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Detection and typing of *Lactobacillus parabuchneri*, a microorganism with harmful and detrimental effects in cheese

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Introduction

- *Lactobacillus parabuchneri* (formerly known as *L. buchneri*) has been isolated from cheese implicated in an outbreak of histamine poisoning (Sumner et al. 1985)
- 2012: Cheddar cheese containing 1227 mg/kg of histamine was notified as food poisoning at the RASFF (Rapid Alert System for Food and Feed)
- 75 mg of histamine per meal may cause symptoms like diarrhea and headache
- Setting a limit for the histamine level in cheese is under discussion (Switzerland and EU)

Identification of contamination sources of histamine producers and the factors influencing histamine accumulation is crucial



L. parabuchneri causes high histamine content in raw milk cheeses

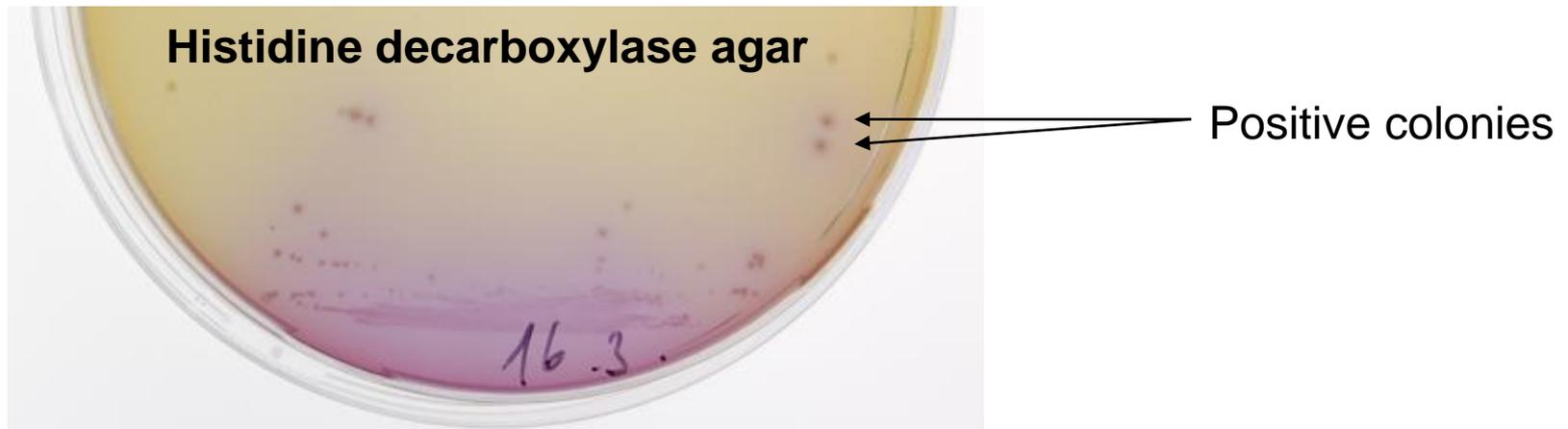
- Good correlation between *hdc* gene (Coton 2005) and the presence of *Lactobacillus buchneri/parabuchneri* (Schmidt 2008) in raw milk cheeses

	N	<i>hdc</i>	<i>L. buchneri</i> <i>L. parabuchneri</i>
Emmental	6	5	5
Gruyère	10	0	0
Tête de moine	8	8	8
Sbrinz	15	1	2
Tilsit	24	22	24
Appenzeller	6	2	4



