22 Noviembre 2023

Aplicaciones de las nuevas tecnologías de secuenciación en seguridad y fraude alimentario

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Introducing Illumina

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Illumina is a global leader in DNA sequencing and array-based technologies, serving customers in the research, clinical and applied markets. Our products are used for applications in the life sciences, oncology, reproductive health, agriculture and other emerging segments.

$4.5 billion (2023)
Annual revenue

~9,100
Number of employees

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1998
Year founded

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OUR MISSION

Improve human health by unlocking the power of the genome

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Genomics applications in Food Science

Integrated in many processes and steps across the food chain

NGS and arrays are used in all the steps from gene to plate

Breeding & Selection	Production	Processing	Consumption
Research	Veterinary Diagnostics	Food Safety	Tracing and Surveillance
Genomic Breeding	Environmental Testing	Food Authenticity	
Parentage		Food Traceability	
		Spoilage	

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The power of NGS

2

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Comparación de tecnologías de biología molecular

q/RT-PCR

- \+ Alta sensibilidad
  Instrumento presente en muchos laboratorios
- − Menor resolución
  Detección de 1 o pocos organismos por muestra

Sanger/CE

- \+ Coste efectivo para tramos pequeños de AND
  Técnica muy conocida
- − Baja sensibilidad (<20%)
  Poco escalable
  Detección de 1 organismo por muestra

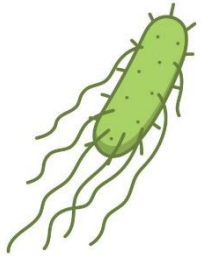
Next-gen Sequencing

- \+ Alta resolución y sensibilidad
  Capacidad de procesar varias muestras y detectar cientos de organismos por muestra
- − Precio muy relacionado al número de muestras

