

An investigation of biogenic amine formation
in cheese, linking microbial community
composition, including the presence
of *Lactobacillus parabuchneri*, to food safety
risks and structural defects

Igor Baroja-Careaga



The Science of Artisan Cheese

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ESNEKI Dairy Centre: our main activities

- Applied research for Farmhouse and Artisan Cheese & Dairy Producers
- Development of innovative cheeses and dairy products
- Physicochemical and microbiological characterization of milk for cheesemaking suitability assessment
- Technological problem solving
- Application of metagenomics to dairy product safety



Milk made for the bottle, not for the vat

Why raw milk quality is a starting point, and not a given, for Basque cheesemakers

01

Milk produced to be drunk

Our region has a long-standing tradition of sheep's milk cheese production, while cow's milk has traditionally been used for industrial dairy processing (destined for sale as liquid drinking milk, not for cheesemaking).

•

02

Quality not optimised for cheesemaking

Consequently, the milk reaching the cheese vat does not consistently meet the physicochemical and microbiological requirements for cheesemaking, which can compromise coagulation properties, processing performance and final cheese quality.

03

Still work to be done

In this context, ensuring the suitability of milk for cheesemaking has become a key challenge for the regional dairy sector. Achieving this goal often requires substantial changes in herd management and feeding strategies in order to obtain milk for optimal cheesemaking performance.

Usual problems

- Unexpected gas-related defects



Cracks and splits
disrupting the paste

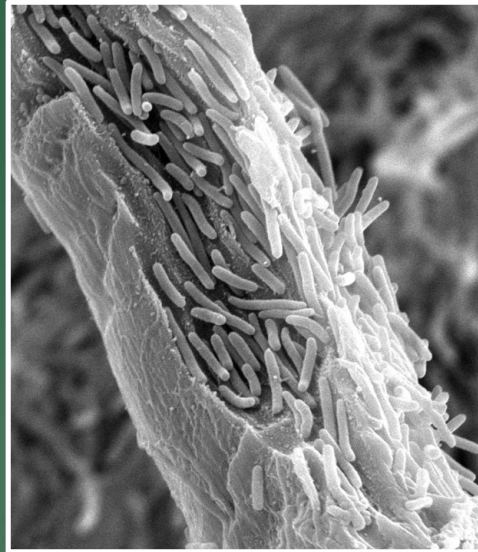


Irregular eyes

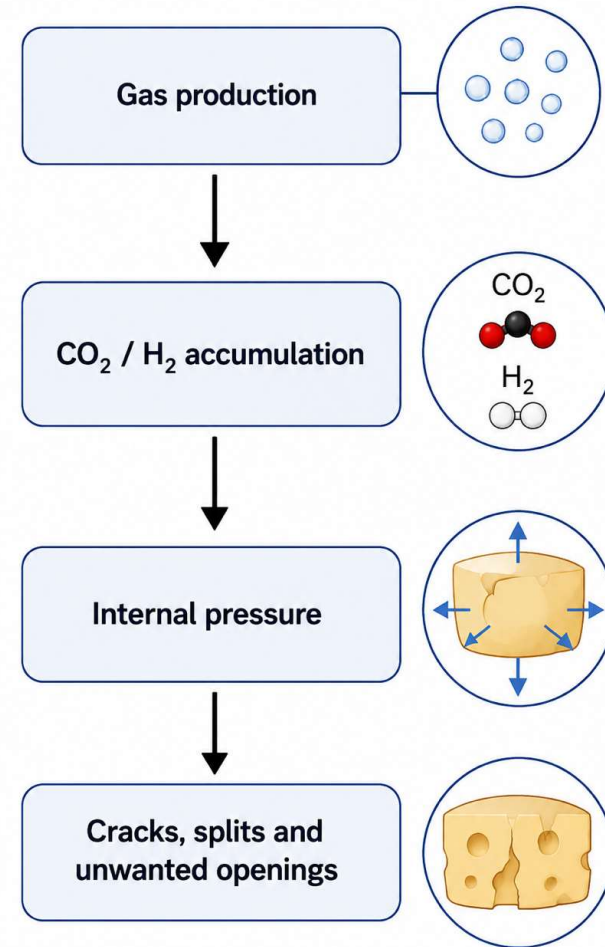


Small, scattered gas
packets

The usual suspect



Clostridium tyrobutyricum



But something didn't add up...

