

# Inhibition of *Clostridioides difficile* by lactic acid bacteria



CATÓLICA  
ESCOLA SUPERIOR  
DE BIOTECNOLOGIA

PORTO

MICROBIOTECH 19

CONGRESS OF MICROBIOLOGY  
AND BIOTECHNOLOGY 2019

Joana Barbosa & Paula Teixeira

Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado

Escola Superior de Biotecnologia, Rua Diogo Botelho, 1327, 4169-005, Porto, Portugal.

\*Corresponding author: jbarbosa@porto.ucp.pt

## Introduction

*Clostridioides difficile* infections have been associated with the antibiotics use (altering the intestinal microbiota, enabling the proliferation and toxins segregation by *C. difficile*).<sup>[1,2]</sup> Moreover, the increase of antibiotic-resistance of hypervirulent strains turns imperative finding alternative therapeutics with preventive and/or prophylactic effects, as the use of probiotics. Lactic acid bacteria are considered as a major group of probiotic bacteria and their ingestion has been pointed out to confer a range of health benefits.<sup>[3]</sup> There are several mechanisms by which probiotic strains may exert their inhibitory activity towards undesired enteropathogens: secretion of different metabolites as lactic acid, hydrogen peroxide, short-chain fatty acids, bacteriocins, proteases, etc.<sup>[4]</sup> Limited information is available on the ability of different probiotic bacteria used in food products to inhibit *Clostridium difficile*.

## Objective

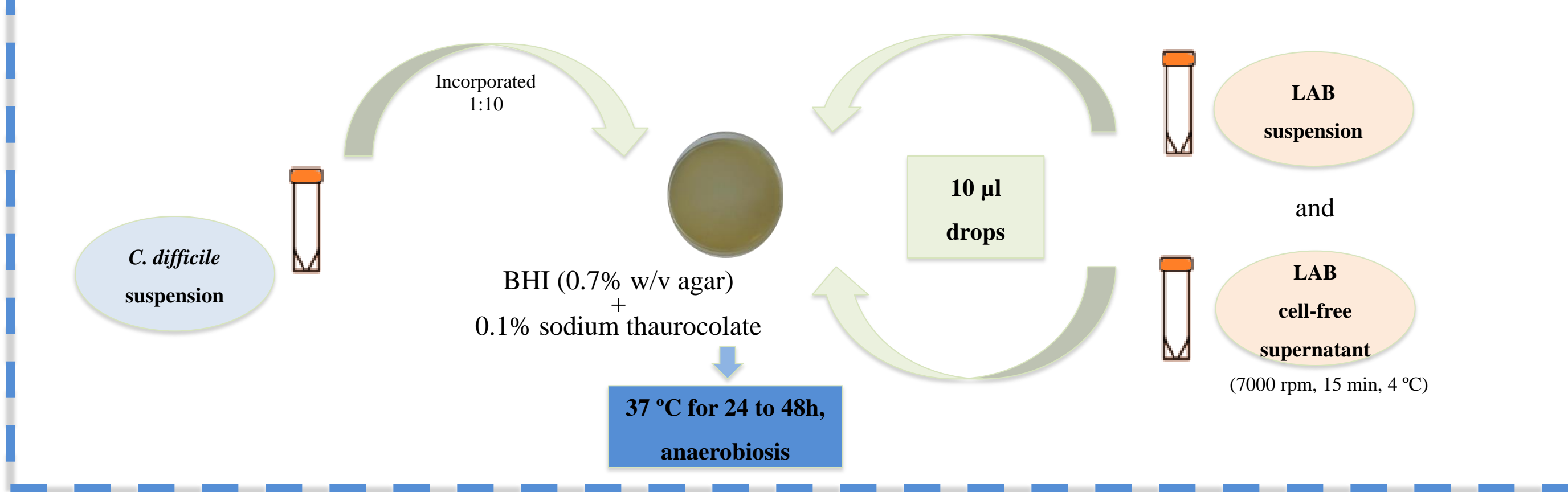
The objective of this study was to test the anti-clostridial activity of 450 lactic acid bacteria (isolated from various food products) against 5 *C. difficile* strains. For those lactic acid bacteria demonstrating inhibitory activity, the mechanisms of inhibition was investigated.

