Inhibition of the foodborne pathogen *Listeria monocytogenes* by a bacteriocinogenic *Leuconostoc lactis* strain

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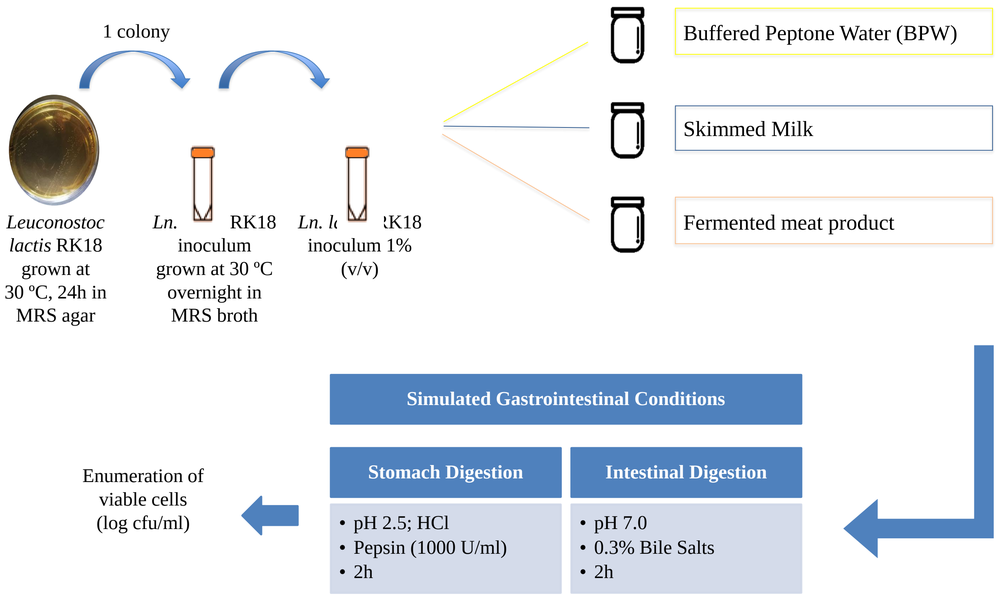
Introduction and Objective

Gastrointestinal disorders are responsible for approximately one million deaths each year across Europe as a consequence of poor diet, substantial morbidity and stress.[1] The use of probiotics can restore the composition and function of gut microbiota, which may enhance immunity and disease resistance [2]. Lactic acid bacteria strains have been recognized as probiotics and their tolerance to salts, acids and enzymes as well as their ability to adhere to intestinal cells have been highly exploited [3]. In addition, the secretion of antimicrobial compounds as bacteriocins can be one of the main characteristics for effective competitive exclusion of pathogens in the gut playing a positive role in human health.

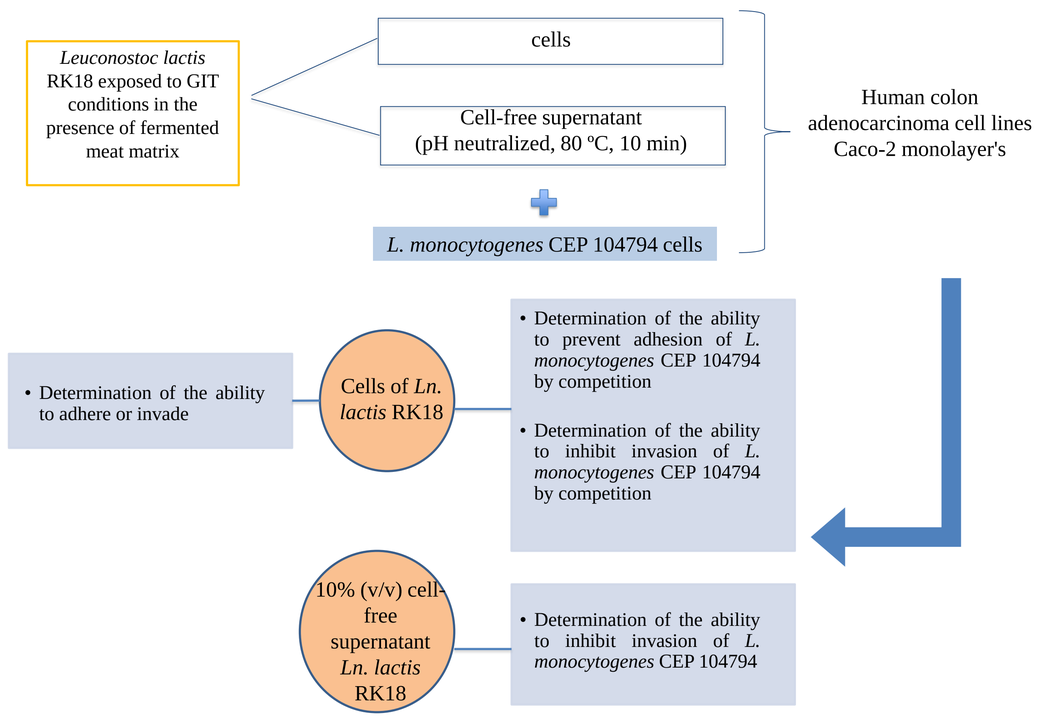
The objective of this study was to determine the ability of a bacteriocinogenic *Leuconostoc lactis* RK18 to survive through a simulated gastrointestinal tract (GIT) in the presence of different food matrices. For the survivors, also their ability to adhere to colon cell lines Caco-2 and simultaneously to prevent the adhesion/invasion of *Listeria monocytogenes* CEP 104794 cells by competitive exclusion or proteinaceous compounds was evaluated.

