

Title	Developing spore-forming gut bacteria as model organisms
Authors	Faherty-McGrath, Louisa B.;van Sinderen, D.;Browne, H.P.
Publication date	2026-08
Original Citation	Faherty-McGrath, L B, van Sinderen, D & Browne, H P 2026, 'Developing spore-forming gut bacteria as model organisms', Trends in Microbiology, vol. 34, no. 8, pp. 840-850. https://doi.org/10.1016/j.tim.2026.04.006
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://www.scopus.com/pages/publications/105037771671 - 10.1016/j.tim.2026.04.006https://www.scopus.com/pages/publications/105037771671?origin=resultslist
Rights	© 2026, The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). - https://creativecommons.org/licenses/by/4.0/
Download date	2026-10-28 14:15:28
Item downloaded from	https://hdl.handle.net/10468/18772

Opinion

Developing spore-forming gut bacteria as model organisms

Louisa B. Faherty-McGrath^{1,2}, Douwe van Sinderen^{1,2}, and Hilary P. Browne^{1,2,*}

Anaerobic gut bacteria have highly evolved functions that promote transmission between human hosts. These include resilient spores produced by many Bacillota (formerly Firmicutes) bacteria, which are tolerant to extended ambient oxygen exposure. Recent culturing and genomic studies have revealed the taxonomic diversity of gut spore-formers and their prevalence in human populations. However, due to a reliance on spore-forming model organisms that are not representative of commensal gut bacteria, we still have a limited understanding of gut microbiome-specific sporulation processes and their underlying genetics. In this opinion article, we outline a rational path for development of new model organisms derived from commensal spore-forming gut bacteria in order to obtain fundamental insights into sporulation and to provide a discovery platform for novel probiotic or microbiome-based therapeutic development.

Sporulation in the human intestinal microbiome

Bacteria constantly encounter stress, including temperature changes, excess or insufficient oxygen, shortage of nutrients, and predation [1]. To counter these challenges, sophisticated dormancy mechanisms ensure long-term survival by arresting growth and metabolic activity until more favourable conditions occur. The production of highly stress-resilient, metabolically dormant structures, such as endospores (spores for short), by members of the Bacillota phylum enhances survival by protecting bacterial DNA and retaining cellular integrity in a dehydrated core surrounded by a cross-linked peptidoglycan cortex and an outer coat [2,3]. While metabolically dormant, spores can quickly recognise environmental signals, resulting in germination and outgrowth of a newly formed vegetative cell [4,5]. Spore-forming bacteria are found in many habitats, including soil, marine sediments, thermal vents, and host microbiomes [6,7]. Their resilience is remarkable, with spores of some species surviving exposure to outer space, as well as temperatures and pressures fatal to humans; hence, spores are considered one of the most durable biological entities [8].

Sporulation is a prevalent phenotype among bacterial members of the human gut microbiome [7,9–12]. Spores promote transmission by providing a stable, dormant state tolerant to environmental challenges encountered during transmission between hosts. As a result, sporulation is associated with greater transmissibility of gut microbiome species, as measured by prevalence [7,13,14]. These spore-formers are predominantly health-associated, with species-specific abilities to protect against pathogen infection, impact gut motility through the promotion of host biosynthesis of serotonin, and modulate the host immune system by inducing regulatory T cells through short-chain fatty acid production [15–19]. The ability to maintain bacterial viability as an extremely durable spore also gives spore-formers advantages over non-spore-forming bacteria for development as probiotics or microbiome-based therapeutics, including survival during formulation, long-term storage without a cold chain or

Highlights

Sporulation is a widespread phenotype in the human gut microbiome, promoting the transmission of commensal anaerobic bacteria.

Spore-forming gut bacteria have potential as probiotics or microbiome-based therapeutics due to their beneficial properties, including pathogen inhibition. Durable spores also enhance survival and maintain viability during formulation and storage.

Most knowledge on gut spore-formers relies on *Bacillus subtilis* and *Clostridioides difficile*. We propose that these are unsuitable as models for commensal gut bacteria, as they are not commonly found in the gut (*B. subtilis*), most strains are pathogenic (*C. difficile*), or they do not have known health associations compared with more prevalent gut commensal bacteria.

We outline a path to develop novel gut commensal spore-formers as model organisms, which can provide a means to better understand gut sporulation processes and serve as a resource for therapeutic applications.

¹School of Microbiology, University College Cork, Cork, Ireland

²APC Microbiome Ireland, University College Cork, Cork, Ireland

*Correspondence: hilary.browne@ucc.ie (H.P. Browne).

lyophilisation, and intact (i.e., viable with retained cellular integrity) delivery to the intestinal target site [20].

However, to realise their therapeutic benefits, a greater understanding of gut spore-former biology is required, including their metabolic capabilities, microbiome community interactions, as well as host interactions and sporulation processes. Due to their role in disease, several spore-forming species are well studied, including food-associated pathogens such as *Clostridium perfringens* and *Bacillus cereus* [21,22]. *C. perfringens* is also a risk factor for necrotising enterocolitis in infants [23]. The best-studied gut-associated spore-former, *C. difficile* (formerly *Clostridium difficile*), currently acts as a guidepost for characterising gut microbiome sporulation. However, the pathogenicity of these species makes them unsuitable as model organisms for gut commensal spore-formers. Although non-toxicogenic strains of *C. difficile* exist, they are not as strongly associated with human health compared with more prevalent spore-forming species found in the gut [23–25]. More broadly, *B. subtilis* is the best-studied spore-former and a commonly consumed probiotic [3,26,27]. However, as we will outline in the subsequent sections, *B. subtilis* is phylogenetically distinct from prevalent gut spore-formers and is not commonly detected in the gut. Therefore, we feel that there is an obvious and imperative need for new commensal gut microbiome spore-forming model organisms.

In this opinion article, we discuss current spore-former model organisms and their purported probiotic characteristics. We outline a path for isolating and characterising commensal gut spore-formers that, based on ecological principles and phylogenetic placement, may act as new and relevant model organisms. We also discuss the knowledge gaps these species can address in terms of understanding sporulation in the gut microbiome and their therapeutic potential.

