basque dairy: tradition inspiring innovation

FACE NETWORK 2026

esneki zentroa Leartiker

MARKINA - XEMEIN

SESSION 3

DNA in dairy

“Practical application of metagenomic techniques to adjust cheesemaking and ripening processes, as well as to ensure the safety of dairy products”



Igor Baroja Careaga

FACE Network 2026 · 19-21 October · Markina-Xemein

Molecular biology at Esneki Zentroa



LABORATORIES AND PILOT PLANT

Microbiology, physicochemistry and molecular biology working together

UNDERSTANDING CHEESE THROUGH DNA

Investigating what happens in cheese and during its production

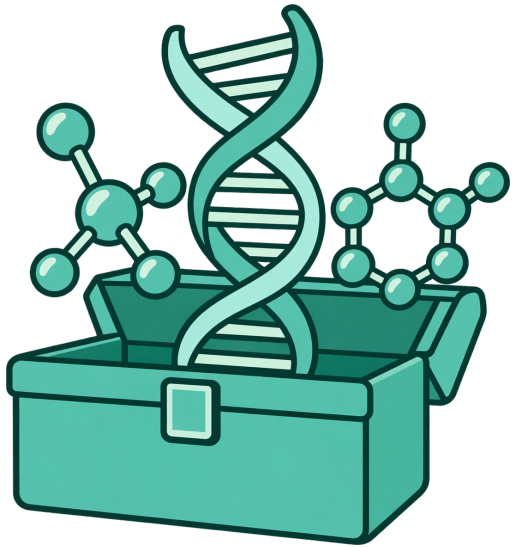
DNA EXTRACTION AND ANALYSIS

First extract the DNA, then analyse the information carried by genes

01

The molecular toolbox

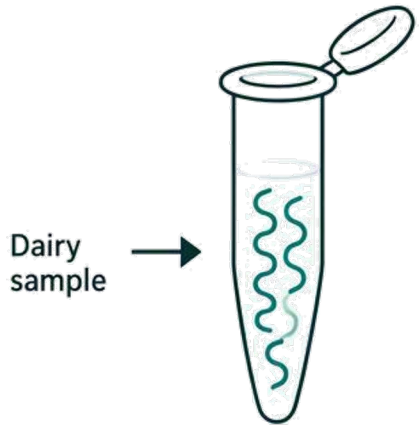
PCR/qPCR, community profiling and whole-genome sequencing



PCR detects a chosen DNA target

1 Extract DNA

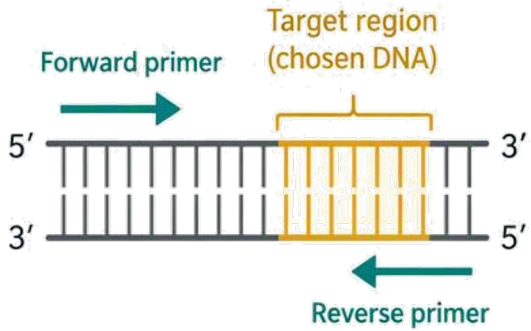
DNA is extracted from the dairy sample.



Tube contains mixed DNA (from bacteria, milk, starter cultures, etc.).

2 Select a target

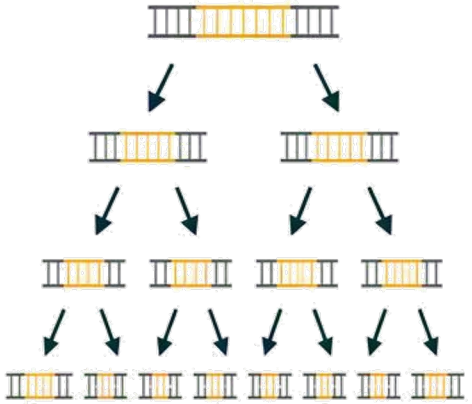
Primers bind to sequences that flank the chosen target region.



Primers (short DNA sequences) define the target to be amplified.

3 Make copies

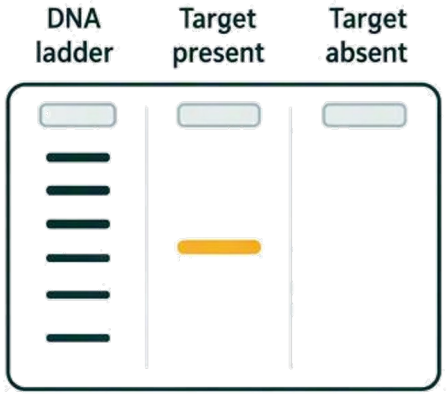
Thermal cycling makes millions of copies of only the target DNA fragment.



Only the selected target is amplified.

4 Detect the target

Amplified DNA is detected (e.g., by gel electrophoresis).

