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Citation for final published version:

O'Toole, Paul W., Marchesi, Julian Roberto and Hill, Colin 2017. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nature Microbiology* 2 (5) , 17057. 10.1038/nmicrobiol.2017.57 file

Publishers page: <http://dx.doi.org/10.1038/nmicrobiol.2017.57>  
<<http://dx.doi.org/10.1038/nmicrobiol.2017.57>>

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1 **Next Generation Probiotics; transitioning from probiotics to Live**  
2 **Biotherapeutics**

3

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13

14 **Abstract**

15 The leading probiotics currently available to consumers are generally drawn from a narrow range of  
16 organisms. Knowledge of the gut microbiota and its constituent actors is changing this paradigm,  
17 particularly given the phylogenetic range and relatively unknown characteristics of the organisms  
18 under investigation as novel therapeutics. For this reason, and because their development is likely to  
19 be more amenable to a pharmaceutical than a food delivery route, these organisms are often  
20 operationally referred to as Next Generation Probiotics, a concept which overlaps with the newly  
21 emerging concept of Live Biotherapeutic Products. The latter is a class of organisms developed  
22 exclusively for pharmaceutical application. In this perspective we discuss what lessons have been  
23 learned from working with traditional probiotics, explore the kinds of organisms likely to be used as  
24 novel microbial therapeutics, discuss the regulatory framework required, and propose how scientists  
25 may meet this challenge.

26

## 27 Introduction

28 Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer  
29 a health benefit on the host”<sup>1</sup>. Probiotics have a centuries-long history of safe use (Fig. 1) but have  
30 only been recognised as being of economic value during the 20<sup>th</sup> century. The global probiotics market  
31 is projected to reach a turn-over value of USD\$46.55 billion by 2020  
32 (<http://www.marketsandmarkets.com/PressReleases/probiotics.asp>), and is dominated by food  
33 companies, nutritional supplement companies, and dedicated probiotic production companies. The  
34 probiotic organisms that feature in these products have been mainly sourced from the gut or from  
35 traditional fermented foods such as pickles, yoghurts, and kefir grains. Thus the majority of the  
36 probiotics sold and used both in probiotic research and commercial probiotic development are from  
37 a limited list of genera, which mainly include *Lactobacillus* spp. and *Bifidobacterium* spp. The more  
38 commonly exploited strains/species among the lactobacilli and bifidobacteria have been accepted as  
39 having Generally Regarded as Safe (GRAS) status in the United States  
40 (<http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices>) or have been granted Qualified  
41 Presumption of Safety status by the European Food Safety Authority (EFSA)<sup>2</sup>. Other probiotics  
42 currently available in the marketplace include *Saccharomyces*, *Bacillus* spp., *Escherichia coli*,  
43 enterococci and *Weissella* spp. We consider it likely that these organisms will continue to be  
44 developed and regulated under the current mechanisms for probiotics rather than the novel pathways  
45 discussed below.

46 With the development of better culturing methodologies, more affordable genome and  
47 metagenome sequencing and more powerful tools to edit and modify bacterial genomes, we are now  
48 on the cusp of a new era in probiotic research, one which allows us to develop bespoke probiotics that  
49 address specific consumer needs and issues. The knowledge of the composition and function of the  
50 human gut microbiome, also accelerated by massively parallel sequencing, has dramatically extended  
51 the range of organisms with potential health benefits, although many of these are still at the very early  
52 stage of mechanistic investigation (Table 1). These organisms are sometimes referred to as “Next  
53 Generation Probiotics” but may also be termed “Live Biotherapeutic Products” (LBPs<sup>3</sup>) in the context  
54 of a new regulatory framework in the USA (see below). Both academic and industry scientists are  
55 faced by a set of challenges which partly mirror those faced in recent decades by those engaged in  
56 probiotic research, but which have additional distinguishing issues that may facilitate or complicate  
57 their commercial development. There are many other candidate therapeutic organisms in various  
58 phases of development in the burgeoning microbiome-based biopharma sector but Table 1 entries  
59 are restricted to selected examples that have been published, and preferably tested in humans.

