

# Screening of bacteriocinogenic lactic acid bacteria cultures and their characterization as potential probiotics



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PORTO

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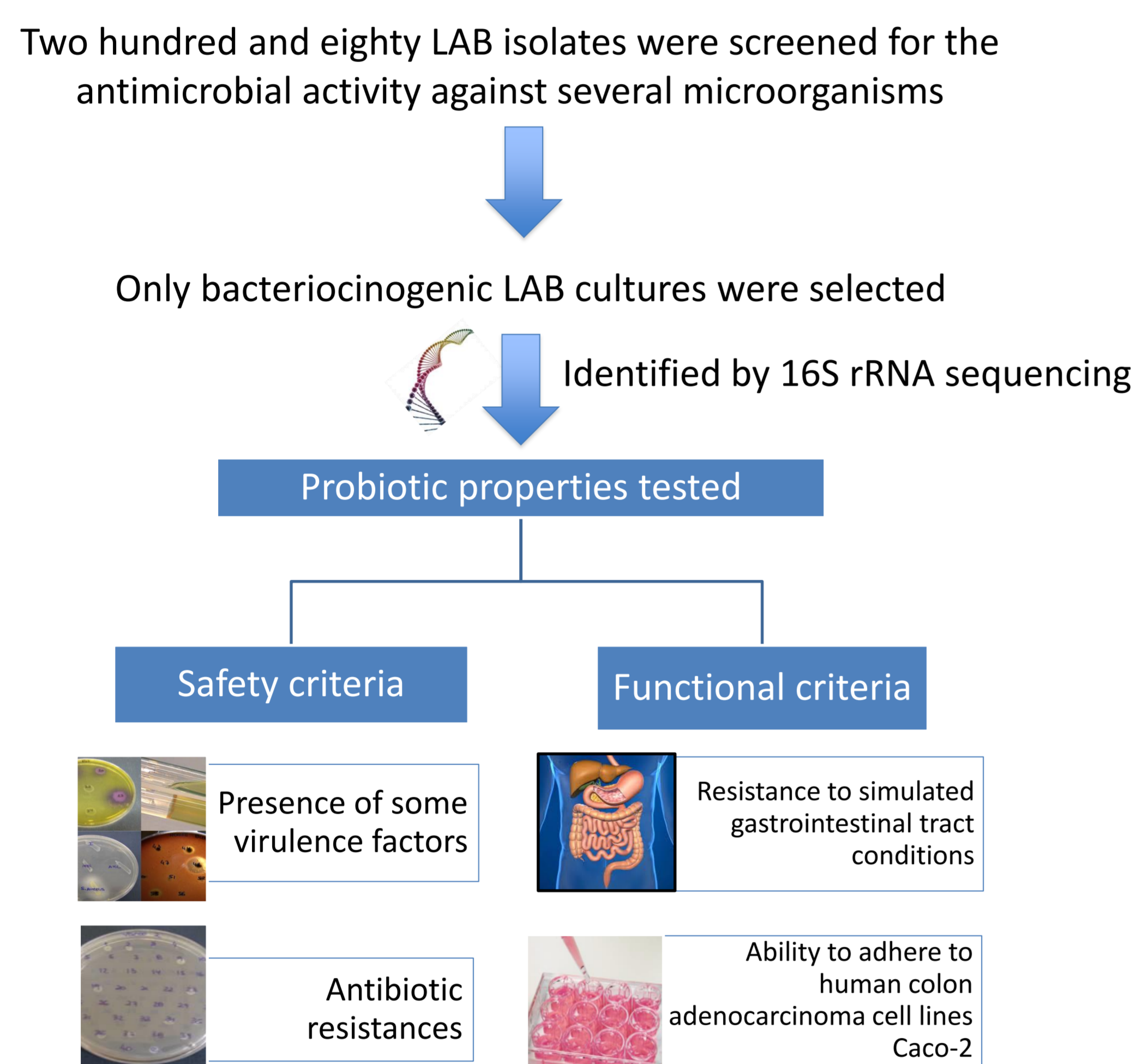
## Introduction

Ingestion of probiotic lactic acid bacteria (LAB) has been pointed out to confer a range of health benefits, including the immune system stimulation, reduction of infectious illness, among others (Harzallah and Belhadj 2013). Food products containing probiotics should follow the guidelines established by FAO/WHO (2001). In general, probiotics have to be harmless to the host, they cannot be pathogenic, mutagenic or carcinogenic, allergenic or provoke any type of adverse reactions.

