

Identification et caractérisation de microorganismes isolés de produits laitiers non conformes et/ou atypiques à la suite d'un test de maintien de qualité permettant la complétion d'une collection

LABORATOIRE
JULIE JEAN

Mariam Richter, Éric Jubinville, Valérie Goulet-Beaulieu and Julie Jean



UNIVERSITÉ
LAVAL

Faculty of agriculture and food sciences, Institute of nutrition and functional foods, Université Laval, Québec, Canada

INTRODUCTION

Quebec's dairy industry represents around 50% of Canada's¹ (transformation receipts estimated at \$2,864 billions in 2021 for Quebec)¹

Challenges of the industry resulting in **quality problems, economic loss and food waste**:

- Perishable products that deteriorate non-compliant products, atypical products²
- Microorganisms that alter dairy products or cause nonconformity: bacteria, moulds, yeasts²

Project objectives:

- Identify new microbial isolates obtained from dairy products by keeping quality tests
- Characterization of the sensitivity of these isolates towards heat, antibiotics, disinfectants and their capacity of biofilm creation
- Complete a documented collection of undesirable microorganisms responsible for atypical or non-compliant dairy products

Problems of the dairy products

- **Non-compliant:** Does not respect the specification of a product established by norms (governmental ones: ACIA in Canada, MAPAQ in Quebec)³
- **Atypical:** Differs from the established standards by physico-chemical aspects such as taste, texture, appearance or other organoleptic markers of the finished product^{2,4}

Non-compliant³:
Simple plate count (SPC)
 2.5×10^4 CFU/mL
Coliform count:
10 CFU/mL
Atypical⁴: 10^6 CFU/mL

Keeping quality tests in the dairy industry⁴:
▪ Predict the shelf life of dairy products
▪ Use slightly provocative conditions such as temperatures above 4 °C

METHODOLOGY

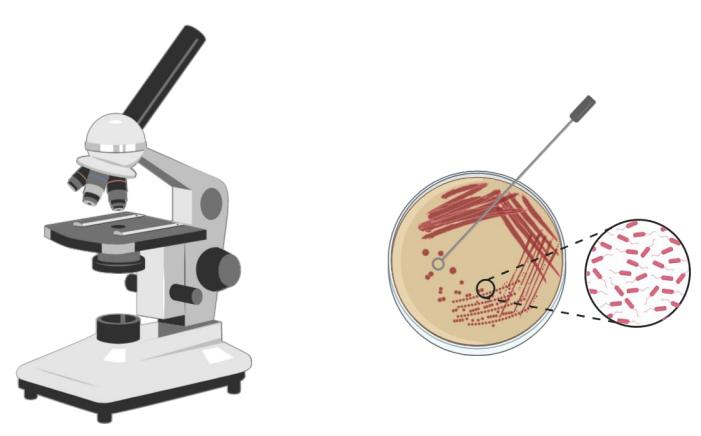
1. Isolation of microorganisms

Table 1: Keeping quality tests⁴

Tests	Preincubation time	Preincubation temperature
Moseley (modified)	7 days	7.2 °C
Virginia shelf-life	18 hours	21 °C
<i>Paenibacillus</i>	48 hours	13 °C

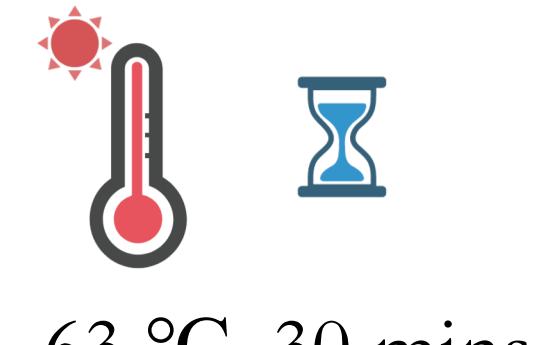
2. Identification of microorganisms

- By culture on selective media
- By MALDI-TOF
- By Sanger sequencing of 16S region