Isolation of *L. parabuchneri*

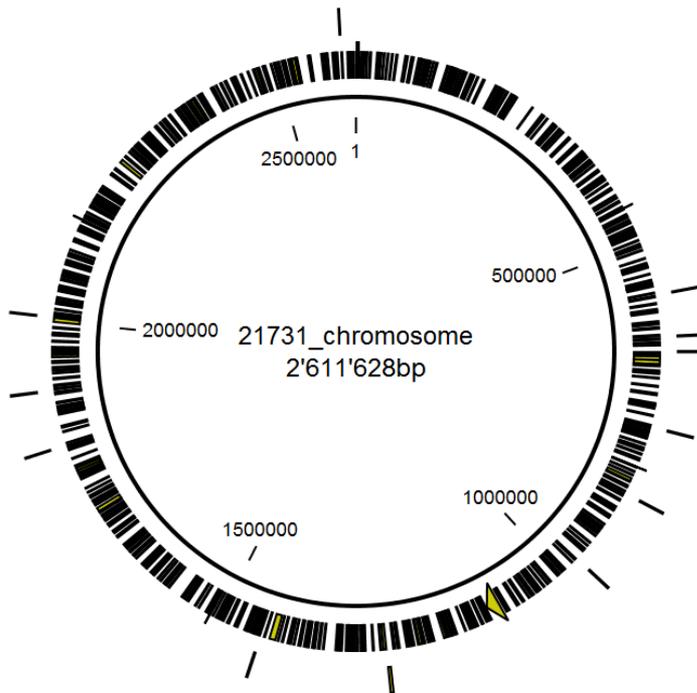
- *L. parabuchneri* could be repeatedly isolated from cheese with high histamine content (hard and semi-hard raw milk cheeses)



Development of detection and typing methods
for *Lactobacillus parabuchneri*

Sequencing of the genome

- PacBio and Illumina: FAM21731 isolated from Emmental cheese
- Ion Torrent PGM: 12 isolates from various origins

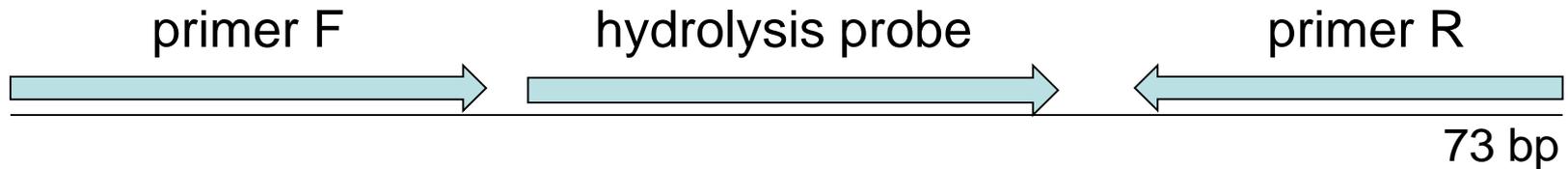


Complete chromosome (2.6 Mb)

- genome comparison revealed a unique sequence for *L. parabuchneri*
- Highly conserved
- single copy gene



