A critical evaluation of the factors affecting the survival and persistence of beneficial bacteria in healthy adults

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Abstract

The efficacy of a probiotic depends on its ability to survive and persist in the digestive tract. Regulatory agencies around the world recommend minimum dosages in order for a product to be termed a probiotic. However, the effect of dosage on the survival of the bacteria in the gut – the primary objective of probiotic administration – has not been critically evaluated. We performed a systematic literature review to assess the available data on the survival rate, during gastrointestinal transit, of probiotic bacteria that were orally administered to healthy adults. We also evaluated the persistence of the administered strain(s) after discontinuation of treatment and the potential role played by the food matrix in which probiotics have been administered. From a regulatory perspective, the profile of the target population is key to establishing the efficacy of probiotics. Therefore, we focussed on subjects without disease conditions. We evaluated 17 studies of single strains and 13 studies of multi-strain products, which reported survival and persistence outcomes. Persistence in the gut and recovery from stool were strain dependent. When the administered dose was higher than 10^{10} cfu/day, the probiotic could be recovered from stool regardless of the strain used. Treatment duration did not affect faecal recovery. Thus, dosage recommendations for probiotics by regulatory agencies are lower than that required for a strain to survive, persist and be efficacious in the gut.

Keywords: gastrointestinal tract transit, multi-strain, single strain

1. Introduction

Between 2001 and 2002, an Expert Panel Consultation convened by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) established guidelines for the evaluation of health and nutritional properties of probiotics in food (FAO/WHO, 2001). These documents provided the widely accepted (Hill *et al.*, 2014) definition of probiotics: ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’.

However, these documents contain two additional, but less known, key statements (FAO/WHO, 2001):

1. ‘For use in foods, probiotic microorganisms should not only be capable of surviving passage through the digestive tract, but also have the capability to proliferate in the gut.’

2. ‘It is the ability to remain viable at the target site and to be effective, that should be verified for each potentially probiotic strain.’

The ability to survive and persist in the digestive tract forms the basis of the FAO/WHO guidelines. Regulatory agencies all over the world have used these statements as references. For instance, the European Food Safety Authority (EFSA, 2016), the US Food and Drug Administration (FDA), and Health Canada have used them as templates for their own guidelines on probiotics, as have agencies in China, India, Brazil, Argentina, and other nations. Some of these national guidelines have also recommended specific dosages for probiotics. Italy’s Ministry of Health requires that a product termed ‘probiotic’ contain a minimum dose of 10^9 viable bacteria/day (Ministero della Salute, 2013). In China, the State Administration for Market Regulation issued a document (CIRS, 2019) which stated that: ‘The number of

viable bacteria of each kind of probiotic health food during its shelf life shall not be less than 10⁶ cfu/ml (g). However, the rationale behind these dosage recommendations has not been fully justified through empirical studies.

Despite extensive research on the health benefits of probiotic bacteria (Sánchez *et al.*, 2017; Sanders *et al.*, 2019; Szajewska and Hojsak, 2020), there is no consensus on the dose to be administered that would allow the establishment and persistence of these strains in the gut after treatment has ended.

The potential relationship between the administered dose and physiological effects of probiotics has been described previously (Ouwehand, 2017). However, this review lacked a critical evaluation of the reported survival/persistence rates of the administered bacteria and the observed probiotic outcomes – a ‘dose-response’ evaluation. The review also focused on clinical effects in subjects who had underlying pathological conditions.

In recent years, studies have tested the efficacy and safety of ‘modified’ probiotics, such as those that have been heat-killed or those that contain substances secreted by probiotics (termed ‘postbiotics’). Evidence for their efficacy is still scarce in human trials. A systematic review found that in the majority of trials conducted to date, ‘modified’ probiotics were not significantly different from conventional probiotics in terms of efficacy, and were advantageous only in two specific disease states: adult obesity and chronic diarrhoea (Zorzela *et al.*, 2017).

In this review, we focus on the ability of viable probiotic strains to survive and reproduce in the gastrointestinal tract of healthy adults. We summarise and evaluate the available data on dose-response outcomes of clinical studies. Results from *in vitro* studies or animal models, and studies conducted in paediatric subjects or those fed with infant formula will not be discussed here.

2. Materials and methods

To find relevant literature, a PubMed and Scopus search was performed using ‘probiotics’ and ‘humans’ as the main key words, combined with ‘survival’ and ‘persistence’ in two waves of search for each database. The filter ‘clinical trial’ was also applied in a further wave of search; however, results obtained without this filter were also retained for further analyses. Only manuscripts published in English as full texts in peer reviewed journals were retained.

