

Mapeo de superficies para la detección de *hot spots* microbianos persistentes en entornos alimentarios

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VII Fòrum BSA

ESTRUCTURA

1

¿Qué buscamos?

Persistencia

Patógenos persistentes en instalaciones alimentarias

Hotspots microbianos

2

¿Cómo buscamos?

Metagenómica dirigida al 16S

3

¿Dónde buscamos?

Mapeo de instalaciones

Toma de decisiones

ESTRUCTURA

1

¿Qué buscamos?

Persistencia

Patógenos persistentes en instalaciones alimentarias

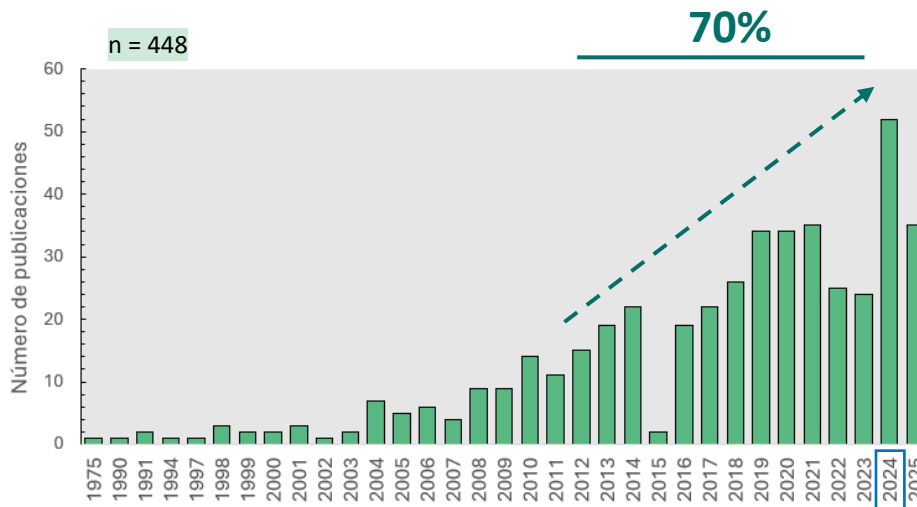
Hotspots microbianos

¿Qué buscamos?

PERSISTENCIA



Capacidad para sobrevivir durante periodos prolongados de tiempo en una superficie Carpentier & Cerf (2011)



SCIENTIFIC OPINION

EFSA JOURNAL

Persistence of microbiological hazards in food and feed production and processing environments

EFSA Panel on Biological Hazards (BIOHAZ)

Figura 1. Análisis bibliométrico en la base de datos PubMed con los términos “*foodborne pathogens*” AND “*persistance*” AND “*food industry*”.

¿Qué buscamos?

PATÓGENOS PERSISTENTES EN INSTALACIONES ALIMENTARIAS

SCIENTIFIC OPINION

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Abstract

Listeria monocytogenes (in the meat, fish and seafood, dairy and fruit and vegetable sectors), *Salmonella enterica* (in the feed, meat, egg and low moisture food sectors) and *Cronobacter sakazakii* (in the low moisture food sector) were identified as the bacterial food safety hazards most relevant to public health that are associated with persistence in the food and feed processing environment (FFPE). There is a wide range of subtypes of these hazards involved in persistence in the FFPE. While some specific subtypes are more commonly reported as persistent, it is currently not possible to identify universal markers (i.e. genetic determinants) for this trait. Common risk factors for persistence in the FFPE are inadequate zoning and hygiene barriers; lack of hygienic design of equipment and machines; and inadequate cleaning and disinfection. A well-designed environmental sampling and testing programme is the most effective strategy to identify contamination sources and detect potentially persistent hazards. The establishment of hygienic barriers and measures within the food safety management system, during implementation of hazard analysis and critical control points, is key to prevent and/or control bacterial persistence in the FFPE. Once persistence is suspected in a plant, a 'seek-and-destroy' approach is frequently recommended, including intensified monitoring, the introduction of control measures and the continuation of the intensified monitoring. Successful actions triggered by persistence of *L. monocytogenes* are described, as well as interventions with direct bactericidal activity. These interventions could be efficient if properly validated, correctly applied and verified under industrial conditions. Perspectives are provided for performing a risk assessment for relevant combinations of hazard and food sector to assess the relative public health risk that can be associated with persistence, based on bottom-up and top-down approaches. Knowledge gaps related to bacterial food safety hazards associated with persistence in the FFPE and priorities for future research are provided.