Sporulation in the model spore-formers *B. subtilis* and *C. difficile*

Most of our knowledge of sporulation genes and gut sporulation processes in commensal gut bacteria is based on comparative genomic studies using *B. subtilis* and *C. difficile* [7,28–30]. *B. subtilis* (family Bacillaceae) is a facultative anaerobe and one of the most intensively studied bacterial species based on published literature, as well as the best-studied spore-former due to its extensive history of research, natural genetic competence, and diverse molecular toolbox [26,31]. Both detected and metabolically active in multiple environments, including soil and other environmental samples, recent studies have also recovered *B. subtilis* strains from the human gut [26,32,33]. *C. difficile* (family Peptostreptococcaceae) is a strict anaerobe and a major nosocomial pathogen, being the leading cause of hospital-associated diarrhoea. *C. difficile* spores are resistant to oxygen and disinfectants, ensuring their persistence and promoting transmission in clinical settings [2,5,34]. Depletion of the commensal intestinal microbiota through antibiotic consumption in hospitalised patients reduces colonisation resistance, allowing *C. difficile* to exploit available niches with consequent gut colonisation.

Sporulation is an energetically expensive biological process, with entry into sporulation tightly controlled through a phosphorelay system, in which histidine kinases, phosphotransferases, and phosphatases modulate the level of phosphorylated response regulator Spo0A (Spo0A-P). Spo0A is the master regulator of sporulation, and accumulation of Spo0A-P beyond a critical threshold triggers the sequential activation of four cell compartment-specific RNA polymerase sigma (σ) factors (σ^F , σ^E , σ^G , and σ^K) that coordinate the spatiotemporal transcription of downstream sporulation genes, ultimately resulting in lysis of the mother cell and release of the mature spore. Figure 1 provides an overview of some key sporulation genes shared between, and unique to, *C. difficile* and *B. subtilis*.

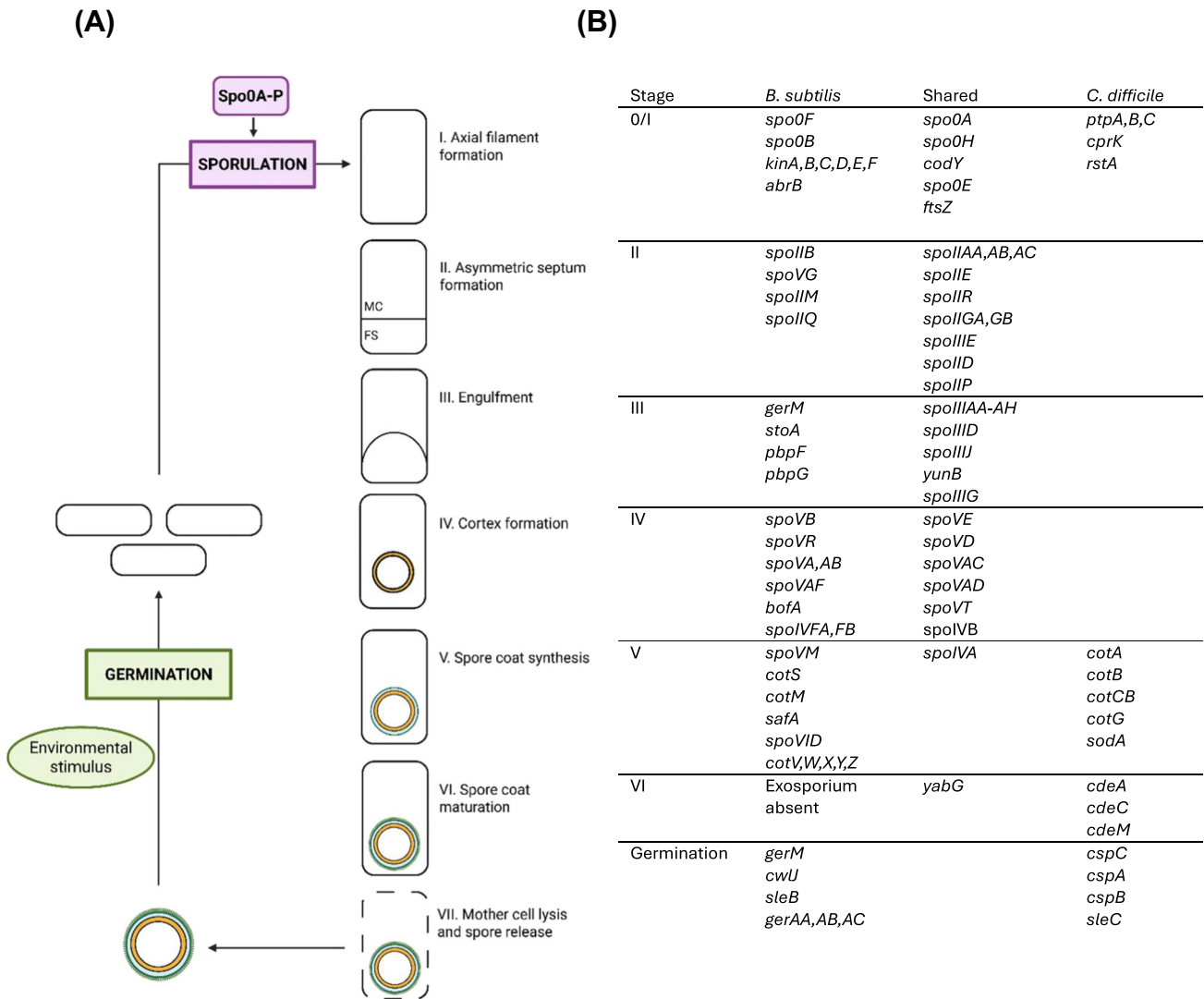


Figure 1. (A) Schematic overview of sporulation stages and key sporulation genes. Spore germination leads to outgrowth of a vegetative cell capable of forming a new spore and completing the sporulation life cycle. Spo0A-P is phosphorylated Spo0A. (B) Conservation of sporulation genes associated with different sporulation stages in *B. subtilis* and *C. difficile*, highlighting genes that are shared and unique to both species. This list illustrates the commonalities and differences in sporulation gene architecture between the two species and includes a subset of the total number of genes required to form and germinate a spore. Stage 0 (sporulation onset) and stage I (axial filament formation) are combined. This figure was created using BioRender (<https://BioRender.com/nravg7s>). FS: forespore; MC: mother cell.