Manuscripts dealing with non-prokaryotic probiotics, such as yeast, were excluded from this review. Because the scope of this review was limited to healthy subjects, all data obtained from clinical studies involving subjects with pathological conditions were discarded. Studies in

which probiotics were co-administered with prebiotics and those that did not identify the strain of the probiotic were excluded. Studies that did not clearly describe the treatment regimen (dose or length of treatment) were also omitted. The retrieved publications were then classified into those dealing with a single strain and those dealing with multi-strain products.

Doses are presented as colony forming units (cfu), and represent cfu/day. For practical reasons, the new taxonomic nomenclature of the *Lactobacillus* genus (Zheng *et al.*, 2020) has not been used; instead, we use the old genus names.

3. Results

The literature search in each database using the relevant keywords yielded a large number of studies (Table 1 shows the output after removing duplicate results). After independent checking of the abstracts, we selected 31 studies, reported in 28 publications, for the analysis. Two publications (Plaza-Díaz *et al.*, 2013; Prilassnig *et al.*, 2007), which report data on single and multi-strain products, were considered in both categories. Most studies expressed faecal recovery and persistence as either cfus or the number of subjects testing positive for the strain.

Identification of probiotic strains in faecal samples

Most studies used strain-specific methods to identify the administered probiotic strain in faeces. The most common methods included strain-specific PCR and rapid amplification of polymorphic DNA (RAPD) genotyping (Arioli *et al.*, 2018; Dommels *et al.*, 2009; Ghelardi *et al.*, 2015; Hutt *et al.*, 2011; Mai *et al.*, 2017; Matto *et al.*, 2006; Plaza-Díaz *et al.*, 2013; Prilassnig *et al.*, 2007; Rattanaprasert *et al.*, 2014; Saxelin *et al.*, 2010; Taverniti *et al.*, 2019).

Saxelin *et al.* (1995) and Alander *et al.* (1999) identified *Lactobacillus rhamnosus* GG by colony morphology and the strain’s inability to ferment lactose. Four studies employed strain-specific probes in colony hybridisation or dot-blot hybridisation assays (De Champs *et al.*, 2003; Donnet-Hughes *et al.*, 1999; Shinoda *et al.*, 2001; Wassenaar *et al.*, 2014). Vesa *et al.* (2000) used mutant strains resistant to rifampicin and streptomycin, with fermentation profiles

Table 1. Results of the literature search, using the keywords ‘survival’ and ‘persistence’ with the filter ‘clinical trials’, to retrieve studies dealing with faecal recovery of probiotics.

	PubMed	Scopus	Applying the filter ‘clinical trials’
Survival	1,970	1,298	158
Persistence	494	176	89

identical to those of the original strains; this allowed their detection on De Man, Rogosa and Sharpe (MRS) media containing these antibiotics.

Larsen *et al.* (2006) also used MRS media with antibiotics specific to *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus paracasei* subsp. *paracasei* (CRL-431). They confirmed the identity of the resultant colonies using pulsed-field gel electrophoresis and fluorescent whole cell hybridisation. Lund *et al.* (2002) used pulsed-field gel electrophoresis to compare banding patterns between the *Enterococcus faecium* strain from the Causido® culture that they administered and those that they isolated from faeces. Jacobsen *et al.* (1999) and Klingberg and Budde (2006) used strain-specific Internal Transcribed Spacer region (ITS)-PCR and restriction enzyme analysis followed by pulsed-field gel electrophoresis to identify the probiotic strains they isolated in their studies.

Khine *et al.* (2019) used a strain-specific monoclonal antibody in an enzyme-linked immunosorbent assay (ELISA) to detect *Lactobacillus casei* Shirota. Van Bokhorst-Van de Veen *et al.* (2012) amplified and sequenced a variable intergenic region to distinguish between the probiotic strains isolated from faeces samples. Five studies did not conduct strain-level testing to identify the administered probiotics in faeces (Ahmed *et al.*, 2007; Hanifi *et al.*, 2015; Plaza-Díaz *et al.*, 2013; Rochet *et al.*, 2008; Smith *et al.*, 2011).

Survival and persistence of probiotics administered as a single strain

We retrieved 17 publications reporting survival and/or persistence outcomes of clinical studies performed in a total of 432 healthy humans with single strains (mainly belonging to *Lactobacillus* or *Bifidobacterium* genera; Table 2). Of these, 19 subjects from two studies participated in cross-over feeding studies (De Champs *et al.*, 2003; Prilassnig *et al.*, 2007). Five studies reported dose-response measurements for a total of 195 subjects (Ahmed *et al.*, 2007; De Champs *et al.*, 2003; Donnet-Hughes *et al.*, 1999; Hanifi *et al.*, 2015; Saxelin *et al.*, 1995). One study involved administration of the same dosage daily or on alternate days to nine subjects each (Smith *et al.*, 2011).