KEY WORDS

cleaning and disinfection, *Cronobacter sakazakii*, food processing, interventions, *Listeria monocytogenes*, Persistence, risk factors, *Salmonella enterica*, subtypes



Microorganismo	Sectores afectados
<i>Listeria monocytogenes</i>	Cárnicos, pescados y mariscos, lácteos, frutas y hortalizas
<i>Salmonella enterica</i>	Piensos, cárnicos, huevos, alimentos de baja humedad
<i>Cronobacter sakazakii</i>	Alimentos de baja humedad, especialmente en fórmulas infantiles

¿Qué buscamos?

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ORIGEN CEPAS PERSISTENTES

- Zonas de refugio de las bacterias
- Supervivencia desinfección
 - Concentraciones subletales de desinfectante
- Mecanismos fisiológicos y genéticos
 - Formas VBNC
- Formación de biofilms
 - Microbiota residente

2



DETECCIÓN

- Localización de puntos
- Evaluación
- Toma de decisiones

3



CONTROL

- Limpieza y desinfección
- Tratamiento de choque
- Desarrollo de alternativas

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¿Qué buscamos?

HOTSPOTS MICROBIANOS

Adhesión, protección y transferencia
BIOFILMS

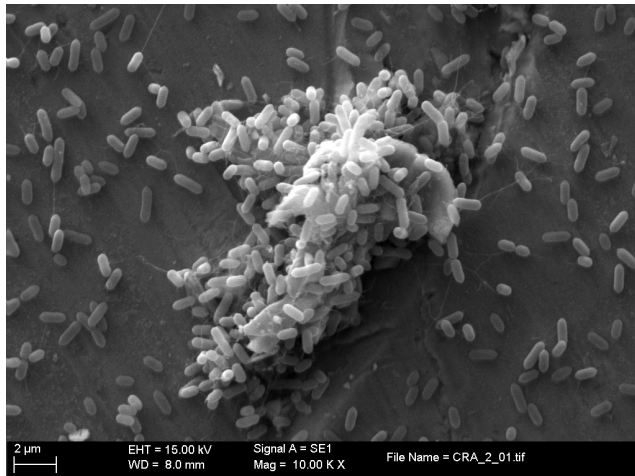


Figura 2. Células de *L. monocytogenes* en estado sésil protegiéndose en una zona dañada de una superficie de acero inoxidable.

Ripolles-Avila (2018)

VBNC

reto para la industria alimentaria

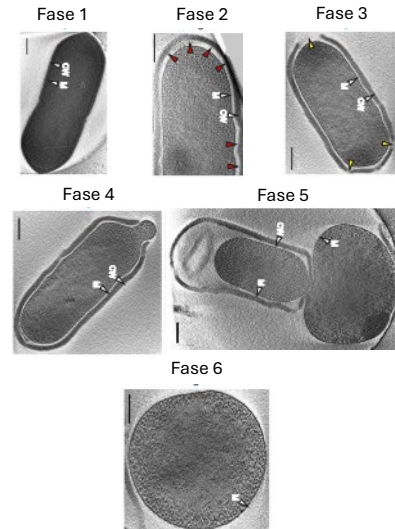


Figura 3. Transformación celular de *L. monocytogenes* a estado VBNC.

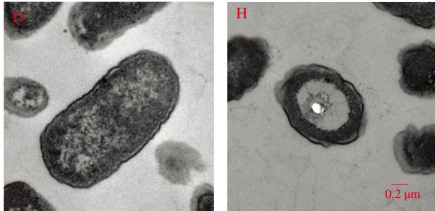
Carvalho et al., (2024)

¿Qué buscamos?

