



The pros, cons, and many unknowns of probiotics

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1 **The pros, the cons, and the many unknowns of probiotics**

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44 **Abstract**

45 Consumption of over-the-counter probiotics has been globalized in recent years. Emerging
46 health trends, extensive commercial endorsement, and conflicting clinical results have led to a
47 highly polarized state, in which, on the one hand, probiotics use has been greatly popularized by
48 the general public, but on the other hand many proposed probiotics health indications remain
49 non-sufficiently substantiated, and are accompanied by a highly debated medical literature.
50 Emerging insights from the microbiome field now enable a re-assessment of probiotics gut
51 colonization, strain-level activity, interactions with the indigenous microbiome, safety and
52 impacts on the eukaryotic host, in reaching more comprehensive conclusions on physiological
53 effects and potentially useful medical indications. In this perspective, we will highlight key
54 advances, challenges, and limitations in striving towards an unbiased interpretation of the large,
55 but often debatable data regarding over-the-counter probiotics, and propose avenues to
56 improve the quality of evidence, transparency, public awareness, and regulation of their use.

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81 **Introduction**

82 The concept of oral consumption of microorganisms as means of inducing health benefits has
83 intrigued humans for centuries. The term ‘probiotics’ first appeared in this context in 1974 and
84 conceptually evolved to the current common definition suggested by the FAO/WHO in 2002:
85 “live microorganisms, which, when administered in adequate amounts, confer a health benefit
86 on the host”¹. Nowadays, over-the-counter microbial therapy constitutes a constantly growing
87 multi-billion-dollar industry² and is one of the most commonly consumed forms of food
88 supplements worldwide³. Probiotics are supplemented to foods such as yogurt, cheese, ice
89 cream, snacks and nutritional bars, breakfast cereals, and infant formulas, as well as cosmetic
90 products, and are also commercialized in the form of lyophilized pills⁴. Probiotics consumption
91 is widely supported by physicians⁵, and specifically gastroenterologists⁶. This popularity
92 notwithstanding, data from decades of research on the efficacy of probiotics in treating or
93 preventing disease often points towards opposing conclusions, and remains conflicting, debated
94 and confusing in many cases. Moreover, the major medical regulatory authorities, such as the
95 European Food Safety Authority⁷ or the US Food and Drug Administration⁸, have yet to approve
96 any probiotic formulation as a medical intervention modality. As a result, probiotics marketing
97 as dietary supplements is often driven by properties such as safety, viability in the GI tract and
98 lack of impact on food taste, rather than by unequivocal health-promoting effects⁹. This
99 confusing state merits seeking better evidence-based proofs of probiotics impacts on humans
100 and their adverse effects¹⁰. In this perspective, we will highlight and discuss some of the major
101 prospects and limitations of the current approach to probiotics, present challenges in
102 interpretation of available data, and suggest possible strategies to clarify these issues and
103 transform probiotics into a more reproducible and universally accepted measurement-based
104 approach.

105 In providing this critical perspective, we would like to emphasize that the reviewed over-the-
106 counter microbial interventions will be termed ‘probiotics’ regardless of their benefit, efficacy
107 or lack thereof. Importantly, while aiming to offer a critical overview of the state of probiotics,
108 we do not wish to ‘throw the baby out with the bathwater’. The uncertainty created by some of
109 the opposing evidence with regards to probiotics notwithstanding, we conceptually believe that
110 rigorous research and regulation has a promising potential of materializing into an effective
111 medical intervention in selected indications, some of which are exemplified below. Of note, this
112 perspective is not aimed at reviewing investigational, non-commercially available “next
113 generation” microbial therapy approaches that are being proposed as interventions in various
114 medical indications. These are discussed elsewhere^{11,12}.

115 It is unrealistic to include all probiotics studies and their suggested indications in one
116 perspective. Therefore, we will highlight notable examples to discuss **A**. The ‘knowns’ and
117 challenges with respect to strength of evidence and clinical interpretation of studies assessing

118 health benefits of probiotics. **B.** Suggested mechanisms of probiotics, touching upon the gut
119 colonization debate **C.** Interactions of probiotic strains with the gut microbiome. **D.** Safety, and
120 **E.** Future directions.

121

122 **Clinical efficacy**

123 The effects of probiotics on humans have been extensively studied both by scientists and the
124 food and drug industry for decades, leading to multiple suggested prophylactic and therapeutic
125 health indications and claims, including prevention or treatment of acute, antibiotic-associated,
126 and *Clostridium difficile*-associated diarrhea, amelioration of inflammatory bowel disease and
127 irritable bowel syndrome and risk reduction for neonatal late-onset sepsis and necrotizing
128 enterocolitis. Other claims include, among many others, eradication of *Helicobacter pylori*,
129 reduction in incidence and severity of respiratory infections, alleviation of depression,
130 prevention or treatment of atopic dermatitis and reduction of cardiovascular risk factors
131 associated with cardiometabolic syndrome¹³. Regretfully, despite the fact that some clinical
132 trials related to the above health claims are of high methodological quality and validity¹⁴⁻¹⁸,
133 careful examination of the large body of evidence reveals that, for most of the above indications,
134 there are also studies of similarly high methodological quality featuring negative or opposing
135 results, collectively leading to conflicting, ambiguous and debatable overall conclusions.