Not all defective cheeses
showed the same
phenotype

Gas-related defects

Spicy / burning sensory
notes

Biogenic amines
accumulation

The question:

Could another microorganism be involved?

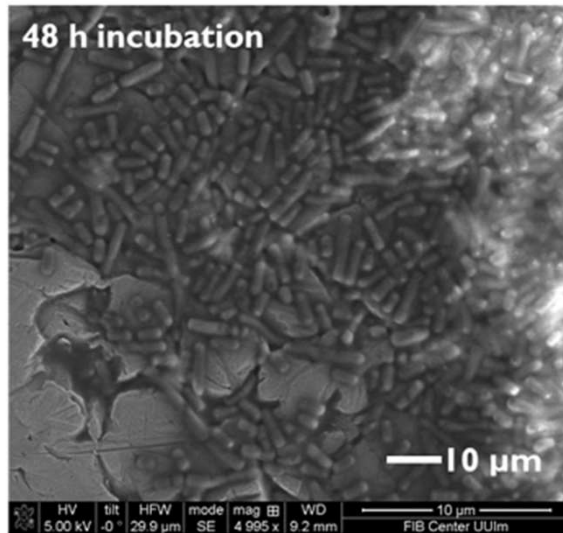
A new suspect emerged

Lentilactobacillus parabuchneri

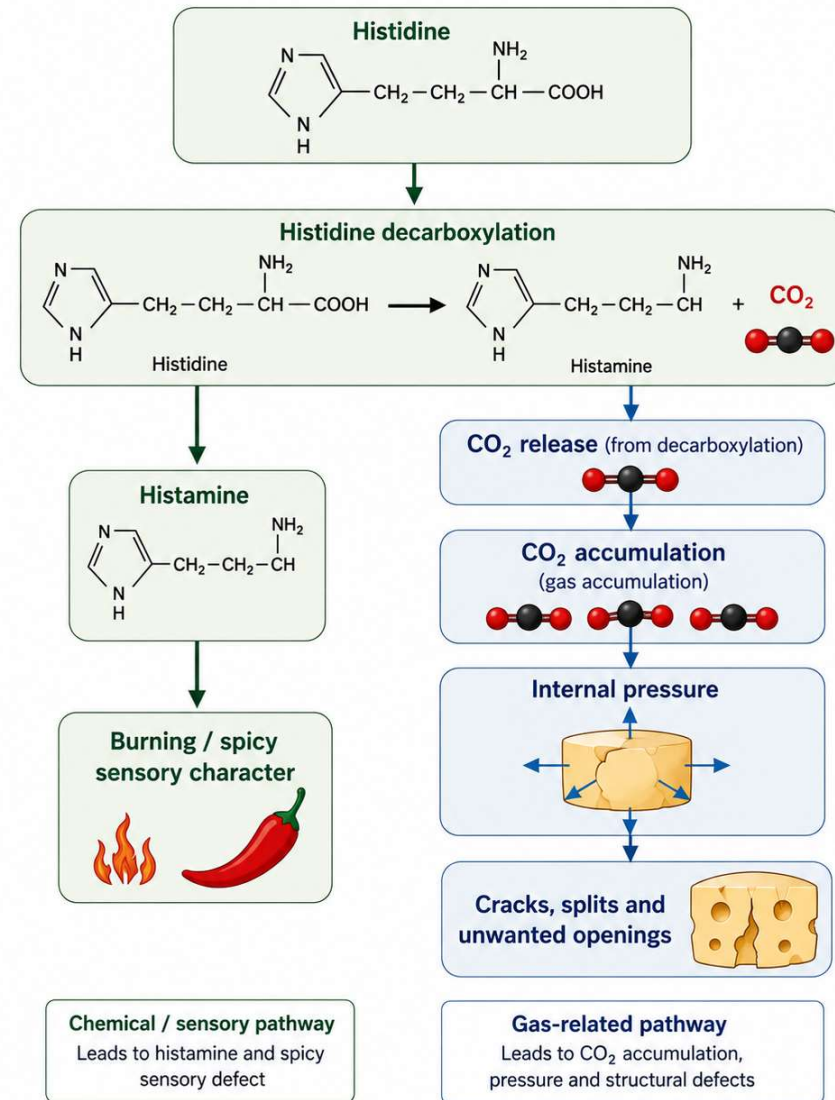
- Cheese-associated bacterium
- Heterofermentative
- Histamine-producing strains
 - Associated with gas formation



Why was *L. parabuchneri* suspicious?



