2014; Irlinger et al., 2015). The quantity of *C. jadinii* in the cheese

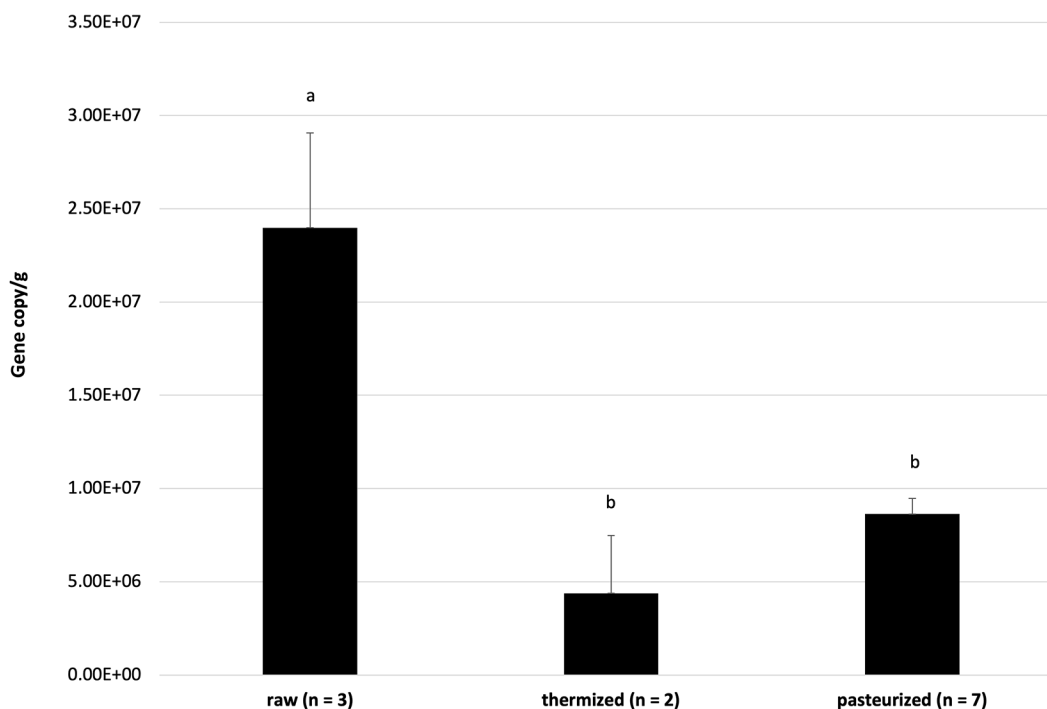
**Table 6.** Quantitative PCR of *Kazachstania servazzii* in specialty cheeses

Type of rind and milk heat treatment	Cheese number (cheese plant) <sup>1</sup>	Rind (gene copies/cm <sup>2</sup> )	Core (gene copies/g)
Washed	Pasteurized		
	C12 (F)	<LOD <sup>2</sup>	$0.9 \pm 1.6 \times 10^5$
Natural	Thermized		
	C28	$1.89 \pm 0.51 \times 10^3$	<LOD
	C29 (H)	$0.6 \pm 1.0 \times 10^1$	<LOD

<sup>1</sup>Cheese samples in which *Kazachstania servazzii* was not detected (in both the rind and core) are not presented. Cheeses with the same letter (A to H) were produced by the same cheese plant. Means and SE were calculated from means of each of 3 biological replicates (3 technical replicates were performed per biological replicate) for the rinds of each cheese variety, and from the means of values obtained from 3 technical replicates for each core (1 biological replicate was sampled per cheese variety).

<sup>2</sup>LOD = limit of detection, determined using serial dilutions, and set to 3 gene copies per PCR reaction.





**Figure 3.** Quantitative PCR enumeration (gene copies/g) of *Pichia kudriavzevii* in the cores of bloomy-rind cheeses. Values were calculated from means of each variety of cheese (all 3 biological replicates of each individual cheese were included). Different letters (a, b) indicate significant differences ( $P < 0.05$ ) among core samples. Error bars represent SE.

core increased with milk thermization compared with cheese from raw and pasteurized milk ( $P < 0.01$ ; Figure 1). Conversely, *P. kudriavzevii* was more abundant in bloomy-rind cheese made from raw milk ( $P < 0.05$ ; Figure 3). However, caution should be exercised in the interpretation of these results because initial concentrations of yeasts in the milk were not established for the cheeses studied. Changes in indigenous yeast counts from the raw milk to the ripened cheese surface and core should be further studied to provide insight into the sources and effects of these microorganisms.

