Probiotic and technological characteristics of microorganisms isolated from a natural ecosystem, the Kefir grain.

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Kefir

Fermented milk from the Caucasian mountains

Described as a food in:

- Código Alimentario Argentino Art 576 - (Res. MSyAS N° 295 del 14.04.99)
- International Dairy Federation Bulletin

Kefir was use in

* Treatment of gastrointestinal disorders
* Treatment of respiratory disorders

Kefir properties:

- Antibacterial, antifungic and antitumoral (*in vitro* and in animals)
- Immunomodolatory (in animals)

↓

Probiotic fermented milk

"Kefir: a millenary fermented milk with probiotic properties"

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Industrial production in east Europe and Asia

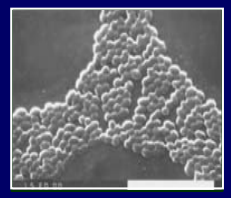
Homemade: with grains

Kefir grains

- ✓ Matrix polysaccharide-protein
- ✓ Yeast
- ✓ Lactic Acid Bacteria
- ✓ Acetic Acid Bacteria

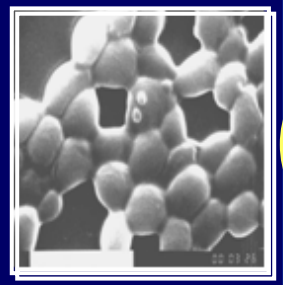
10^8 – 10^9 microorganisms/gram

Acetobacter



Lactobacillus
Lactococcus
Leuconostoc

Saccharomyces
Candida
Kluyveromyces
Torula

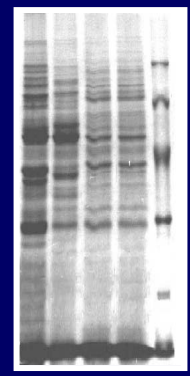


Source of strains safe and potentially probiotic

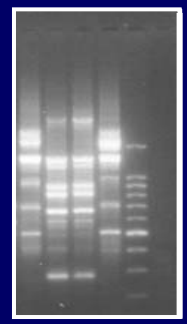
Identification of isolates

-Sugar fermentation

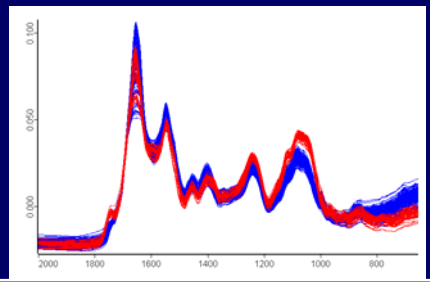
-Whole-cell proteins by SDS-PAGE

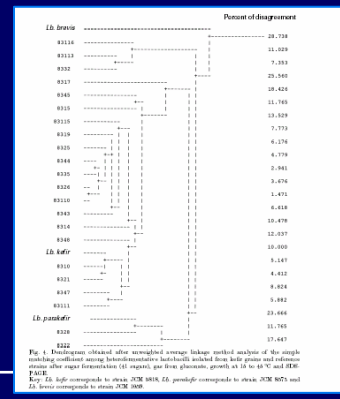


- Sequence of spacer 16S-23S rRNA
- ARDRA
- RAPD-PCR



- FT-IR





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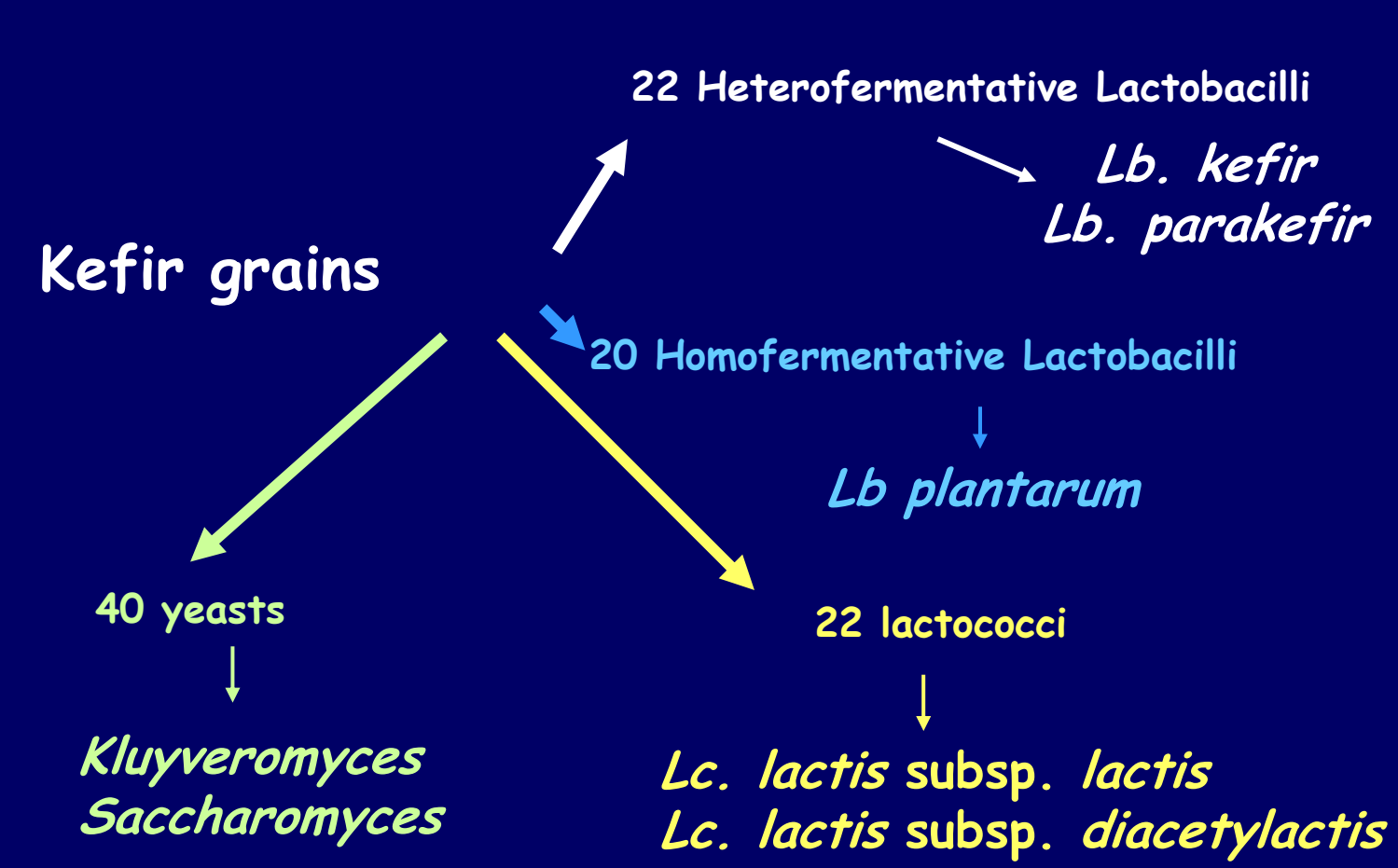
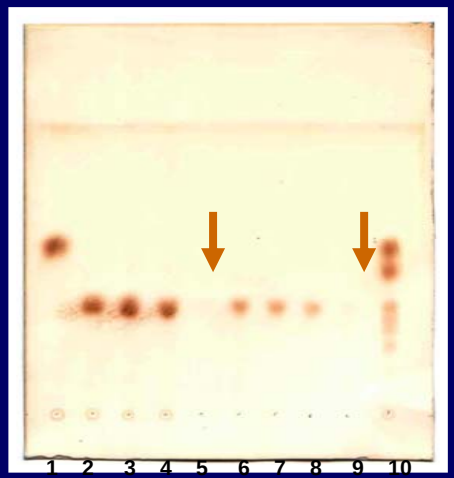


Selection of 5 strains resistant to gastrointestinal conditions : *Lb. plantarum* CIDCA 83114, *Lb. kefir* CIDCA 8348, *Lc. lactis* CIDCA 8221, *Sc. cerevisiae* CIDCA 8112, *K. marxianus* CIDCA 8154