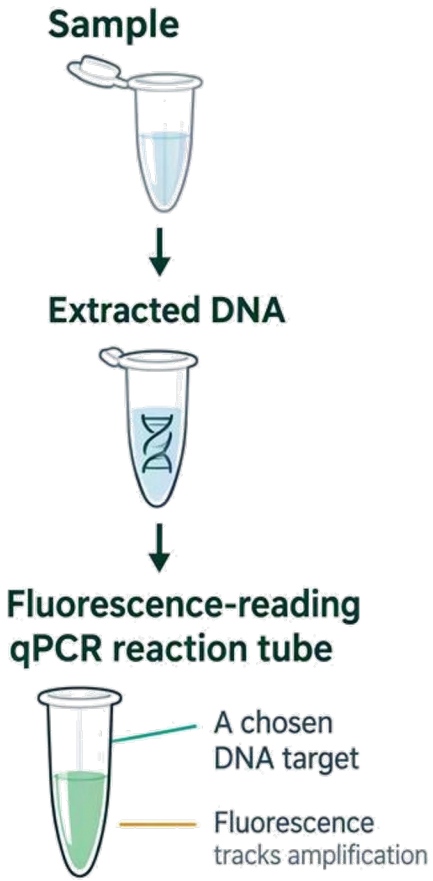


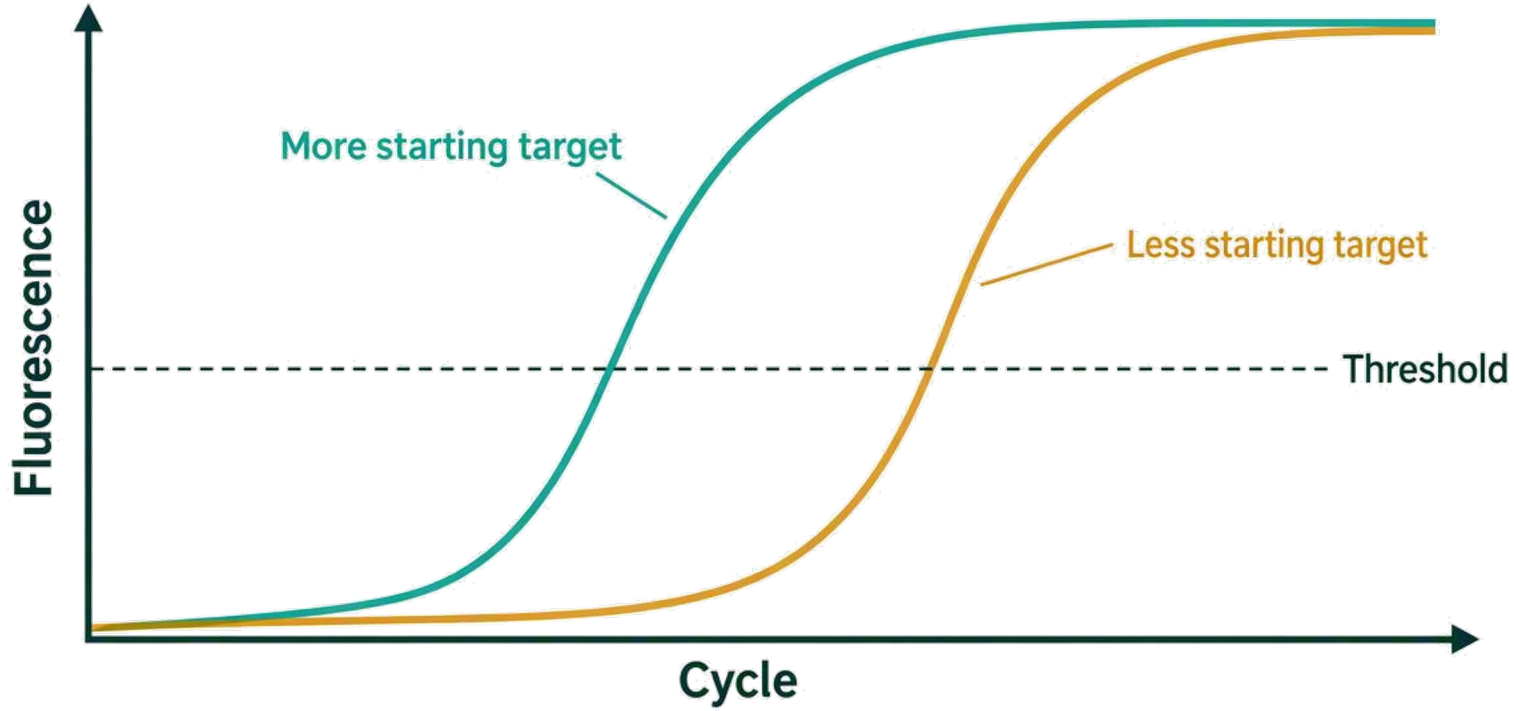
Controls are essential.
Positive control shows a band.
Negative control shows no band.

