# *In situ* phytate degradation by *Bifidobacterium* spp. as a novel approach to tackle micronutrients deficiencies in early life

Di Stefano E.[1,3], James K.[1], Bottacini F.[1,7], Esteban Torres M.[1], Derrien M.[2], Boniface-Guiraud A.[2], MacSharry J.[1,3,4], Bourdet-Sicard R.[2], Cotter P.D.[1,5], McAuliff F.M.[6], van Sinderen D.[1,3]

[1] APC Microbiome Institute, University College Cork, Ireland; [2] Danone Global Research & Innovation Center, Gif sur Yvette, France; [3] School of Microbiology, University College Cork, Ireland; [4] School of Medicine, University College Cork, Ireland; [5] Teagasc Food Research Centre, Moorepark, Ireland; [6]UCD Perinatal Research Centre, University College Dublin, National Maternity Hospital, Dublin, Ireland, [7]Department of Biological Sciences, Munster Technological University, Cork, Ireland

## INTRODUCTION

Phytic acid is the main form of phosphorous storage in plant seeds. Due to its polyanionic nature, phytic acid chelates mineral cations such as iron, zinc, calcium and magnesium, both in food matrices and in the stomach, resulting in insoluble phytate salts. Monogastric animals, like humans, lack sufficient intestinal phytase enzymes to break down phytates, which pass undigested through the gastrointestinal tract, impeding the absorption minerals complexed to it. In low- and middle-income countries (LMICs), where complementary foods are mainly plant-based, such as cereal or pulse porridges, phytate significantly contributes to micronutrient deficiencies, especially in weaning children.

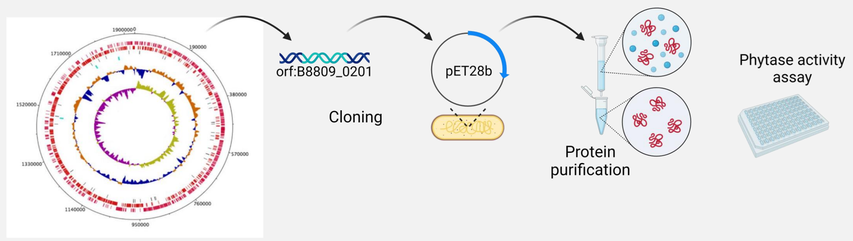
Phytase encoding genes have been identified in a variety of microorganisms (e.g, *Aspergillus niger*, *E.coli*), however only a few phytase degrading strains have been identified among Bifidobacterium spp., with *B. longum* subsp. *infantis* ATCC15697 being among the most active Bifidobacterium described. Being coloniser of the infant gut, Bifidobacteria could find valuable applications as probiotics for early life nutrition. Sufficient phytase activity in the gut could allow for *in situ* phytate degradation and offer an innovative approach to improve mineral absorption from plant-foods.

## OBJECTIVES

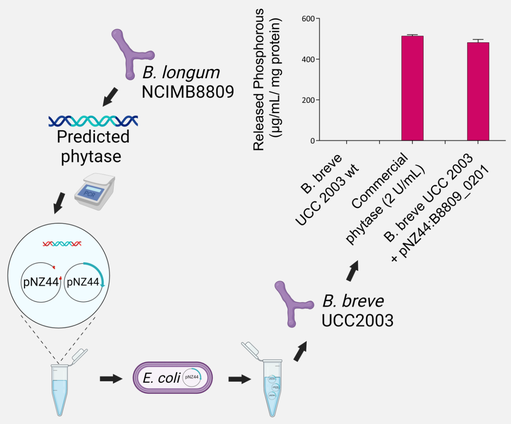
- To screen a collection of 50 human-derived *Bifidobacterium* spp. strains (species *B. longum, B. animalis, B. catenulatum, B. pseudocatenulatum, B. breve, B. bifidum*) for phytase activity *in vitro*.
- To sequence and annotate strains exhibiting phytase activity *in vitro*, to allow bioinformatic identification of candidate phytase genes.
- To test purified proteins encoded by candidate phytases identified by the bioinformatic approach for their phytase and phosphatase activity *in vitro*; Genes confirmed to encode phytases were further cloned and heterologously expressed in the host *B. breve* UCC2003, which lack phytase activity, and the resulting recombinant strains were assessed for their phytase activity.

## METHODS

1. *SCREENING BIFIDOBACTERIUM SPP. BANK*
   - Strains cultured in low-phosphate De Man–Rogosa–Sharpe medium
   - For *in vitro* phytase activity assessment, cells were washed and resuspended in sodium acetate buffer (50 mM, pH 5.5) and incubated with 0.75 mM sodium phytate (3 h). Released phosphate measured by colorimetric reaction with ammonium molybdate measured at $OD_{650}$ nm.
2. *BIOINFORMATIC SEARCH PHYTASE ENCODING GENES*
   - Phytase producing strains were sequenced and the resulting chromosomes were annotated.
   - Candidate phytase genes in *Bifidobacterium* strains were identified based on PFAM entries and experimentally characterized phytases from literature.
3. *PHYTASE ENCODING GENES TESTING & PROTEINS CHARACTERIZATION*