Specificity of the detection system

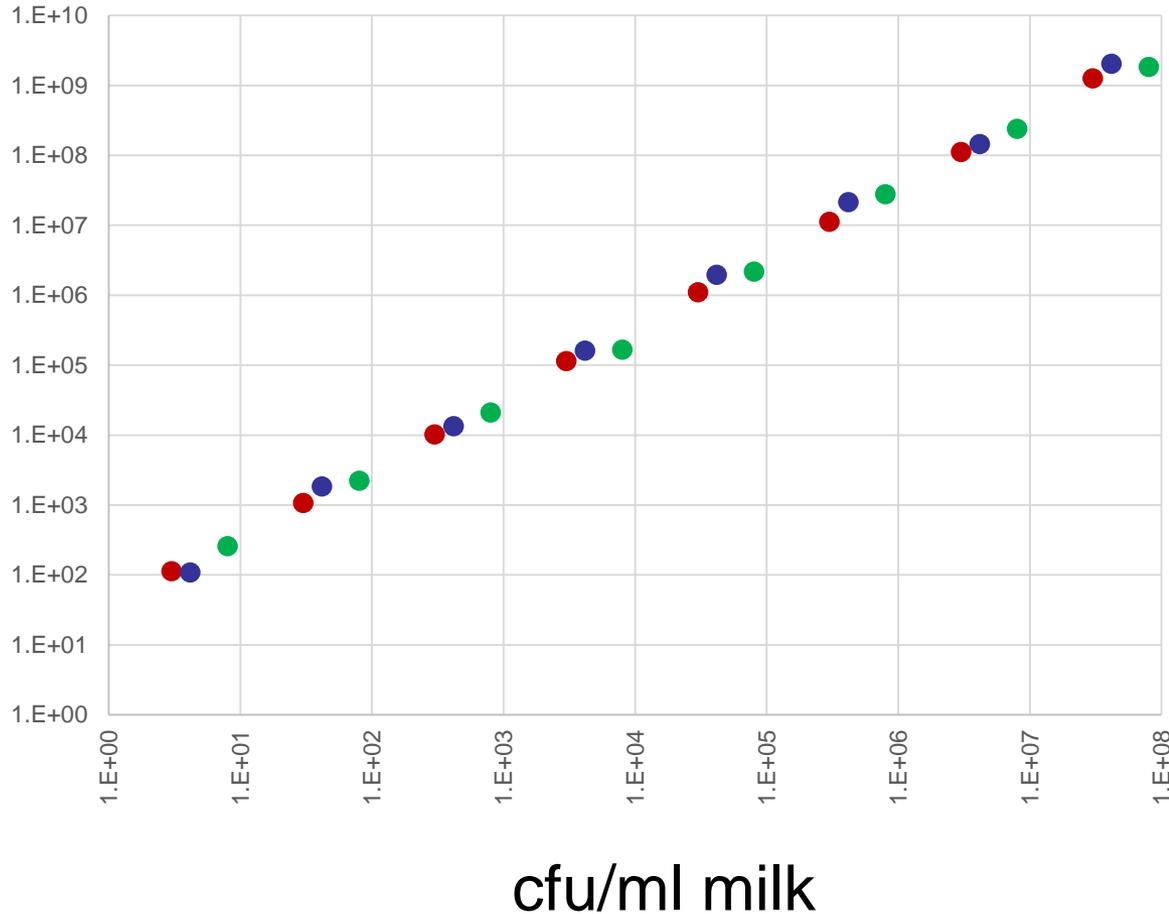


- All *Lactobacillus parabuchneri* isolates were positives (N=200)
- All other species were negatives
 - *L. buchneri* (N=8), *L. hilgardii*, *L. malefermentans*
 - *L. casei*, *L. delbrueckii* ssp. *lactis*, *L. plantarum*, *L. paraplantarum*, *L. rhamnosus*
 - *Lactococcus lactis* ssp. *cremoris*, *L. lactis* ssp. *diacetylactis*, *L. lactis* ssp. *lactis*
 - *Leuconostoc mesenteroides*, *L. lactis*
 - *Pediococcus acidilactici*, *P. pentosaceus*
 - *Streptococcus thermophilus*
 - *Enterococcus faecalis*, *E. faecium*, *E. durans*



Quantification of *L. parabuchneri* in raw milk (triplicate)

