

# Aplicaciones de los exopolisacáridos producidos por Bacterias Ácido Lácticas (BAL) en la industria quesera: retos y oportunidades

Dra. Claudia Yuritzi Figueroa Hernández

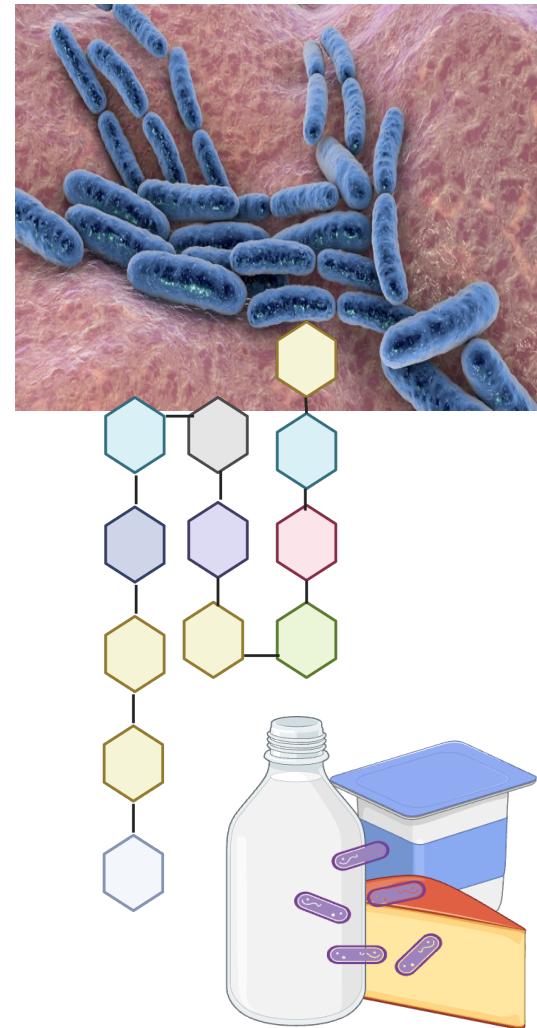
Dr. Hugo Sergio García Galindo

TECNM-Instituto Tecnológico de Veracruz



# Contenido de la presentación

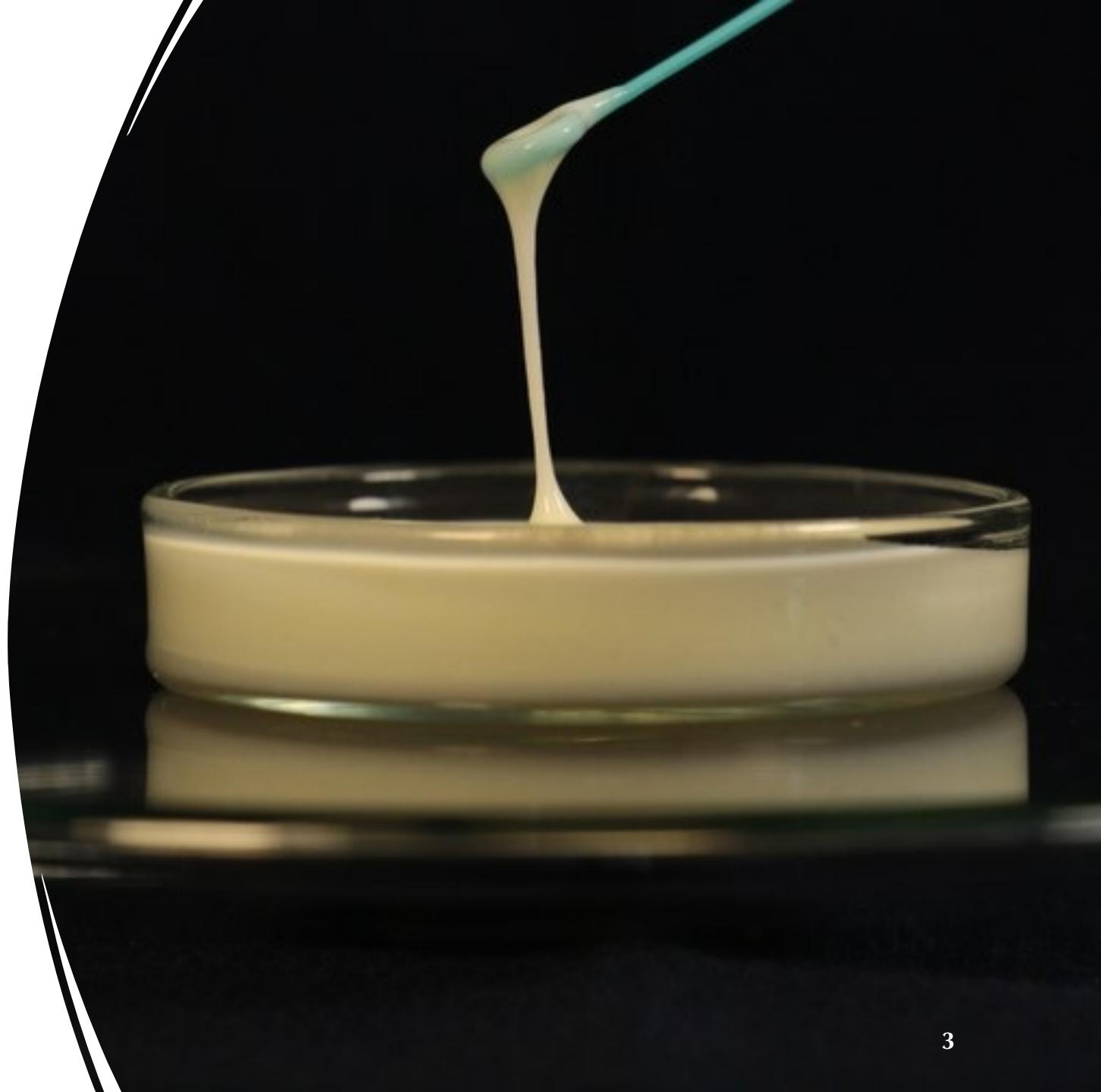
- ✓ **Introducción**
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- ✓ **Clasificación de los EPS producidos por las BAL**
- ✓ **Principales HoPSs producidos por las BAL**
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- ✓ **Síntesis y caracterización de EPS de BAL**
- ✓ **Principales efectos de la incorporación de EPS en los productos lácteos**
- ✓ **Estudios de aplicación de EPS en la formulación de quesos**
- ✓ **Retos y oportunidades**





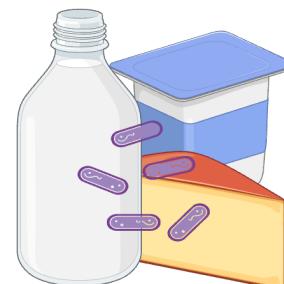
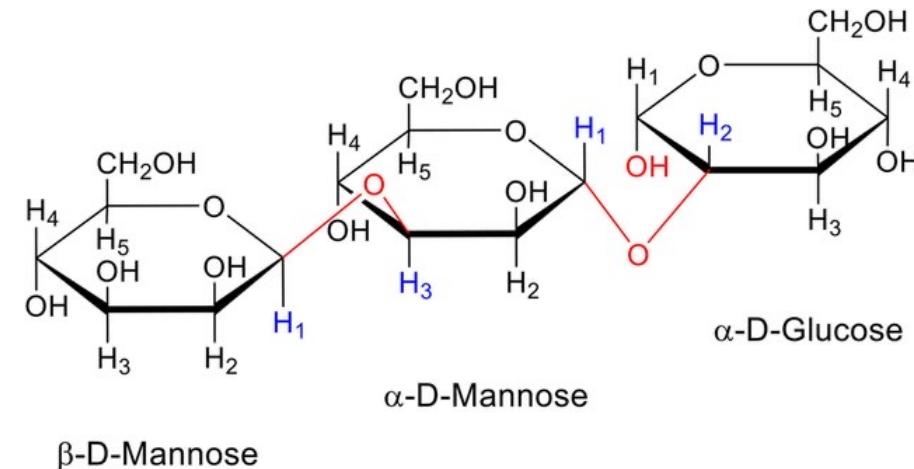
# Introducción

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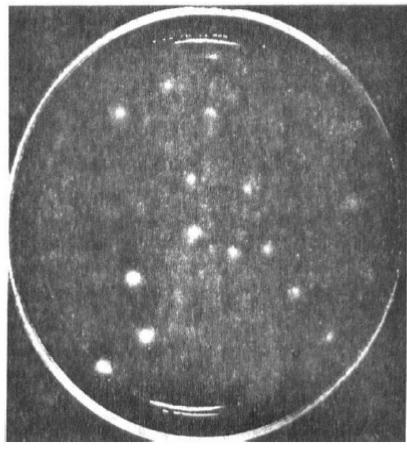
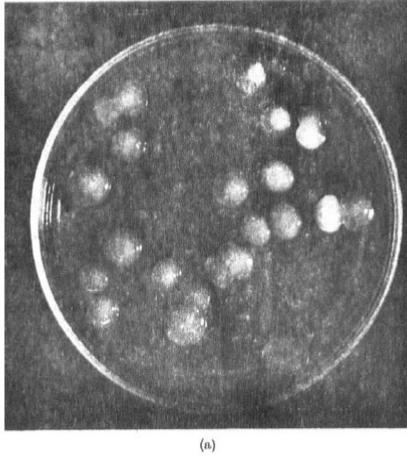


# ¿Qué es un exopolisacárido?

- ✓ Sustancias poliméricas de alto PM constituidas por carbohidratos sustituidos con proteínas, ADN, fosfolípidos y otros compuestos (acetato, glicerol, piruvato, carboxilato, succinatos y fosfatos).
- ✓ Metabolitos de hongos, **bacterias**, algas y levaduras, y son considerados como compuestos de valor agregado.



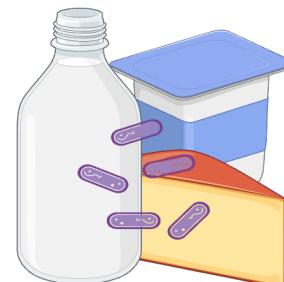
# Exopolisacáridos- primeros estudios



Fta. 1. (a) Mucoid colonies and (b) non-mucoid colonies of wild-type *Klebsiella aerogenes* grown on nutrient agar (72 hr. at 35°).

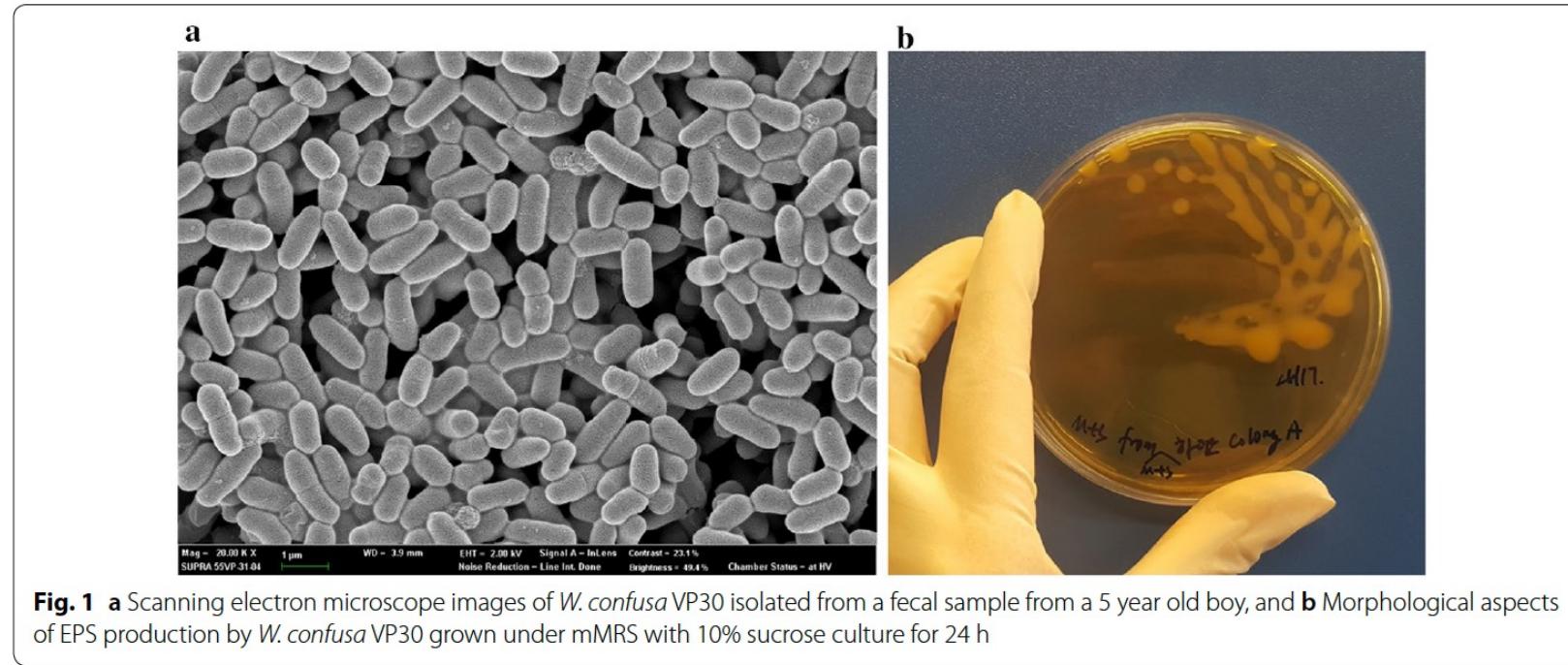
- ✓ El término fue introducido por primera vez por Sutherland en 1972 para designar los biopolímeros de carbohidratos de alto PM producidos por microorganismos.

Sutherland, 1972

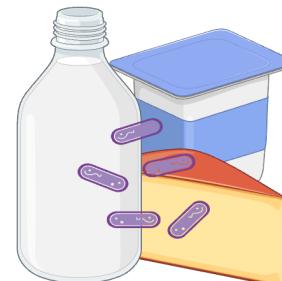


# EPS bacterianos comparados con EPS de otras fuentes

- ✓ Los EPS producidos por bacterias pueden obtenerse a escala industrial sin importar la estación ni las condiciones meteorológicas. Son limos extracelulares que se liberan fácilmente de las células al medio.



W: *Weisella*

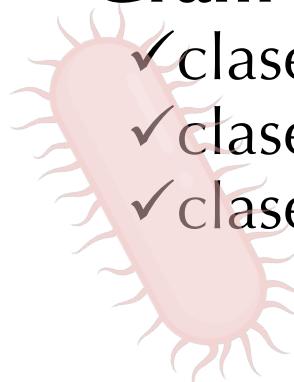


# Principales bacterias productoras de exopolisacáridos

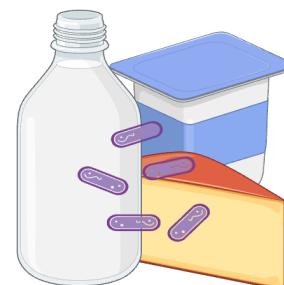
## ✓ Gram negativas

- ✓ clase **Alphaproteobacteria**: *Acetobacter*, *Gluconobacter*,  
*Gluconacetobacter*, *Komagataeibacter*, *Kozakia*, *Neoasaia*, *Agrobacterium*,  
*Rhizobium* y *Zymomonas*
- ✓ clase **Betaproteobacteria**: *Alcaligenes* y *Achromobacter*
- ✓ clase **Gammaproteobacteria**: *Azotobacter*, *Pseudomonas*, *Enterobacter*,  
*Alteromonas*, *Pseudoalteromonas*, *Xanthomonas*, *Halomonas*, *Erwinia*,  
*Vibrio* y *Klebsiella*.

## ✓ Gram-positivas

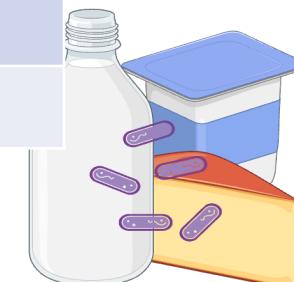


- ✓ clase **Bacilli**: *Bacillus*, *Paenibacillus*, **Lactobacillus**, **Leuconostoc**
- ✓ clase **Clostridia**: *Sarcina*
- ✓ clase **Actinomycetia**: *Bifidobacterium* y *Rhodococcus*.