- 5 candidate phytase encoding genes cloned in *E.coli* BL21 (IPTG-inducible heterologous expression vector plasmid pET28b) and overexpressed (NZY Auto-induction LB medium). His-tagged proteins purified with Ni-NTA column; protein purity and size were confirmed by SDS-PAGE
- Phosphatase and phytase activity: purified proteins (0.2 mg/mL) incubated with 3 mM of substrates phytic acid sodium salt, para-nitrophenylphosphate (PNPP), 5-Bromo-4-chloro-3-indolyl phosphate (XP), or O-phospho-L-tyrosine (PLT) for 1h, 37°C. Released phosphate was measured by colorimetric reaction as described above




- Phytase activity of phytase encoding genes was tested in *B. breve* UCC2003 (vectors pNZ44, pBC1.2)

## RESULTS

**Fig. 1: Phytase activity** of 55 *Bifidobacterium* spp. strains (µg/mL phosphate /mg protein ). High: > 10. Moderate: 5-10. Low: 1-5. Absent: <1

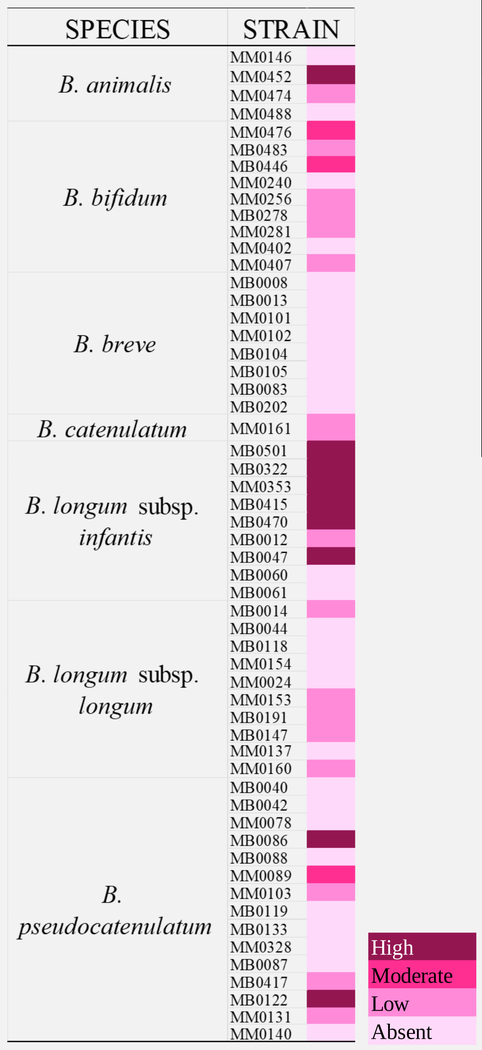
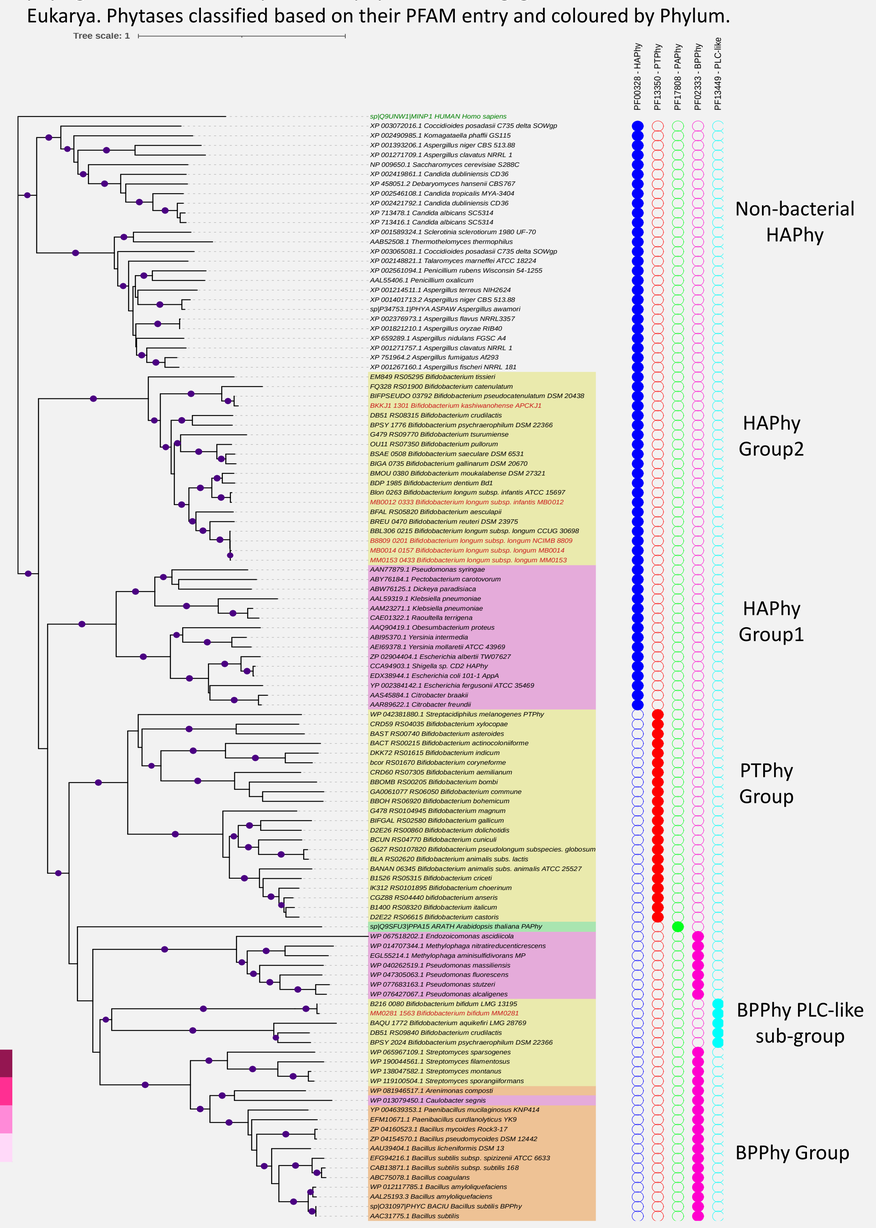