60 Expanding this parsimonious list will require completion of pre-clinical safety trials, and safety and  
61 efficacy trials in humans.

62

### 63 **What is a Next Generation Probiotic?**

64 Next Generation Probiotics (NGPs) obviously conform to the normal definition of a probiotic, but in  
65 this discussion we are primarily referring to those microbes which have not been used to date as  
66 agents to promote health, and which are more likely to be delivered under a drug regulatory  
67 framework (Fig. 2). NGPs also fit well within the US Food and Drug Administration definition of Live  
68 Biotherapeutic Product: “a biological product that: 1) contains live organisms, such as bacteria; 2) is  
69 applicable to the prevention, treatment, or cure of a disease or condition of human beings; and 3) is  
70 not a vaccine.”

71 (<http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/General/UCM292704.pdf>).

73 Given that the term LBP is now a formally recognised concept, at least in the USA, one may reasonably  
74 question if a term such as NGP is necessary at all. We suggest that at this juncture that classifying  
75 certain microbes as NGPs can serve a useful purpose, in that the term emphasises that they differ  
76 from traditional probiotics in how they are likely to be viewed by regulators, and recognises the  
77 likelihood that NGPs will also include genetically modified microorganisms (GMMs). Probiotics have  
78 been largely included in food delivery vehicles or as supplements, marketed and regulated as foods or  
79 functional foods, and are clearly positioned in consumer perception a long way from the controversial  
80 issue of GMMs or Genetically Modified Food. Since the likely route to market for LBPs and NGPs will  
81 follow a path marked by studies of preclinical mode of action, safety, pharmacokinetics,  
82 pharmacodynamics, phase 1-3 trials, accompanied by passing appropriately timed regulatory approval  
83 hurdles (see below), it seems that referring to these organisms as simply “probiotics” will generate  
84 confusion rather than clarity, to scientists and consumers alike.

85 It is also worth considering if both terms NGP and LPB are different and necessary. The differences are  
86 mainly but not exclusively operational ones; NGPs tend to be investigated by laboratories previously  
87 engaged in probiotic and microbiome research and often have a development trajectory based on the  
88 probiotic experience in the laboratory; LBPs tend to be investigated by start-up biotechnology  
89 companies or pharmaceutical companies with the expressed intention of seeking approval for  
90 pharmaceutical marketing. GM probiotics arguably span both label domains, with there being a  
91 reasonable case that calling them LBPs rather than NGPs is less likely to erode consumer confidence

92 that probiotics are simple unmodified organisms. We suggest that NGP is a reasonable attempt to  
93 mark the transition from traditional microbes with long histories of safe use, to untried microbes with  
94 no such historical acceptance. In time, we believe that the term NGP will disappear and its members  
95 will either merge with current probiotics or will take a pharmaceutical route to market, in which case  
96 they would be developed as LBPs.

#### 97 **Examples of current NGP candidates**

98 A scan of the primary literature for the period of 2000-2016 using the term “probiotic\*” reveals 16,064  
99 articles, 9,811 of which contain the word *Lactobacillus* and 3,463 *Bifidobacterium*, either in the title  
100 or abstract. The majority of papers that mentioned non-canonical probiotic genera, for example  
101 *Clostridium* or *Bacteroides*, did so in the context of these genera being pathogenic strains to be  
102 modulated by the consumption of the probiotic, rather than as actual probiotics. Furthermore, any  
103 connotations of the term with other genera such as *Faecalibacterium* or *Akkermansia* were very rare.  
104 Where non lactobacilli or bifidobacterial probiotics were mentioned, it is evident that there are two  
105 strategies being employed to develop them as NGPs. As with current probiotics, one strategy involves  
106 associating the presence or absence of a specific strain with a health phenotype and exploring whether  
107 the chosen strain, when administered in sufficient quantities, can recapitulate the health phenotype.  
108 The second strategy is to adopt a well-characterised probiotic strain and use them as delivery vehicles  
109 for a specific molecule, again choosing the molecule to be delivered based on either a strong  
110 association or some mechanistic insight which shows that addition of the molecule would abrogate  
111 the disease phenotype and thus promote health.