## CONCLUSIONS

Using species-specific primers and probes, we developed a highly specific and sensitive real-time quantitative PCR method to analyze the presence and distribution of *Cyberlindnera jadinii*, *Pichia kudriavzevii*, and *Kazachstania servazzii* in specialty cheeses. Results confirmed that these 3 indigenous yeasts are present in different commercial cheeses produced in the province of Québec and revealed that *C. jadinii* and *P. kudriavzevii* are found in especially large quantities in the cheese core. This study highlights the importance of research not only on the microbial dynamics of the cheese core

but also on the effects of these indigenous yeast species on the physicochemical properties of specialty cheeses.

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## REFERENCES

- Al-Samarrai, T. H., and J. Schmid. 2000. A simple method for extraction of fungal genomic DNA. *Lett. Appl. Microbiol.* 30:53–56. <https://doi.org/10.1046/j.1472-765x.2000.00664.x>.
- Beresford, T., and A. Williams. 2004. The microbiology of cheese ripening. Pages 287–318 in *Cheese: Chemistry, Physics and Microbiology*. 3rd ed. Vol. 1. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. Elsevier.
- Bokulich, N. A., and D. A. Mills. 2013. Facility-specific “house” microbiome drives microbial landscapes of artisan cheesemaking plants.

- Appl. Environ. Microbiol. 79:5214–5223. <https://doi.org/10.1128/AEM.00934-13>.
- Borelli, B. M., E. G. Ferreira, I. C. A. Lacerda, G. R. Franco, and C. A. Rosa. 2006. Yeast populations associated with the artisanal cheese produced in the region of Serra da Canastra, Brazil. *World J. Microbiol. Biotechnol.* 22:1115–1119. <https://doi.org/10.1007/s11274-006-9151-3>.
- Büchl, N. R., and H. Seiler. 2011. Yeasts and molds: Yeasts in milk and dairy products. Pages 744–753 in *Encyclopedia of Dairy Sciences*. 2nd ed. J. W. Fuquay, P. F. Fox, and P. L. H. McSweeney, ed. Elsevier.
- Chamba, J.-F., and F. Irlinger. 2004. Secondary and adjunct cultures. Pages 191–206 in *Cheese: Chemistry, Physics and Microbiology*. 3rd ed. Vol. 1. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. Elsevier.
- Chen, L., J. Cui, Q. Ding, Y. Ma, L. Chen, J. Dong, T. Jiang, and J.-L. Maubois. 2012. The effect of yeast species from raw milk in China on proteolysis and aroma compound formation in Camembert-type cheese. *Food Bioprocess Technol.* 5:2548–2556. <https://doi.org/10.1007/s11947-011-0589-4>.
- Chombo-Morales, P., M. Kirchmayr, A. Gschaedler, E. Lugo-Cervantes, and S. Villanueva-Rodríguez. 2016. Effects of controlling ripening conditions on the dynamics of the native microbial population of Mexican artisanal Cotija cheese assessed by PCR-DGGE. *Lebensm. Wiss. Technol.* 65:1153–1161. <https://doi.org/10.1016/j.lwt.2015.09.044>.
- Cogan, T. M., M. Hohenegger, M. Guéguen, R. Beduhn, E. Jamet, S. Goerges, M. Goodfellow, J. Mounier, S. Larpin, H. Sebastiani, J. Swings, N. Bora, M. Vancanneyt, J.-F. Chamba, M. C. Rea, S. Scherer, N. Desmasures, A. C. Ward, R. Gelsomino, and F. Irlinger. 2014. Biodiversity of the surface microbial consortia from Limburger, Reblochon, Livarot, Tilsit, and Gubbeen cheeses. Pages 219–250 in *Cheese and Microbes*. C. W. Donnelly, ed. ASM Press.
- Del Mónaco, S. M., M. E. Rodríguez, and C. A. Lopes. 2016. *Pichia kudriavzevii* as a representative yeast of North Patagonian wine-making terroir. *Int. J. Food Microbiol.* 230:31–39. <https://doi.org/10.1016/j.ijfoodmicro.2016.04.017>.
- Delcenserie, V., B. Taminiau, L. Delhalle, C. Nezer, P. Doyen, S. Crevecoeur, D. Roussey, N. Korsak, and G. Daube. 2014. Microbiota characterization of a Belgian protected designation of origin cheese, Herve cheese, using metagenomic analysis. *J. Dairy Sci.* 97:6046–6056. <https://doi.org/10.3168/jds.2014-8225>.
- Dugat-Bony, E., L. Garnier, J. Denonfoux, S. Ferreira, A.-S. Sarthou, P. Bonnarme, and F. Irlinger. 2016. Highlighting the microbial diversity of 12 French cheese varieties. *Int. J. Food Microbiol.* 238:265–273. <https://doi.org/10.1016/j.ijfoodmicro.2016.09.026>.
- Fleet, G. H. 2011. Yeast spoilage of foods and beverages. Pages 53–63 in *The Yeasts—A Taxonomic Study*. 5th ed. Vol. 1. C. P. Kurtzman, J. W. Fell, and T. Boekhout, ed. Elsevier.
- Fox, P. F., and P. L. H. McSweeney. 2004. Cheese: An overview. Pages 1–18 in *Cheese: Chemistry, Physics and Microbiology*. 3rd ed. Vol. 1. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. Elsevier.
- Gonçalves Dos Santos, M. T. P., M. J. Benito, M. G. Córdoba, N. Alvarenga, and S. Ruiz-Moyano Seco de Herrera. 2017. Yeast community in traditional Portuguese Serpa cheese by culture-dependent and -independent DNA approaches. *Int. J. Food Microbiol.* 262:63–70. <https://doi.org/10.1016/j.ijfoodmicro.2017.09.013>.