**Fig. 2: Classification of predicted Bifidobacterium phytases**. Neighbour-joining phylogenetic tree of the predicted phytase-encoding genes in various Bacteria and Eukarya. Phytases classified based on their PFAM entry and coloured by Phylum.




| Strain | Gene locus tag | Protein size (AA) |
|---|---|---|
| *B. longum longum* NCIMB8809 | B8809_0201 | 618 |
| *B. longum infantis* MB0047 | MB0047_0847 | 623 |
| *B. bifidum* MM0281 | MM0281_1563 | 829 |
| *B. kashiwanohense* APCKJ1 | BKKJ1_1301 | 637 |
| | BKKJ1_1001 | 125 |

**Table 1: Candidate phytase encoding genes.** Identification was based on PFAM entries and experimentally characterized phytases.

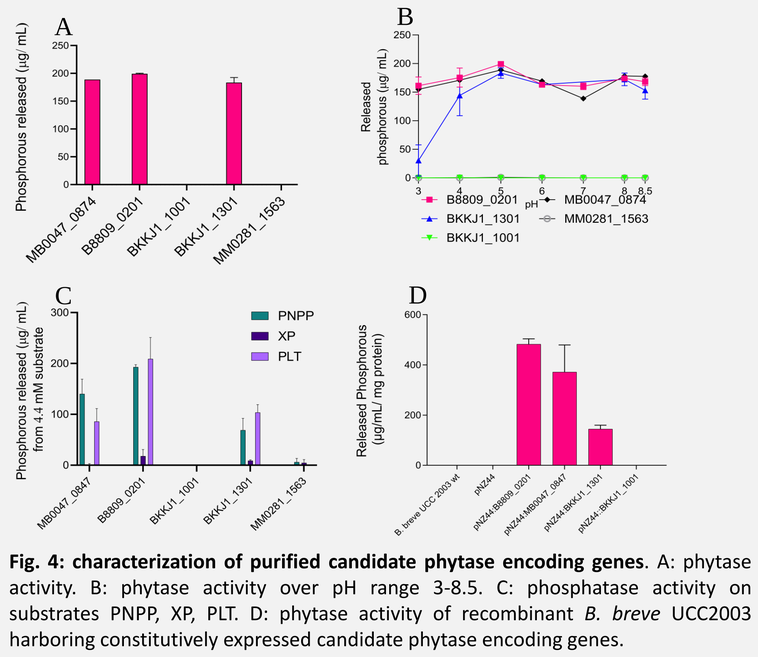



**Fig. 4: characterization of purified candidate phytase encoding genes**. A: phytase activity. B: phytase activity over pH range 3-8.5. C: phosphatase activity on substrates PNPP, XP, PLT. D: phytase activity of recombinant *B. breve* UCC2003 harboring constitutively expressed candidate phytase encoding genes.

## CONCLUSIONS

- In this study, we screened a bank of 50 human-derived *Bifidobacterium* spp. for *in vitro* phytase activity; 9 strains exhibited high activity, comparable to the previously described active *B. longum* subsp. *infantis* ATCC15697. The most active strains belonged to the species *B. longum, B. animalis and B. pseudocatenulatum.* No active strains were identified among the tested *B.breve* strains.
- Genes encoding phytases were identified in *B. longum* subsp. *longum* NCIMB 8809, *B. longum* subsp*. infantis* MB0047, *B. bifidum* MM0281 and *B. catenulatum* subsp. *kashiwanohense* APCKJ1 based on PFAM entries and previously characterized phytases. *B. longum* and *B. catenulatum* candidates were confirmed to be phytases, while no active pytase encoding gene was identified in *B. bifidum*.
- These findings deepen our understanding of phytase distribution and activity in the *Bifidobacterium* genus, laying the groundwork for tailored solutions to enhance micronutrient absorption from phytate-rich foods.

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