Bajrami et al. 2022



Our hypothesis

Could *Lentilactobacillus parabuchneri* link histamine accumulation with gas-related cheese defects?

We predicted: $\uparrow$ *L. parabuchneri* $\rightarrow$ $\uparrow$ histamine, and potentially $\uparrow$ risk of gas-related and sensory defects

So we researched



Cheese samples: 2025 and 2026 production, selected according to phenotype (visual defects, spicy / burning notes, suspected histamine accumulation, reference cheeses)

Three analytical approaches

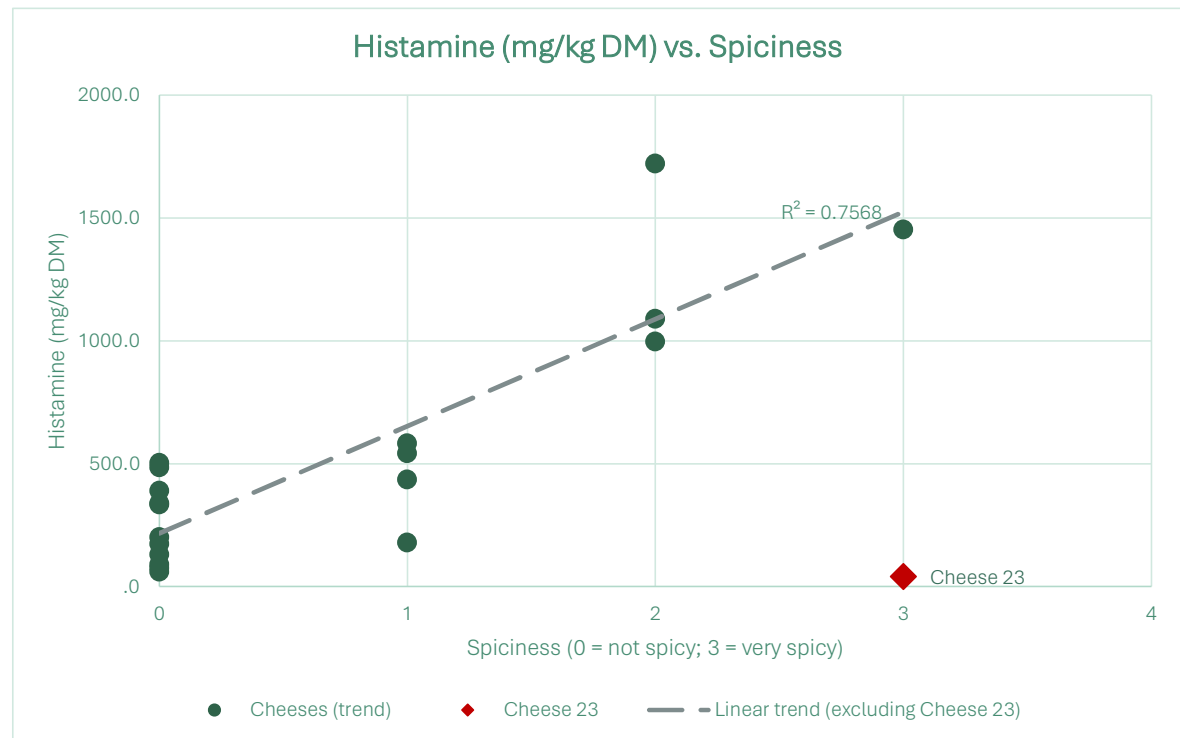
- 1. Histamine quantification — what happened?
- 2. *L. parabuchneri* qPCR — is our suspect there?
- 3. 16S rRNA sequencing — what is the microbial context?

First clue

Does the phenotype correspond to histamine accumulation?

Main finding: spicy / burning cheeses showed higher histamine concentrations.

Although the trend is clear, one cheese does not follow it: cheese 23, an Emmental, with only 40 mg of histamine but a spiciness level of 3.