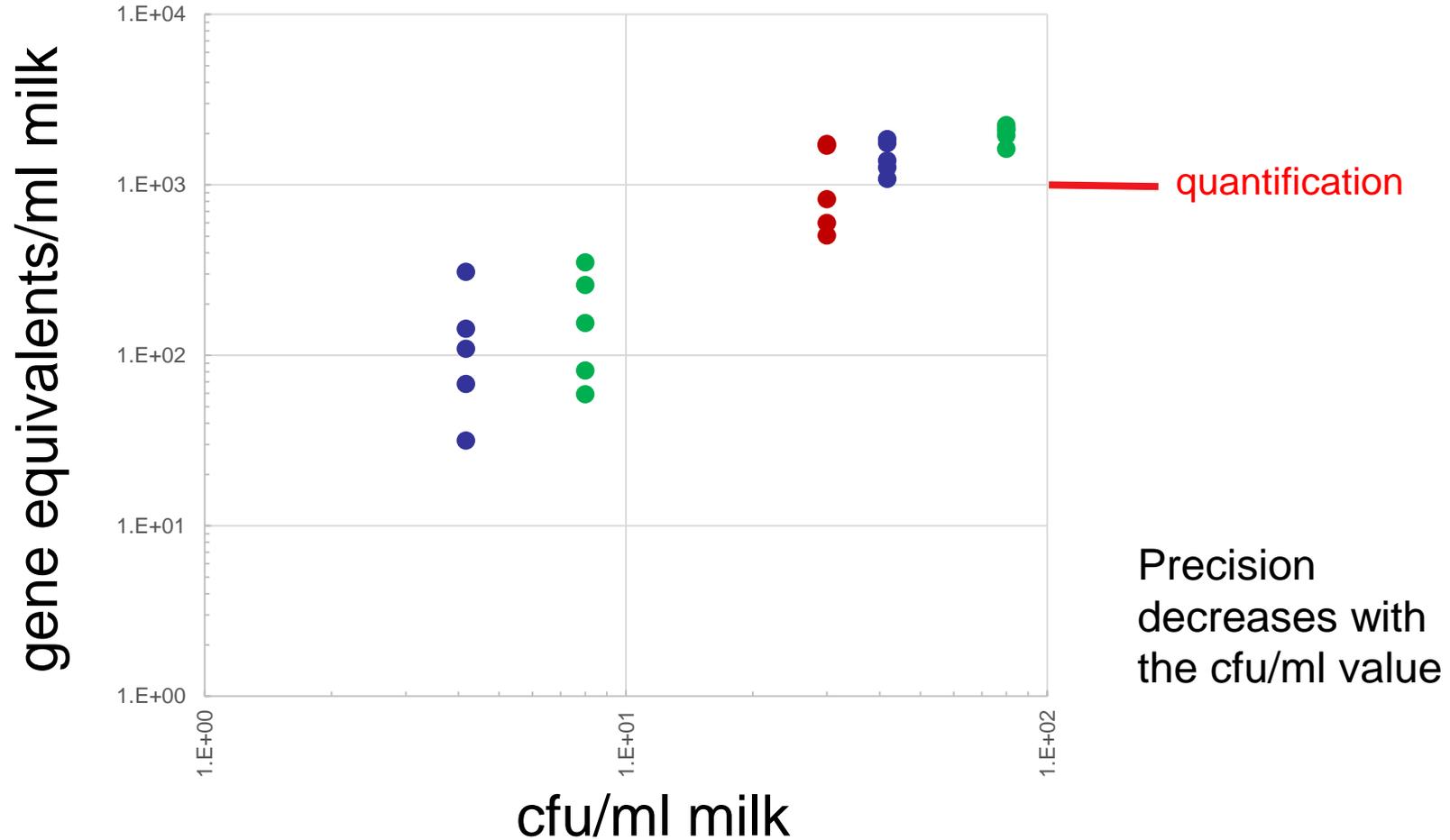
gene equivalents/ml milk



- The method is:
- quantitative
 - sensitive
 - reproducible



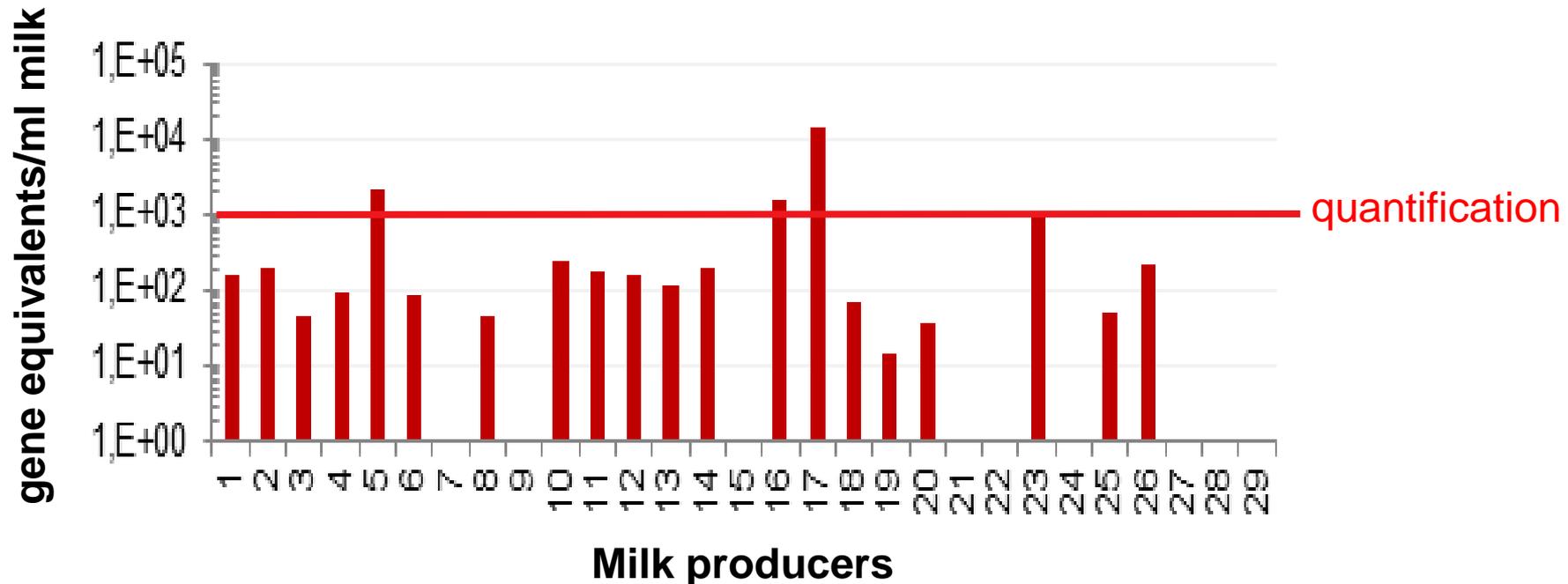
Precision at low target concentrations (five DNA extractions)