112 The two most abundant families in the colon are *Bacteroidales* and *Clostridiales*. The former are being  
113 explored as potentially novel second-generation probiotics. For example, Deng and colleagues <sup>4</sup>  
114 isolated *B. fragilis* strain ZY-312 from the faeces of a healthy breastfed infant and proceeded to show  
115 that the organism possessed potentially health promoting phenotypes when incubated with  
116 colonocytes and macrophages. These phenotypes include the promotion of the production of  
117 microbicidal molecules and phagocytic functions in macrophages. However, these functions appear  
118 to be strain dependent; for example *B. fragilis* has been reported to make fragilysin <sup>5,6</sup> which has been  
119 implicated as a risk factor for developing colorectal cancer <sup>7</sup>, which would not be a desirable trait in a  
120 next-generation probiotic. The bacterial polysaccharide, PSA, which was reported in 2005 <sup>8</sup> is another  
121 probiotic feature of *B. fragilis*. PSA is part of a larger family of zwitterionic polysaccharides (ZPS) and  
122 has been reported to play an immunomodulatory role, and depending on the type of polysaccharide,  
123 this can be either immunoregulatory or pro-inflammatory. These results show that it is important to

124 identify the strain being used because its health promoting features will be closely aligned to its  
125 evolutionary history, a feature which is also true for traditional probiotics.

126 *Bacteroides xyloxydans* DSM 23964 has also been considered an NGP. It was isolated from human  
127 faeces, and does not encode the *Bacteroides fragilis* enterotoxin or produce PSA<sup>9</sup>. It has been shown  
128 to be tolerated in Phase I trials<sup>9</sup>, and in a later study in humans the same team showed that the heat  
129 inactivated preparation of this organism was able to increase the levels of Thomsen-Friedenreich (TF $\alpha$ )  
130 specific IgM antibodies in a manner which was dose-dependent and time constrained<sup>10</sup>. The authors  
131 speculated that an increase in these antibodies would promote a more robust response to cancer and  
132 thus ameliorate the host's own cancer immune surveillance system<sup>10</sup>. However, by heat inactivating  
133 the organism they are effectively contravening what is one of the defining characteristics of probiotics;  
134 that it must be a living organism. Furthermore, the desired outcome, to prevent cancer, is a difficult  
135 one to prove, as it will require large cohorts prospectively studied over 20-30 years to assess efficacy.  
136 Other *Bacteroides* spp. have also been considered as potential NGPs; *Bacteroides dorei* D8, has been  
137 shown to convert cholesterol to coprostanol *in vitro*, and may be considered as a probiotic in the  
138 context of the cholesterol-CVD axis; *B. acidifaciens* has been shown to increase IgA in gnotobiotic mice  
139 mono-associated with the bacterium<sup>11</sup> and a strain of *B. ovatus*, when fed to mice, increased levels of  
140 anti-TF $\alpha$  IgM and IgG antibodies.

141 The other common genus found in the colon, *Clostridium*, has not yet been explored to the same  
142 extent as the *Bacteroides* species complex. One strain, *Clostridium butyricum* MIYAIRI 588 (CBM 588;  
143 also referred to as *C. butyricum* FERM BP-2789), has been studied for over 50 years, mainly in Asia.  
144 From the limited number of publications it appears that this organism has been used to treat  
145 *Clostridium difficile* infections<sup>12</sup>, *Helicobacter pylori* infections<sup>13</sup>, cholesterol levels<sup>14,15</sup> and cancer<sup>16</sup>.