Methods

A. Survival of *Ln. lactis* RK18 through simulated gastrointestinal tract



B. Ability to adhere and/or invade human colon adenocarcinoma cells by *Ln. lactis* RK18 and to prevent the adhesion and/or invasion by *Listeria monocytogenes* CEP 104794



Results

A. Survival of *Ln. lactis* RK18 through simulated gastrointestinal tract

Results obtained for the survival of *Ln. lactis* RK18 through simulated gastric and intestinal digestions, for both matrices and control are shown in Table 1.

Table 1. Logarithmic reduction of *Ln. lactis* RK18 through simulation of gastrointestinal tract conditions

Matrix	Log N/N_0		
	0 min	60 min[a]	120 min[b]
BPW	0.00±0.00	-0.83±0.94	-6.30±120*
Skimmed milk	0.00±0.00	0.21±0.38	-6.71±1.01*
Fermented meat	0.00±0.00	-0.09±0.07	-1.37±0.22

Survival is represented as the mean of the logarithmic reduction: log (N/N0) ±the standard error of the mean; N is the CFU/mL at each sampling time; N_0 is the CFU/mL at time zero.
[a]Survival after exposure to pH 3.0 in the presence of pepsin.
[b]Survival after exposure to pH 3.0 in the presence of pepsin and subsequent exposure to bile salts at pH 7.0.
*Isolate was reduced to values below the detection limit of the enumeration technique

- The exposure to the stomach conditions (60 min) did not cause any significant reduction of *Ln. lactis* RK18.
- The exposure to the small intestine conditions (120 min) caused a greater reduction in viable cells tested in BPW and skimmed milk.
- Survival of *Ln. lactis* RK18 was only observed when protected by the fermented meat matrix with reductions lower than 1.5 log cfu/ml.

B. Ability to adhere and/or invade human colon adenocarcinoma cells by *Ln. lactis* RK18 and to prevent the adhesion and/or invasion by *Listeria monocytogenes* CEP 104794

- *Leuconostoc lactis* RK18 cells were able to adhere, but not invade intestinal Caco-2 cells (figure 1).
- Apparently, gastrointestinal conditions may influence the ability of *Ln. lactis* RK18 cells to adhere to Caco-2 cells, even in the presence of a food matrix.

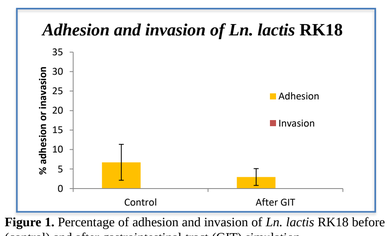


Figure 1. Percentage of adhesion and invasion of *Ln. lactis* RK18 before (control) and after gastrointestinal tract (GIT) simulation

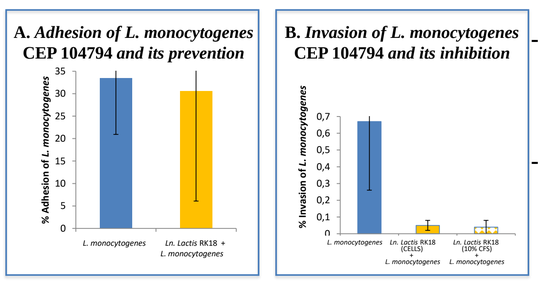


Figure 2. Percentage of *L. monocytogenes* CEP 104794 adhesion and its prevention by *Ln. lactis* RK18 cells (**A**) and percentage of *L. monocytogenes* CEP 104794 invasion and its inhibition by *Ln. lactis* RK18 cells or 10% CFS (**B**).

- Percentage of *L. monocytogenes* CEP 104794 adhesion to Caco-2 cells were high (33.5%), but the presence of *Ln. lactis* RK18 allowed a decrease in *L. monocytogenes* adhesion (figure 2A).
- *Listeria monocytogenes* cells were able to invade Caco-2 cells, but although the low invasion percentage of *L. monocytogenes* (0.67%), the presence of *Ln. lactis* RK18, cells or 10% of its treated supernatant (CFS), inhibited *L. monocytogenes* CEP 104794 invasion capacity *in vitro* (figure 2B).

Conclusion and Relevance

With this preliminary study, it was shown that a bacteriocinogenic culture of *Ln. lactis* RK18 presented functional properties combined with the ability to exert its inhibitory effect against the foodborne pathogen *L. monocytogenes* into intestinal cells.

References

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[3] Botes. M., Loos. B., Reenen. C. A. V., Dicks. L. M. T. 2008. Adhesion of the probiotic strains *Enterococcus mundii* ST4SA and *Lactobacillus plantarum* 423 to Caco-2 cells under conditions simulating the intestinal tract. and in the presence of antibiotics and anti-inflammatory medicaments. *Archives of Microbiology* 190(5): 573-584.

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