Among the single-strain studies, the administered daily dose ranged between 10^7 cfu and 10^{12} cfu and faecal recovery ranged between 0 and 10^{12} cfu (Table 2). Among studies that conducted strain-specific identification, recovery during treatment ranged between 0 and 10^8 cfu. Probiotic strains were successfully recovered from stool during the treatment period in all studies, with the exception of three out of the six products tested by Prilassnig *et al.* (2007). Of these, two products were single strain probiotics: *L. casei* var. *rhamnosus* (Antibiofilus®) and *L. casei* Immunitas (Actimel®).

Among publications reporting dose responses, two studies dealing with lactobacilli (Donnet-Hughes *et al.*, 1999; Saxelin *et al.*, 1995) found a linear relationship between dosage and persistence during treatment (Table 2). A study (Hanifi *et al.*, 2015) that focused on the spore former, *Bacillus subtilis* RO179, reported a linear relationship between the administered doses (10^8 , 10^9 , and 10^{10} cfu) and faecal recovery.

Lactobacillus strains appear to persist in the intestines over a maximum length of two weeks (Alander *et al.*, 1999) after the cessation of probiotics, with most being recovered up to 5–7 days after treatment (Table 2). A notable exception is *L. casei* Lcr35, which was observed three weeks after treatment ended (De Champs *et al.*, 2003). The administered probiotic strains did not persist in 100% of treated subjects in any of the studies considered.

Survival and persistence of multi-strain probiotics

We retrieved 13 publications reporting clinical studies, performed in 260 healthy adults, which recorded the survival and/or persistence outcomes of a mixture of strains, mainly belonging to *Lactobacillus* or *Bifidobacterium* genera (Table 3). Of these, 44 subjects participated in cross-over feeding studies (Ghelardi *et al.*, 2015; Klingberg and Budde, 2006; Prilassnig *et al.*, 2007). Several authors reported presence and persistence for only some of the strains that they administered (Table 3).

Three studies used a mixture of different *Lactobacillus* strains (Hutt *et al.*, 2011; Klingberg and Budde, 2006; Van Bokhorst-Van de Veen *et al.*, 2012), while six others used a mixture of *Lactobacillus* and *Bifidobacterium* (Larsen *et al.*, 2006; Mai *et al.*, 2017; Matto *et al.*, 2006; Plaza-Díaz *et al.*, 2013; Prilassnig *et al.*, 2007; Taverniti *et al.*, 2019). Saxelin *et al.* (2010) used a mixture containing more than two genera, whereas Wassenaar *et al.* (2014) used a mixture of six strains of *Escherichia coli* and Ghelardi *et al.* (2015) used four strains of *Bacillus clausii*. The daily administered doses ranged from 10^7 to 10^{11} cfu. Treatment duration varied from one-shot treatment (Ghelardi *et al.*, 2015; Wassenaar *et al.*, 2014) to continuous treatment over 30 days (Plaza-Díaz *et al.*, 2013). Faecal recovery ranged between 10^3 and 10^9 cfu.

In a study dealing with four strains of *B. clausii* (Ghelardi *et al.*, 2015), 20 volunteers were given one of two formulations (vials and capsules; ten per arm) of the same probiotic at the same dosage (10^9 cfu/day), to determine the bioequivalence of the formulations. In a unique approach, the authors calculated the total amount of each of the four strains (taking into consideration the total faecal output) excreted by each volunteer after the one-shot administration through vials or capsules. The probiotic was recovered from faeces in both study arms, up to 12 days after the single administration. Thus, the formulation had little to no effect on the recovery

Table 2. Seventeen studies reporting survival and/or persistence outcomes of clinical studies using single strains in healthy subjects.