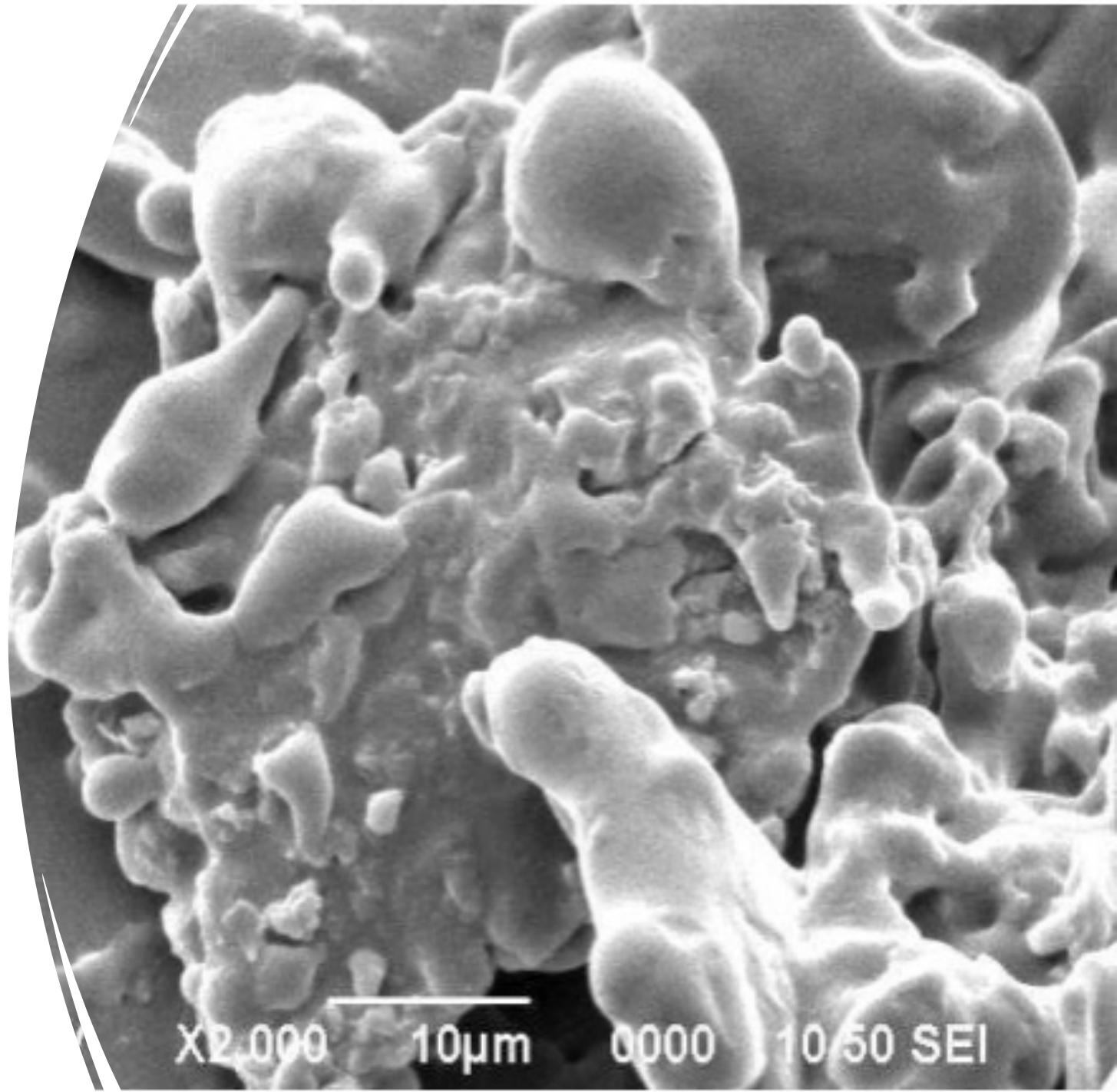


# Principales EPS bacterianos y sus aplicaciones en la industria alimentaria y farmacéutica

EPS de origen microbiano	Aplicaciones	Microorganismo productor
Xantana	Agente de estabilización de emulsiones y suspensiones en la industria alimentaria	<i>Xanthomonas campestris</i>
	Estabilizador de espuma en la industria alimentaria	
Ácido hialurónico	Agente hidratante en productos farmacéuticos y cosméticos	<i>Streptococcus zooepidemicus</i>
Dextrano	Agente espesante o gelificante, texturizante y estabilizante para emulsiones como el helado, jarabes, mermeladas, jugos, etc.	<i>Leuconostoc mesenteroides</i> y <i>Weisella cibaria</i>
Gelano	Agente gelificante utilizado en la industria alimentaria	<i>Sphingomonas elodea</i>
Curdlano	Agente gelificante	<i>Alcaligenes faecalis</i>

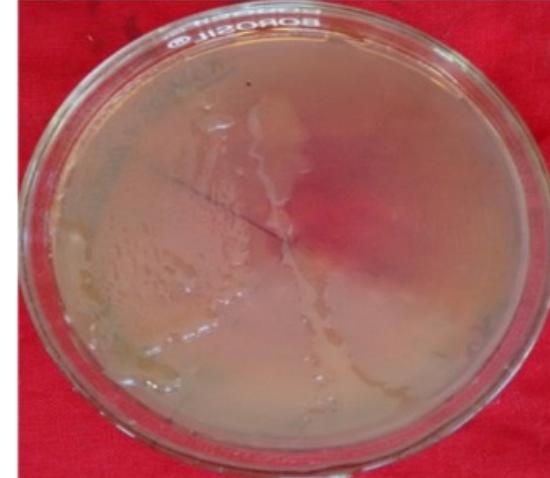


# BAL productoras de EPS



# BAL productoras de EPS

- ✓ Las BAL son microorganismos generalmente reconocidos como seguros (GRAS) tienen la capacidad de producir una amplia gama de EPS sin representar ningún riesgo para la salud.



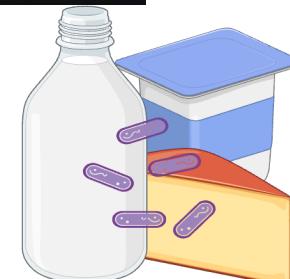
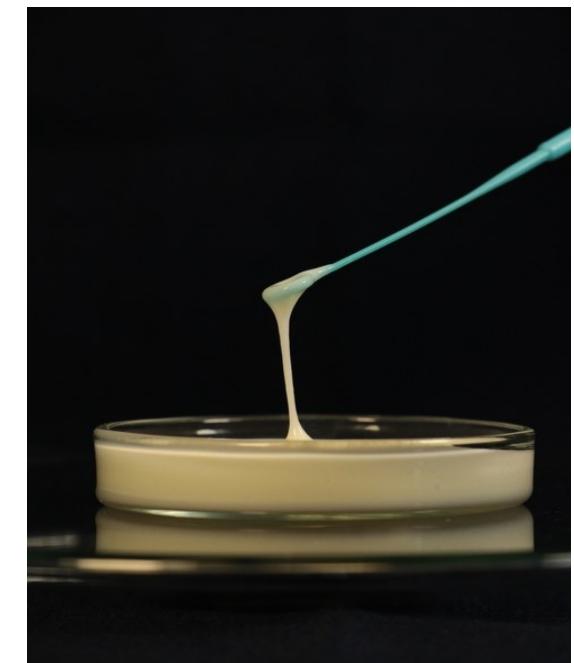
(a)

*Lpb.  
paraplantarum*



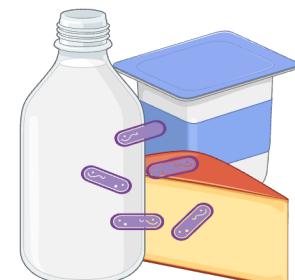
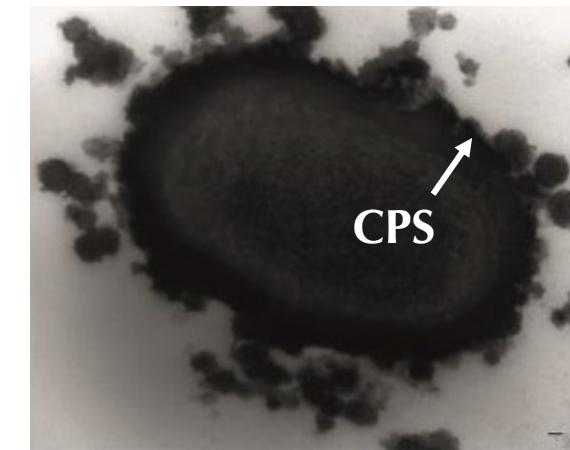
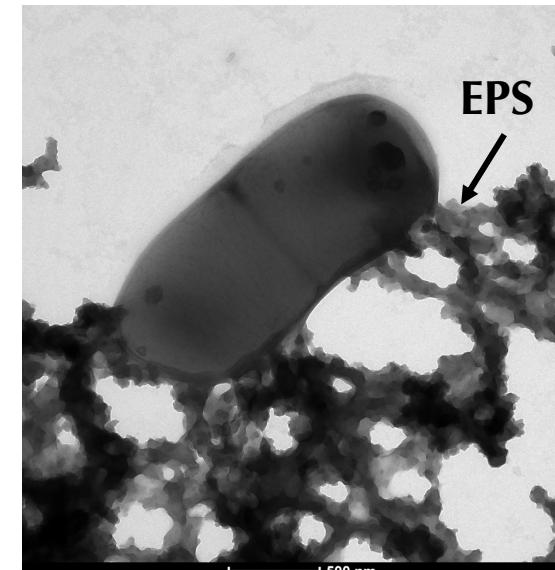
(b)

EPS producido por *Lpb.  
paraplantarum*



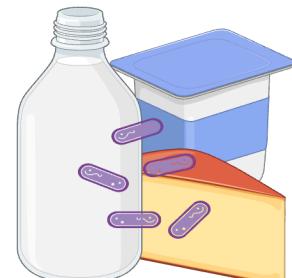
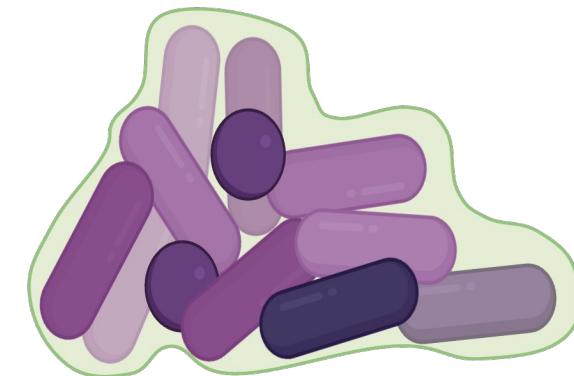
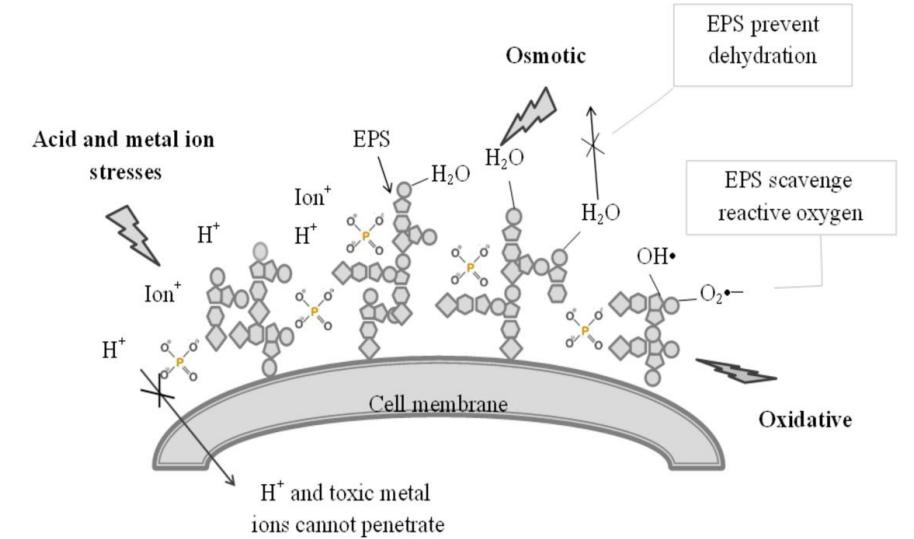
# BAL productoras de EPS

- ✓ Los EPS pueden ser secretados al exterior en forma de limo (EPS limoso) o bien pueden adherirse a la superficie bacteriana formando EPS capsulares (CPS).



# Funciones fisiológicas de los EPS

- ✓ **Protección del microorganismo frente a las condiciones adversas de su entorno, como la deshidratación, el estrés ácido o temperaturas desfavorables**
- ✓ **Brinda protección a la bacteria frente a mo's patógenos o antibióticos**
- ✓ **Brinda protección contra enzimas gástricas y pancreáticas**
- ✓ **Contribuyen a la formación de biopelículas**



# EPS en productos lácteos fermentados



Producto: Kéfir  
Microorganismo:  
*Lb. kefiranofaciens*  
EPS: Kefiran

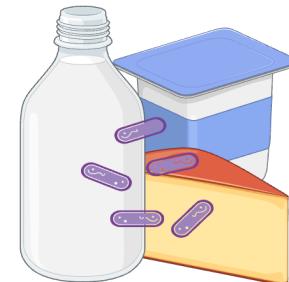


Producto: Viili  
Microorganismo: *Lc. lactis* subsp. *cremoris*  
EPS: Villian



Producto: Långfil  
Microorganismos: *Lc. lactis*  
biovar *longi* y  
*Leu. mesenteroides* subsp.  
*cremoris*

EPS: Heteropolisacárido  
capsular compuesto:  
ramnosa, glucosa,  
galactosa  
glicerol y fósforo

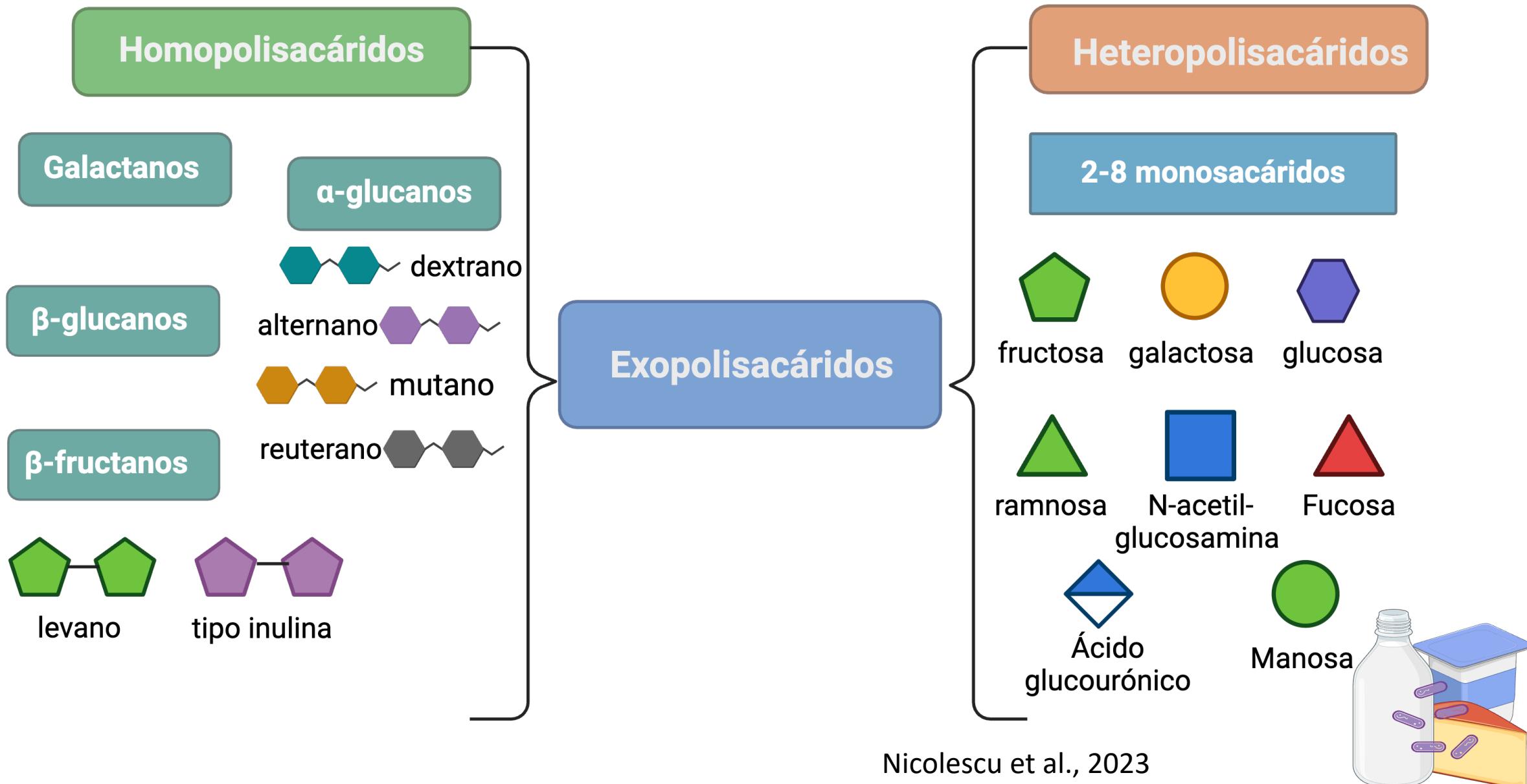


# Clasificación de los EPS producidos por las BAL

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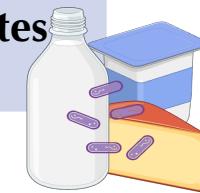