While *spo0A* and the genes encoding the sporulation-specific sigma factors are conserved in all spore-former genomes analysed to date, other sporulation genes are not, thus providing evolutionary flexibility to adapt to different environments. For example, the multicomponent phosphorelay system required for Spo0A activation in *B. subtilis* is not conserved in many Clostridia. In addition, the majority of sporulation genes are not conserved between *C. difficile* and *B. subtilis*, and only approximately 25% of spore coat proteins are conserved between both species [28,29,35]. These proteins are important for environmental interactions and adhesion to external surfaces, and this divergence reflects the different ecological niches these species typically occupy. Furthermore, the exosporium layer surrounding the spore coat is absent in

B. subtilis, despite being present in *B. cereus*, *B. anthracis*, *B. thuringiensis*, and *C. difficile* [36]. Bacteriophages can also contain sporulation genes, including *spo0A*, providing a means to potentially regulate sporulation and disseminate sporulation genes within the gut microbiome [37]. Future studies of spore-formers in distinct taxonomic families based on new model organisms are likely to reveal additional differences in sporulation gene content compared with *B. subtilis* and *C. difficile* [29].

Spores generally remain dormant until they encounter small molecules detected by spore receptors, initiating a cascade of germination events and restoring active metabolism and outgrowth of the vegetative cell [2,5]. Germinants include physical factors such as hydrostatic pressure, nutrients such as sugars and amino acids, and non-nutrients such as bile salts [5,7,38–40]. CspC, along with CspA, are the germinant receptors in *C. difficile* that form a heterodimer in response to bile acids such as taurocholate and amino acid cogerminants. This leads to activation of the protease CspB and subsequently the cortex-lytic enzyme SleC, resulting in spore cortex degradation, spore core hydration, and outgrowth of the vegetative cell [41]. However, *C. difficile* lacks the Ger germinant receptors found in many other spore-formers, including *B. subtilis* [29,41,42] (Figure 1). These function as transmembrane channels that release ions in response to nutrients, leading to germination [43]. Other gut spore-formers, such as *Clostridium innocuum* and *Erysipelatoclostridium ramosum* (family Erysipelotrichaceae), also lack GerB and GerC [29]. This further illustrates differences in germination processes between phylogenetically diverse gut bacteria.

Gut spore-formers beyond *C. difficile* also readily germinate in response to intestinal bile salts, highlighting shared evolutionary adaptations to the intestinal environment. Different germination responses are observed between species depending on the bile acid encountered. For example, 16S rRNA amplicon-based identification of taxa from ethanol-treated stool demonstrated that glycocholate was a more potent germinant compared with taurocholate, whereas our previous work using six purified isolates showed taurocholate to be more effective [7,38]. While methodologically different, this still highlights poorly understood adaptations of gut bacteria to bile acid-induced germination and the requirement for new gut model spore-formers.

Current spore-forming probiotics and microbiome therapeutics

Members of the *Bacillus* genus, including *B. subtilis* and *Bacillus coagulans*, are currently the most widely consumed spore-forming probiotics, reflecting their long and safe use in food manufacturing and generally recognised as safe (GRAS) status (Table 1). Enterogermina (produced by Sanofi/Opella), an over-the-counter *Bacillus clausii* spore-based supplement for the prevention of infantile diarrhoea, marked the first use of the genus *Bacillus* as a probiotic in 1958 [27]. The proposed health benefits of *Bacillus* probiotics, primarily demonstrated through *in vitro* studies or animal models, include colonisation resistance mechanisms to inhibit pathogens, host immunomodulation, and reduction of inflammation [27,44].

While it is difficult to establish the provenance of the exact strains used as probiotics listed in Table 1, these *Bacillus* species are rarely detected in the human gut microbiome. In four recent studies using large-scale culturing approaches from European, North American, and Asian populations, only 48 *Bacillus* isolates were retrieved by cultivation from a total of 16 601 isolates (thus representing 0.3%) [9,45–47]. Furthermore, metagenome studies profiling gut microbiomes of distinct human populations demonstrate that *Bacillus* species are not common gut colonisers. *Bacillus* species were detected in less than 0.16% of a large Dutch cohort of 8208 individuals and were not detected in metagenomes from 1801 individuals from Burkina Faso, Ghana, Kenya, and South Africa using read mapping approaches [48,49]. Based on this, we conclude

Table 1. Spore-based probiotics currently on the market

Product	Claims	Species	Dose (CFU/ml)
Bio-Kult Everyday	Digestive and immune support	<i>B. subtilis</i> PXN® 21® and 13 other bacterial strains	2×10^9
Lactoflorene Plus	Gut microbiome restoration	<i>B. coagulans</i> BC513 and three other bacterial strains	4×10^7
Primal Defense Ultra	Promotes healthy digestion	<i>B. subtilis</i> and 12 other species	5×10^9
Gut Health All-in-one	Supports regular digestion	<i>B. coagulans</i> (MTCC 5856)	2×10^9
Youtheory Spore Probiotic	Supports digestive health	<i>B. clausii</i> (MTCC 5980) <i>B. subtilis</i> (MTCC 5981) <i>B. coagulans</i> (MTCC 5856)	6×10^9
MegaSporeBiotic	Maintains a healthy gut barrier and supports immune function	<i>B. subtilis</i> (HU58) <i>B. coagulans</i> (SC-208) <i>B. clausii</i> (SC-109) <i>Bacillus licheniformis</i> (SL-307) <i>Bacillus indicus</i> (HU36)	4×10^9
Clinical Daily Smart Defense Mega Spore Probiotic	Advanced immune and digestive support	<i>B. subtilis</i> DE111® and six other species	5.75×10^9
Cura Sporebiotics	Reduces gut inflammation, supports immune health, and gut microbiome restoration	<i>B. subtilis</i> (HU58) <i>B. coagulans</i> (SC-208) <i>B. clausii</i> (SC-109)	3×10^9
Spore-Based Bio Cultures	Supports digestive and immune health	<i>B. coagulans</i> (MTCC 5856)	2×10^9
Digestive Advantage® Daily Probiotic 4 in 1	Helps with immune health, occasional bloating and diarrhoea, and minor abdominal discomfort	<i>B. coagulans</i> GBI-30, 6086 (BC30)	2×10^9