Strain	Daily dose(s) in cfu unless otherwise mentioned	Treatment (days)	Subjects	Recovery during treatment	Persistence after treatment	Reference
<i>Lactobacillus rhamnosus</i> GG	dose A: 1.6×10^8 ; dose B: 1.2×10^{10} ; gelatin capsule	7	20	10^5 to 10^6 for dose B; 10^3 in one sample for dose A	NA ^a	Saxelin <i>et al.</i> , 1995
<i>Lactobacillus johnsonii</i> La1	low dose: approx. 10^8 ; high dose: approx. 10^9	21 + follow up	42	low dose: 10^4 ; high dose: 10^5	negative detection 7 days after end of treatment	Donnet-Hughs <i>et al.</i> , 1999
<i>L. rhamnosus</i> GG	6×10^{10}	12 + follow up	21	strain was detected in all volunteers at approx. 10^6 cfu	8 out of 21 positive one week after discontinuation at 10^3 - 10^5 cfu	Alander <i>et al.</i> , 1999
<i>Lactobacillus fermentum</i> KLD, <i>Lactobacillus plantarum</i> NCIMB 8826, <i>Lactococcus lactis</i> MG 1363	10^8 of <i>L. plantarum</i> in fermented milk	7 + 21 follow up	18 (6 with <i>L. plantarum</i>)	<i>L. plantarum</i> NCIMB 8826: 10^8 ; the other strains were not tested	not detected in faeces two weeks after treatment ended	Vesa <i>et al.</i> , 2000
<i>Enterococcus faecium</i> Causido®	4.5 - 7.5×10^9	10	10 (+10 receiving concomitant vancomycin)	10^3 - 10^6 cfu (8/10 positive)	all subjects negative 3 weeks after treatment ended	Lund <i>et al.</i> , 2002
<i>Lactobacillus helveticus</i> CP53	one shot 10^{11} cells	5	7	10^4 - 10^8 cfu in 4/7 subjects	NA ^a	Shinoda <i>et al.</i> , 2001
<i>Lactobacillus casei</i> subsp. <i>rhamnosus</i> Lcr35	dose A: 10^8 ; dose B: 10^{10} ; dose C: 10^{12}	7 + 7	12 (cross-over)	10^4 / 10^5 (not related to the dose)	10^3 - 10^6 at seven days after treatment ended	De Champs <i>et al.</i> , 2003
<i>Bifidobacterium lactis</i> HN019	high: 5×10^9 ; medium: 1×10^9 ; low: 6.5×10^7	28	80 (20 in each dosage + 20 placebo)	high: 10^9 ; medium: 10^9 ; low: 10^9 b; (total bifidobacteria levels)	NA ^a	Ahmed <i>et al.</i> , 2007
<i>L. casei</i> var. <i>rhamnosus</i> (Antibiophilus®), <i>L. casei</i> Immunitas (Actimel®)	1×10^9 cells (Antibiophilus), 1×10^{10} cells (Actimel); each product administered separately	7	7 (cross-over)	no recovery in 5 subjects for each product	NA ^a	Prilassnig <i>et al.</i> , 2007
<i>L. rhamnosus</i> GG, <i>Lactobacillus reuteri</i> DSM 17938	1×10^9 <i>L. reuteri</i> 17938, 5×10^9 <i>L. rhamnosus</i> GG; each strain administered separately	21	29	In the range of 10^6 per strain	NA ^a	Dommels <i>et al.</i> , 2009
<i>L. casei</i> DN-114 001 ^{nif}	approx. 10^{10}	8	7	Bacteria-equivalents of <i>L. paracasei</i> group 2 log higher than at the start	Recovery up to 3 days; absence after 7 days	Rochet <i>et al.</i> , 2008

Table 2. Continued.

Strain	Daily dose(s) in cfu unless otherwise mentioned	Treatment (days)	Subjects	Recovery during treatment	Persistence after treatment	Reference
<i>L. reuteri</i> DSM 17938	10 ⁹	A: 7 every day; B: 7, on alternate days	A: 9; B: 9	approx. 10 ⁵ in all subjects	A: 4/9 and 2/9 positive after 1 and 2 weeks, respectively; B: 3/9 and 2/9 positive after 1 and 2 weeks, respectively	Smith <i>et al.</i> , 2011
<i>L. reuteri</i> DSM 17938	5×10 ⁸	7 ^c	15	10 ⁸	after 2 days: 10 ⁷ ; after 4 days: not detectable	Rattanaprasert <i>et al.</i> , 2014
<i>L. paracasei</i> CNCM I-4034; <i>L. rhamnosus</i> CNCM I-4036; <i>Bifidobacterium breve</i> CNCM I-4035	9×10 ⁹	30	100 total (60 receiving single strain, 20 multistrain, and 20 placebo)	not identified at the strain level	only <i>L. rhamnosus</i> CNCM I-4036 plate counted and identified 14 days after treatment	Plaza-Diaz <i>et al.</i> , 2013
<i>Bacillus subtilis</i> RO179	dose A: 0.1×10 ⁹ ; dose B: 1.0×10 ⁹ ; dose C: 10×10 ⁹	28	81 (20 placebo)	dose A: 10 ⁴ ; dose B: 10 ⁵ ; dose C: 10 ⁶ ^b	after 7 days: dose B: 10 ^{0.8} ; dose C: 10 ^{2.1}	Hanifi <i>et al.</i> , 2015
<i>L. paracasei</i> CNCM I-1572 (DG)	8×10 ⁹	7	20	10 ^{3.6} -10 ^{6.7} cfu/g in all volunteers	max. 5 days	Arioli <i>et al.</i> , 2018
<i>L. casei</i> Shirota (YAKULT®)	1.0×10 ¹⁰	14	21	day 7: 10 ⁷ ; day 14: 10 ⁷ ^b	no positive subjects after 14 days	Khine <i>et al.</i> , 2019
^a Faecal persistence data were not collected after the end of treatment; NA = not available.						
^b Reported as log cfu in the original publication.						
^c Only subjects in the cross-over section with the strain without prebiotic supplementation.						