# Clasificación de los EPS producidos por BAL



# Principales características de los HoPSS y HePSS

Propiedades	Homopolisacáridos (HoPSS)	Heteropolisacáridos (HePSS)
Composición	Un solo monosacárido	Más de uno
	Principalmente compuestos por glucosa y fructosa	Principalmente compuestos por glucosa, galactosa, ramnosa
	No existen otros grupos	Existen otros grupos como acetilos y fosfatos
	No contiene grupos cargados	Puede contener grupos cargados
Tipo de enlaces	Tipo $\alpha$ o $\beta$	Tipo $\alpha$ o $\beta$
	Principalmente cadenas lineales, pero pueden tener ramificaciones	Principalmente ramificados
Producción y Sustrato utilizado	Producidos extracelularmente utilizando sacarosa o almidón	Producidos intracelularmente de intermediarios como UDP-glucosa y UDP-galactosa
Peso molecular	$> 10^6$ Da	$10^4$ - $10^6$ Da
Microorganismos productores	<i>Lactobacillus</i> , <i>Leuconostoc</i> , <i>Streptococcus</i> , <i>Oenococcus</i> , <i>Pediococcus</i> y <i>Weisella</i>	<i>Lactobacillus</i> , <i>Streptococcus</i> , y <i>Lactococcus</i>
Rendimiento	Generalmente producidos en altas cantidades (g/L)	Generalmente producidos en bajas cantidades (mg/L)
Funcionalidad	Pueden actuar como hidrocoloides, agentes ligadores de agua y prebióticos	Pueden actuar como moduladores inmunes, agentes anticancerígenos y antioxidantes

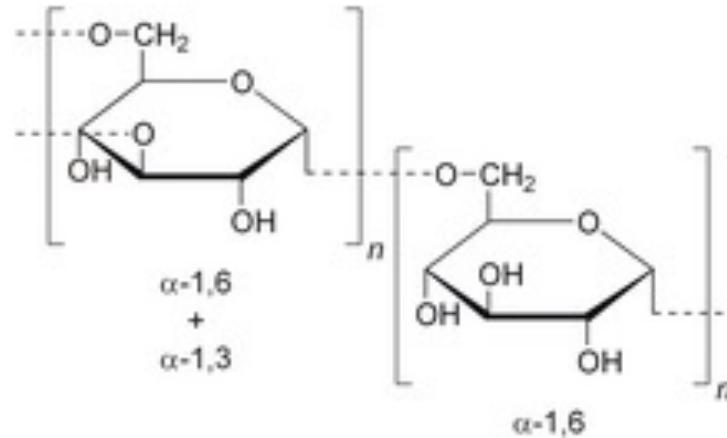


# Principales HoPSs producidos por las BAL

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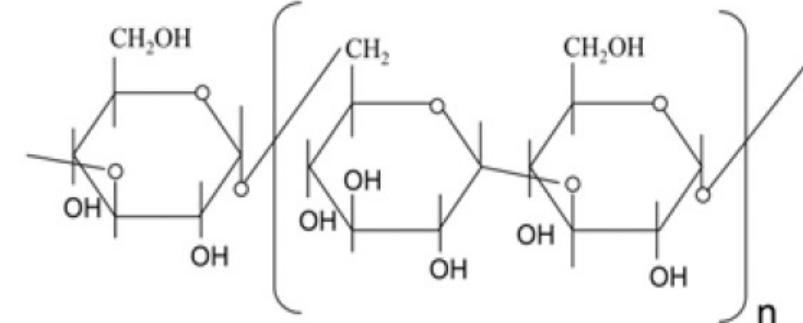


# Principales HoPSs producidos por cepas de BAL



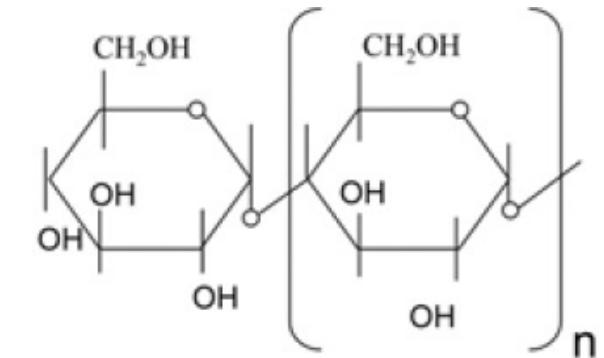
## Dextrano

BAL productoras: *Leu. mesenteroides*, *Leu. pseudomesenteroides*, *Leu. citreum*, *Lb. kefiranofaciens*, *Lcb. casei*, *Lat. curvatus*, *Lat. sakei*, *Lim. fermentum*, *Lim. reuteri*, *P. pentosaceus*, *W. cibaria* y *W. confusa*



## Alternano

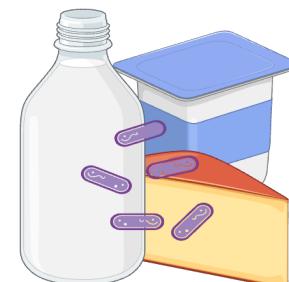
BAL productoras: *Leu. mesenteroides*, *Leu. citreum*



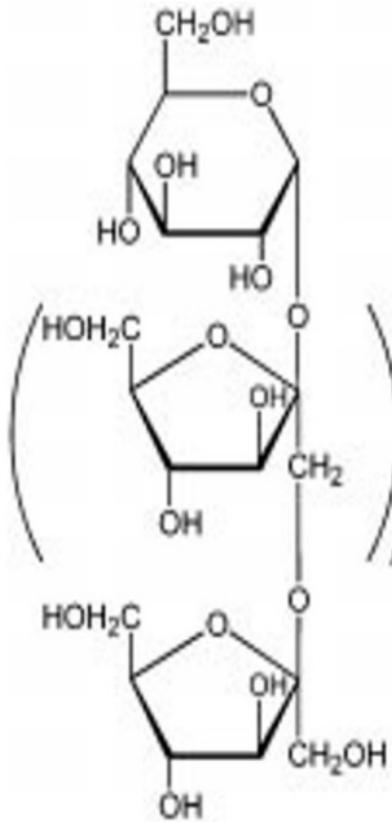
## Reuterano

BAL productoras: *Lim. reuteri*

## $\alpha$ -Glucanos

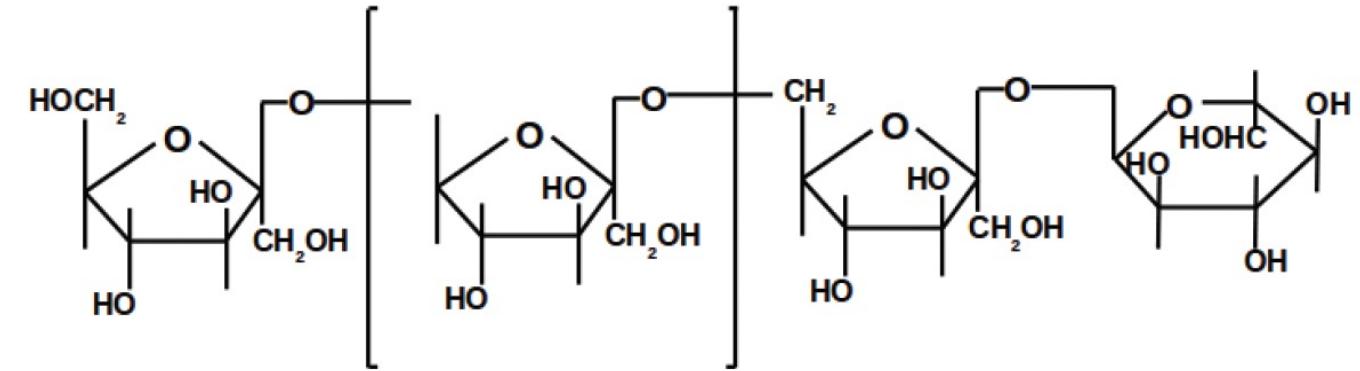


# Principales HoPSs producidos por cepas de BAL



**Inulina**

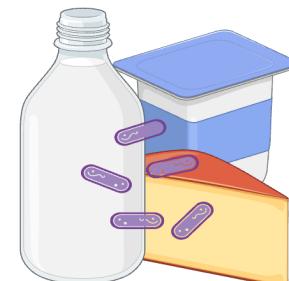
BAL productoras: *Lb. johnsonii*, *Lim. reuteri*, *Leu. citreum*



**Levano**

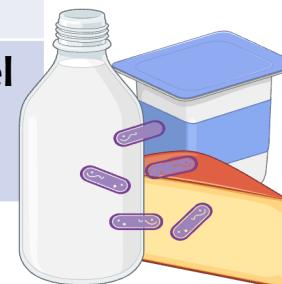
BAL productores: *Lim. reuteri*,  
*Lim. frumenti*, *F. sanfranciscensis*, *Leu. mesenteroides*, *S. salivarius*

**$\beta$ -Fructanos**



# Efecto de la incorporación de HoPSs producidos por BAL en alimentos

Clasificación	HoPSs/Composición/PM	Efecto de su incorporación en alimentos
$\alpha$ -Glucanos	Dextrano $\alpha$ -D-GlcP (1 → 6) Mw: $10^3$ – $10^7$ Da	Mejora la suavidad, la textura de la miga y el volumen de los panes en productos de panadería
		Evita la cristalización, mejora la retención de humedad y aumenta la viscosidad en la fabricación de dulces o helados
		Coadyuvante, emulsionante y estabilizador
	Alternano $\alpha$ -D-GlcP (1 → 3)/ $\alpha$ -D-GlcP (1 → 6) Mw: > $10^6$ Da	Edulcorante en productos de confitería
		Estabilizante y emulsionante
		Prebióticos
	Reuterano $\alpha$ -D-GlcP (1 → 4) Mw: $10^7$ Da	Utilizado en productos de panadería, mejora la suavidad y retrasa el endurecimiento
	Inulina $\beta$ -D-FruP (2 → 1) Mw: $10^3$ – $10^7$ Da	Prebiótico y sustituto del azúcar (en combinación con otros edulcorantes), sustituto de la grasa y modificador de la textura en productos lácteos bajos en grasa. Mejora la sensación en boca y la cremosidad
	Levano $\beta$ -D-FruP (2 → 6) Mw: $10^4$ – $10^8$ Da	Prebióticos, emulsionantes, estabilizadores y espesantes. Aumenta el volumen, retarda el endurecimiento y mejora textura, suavidad y textura de la miga en productos de panadería



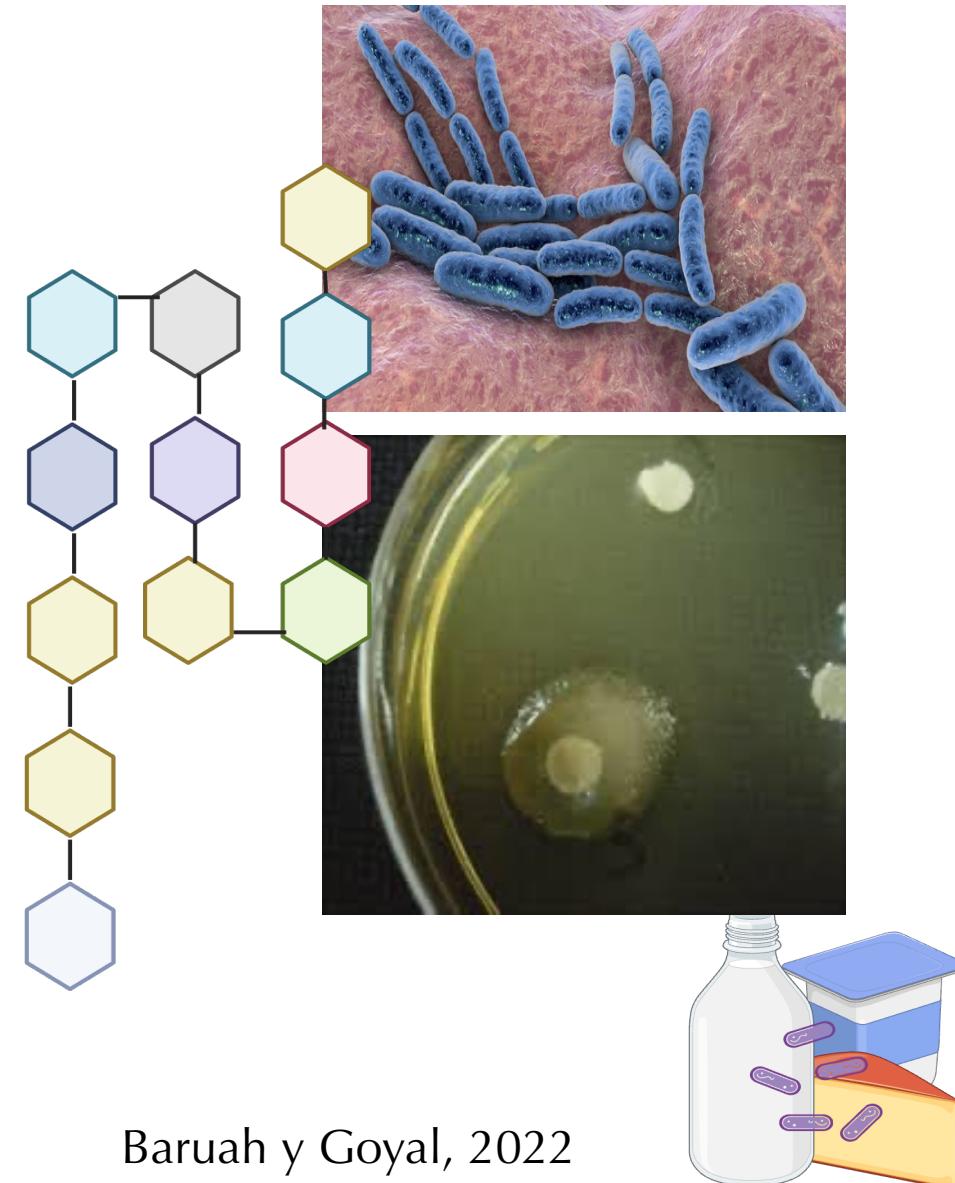
# Principales HePSs producidos por las BAL

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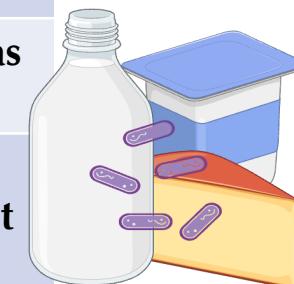
# Principales BAL productoras de HePSs

Género	Especies
<i>Lactococcus</i>	<i>Lc. lactis subsp. cremoris</i>
<i>Lactobacillus</i>	<i>Lb. acidophilus, Lb. delbrueckii subsp. bulgaricus, Lb. helveticus</i>
<i>Latilactobacillus</i>	<i>Lat. curvatus, Lat. sakei</i>
<i>Lacticaseibacillus</i>	<i>Lcb. paracasei, Lcb. rhamnosus</i>
<i>Fructolactobacillus</i>	<i>F. sanfranciscensis</i>
<i>Streptococcus</i>	<i>S. macedonius, S. termophilus</i>



# Efecto de la incorporación de HePSs producidos por BAL en alimentos

BAL	Composición monomérica y PM (Da) del HePS	Propiedades tecno-funcionales
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> DGCC291	Glucosa, galactosa $1.4 \times 10^6$	Incrementan la viscosidad y disminuyen la sinéresis en yogurt
<i>Lb. helveticus</i>	Glucosa, manosa, galactosa, ramnosa, arabinosa $1.8 \times 10^5$	Agente antioxidante y prebiótico
<i>Lentilactobacillus kefiri</i>	Kefiran: glucosa, galactosa $7.6 \times 10^5$	Prebiótico, agente gelificante y texturizante
<i>Lpb. plantarum</i> C70	Arabinosa, manosa, glucosa, galactosa $3.8 \times 10^5$	Incrementa la textura y las propiedades reológicas
<i>Lpb. plantarum</i> C88	Glucosa, galactosa $1.2 \times 10^6$	Antioxidante y prebiótico
<i>Lpb. plantarum</i> JLK0142	Glucosa, galactosa $1.3 \times 10^5$	Aumenta la calidad de los quesos bajos en grasa
<i>Lpb. plantarum</i> YW11	Glucosa, galactosa $1.1 \times 10^5$	Incrementa la viscosidad en la leche desnatada
<i>S. thermophilus</i> AR333	Galactosa, glucosa, galactosamina $3.1 \times 10^5$	Incrementa la calidad en leches fermentadas
<i>S. thermophilus</i> NIZO 2104	Galactosa, ribosa, glucosa, N-acetil-galactosamina $9.0 \times 10^5$	Aumenta la viscosidad y el módulo de elasticidad, disminuye la sinéresis en yogurt