Dairy example: test for a bacterial marker or an amine-related gene.

⚠ A DNA signal does not prove that cells are alive.

qPCR follows DNA amplification in real time



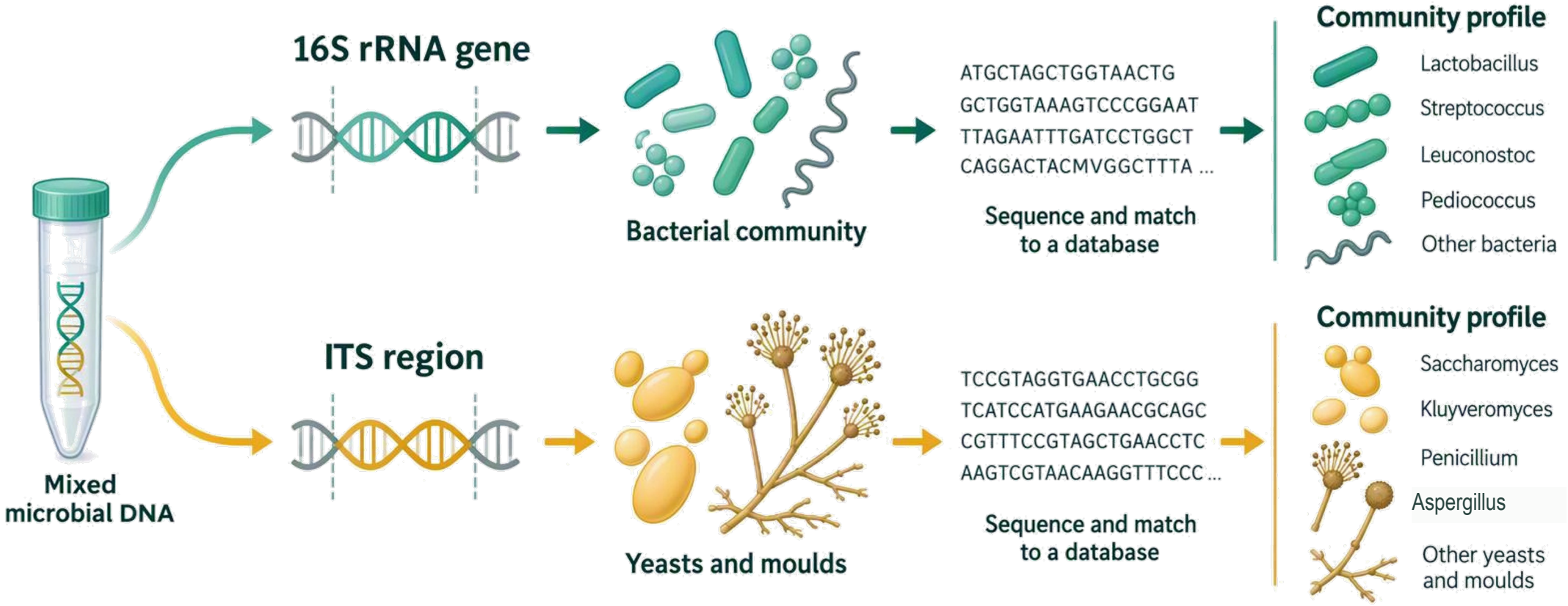


Earlier signal usually means more starting target within the same validated assay.

Calibration estimates target quantity. Controls check inhibition.

Conceptual curves, not experimental data. DNA quantity alone does not establish viability.

16S and ITS reveal different communities



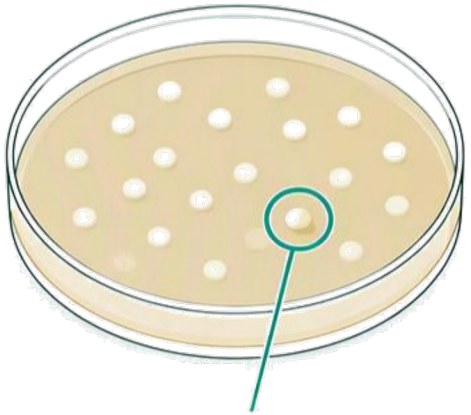
Compare milk, cheese, batches and ripening stages

Relative composition, not a direct count of living cells. Species resolution varies.