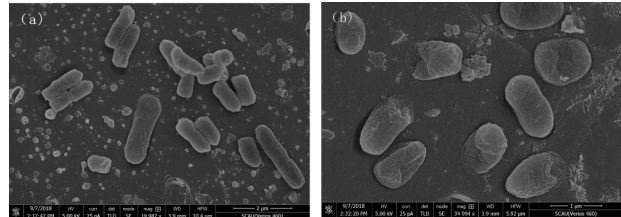
HOTSPOTS MICROBIANOS

VBNC

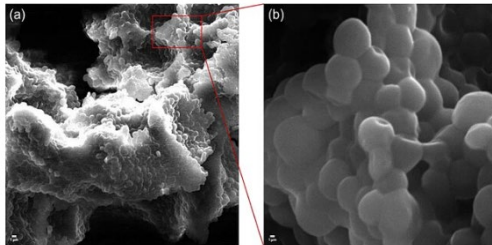
reto para la industria alimentaria



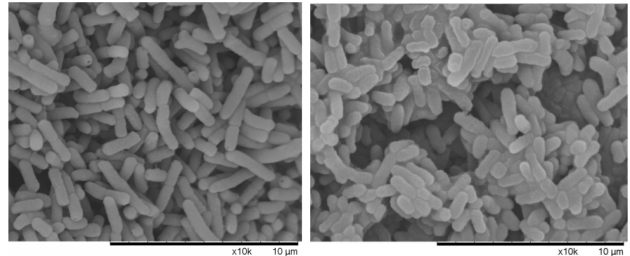
Salmonella enterica
Wang et al., (2025)



Cronobacter sakazakii
Zhang et al., (2020)



Vibrio parahaemolyticus
Ramesh et al., (2024)



Escherichia coli O157:H7
Wei & Zhao (2018)

ESTRUCTURA

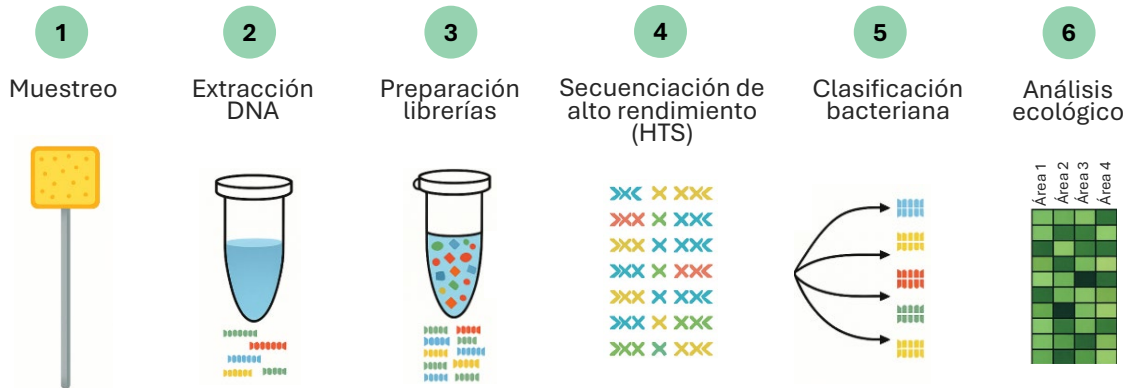
2

¿Cómo buscamos?

Metagenómica dirigida al 16S

¿Cómo buscamos?

METAGENÓMICA DIRIGIDA AL 16S



**Este enfoque permite:*

- Detectar patógenos persistentes de baja abundancia
- Visualizar microbiota residente que favorece la persistencia
- Comparar zonas con/sin contaminación crónica
- Guiar medidas correctivas en entornos industriales

Modificado de Effendi et al., (2022)

ESTRUCTURA

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¿Dónde buscamos?