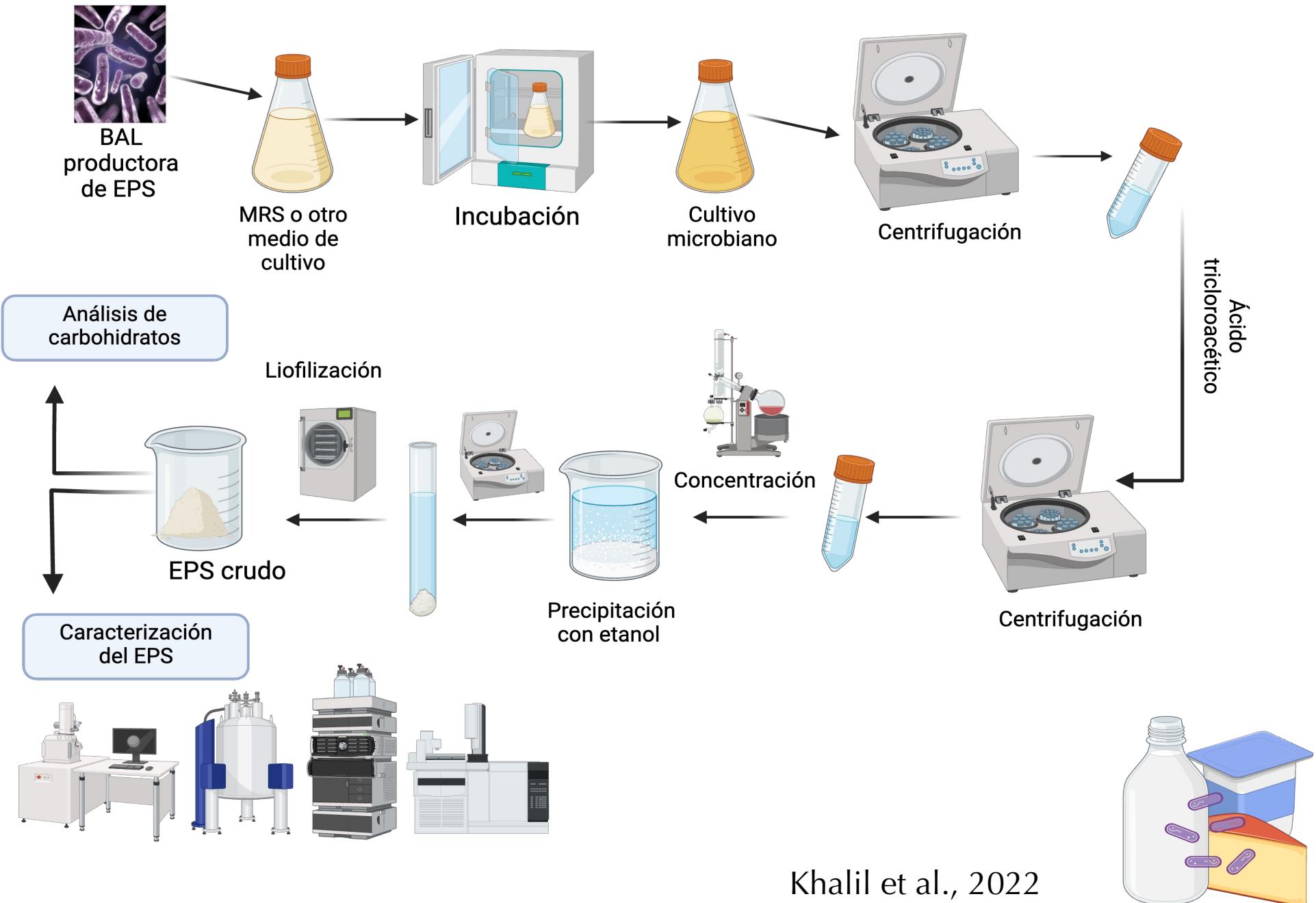
# Síntesis y caracterización de EPS

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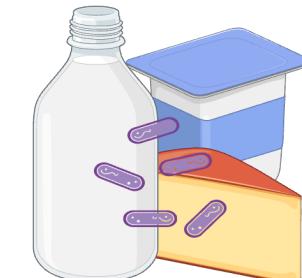
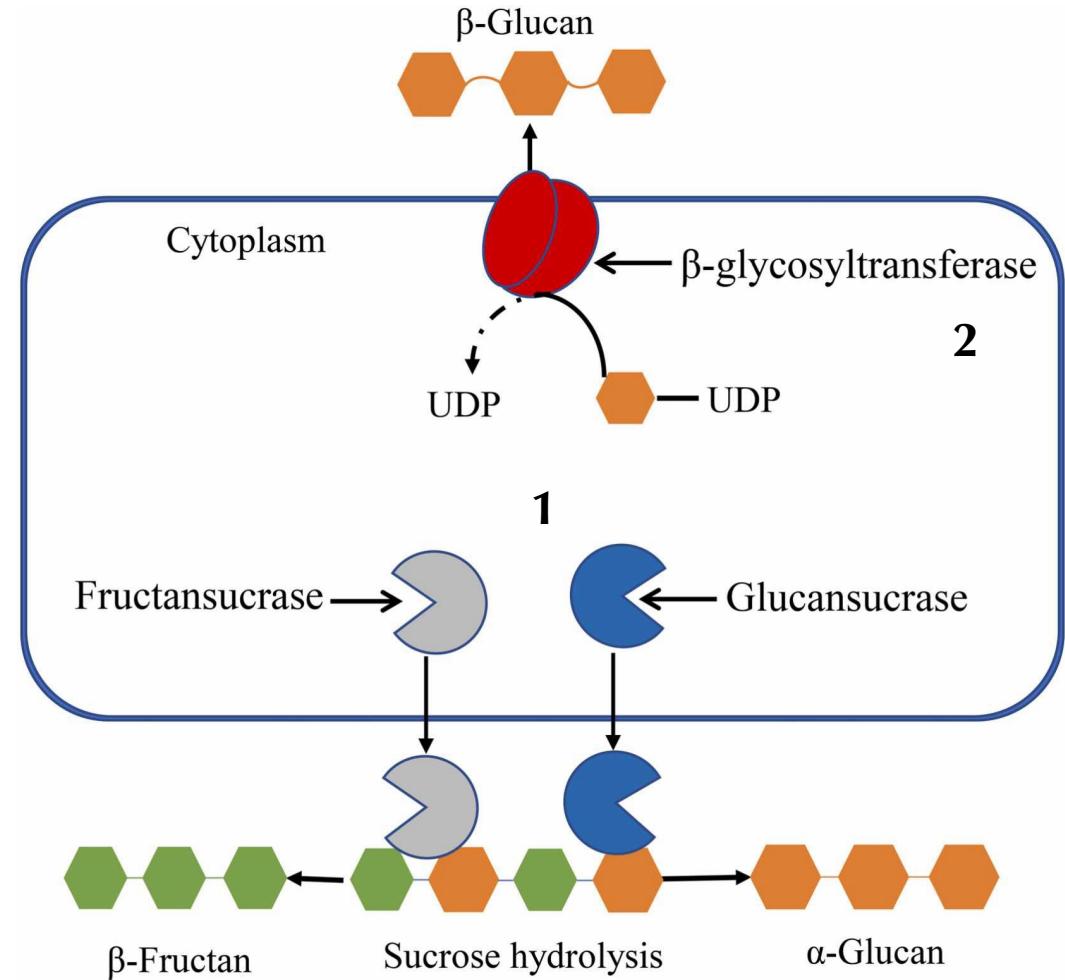
# Metodología general de síntesis y caracterización de EPS de BAL



# Síntesis de HoPSs en las BAL

Las BAL utilizan la vía de síntesis extracelular para sintetizar HoPSs. Esta vía consta de dos pasos:

- 1) **Hidrólisis de un sustrato: como sacarosa**
- 2) **Unión de los residuos de monosacáridos a la cadena aceptora del aglicano, seguido de la liberación directa de las cadenas polimerizadas al ambiente extracelular**

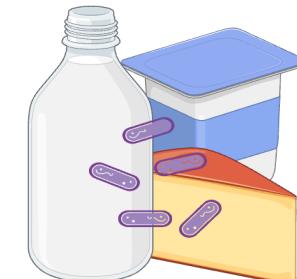
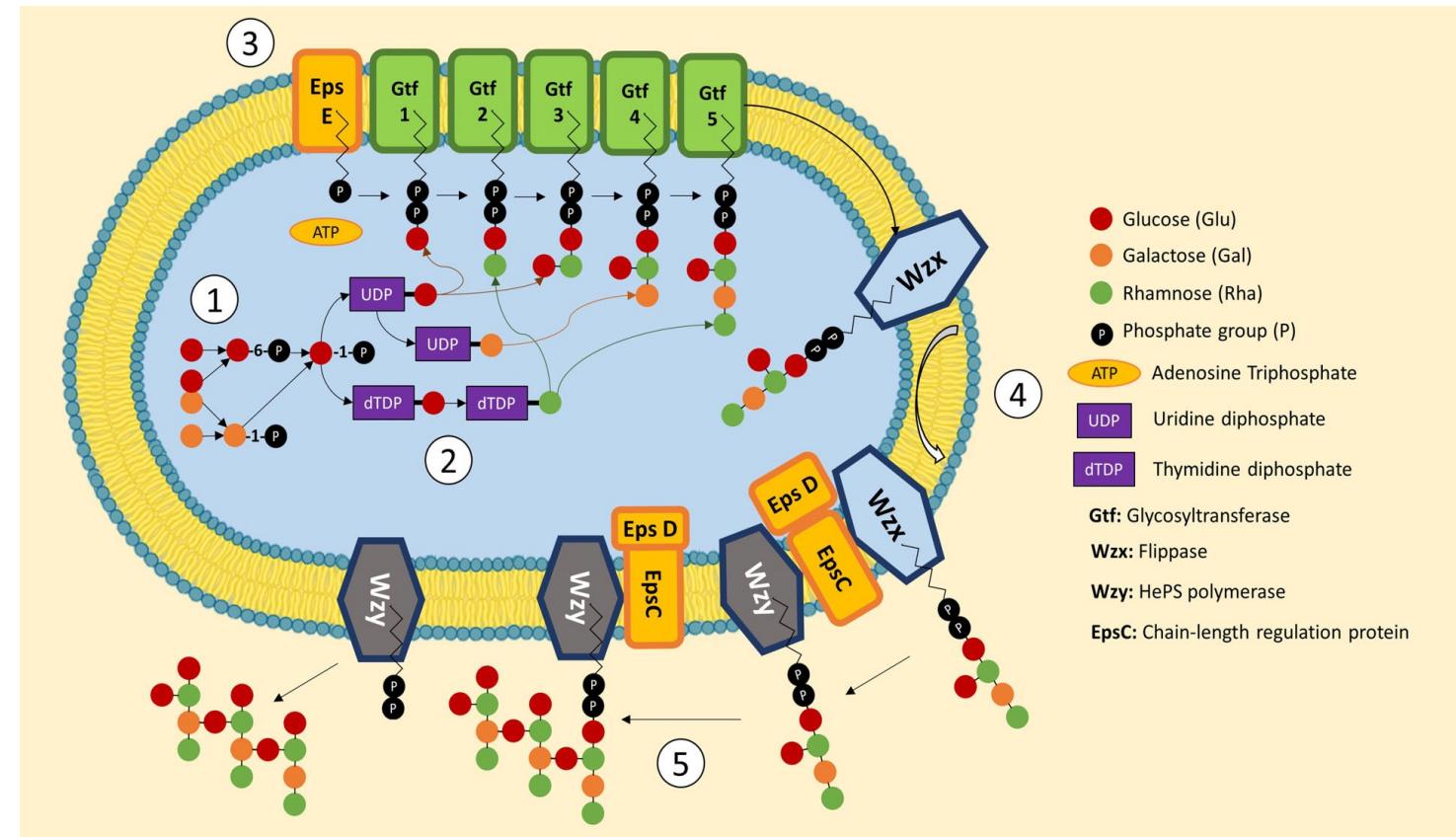




# Síntesis de HePSs en las BAL

Se realiza a través de la vía Wzy-dependiente, la cual consta de cinco fases principales

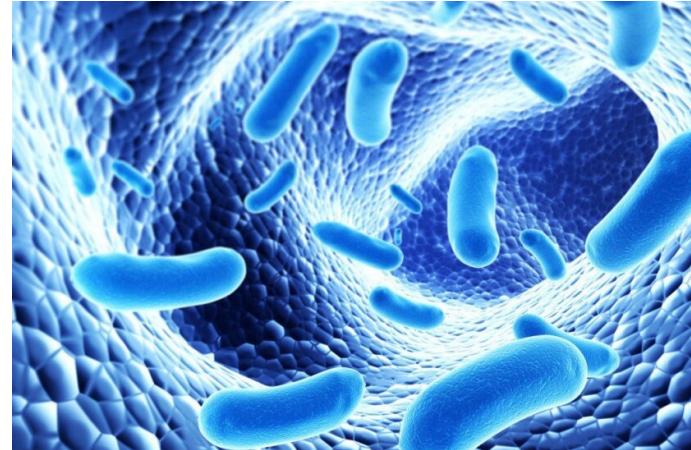
1. Transporte de azúcares al citoplasma
2. Síntesis de nucleótidos de azúcar
3. Formación de unidades repetitivas y unión al lípido acarreador de glicósidos
4. Translocación de unidades repetitivas a la superficie extracelular
5. Polimerización extracelular de heteropolisacáridos



# Principales factores que influyen en la síntesis de EPS (HoPSs y HePSs)

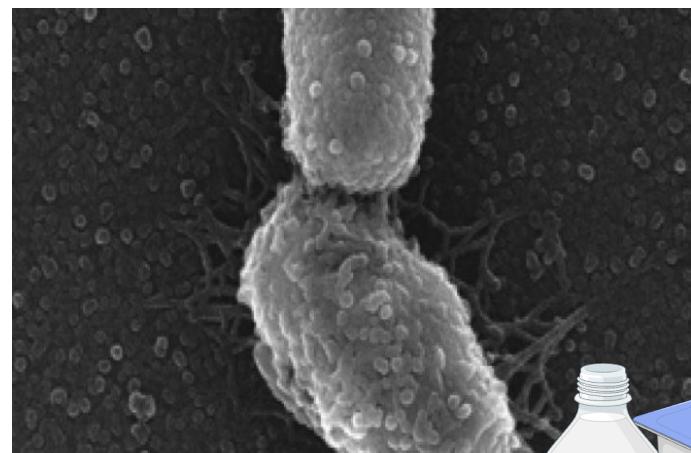
## ✓ Factores microbiológicos

- Microorganismos productores de EPS (HopSSs y HePSs)- **Especies/cepas**
- Crecimiento microbiano- **Fase exponencial/Estacionaria**
- Interacciones microbianas- **Sinergismo/ Antagonismo**



## ✓ Composición del medio de cultivo

- Fuente de carbono – **Glucosa/Lactosa/Sacarosa, etc.**
- Fuente de nitrógeno **Orgánico/Inorgánico**



## ✓ Parámetros fisicoquímicos

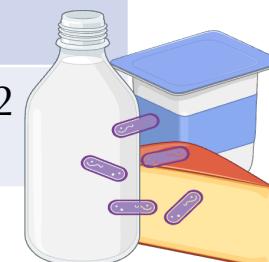
- pH
- Temperatura
- Nivel de oxigenación
- Tiempo de incubación



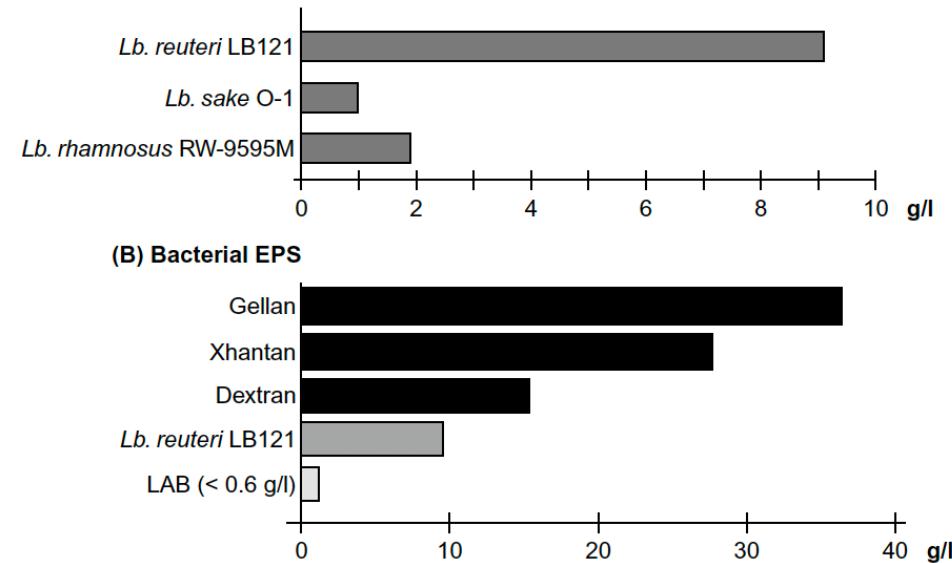
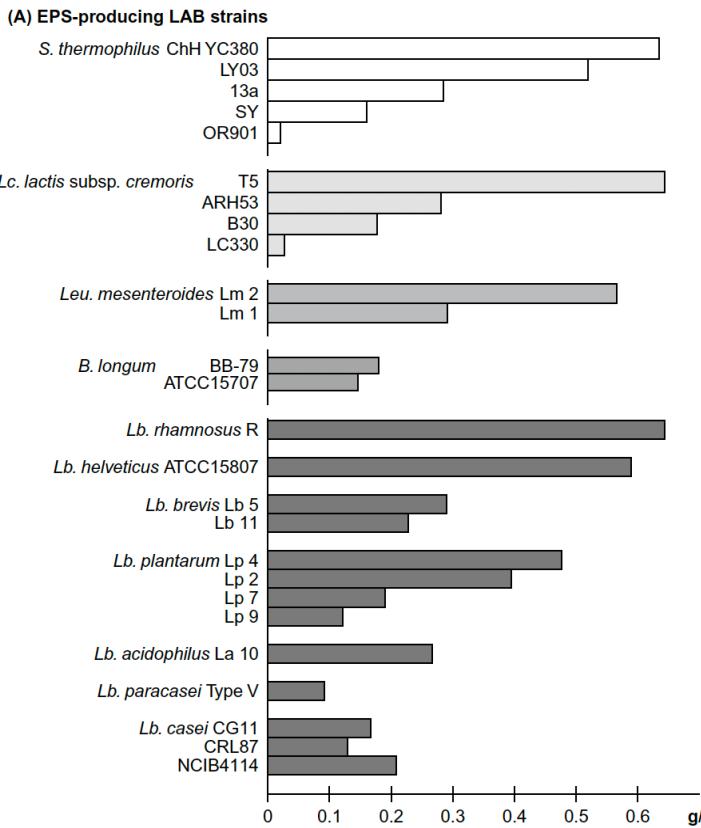
# Condiciones de cultivo para la síntesis de EPS por BAL