## Methods

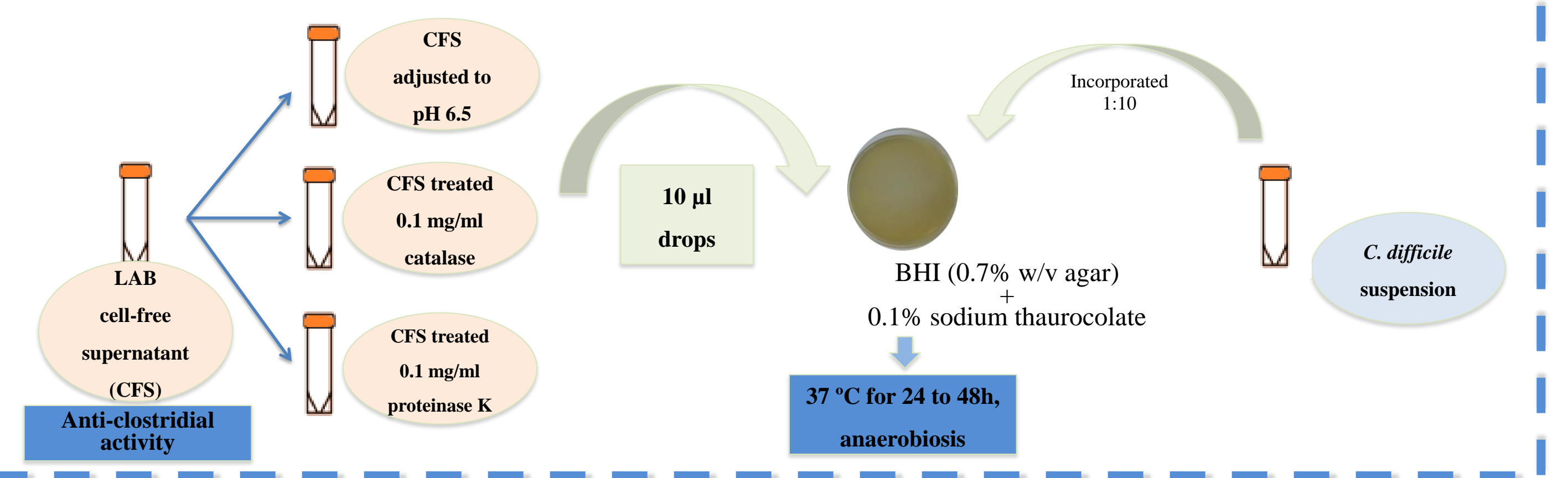
### *Clostridioides difficile* and lactic acid bacteria strains and preparation of inocula

Anticlostridial activity of 450 lactic acid bacteria (LAB) isolated from various food products (fermented meat products, smoked salmon, cheese, seafood, etc) were tested against five *C. difficile* strains (four gently provided by Hospital de S. Marcos, Braga: *C. difficile* H63866, *C. difficile* I805452, *C. difficile* I805937, *C. difficile* V315638 and one from German collection of microorganisms and cell cultures: *C. difficile* DSMZ 1296). Each LAB was cultivated in de Man, Rogosa and Sharpe agar plates (MRS agar) at 30 °C for 24h and, subsequently, one colony of each isolate was transferred to 10 ml of MRS broth. Each *C. difficile* strain was cultivated twice in Brain Heart Infusion broth (BHI broth) at 37 °C for 24 h under anaerobic conditions.

### A. Anti-clostridial activity of lactic acid bacteria strains



### B. Inactivation mechanisms of lactic acid bacteria with anti-clostridial activity



## Results

### A. Anti-clostridial activity of lactic acid bacteria strains

The percentage of lactic acid bacteria with anti-clostridial activity is presented in figures 1A and 1B.

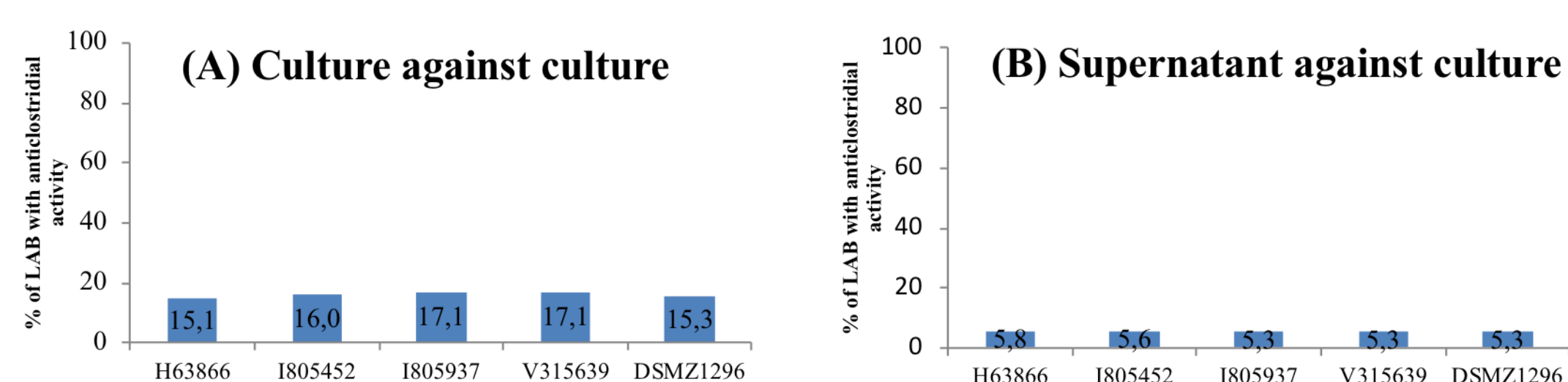


Figure 1. Percentage of LAB with anticlostridial activity against each *C. difficile* strain: (A) culture of LAB and (B) LAB cell-free supernatant against *C. difficile* culture.