Kluyveromyces marxianus CIDCA 8154

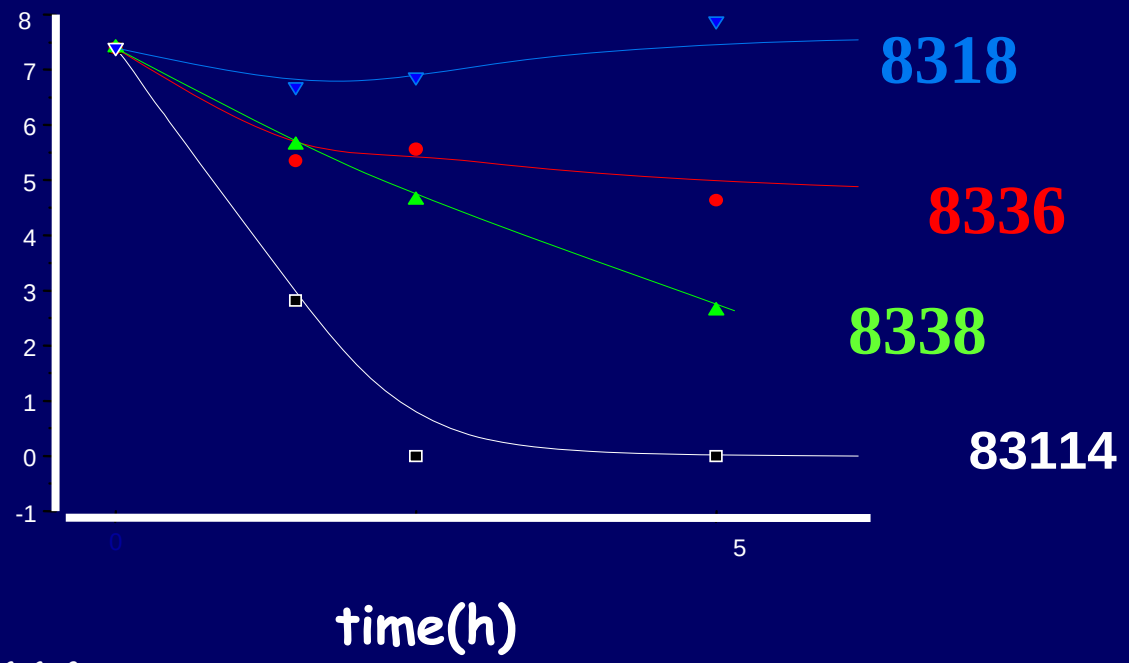


Thin layer chromatography (TLC)

1- Glucose
2- Lactose
3- Milk (diluted 1:10)
4- Milk fermented with *Lb. plantarum* 83114
5- FM with yeast 8154
6- 83114 + 8154 (1: 1)
7- 83114 + 8154 (1: 3)
8- 83114 + 8154 (1: 5)
9- 83114 + 8154 (1: 8)
10- Commercial milk without lactose (diluted 1:10)

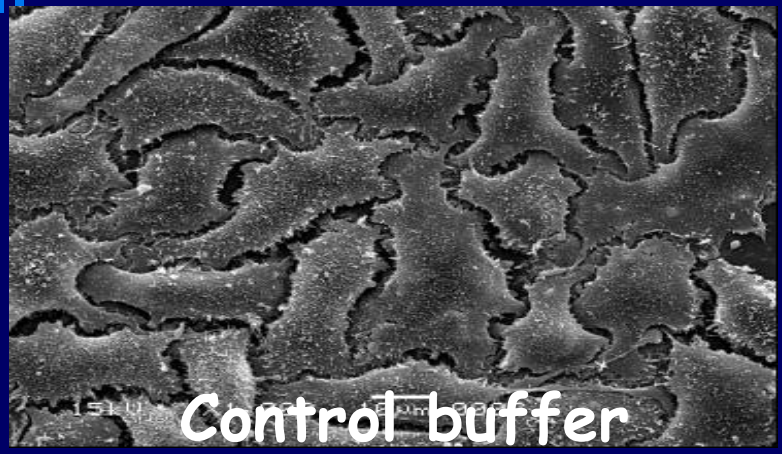
Milk fermented with	Sugar content (g/100ml) ± DS*
L. plantarum (48 hs)	3,91 ± 0,13
K. marxianus (48 hs)	0,07 ± 0,02
L. plantarum + *K. marxianus* (48 hs)	0,07 ± 0,01
Non fermented milk	4,03 ± 0,46
Comercial lactose-free milk	0,9

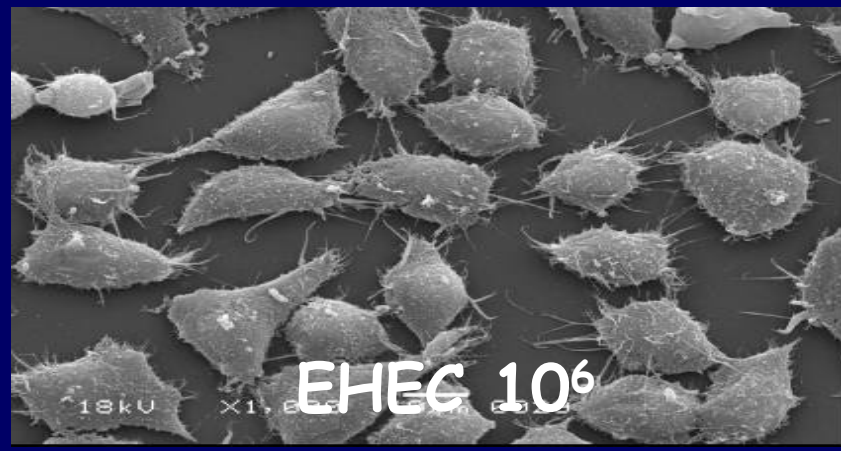
Bactericidal effect of SCS of *Lb. plantarum* against EHEC 71935