Comparación de tecnologías de biología molecular

q/RT-PCR

Presence/ absence results



Salmonella enterica

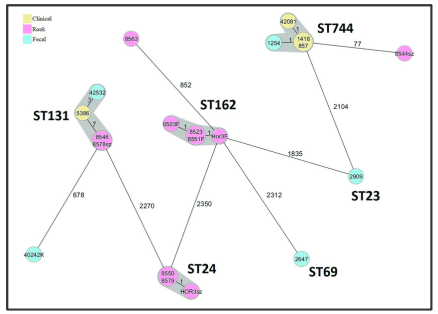
Sanger/CE

MLST of 7 loci for Typing

Salmonella entérica subsp. enterica

Next-Gen Sequencing

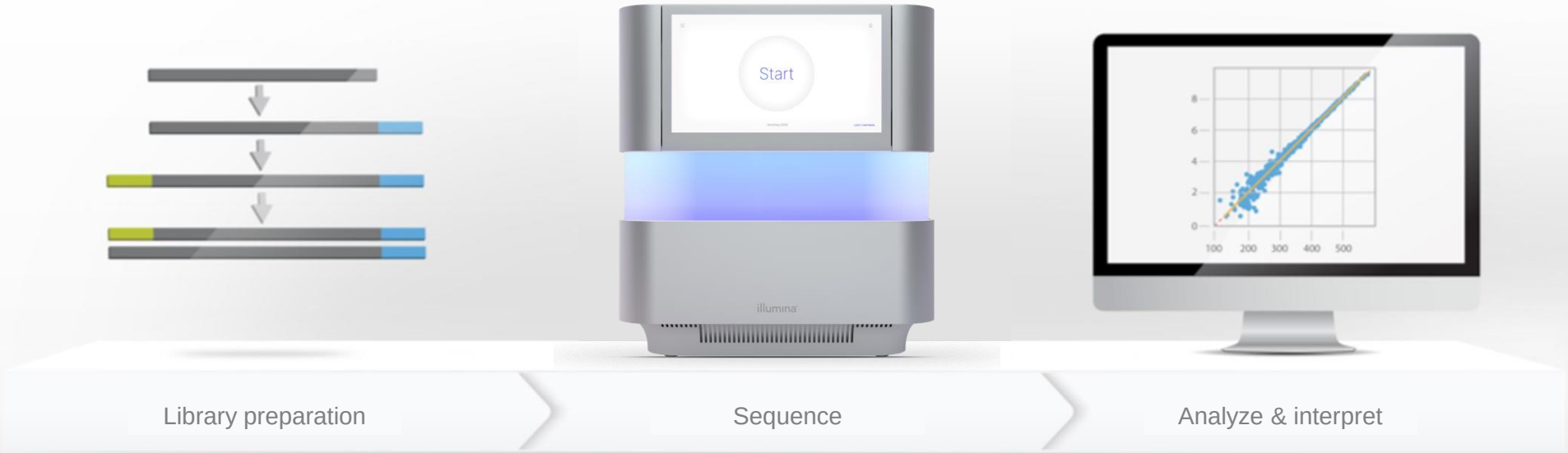
wgMLST of the Whole genome



Full ID for outbreak investigation, serotyping, AMR

The NGS approach

Integrated workflows from prepared sample to answer



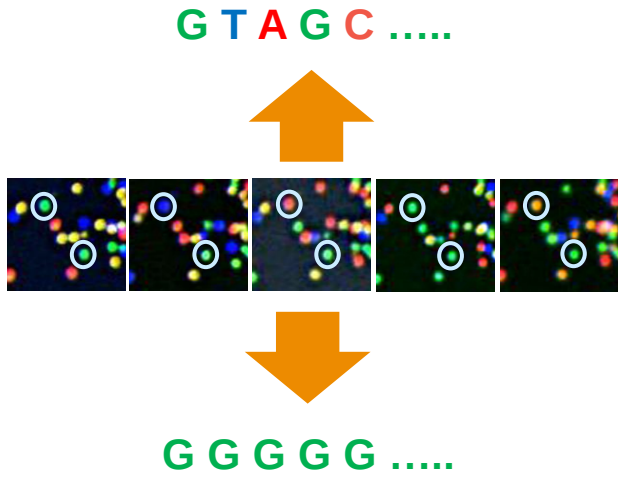
Flexible workflow solutions enable markets

- Simplified library prep
- Custom content
- Flexible, accessible sequencing
- Integrated analysis

Analysis

Primary analysis

- Runs in parallel to the sequencing
- Uses the images to provide base calls of the reads

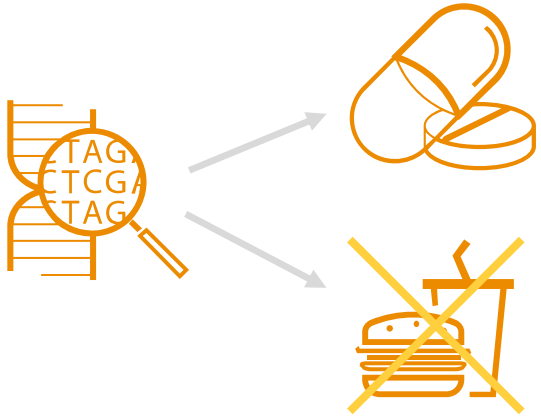


Secondary analysis

- Maps the reads to the reference genome (alignment)
- Analyses the mapped reads for any differences compared to the reference genome (variant calling)

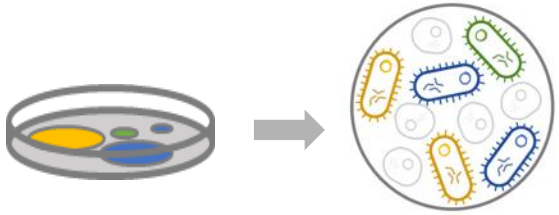
Interpretation

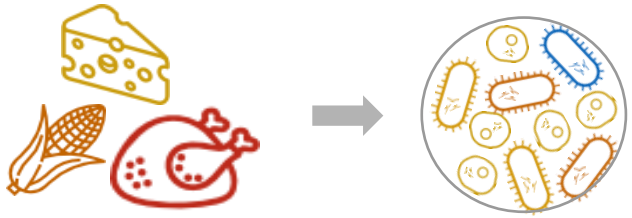
- Leads to knowledge and insights into basic biology
- Potential causes of genetic disease
- Possible treatment or prevention



https://emea.illumina.com/informatics/sequencing-data-analysis.html

Applications for Culture vs. Culture-Free Sequencing





Whole-Genome Sequencing (WGS)	Amplicon Sequencing	Shotgun metagenomics
Sequencing a **cultured isolate** for taxonomy, evolution, and function	Sequencing **specific genes** for taxonomy and relative abundance	Sequencing **"everything"** in a sample for taxonomy, abundance, and **function**