146 One of the most abundant species to be found in the large intestine is *Faecalibacterium prausnitzii*,  
147 which has been reported to be depleted in individuals with inflammatory bowel disease<sup>17</sup>. Therefore,  
148 it seems reasonable that if there was a causal link between disease status and the absence of this  
149 organism, then by simply feeding it to the individual its health promoting features should be restored  
150 and thus it may be considered an NGP. However, there is no evidence, either published or deposited  
151 at ClinicalTrials.gov, for this organism's efficacy as a probiotic to be able to reverse the symptoms of  
152 IBD when fed to humans. In animal models, evidence is available and feeding animals with *F.*  
153 *prausnitzii* does lead to or associate with induction of anti-inflammatory cytokines<sup>18</sup> or reduction of  
154 pro-inflammatory cytokines<sup>19</sup> in induced models of colitis/IBD.

155 An alternative route to developing some NGPs is to take GRAS organisms or commensals and use them  
156 as a delivery vehicle for a bioactive molecule. In this approach the bacterial "vehicle" is known not to

157 produce any virulence factors and will be tolerated by the host and if chosen carefully, may not even  
158 colonise the host. Two groups have used *Lactococcus lactis* strains (not normally considered to be  
159 probiotics) as their vehicle for delivering a range of anti-inflammatory molecules. *L. lactis* was  
160 engineered to deliver the serine protease inhibitor, elafin, and shown that in an animal model of colitis  
161 administration of the GMO reduced elastolytic activity and inflammation <sup>20</sup>. Another laboratory  
162 engineered *L. lactis* to deliver several different human molecules, most notably IL-10 <sup>21</sup> for controlling  
163 allergen sensitivity and Trefoil Factor 1 <sup>22</sup> to treat oral mucositis, with other examples being covered  
164 in more detail elsewhere <sup>23</sup>. While these approaches used a GRAS food-derived bacterium as their  
165 delivery vehicle, the common colonic bacterium *Bacteroides ovatus* has been employed as a host to  
166 express and produce either murine IL-2 <sup>24</sup>, keratinocyte growth factor-2 (KGF-2) <sup>25</sup> or TGF- $\beta$ 1 <sup>26</sup>, all  
167 under the control of a xylan inducible promoter, which was re-purposed from its original task of driving  
168 expression of the *B. ovatus* xylanase gene <sup>27</sup>. In one animal trial, TGF- $\beta$ 1-producing *B. ovatus* was  
169 administered to mice with DSS-colitis, and induced production of the TGF- $\beta$ 1 *in situ*, by inclusion of  
170 xylan in the drinking water. The authors concluded that this GMO was able to significantly improve  
171 the clinical scores and accelerate healing, and stated that the results “are comparable and most cases  
172 superior to that achieved by conventional steroid therapy” <sup>27</sup>.