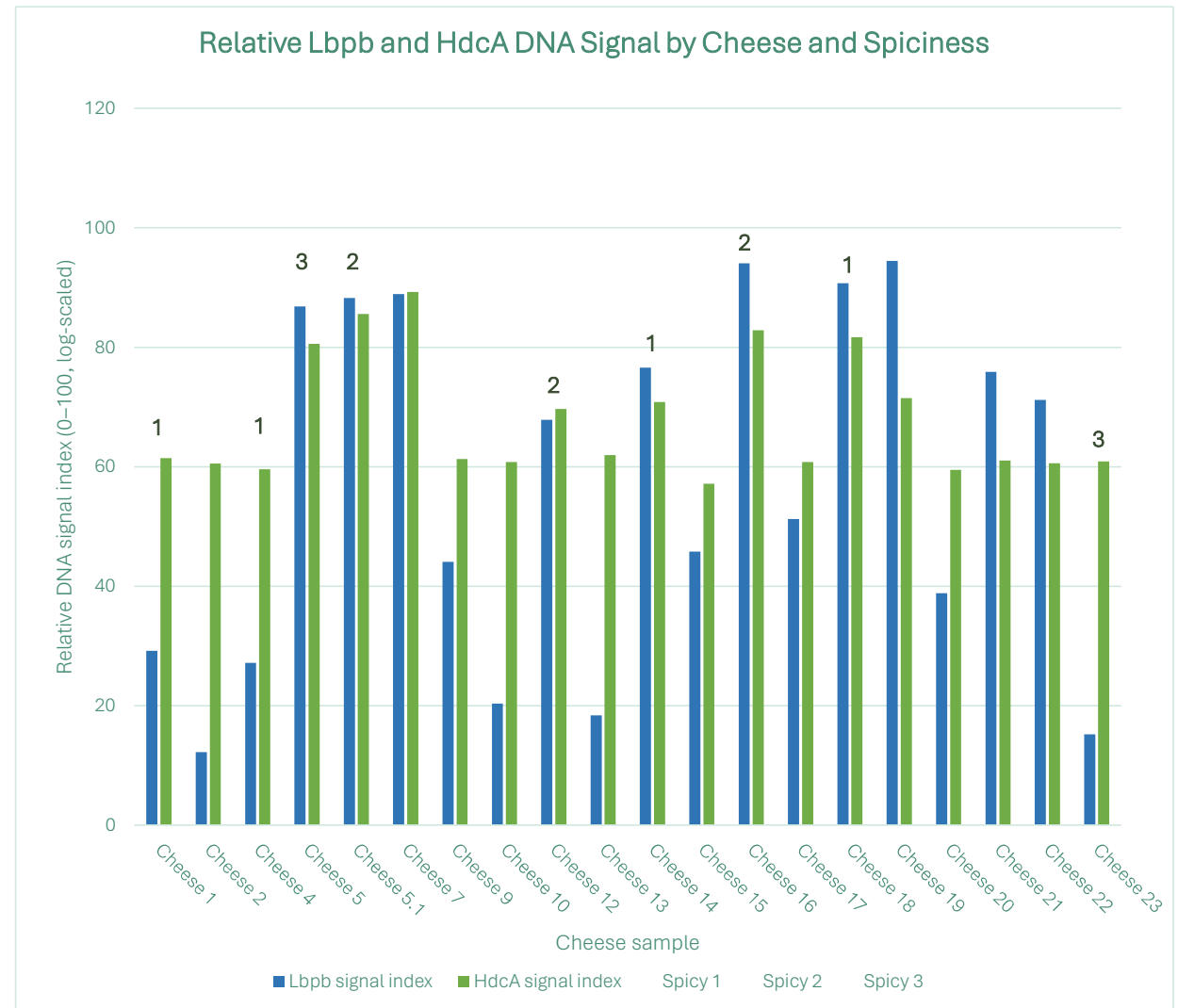
Second clue

Is our suspect actually there?

L. parabuchneri (Lbpb) and histidine decarboxylase (hdcA) genes quantified by qPCR, log₁₀ gene copies / g cheese

In several cheeses the hdcA signal (green) is much higher than the species-specific Lbpb signal (blue), pointing to other histamine-producing bacteria.

Here the spicy phenotype is not clearly related to the abundance of these genes.