WGS examines one isolate in detail

1 Select one isolate

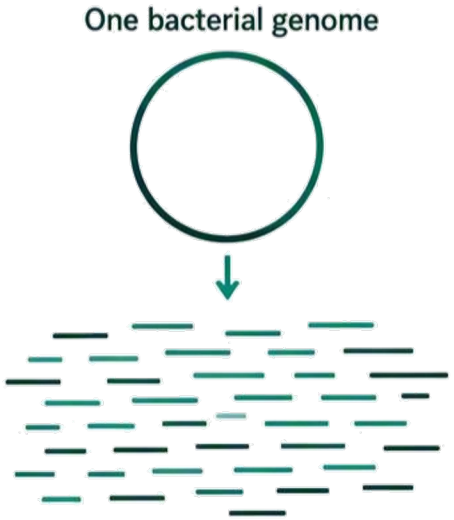
Choose a single bacterial colony from a plate.



One colony.
One isolate.

2 Sequence its DNA

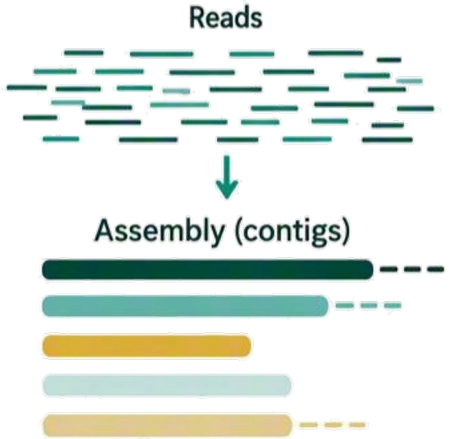
Sequence the DNA from this isolate.



Many short DNA reads

3 Analyse the genome

Assemble the reads into several DNA segments (contigs).



Draft assembly: multiple contigs, not a guaranteed closed genome.

4 Interpret the strain

Use the genome to answer key questions.

Identity
What is this isolate?

Strain comparison
How does it relate to other strains?

Genes of interest
Which genes and functions are present?

DNA results need biological context

- Detectable DNA may come from living or dead cells
- A gene can be present without producing its expected effect
- Relative abundance differs from an absolute microbial count
- Sampling and controls determine how confidently we interpret a result

Context

Culture, chemistry and process knowledge

The strongest conclusion combines molecular evidence with measured behaviour

02

Evidence from our dairy projects

BLUECARE, GAZTAMINA, ELAMINA, SHIGA and GAZTANOLA

BLUECARE followed the blue-cheese microbiome





16S + ITS

Bacteria, yeasts and moulds through ripening

QUESTION

How does blue-cheese microbiota change during ripening?