that the majority of *Bacillus* species are not prevalent in the human gut microbiome and lack functional adaptations needed for stable colonisation, unlike more prevalent species.

While probiotics, by definition, do not have to colonise, for some desired outcomes, it is reasonable to assume colonisation to sufficient abundance levels and temporal scales is required to exert a therapeutic effect (e.g., to produce sufficient levels of an antimicrobial peptide or an immunomodulatory molecule) [50]. For example, FDA-approved, human microbiome-derived spores are currently in use as live biotherapeutic products (LBPs) with demonstrated colonisation (or engraftment) to prevent recurrent *C. difficile* infection (rCDI) following antibiotic treatment [51]. VOWST contains purified intestinal spores in an oral capsule and resolves infection through several proposed mechanisms, including depletion of primary bile acids, enrichment of secondary bile acids, and increased short-chain fatty acid production [52]. As VOWST contains fractionated spores, it can be stored for up to 36 months at room temperature without the need for lyophilisation or freeze-drying to preserve bacterial viability. In contrast, REBYOTA, another FDA-approved live faecal microbiota-based therapeutic used for the prevention of rCDI that lacks spores, requires thawing prior to use and must be stored at 2–8°C, with a shelf life of up to 5 days [53]. VOWST highlights the benefits of using human-derived spores to resolve infection and underscores the value of developing new model spore-formers that have future therapeutic potential.

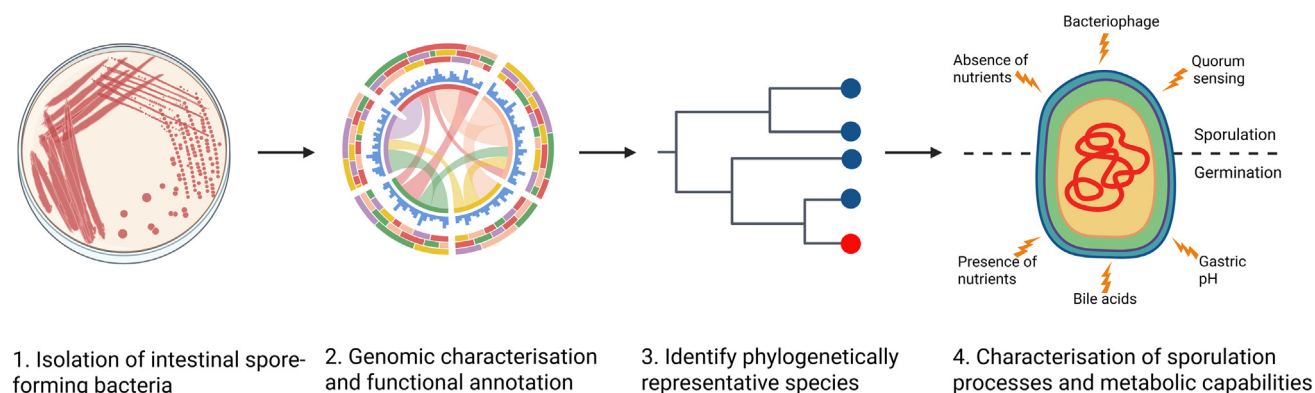
Given their low prevalence in the gut microbiome of human populations, we suggest *Bacillus* species are unsuitable to act as model organisms of commensal spore-forming gut bacteria, as they lack sufficient ecological relevance and are not as impactful for human health compared

with species more commonly found in the gut environment. Using the same culture-based and metagenome studies, *C. difficile* is also present at very low levels (0.08% prevalence in culturing studies and absent from the metagenomics-based studies); hence, nontoxigenic *C. difficile* strains are also unsuitable. A more appropriate option is to study one or more spore-forming species that are (i) highly adapted to the gut, (ii) prevalent in human populations, and (iii) associated with human health. Detailed characterisation can then pave the way for probiotic or therapeutic development. Next, based on these criteria, we outline a rational path for the selection of new model organisms to study gut sporulation and the role of spore-formers in human health.

Rational selection of species as model commensal gut spore-formers

Model organisms share several features that make them suitable for extensive research, including a long history of use generating increased knowledge over time, ease of growth under laboratory conditions, and amenability to genetic manipulation [54]. They can act as exemplars of important closely related organisms; for example, *B. subtilis* is used to understand the biology of *Bacillus* pathogens such as *B. anthracis* [26]. To provide ecological relevance, model organisms should also ideally be prevalent in the environment under study. Using these criteria, Figure 2 outlines a workflow for the development of commensal gut spore-formers as model organisms. While there is some overlap of common features required for therapeutic and model organism development (e.g., ease of use and consistent high spore yields), we also highlight distinguishing features where appropriate (e.g., for therapeutic development, the lack of virulence factors and amenability to industrial formulation will be more relevant).