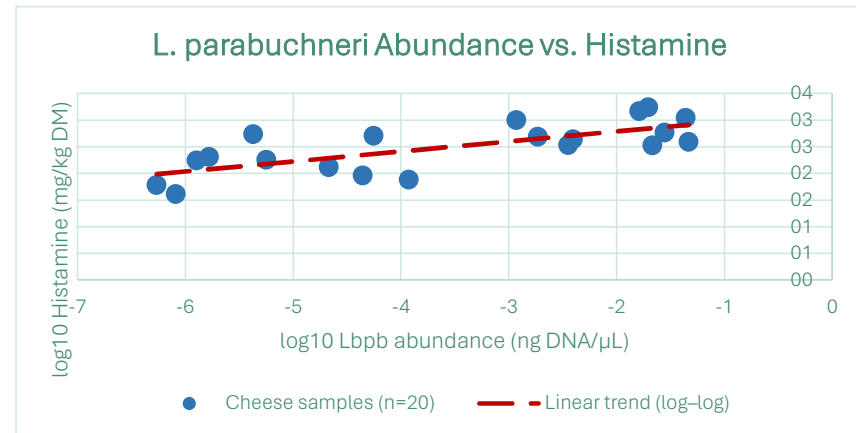
Third clue

Do *L. parabuchneri* and histamine move together?

Pearson correlation on log10-transformed values: $r = 0.725$, $p = 0.000297$, $n = 20$

Abundance and histamine move together strongly and significantly; no sample exclusion is required.

Lbpb is used here because it reflects abundance, while hdcA only indicates histamine-production potential.



	r	p
Lbpb	0,725	0,000297
HdcA	0,624	0,003292

Fourth clue

But cheese is not a single microorganism: 16S rRNA sequencing — who else is there?

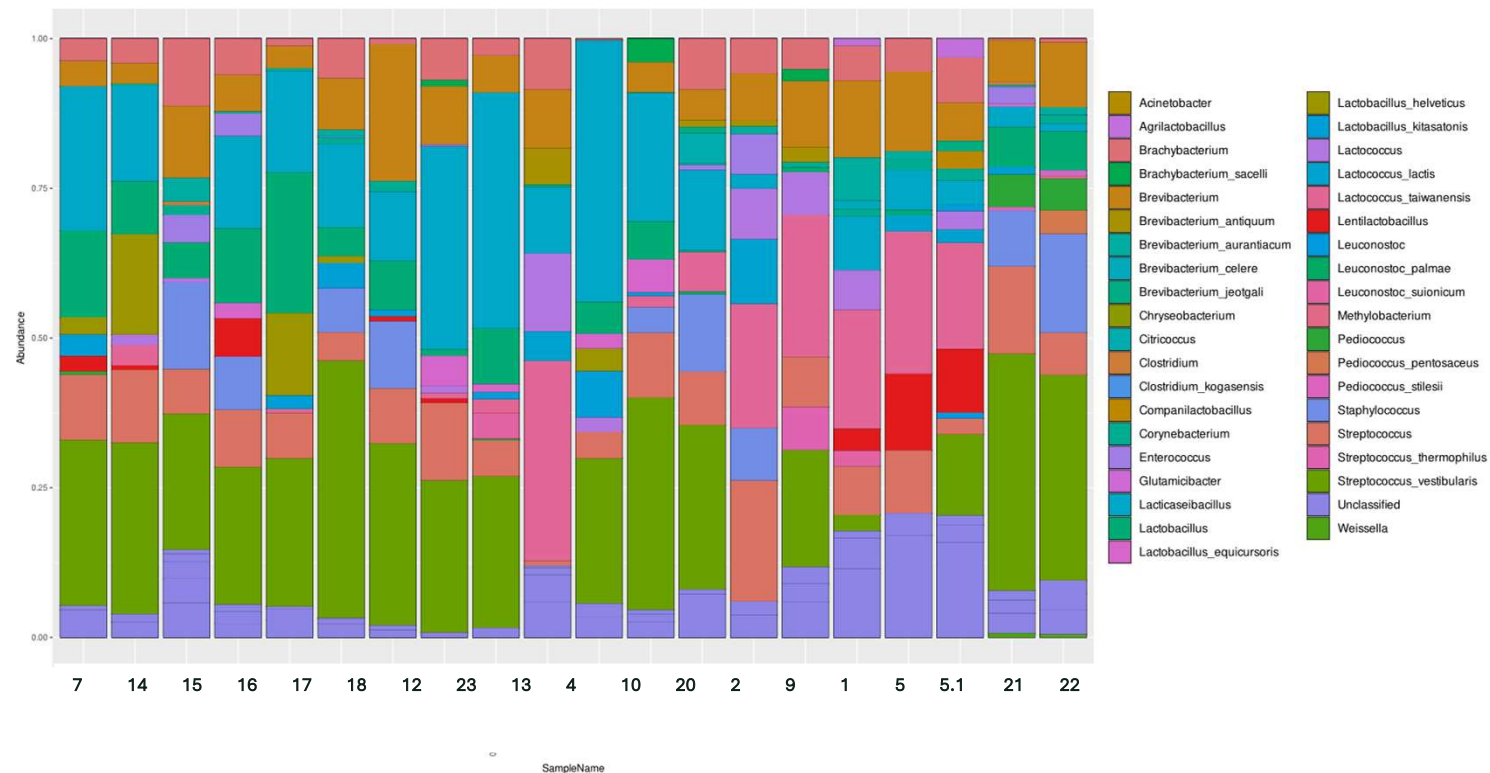
Bacteriome of all samples characterised by 16S rRNA sequencing