Detection of *L. parabuchneri* in raw milk

Case study: Milk from suppliers of a cheese manufacturer producing cheese with high histamine content

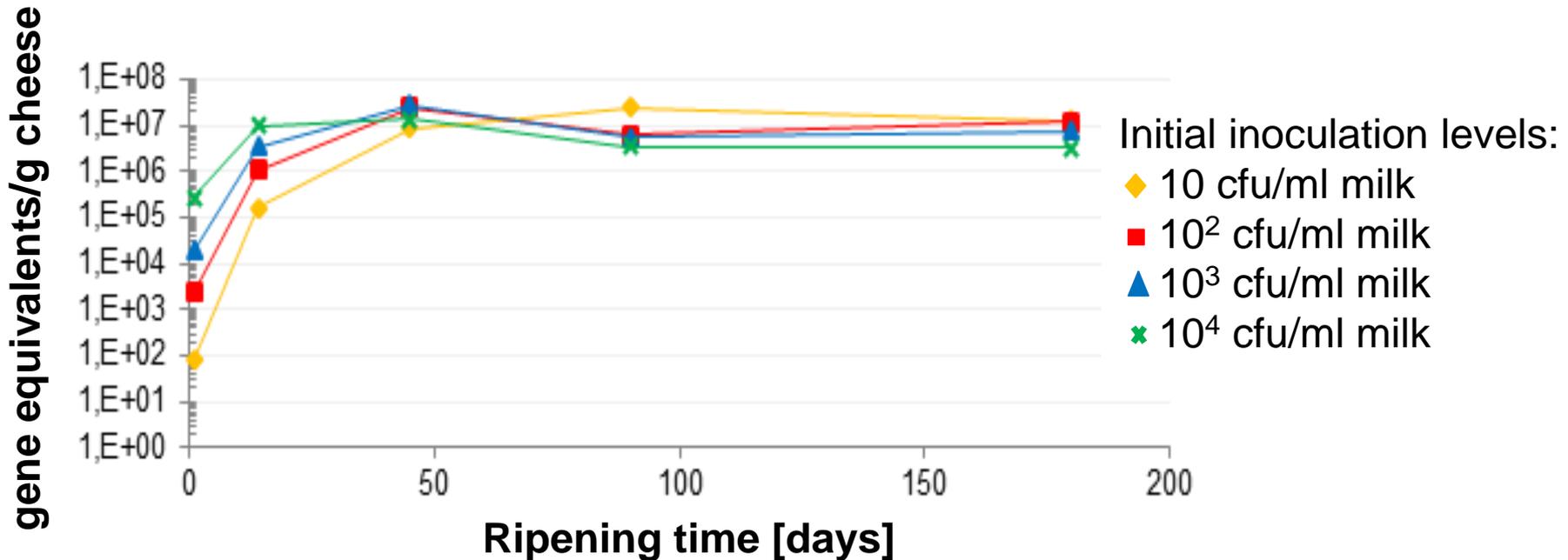


- Identification of producers with high loads of *L. parabuchneri*
- Most of the samples are at the limit of detection