- Food outbreaks, identify source
- Monitor for pathogens in product and facilities
- Differentiate resident, persistent, and transient pathogens
- Virulence factors, AR genes, and other genetic markers of interest
- Maintain or enhance food quality (starter cultures)

- Community profiling to predict pathogen, spoilage risk, and allergen
- Detect genetic modifications (GMOs)
- Authenticate high value foods (olive oil)
- Verify speciation (kosher, halal)
- Procure correct ingredients for final product (gluten free)

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Problemas relacionados con la alimentación tienen un gran impacto socio-econòmico

La genómica puede ayudar a prevenir o reducir ese impacto

Seguridad Alimentaria

El costo estimado de los incidentes de seguridad alimentaria para la economía de los Estados Unidos es de alrededor de **$7 mil millones por año**, que proviene de notificar a los consumidores, retirar alimentos de los estantes y pagar daños como resultado de demandas.

Economic Impact of Food Safety Outbreaks on Food Businesses. Hussain MA, et al. Foods. 2013. PMID: 28239140

Fraude Alimentario

Las estimaciones de los expertos han encontrado que el fraude alimentario **afecta al 1% de la industria alimentaria mundial** a un costo de alrededor de 10 a 15 mil millones $ al año, aunque algunas estimaciones de expertos más recientes sitúan el costo en $ 40 mil millones al año.

FDA: Economically Motivated Adulteration (Food Fraud)

Producción Alimentaria

La producción agrícola y la seguridad alimentaria siguen siendo **una de las principales preocupaciones mundiales**. La investigación y los comentarios en este número de Nature destacan las diversas amenazas a nuestras frutas y verduras y los efectos de arrastre de las crisis alimentarias.

Genomics and our future food security. Editorial. Nat Genet. 2019. PMID: 30705438

NGS applications in Food Science

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Food safety

Food safety applications:

Using NGS to Assess Food Pathogen Outbreak

20 minutos Actualidad Nacional Internacional Deportes Cultura Opinión Más

Una misma cepa de listeria procedente de la carne La Mechá ha contagiado a 144 pacientes

EFE 12.09.2019 - 15:21H

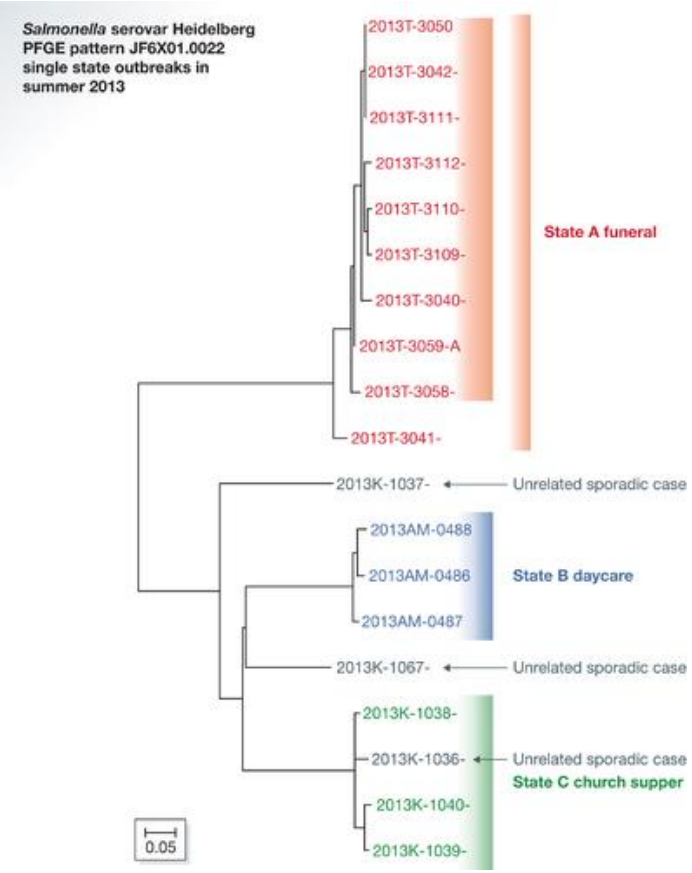
GOBIERNO DE ESPAÑA | MINISTERIO DE CIENCIA E INNOVACIÓN | Instituto de Salud Carlos III