WHAT WE SAW

Profiles varied with stage, batch and storage conditions

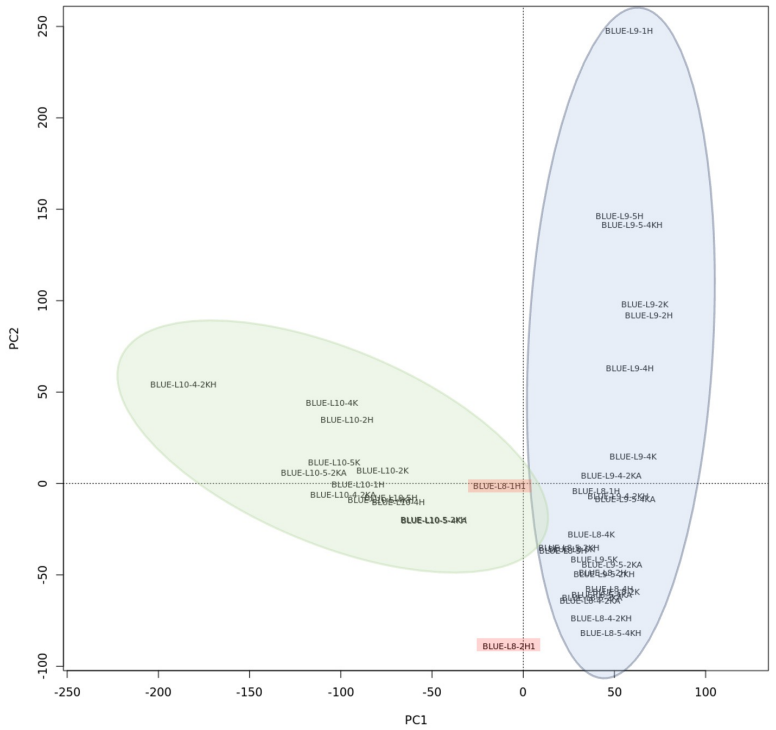
INTERPRETATION

Run effects and fungal identification limited some comparisons

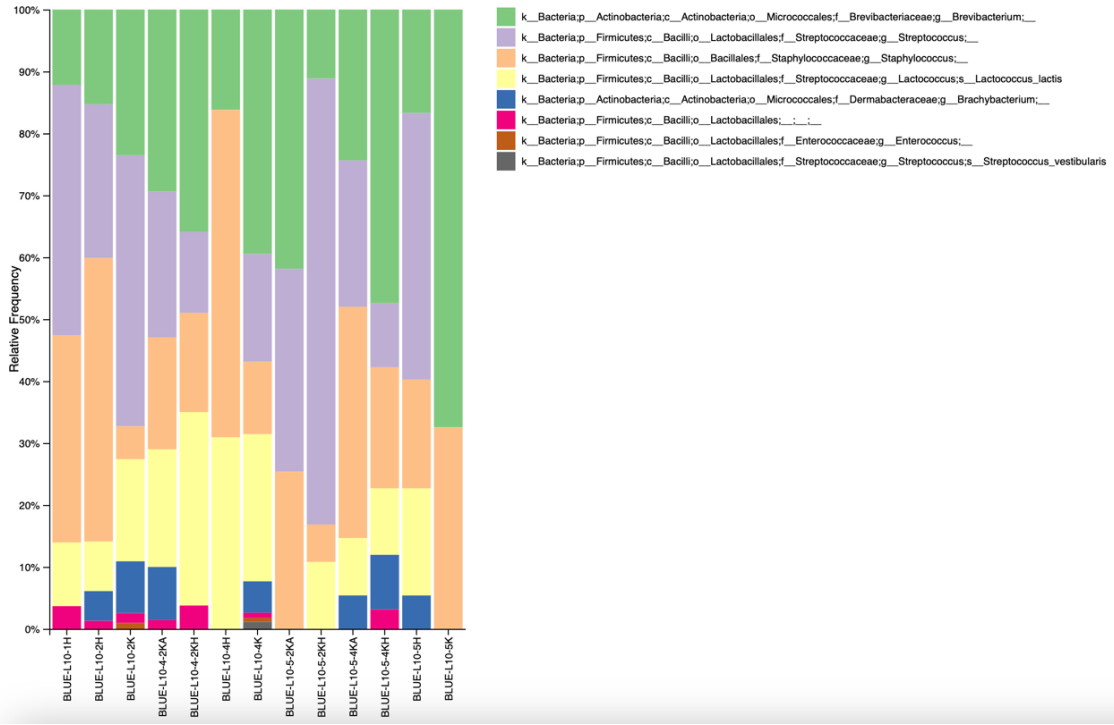
BLUECARE: the largest differences were between batches

The strongest contrasts involved ITS profiles: moulds and yeasts in blue cheese.

THE MAIN SEPARATION WAS BETWEEN BATCHES



16S: BACTERIAL COMPOSITION ALSO VARIED



Within each batch, bacterial and fungal profiles also differed between wrappings.

GAZTANOLA: microbial communities on ripening supports



Ripening trials on different supports

THE COMPARISON

Wood and alternative supports in pilot and working dairies

THE QUESTION

Do different surfaces host different microbial communities?

THE MOLECULAR APPROACH

16S for bacteria

ITS for moulds and yeasts

GAZTANOLA found different communities across surfaces

16S and ITS profiling compared bacteria, moulds and yeasts on ripening supports.

16S: BACTERIA



ITS: MOULDS AND YEASTS



WHICH DIFFERENCES ARE SIGNIFICANT?