173

174



175 **Table 1. Selected examples of Next Generation Probiotics**

176

177

Organism	Type	Disease Target	Level of Evidence	Study type	Ref
<i>Bacteroides xylanisolvens</i> DSM 23694	Natural (human)	Cancer	Medium: safety in humans has been established, while levels of T $\alpha$ specific-IgM have been shown to be elevated in humans.	In human	10
<i>B. ovatus</i> D-6	Natural (human)	Cancer	Low to medium: increases levels of murine T $\alpha$ specific-IgM and IgG.	Pre clinical in mice	28
<i>B. ovatus</i> V975	GMO (originally from human gut samples) expressing Human keratinocyte growth factor-2 (KGF-2)	Intestinal Inflammation	Medium: Shows abrogation of symptoms of DSS induced in murine colitis model.	Pre clinical in mice	25
<i>B. ovatus</i> V975	GMO expressing Human transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1)	Intestinal inflammation	Medium: Shows abrogation of symptoms of DSS induced in murine colitis model.	Pre clinical in mice	26
<i>B. dorei</i> D8	Natural (human)	Heart disease	Low, depletion of cholesterol in vitro	Pre clinical <i>in vitro</i>	29
<i>B. fragilis</i> ZY-312	Natural (human)	Clearance of infectious agents	Low: data only in vitro.	Pre clinical <i>in vitro</i>	4
<i>B. acidifaciens</i> JCM 10556(T)	Natural (mouse)	Clearance of infectious agents	Low-medium: Increases IgA levels in the large intestine of gnotobiotic mice.	Pre clinical in mice	11
<i>Clostridium butyricum</i> MIYAIRI 588	Natural (human)	Multiple targets including cancer, inflammation and infectious agents	Low-Medium: Evidence gathered for claims in human and animals trials	In human	12-16,30-42
<i>Faecalibacterium prausnitzii</i>	Natural (human)	Mainly IBD, but also asthma, eczema and Type II diabetes	Low to Medium: Mainly focused animal models of colitis and in associative studies	Pre clinical in mice and <i>in vitro</i>	18,43,44
<i>L. lactis</i> ::elafin	GMO (Host isolated from food)	Mainly inflammatory disease such as IBD	Medium: Good evidence from animal models of IBD	Pre clinical in mice	20
<i>L. lactis</i> :: Trefoil Factor 1 or IL-10	GMO (Host isolated from food)	Allergen sensitivity and autoimmune diseases – Type I Diabetes	Medium: Mainly animal based efficacy.	In humans Phase I trial	23

178

179

180

181 **Issues facing the development and marketing of NGPs and LBPs**

182

183 *Current EFSA and FDA positions on probiotics and LBPs*

184 The existing regulatory positions for probiotics are not consistent across all jurisdictions, and so we  
185 will briefly summarise the current situation in the United States and the European Union. When  
186 considering regulatory positions on probiotics, it is important to recognize that probiotics can be  
187 utilized in a variety of different product types. Probiotics can be delivered in the form of conventional  
188 foods, infant formula, pet foods, dietary supplements, drugs, cosmetics and even medical devices<sup>1</sup>.  
189 The regulatory requirements and types of allowable claims for each of these products differ. Most  
190 probiotics today are components of either foods or dietary supplements.

191 In the European Union the responsible regulatory agency is the European Food Safety Authority  
192 (EFSA). The EFSA Panel on Dietetic Products, Nutrition and Allergies has evaluated over 400 probiotic  
193 applications, but has not reached a positive opinion on any health claims. Indeed, even the use of the  
194 term ‘probiotic’ has been effectively outlawed by an amendment which regulates the use of ‘generic  
195 descriptors’<sup>45</sup>. It is not clear whether any NGPs would be subjected to any additional regulatory  
196 scrutiny, but any genetically modified microbes would also have to be approved by the EFSA Panel on  
197 Genetically Modified Organisms, while the authorisation of any microbe as a drug would have to be  
198 authorised by the European Medicines Agency.

199 In the United States, regulatory authorities do not use the term ‘probiotic’. Even though precisely  
200 defined<sup>1</sup>, they instead use the term live microbial ingredients, when referring to ingredients in foods  
201 or dietary supplements, or live biotherapeutic agents when referring to use as a drug. With regard to  
202 claims in the United States, claims that a product can diagnose, cure, mitigate, treat, or prevent  
203 disease are only allowed on drugs. Health benefit claims for foods or dietary supplements are of two  
204 types. The first type, an approved Health Claim, has not been used for probiotics. This claim relates to  
205 the ability of the food or supplement to reduce the risk of disease. This claim must be approved by  
206 the FDA or an authoritative body (such as the Institute of Medicine). The second type of claim is the  
207 structure/function claim. Such claims relate the probiotic to the normal structure and function of the  
208 healthy human body. Recently, in the context of infant formula, the FDA expressed the opinion in a  
209 draft guidance that such claims are acceptable on dietary supplements, but that such claims on foods  
210 must relate to the taste, aroma or nutritive function of the food<sup>46</sup>.