Effect of the inoculation level in vat milk

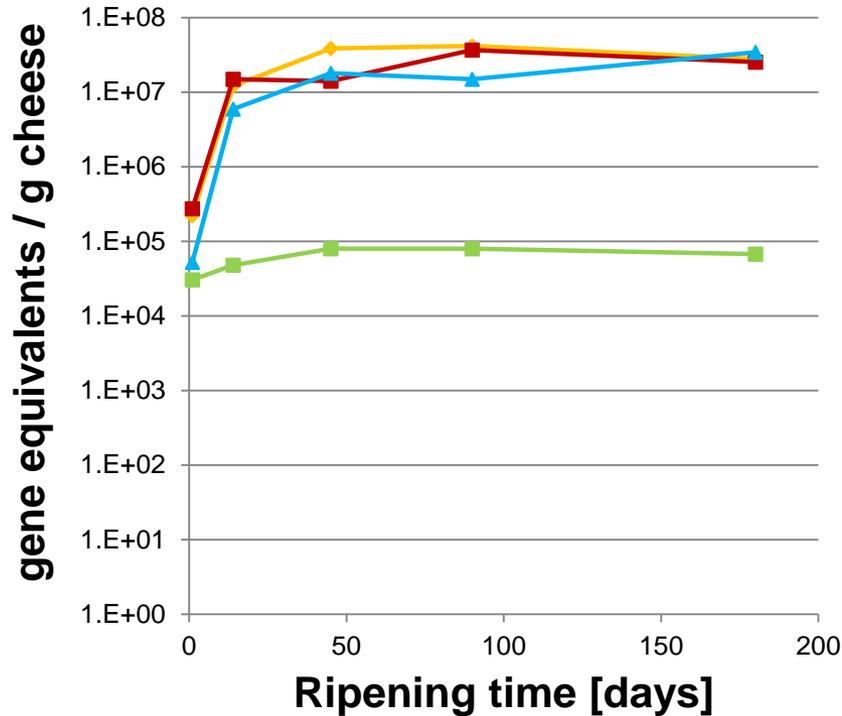
Cheese experiment: Growth of *L. parabuchneri* in experimental semi-hard cheeses



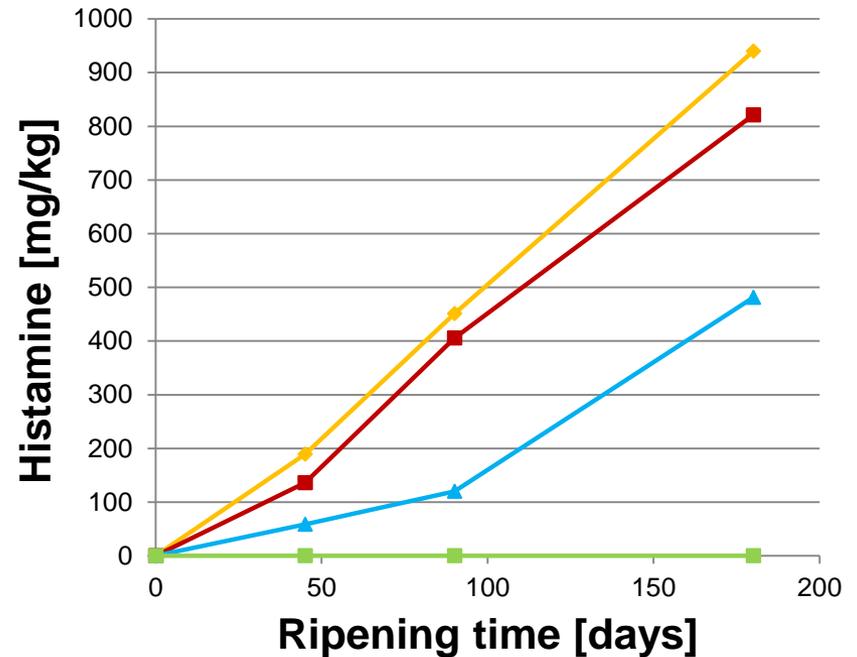
After 45 days *L. parabuchneri* reached independently of the initial contamination level in a concentration of about 10⁷ cfu/g