The first step (step 1) is the isolation of spore-forming bacteria from faecal samples (i.e., of presumed gut origin). Recent studies have isolated over 150 different bacterial species that produce ethanol-resistant spores from faeces [7,9,45]. Immersion of faecal samples in ethanol kills vegetative cells, and only ethanol-resistant spores survive. Following ethanol shock, plating of faecal samples on rich nutrient agar, such as yeast casitone fatty acid (YCFA) or Gifu anaerobic medium (GAM), with appropriate germinants such as bile acids, leads to the outgrowth of spores, allowing their isolation as pure colonies [38]. While ethanol is extremely effective at killing non-spore-formers, this approach may kill ethanol-sensitive spores that are still hardy enough to tolerate environmental insults encountered during transmission. Exposure to high temperatures (such as 80°C for 30 min) is an alternative approach to select for spores [55]. Many of these isolates



Trends in Microbiology

Figure 2. Workflow for developing commensal gut spore-forming bacteria as model organisms. The workflow consists of four distinct steps: (1) isolation of spore-forming bacteria, (2) genomic characterisation and functional annotation, (3) identification of phylogenetically representative species, and (4) characterisation of sporulation processes and metabolic capabilities. This figure was created using BioRender (<https://BioRender.com/ol3jn3h>).

have been deposited in public international repositories, ensuring long-term and easy access for researchers [7,45,47,56].

Following isolation, whole genome sequencing permits accurate taxonomic identification and functional profiling to characterise encoded metabolic pathways and sporulation gene content (step 2). For example, the commercial probiotic Enterogermina was originally reported to contain *B. subtilis*; however, using genome sequencing, it was later determined to contain *B. clausii*, a more distantly related *Bacillus* species [27,57]. For probiotic or therapeutic development, both isolates and genomes enable screening for the presence of virulence factors and antimicrobial resistance genes, which are necessary to ensure safety for human consumption, as well as health-associated properties.

The next step (step 3) is to identify species that are phylogenetically representative and prevalent, as findings from these species can be applied to other closely related species. Type strains may be unsuitable, as they are described as the first isolate designated for a new species without necessarily being phylogenetically representative. Extracting the Bacillota phylogeny from the Genome Taxonomy Database (GTDB) using AnnoTree reveals extensive diversity, with nearly 18 000 different species and 170 taxonomic orders (Figure 3) [58,59]. Within these orders are spore-forming species that colonise multiple environments, including deep-sea vents, soil, salt plains, and multicellular eukaryotic hosts, including humans [6]. Sporulation is not conserved across the Bacillota, with clade-specific and even species-specific inability to form spores. For

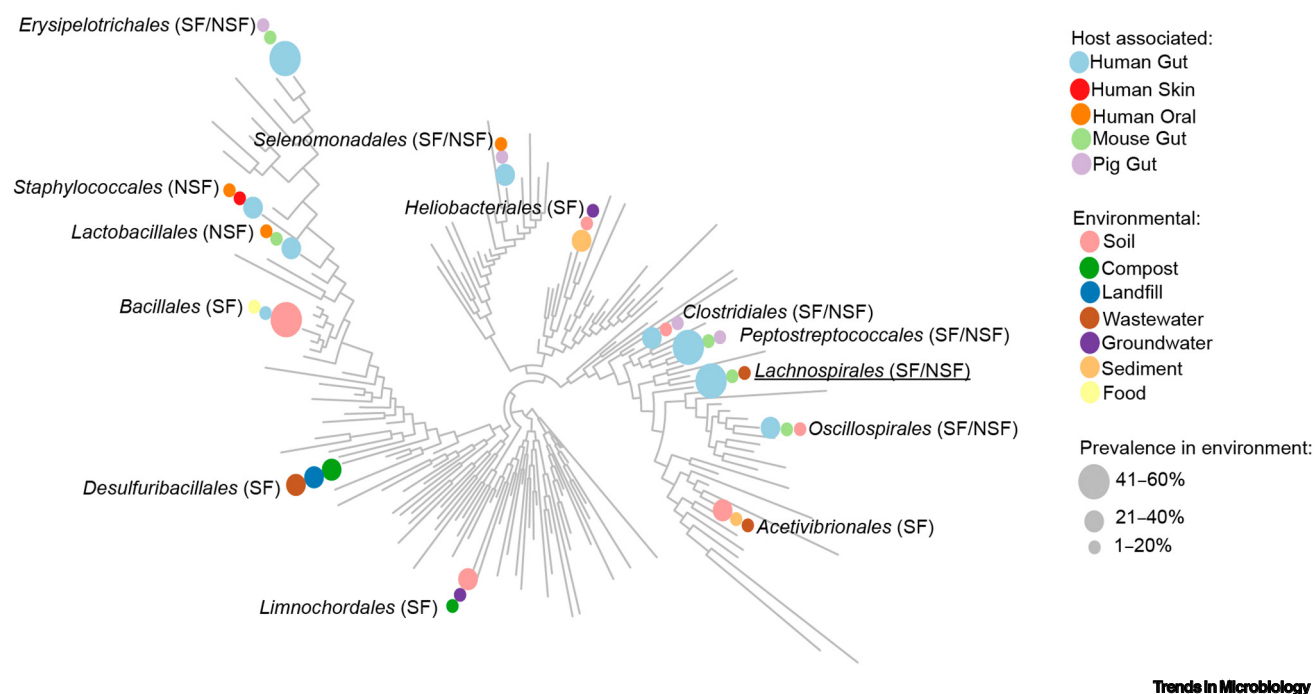


Figure 3. Phylogenomics and environmental prevalence identify species within the Lachnospirales order as candidates for commensal gut spore-forming model organisms. GTDB phylogeny of the Bacillota phylum was extracted using AnnoTree and presented at the taxonomic level of order. Specific orders are highlighted by name, and their source environments were determined using prevalence information in Sandpiper, a resource for screening metagenome databases. The size of the bubble indicates prevalence in a particular environment; only the three most prevalent environments for the selected orders are presented. Bubble colour indicates environment. The *Lachnospirales* (underlined) contain species identified as potential new model spore-formers. GTDB: Genome Taxonomy Database; NSF: non-spore-forming; SF: spore-forming.

example, the Erysipelotrichales order contains both spore-forming and non-spore-forming species present in the gut.