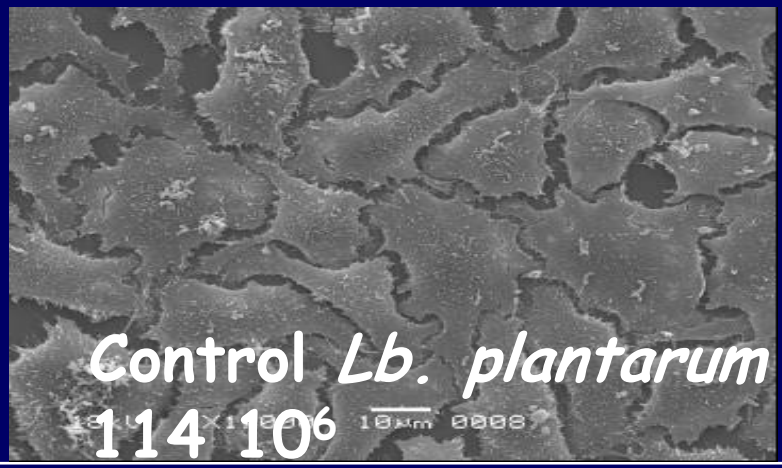


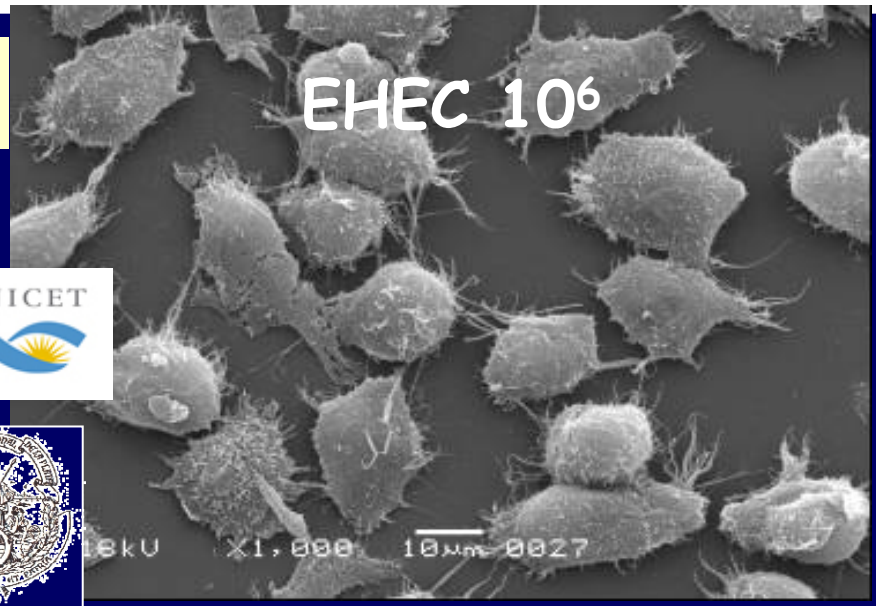
Strain CIDCA 83114

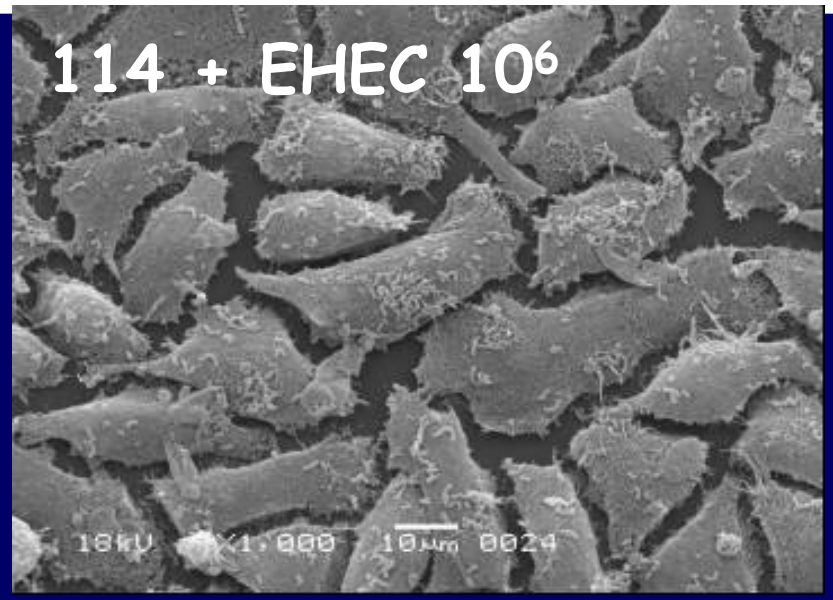
SEM of Hep-2 monolayer incubated during 3 h with:

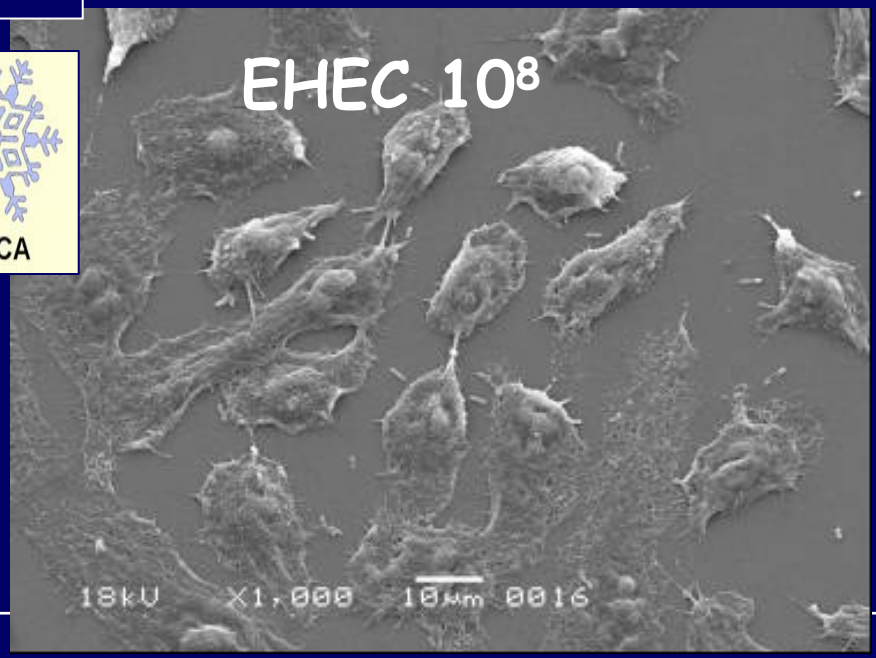












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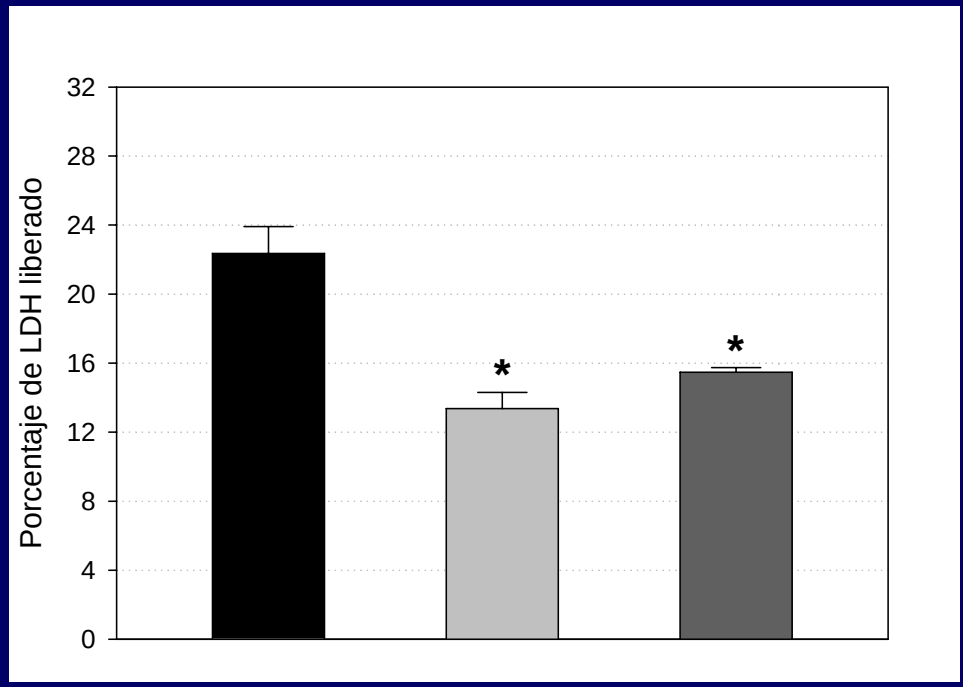
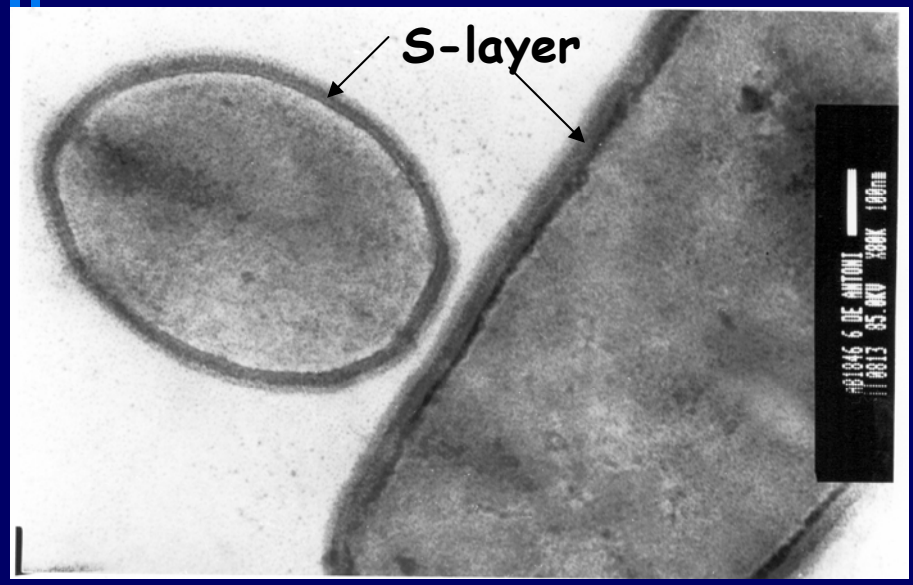
LDH liberated by Vero cells by EHEC SCS preincubated or not with *Lb. plantarum* CIDCA 83114 and their isolated walls