rate of the strains. There were more volunteers from whom faecal recovery was equal to or higher than the administered dose, and fewer volunteers in whom recovery was lower than the administered dose. Thus, the probiotic survives passage through the gastrointestinal tract. In particular, strain O/C exhibited the potential to multiply in the gut: ten volunteers, irrespective of the study arm, had higher spore recovery than ingestion. Five volunteers showed the same behaviour for the strains N/R and four volunteers for strain SIN. None of the volunteers showed an increase in the number of recovered T strain when compared to administered dose. The predominant strain in both formulations, N/R, displayed the longest persistence.

We retrieved three dose response studies (Larsen *et al.*, 2006; Taverniti *et al.*, 2019; Van Bokhorst-Van de Veen *et al.*, 2012), which deal with different formulations (capsules, yoghurt, cheese, and encapsulated cells), dosages, and treatment times. The faecal recovery of *B. animalis* subsp. *lactis* BB-

12 increased significantly ($P=0.001$) with an increase in dose and persisted in a small number of subjects beyond the treatment period (Larsen *et al.*, 2006). The analysis reported by Van Bokhorst-Van de Veen and colleagues (2012) did not allow them to conclude on the effect of the dosage of probiotics. However, Taverniti and colleagues (2019) identified a clear dose-dependence between the amount of recovered strains (one *Bifidobacterium* and three *Lactobacillus* strains) and the administered dosages. The higher dosage resulted in the recovery of 10⁶ to 10⁹ cfu/g faeces of the probiotic in all twenty subjects.

Persistence beyond treatment of multi-strain probiotics closely resembled that of single-strain products, ranging from three days (Hutt *et al.*, 2011) to 17 days (Saxelin *et al.*, 2010). Not all treated subjects had persistent strains in stool, with the notable exception of the mix used by Taverniti *et al.* (2019), in which all three strains were recovered from subjects administered the higher dose.

Table 3. Thirteen studies reporting survival and/or persistence outcomes of clinical studies using multi-strain products in healthy subjects.