We have previously shown that the loss of sporulation in gut Erysipelotrichales is associated with features of host adaptation, including genome reduction and a more restricted carbohydrate metabolism profile [11]. The loss of sporulation in these bacteria is linked with colonisation to higher abundance levels compared with spore-formers. This suggests an evolutionary trade off, where the lower intestinal abundance of spore-formers increases the chances of expulsion or extinction within a host, which is compensated by the ability to survive as a spore. In contrast, the higher abundance levels of species that have lost the ability to sporulate indicate greater levels of host adaptation linked to genome reduction and a greater reliance on within-host persistence. Thus, depending on the question of interest (e.g., maintenance vs. loss of sporulation), different species within the Bacillota phylogeny will be more relevant.

We assigned environment-specific metagenome prevalence using Sandpiper to identify three highly prevalent human gut taxonomic orders (>41% prevalence in human gut metagenome datasets): Peptostreptococcales, Lachnospirales, and Erysipelotrichales [60]. The Peptostreptococcales contain species such as pathogenic *C. difficile* and commensal *Intestinibacter bartlettii*. The Erysipelotrichales contain gut spore-forming species such as *C. innocuum* and non-spore-forming *Catenibacterium mitsuokai*. Of these three orders, the Lachnospirales contain the highest number of gut spore-formers, including highly prevalent species from *Roseburia* (e.g., *R. hominis* and *R. intestinalis*), *Blautia* (e.g., *B. wexlerae* and *B. producta*), *Anaerostipes* (e.g., *A. caccae*), and *Coprococcus* (e.g., *C. eutactus*) genera, all contained within the Lachnospiraceae family [61–63]. Considering our workflow described earlier, species from these genera have been isolated, whole genome sequenced, and deposited in public culture collections. Some are demonstrated spore-formers, are prevalent in human populations, and are associated with beneficial functions, including pathogen inhibition by bacteriocin production, cross-feeding with other gut microbiome species, reduced risk of type II diabetes, and lower rates of food allergies [15,64–66]. Taken together, these are credible candidates for future gut spore-forming model organisms.

Having identified potentially suitable species, the final step (step 4) is characterisation of sporulation processes and metabolic capabilities. Species that produce resilient and stable spores in large numbers will enable downstream functional profiling. This will also be a requirement for scaling up during the manufacturing and production of probiotics. However, based on our own unpublished research, we have observed that some species produce spores (i.e., they were isolated from ethanol-treated stool samples), but sporulation cannot be induced *in vitro* (i.e., isolate colonies are not recovered after ethanol treatment in pure culture). This highlights a knowledge gap where we are currently unable to replicate the necessary conditions for sporulation in the laboratory. Potential intestinal sporulation triggers could include host mucus, bile acids, nutrients, bacterial interactions, quorum sensing, or bacteriophage. Until these triggers are elucidated, some species we highlight as potential new model organisms may yet prove to be unsuitable due to a lack of or low numbers of produced spores.

Finally, long-term genetic tools are required to understand gene functions underlying metabolic functions and sporulation. Vectors for genetic manipulation of spore-formers are available, such as the ClosTron gene knockout system, developed from the original TargeTron system and based on group II introns, and the plasmid-based pMTL80000 module, as well as versatile transposons (TNs) to generate TN-labelled and tractable mutant libraries [67–70]. More recently, genetic manipulation of *Lachnospiraceae* species has been demonstrated using conjugative plasmids and the

development of clustered regularly interspaced short palindromic repeats interference (CRISPRi) and group II intron-based genetic tools [71,72]. However, the widespread implementation of these tools is limited and remains a considerable hurdle to establishing these species as model organisms. The availability of genomes and isolates provides starting material for the development of new vectors and overcoming obstacles to genetic manipulation, such as restriction–modification systems.

We propose that, in the short term, research should focus on those species satisfying the criteria we have outlined earlier (phylogenetically representative, whole genome sequenced isolates associated with human health), as genetic tools are not required to characterise their metabolism, host interactions, and intestinal triggers of sporulation and germination. In the long term, the goal should be the development of genetic tools for these *Lachnospiraceae* species. A pragmatic approach will involve the selection of species from different genera and then focusing on those for which effective genetic tools become available to validate metabolism, host interactions, and sporulation-related discoveries.

Concluding remarks

Spores are an ancient and incredibly resilient phenotype that have made a major contribution to bacterial diversity, survival, and colonisation of different environments. While research on gut spore-formers has focused on enteric pathogens such as *C. difficile*, it is increasingly evident that many health-promoting gut bacterial species are capable of spore formation and use spores to effectively transmit between hosts. As human gut spore-formers remain understudied, there are many outstanding questions related to their intestinal ecology and spore production (see [Outstanding questions](#)). Developing new commensal gut model spore-formers will improve our understanding of the diversity of sporulation gene architecture within the gut microbiome, the metabolic capabilities of spore-formers and their host interactions, and the extent of sporulation and germination triggers. Understanding the processes that underpin the sporulation life cycle and ecology of spore-formers will provide new insights into the mechanisms by which our intestinal microbiota transmit between hosts and create new opportunities for translational research applications.

Acknowledgments

We acknowledge funding support from Science Foundation Ireland (SFI; now Research Ireland) under grant no. 12/RC/2273 and 16/SP/3827. H.P.B. is funded by the European Research Council starter grant: SYNergize 101115994.

Declaration of interests

The authors declare no competing interests.