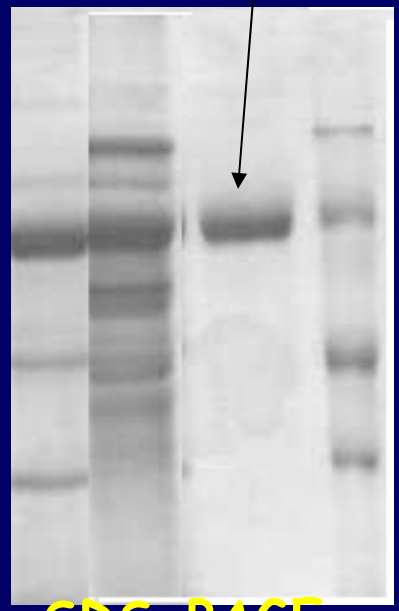




Lb. kefir

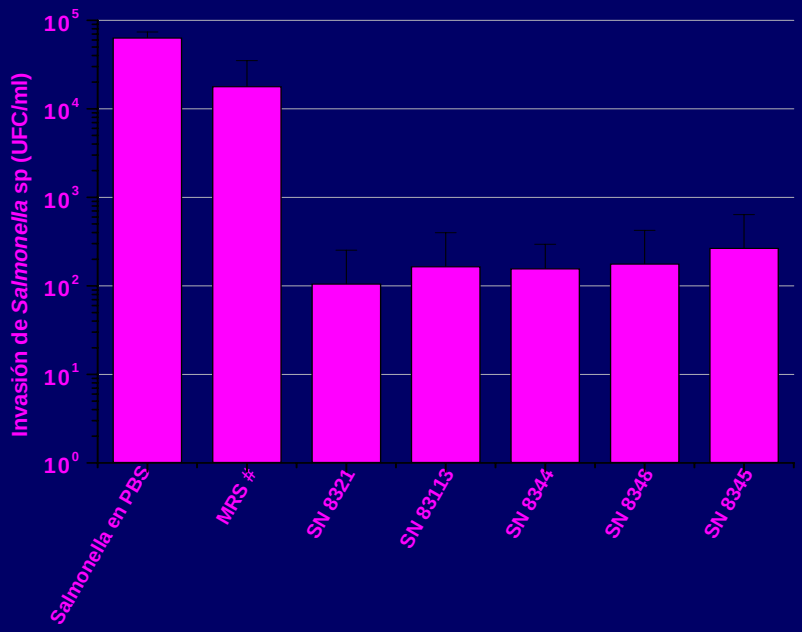
S-layer: Hydrophobic protein
66-71 kDa

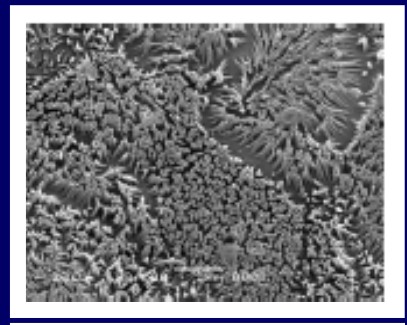
S-layer protein in SCS:
0.1 mg/ml



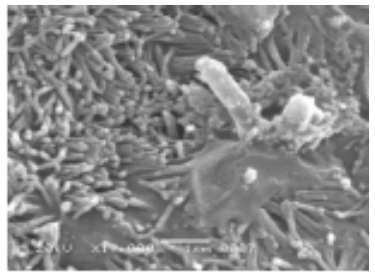
SDS-PAGE

Invation inhibition of Caco-2 cells by *Salmonella* preincubated with *Lb. kefir* SCS