211 Importantly to the context of development of NGPs, the FDA position on what constitutes a ‘new  
212 dietary ingredient’ must be considered. In August 2016, the FDA published a draft guidance on this

213 topic<sup>47</sup>. This draft contains the statement: “Bacteria that have never been consumed as food are  
214 unlikely to be dietary ingredients.” In short, any probiotics on the market prior to the adoption of the  
215 dietary supplement regulations (Dietary Supplement Health and Education Act of 1994) in October 15,  
216 1994 can be grandfathered in as a dietary supplement ingredient. However, the FDA does not provide  
217 a direct path to a dietary supplement for any novel probiotics. If an NGP is first marketed in food, it is  
218 considered a dietary ingredient, and then has a path to become a dietary supplement. This is a  
219 cumbersome, indirect pathway that will likely result in any microorganisms being developed instead  
220 as LBPs.

221 As stated earlier, the FDA Center for Biologic Evaluation and Research (CBER) defined a live  
222 biotherapeutic product (LBP) as ‘*a biological product that: 1) contains live organisms, such as bacteria;*  
223 *2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and 3)*  
224 *is not a vaccine*’<sup>48</sup>. This would appear to be a very useful category which could be exploited for novel  
225 microbes ‘mined’ from the microbiota. CBER requires a very detailed characterisation of any  
226 microorganisms in this category, similar to that required for vaccines. LBPs would have to be produced  
227 to Good Manufacturing Practice (GMP) standards. CBER also allows for the development of  
228 recombinant LBPs, composed of microorganisms that have been genetically modified through the  
229 purposeful addition, deletion, or modification of genetic material. The path for conducting human  
230 research on LBPs is clear, though we know of no examples that have completed it yet. The  
231 Investigational New Drug (IND) process must be followed. Over past years, the FDA had considered  
232 essentially all probiotic research to be drug research. Under the auspices of the International Scientific  
233 Association for Probiotics and Prebiotics (ISAPP), several researchers challenged FDA on this position,  
234 demonstrating the negative impact it has had on the conduct of human research on probiotics in the  
235 United States as well as pointing out that such research on foods or dietary supplements is legal under  
236 U.S. law<sup>49</sup>. Recently, the FDA relaxed their position, seemingly to provide a path for human research  
237 on probiotic foods or dietary supplements without needing an Investigational New Drug (IND)  
238 approval<sup>50</sup>.

239 While EFSA is the competent authority for legislating and oversight with regard to probiotics,  
240 The European Directorate for the Quality of Medicines (EDQM) enables the development,  
241 implementation and monitoring of the application of quality standards for safe medicines and their  
242 use (<https://www.edqm.eu/en/EDQM-mission-values-604.html>). The EDQM appointed a Live  
243 Biotherapeutic Products Working Party in 2014, to develop a monograph for Live Biotherapeutic  
244 Products (LBPs). The purpose of this monograph will be to harmonise quality standards for LBPs as  
245 biological medicinal products and it is expected to be enacted shortly.

246

247 *What do proponents of LBPs need to demonstrate?*

248 According to FDA regulations all LBP applications must include a ‘description of the drug substance’,  
249 to include the biological name and strain designations; the original source of cells from which the drug  
250 substance was derived; the culture/passage history of the strains; a description of the clinical health  
251 of the donor; a summary of the phenotype and genotype of the product strains; and documentation  
252 and summary of modifications, if any, to the LBP, e.g., intentional introduction of foreign genes or  
253 mutations, along with details of the genetic construction. These demands should be possible for most  
254 LBPs isolated from the microbiome, although providing a complete description of the precise  
255 culture/passage history of the strains may be challenging for strains isolated a number of years ago.