BAL	Medio de cultivo	Temperatura (°C)	Tiempo (h)	pH	Rendimiento (mg/L)	Referencia
<i>Lcb. rhamnosus</i> 9595 M	MBM	32-37	72	6	~1000	Dupont et al., 2000
<i>Lb. delbrueckii bulgaricus</i> RR	Suero de leche	38	24-28	5	95-110	Briczinski y Roberts, 2002
<i>Lcb. rhamnosus</i> R	MBM	37	72	6	500	Pham et al., 2000
<i>Lb. delbrueckii bulgaricus</i>	Leche	42	24	/	110	Bouzar et al., 1996
<i>Lb. delbrueckii bulgaricus</i>	MRS	40	18	/	263	Aslim et al., 2005
<i>Lcb. rhamnosus</i> GG	Leche	37	20	/	80	Faber et al., 2001
<i>Lcb. casei</i> CG11	MBM	25	48	/	130	Cerninig et al., 1994
<i>Lb. helveticus</i>	Leche descremada	37	60	5	730	Ling y Cheng, 2007
<i>Lcb. delbrueckii bulgaricus</i>	Suero libre de proteína	37	18	6	800	Shanen y Bravon, 2007
<i>Lcb. rhamnosus</i> 9595	Permeado de suero de leche	37	24	6	2775	Macedo et al., 2002

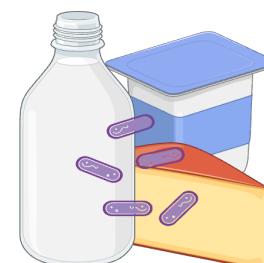
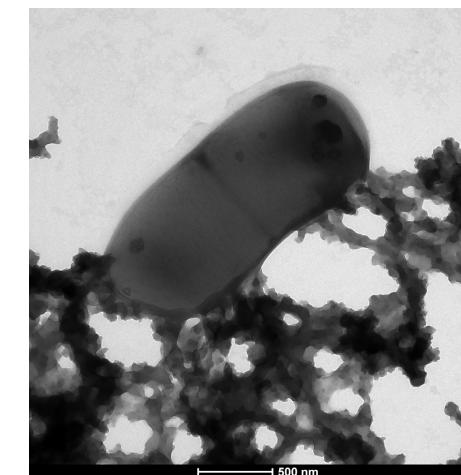
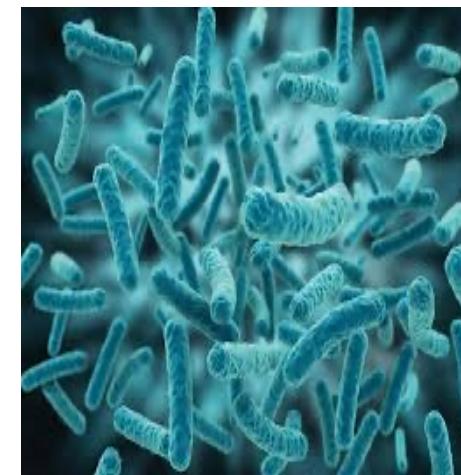
MBM: Medio Basal mínimo



# Comparación del rendimiento en la producción de EPS



**FIGURE 45.1** Comparación del rendimiento en la producción de EPS (g/l) obtenido de varias cepas de LAB productoras de EPS (A) y de otras bacterias productoras de EPS para aplicaciones alimentarias (B). El gelan es producido por *Sphingomonas paucimobilis* y xantán por *Xanthomonas campestris*, ambas especies Gram-negativas, mientras que el dextrano es producido por la bacteria Gram-positiva LAB, *Leuconostoc mesenteroides*.



# Caracterización de EPS producidos por BAL

Técnica	Información obtenida	Referencia
Resonancia Magnética Nuclear ( <sup>1</sup> H y <sup>13</sup> C)	Configuración molecular, enlaces y enlaces cruzados, orientación de la molécula de azúcar, análisis de la estructura.	Kavitake et al., 2016; Ismail et al., 2010
Microscopía electrónica de barrido (SEM) y Microscopía electrónica de transmisión (TEM)	Aspecto de los gránulos, microestructura y morfología de la superficie	Wang et al., 2010
Microscopía de fuerza atómica (AFM)	Conformación y estructura molecular	Ahmed et al., 2013
Difracción de rayos X	Estructura del monosacárido	Andhare et al., 2014
GC-MS	Composición y configuración de los monosacáridos	Wang et al., 2014
Microscopía electrónica por transformada de Fourier (FTIR), espectros de Raman	Detección de grupos estructurales y funcionales, identificación de compuestos específicos	Wang et al., 2014; Landersjö et al., 2002
Cromatografía líquida de alta presión (HPLC)	Purificación e identificación de monosacáridos	Kavitake et al., 2016; Sathiyanarayanan et al., 2015
Análisis Termogravimétrico (TGA) y Calorimetría diferencial de barrido (DSC)	Estabilidad térmica (efecto de la temperatura en la pérdida de peso)	Kavitake et al., 2016, 2020

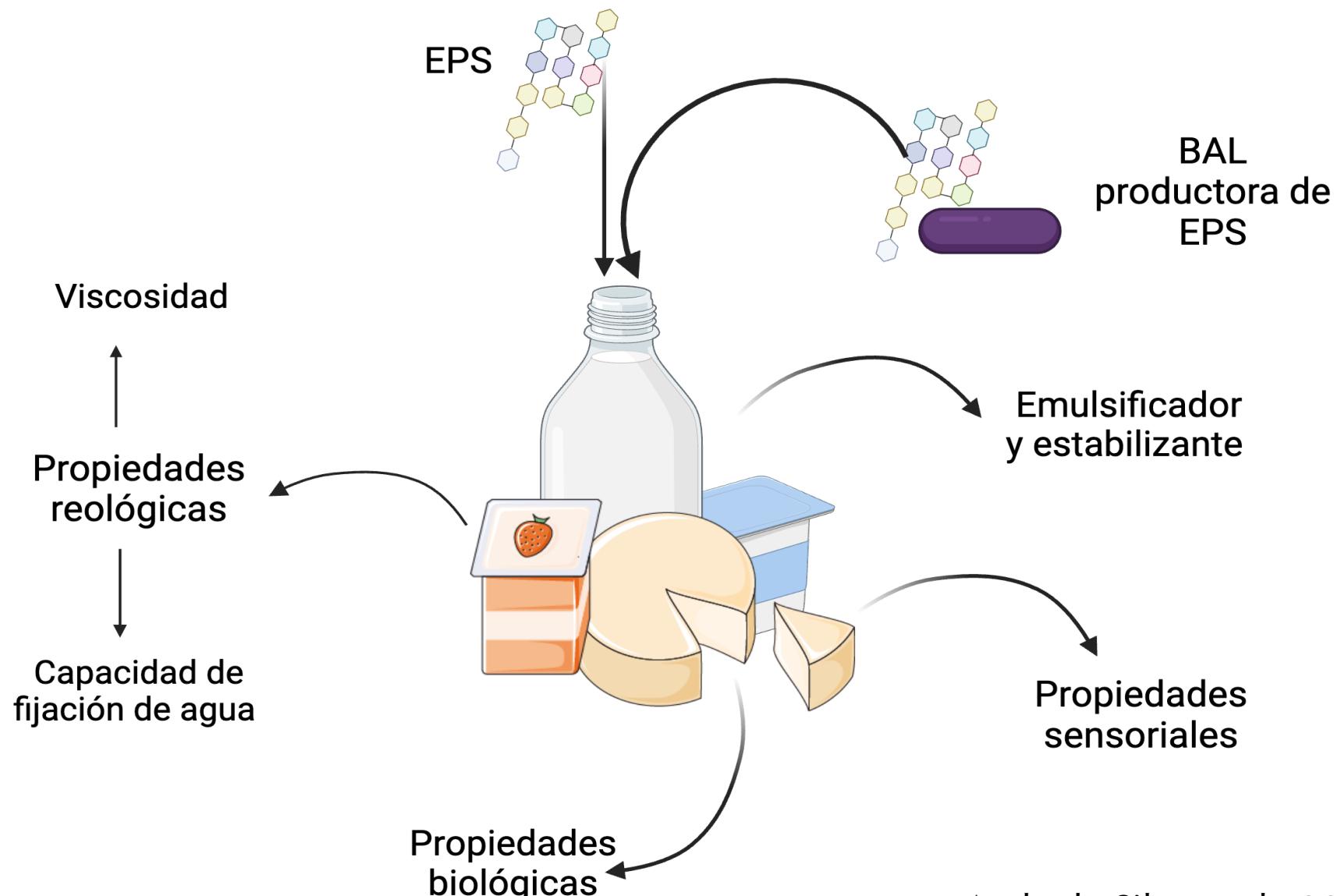




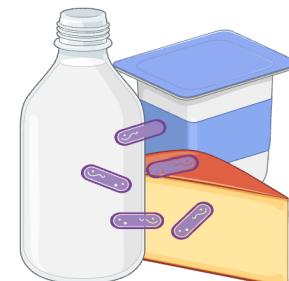
# Principales efectos de la incorporación de EPS en los productos lácteos



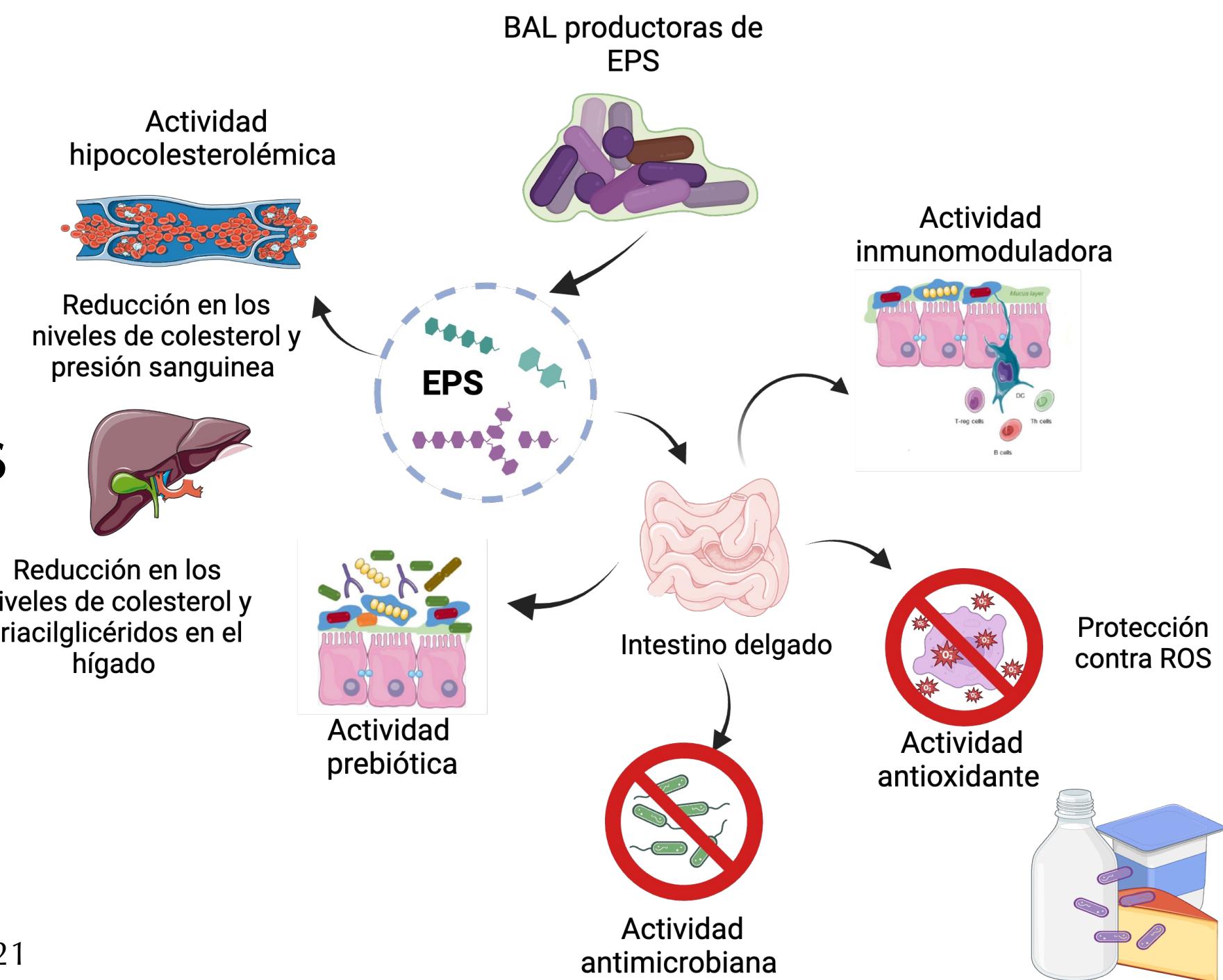
# Efecto de los EPS en las propiedades tecno-funcionales de los productos lácteos



Andrade-Silva et al., 2019

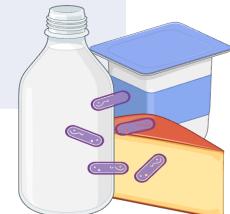


# Actividades biológicas de los EPS



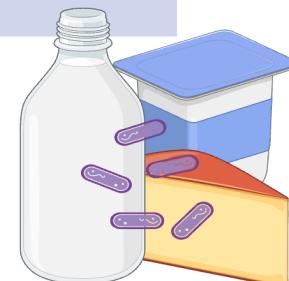
# Efectos de los EPS en los productos lácteos - Ejemplos

Microorganismo productor de EPS	Alimento	Efectos principales	Referencias
<i>S. thermophilus</i>	Ayran - Bebida turca a base de yogurt	Incrementa la viscosidad aparente	Yilmaz et al., 2015
<i>S. thermophilus</i>	Queso italiano bajo en grasa tipo Caciotta	Incrementa el sabor, gusto y la masticabilidad	Di Cagno et al., 2014
<i>S. thermophilus</i> ST-5581, ST-4239 y <i>Lc. lactis</i> subsp. <i>cremoris</i> JFR1	Leche fermentada	Disminuye la sinéresis, incrementa la viscosidad, disminuye la incidencia de cáncer de colon y la actividad de la enzima ciclooxygenasa-2	Purohit et al., 2009
<i>S. thermophilus</i> ASCC 1275	Yogurt batido	Disminuye firmeza y sinéresis	Amatayakul et al, 2006
<i>S. thermophilus</i> zlw TM11	Yogurt	Aumenta la textura del yogurt y disminuye la separación del suero	Han et al., 2016
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 147 y 2214	Leche fermentada	Aumenta viscosidad, disminuye sinéresis, y aumenta la capacidad de fijación de agua	Bancalari et al., 2020



# Efectos de los EPS en los productos lácteos- Ejemplos

Microorganismo productor de EPS	Alimento	Efectos principales	Referencias
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> CNRZ 1187 y 416	Leche fermentada	Aumenta viscosidad	Petry et al., 2003
<i>Lpb. plantarum</i> KX881772 y KX881779	Queso Akawi bajo en grasa	Aumenta la capacidad de fijación de agua, incrementa la estructura elástica, reduce la dureza y adhesividad, genera altos puntajes en apariencia y calidad global	Ayyash et al., 2018
<i>Lim. fermentum</i> Lf2	Yogurt sin grasa	Incrementa la viscosidad aparente y aumenta la capacidad de retención de agua	Ale et al., 2016
<i>Lim. mucosae</i> DPC 6426	Yogurt bajo en grasa	Aumenta la viscosidad y reduce la sinéresis	London et al., 2015
<i>Lcb. rhamnosus</i> JAAS8	Yogurt	Incrementa la viscosidad aparente y la capacidad de fijación de agua	Yang et al., 2010



# Estudios de aplicación de EPS en la formulación de quesos

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# Estudios de aplicación de EPS en la formulación de quesos

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## Improvement of Texture and Structure of Reduced-Fat Cheddar Cheese by Exopolysaccharide-Producing Lactococci

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**Table 4.** Changes in textural characteristics and protein-occupied area (mean  $\pm$  SD) in cheese matrix during ripening of experimental full- and reduced-fat Cheddar cheeses