Strains	Daily dose(s) in cfu	Treatment (days)	Treated subjects	Recovery during treatment	Persistence after treatment	Reference
<i>Lactobacillus rhamnosus</i> 19070-2, <i>Lactobacillus reuteri</i> DSM 12246, <i>L. rhamnosus</i> LGG, <i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i> CHCC 2329, <i>Lactobacillus casei</i> subsp. <i>alactus</i> CHCC 3137	10 ¹⁰ twice a day of either Mix 1 (<i>L. rhamnosus</i> 19070-2 and <i>L. reuteri</i> DSM 12246) or Mix 2 (<i>L. rhamnosus</i> LGG, <i>L. delbrueckii</i> subsp. <i>lactis</i> CHCC 2329 and <i>L. casei</i> subsp. <i>alactus</i> CHCC 3137)	18	12	10 ⁵ to 10 ⁸ cells/g in some subjects	<i>L. reuteri</i> DSM 12246 (in one case) and <i>L. rhamnosus</i> 19070-2 and <i>L. rhamnosus</i> GG (two cases each) reisolated at five days after treatment ended	Jacobsen <i>et al.</i> , 1999
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BB-12); <i>L. paracasei</i> subsp. <i>paracasei</i> CRL-431)	A: 10 ⁸ ; B: 10 ⁹ ; C: 10 ¹⁰ ; D: 10 ¹¹ ; E: placebo	21 + 14 follow up	75 (15 in each group)	CRL-431 never isolated. BB-12 recovery strongly dose-dependent: A: 3/14; B: 7/15; C: 7/15; D: 13/15	B: 1/15; D: 2/15 positive at the end of the wash out period; remaining groups: 0/15	Larsen <i>et al.</i> , 2006
3 <i>Lactobacillus plantarum</i> strains (MF1291, MF1298, DC13), <i>Lactobacillus pentosus</i> MF1300, <i>Lactobacillus salivarius</i> DC5	Mix A: MF1291 + MF1298; Mix B: MF1300 + DC5 + DC13; MF1298 in a fermented sausage; 6×10 ⁹ per strain, twice a day	18 + 12 follow up	17 cross-over	MF1298: 4/17; DC13: 3/17 subjects in the range 10 ³ -10 ⁸ cfu/g	MF1298 present in 1 subject 6 days after end of treatment; DC13 present in 1 subject 6 and 12 days after end of treatment	Klingberg and Budde, 2006
<i>L. paracasei</i> F19, <i>Lactobacillus acidophilus</i> NCFB 1748, <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BB-12 (Dofilus ABC yogurt)	2×10 ¹⁰ per strain	10 + up to 19 follow up	14	F19: 14/14 (10 ^{5.5}); BB-12: 11/14 (10 ^{6.9}); NCFB 1748: 3/14 (10 ^{5.2}) ^a	F19: 2/10 after 4 days (10 ⁴ -10 ⁵); BB-12: 4/10 after 4 days (10 ⁶); 1/10 after 9 days (10 ⁴); 1/5 after 19 days (10 ⁷); NCFB 1748: 1/14 after 4 days (10 ^{4.9}); 2/10 after 9 days (10 ⁵)	Matto <i>et al.</i> , 2006
<i>L. acidophilus</i> combined with <i>Bifidobacterium infantis</i> (Infloran®), <i>Lactobacillus gasseri</i> combined with <i>Bifidobacterium longum</i> (Omniflora®)	Infloran: 10 ⁹ per strain; Omniflora: 10 ⁸ per strain	7	7 cross-over	1/6 positive subject for Infloran only at the end of the treatment	NA ^b	Prilassnig <i>et al.</i> , 2007
<i>L. rhamnosus</i> GG and LC705, <i>B. animalis</i> subsp. <i>lactis</i> BB-12, <i>Propionibacterium freudenreichii</i> subsp. <i>shermanii</i> JS	comparison among 3 matrices: yoghurt, cheese, capsules; total cfu for the 3 strains: 10 ¹⁰	14 + 21 follow up	36	all strains detected as genome copies at the end of treatment	median number of days after treatment when each strain was detected: LGG: 17; LC705: 5; BB-12: 7; PJS: 7	Saxelin <i>et al.</i> , 2010
<i>L. gasseri</i> 177 and E16B7, <i>L. fermentum</i> 338-1-1, <i>L. acidophilus</i> 821-3, <i>L. paracasei</i> 317	10 ¹⁰ per strain	5 + 15 follow up	14	all strains recovered at the end of the treatment at about 10 ⁶ cfu/g each	<i>L. acidophilus</i> 821-3 was detected in 4/9 subjects three days after treatment ended (10 ^{4.3} to 10 ⁷ cfu/g) and in 2/9 subjects five days after treatment ended (10 ^{8.3} and 10 ^{3.9} cfu/g)	Hutt <i>et al.</i> , 2011

Table 3. Continued.

Strains	Daily dose(s) in cfu	Treatment (days)	Treated subjects	Recovery during treatment	Persistence after treatment	Reference
21 strains of <i>L. plantarum</i> in mixes containing 10 strains each	10 ¹¹ total amount of each mixture; the WCFS1 strain was included as a 10-fold dilution series in four steps	10 + 21 follow up	10	One strain (WCFS1) identified by RT-PCR after 7 days	Only genomic detection	Van Bokhorst-Van de Veen <i>et al.</i> , 2012
<i>L. paracasei</i> CNCM I-4034, <i>Bifidobacterium breve</i> CNCM I-4035, <i>L. rhamnosus</i> CNCM I-4036	9×10 ⁹ per strain	30 + 15 follow up	20	isolates not identified at the strain level	<i>L. rhamnosus</i> CNCM I-4036; recovered from stools at day 30 (level of presence unknown)	Plaza-Diaz <i>et al.</i> , 2013
6 strains <i>Escherichia coli</i> (Symbioflor®)	1 volunteer: 2×10 ⁸ ; 2 volunteers: 1×10 ⁹ ; 2 volunteers: 2×10 ⁹	One-shot + 210 follow up	5	NA	in the observation period, the mean values of the probiotic <i>E. coli</i> outnumbered those of host <i>E. coli</i> in 3/5 subjects	Wassenaar <i>et al.</i> , 2014
4 strains (O/C, N/R, SIN and T) of <i>Bacillus clausii</i> (Enterogermina®)	2×10 ⁹ total count of the 4 strains	One-shot in cross-over	20 cross-over	10 ⁹ cfu one day after the one-shot administration	detected till day 12	Ghelardi <i>et al.</i> , 2015
<i>L. acidophilus</i> NCFM, <i>B. longum</i> BB536	10 ⁹ per strain; two matrices (gelatin vs micro-encapsulated)	12 + 7 follow up	12	increased recovery of lactobacilli with consumption of encapsulated probiotic	increase lasts less than 7 days	Mai <i>et al.</i> , 2017
<i>B. animalis</i> subsp. <i>lactis</i> BI-04, <i>L. acidophilus</i> La-14, <i>L. plantarum</i> SDZ-11, <i>L. paracasei</i> SDZ-22	dose A (7×10 ⁹ cells): BI-04 and La-14: 10 ⁹ ; SDZ-11: 10 ⁸ ; SDZ-22: 10 ⁷ ; dose B (7×10 ¹⁰ cells): each strain 10 times higher than in dose A	14 + 14 follow up	40	dose A: 15/20 positive; dose B: 20/20 positive on the last day of treatment (10 ⁶ -10 ⁹ cfu/g)	only qPCR data available	Taverniti <i>et al.</i> , 2019
^a Reported as log cfu in the original publication.						
^b Faecal persistence data were not collected after the end of treatment.						