References

1. Lennon, J.T. and Jones, S.E. (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat. Rev. Microbiol.* 9, 119–130
2. Paredes-Sabja, D. *et al.* (2014) *Clostridium difficile* spore biology: sporulation, germination, and spore structural proteins. *Trends Microbiol.* 22, 406–416
3. Errington, J. (2003) Regulation of endospore formation in *Bacillus subtilis*. *Nat. Rev. Microbiol.* 1, 117–126
4. Shen, A. (2015) A gut odyssey: the impact of the microbiota on *Clostridium difficile* spore formation and germination. *PLoS Pathog.* 11, e1005157
5. Vinay, G. *et al.* (2025) Bile acids as germinants for *Clostridioides difficile* spores, evidence of adaptation to the gut? *FEMS Microbiol. Rev.* 49, fuaf005
6. Hutchison, E.A. *et al.* (2014) Sporulation in bacteria: beyond the standard model. *Microbiol. Spectrum* 2 TBS-0013–2012
7. Browne, H.P. *et al.* (2016) Culturing of ‘unculturable’ human microbiota reveals novel taxa and extensive sporulation. *Nature* 533, 543–546
8. Deshevaya, E.A. *et al.* (2024) Survival of microorganisms during two-year exposure in outer space near the ISS. *Sci. Rep.* 14, 334
9. Poyet, M. *et al.* (2019) A library of human gut bacterial isolates paired with longitudinal multiomics data enables mechanistic microbiome research. *Nat. Med.* 25, 1442–1452
10. Kearney, S.M. *et al.* (2018) Endospores and other lysis-resistant bacteria comprise a widely shared core community within the human microbiota. *ISME J.* 12, 2403–2416
11. Browne, H.P. *et al.* (2021) Host adaptation in gut Firmicutes is associated with sporulation loss and altered transmission cycle. *Genome Biol.* 22, 204
12. Appert, O. *et al.* (2020) Initial butyrate producers during infant gut microbiota development are endospore formers. *Environ. Microbiol.* 22, 3909–3921
13. Valles-Colomer, M. *et al.* (2023) The person-to-person transmission landscape of the gut and oral microbiomes. *Nature* 614, 125–135
14. Hildebrand, F. *et al.* (2021) Dispersal strategies shape persistence and evolution of human gut bacteria. *Cell Host Microbe* 29, 1167–1176.e9

Outstanding questions

What induces sporulation in commensal gut bacteria?

How does the genetic architecture of sporulation differ between gut bacteria, and how does this drive adaptation to niches within the gut?

How do spore resilience and spore production within individual species relate to transmission ability and prevalence in global human populations?

What is the extent of germination triggers in commensal gut bacteria?

How resistant are human gut spores?