Lagina	P_L4_G_Eg_3H	P_L4_G_Eg_3H	P_L4_G_Ku_3H	P_L4_G_Ku_3H	P_L4_G_Es_3H	P_L4_G_Es_3H_2_ITS		P-Value	Diferencia Significativa
k__Bacteria|p__Actinomycetota|c__Actinomycetia|o__Micrococcales|f__Brevibacteriaceae|g__Brevibacterium	40.13	41.04	30.49	29.80	25.41	36.69		0.185	No
k__Bacteria|p__Bacillota|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Staphylococcus	27.69	18.42	32.30	15.89	22.75	17.27		0.272	No
k__Fungi|p__Ascomycota|c__Sordariomycetes|o__Hypocreales	24.93	24.92	13.75	12.97	19.62	28.04		0.073	No
k__Fungi|p__Mucoromycota|c__Mucoromycetes|o__Mucorales|f__Mucoraceae|g__Mucor|s__Mucor_lanceolatus	39.35	37.18	12.85	11.60	13.11	9.47	*	0.002	Sí
k__Bacteria|p__Bacillota|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Lactococcus|s__Lactococcus_lactis	19.26	19.37	24.12	29.72	11.23	10.83	*	0.014	Sí
k__Bacteria|p__Actinomycetota|c__Actinomycetia|o__Micrococcales|f__Dermabacteraceae|g__Brachybacterium	11.19	21.17	4.11	4.08	24.12	19.52		0.065	No
k__Fungi|p__Ascomycota|c__Sordariomycetes|o__Hypocreales|f__Hypocreaceae|g__Pseudoacremonium|s__Pseudoacremonium_sacchari	20.27	20.31	10.34	11.88	7.32	9.00	*	0.019	Sí
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_aurantiogriseum	3.18	3.81	16.49	15.82	10.01	10.52	*	0.001	Sí
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_thymicola	3.13	3.79	16.07	12.07	9.12	11.19	*	0.003	Sí
k__Fungi|p__Ascomycota|c__Sordariomycetes|o__Hypocreales|f__Hypocreaceae|g__Acremonium|s__Acremonium_blochii	1.73	1.66	0.04	0.05	27.44	15.75	*	0	Sí
k__Bacteria|p__Actinomycetota|c__Actinomycetia|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Saccharopolyspora|s__Saccharopolyspora_glor	1.09	0.00	8.97	11.54	0.00	0.00	*	0	Sí
k__Fungi|p__Ascomycota|c__Saccharomycetes|o__Saccharomycetales|f__Debaryomycetaceae|g__Debaryomyces|s__Debaryomyces_prosopidis	0.25	0.24	6.11	9.99	2.34	1.75	*	0.045	Sí
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_coccotrypicola	0.89	0.97	4.40	6.39	2.66	2.73		0.067	No
k__Bacteria|p__Actinomycetota|c__Actinomycetia|o__Micrococcales|f__Promicromonosporaceae|g__Promicromonospora	0.46	0.00	0.00	3.40	6.47	6.05	*	0.012	Sí
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Aspergillus|s__Aspergillus_versicolor	0.19	0.17	4.17	3.90	0.23	0.23		0.089	No
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_gladioli	0.48	0.61	2.32	2.08	1.44	1.53		0.073	No
k__Bacteria|p__Actinomycetota|c__Actinomycetia|o__Micrococcales|f__Micrococcaceae|g__Glutamicibacter	0.00	0.00	0.00	3.56	1.64	3.11	*	0.001	Sí
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Aspergillus|s__Aspergillus_sydowii	0.14	0.16	3.42	3.24	0.21	0.29	*	0.045	Sí
k__Fungi|p__Ascomycota|c__Sordariomycetes|o_incertaesedis|f_incertaesedis|g__Wallrothiella|s__Wallrothiella_subiculosa	0.33	0.50	1.69	1.89	1.21	1.29		0.067	No
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_solitum	0.35	0.45	2.18	1.52	0.97	1.23	*	0.045	Sí
k__Bacteria|p__Actinomycetota|c__Actinomycetia|o__Micrococcales|f__Micrococcaceae|g__Micrococcus|s__Micrococcus_terreus	0.00	0.00	0.00	0.00	3.07	2.81	*	0.001	Sí
k__Bacteria|p__Actinomycetota|c__Actinomycetia|o__Micrococcales|f__Micrococcaceae|g__Glutamicibacter|s__Glutamicibacter_bergerei	0.00	0.00	0.00	0.00	3.36	2.34	*	0.001	Sí
k__Fungi|p__Ascomycota|c__Sordariomycetes|o__Hypocreales|f__Hypocreaceae|g__Acremonium	0.18	0.19	0.11	0.13	1.18	2.06	*	0.045	Sí
k__Fungi|p__Ascomycota|c__Sordariomycetes|o__Hypocreales|f__Hypocreaceae|g__Acremonium|s__Acremonium_alternatum	1.43	1.79	0.01	0.02	0.12	0.21		0.067	No
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Aspergillus|s__Aspergillus_hongkongensis	0.06	0.05	1.60	1.69	0.07	0.08	*	0.045	Sí
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptomycetales|f__Streptomycetaceae|g__Streptomyces	0.00	0.00	0.00	0.00	1.95	1.38	*	0.001	Sí
k__Fungi|p__Ascomycota|c__Leotiomycetes|o__Erysiphales|f__Erysiphaceae|g__Microidium|s__Microidium_phyllanthi	0.39	0.23	1.14	0.80	0.29	0.48		0.067	No
k__Fungi|p__Ascomycota|c__Sordariomycetes|o__Hypocreales|f__Hypocreaceae|g__Acremonium|s__Acremonium_spinosum	0.05	0.07	0.09	0.06	0.83	1.67		0.045	Sí
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_catenatum	1.29	1.26	0.00	0.00	0.01	0.02		0.067	No
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_osmophilum	0.16	0.20	0.58	0.63	0.35	0.36		0.045	Sí
k__Fungi|p__Ascomycota|c__Sordariomycetes|o__Hypocreales|f__Hypocreaceae|g__Acremonium|s__Acremonium_hennebertii	0.53	0.61	0.28	0.36	0.20	0.20		0.067	No
k__Bacteria|p__Bacillota|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Staphylococcus|s__Staphylococcus_equorum	0.18	0.00	0.00	1.63	0.00	0.00		0.045	Sí
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_sclerotigenum	0.09	0.08	0.35	0.52	0.23	0.25		0.067	No
k__Fungi|p__Ascomycota|c__Saccharomycetes|o__Saccharomycetales|f__Debaryomycetaceae|g__Debaryomyces|s__Debaryomyces_hansenii	0.03	0.02	0.47	0.71	0.12	0.15		0.045	Sí
k__Fungi|p__Ascomycota|c__Saccharomycetes|o__Saccharomycetales|f__Debaryomycetaceae|g__Candida|s__Candida_parapsilosis	0.06	0.08	0.02	0.04	0.15	0.44		0.067	No
k__Fungi|p__Ascomycota|c__Eurotiomycetes|o__Eurotiales|f__Aspergillaceae|g__Penicillium|s__Penicillium_polonicum	0.03	0.05	0.15	0.16	0.09	0.12		0.045	Sí
k__Fungi|p__Ascomycota|c__Coniocybomycetes|o__Coniocybales|f__Coniocybaceae|g__Chaenotheca|s__Chaenotheca_brunneola	0.06	0.04	0.18	0.13	0.06	0.11		0.067	No
k__Fungi|p__Ascomycota|c__Sordariomycetes|o__Hypocreales|f__Cordycipitaceae|g__Leptobacillium|s__Verticillium_leptobactrum	0.01	0.02	0.13	0.18	0.05	0.03		0.045	Sí
k__Fungi|p__Ascomycota|c__Saccharomycetes|o__Saccharomycetales|f__Debaryomycetaceae|g__Debaryomyces|s__Debaryomyces_coudertii	0.00	0.00	0.14	0.18	0.04	0.04		0.067	No
k__Bacteria|p__Actinomycetota|c__Actinomycetia|o__Micrococcales|f__Micrococcaceae|g__Kocuria	0.00	0.00	0.00	0.38	0.00	0.00		0.045	Sí