Matrix impact on survival and persistence

The impact of the food matrix on survival and persistence was reported by Ghelardi *et al.* (2015), Saxelin *et al.* (2010) and Mai *et al.* (2017). While Ghelardi *et al.* (2015) reported no matrix effect, Saxelin *et al.* (2010) concluded that the administration of probiotic mixtures in cheese negatively affected the faecal counts of *Propionibacterium* spp. and *Bifidobacterium* spp. The possible reasons for this difference were not addressed by the authors. On the other hand, a difference between gelatine and microencapsulation, with the latter showing superior outcomes, was reported by Mai *et al.* (2017).

4. Discussion

In line with FAO/WHO statements (FAO/WHO, 2002) and Hill *et al.* (2014), our review highlights the importance of strain specificity in the survival and persistence of probiotic bacteria belonging to different genera: *Lactobacillus*, *Bifidobacterium*, and *Bacillus*. The diversity of methods employed to detect probiotic strains in faeces makes it challenging to draw direct comparisons between studies. Future studies using standardised methods and a larger sample size will offer more clarity on the dose-response relationship of probiotics. Nevertheless, an overall assessment of faecal recovery studies performed with single strain products suggests that two different groups can be identified:

1. Recovery amount between 10^4 and 10^5 cfu/g (Arioli *et al.*, 2018; De Champs *et al.*, 2003; Donnet-Hughes *et al.*, 1999; Smith *et al.*, 2011).
2. Recovery amount higher than 10^5 cfu/g (Ahmed *et al.*, 2007; Alander *et al.*, 1999; Dommels *et al.*, 2009; Hanifi *et al.*, 2015; Khine *et al.*, 2019; Lund *et al.*, 2002; Rattanaprasert *et al.*, 2014; Saxelin *et al.*, 1995; Vesa *et al.*, 2000).

Multi-strain products also fall into these two categories:

1. Recovery amount between 10^4 and 10^5 cfu/g (Klingberg and Budde, 2006; Matto *et al.*, 2006).
2. Recovery amount higher than 10^5 cfu/g (Ghelardi *et al.*, 2015; Jacobsen *et al.*, 1999; Taverniti *et al.*, 2019).

A daily dose lower than 10^9 cfu always resulted in a percentage of subjects testing negative for the administered strains. However, even very high doses (10^{12} cfu) did not lead to recovery of the probiotics in all participants (De Champs *et al.*, 2003). This highlights the presence of healthy adults who can be termed 'non-responders' and the 'colonisation resistance' exerted by bacteria already present in their gut.

With the exception of Ahmed *et al.* (2007), Rochet *et al.* (2008) and Rattanaprasert *et al.* (2014), in all other single-strain studies, the amount of probiotic recovered from faeces was lower than that administered, and ranged between 10^3 and 10^8 cfu. The Ahmed *et al.* (2007) study counted total bifidobacteria levels in faeces, which may account for the similar faecal counts (10^9 cfu) in the high, medium and low dose groups. The Rochet *et al.* (2008) study used temporal temperature gradient gel electrophoresis using primers specific to the *Lactobacillus-Pediococcus-Leuconostoc-Weissella* group and real-time PCR using primers specific to the *L. paracasei* group, to which the administered probiotic belongs. Therefore, the faecal recovery in this study represents a general increase in gut bacteria belonging to the *L. paracasei* group and not a specific increase of the administered strain. In the Rattanaprasert *et al.* (2014) study, the amount of the probiotic strain administered was equal to that recovered from stool. However, this strain did not colonise or persist in the gut of subjects beyond the treatment period.