- Irlinger, F., S. Layec, S. Hélinck, and E. Dugat-Bony. 2015. Cheese rind microbial communities: Diversity, composition and origin. *FEMS Microbiol. Lett.* 362:1–11. <https://doi.org/10.1093/femsle/fnu015>.
- Koné, M. K., S. T. Guéhi, N. Durand, L. Ban-Koffi, L. Berthiot, A. F. Tachon, K. Brou, R. Boulanger, and D. Montet. 2016. Contribution of predominant yeasts to the occurrence of aroma compounds during cocoa bean fermentation. *Food Res. Int.* 89:910–917. <https://doi.org/10.1016/j.foodres.2016.04.010>.
- Kurtzman, C. P. 2011a. *Lindnera* Kurtzman, Robnett & Basehoar-Powers (2008). Pages 521–543 in *The Yeasts—A Taxonomic Study*. 5th ed. Vol. 1. C. P. Kurtzman, J. W. Fell and T. Boekhout, ed. Elsevier.
- Kurtzman, C. P. 2011b. *Pichia* E.C. Hansen (1904). Pages 685–707 in *The Yeasts—A Taxonomic Study*. 5th ed. Vol. 1. C. P. Kurtzman, J. W. Fell and T. Boekhout, ed. Elsevier.
- Larpin-Laborde, S., M. Imran, C. Bonaïti, N. Bora, R. Gelsomino, S. Goerges, F. Irlinger, M. Goodfellow, A. C. Ward, M. Vancanneyt, J. Swings, S. Scherer, M. Guéguen, and N. Desmasures. 2011. Surface microbial consortia from Livarot, a French smear-ripened cheese. *Can. J. Microbiol.* 57:651–660. <https://doi.org/10.1139/w11-050>.
- Lavoie, K., M. Touchette, D. St-Gelais, and S. Labrie. 2012. Characterization of the fungal microflora in raw milk and specialty cheeses of the province of Quebec. *Dairy Sci. Technol.* 92:455–468. <https://doi.org/10.1007/s13594-011-0051-4>.
- Lessard, M.-H., G. Bélanger, D. St-Gelais, and S. Labrie. 2012. The composition of Camembert cheese-ripening cultures modulates both mycelial growth and appearance. *Appl. Environ. Microbiol.* 78:1813–1819. <https://doi.org/10.1128/AEM.06645-11>.
- Lessard, M.-H., C. Viel, B. Boyle, D. St-Gelais, and S. Labrie. 2014. Metatranscriptome analysis of fungal strains *Penicillium camemberti* and *Geotrichum candidum* reveal cheese matrix breakdown and potential development of sensory properties of ripened Camembert-type cheese. *BMC Genomics* 15:235. <https://doi.org/10.1186/1471-2164-15-235>.
- Lopandic, K., S. Zelger, L. K. Bánszky, F. Eliskases-Lechner, and H. Prillinger. 2006. Identification of yeasts associated with milk products using traditional and molecular techniques. *Food Microbiol.* 23:341–350. <https://doi.org/10.1016/j.fm.2005.05.001>.
- Lyumugabe, F., J. P. Uvisenga, E. B. Songa, and P. Thonart. 2014. Production of traditional sorghum beer “Ikigage” using *Saccharomyces cerevisiae*, *Lactobacillus fermentum* and *Issateckenkia orientalis* as starter cultures. *Food Nutr. Sci.* 5:507–515. <https://doi.org/10.4236/fns.2014.56060>.
- Mei, J., Q. Guo, Y. Wu, and Y. Li. 2014. Microbial diversity of a Camembert-type cheese using freeze-dried Tibetan kefir coculture as starter culture by culture-freeze and culture-independent methods. *PLoS One* 9:e111648. <https://doi.org/10.1371/journal.pone.0111648>.
- Pangallo, D., N. Šaková, J. Koreňová, A. Puškárová, L. Kraková, L. Valík, and T. Kuchta. 2014. Microbial diversity and dynamics during the production of May bryndza cheese. *Int. J. Food Microbiol.* 170:38–43. <https://doi.org/10.1016/j.ijfoodmicro.2013.10.015>.
- Prillinger, H., O. Molnar, F. Eliskases-Lechner, and K. Lopandic. 1999. Phenotypic and genotypic identification of yeasts from cheese. *Antonie van Leeuwenhoek* 75:267–283. <https://doi.org/10.1023/A:1001889917533>.
- Romero-Gil, V., L. Rejano-Zapata, A. Garrido-Fernández, and F. N. Arroyo-López. 2016. Effect of zinc formulations, sodium chloride, and hydroxytyrosol on the growth/no-growth boundaries of table olive related yeasts. *Food Microbiol.* 57:71–80. <https://doi.org/10.1016/j.fm.2016.01.007>.
- Schmidt, J. L., J. Lenoir, and M. Schmidt. 1980. Contribution à l'étude de la flore levure du fromage de Camembert (II). *Lait* 60:272–282. <https://doi.org/10.1051/lait:1980595-59616>.
- Silva, C. F., R. F. Schwan, E. Sousa Dias, and A. E. Wheals. 2000. Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. *Int. J. Food Microbiol.* 60:251–260. [https://doi.org/10.1016/S0168-1605\(00\)00315-9](https://doi.org/10.1016/S0168-1605(00)00315-9).
- Succi, M., A. Reale, C. Andrighetto, A. Lombardi, E. Sorrentino, and R. Coppola. 2003. Presence of yeasts in southern Italian sourdoughs from *Triticum aestivum* flour. *FEMS Microbiol. Lett.* 225:143–148. [https://doi.org/10.1016/S0378-1097\(03\)00500-7](https://doi.org/10.1016/S0378-1097(03)00500-7).
- Tominaga, T. 2004. Rapid identification of pickle yeasts by fluorescent PCR and microtemperature-gradient gel electrophoresis. *FEMS Microbiol. Lett.* 238:43–48. <https://doi.org/10.1111/j.1574-6968.2004.tb09735.x>.
- Tornai-Lehoczi, J. 2003. CHROMagar *Candida* medium as a practical tool for the differentiation and presumptive identification of yeast