Cheese <sup>1</sup>	Cheese age (mo)	Textural characteristics <sup>2</sup>				
		Hardness, N	Fracturability, N	Springiness	Cohesiveness	Protein-occupied area, % <sup>3</sup>
CFF	0	27.2 $\pm$ 1.9 <sup>i</sup>	25.8 $\pm$ 2.4 <sup>b</sup>	0.598 $\pm$ 0.051 <sup>e</sup>	0.278 $\pm$ 0.025 <sup>a</sup>	46.3 $\pm$ 3.4 <sup>g</sup>
	1	26.0 $\pm$ 2.5 <sup>j</sup>	14.6 $\pm$ 1.4 <sup>ijk</sup>	0.281 $\pm$ 0.088 <sup>n</sup>	0.182 $\pm$ 0.023 <sup>o</sup>	ND <sup>d</sup>
	3	31.9 $\pm$ 2.2 <sup>fg</sup>	14.6 $\pm$ 1.9 <sup>ijk</sup>	0.233 $\pm$ 0.047 <sup>s</sup>	0.194 $\pm$ 0.011 <sup>l</sup>	ND
	6	33.8 $\pm$ 3.2 <sup>d</sup>	15.7 $\pm$ 1.3 <sup>hi</sup>	0.216 $\pm$ 0.041 <sup>u</sup>	0.180 $\pm$ 0.006 <sup>p</sup>	57.4 $\pm$ 3.6 <sup>cd</sup>
CLF	0	37.8 $\pm$ 3.6 <sup>b</sup>	29.2 $\pm$ 2.7 <sup>a</sup>	0.726 $\pm$ 0.060 <sup>b</sup>	0.272 $\pm$ 0.029 <sup>b</sup>	58.0 $\pm$ 2.5 <sup>cd</sup>
	1	33.8 $\pm$ 3.2 <sup>de</sup>	23.5 $\pm$ 2.6 <sup>c</sup>	0.386 $\pm$ 0.038 <sup>g</sup>	0.236 $\pm$ 0.012 <sup>e</sup>	ND
	3	35.9 $\pm$ 1.8 <sup>c</sup>	17.5 $\pm$ 2.6 <sup>def</sup>	0.277 $\pm$ 0.05 <sup>o</sup>	0.203 $\pm$ 0.010 <sup>k</sup>	ND
	6	38.0 $\pm$ 3.3 <sup>b</sup>	17.9 $\pm$ 2.0 <sup>de</sup>	0.260 $\pm$ 0.027 <sup>p</sup>	0.187 $\pm$ 0.008 <sup>n</sup>	67.3 $\pm$ 2.9 <sup>a</sup>
SMQ	0	32.4 $\pm$ 2.4 <sup>ef</sup>	23.6 $\pm$ 2.4 <sup>c</sup>	0.708 $\pm$ 0.061 <sup>e</sup>	0.229 $\pm$ 0.021 <sup>f</sup>	55.6 $\pm$ 3.0 <sup>de</sup>
	1	30.6 $\pm$ 2.9 <sup>gh</sup>	17.1 $\pm$ 1.4 <sup>efg</sup>	0.331 $\pm$ 0.039 <sup>j</sup>	0.212 $\pm$ 0.008 <sup>h</sup>	ND
	3	34.9 $\pm$ 2.6 <sup>cd</sup>	18.2 $\pm$ 2.5 <sup>de</sup>	0.277 $\pm$ 0.041 <sup>q</sup>	0.210 $\pm$ 0.018 <sup>i</sup>	ND
	6	39.2 $\pm$ 2.7 <sup>ab</sup>	18.7 $\pm$ 2.0 <sup>d</sup>	0.243 $\pm$ 0.037 <sup>q</sup>	0.207 $\pm$ 0.013 <sup>j</sup>	63.2 $\pm$ 2.6 <sup>ab</sup>
JRF	0	24.0 $\pm$ 2.8 <sup>jk</sup>	10.0 $\pm$ 0.4 <sup>l</sup>	0.646 $\pm$ 0.044 <sup>d</sup>	0.242 $\pm$ 0.022 <sup>d</sup>	52.6 $\pm$ 2.6 <sup>ef</sup>
	1	20.7 $\pm$ 1.8 <sup>l</sup>	10.2 $\pm$ 1.5 <sup>l</sup>	0.337 $\pm$ 0.047 <sup>i</sup>	0.195 $\pm$ 0.011 <sup>ll</sup>	ND
	3	26.6 $\pm$ 2.6 <sup>i</sup>	11.3 $\pm$ 0.9 <sup>l</sup>	0.225 $\pm$ 0.032 <sup>t</sup>	0.194 $\pm$ 0.008 <sup>l</sup>	ND
	6	32.3 $\pm$ 2.9 <sup>efg</sup>	13.3 $\pm$ 1.4 <sup>k</sup>	0.213 $\pm$ 0.037 <sup>v</sup>	0.186 $\pm$ 0.009 <sup>n</sup>	58.8 $\pm$ 2.0 <sup>ed</sup>
SMJF	0	24.7 $\pm$ 2.4 <sup>k</sup>	17.7 $\pm$ 1.0 <sup>de</sup>	0.538 $\pm$ 0.077 <sup>f</sup>	0.249 $\pm$ 0.017 <sup>c</sup>	50.7 $\pm$ 2.9 <sup>fg</sup>
	1	23.2 $\pm$ 1.7 <sup>k</sup>	13.3 $\pm$ 1.0 <sup>k</sup>	0.300 $\pm$ 0.044 <sup>l</sup>	0.194 $\pm$ 0.013 <sup>l</sup>	ND
	3	35.4 $\pm$ 2.6 <sup>e</sup>	14.1 $\pm$ 1.7 <sup>jk</sup>	0.285 $\pm$ 0.071 <sup>m</sup>	0.190 $\pm$ 0.015 <sup>m</sup>	ND
	6	40.5 $\pm$ 3.2 <sup>a</sup>	15.3 $\pm$ 1.5 <sup>hij</sup>	0.259 $\pm$ 0.048 <sup>p</sup>	0.167 $\pm$ 0.014 <sup>q</sup>	60.8 $\pm$ 1.7 <sup>bc</sup>
LEC	0	33.2 $\pm$ 2.1 <sup>ef</sup>	24.9 $\pm$ 2.0 <sup>b</sup>	0.804 $\pm$ 0.045 <sup>a</sup>	0.237 $\pm$ 0.018 <sup>e</sup>	57.1 $\pm$ 2.4 <sup>cd</sup>
	1	24.3 $\pm$ 0.4 <sup>k</sup>	24.0 $\pm$ 2.8 <sup>bc</sup>	0.369 $\pm$ 0.054 <sup>h</sup>	0.219 $\pm$ 0.011 <sup>g</sup>	ND
	3	29.9 $\pm$ 1.7 <sup>fg</sup>	16.0 $\pm$ 0.9 <sup>gh</sup>	0.304 $\pm$ 0.037 <sup>k</sup>	0.213 $\pm$ 0.006 <sup>h</sup>	ND
	6	38.5 $\pm$ 3.4 <sup>b</sup>	16.2 $\pm$ 1.3 <sup>fg</sup>	0.238 $\pm$ 0.043 <sup>r</sup>	0.189 $\pm$ 0.013 <sup>m</sup>	66.6 $\pm$ 1.8 <sup>a</sup>

<sup>a–v</sup>Superscript letters following numbers in the same column denote significant differences ( $P \leq 0.05$ ).

<sup>1</sup>The 6 experimental cheeses are as follows: CFF = control cheese made with full-fat milk with EPS-nonproducing starter; CLF = reduced-fat cheese with EPS-nonproducing starter; SMQ = reduced-fat cheese with capsular EPS-producing strain; JRF = reduced-fat cheese with ropy EPS-producing strain; SMJF = reduced-fat cheese with capsular and ropy EPS-producing strains (1:1 mix); and LEC = cheese with 0.2% lecithin added, with EPS-nonproducing starter.

**Table 2.** Cheese yield and composition on the day of manufacture<sup>1</sup>

Parameters	Cheese <sup>2</sup>					
	CFF	CLF	SMQ	JRF	SMJF	LEC
Yield, kg/100 kg)	11.73 $\pm$ 0.21 <sup>a</sup>	9.48 $\pm$ 0.17 <sup>d</sup>	9.76 $\pm$ 0.03 <sup>c</sup>	10.67 $\pm$ 0.14 <sup>b</sup>	10.48 $\pm$ 0.14 <sup>b</sup>	9.85 $\pm$ 0.01 <sup>bc</sup>
Fat, <sup>3</sup> %	30.75 $\pm$ 0.35 <sup>a</sup>	15.50 $\pm$ 0.03 <sup>b</sup>	15.00 $\pm$ 0.25 <sup>b</sup>	15.00 $\pm$ 0.00 <sup>b</sup>	14.75 $\pm$ 0.35 <sup>b</sup>	15.25 $\pm$ 0.35 <sup>b</sup>
Protein, %	23.77 $\pm$ 0.57 <sup>d</sup>	28.63 $\pm$ 0.23 <sup>a</sup>	27.06 $\pm$ 0.38 <sup>b</sup>	25.26 $\pm$ 0.66 <sup>c</sup>	25.60 $\pm$ 0.11 <sup>c</sup>	27.13 $\pm$ 0.26 <sup>b</sup>
Ash, %	2.85 $\pm$ 0.09 <sup>d</sup>	3.40 $\pm$ 0.13 <sup>a</sup>	3.16 $\pm$ 0.10 <sup>b</sup>	2.80 $\pm$ 0.10 <sup>d</sup>	3.04 $\pm$ 0.11 <sup>c</sup>	3.24 $\pm$ 0.15 <sup>b</sup>

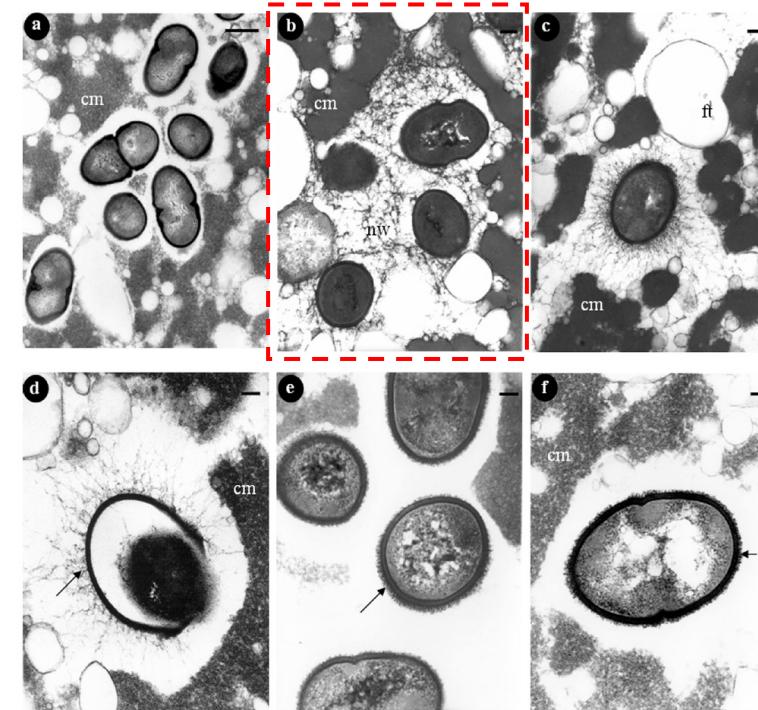
<sup>a–d</sup>Superscript letters following numbers in the same row denote significant differences ( $P \leq 0.05$ ).

<sup>1</sup>Samples were taken after pressing. Data are means  $\pm$  standard deviation.

<sup>2</sup>The 6 experimental cheeses are as follows: CFF = control cheese made with full-fat milk with EPS-nonproducing starter; CLF = reduced-fat cheese with EPS-nonproducing starter; SMQ = reduced-fat cheese with capsular EPS-producing strain; JRF = reduced-fat cheese with ropy EPS-producing strain; SMJF = reduced-fat cheese with capsular and ropy EPS-producing strains (1:1 mix); and LEC = cheese with 0.2% lecithin added, with EPS-nonproducing starter.

<sup>3</sup>Wet weight basis determination.

**Las cepas productoras de exopolisacáridos pueden contribuir a la modificación de la textura y la microestructura del queso**



**Figure 2.** Transmission electron micrographs showing exopolysaccharide (EPS)-producing lactococci in reduced-fat Cheddar cheese matrix at the beginning and after 6 mo of ripening. Panels show EPS-nonproducing lactococci in residual whey pockets in 0-d-old (B, C) and 6-mo-old (D, E, F) JRF cheese matrix. CLF = reduced-fat cheese made with EPS-nonproducing starter culture; JRF = cheese made with starter culture containing ropy strain JRF-1; cm = casein matrix, ft = fat globule, and nw = EPS network-like structure; arrows point to cell-attached EPS. Bars indicate 500 nm (a), 200 nm (b, c), and 100 nm (d, e, f).

# Estudios de aplicación de EPS en la formulación de quesos

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## Effect of exopolysaccharide produced by isogenic strains of *Lactococcus lactis* on half-fat Cheddar cheese

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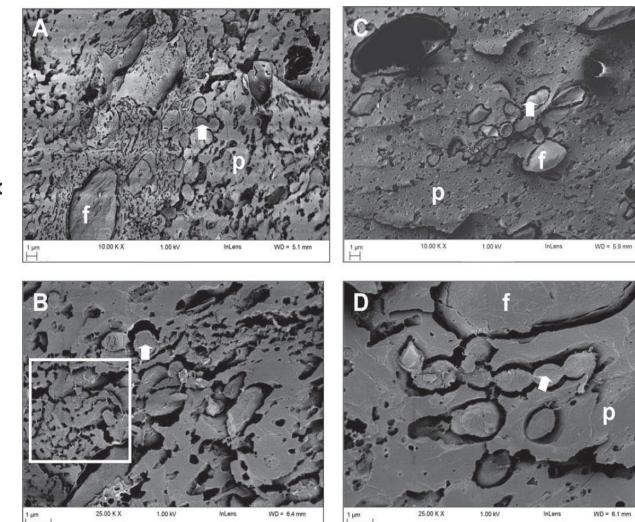
**Table 1.** Cheese yields and composition of half-fat Cheddar cheese manufactured with EPS<sup>+</sup> and EPS<sup>-</sup> starters<sup>1</sup> at d 28 of ripening

Composition <sup>2</sup>	Starter	
	EPS <sup>+</sup>	EPS <sup>-</sup>
Moisture (%), wt/wt	46.11 <sup>a</sup>	41.74 <sup>b</sup>
Fat (%), wt/wt	15.79 <sup>a</sup>	17.17 <sup>b</sup>
Protein (%), wt/wt	32.90 <sup>a</sup>	33.86 <sup>a</sup>
Salt (%), wt/wt	1.73 <sup>a</sup>	1.86 <sup>a</sup>
Ash (%), wt/wt	4.23 <sup>a</sup>	4.73 <sup>a</sup>
Ca (mg/100 g)	907 <sup>a</sup>	1,023 <sup>b</sup>
pH	5.14 <sup>a</sup>	5.36 <sup>b</sup>
MNFS (%), wt/wt	54.28 <sup>a</sup>	50.80 <sup>b</sup>
FDM (%), wt/wt	28.91 <sup>a</sup>	29.81 <sup>a</sup>
S/M (%), wt/wt	3.75 <sup>a</sup>	4.45 <sup>b</sup>
Calcium:protein ratio (mg/g)	28.68 <sup>a</sup>	29.20 <sup>a</sup>
Y <sub>a</sub> (%)	8.02 <sup>a</sup>	7.42 <sup>b</sup>
Y <sub>afpm</sub> (%)	8.33 <sup>a</sup>	7.69 <sup>b</sup>
Y <sub>mfpam</sub> (%)	7.38 <sup>a</sup>	7.25 <sup>a</sup>

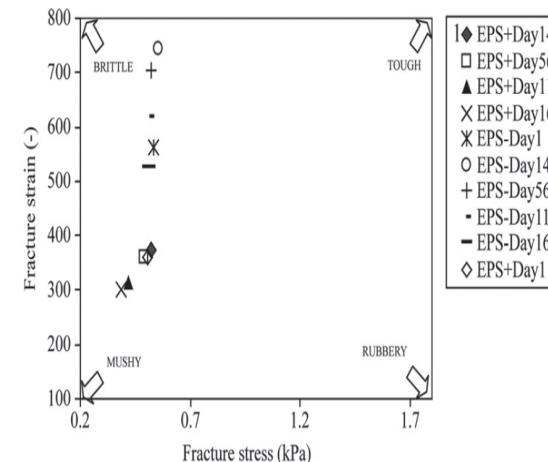
<sup>a,b</sup>Values within a row not sharing a common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>EPS<sup>+</sup> = cheese manufactured with a starter producing an exopolysaccharide (EPS); EPS<sup>-</sup> = cheese manufactured with a starter not producing an EPS.