French officials confirm some E. coli cases linked to Nestlé pizza

·

75 pacientes, 41 con Síndrome Urémico Hemolítico.
2 niños fallecidos.
Posible origen en harina.
Detección foco en Marzo 2022.
Primeros enfermos en Enero

CDC discovers Listeria outbreak linked to enoki mushrooms



Aplicaciones en la seguridad alimentaria

La tecnología NGS puede ser una aproximación reactiva y proactiva

- **Herramienta industrial para monitorizar ingredientes**
- **Permite evaluar la eficiencia de prevenir y de los controles sanitarios**
- **Se usa para determinar la persistencia de patógenos en el ambiente**
- **Posible indicador de resistencias antimicrobianas**

Routine testing

(qPCR, Microbiology)

Positive sample

WGS
- Species characterization
- Outbreak management

Food authenticity and traceability

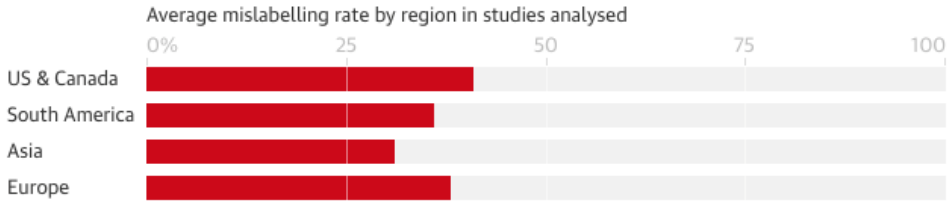
Food fraud is widespread

Adulteration, mixing, or mislabeling is extremely common globally

The Guardian

Revealed: seafood fraud happening on a vast global scale

Guardian analysis of 44 studies finds nearly 40% of 9,000 products from restaurants, markets and fishmongers were mislabelled



Guardian graphic. Source: Guardian review of 44 seafood studies published since 2018

A Guardian Seascape analysis of 44 recent studies of more than 9,000 seafood samples from restaurants, fishmongers and supermarkets in more than 30 countries found that 36% were mislabelled, exposing seafood fraud on a vast global scale.

The Guardian, March 15th 2021 | https://www.theguardian.com/environment/2021/mar/15/revealed-seafood-happening-on-a-vast-global-scale

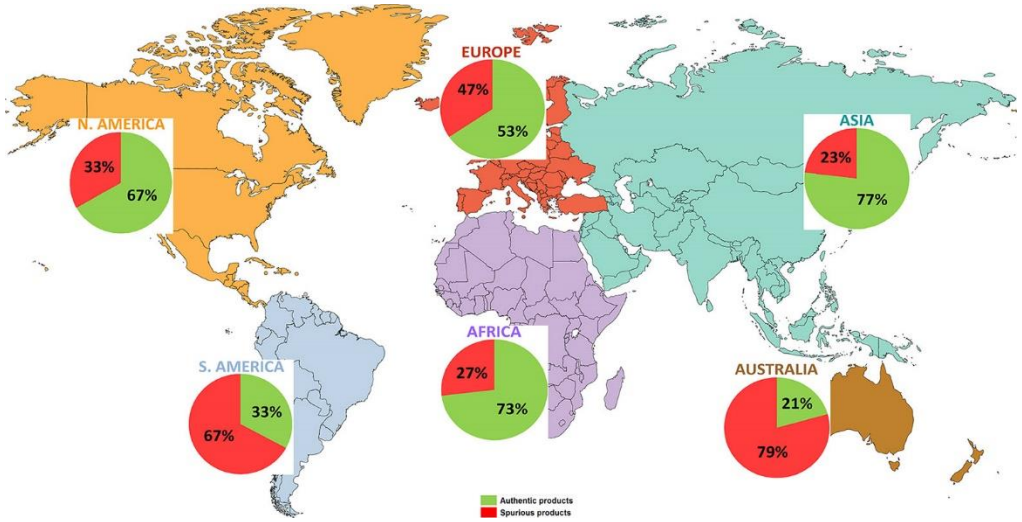
REVIEW ARTICLE

Front. Pharmacol., 24 October 2019 | https://doi.org/10.3389/fphar.2019.01227

The DNA-Based Authentication of Commercial Herbal Products Reveals Their Globally Widespread Adulteration

Mihael Cristin Ichim*

"Stejarul" Research Centre for Biological Sciences, National Institute of Research and Development for Biological Sciences, Piatra Neamt, Romania



Front. Pharmacol., 24 October 2019 | https://doi.org/10.3389/fphar.2019.01227

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Food fraud is widespread

Adulteration, mixing, or mislabeling is extremely common globally

Why NGS in Food authenticity?