Differences were particularly evident in fungal communities.

Community profiles guide which organisms to study next.

GAZTAMINA revealed the problem. ELAMINA pursued solutions



Cheese sample from the linked GAZTAMINA–ELAMINA proyect.

1 WHAT WAS IN THE CHEESE?

Chemical analysis measured biogenic amines in commercial cheeses

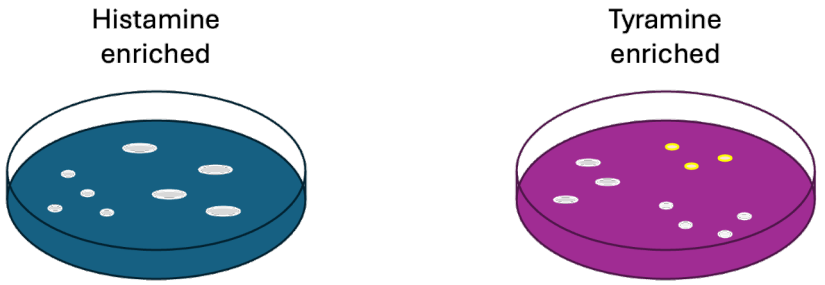
2 WHICH BACTERIA COULD PRODUCE THEM?

The findings prompted a search for amine-production genes

3 COULD WE REDUCE THEM?

ELAMINA investigated candidate cultures and tested them in cheese

PCR searched for genes linked to amine production



	Small white	Large white	Yellow	Dry white	Medium White
Colonies	54	64	26	6	17
HdcA +	0 %	0 %	0 %	0 %	0 %
TdcA +	87.0 %	100 %	11.5 %	50.0 %	35.3 %

76.7% tdcA-positive colonies in the reported screen

THE QUESTION

Did the isolated bacteria carry genes for amine production?

THE TOOL: TARGETED PCR

We screened hdcA and tdcA, targets linked to histamine and tyramine

WHAT THE RESULT TELLS US

Gene detection indicates potential. Chemistry measures the amines

ELAMINA used WGS to investigate candidate cultures

KNOWN ABILITY, UNKNOWN MECHANISM

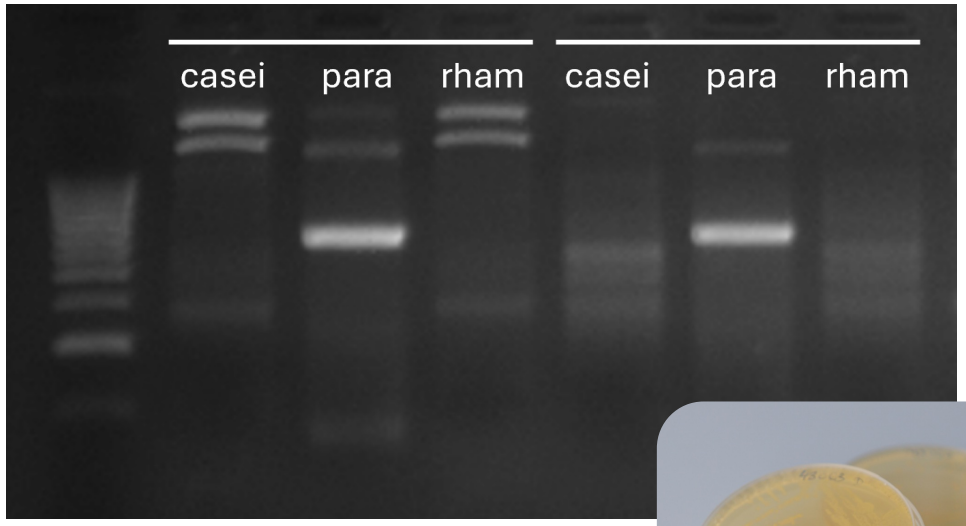
Two strains with reported reducing ability.
WGS explored their genes