<sup>2</sup>MNFS = moisture in the nonfat substances; FDM = fat in DM; S/M = salt in moisture; Y<sub>a</sub> = kg of cheese per 100 kg of milk; Y<sub>afpm</sub> = kg of cheese obtained per 100 kg of milk normalized to a reference level of 1.5% (wt/wt) fat and 3.3% (wt/wt) protein; Y<sub>mfpam</sub> = moisture-adjusted cheese yield per 100 kg of cheese milk adjusted to reference levels of fat (3.4%, wt/wt), protein (3.3%, wt/wt), and moisture (38.5%, wt/wt).



**Figure 3.** Cryogenic-scanning electron micrographs of 1-d-old half-fat Cheddar cheeses manufactured using either an exopolysaccharide (EPS)-producing culture (A and B) or its isogenic non-EPS-producing variant (C and D). Arrow indicates bacterial cells; square indicates the network areas of protein-EPS interaction; f = fat globule; p = protein.



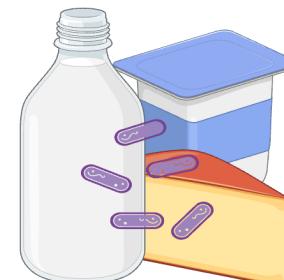
**Figure 5.** Texture map of half-fat Cheddar cheeses as described by Hammann and MacDonald (1992) and Lanier (1998). Arrows indicate increase in that textural attribute. EPS<sup>+</sup> = cheese manufactured with a starter producing an exopolysaccharide (EPS); EPS<sup>-</sup> = cheese manufactured with a starter not producing an EPS, followed by the ripening time at which they were analyzed.

**Table 3.** Trained panel descriptive flavor and texture profiles of half-fat Cheddar cheeses made with EPS<sup>+</sup> and EPS<sup>-</sup> starters<sup>1</sup> at 6 mo of ripening

Item	EPS <sup>+</sup>	EPS <sup>-</sup>
Flavor attribute <sup>2</sup>		
Cooked	2.6 <sup>a</sup>	2.6 <sup>a</sup>
Whey	2.0 <sup>a</sup>	1.9 <sup>a</sup>
Milkfat	1.7 <sup>a</sup>	1.4 <sup>b</sup>
Sulfur	1.7 <sup>b</sup>	2.2 <sup>a</sup>
Brothy	2.6 <sup>b</sup>	2.9 <sup>a</sup>
Nutty	ND <sup>3</sup>	1.0
Cowy	1.7 <sup>a</sup>	1.5 <sup>a</sup>
Mothball	1.1 <sup>b</sup>	1.5 <sup>a</sup>
Sweet	2.1 <sup>a</sup>	2.0 <sup>a</sup>
Sour	3.2 <sup>a</sup>	2.9 <sup>b</sup>
Bitter	0.6 <sup>a</sup>	1.0 <sup>a</sup>
Salty	3.2 <sup>a</sup>	3.2 <sup>a</sup>
Umami	1.9 <sup>a</sup>	1.8 <sup>a</sup>
Texture attribute <sup>4</sup>		
Hfirmness	12.8 <sup>b</sup>	13.7 <sup>a</sup>
Hspringiness	12.6 <sup>a</sup>	12.9 <sup>a</sup>
Hrate of recovery	10.0 <sup>b</sup>	13.0 <sup>a</sup>
Firmness	9.9 <sup>b</sup>	11.6 <sup>a</sup>
Fracturability	8.1 <sup>a</sup>	8.4 <sup>a</sup>
Degree of breakdown	4.2 <sup>a</sup>	2.2 <sup>b</sup>
Cohesiveness	4.9 <sup>a</sup>	4.1 <sup>b</sup>
Adhesiveness	4.8 <sup>a</sup>	4.2 <sup>b</sup>
Smoothness of mass	4.9 <sup>a</sup>	4.2 <sup>b</sup>
Smoothness of mouthcoating	6.6 <sup>a</sup>	3.6 <sup>b</sup>

<sup>a,b</sup>Values within a column not sharing a common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>EPS<sup>+</sup> = cheese manufactured with a starter producing an exopolysaccharide (EPS); EPS<sup>-</sup> = cheese manufactured with a starter not producing an EPS.





# Estudios de aplicación de EPS en la formulación de quesos

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## Assessing the yield, microstructure, and texture properties of miniature Chihuahua-type cheese manufactured with a phospholipase A<sub>1</sub> and exopolysaccharide-producing bacteria

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Table 2. Effect of adding phospholipase A<sub>1</sub> (PL-A<sub>1</sub>) or exopolysaccharide (EPS)-producing bacteria, or both, to cheese milk on the chemical composition of miniature Chihuahua cheeses 10 d after their manufacture

Item <sup>1</sup>	Treatment <sup>2</sup>				SEM
	Control (n = 4)	PL-A <sub>1</sub> (n = 4)	EPS (n = 4)	EPS + PL-A <sub>1</sub> (n = 4)	
<b>Cheese</b>					
Cheese weight (g)	20.61 <sup>c</sup>	25.50 <sup>b</sup>	26.25 <sup>a</sup>	25.82 <sup>ab</sup>	0.242
pH	5.44 <sup>b</sup>	5.32 <sup>ab</sup>	5.34 <sup>ab</sup>	5.20 <sup>a</sup>	0.042
<i>a</i> <sub>w</sub>	0.970 <sup>a</sup>	0.972 <sup>ab</sup>	0.978 <sup>b</sup>	0.975 <sup>ab</sup>	0.002
Moisture (g/100 g)	43.09 <sup>a</sup>	43.42 <sup>ab</sup>	48.28 <sup>c</sup>	45.79 <sup>bc</sup>	0.614
Fat (g/100 g)	30.56 <sup>ab</sup>	29.43 <sup>a</sup>	30.45 <sup>ab</sup>	31.32 <sup>b</sup>	0.296
Protein (g/100 g)	21.87 <sup>a</sup>	25.67 <sup>b</sup>	22.84 <sup>a</sup>	23.62 <sup>ab</sup>	0.589
Ash (g/100 g)	3.04 <sup>ab</sup>	3.19 <sup>a</sup>	2.71 <sup>c</sup>	2.73 <sup>bc</sup>	0.081
TS (g/100 g)	56.91 <sup>a</sup>	56.58 <sup>ab</sup>	51.79 <sup>c</sup>	54.28 <sup>bc</sup>	0.614
FDM (%)	53.79 <sup>b</sup>	52.03 <sup>b</sup>	58.90 <sup>a</sup>	57.71 <sup>a</sup>	0.832
PDM (%)	38.47 <sup>b</sup>	45.39 <sup>a</sup>	44.17 <sup>a</sup>	43.50 <sup>a</sup>	1.102
<b>Whey</b>					
Weight of bulk whey (g)	217.36 <sup>a</sup>	212.66 <sup>b</sup>	211.76 <sup>b</sup>	213.26 <sup>b</sup>	0.560
Ionic calcium (mM × 10 <sup>-3</sup> )	26.63 <sup>a</sup>	17.87 <sup>b</sup>	25.55 <sup>a</sup>	14.84 <sup>b</sup>	2.472
Fat (g/100 g)	0.350 <sup>a</sup>	0.192 <sup>ab</sup>	0.097 <sup>b</sup>	0.227 <sup>ab</sup>	0.056
Fat lost in whey (FL%)	10.89 <sup>a</sup>	5.23 <sup>ab</sup>	2.75 <sup>b</sup>	3.40 <sup>ab</sup>	1.86

<sup>a-c</sup>Means within a row with different superscript letters differ (*P* < 0.05).

<sup>1</sup>*a*<sub>w</sub> = water activity; FDM = fat in DM; PDM = protein in DM.

<sup>2</sup>Control = cheese milk without EPS-producing culture or PL-A<sub>1</sub>; PL-A<sub>1</sub> = cheese milk with added PL-A<sub>1</sub> (15 Lecitase units/100 g of milk); EPS = cheese milk inoculated at 1% (vol/vol) with an EPS-producing culture; EPS + PL-A<sub>1</sub> = cheese milk with an added EPS-producing culture and PL-A<sub>1</sub>.

Table 3. Effects of adding phospholipase A<sub>1</sub> (PL-A<sub>1</sub>) or exopolysaccharide (EPS)-producing bacteria, or both, in cheese milk on the texture properties of miniature Chihuahua cheeses 10 d after their manufacture

Item	Treatment <sup>1</sup>				SEM
	Control (n = 4)	PL-A <sub>1</sub> (n = 4)	EPS (n = 4)	EPS + PL-A <sub>1</sub> (n = 4)	
Hardness (N)	5.81 <sup>b</sup>	4.07 <sup>a</sup>	4.10 <sup>a</sup>	3.83 <sup>a</sup>	0.331
Fracturability (N)	4.38 <sup>b</sup>	2.08 <sup>a</sup>	2.71 <sup>a</sup>	2.12 <sup>a</sup>	0.339
Springiness (mm)	4.55 <sup>a</sup>	3.64 <sup>a</sup>	3.99 <sup>a</sup>	2.89 <sup>a</sup>	0.463
Chewiness (mJ)	5.79 <sup>a</sup>	2.06 <sup>b</sup>	3.77 <sup>ab</sup>	1.74 <sup>b</sup>	0.771
Cohesiveness	0.222 <sup>a</sup>	0.205 <sup>a</sup>	0.207 <sup>a</sup>	0.171 <sup>a</sup>	0.026
Apparent Young's modulus (kPa)	5.2 <sup>b</sup>	4.7 <sup>c</sup>	5.9 <sup>a</sup>	4.4 <sup>c</sup>	0.280

<sup>a-c</sup>Means within a row with different superscript letters differ (*P* < 0.05).

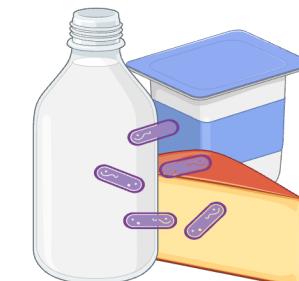
<sup>1</sup>Control = cheese milk without EPS-producing culture or PL-A<sub>1</sub>; PL-A<sub>1</sub> = cheese milk with added PL-A<sub>1</sub> (15 Lecitase units/100 g of milk); EPS = cheese-milk inoculated at 1% (vol/vol) with an EPS-producing culture; EPS + PL-A<sub>1</sub> = cheese milk with an added EPS-producing culture and PL-A<sub>1</sub>.

Table 4. Sensory analysis of Chihuahua cheese manufactured with an exopolysaccharide (EPS)-producing bacteria or phospholipase A<sub>1</sub> (PL-A<sub>1</sub>), or both<sup>1</sup>

Treatment	Rank sum <sup>2</sup>	
	Flavor	Texture
Control cheese	98 <sup>a</sup>	110 <sup>ab</sup>
Cheese made with PL-A <sub>1</sub>	134 <sup>ab</sup>	140 <sup>b</sup>
Cheese made with EPS-producing culture	103 <sup>a</sup>	95 <sup>a</sup>
Cheese made with EPS + PL-A <sub>1</sub>	156 <sup>b</sup>	145 <sup>b</sup>

<sup>a,b</sup>Rank sums within a column with different superscript letters differ, according to the critical difference value between samples (Christensen et al., 2006).

**Los EPS mejoran el rendimiento del queso al aumentar la retención de agua y grasa, pero tienen un efecto negativo en la textura y el sabor del queso Chihuahua.**





# Estudios de aplicación de EPS en la formulación de quesos



Isolation and characterisation of exopolysaccharide-producing *Weissella* and *Lactobacillus* and their application as adjunct cultures in Cheddar cheese

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**Table 1**  
List of strains examined as potential adjuncts, their rRNA typing matches and properties of produced exopolysaccharides (EPS).

Species	Original source	EPS (linkage) and oligosaccharide produced	EPS molecular mass (Da)	Typical EPS yield <sup>a</sup> in EPS-broth (g L <sup>-1</sup> )
<i>Weissella cibaria</i> MG1 (98% identity with <i>W. cibaria</i> ILMC 17609 <sup>T</sup> 16S rRNA gene sequence)	Sourdough	Glucan homopolysaccharide ( $\alpha$ -1,6) and glucooligosaccharide	$5 \times 10^6$ – $4 \times 10^7$	36
<i>Lactobacillus casei</i> C12 (98% identity with <i>L. casei</i> ATCC393 16S rRNA gene sequence)	Cheese	Heteropolysaccharide	$5 \times 10^3$ – $10^5$	0.7
<i>Lactobacillus reuteri</i> ff2hh2 (97% identity with <i>L. reuteri</i> ICM1112 16S rRNA gene sequence)	Porcine gut	Glucan homopolysaccharide ( $\alpha$ -1,6 and $\alpha$ -1,4) and fructooligosaccharide	$10^5$ – $4 \times 10^7$	3.6
<i>Lactobacillus reuteri</i> cc2 (97% identity with <i>L. reuteri</i> ICM1112 16S rRNA gene sequence)	Porcine gut	Glucan homopolysaccharide ( $\alpha$ -1,6 and $\alpha$ -1,4) and fructooligosaccharide	$10^5$	2

<sup>a</sup> Typical yield in EPS-broth (g L<sup>-1</sup>).



**Table 2**  
Cheeses manufactured in this study.<sup>a</sup>

Cheese	Component
A	Control (no adjunct or EPS ingredient)
B	<i>W. cibaria</i> MG1
C	<i>W. cibaria</i> MG1 + 5% (v/v) <sup>b</sup> sucrose
D	<i>L. reuteri</i> cc2
E	<i>L. reuteri</i> cc2 + 4% (v/v) sucrose + 1% (v/v) glucose
F	EPS ingredient, 0.2% (w/v) in cheese–milk

<sup>a</sup> All cheeses contained *L. lactis* R604 starter culture. Adjuncts were added at a final titre of  $10^5$  cfu mL<sup>-1</sup>.

**La utilización de *W. cibaria* en la fabricación de queso aumenta la retención de humedad sin afectar a la proteólisis del queso y, por tanto, a las propiedades características de sabor y aroma del queso Cheddar.**

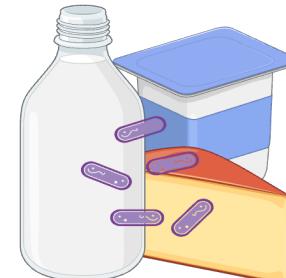
**Table 3**

Microbial counts and gross composition of miniature Cheddar cheeses<sup>a</sup> produced without (control) or with EPS-producing adjuncts or with addition of purified EPS ingredient.<sup>b</sup>

Cheese	Starter count (log cfu g <sup>-1</sup> , day 90)	Adjunct count (log cfu g <sup>-1</sup> , day 7)	Adjunct count (log cfu g <sup>-1</sup> , day 90)	pH (day 90)	Salt (% w/w, day 30)	Moisture (% w/w, day 30)	S/M (% w/w)	FAA (day 90) (mg Leu cheese <sup>-1</sup> )	pH 4.6-soluble protein (day 90) (%)
A	11.05	Not added	Not added	5.09	1.85 ± 0.29	30.3 ± 5.0	6.13 ± 0.71	7.63 ± 2.27	0.65 ± 0.05
B	11.80	5.78	7.57	4.93	1.81 ± 0.14	33.0 ± 6.3	5.68 ± 1.31	8.22 ± 2.31	0.70 ± 0.05
C	10.99	5.73	7.18	4.89	2.05 ± 0.19	32.7 ± 5.4	6.47 ± 1.49	7.79 ± 2.09	0.67 ± 0.08
D	12.10	5.01	7.26	4.93	1.67 ± 0.21	31.1 ± 4.0	5.44 ± 0.89	7.45 ± 1.85	0.70 ± 0.07
E	11.48	5.09	5.85	5.10	2.34 ± 0.38	31.5 ± 5.2	7.54 ± 1.28	7.57 ± 2.09	0.64 ± 0.05
F	12.02	No adjunct	No adjunct	4.96	1.76 ± 0.27	31.9 ± 4.3	5.63 ± 1.38	7.89 ± 3.32	0.68 ± 0.08

<sup>a</sup> Cheeses were: A, control cheese; B–F, experimental cheeses made with the addition of (B) *W. cibaria* MG1, (C) *W. cibaria* MG1 plus substrate carbohydrate, (D) *L. reuteri* cc2, (E) *L. reuteri* cc2 plus substrate carbohydrates, (F) addition of EPS as ingredient. NSLAB counts on control cheeses at day 90 were 7.88 log cfu g<sup>-1</sup>.

<sup>b</sup> Abbreviations are: S/M, salt in moisture; FAAs, free amino acids. No statistically significant difference between samples ( $p > 0.05$ ) was found for pH (day 90), moisture, FAA (day 90) or pH 4.6-soluble protein (day 90); salt was significantly different ( $p < 0.05$ ) between the control and cheeses made with *L. reuteri* cc2 plus substrate carbohydrates (cheese E). Microbial counts are the means of six replicates and compositional data are the means of three replicates. Values shown are means ± standard deviation.



# Estudios de aplicación de EPS en la formulación de quesos

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## EFFECT OF AN EXOPOLYSACCHARIDE-PRODUCING STRAIN OF *STREPTOCOCCUS THERMOPHILUS* ON THE YIELD AND TEXTURE OF MEXICAN MANCHEGO-TYPE CHEESE

Diana Lluis-Arroyo<sup>1</sup>, Angélica Flores-Nájera<sup>1</sup>, Alma Cruz-Guerrero<sup>1</sup>, Francisco Gallardo-Escamilla<sup>1</sup>, Consuelo Lobato-Calleros<sup>2</sup>, Judith Jiménez-Guzmán<sup>1</sup>, and Mariano García-Garibay<sup>1</sup>

Table 1 Cheese yield.

