The pathophysiology of Irritable Bowel Syndrome (IBS) involves increased intestinal permeability, micro-inflammation and gut dysbiosis with associated visceral hypersensitivity. The probiotic mix of Lactobacillus plantarum strains CECT7483 (KABP023) and Pedococcus acidilactici CECT7483 (KABP023) has shown positive clinical outcomes in IBS. However, their exact mechanisms of action have not been elucidated. Postbiotics are probiotic-derived metabolites or cell components that can improve host health. In this work, we sought to characterize said strains about the production of specific postbiotics known to influence gut permeability and inflammation - acetate, acetylcholine (ACH) and polyphosphate (polyP) - as well as their inhibitory activity against bacteria known to be increased in the gut of IBS patients.

### RESULTS

Production of acetate was dependent on carbon source (Fig 1A). The production from glucose was low. The fermentation of xylose increased the acetate production by all the strains while arabinose yielded the greatest concentrations. Acetate production was higher from pentoses than hexose. L. plantarum strains synthesized the largest amounts of ACh whereas P. acidilactici CECT7483 produced low and control strain OD values (Fig 1B). After 6 h of growth, amounts of PolyP granules was observed for the L. plantarum and control strains but not for Pediococcus (Fig 2). Strain CECT7484 produced the highest amounts of polyP followed by CECT 7485 and ATCC 53513. The 3 strains produced antimicrobials against all 6 target bacteria. Consistent with phenotypic analysis, in silico study confirmed the presence of phosphofructokinase (pfk) gene (responsible for acetate synthesis) in the 3 genomes and polysphosphate kinase (ppk) gene (polyp production) in the 2 L. plantarum genomes. Bacteriocin (antimicrobial compound) genes were found in the genomes of the 3 strains, with CECT7484 and CECT7485 (Table 3). Results are summarized in Table 3. Moreover, the 3 strains were susceptible to all the antibiotics tested except P. acidilactici CECT7483 for tetracycline (Table 2). However, genome analysis showed the strains do not harbor genes encoding antibiotic resistances.

### METHODS

We measured the capacity to Biosynthesize acetate from 3 carbon sources: glucose (hexose), xylose and arabinose (pentoses) by colorimetric assay, ACh by HPLC-ESI-MS/MS and polyP granules by fluorescence, using the commercial probiotic Lactobacillus rhamnosus ATCC35103 as control. Inhibitory activity was tested on 6 IBS-associated bacteria (2 enterobacteria, 2 Actinomyces spp. and 2 Streptococcus spp. strains). Supernatants from co-cultures of probiotic and target bacteria were defined and adjusted to pH 7 to only detect organic-acid independent inhibition. Target bacteria were cultured on the supernatants for 16 h and OD was monitored. Genomes were sequenced by HiSeq 2500-3000 and key genes involved in the biosynthesis of these postbiotics were searched. BLASTP search was performed with ≥ 70% identity and ≥ 70% coverage. Bacteriome regions were searched using BAGE3. Additionally, susceptibility of the 3 strains to 8 antibiotics required by European Food Safety Authority (EFSA) were determined according to ISO10262:2010. Genomes were compared against the Card database to search for antibiotic resistance genes.

### CONCLUSIONS

The probiotic strains were proven to produce postbiotic molecules including acetate, polyP, ACh and antimicrobial compounds (potentially bacteriocins) against IBS-associated microorganisms. This unique combination of postbiotics could explain the clinically beneficial effects of the probiotic mix in IBS subjects. In addition, the 3 strains lack of antibiotic resistances traits of concern as required by EFSA.

### REFERENCES


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**Table 3.** Susceptibility profile of the 3 probiotic strains and the control strain. (+, tested; +, +, not tested; **+, tested by two-way ANOVA followed by Bonferroni tests; *+, tested by one-way ANOVA followed by Bonferroni tests; #, not detected; ***, not tested).

**Table 2.** Summary of postbiotics produced by the 3 probiotic strains and the control strain. (+, tested; +, +, not tested)

**Figure 1.** Acute production determined after growing strains overnight in minimal medium with 20 g/l of glucose, xylose or arabinose. ACh biosynthesis was measured in overnight cultures in M9R L. rhamnosus ATCC 53103 was used as control. Statistical differences with control were determined by one-way ANOVA followed by Bonferroni tests. *p<0.01, **p<0.005, ***p<0.0005, ***, not detected.

**Figure 2.** PolyP granules quantified along the growth curve of strains cultured in M9 medium L. rhamnosus ATCC 53103 was used as control. Data adjusted per unit of DO550. If polyP CECT 7483 values were below the limit of detection. Dunnet test was carried out comparing with control (*, p<0.05).

**Figure 3.** Inhibition capacity through the production of antimicrobials of the 3 probiotic strains against IBS-associated microorganisms. Supernatant of the co-culture of probiotic with the microorganism tested was obtained and pH adjusted to 7. Microorganisms were cultured in the supernatants. OD (520 nm) was measured at 16 h. Each bacteria tested was grown in medium as control. Data adjusted by two-way ANOVA followed by Bonferroni tests considering data from all growth curves. *p<0.001.