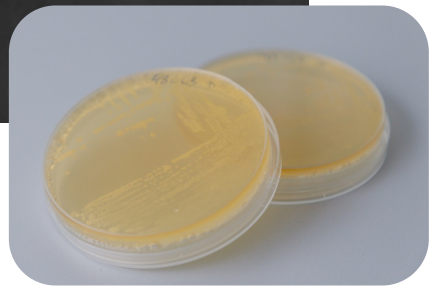
A SURPRISE IN THE GENOME

Identity changed. We recognised a gene explaining amine degradation

BACK TO THE CHEESE

Tyramine reduction was significant.
Histamine reduction was not



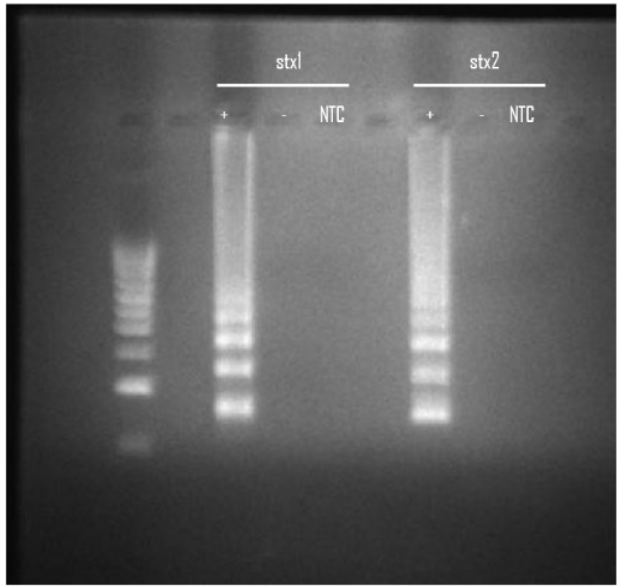


IPLA 4a and IPLA 5b

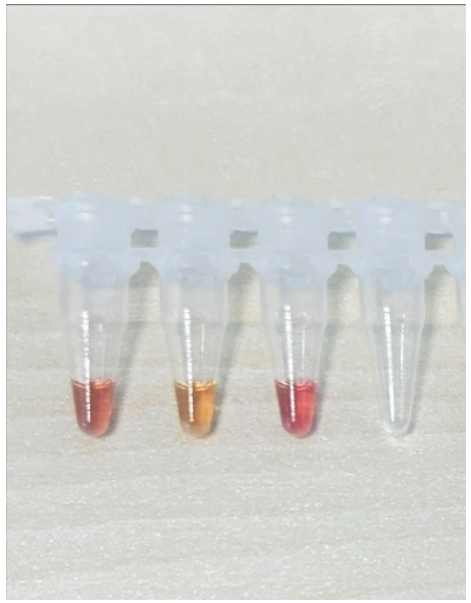
Initially labelled L. casei

WGS identified Lacticaseibacillus paracasei

SHIGA combines genome analysis with detection



Amplification check



Visual readout

GENOMIC FOUNDATION

WGS characterises dairy-associated STEC isolates

TARGETED DETECTION

qPCR work and preliminary LAMP detection of stx1 and stx2

CURRENT STATUS

Promising feasibility. Full dairy-matrix validation remains in progress

The dairy question determines the method

- A known organism or gene: targeted PCR/qPCR
- A changing community: 16S/ITS profiling
- The identity or genetic potential of an isolate: WGS
- A product decision: combine DNA with culture, chemistry and dairy expertise

A clear question and efficient sampling help us obtain useful results

Combining molecular techniques with microbiological and physicochemical analyses helps us support cheesemakers



FACE NETWORK 2026

esneki zentroa Leartiker

MARKINA - XEMEIN

Thank you · Eskerrik asko

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Esneki Zentroa, Leartiker · Markina-Xemein

esneki zentroa Leartiker

Farmhouse and Artisan Cheese & Dairy Producers European Network

UDALA MARKINA XEMEIN

hazi

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EUSKO JAURLARITZA GOBIERNO VASCO

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