	Before salting	After salting	After ripening
Control cheese	11.16 ± 0.19 <sup>a</sup>	11.67 ± 0.07 <sup>c</sup>	9.43 ± 0.04 <sup>d</sup>
Ropy-strain cheese	12.37 ± 0.28 <sup>b</sup>	12.53 ± 0.28 <sup>b</sup>	10.45 ± 0.06 <sup>e</sup>
% enhancement of yield	10.8	7.4	10.9

Different superscript letters indicate significantly different values ( $P < 0.001$ ).

Table 2 Chemical composition of the cheese (g·100 g<sup>-1</sup>).

	TS	Moisture	Fat	Total protein	Fat (dwb)	Total protein (dwb)
Control cheese	59.7 ± 0.5 <sup>a</sup>	40.3 ± 0.5 <sup>a</sup>	30 ± 0.0 <sup>a</sup>	21.0 ± 1.2 <sup>a</sup>	50.3 ± 1.0 <sup>a</sup>	35.3 ± 0.6 <sup>a</sup>
Ropy strain cheese	55.3 ± 0.2 <sup>b</sup>	44.7 ± 0.2 <sup>b</sup>	34 ± 0.0 <sup>b</sup>	19.0 ± 1.0 <sup>b</sup>	61.5 ± 1.0 <sup>b</sup>	34.3 ± 0.1 <sup>b</sup>

TS: total solids; NFS: non-fat solids; dwb: dry weight basis.

Different superscript letters in the same column indicate significantly different values ( $P < 0.005$ ).

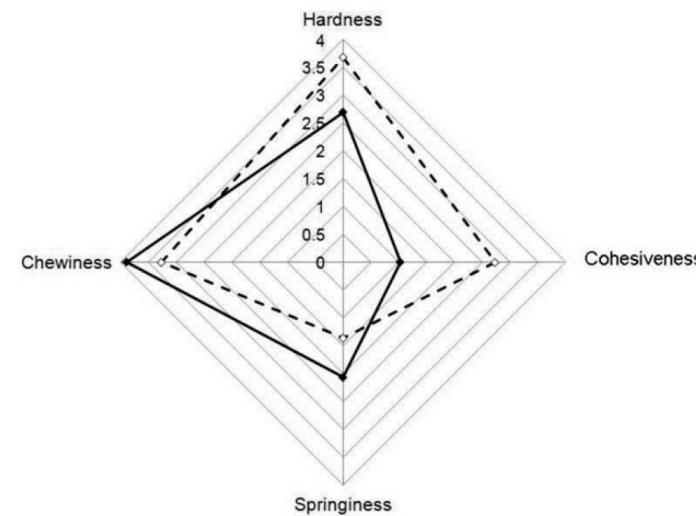
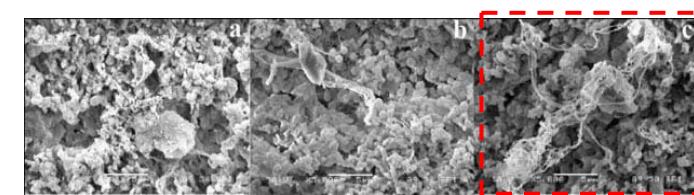
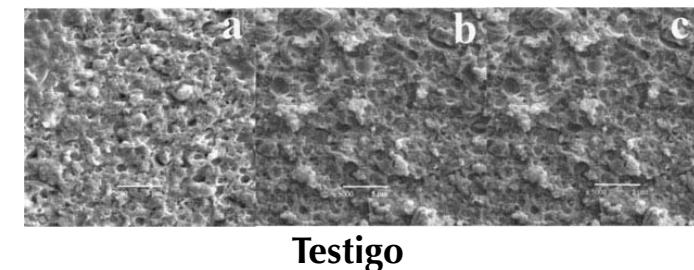


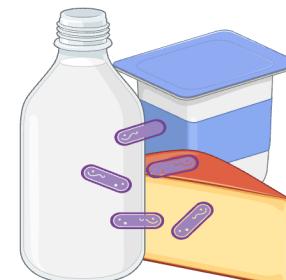
Figure 6 Comparison of the sensory attributes of ropy and control Mexican manchego-type cheese. □, control; ■, ropy.

Table 3 Textural characteristics of ropy and control cheese.

	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (Nmm)
Control cheese	8.67 ± 0.26 <sup>a</sup>	0.15 ± 0.02 <sup>a</sup>	6.90 ± 0.42 <sup>a</sup>	8.96 ± 1.21 <sup>a</sup>
Ropy strain cheese	6.73 ± 0.47 <sup>b</sup>	0.18 ± 0.01 <sup>b</sup>	6.24 ± 0.38 <sup>b</sup>	7.66 ± 1.14 <sup>a</sup>

Different superscript letters in the same column indicate significantly different values ( $P < 0.05$ ).

**El uso de *S. thermophilus* SY-102 en el queso mexicano tipo manchego aumentó la retención de humedad y grasa dentro de la matriz del queso. Esto se reflejó en un cambio de las características texturales, siendo la dureza la que presentó el cambio más significativo**



# Estudios de aplicación de EPS en la formulación de quesos

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## Manufacture of low-fat Cheddar cheese by exopolysaccharide-producing *Lactobacillus plantarum* JLK0142 and its functional properties

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**Table 1.** Gross composition (d 0), water activity (d 90), and pH of low-fat Cheddar cheese (mean  $\pm$  SD; n = 3)

Variable	Cheese <sup>1</sup>		
	A	B	C
Moisture (g/100 g)	36.66 $\pm$ 1.98 <sup>b</sup>	40.59 $\pm$ 2.08 <sup>ab</sup>	43.86 $\pm$ 2.31 <sup>a</sup>
Protein (g/100 g)	22.82 $\pm$ 0.30 <sup>a</sup>	22.08 $\pm$ 0.68 <sup>a</sup>	22.61 $\pm$ 0.46 <sup>a</sup>
Salt (g/100 g)	1.42 $\pm$ 0.08 <sup>b</sup>	1.63 $\pm$ 0.04 <sup>ab</sup>	1.69 $\pm$ 0.12 <sup>a</sup>
FDM <sup>2</sup> (%)	24.67 $\pm$ 0.84 <sup>a</sup>	24.07 $\pm$ 1.63 <sup>a</sup>	23.15 $\pm$ 1.36 <sup>b</sup>
Water activity (d 90)	0.93 $\pm$ 0.03 <sup>a</sup>	0.89 $\pm$ 0.01 <sup>a</sup>	0.91 $\pm$ 0.01 <sup>a</sup>
pH			
d 0	5.62 $\pm$ 0.10 <sup>A,a</sup>	5.66 $\pm$ 0.08 <sup>A,a</sup>	5.64 $\pm$ 0.03 <sup>A,a</sup>
d 30	5.60 $\pm$ 0.03 <sup>A,a</sup>	5.58 $\pm$ 0.12 <sup>AB,a</sup>	5.55 $\pm$ 0.15 <sup>A,a</sup>
d 60	5.56 $\pm$ 0.04 <sup>A,a</sup>	5.51 $\pm$ 0.09 <sup>AB,a</sup>	5.49 $\pm$ 0.07 <sup>A,a</sup>
d 90	5.53 $\pm$ 0.18 <sup>A,a</sup>	5.40 $\pm$ 0.05 <sup>B,a</sup>	5.42 $\pm$ 0.06 <sup>A,a</sup>

<sup>a,b</sup>Means for pH in the same column followed by different superscripts are significantly different ( $P < 0.05$ ).

<sup>a,b</sup>Means in the same row followed by different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup>Cheese: A = control cheese; B = cheese made with addition of purified exopolysaccharide ingredient; C = cheese made with exopolysaccharide-producing *Lactobacillus plantarum* JLK0142 as an adjunct culture.

<sup>2</sup>FDM = fat-in-dry matter.

**Table 3.** Sensory analysis (score) of low-fat Cheddar cheese after ripening for 90 d (mean  $\pm$  SD; n = 3)

Cheese <sup>1</sup>	Sensory attribute			
	Texture	Appearance	Flavor	Overall acceptance
A	6.3 $\pm$ 0.7 <sup>a</sup>	7.1 $\pm$ 0.9 <sup>a</sup>	6.8 $\pm$ 0.5 <sup>a</sup>	7.4 $\pm$ 0.2 <sup>a</sup>
B	6.5 $\pm$ 0.2 <sup>a</sup>	7.5 $\pm$ 0.7 <sup>a</sup>	7.6 $\pm$ 0.3 <sup>b</sup>	7.2 $\pm$ 0.5 <sup>a</sup>
C	7.2 $\pm$ 0.6 <sup>b</sup>	7.3 $\pm$ 0.5 <sup>a</sup>	7.5 $\pm$ 0.4 <sup>b</sup>	7.7 $\pm$ 0.3 <sup>a</sup>

<sup>a,b</sup>Means in the same column followed by different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup>Cheese: A = control cheese; B = cheese made with addition of purified exopolysaccharide ingredient; C = cheese made with exopolysaccharide-producing *Lactobacillus plantarum* JLK0142 as an adjunct culture.

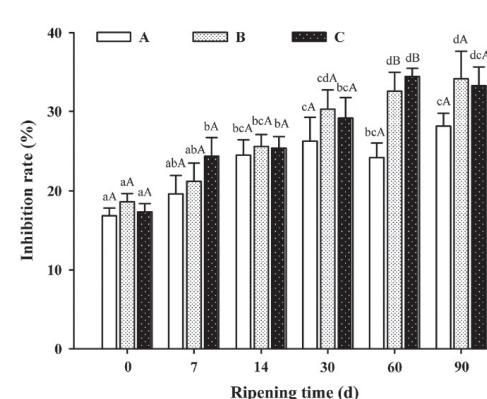
**Table 2.** Texture profile analysis of low-fat Cheddar cheese during 90 d of ripening at 4°C (mean  $\pm$  SD; n = 3)

Variable	Ripening time (d)	Cheese <sup>1</sup>		
		A	B	C
Hardness (kg)	0	4.89 $\pm$ 0.56 <sup>a,A</sup>	3.56 $\pm$ 0.38 <sup>ab,B</sup>	3.12 $\pm$ 0.37 <sup>a,B</sup>
	30	4.15 $\pm$ 0.74 <sup>a,A</sup>	3.02 $\pm$ 0.12 <sup>ab,B</sup>	2.84 $\pm$ 0.20 <sup>b,C</sup>
	60	3.68 $\pm$ 0.22 <sup>ab,A</sup>	2.68 $\pm$ 0.15 <sup>bc,B</sup>	1.89 $\pm$ 0.15 <sup>b,B</sup>
	90	2.67 $\pm$ 0.38 <sup>b,A</sup>	2.17 $\pm$ 0.09 <sup>c,B</sup>	1.53 $\pm$ 0.13 <sup>a,B</sup>
Cohesiveness	0	0.88 $\pm$ 0.01 <sup>a,A</sup>	0.79 $\pm$ 0.03 <sup>ab,AB</sup>	0.76 $\pm$ 0.03 <sup>a,B</sup>
	30	0.82 $\pm$ 0.02 <sup>ab,A</sup>	0.77 $\pm$ 0.01 <sup>bc,B</sup>	0.73 $\pm$ 0.04 <sup>ab,B</sup>
	60	0.79 $\pm$ 0.02 <sup>bc,A</sup>	0.72 $\pm$ 0.02 <sup>bc,B</sup>	0.72 $\pm$ 0.01 <sup>b,B</sup>
	90	0.75 $\pm$ 0.01 <sup>c,A</sup>	0.70 $\pm$ 0.02 <sup>b,B</sup>	0.68 $\pm$ 0.01 <sup>b,B</sup>
Springiness (mm)	0	0.84 $\pm$ 0.03 <sup>a,B</sup>	0.88 $\pm$ 0.01 <sup>a,AB</sup>	0.90 $\pm$ 0.01 <sup>a,A</sup>
	30	0.82 $\pm$ 0.02 <sup>ab,A</sup>	0.86 $\pm$ 0.01 <sup>a,A</sup>	0.85 $\pm$ 0.03 <sup>b,A</sup>
	60	0.77 $\pm$ 0.02 <sup>bc,B</sup>	0.82 $\pm$ 0.02 <sup>b,A</sup>	0.83 $\pm$ 0.01 <sup>bc,A</sup>
	90	0.74 $\pm$ 0.01 <sup>c,C</sup>	0.77 $\pm$ 0.01 <sup>c,B</sup>	0.80 $\pm$ 0.01 <sup>c,A</sup>
Chewiness (mJ)	0	1.78 $\pm$ 0.21 <sup>a,A</sup>	1.52 $\pm$ 0.30 <sup>a,B</sup>	1.46 $\pm$ 0.13 <sup>A</sup>
	30	1.54 $\pm$ 0.17 <sup>a,A</sup>	1.38 $\pm$ 0.15 <sup>ab,A</sup>	1.29 $\pm$ 0.20 <sup>ab,A</sup>
	60	1.06 $\pm$ 0.09 <sup>b,A</sup>	0.94 $\pm$ 0.05 <sup>bc,A</sup>	1.03 $\pm$ 0.03 <sup>bc,A</sup>
	90	0.72 $\pm$ 0.02 <sup>b,B</sup>	0.85 $\pm$ 0.01 <sup>c,A</sup>	0.76 $\pm$ 0.02 <sup>c,B</sup>

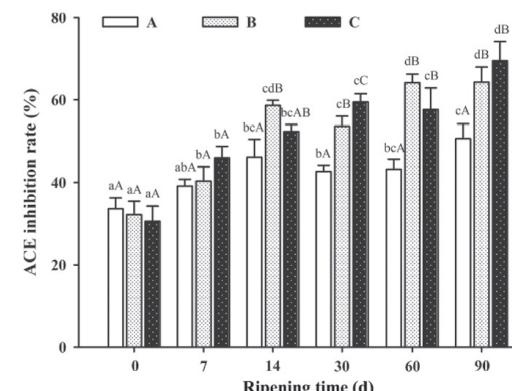
<sup>a-c</sup>Means in the same column and within a variable followed by different superscripts are significantly different ( $P < 0.05$ ).

<sup>A-C</sup>Means in the same row followed by different superscripts are significantly different ( $P < 0.05$ ).

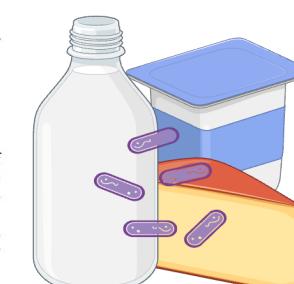
<sup>1</sup>Cheese: A = control cheese; B = cheese made with addition of purified exopolysaccharide ingredient; C = cheese made with exopolysaccharide-producing *Lactobacillus plantarum* JLK0142 as an adjunct culture.



**Figure 6.** Inhibitory activities against human colon cancer HT-29 cells of low-fat Cheddar cheese during 90 d of ripening at 4°C. A = control cheese; B = experimental cheese made with the addition purified exopolysaccharide ingredient; C = experimental cheese made with the addition of exopolysaccharide-producing *Lactobacillus plantarum* JLK0142. Means among different ripening times with different lowercase letters (a-d) are significantly different ( $P < 0.05$ ); mean among different cheeses with uppercase letters (A-B) are significant different ( $P < 0.05$ ). Values shown are mean  $\pm$  SD (n = 3).



**Figure 5.** Angiotensin-converting enzyme (ACE) inhibitory activities of low-fat Cheddar cheese during 90 d of ripening at 4°C. A = control cheese; B = experimental cheese made with the addition of purified exopolysaccharide ingredient; C = experimental cheese made with the addition of exopolysaccharide-producing *Lactobacillus plantarum* JLK0142. Means among different ripening times with different lowercase letters (a-d) are significantly different ( $P < 0.05$ ); means among different cheeses with uppercase letters (A-C) are significant different ( $P < 0.05$ ). Values shown are mean  $\pm$  SD (n = 3).



# Retos y oportunidades

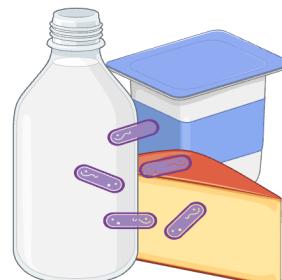
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# Retos y oportunidades

- ✓ Es necesario seleccionar cuidadosamente la cepa y las condiciones de síntesis de EPS, ya que el tipo y la cantidad de EPS, así como las condiciones óptimas de crecimiento de los microorganismos utilizados deben ajustarse al proceso de producción específico y a las características del alimento.
- ✓ Seguir estudiando la relación existente entre las características fisicoquímicas de los EPS y su probable efecto biológico.
- ✓ Demostrar la seguridad y efectos beneficiosos de las cepas de BAL productoras de EPS en la formulación de productos lácteos a través de ensayos clínicos. Por lo tanto, se deben realizar más estudios de intervención para cepas específicas y grupos de población específicos.



A large, colorful watercolor-style splash of ink in shades of pink, purple, yellow, and orange, serving as a background for the text.

*Muchas  
Gracias*