256 Complete ‘characterisation’ of an LBP must also be provided. This comprehensive list includes, *inter*  
257 *alia*, methods for detection and identification, antibiotic resistance, methods used and a justification  
258 for any genetic manipulation, and any support for a mechanism of action. The manufacturer must  
259 also provide a complete and comprehensive description of the manufacturing method and  
260 infrastructure, the materials used in the manufacturing process, and details of any other products  
261 produced in the same facility.

262 LBPs will be subjected to the normal IND requirements as would any other drug substance. Initial  
263 studies in humans will be concerned with safety, and so are likely to involve healthy volunteers to look  
264 for adverse events (see below).

265

## 266 **Production challenges and scale-up**

267 Many of the commercially successful probiotics that currently dominate the marketplace were  
268 selected in large part based on their technological robustness, by which is meant that they withstand  
269 the process of growth, enrichment, freeze-drying or product incorporation, and retain viability during  
270 product shelf-life. The *Bifidobacterium* and *Lactobacillus* species that form the mainstay of the  
271 commercial supply are anaerobic or microaerophilic organisms, but are much less sensitive to  
272 atmospheric oxygen than the strict anaerobes such as *Faecalibacterium prausnitzii*, *Akkermansia*  
273 *muciniphila* and others that are currently being explored as NGPs. Bacterial fermentation is, by  
274 definition, an anaerobic process, but nevertheless current production lines were not developed to  
275 allow harvesting viable bacterial cells with the complete exclusion of oxygen throughout. Even for the  
276 initial product development stage of supporting trials, fermentation of pilot cultures up to 100 litres  
277 is required to prepare inocula for large-scale fermentation in thousand-litre volumes. As a further

278 challenge, the whole process must be performed under GMP conditions that are regulated and  
279 inspected at national level in EU member states. Following fermentation, the microbial cell biomass  
280 requires (typically) to be free-dried, again under strictly anaerobic conditions, followed by microbial  
281 quality control steps (microbial purity, viable cell counts). If being encapsulated, the freeze-dried  
282 material must be milled into an homogenous powder that is tested for galenic properties (powder  
283 characterization, disintegration, dissolution properties). Finally, the powder must be encapsulated in  
284 the absence of oxygen but also with very low water content, with or without excipients or other  
285 agents, typically based on pilot data from intestinal transit studies used to determine how to optimize  
286 viability. This chain of technological stages presents a significant challenge to the large number of  
287 start-up companies aiming to develop novel therapeutics based on anaerobic gut commensals  
288 (reviewed in ref.<sup>51</sup>)

289

## 290 **Conclusions and Action Required**

291 The term probiotic is not a taxonomic one, but refers to functionality. Nothing in the definition of  
292 the term limits the species, genus or even Kingdom from which probiotics can be selected, nor does  
293 it dictate whether they must be naive strains or whether they can have been subjected to any form  
294 of genetic manipulation. Why do we therefore feel the need to use the term 'Next Generation  
295 Probiotics'? We believe that it is highly likely that in the near future the enormous amount of  
296 research on the beneficial impact of the microbiome on human health will lead to the discovery and  
297 development of novel microorganisms derived from our microbial symbionts. In many cases these  
298 may belong to unusual and formerly 'uncharacterised' microorganisms with unusual properties, or  
299 perhaps may even be microorganisms formerly thought of as pathogens or pathobionts. These  
300 developments will present significant challenges for scientific research, for industrial exploitation  
301 and for regulatory agencies. For the moment the term NGP can serve as a useful descriptor for  
302 these 'non-traditional' microbes. Other human commensals developed and approved through a  
303 pharmaceutical route for curing disease or alleviating symptoms will likely retain the LBP moniker.  
304 The success of faecal microbiota transplantation (FMT) for curing diarrhoea associated with  
305 recurrent *Clostridium difficile* infection<sup>52</sup> has provided a conceptual framework for isolating  
306 organisms or consortia that might improve diseases associated with gut microbiota alteration<sup>53</sup>.  
307 These could include GMMs, bacterial spores, or bacteriophages, that would also be more readily  
308 developed as LBPs.