Taxonomy confirms *L. parabuchneri* in the samples where qPCR detected it

Starter cultures dominate each cheese type

Staphylococcus carried in with the raw milk; *Brevibacterium* shared across cheeses ripened in the same chamber

Main finding: no other taxon correlates with the spicy phenotype



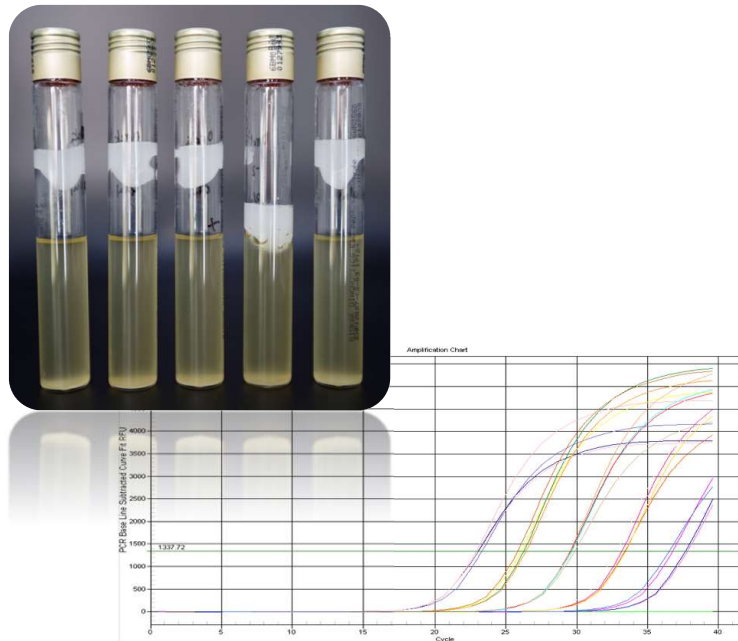
So... is *L. parabuchneri* the culprit?

The clues line up: phenotype → histamine → *L. parabuchneri* → microbial community

- The evidence points towards a role
- Present in affected cheeses
- Associated with histamine accumulation
- Metabolically capable of CO₂ production
- Previously associated with crack formation and burning taste



From suspect to prevention



The next steps

- 1. Complete the analysis of all the cheeses we have, covering different batches and production conditions
- 2. Apply the same approach to look for other bacteria of interest behind the defect
- 3. Characterise the strains: histamine-producing vs. non-producing, growth dynamics and interactions during ripening
- 4. Detection methods already developed, from simple microbiological screening to quantitative analysis of raw milk
- 5. Good results so far, in cooperation with the microbiology laboratory, towards monitoring and prevention

Conclusions

- Gas and sensory defects in artisan cheeses are not fully explained by the usual butyric suspect
- Spicy cheeses accumulate more histamine, and histamine correlates strongly with *L. parabuchneri* abundance ($p < 0.005$)
- HdcA exceeds the species signal in some cheeses: other bacteria also carry histamine-producing potential
- 16S sequencing shows no other taxon linked to the spicy phenotype
- *L. parabuchneri* is a strong suspect, but association is not yet causation
- Raw milk quality and reliable detection are the practical levers for prevention

We started with a cheese defect. We found a new suspect. Now we need to go further.

Thank you