Influence of scalding conditions



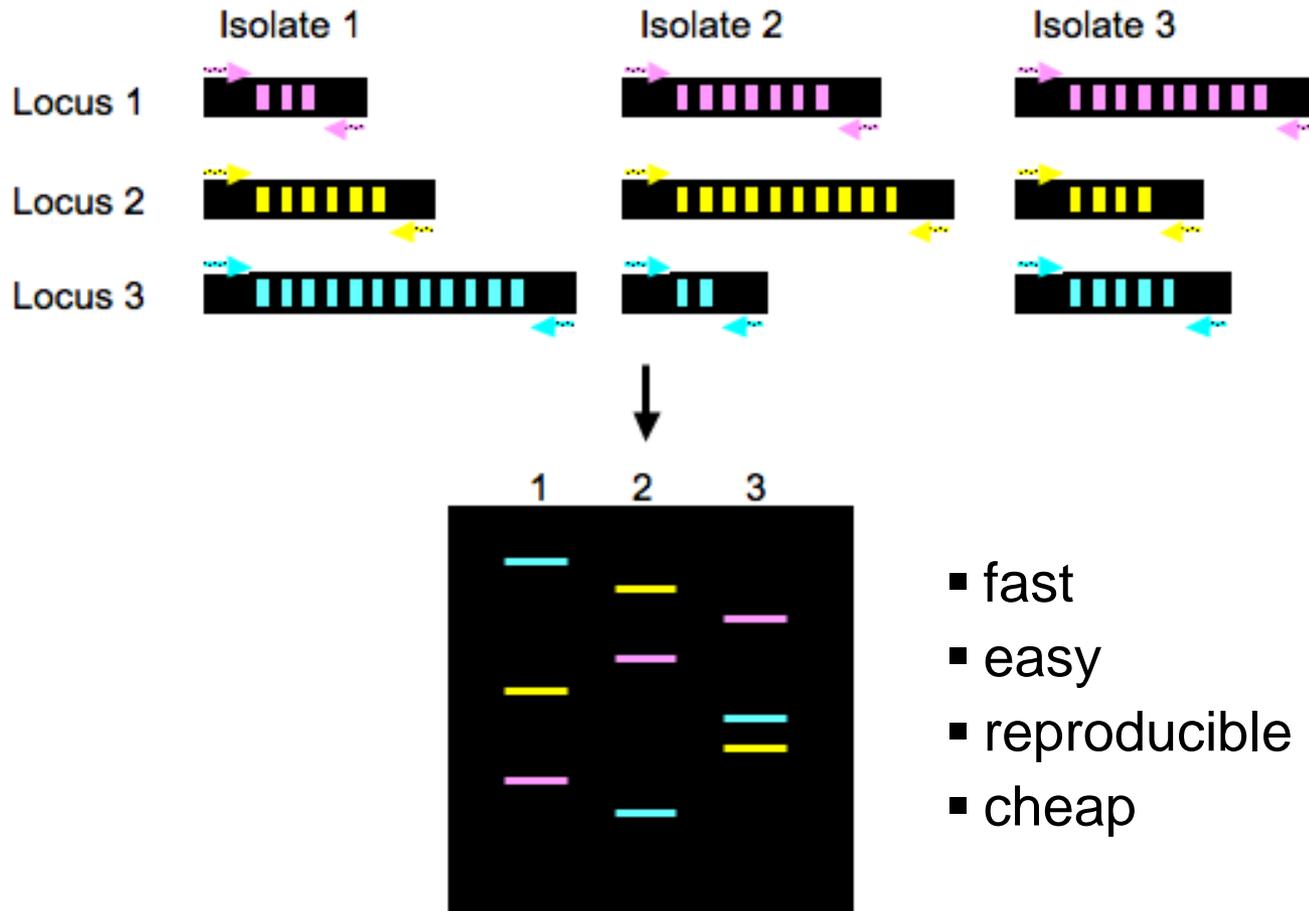
- ◆ 44°C, starter 1
- 48°C, starter 1
- ▲ 52°C, starter 2
- 56°C, starter 2