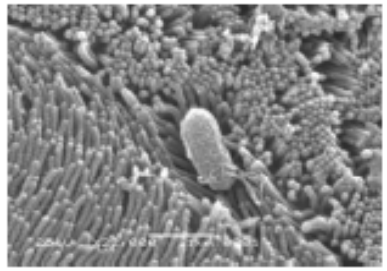




Caco-2

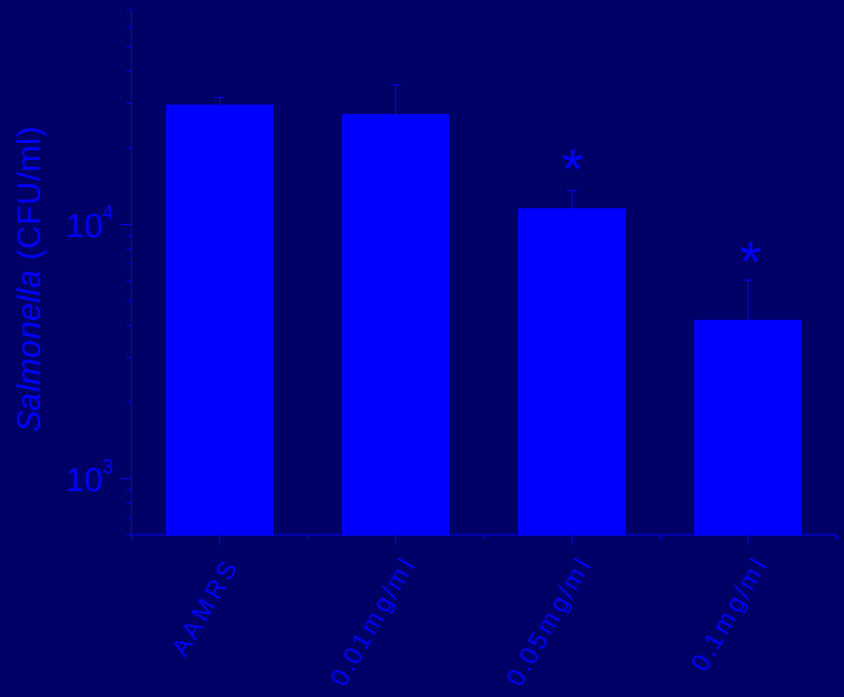


Caco-2 + *Salmonella*



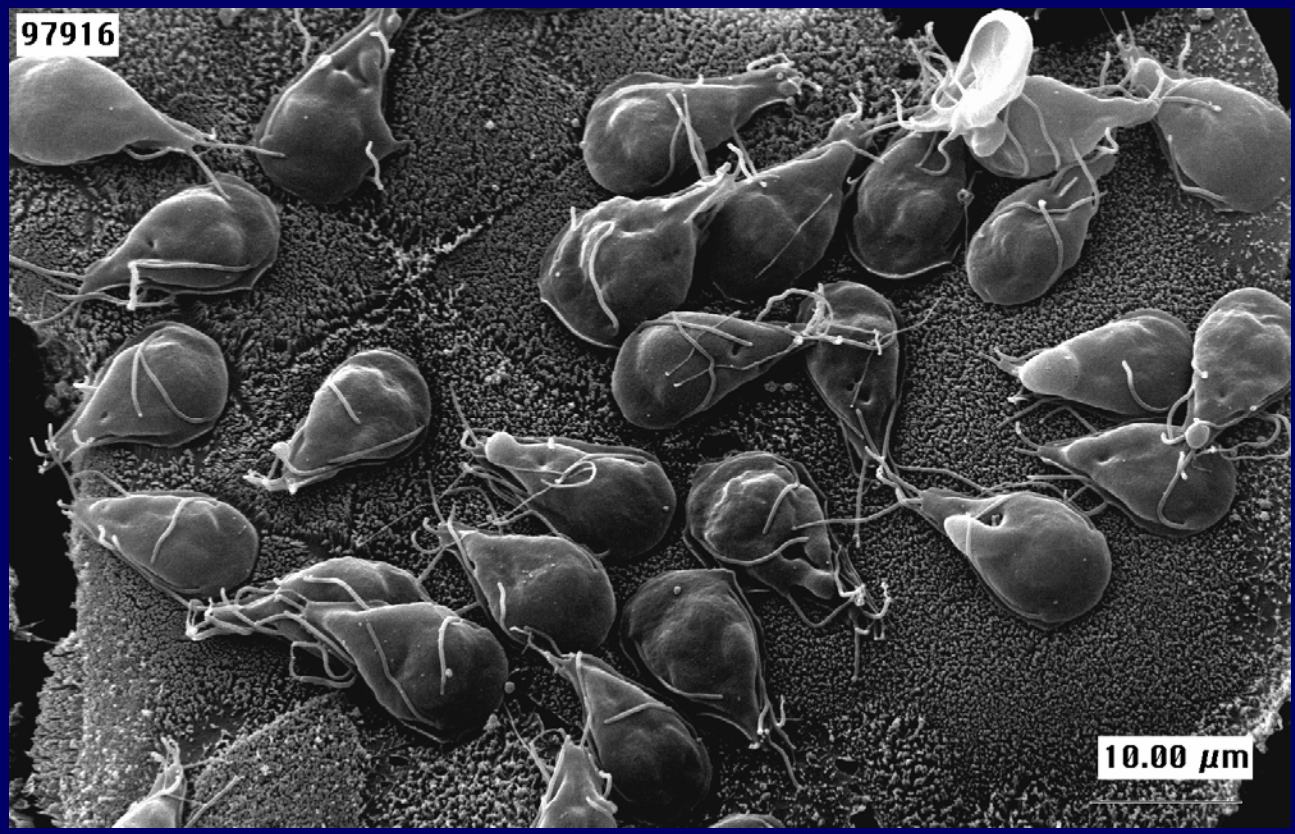
Caco-2 + *Salmonella* + *S-layer*

Invasion inhibition of Caco-2 by *Salmonella* preincubated with S-layer protein



S-layer protein was detected on *Salmonella* surface by Ag-Ac reaction

Adhesion of trophozoites of *Giardia intestinalis* WB on Caco-2 cells



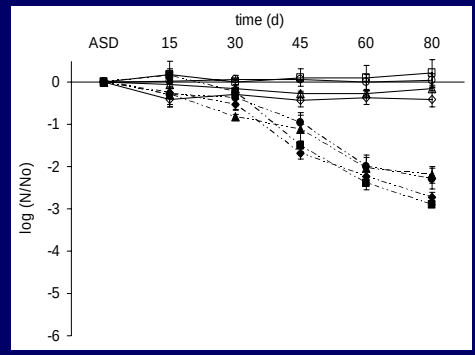
In-vivo assays

Mouse C57BL/6 (S. lipolytica CIDCA 812, L. plantarum CIDCA 83114, L. kefir CIDCA 8348)

	Trofozoites per animal → 2,6 .10^7	
	treatment	**control**
Infection rate (7 days post-inoculation	2/20 (10 %)	8/20 (40 %)
Trofozoites/ml Intestine content in infected animals	1.10^4	6.10^3

Spray drying: microorganismos del tricepa

- Leche en polvo reconstituída al 11%
- Temperaturas de salida: 70°C
- Temperatura de entrada: 180°C
- Presión: 5 Bar



80 días a 6°C el número de microorganismos viables :

L. plantarum CIDCA 83114: 10^{11} UFC/g

L. kefir CIDCA 8348: 10^{8} UFC/g

S. cerevisiae CIDCA 82112: 10^{6} UFC/g


Muchas Gracias