Three studies administered *L. rhamnosus* GG to subjects; of these, only one study found persistence of the administered strain after the end of treatment (Alander *et al.*, 1999). This study compared persistence in faeces with persistence in colonic biopsy samples to show that *L. rhamnosus* GG persists in the gut in most subjects (7 out of 8) for at least a week after the end of treatment, even though it is absent from faeces. Thus, faecal recovery may underestimate persistence in studies of this strain.

Three studies treated subjects with *L. reuteri* DSM 17938 at 10^8 or 10^9 cfu and recovered between 10^5 and 10^8 cfu in faeces. Of these, only the Smith *et al.* (2011) study noted persistence beyond treatment in a small proportion of subjects for two weeks after treatment ended, independent of the dosage administered. This may be due to the 100% study protocol compliance noted by the authors, which may be difficult to replicate in different study settings.

Even though most strains, with the exception of *L. rhamnosus* GG and *L. johnsonii* La1 (Donnet-Hughes *et al.*, 1999; Saxelin *et al.*, 1995), lack a linear relationship between the administered dose and the recovered amount, a minimum dose that allows faecal recovery could be set at 10^8 cfu/day (Donnet-Hughes *et al.*, 1999; Rattanaprasert *et al.*, 2014).

For both categories of products – single strain and multi-strain – the levels in stools were frequently reported to be 10^6 cfu/g faeces. The highest amount, 10^9 cfu/g faeces, reported by Taverniti *et al.* (2019) for vegetative cells, and by Hanifi *et al.* (2015) and Ghelardi *et al.* (2015) for spores, confirm that spores are highly resistant to the harsh conditions of gastrointestinal transit. When comparing the recovery rates of two different spore-formers, a higher amount (10-100 times higher) was observed for *B. clausii* (Ghelardi *et al.*, 2015) than for *B. subtilis* RO179 (Hanifi *et al.*, 2015). Such an effect may be species-specific rather than being related to the duration of treatment; however, further studies comparing similar treatment regimens for these species are warranted. The length of time when strains could be recovered was highly variable: it ranged from five to seven days in most studies that recorded persistence, but could also last as long as 30 or 90 days (Plaza-Diaz *et al.*, 2013; Taverniti *et al.*, 2019).

The selected studies contained several confounding factors. Only adults participated in these studies. The duration of treatment varied between one-shot administration and 30 days, with the majority of studies conducted over a 7- or 14-day period. The number of participants was limited; one third of the studies enrolled ten or fewer participants. However, the overall assessment allows us to draw some conclusions.

Taken together, the dose response studies suggest that a linear relationship may exist between the administered dose and the recovered amount for specific strains. However, this is not generalisable, as there are examples in which the impact of the dose is negligible. The duration of treatment (which varies from one to thirty days) also does not affect faecal recovery or persistence (Tables 3 and 4). Dosing the same products every day or on alternate days does not influence recovery rate (Smith *et al.*, 2011). As suggested by Taverniti and colleagues (2019), a higher dosage may increase the persistence of faecal recovery, but this may

only apply to strains that share common characteristics, such as gastrointestinal resistance, adhesion to the mucosa, and ability to multiply in the gut.

Different *Lactobacillus* sp. have different minimum doses that allow faecal recovery: 10^8 cfu/day for *L. reuteri* DSM 17938 and 10^9 for *L. rhamnosus* GG. This dose effect is apparent up to 10^{10} cfu/day, with strain-dependent variations related to the amount administered. For doses larger than 10^{10} cfu/day, faecal recovery appears to be independent from dosage. The highest faecal recovery reported was 10^9 cfu/g. These data suggest a dose response effect, as reported by Ouwehand *et al.* (2017). However, the results of this analysis also confirm that strains may differ in their ability to survive the gastrointestinal tract and to proliferate in the gut. The dosage and the treatment duration appear to play a secondary role. Similar conclusions apply to *Bifidobacterium* and other species, in spite of the lower number of studies involving these species.

5. Conclusions

Faecal recovery and persistence in the gut are strain-dependent, but doses higher than 10^{10} cfu/day generate a good level of faecal recovery independent of the strain. Faecal recovery and persistence are independent of treatment duration. The food matrix may affect the survival of probiotics, but the number of studies that test this factor are too few to allow us to draw conclusions. In summary, despite variability across strains, current data suggest that the dose required for the majority of strains to survive and persist are higher than the doses recommended for probiotics by regulatory agencies.

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