3. Characterization of microorganisms

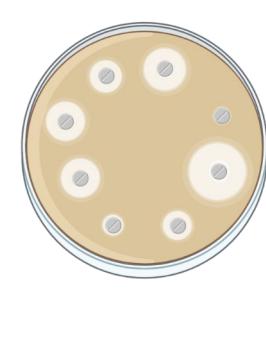
Heat sensitivity



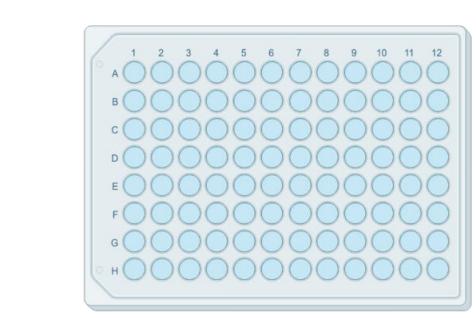
63 °C, 30 mins

Antibiotic sensitivity

- Ampicilline
- Gentamycine
- Tetracycline
- Fosfomycine
- Ceftriaxone
- Ciprofloxacin
- Negative control: antifungal fluconazole



Biofilm formation capacity



- Microplaque 96 wells
- Minimal biofilm eradication concentration (MBEC)

Disinfectant sensitivity

- Peracetic acid
- Positive control: sodium hypochlorite
- Negative control: TSB media



CONCLUSIONS

- Acquisition, identification and characterization of new microbial isolates of keeping quality tests
- Contribution of the collection: « Creation of a documented collection of microorganisms associated with non-compliant or atypical end of code dairy products »
- Remedy the lack of information concerning the undesirable microorganisms in Quebec and Canada dairy products.