309 A suggested development pathway for these products is summarized graphically in Fig. 3.  
310 The most challenging initial task is to identify a candidate LBP. Hypothesis-based approaches to this

311 include identifying organisms whose relative abundance levels are depleted in subjects with a  
312 condition associated with an altered microbiome; organisms that are associated with successful FMT  
313 treatment of a particular condition; organisms already known to modulate the microbiome  
314 composition or function; organisms known to influence a particular host pathway or phenotype  
315 relevant to a particular disease. Alternatively, one may screen a bank of strains for a desired *in vitro*  
316 or *in vivo* activity.

317 The next phase is to characterize the LBP, initially by genome sequencing to screen for transmissible  
318 antibiotic resistance genes, and presumptive virulence factors such as toxins. Unless already  
319 performed during candidate LBP screening, trials in enzyme assays, cell models, animal models or *ex*  
320 *vivo* models are required to confirm phenotype related to the desired LBP effect. Depending on  
321 strain identity and any safety information for that species or closely related species, safety and  
322 toxicity in animal models may require additional focus.

323 The production phase should have already been scoped out so that pilot scale, defined medium,  
324 conditions have been established for rapid GMP scale-up. Establishment of an effective formulation  
325 for delivery will include confirmation of LBP survival and bioavailability upon ingestion. GMP product  
326 approval will be required so that production of batches for human trials may commence.

327 Finally, a typical series of pharmaceutical clinical trials will be implemented. Phase 1 will, for many  
328 LBPs, be a *First in Human* trial and will establish safety, and examine dosage ranges. Phase 2 will  
329 revolve around the primary endpoint expected for the LBP, in small group sizes. Phase 3 will examine  
330 efficacy, side effects, and relative benefits in larger group.

331 Accompanying all of these milestones will be achieving deliverables relevant to seeking regulatory  
332 approval by CBER, EDQM or relevant competent authority. These agencies should (continue to)  
333 engage with relevant stakeholders, especially as legislation is being developed, so that all parties  
334 have a clear understanding of precisely what documentation is required for approval of LBPs for  
335 commercial sale.

336

### 337 **Figure Legends**

338

339 Figure 1. Time-line of selected milestones in the history of probiotics and next-generation probiotics.

340

341 Figure 2. Schematic diagram summarizing some differences in the history and route to market of  
342 probiotics, next-generation probiotics, and Live Biotherapeutic Products.

343

344 Figure 3. Graphical summary of the pathway to regulatory approval for Live Biotherapeutic products.

345

346 **References**

347

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504

### 505 **Acknowledgements**

506 We thank the Panel members of the ISAPP 2016 meeting for stimulating discussions, and Mary Ellen  
507 Sanders for reviewing the section on regulations. The opinions in this article are those of the authors  
508 only, and do not represent a consensus of the ISAPP convened panel. We are grateful to three  
509 anonymous reviewers for constructive criticism that helped refine our thinking.

510

### 511 **Disclosures**

512 PWOT and CH are funded in part by Science Foundation Ireland (APC/SFI/12/RC/2273) in the form of  
513 a research centre which is/has recently been in receipt of research grants from the following  
514 companies: Cremo, Mead Johnson Nutrition, Kerry, General Mills, GE Healthcare, Friesland Campina,  
515 Sigmoid, Alimentary Health, Second Genome, Nutricia, Danone, Janssen, AbbVie, Suntory Morinaga  
516 Milk Industry Ltd, Pfizer Consumer Health, Radisens, 4D Pharma, Crucell, Adare Pharma, Artugen  
517 Therapeutics, Caelus. PWOT is a founder shareholder of Tucana Health Ltd. CH is a founder  
518 shareholder in Artugen therapeutics. These relationships with industry have no bearing on the present  
519 work and neither influenced nor constrained it. JRM has consulted and received payment from Cultech  
520 Ltd, Takeda Pharmaceuticals and Unilever.

521