- Characterization of multiple species at once
- Untargeted detection of thousands of organisms with no requirement for previous knowledge
- Most relieable method for species identification
- Allows better dealing with damaged DNA

DNA Barcoding & Metabarcoding

- Scientific foundations and databases growing
- Can provide composition + origin signatures
- Primers tailorable to specific clades
- Relationship between microbiome and food

international BARCODE OF LIFE

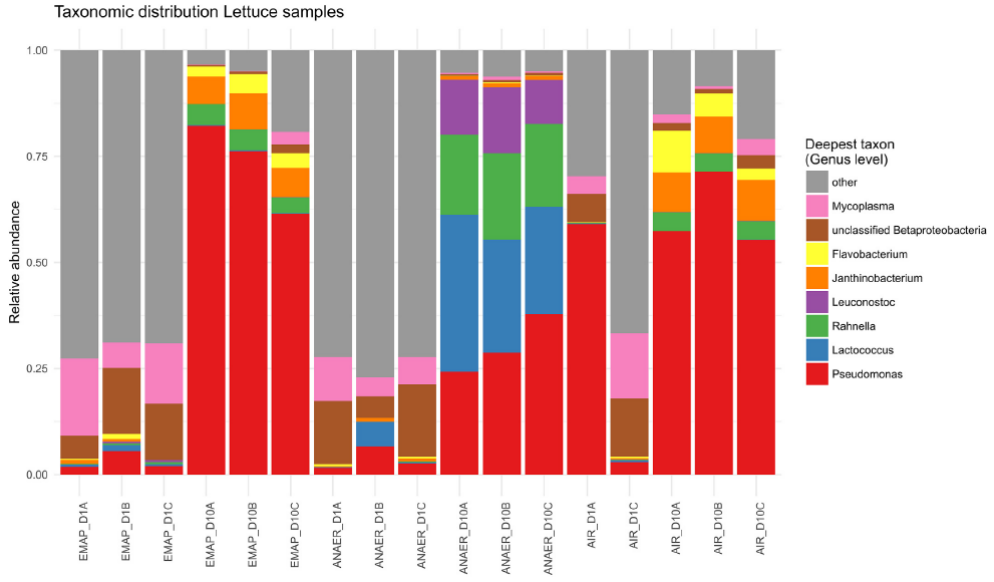
Food production

Food production applications:

Microbial Diversity in Packaging evaluation

Characterization of spoilage microbiota in packaged iceberg lettuce using culture-free amplicon sequencing

- Studied microbial and biochemical contributors to spoilage in fresh cut lettuce
- Microbial analysis of lettuce microbiome under aerobic @ 3%O_2 and perforated, and anaerobic conditions.
- Culture-free amplicon sequencing revealed Pseudomonas spp. as the dominating aerobic spoilers, whereas lactic acid producing bacteria (*Leuconostoc* spp. and *Lactococcus* spp.) dominated anaerobic packaging condition.
- Identification of genera with spoilage capacity to produce off odors, leading to early consumer rejection



Angelos-Gerasimos I. et.al *Characterization of spoilage markers in modified atmosphere packaged iceberg lettuce Intl Journal of Food Micro 279 (2018) 1–13*

Understanding of microbial contributors to spoilage can inform future packaging technologies and design to extend shelf life of lettuce