RESULTS

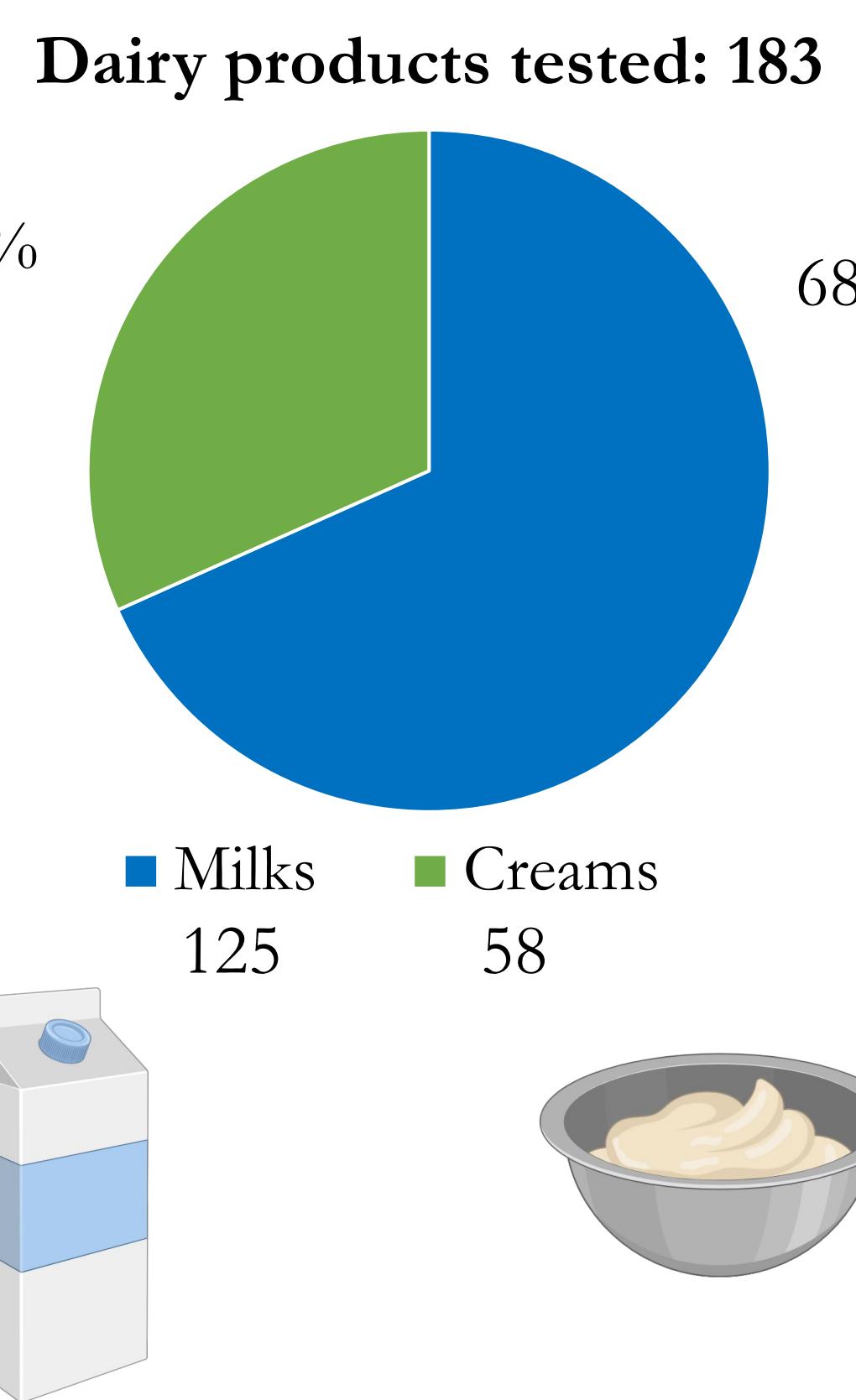


Table 2: Non-compliant or atypical dairy products

Non-compliant for coliform test	Non-compliant for simple plate count (SPC) test	Atypical
13/183 (7.1%)	14/183 (7.7%)	12/183 (6.6%)

Table 3: Non-compliant dairy products from keeping quality tests

Non-compliant for Virginia shelf-life procedure	Non-compliant for Moseley test	Presence of <i>Paenibacillus</i>
19/183 (10.4%)	26/183 (14.2%)	60/183 (32.8%)

Paenibacillus test identified most microorganisms

3 *Bacillus* identified by *Paenibacillus* test

3 *Paenibacillus* identified by *Paenibacillus* test

Further identification by Sanger sequencing

Table 4: MALDI-TOF identification for 12 dairy products

MALDI-TOF Identification	Isolated from keeping quality test		
	<i>Paenibacillus</i>	Virginia	Moseley
<i>Bacillus pumilus</i>	7	0	1
<i>Bacillus cereus</i>	1	0	0
<i>Bacillus thuringiensis</i>	1	0	0
<i>Paenibacillus amylolyticus</i>	1	0	0
<i>Paenibacillus odorifer</i>	6	1	0
<i>Paenibacillus etheri</i>	2	0	0
<i>Solibacillus silvestris</i>	1	0	0
<i>Viridibacillus neidei</i>	0	0	1
<i>Psychrobacillus psychrotolerans</i>	1	0	0
<i>Pseudoclavibacter helvolus</i>	1	0	1
<i>Pseudomonas fluorescens</i> group	0	0	1
<i>Staphylococcus haemolyticus</i>	1	0	0
<i>Micrococcus luteus</i>	1	0	0
<i>Bacilles Gram +</i> No identification	0	1	0

PERSPECTIVES

- Creation of reference points for the dairy industry to develop adapted solutions that are more specific.
- Diminish costs caused by non-compliant or atypical products.
- Reduce food waste and therefore the environmental impact.

FURTHER INFORMATIONS

REFERENCES

1. Agriculture Ministry of Quebec. (2022) *Production laitière (lait de vache)*. Government of Québec, cited 2023 18th September ; Available from: <https://www.quebec.ca/agriculture-environnement-et-ressources-naturelles/agriculture/industrie-agricole-au-quebec/productions-agricoles/production-lait-vache/>
2. Vuilleard, J.-C. (2019) Foundation of dairy technology of Quebec, *Science et technologie du lait*. 3e édition, pp. 515. Presses of Laval University,
3. Gouvernement du Canada. (2005) *Code national sur les produits laitiers* Exigences pour la production et la transformation du lait. Gouvernement du Canada, cited 2023 february 17th; Available from : https://agriculture.canada.ca/sites/default/files/legacy/resources/prod/dairy/pdf/Code_national_sur_les_produits_laitiers_Partie_II-III-juillet_2005.pdf
4. WHITE, C. H. (1993) Rapid Methods for Estimation and Prediction of Shelf-Life of Milk and Dairy Products. *J Dairy Sci* 76:3126-3132

ACKNOWLEDGEMENTS

Novalait
Catalyseur de recherche

Images by Biorender.com



LABORATOIRE
JULIE JEAN

VIRLOGIE
BIOFILMS
MICROBIOLOGIE
ALIMENTAIRE
UNIVERSITÉ
LAVAL



INAF
INSTITUT SUR LA
NUTRITION ET LES
ALIMENTS FONCTIONNELS