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Toma de decisiones

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MAPEO DE INSTALACIONES

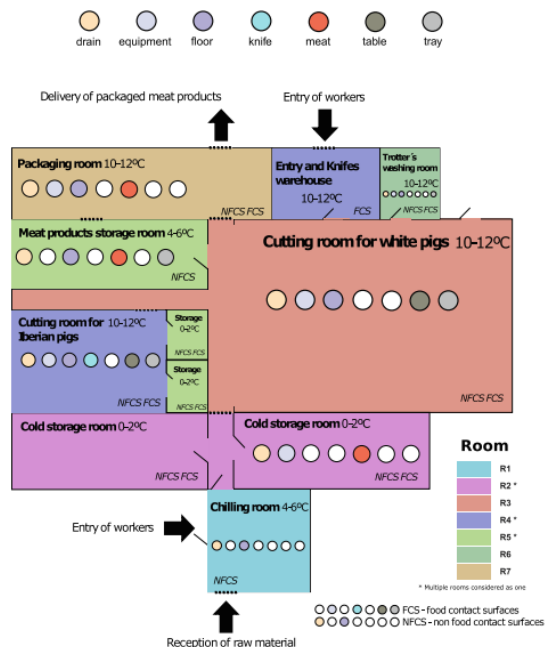
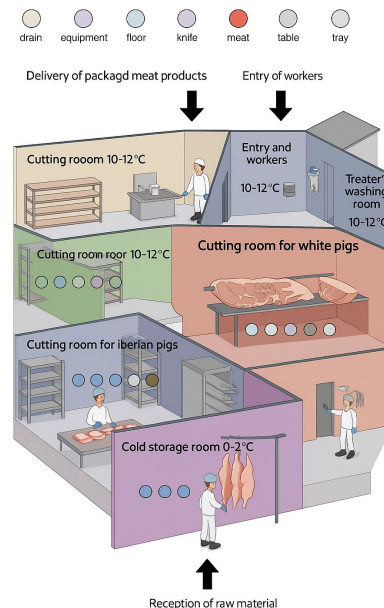


Figura 4. Mapa esquemático de una planta de procesamiento de carne.

Cobo-Díaz et al., (2021)



*Imagen generada por ChatGPT

Localizar zonas críticas
PUNTOS DE ALERTA

↳ ¿Por qué?

¿Dónde buscamos?

MAPEO DE INSTALACIONES

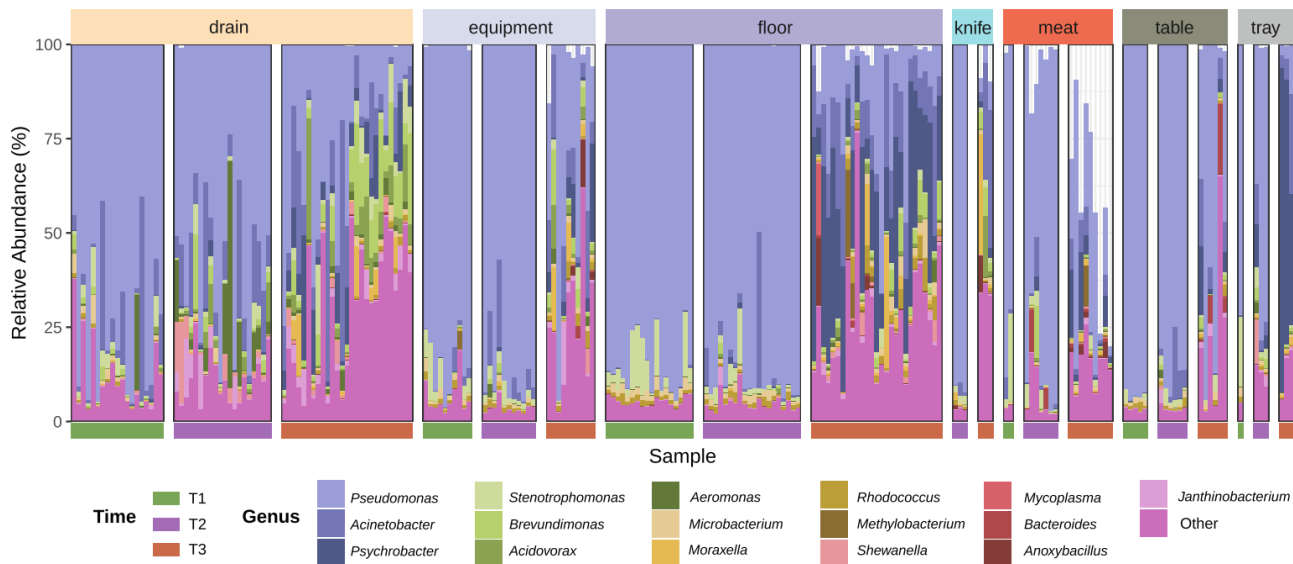
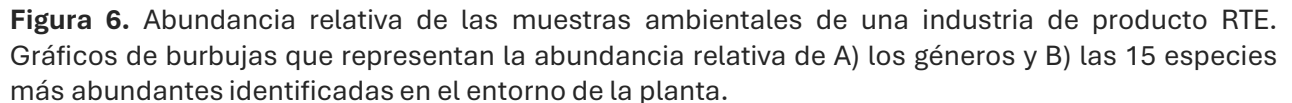


Figura 5. Abundancia relativa de los 16 géneros bacterianos más relevantes (abundancia total promedio > 1%). Cada barra representa una muestra, y las muestras están agrupadas según la superficie y los tiempos evaluados.