### B. Inactivation mechanisms of lactic acid bacteria with anti-clostridial activity

The results obtained for the antimicrobial activity of 26 lactic acid bacteria against five *C. difficile* strains are shown in Table 1. In figure 2 is shown an image of inhibition halo zones obtained.

Table 1. Inhibitory activity of cell-free supernatants and neutralized cell-free supernatants of lactic acid bacteria against *C. difficile* cultures

LAB	Neutralized supernatant against culture					Neutralized supernatant treated with catalase against culture					Neutralized supernatant treated with proteinase K against culture				
	C/1805937	C/1805452	C/1805937	C/V315639	C/DSMZ1296	C/1805937	C/1805452	C/1805937	C/V315639	C/DSMZ1296	C/1805937	C/1805452	C/1805937	C/V315639	C/DSMZ1296
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

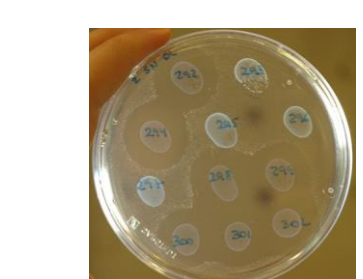


Figure 2. Translucent halo zones obtained for inhibitions of supernatant of several LAB against *C. difficile* culture I805452.

Legend:  
(+) = presence of translucent halo zone  
(-) = absence of translucent halo zone

## Discussion

- Of the 450 lactic acid bacteria, 15.1% (68) inhibited the isolate *C. difficile* H63866, 16% (72) *C. difficile* I805452, 17.1% (77) *C. difficile* I805937 and V315639 and 15.3% (69) *C. difficile* DSMZ1296, as a result of competition between cells (culture against culture).
- When the cells were centrifuged and the untreated cell-free supernatant was used, 26 LAB maintained their anticlostridial activity.
- After treatment of cell-free supernatant (CFS) of the 26 selected LAB it was proposed that *C. difficile* was inhibited:
  - by the action of **lactic acid** (12 isolates);
  - by the production of **hydrogen peroxide** (3 isolates);
  - by the action of **other nature compounds, other than bacteriocins** since, their activity was not lost after the digestion with the proteolytic enzyme proteinase K (6 isolates);
  - by the presence of **proteinaceous compounds**, since their activity was lost after the digestion with the proteolytic enzyme proteinase K (5 isolates). This suggests that their inhibitory mechanism may be bacteriocinogenic.

## Conclusion and Relevance

In conclusion, we have identified food LAB isolates displaying a clear inhibitory activity against five *C. difficile* strains. Although further *in vitro* tests are still needed, such as the ability of selected LAB to inhibit the invasion of *C. difficile* into intestinal Caco-2 cells, these LAB isolates may be used as part of potential anti-*C. difficile* strategies as alternative to antibiotics or as preventive of *C. difficile* infections.

## References

- [1] Bartlett JG (2006) Narrative review: the new epidemic of *Clostridium difficile*-associated enteric disease. *Ann Intern Med* 145: 758-764.
- [2] Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J Jr. (1995) *Clostridium difficile*-associated diarrhea and colitis. *Infect Control Hosp Epidemiol* 16: 459-477.
- [3] Sathyabama S, Vijayabharathi R, Devi PB, Kumar MR, Priyadarisini VB (2012) Screening for probiotic properties of strains isolated from feces of various human groups. *J Microbiol* 50: 603-612.
- [4] Zhang YC, Zhang LW, Ma W, Yi HX, Yang X, Du M, Shan YJ, Han X, Zhang LL (2012) Screening of probiotic lactobacilli for inhibition of *Shigella sonnei* and the macromolecules involved in inhibition. *Anaerobe* 18: 498-503.

## Acknowledgements

This work was supported by funding from the National Funds from the Fundação para a Ciência e a Tecnologia (FCT) through project UID/Multi/50016/2019. Financial support for author J. Barbosa was provided by a post-doctoral fellowship SFRH/BPD/113303/2015 (FCT).



FCT Fundação para a Ciência e a Tecnologia