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- species isolated from salads. *Int. J. Food Microbiol.* 86:189–200. [https://doi.org/10.1016/S0168-1605\(03\)00256-3](https://doi.org/10.1016/S0168-1605(03)00256-3).
- Valdés-Stauber, N., S. Scherer, and H. Seiler. 1997. Identification of yeasts and coryneform bacteria from the surface microflora of brick cheeses. *Int. J. Food Microbiol.* 34:115–129. [https://doi.org/10.1016/S0168-1605\(96\)01171-3](https://doi.org/10.1016/S0168-1605(96)01171-3).
- Vaughan-Martini, A., M.-A. Lachance, and C. P. Kurtzman. 2011. The Yeasts: *Kazachstania* Zubkova (1971). Pages 439–470 in *The Yeasts—A Taxonomic Study*, 5th ed. Vol. 2. C. P. Kurtzman, J. W. Fell and T. Boekhout, ed. Elsevier.
- Wolfe, B. E., J. E. Button, M. Santarelli, and R. J. Dutton. 2014. Cheese rind communities provide tractable systems for *In situ* and *In vitro* studies of microbial diversity. *Cell* 158:422–433. <https://doi.org/10.1016/j.cell.2014.05.041>.
- Wyder, M.-T. M.-T., and Z. Puhán. 1999. Role of selected yeasts in cheese ripening: An evaluation in aseptic cheese curd slurries. *Int. Dairy J.* 9:117–124. [https://doi.org/10.1016/S0958-6946\(99\)00032-1](https://doi.org/10.1016/S0958-6946(99)00032-1).

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