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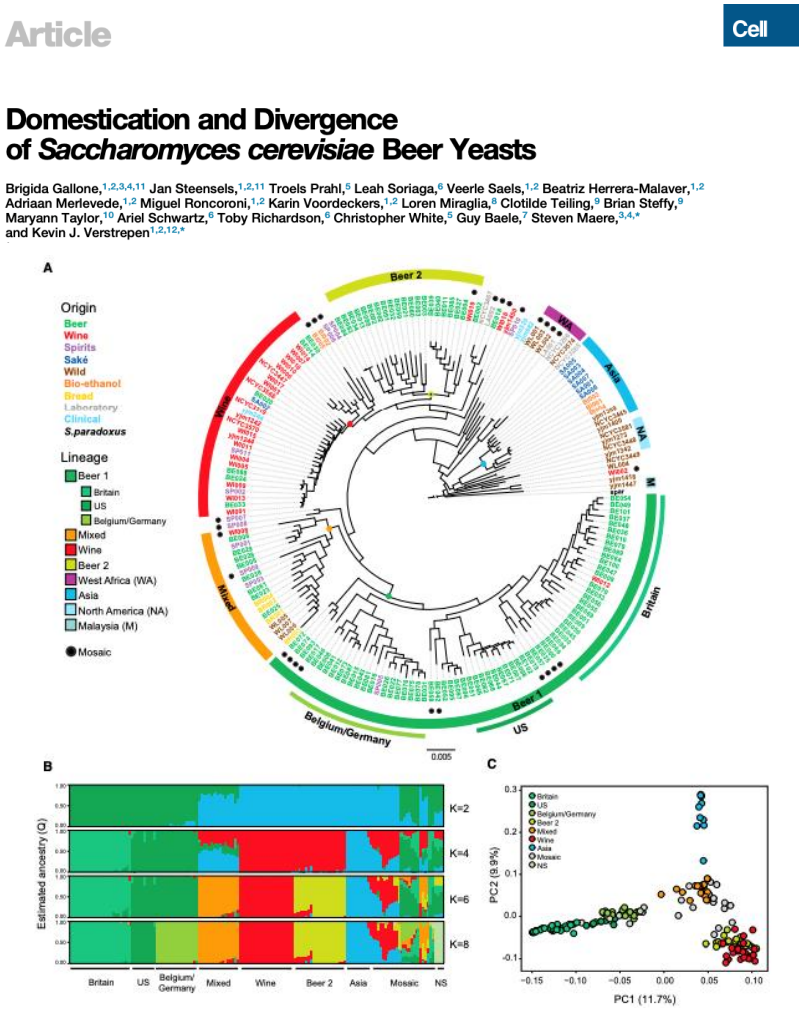
Food production applications:

The secret behind the beer: the genetics of industrial yeasts



Article

Cell

Domestication and Divergence of *Saccharomyces cerevisiae* Beer Yeasts

Brigida Gallone,[1,2,3,4,11] Jan Steensels,[1,2,11] Troels Prahl,[5] Leah Soriaga,[6] Veerle Saels,[1,2] Beatriz Herrera-Malaver,[1,2] Adriaan Merlevede,[1,2] Miguel Roncoroni,[1,2] Karin Voordeckers,[1,2] Loren Miraglia,[8] Clotilde Teiling,[9] Brian Steffy,[9] Maryann Taylor,[10] Ariel Schwartz,[6] Toby Richardson,[6] Christopher White,[5] Guy Baele,[7] Steven Maere,[3,4,*] and Kevin J. Verstrepen[1,2,12,*]

KEY HIGHLIGHTS

- **Analyzed the genomes and phenomes of 157 industrial *Saccharomyces cerevisiae* yeasts.**
- Present-day industrial yeasts originate from only a few domesticated ancestors
- **Beer yeasts show strong genetic and phenotypic hallmarks of domestication**
- Domestication of industrial yeasts predates microbe discovery
- The genome sequences, phylogenetic tree, and phenome data can be used to set up marker-assisted breeding schemes similar to those routinely used for the breeding of superior crops and livestock

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Gallone B, Steensels J, Prahl T *et al*. Domestication and Divergence of Saccharomyces cerevisiae Beer Yeasts. Cell. 2016 Sep 8;166(6):1397-1410.e16. doi: 10.1016/j.cell.2016.08.020.

The microbiota can influence cheese quality

Shotgun metagenomics to characterize the bacterial community

frontiers in Microbiology

REVIEW

Sequencing of the Cheese Microbiome and Its Relevance to Industry

Bhagya. R. Yeluri Jonnala[1,2], Paul L. H. McSweeney[1], Jeremiah J. Sheehan[2] and Paul D. Cotter[2,3]*

[1] Food and Nutrition Department, University College Cork, Cork, Ireland, [2] Teagasc Food Research Centre, Fermoy, Ireland, [3] APC Microbiome Ireland, Cork, Ireland

The microbiota of cheese plays a key role in determining its organoleptic and other physico-chemical properties. It is essential to understand the various contributions, positive or negative, of these microbial components in order to promote the growth of desirable taxa and, thus, characteristics. The recent application of high throughput DNA sequencing (HTS) facilitates an even more accurate identification of these microbes, and their functional properties, and has the potential to reveal those microbes, and associated pathways, responsible for favorable or unfavorable characteristics. This technology also facilitates a detailed analysis of the composition and functional potential of the microbiota of milk, curd, whey, mixed starters, processing environments, and how these contribute to the final cheese microbiota, and associated characteristics. Ultimately, this information can be harnessed by producers to optimize the quality, safety, and commercial value of their products. In this review we highlight a number of key studies in which HTS was employed to study the cheese microbiota, and pay particular attention to those of greatest relevance to industry.