Cobo-Díaz et al., (2021)



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¿Dónde buscamos?

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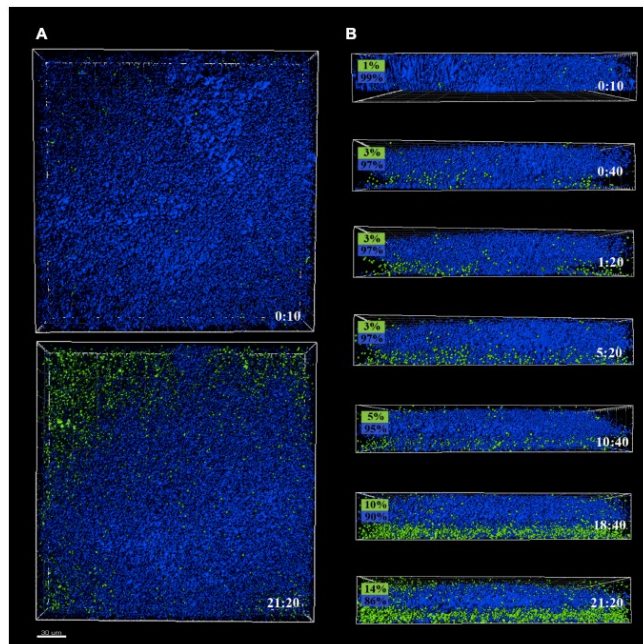
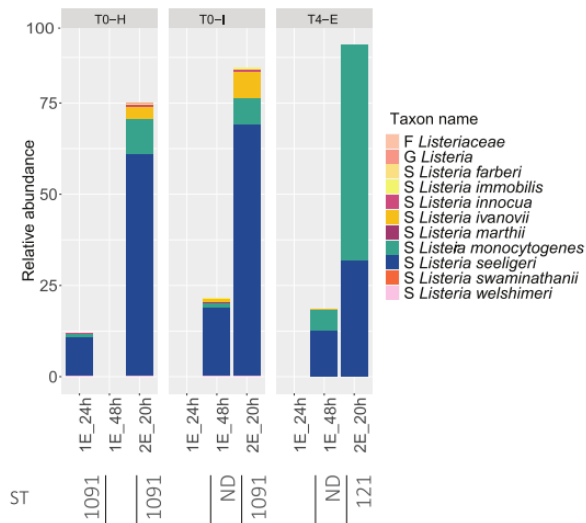


Figura 7. Imágenes de CLSM de diferentes secciones de biofilms de *P. fluorescens* (A-parte superior) y mixto con *L. monocytogenes* Scott A (A-parte inferior) formados a 48 h y a 20°C. Las células de *P. fluorescens* aparecen en azul (DAPI) y las células verdes corresponden a las etiquetas de GFP *L. monocytogenes*.

Puga et al., (2018)

¿Dónde buscamos?

MAPEO DE INSTALACIONES



→ Taxones no detectados por metagenómica
< 1%

métodos dependientes de cultivo



MUESTREO

Procedimiento extracción ADN

Barcenilla et al., (2024)

Figura 8. Dinámica microbiana de los enriquecimientos de *Listeria* spp. en muestras positivas para *L. monocytogenes*.

Díaz et al., (2025)

¿Dónde buscamos?

TOMA DE DECISIONES

take-home messages



Cuidado con las bacterias en estado VBNC



Muestreo activo y con fricción adecuada



No confiar solo en cultivos negativos



Vigilancia focalizada en puntos críticos históricos



Analizar la microbiota residente



Rediseñar limpieza según datos microbianos

The background of the slide features a stylized DNA double helix structure. The helix is composed of two strands, one on the left and one on the right, rendered in various shades of teal and green. The strands are connected by horizontal rungs, also in similar shades, creating a three-dimensional effect. The overall color palette is a range of greens, from light mint to deep forest green.

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MUCHAS GRACIAS POR SU ATENCIÓN

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