For commercial purposes, potential probiotics need to be subjected to several tests: i) to the level of safety: origin, screening and selection of probiotics in terms of phenotype and genotype pathogenicity; ii) to the level of functionality: probiotics should be tolerant to acid and bile and possess intestinal epithelial adhesion properties; iii) to the level of technology: tests for genetically stable strains are required for large-scale production and iv) to the physiological level: immunomodulation, cholesterol metabolism, antimutagenic and anticarcinogenic properties are needed assays.

The aim of this study was to select different bacteriocinogenic LAB isolated from different food products and study some of their probiotic characteristics in terms of safety, functional and physiological properties.

## Methods



## Discussion & Conclusion

From all the 280 LAB isolates tested, only seven showed bacteriocinogenic activity and were selected for further studies. These isolates were identified as *P. pentosaceus* (6) and *L. plantarum* (1). All the isolates lacked intrinsic virulence factors and no antibiotic resistances were detected phenotypically. Also, all the isolates survived through the simulated gastrointestinal tract conditions, except *L. plantarum* R23, which growth was reduced in more than 2 log cycles. On the other hand, only *P. pentosaceus* CFF4 was able to adhere to Caco-2 cells (11 % of adherence after the simulated gastrointestinal tract passage).

In conclusion, this study demonstrated that, at least one isolate, *P. pentosaceus* CFF4, presented the most attributes to be a potential probiotic bacterium; however, more studies must be carried out to validate this potential.

## Results

- From 280 LAB isolates, 85 inhibited some target microorganisms by cell to cell competition, 31 by low pH (data not shown) and seven by production of proteinaceous compounds.

Table 1. Identification of seven LAB isolates by 16S rRNA sequencing

Food	Isolate	Identification	16S rRNA sequencing similarity
arugula	R23	<i>Lactobacillus plantarum</i>	99%
cheese	Q42	<i>Pediococcus pentosaceus</i>	99%
cheese	Q43	<i>Pediococcus pentosaceus</i>	99%
fermented shrimp	CFF4	<i>Pediococcus pentosaceus</i>	100%
fermented shrimp	CFF5	<i>Pediococcus pentosaceus</i>	100%
fermented shrimp	CFF51	<i>Pediococcus pentosaceus</i>	100%
fermented shrimp	CFF202	<i>Pediococcus pentosaceus</i>	100%

### Safety criteria of potential probiotics

None of the virulence factors tested (biogenic amines and hydrolytic enzymes) were produced by the seven LAB isolates, which also did not present any of the virulence gene tested (data not shown).

All LAB isolates were inhibited at concentrations equal or lower than the established cut-off value for all antibiotics tested (EFSA 2012), i.e. all isolates were sensitive to all antibiotics.

### Functional criteria of potential probiotics

Table 2. Survival of seven LAB isolates through simulated gastrointestinal tract conditions

Isolate	log CFU/ml (a)		
	0 min	60 min (b)	120 min (c)
R23	6.82 ± 0.06	4.49 ± 0.04	4.56 ± 0.05
Q42	7.16 ± 0.02	6.82 ± 0.06	6.64 ± 0.09
Q43	6.87 ± 0.20	7.18 ± 0.20	6.65 ± 0.09
CFF4	7.25 ± 0.12	7.12 ± 0.03	6.90 ± 0.22
CFF5	7.25 ± 0.03	7.02 ± 0.17	7.17 ± 0.13
CFF51	7.06 ± 0.27	7.30 ± 0.19	7.04 ± 0.05
CFF202	7.12 ± 0.07	7.07 ± 0.16	6.87 ± 0.06

(a) Survival is represented as the mean of the log CFU/ml ± the standard error of the mean.

(b) Survival after exposure to pH 2.5 in the presence of pepsin.

(c) Survival after exposure to pH 2.5 in the presences of pepsin and subsequent exposure to bile salts at pH 7.0.

After exposure to gastrointestinal tract conditions, none of the isolates were able to adhere to Caco-2 cells (data not shown), with the exception of *P. pentosaceus* CFF4, for which 11% adhesion was observed.

## Bibliography

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