Experimental cheese cooked at 52°C showed reduced histamine content despite of similar growth of *L. parabuchneri*



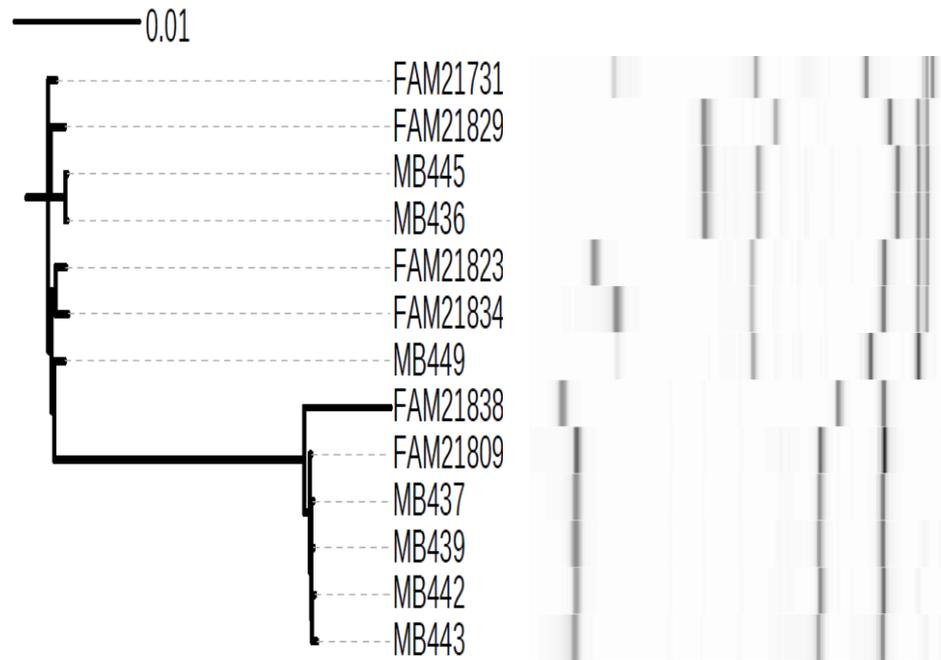
Typing by multiplex PCR: Principle



- fast
- easy
- reproducible
- cheap

Tandem repeats finder tool: G. Benson (1999)

Typing by multiplex PCR: Discrimination

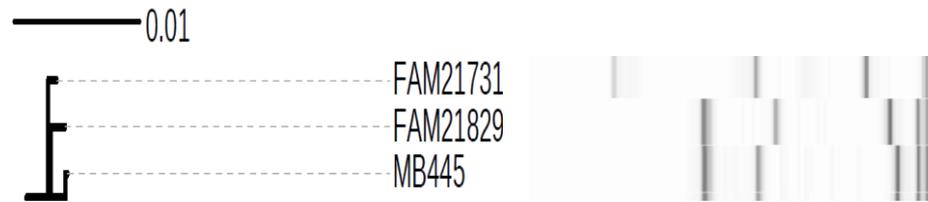


Core genome
(based on 1718 ortho-
logous gene clusters)

Multiplex PCR

- Gene sequences of the core genome of *L. parabuchneri* are highly conserved
- Our typing method has a great discrimination power

Typing by multiplex PCR: Discrimination



 **Typing and tracing of *Lactobacillus parabuchneri* in raw milk and cheese**
Patrizia Ascone, Agroscope IFS, 15:40h

Core genome
(based on 1718 orthologous gene clusters)

Multiplex PCR

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Summary

- *L. parabuchneri* is responsible for histamine production in most hard and semi-hard raw milk cheeses
- The newly developed qPCR method is quantitative, sensitive and reproducible
- Contaminations of *L. parabuchneri* in milk and cheese can be easily detected
- The newly developed typing method is fast, reproducible and discriminant



Acknowledgments

- Daniel Wechsler
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- Karl Schafroth
- Patrizia Ascone





Thank you for your attention



Agroscope good food, healthy environment