15. Kim, S.G. *et al.* (2019) Microbiota-derived lantibiotic restores resistance against vancomycin-resistant *Enterococcus*. *Nature* 572, 665–669
16. Buffie, C.G. *et al.* (2015) Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. *Nature* 517, 205–208
17. Atarashi, K. *et al.* (2013) T_{reg} induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* 500, 232–236
18. Furusawa, Y. *et al.* (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504, 446–450
19. Yano, J.M. *et al.* (2015) Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 161, 264–276
20. O'Toole, P.W. *et al.* (2017) Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nat. Microbiol.* 2, 17057
21. Bintis, T. (2017) Foodborne pathogens. *AIMS Microbiol.* 3, 529–563
22. Li, J. *et al.* (2016) *Clostridium perfringens* sporulation and sporulation-associated toxin production. *Microbiol. Spectrum* 4 TBS-0022-2015
23. Kiu, R. *et al.* (2023) Particular genomic and virulence traits associated with preterm infant-derived toxigenic *Clostridium perfringens* strains. *Nat. Microbiol.* 8, 1160–1175
24. Chapman, J.A. *et al.* (2026) *Clostridia* from preterm infants metabolize human milk oligosaccharides to suppress pathobionts and modulate intestinal function in organoids. *Nat. Microbiol.* 11, 940–959
25. Gerding, D.N. *et al.* (2018) Non-toxigenic *Clostridioides* (formerly *Clostridium*) *difficile* for prevention of *C. difficile* infection: from bench to bedside back to bench and back to bedside. *Front. Microbiol.* 9, 1700
26. Stülke, Jörg *et al.* (2023) *Bacillus subtilis*, a Swiss army knife in science and biotechnology. *J. Bacteriol.* 205, e00102
27. Cutting, S.M. (2011) *Bacillus* probiotics. *Food Microbiol.* 28, 214–220
28. Ramos-Silva, P. *et al.* (2019) From root to tips: sporulation evolution and specialization in *Bacillus subtilis* and the intestinal pathogen *Clostridioides difficile*. *Mol. Biol. Evol.* <https://doi.org/10.1093/molbev/msz175>
29. Galperin, M.Y. *et al.* (2022) Conservation and evolution of the sporulation gene set in diverse members of the Firmicutes. *J. Bacteriol.* 204, e0007922
30. Yutin, N. and Galperin, M.Y. (2013) A genomic update on clostridial phylogeny: Gram-negative spore-formers and other misplaced clostridia. *Environ. Microbiol.* 15, 2631–2641
31. Jensen, P.A. (2025) Ten species comprise half of the bacteriology literature, leaving most species unstudied. *bioRxiv* <https://doi.org/10.1101/2025.01.04.631297>
32. Hong, H.A. *et al.* (2009) *Bacillus subtilis* isolated from the human gastrointestinal tract. *Res. Microbiol.* 160, 134–143
33. Ma, B. *et al.* (2023) A genomic catalogue of soil microbiomes boosts mining of biodiversity and genetic resources. *Nat. Commun.* 14, 7318
34. Kumar, N. *et al.* (2019) Adaptation of host transmission cycle during *Clostridium difficile* speciation. *Nat. Genet.* 51, 1315–1320
35. Driks, A. and Eichenberger, P. (2016) The spore coat. *Microbiol. Spectrum* 4 TBS-0023-2016
36. Henriques, A.O. and Moran, C.P. (2007) Structure, assembly, and function of the spore surface layers. *Ann. Rev. Microbiol.* 61, 555–588
37. Schwartz, D.A. *et al.* (2023) Human-gut phages harbor sporulation genes. *mBio* 14, e0018223
38. Tanaka, M. *et al.* (2020) Cultural isolation of spore-forming bacteria in human feces using bile acids. *Sci. Rep.* 10, 15041
39. Sorg, Joseph A. and Sonenshein Abraham, L. (2008) Bile salts and glycine as cogerminants for *Clostridium difficile* spores. *J. Bacteriol.* 190, 2505–2512
40. Wilson, K.H. *et al.* (1982) Use of sodium taurocholate to enhance spore recovery on a medium selective for *Clostridium difficile*. *J. Clin. Microbiol.* 15, 443–446
41. McNellis, M.E. *et al.* (2026) The CspC:CspA heterodimer transduces germinant and co-germinant signals during *Clostridioides difficile* spore germination. *PLoS Biol.* 24, e3003610
42. Francis, M.B. *et al.* (2013) Bile acid recognition by the *Clostridium difficile* germinant receptor, CspC, is important for establishing infection. *PLoS Pathog.* 9, e1003356
43. Gao, Y. *et al.* (2023) Bacterial spore germination receptors are nutrient-gated ion channels. *Science* 380, 387–391
44. Piewngam, P. *et al.* (2018) Pathogen elimination by probiotic *Bacillus* via signalling interference. *Nature* 562, 532–537
45. Forster, S.C. *et al.* (2019) A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nat. Biotechnol.* 37, 186–192
46. Liu, C. *et al.* (2021) Enlightening the taxonomy darkness of human gut microbiomes with a cultured biobank. *Microbiome* 9, 119
47. Hitch, T.C.A. *et al.* (2025) HIBC: a publicly available collection of bacterial strains isolated from the human gut. *Nat. Commun.* 16, 4203
48. Maghini, D.G. *et al.* (2025) Expanding the human gut microbiome atlas of Africa. *Nature* 638, 718–728
49. Gacesa, R. *et al.* (2022) Environmental factors shaping the gut microbiome in a Dutch population. *Nature* 604, 732–739
50. Suez, J. *et al.* (2019) The pros, cons, and many unknowns of probiotics. *Nat. Med.* 25, 716–729
51. Feuerstadt, P. *et al.* (2022) SER-109, an oral microbiome therapy for recurrent *Clostridioides difficile* infection. *N. Engl. J. Med.* 386, 220–229
52. Bryant, J.A. *et al.* (2026) The impact of an oral purified microbiome therapeutic on the gastrointestinal microbiome. *Nat. Med.* 32, 186–196
53. Gonzales-Luna, A.J. *et al.* (2023) Emerging options for the prevention and management of *Clostridioides difficile* infection. *Drugs* 83, 105–116
54. Blount, Z.D. (2015) The unexhausted potential of *E. coli*. *eLife* 4, e05826
55. Setlow, P. (2019) Observations on research with spores of Bacillales and Clostridiales species. *J. Appl. Microbiol.* 126, 348–358
56. Goodman, A.L. *et al.* (2011) Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc. Natl. Acad. Sci. U. S. A.* 108, 6252–6257
57. Green, D.H. *et al.* (1999) Characterization of two *Bacillus* probiotics. *Appl. Environ. Microbiol.* 65, 4288–4291
58. Mendler, K. *et al.* (2019) AnnoTree: visualization and exploration of a functionally annotated microbial tree of life. *Nucleic Acids Res.* 47, 4442–4448
59. Parks, D.H. *et al.* (2018) A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat. Biotechnol.* 36, 996–1004
60. Woodcroft, B.J. *et al.* (2025) Comprehensive taxonomic identification of microbial species in metagenomic data using SingleM and Sandpiper. *Nat. Biotechnol.* Published online July 16, 2025. <https://doi.org/10.1038/s41587-025-02738-1>
61. Sorbara, M.T. *et al.* (2020) Functional and genomic variation between human-derived isolates of *Lachnospiraceae* reveals inter- and intra-species diversity. *Cell Host Microbe* 28, 134–146.e4
62. Mukhopadhyay, I. *et al.* (2025) Novel insights into carbohydrate utilisation, antimicrobial resistance, and sporulation potential in *Roseburia intestinalis* isolates across diverse geographical locations. *Gut Microbes* 17, 2473516
63. Liu, C. *et al.* (2008) Reclassification of *Clostridium coccoides*, *Ruminococcus hansenii*, *Ruminococcus hydrogenotrophicus*, *Ruminococcus luti*, *Ruminococcus productus* and *Ruminococcus schinkii* as *Blautia coccoides* gen. nov., comb. nov., *Blautia hansenii* comb. nov., *Blautia hydrogenotrophica* comb. nov., *Blautia luti* comb. nov., *Blautia producta* comb. nov., *Blautia schinkii* comb. nov. and description of *Blautia wexlerae* sp. nov., isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 58, 1896–1902
64. Hosomi, K. *et al.* (2022) Oral administration of *Blautia wexlerae* ameliorates obesity and type 2 diabetes via metabolic remodeling of the gut microbiota. *Nat. Commun.* 13, 4477
65. Hesser, L.A. *et al.* (2024) A synbiotic of *Anaerostipes caccae* and lactulose prevents and treats food allergy in mice. *Cell Host Microbe* 32, 1163–1176.e6

66. Pichler, M.J. *et al.* (2020) Butyrate producing colonic Clostridiales metabolise human milk oligosaccharides and cross feed on mucin via conserved pathways. *Nat. Commun.* 11, 3285
67. Heap, J.T. *et al.* (2010) The ClosTron: mutagenesis in *Clostridium* refined and streamlined. *J. Microbiol. Methods* 80, 49–55
68. Landry, B.P. and Tabor, J.J. (2017) Engineering diagnostic and therapeutic gut bacteria. *Microbiol. Spectrum* 5 BAD-0020-2017
69. Heap, J.T. *et al.* (2007) The ClosTron: a universal gene knock-out system for the genus *Clostridium*. *J. Microbiol. Methods* 70, 452–464
70. Karberg, M. *et al.* (2001) Group II introns as controllable gene targeting vectors for genetic manipulation of bacteria. *Nat. Biotechnol.* 19, 1162–1167
71. Jin, W.-B. *et al.* (2022) Genetic manipulation of gut microbes enables single-gene interrogation in a complex microbiome. *Cell* 185, 547–562.e22
72. Sheridan, P.O. *et al.* (2019) Heterologous gene expression in the human gut bacteria *Eubacterium rectale* and *Roseburia inulinivorans* by means of conjugative plasmids. *Anaerobe* 59, 131–140