Keywords: high throughput sequencing, cheese, microbiota, sensory characteristics, metatranscriptomics, industry

OPEN ACCESS

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INTRODUCTION

Cheese has a diverse microbial community, which indeed can vary within the cheese from the core to the surface that is greatly influenced by manufacturing including ripening conditions. Understanding the composition of this community (microbiota), and its impact on the quality and safety of cheese products, is of critical importance. In addition to, in the majority of cases, consciously added starter and adjunct bacteria (which are added as a supplement), cheese contains a heterogeneous variety of other, non-starter, microorganisms. These various microorganisms can play vital roles in the development of the organoleptic properties of cheese (Fox et al., 2000), nutrient composition, shelf-life, and safety.

Historically, culture-based microbiology techniques were used to gain an understanding of the microbial component of cheese. However, it has become increasingly clear that this approach can be limited in its ability to detect "difficult-to-culture" or sub-dominant microorganisms, thereby potentially providing misleading results. As a result, culture-independent approaches have become

Abbreviations: HTS, High Throughput Sequencing; NGS, Next Generation Sequencing; PDO, Protected designation of Origin; CIP, Cleaning-in-Place; NMC, Natural Milk Starter Cultures; NWC, Natural Whey Cultures; VOC, Volatile Compounds.

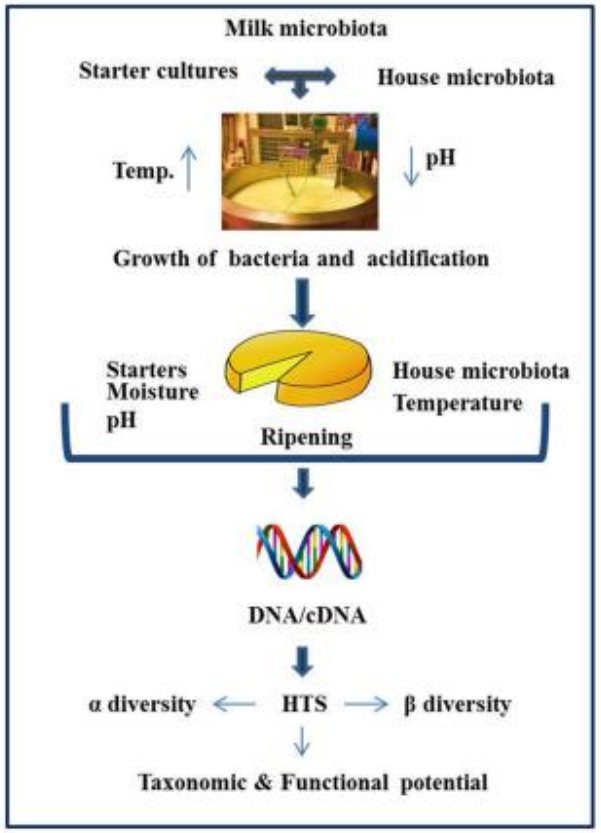

FIGURE 1 | Schematic representation of factors influencing the cheese microbiota, as revealed by HTS.

KEY HIGHLIGHTS

- Cheese has a diverse microbial community and understanding its composition, and its impact on the quality and safety of cheese products, is of critical importance.
- The Microbiota composition is influenced by several factors during cheese production
- The Microbiota play vital roles in the development of the organoleptic properties of cheese, nutrient composition, shelf-life, and safety.
- HTS is a powerful technique that can be used to provide a detailed insight into the microbiology of dairy related samples.

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Yeluri Jonnala BR, McSweeney PLH, Sheehan JJ, Cotter PD. Sequencing of the Cheese Microbiome and Its Relevance to Industry. Front Microbiol. 2018 May 23;9:1020. doi: 10.3389/fmicb.2018.01020.

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