136 This confusing situation may stem from a number of reasons, including the fact that many of the
137 probiotics trial readouts are based on empiric clinical data that varies in its collection
138 methodology, clinical end-points, and analytical rigor. Many reports use of qualitative, self-
139 reported parameters of “well-being”^{19,20}, others provide quantification of markers that do not
140 necessarily have clinical significance, for example reduction of C-reactive protein (CRP) in
141 healthy individuals²¹, or elevation of glucose-stimulated glucagon-like peptide 1 (GLP-1) in
142 glucose-tolerant individuals²². Likewise, a great variability exists as to the systems analyzed,
143 ranging from cell cultures, *in vitro* studies, animal models and human studies spanning
144 observational or randomized, placebo-controlled trials. At times, even within high quality
145 placebo-controlled studies, probiotics putative benefits are conflicting between trials^{23,24}.

146 Another contributor to the variability between probiotics studies is the profusion of studied
147 microorganisms. With observations made over a century ago^{25,26}, the dominant microorganisms
148 used in the probiotics industry even nowadays belong to two genera: *Lactobacillus* and
149 *Bifidobacterium*. Each of these genera includes multiple species, subspecies and strains that
150 feature with both class effects and, in some cases, distinct strain-specific traits. Additional
151 common microorganisms used in the probiotics industry include *Lactococcus spp.*, *Streptococcus*
152 *thermophilus*, *E. coli* Nissle 1917, and the yeast *Saccharomyces boulardii*²⁷. Importantly, some
153 health benefits may require interaction between different strains, contrasting with the current
154 approach of considering probiotics as a homogenous therapeutic entity.

155 To counteract the above methodological and analytical limitations and to overcome
156 underpowered findings researchers and clinicians frequently integrate results from multiple
157 studies in the form of systematic reviews and meta-analyses. The use of such tools may be
158 highly useful in revealing general trends, however it may also be susceptible to biases that can
159 be introduced in each analytical step²⁸, such as obscuring actual effects or their lack thereof by
160 outlier studies that dominate the collective results and artificially resolving contradictory
161 trends. In particular, meta-analyses concerning probiotics tend, at times, to group studies
162 testing various unrelated supplemented bacteria under the same umbrella, thereby risking
163 over- or misinterpretation of results^{29,30}. Consequently, even meta-analyses addressing similar
164 topics may conflict each other^{31,32}. Thus, in our view, meta-analyses can complement, but not
165 replace high-quality, large-scale, multi-center, randomized controlled clinical trials.

166 Moreover, unlike animal models, humans are highly heterogeneous in terms of diet, age range,
167 genetic background and their gut microbiome configuration, and may therefore respond
168 differently to the same intervention (**Fig. 1**). Nevertheless, these readily measurable
169 personalization issues have not been sufficiently addressed in the probiotics literature. As
170 described in the 'Gut colonization' section below, humans feature a differential and highly
171 personalized gut colonization capacity for probiotics, which may drive differential probiotics
172 effects on the host and/or on its indigenous gut microbiome.

173 Finally, many of the probiotics studies are linked, funded, initiated and endorsed by commercial
174 entities of the probiotic industry or by professional lobby groups heavily associated and funded
175 by the same industry³³. While this reality by itself does not necessarily compromise the validity
176 of such studies, there is a need and interest in independent corroboration of efficacy claims to
177 be reproduced through non-affiliated research by scientific and medical entities. Examples of
178 some of the most notable suggested probiotics indications include:

179

180 **Acute gastroenteritis.** Probiotics have been suggested to be effective as preventive or
181 therapeutic means in various pediatric and adult etiologies manifesting as acute diarrhea.
182 Several meta-analyses and systematic reviews indicate that some preparations³⁴, especially
183 those containing *S. boulardii*³⁵, *Lactobacillus rhamnosus GG (LGG)*³⁶ and other strains within the
184 *Lactobacillus* genus³⁷ may ameliorate acute diarrhea in children and shorten its duration by
185 approximately one day. Likewise, probiotics have been shown effective in the prevention and
186 treatment of acute diarrhea in adults, and various preparations, in particular *S. boulardii* and *L.*
187 *rhamnosus*, have been suggested to improve antibiotic-associated diarrhea both in healthy
188 children^{38,39}, adults^{40,41}, and in hospitalized patients⁴². In contrast, other studies and meta-
189 analyses have shown contradictory results as for diarrhea prevention in children⁴³, adults²³, and
190 in the elderly^{41,44}. Notably, the results of two recent high-quality, large-scale, multi-center,
191 randomized placebo-controlled trials assessing treatment with *L. rhamnosus* (LGG or R0011)

192 with or without *L. helveticus* R0052 in over 1800 children presenting to the emergency
193 department with acute gastroenteritis demonstrated no clinical benefits^{45,46}. One meta-analysis
194 in children has noted that the quality of evidence with regard to this indication was low to very
195 low⁴⁷, leading to the omission of probiotics from one clinical management guidelines⁴⁸, whereas
196 another still advocates the use of *LGG* and *S. boulardii* while stating that the evidence upon
197 which these recommendations are based is of low quality⁴⁹. Notwithstanding the dispute, many
198 parents “self-treat” their children, when contracted with gastroenteritis, with “functional foods”
199 containing probiotics⁵⁰.

200

201 ***Clostridium difficile-associated diarrhea (CDAD)***. *Clostridium difficile* thrives in the gut when
202 microbiome-conferred colonization resistance is compromised, such as upon antibiotics
203 treatment in hospitalized patients, thereby causing a disease that can range in severity from
204 mild diarrhea to a life-threatening condition termed pseudo-membranous colitis. Several meta-
205 analyses have shown a cumulative beneficial outcome for probiotics in preventing *C. difficile*
206 infection or its associated morbidity⁵¹, especially when administered close to antibiotics
207 exposure⁵². A follow up 2017 meta-analysis further supported moderate beneficial evidence,
208 but indicated a considerable heterogeneity between trials, and utilized a post-hoc analysis that
209 suggested no significant effect to probiotics on CDAD in trials featuring low and moderate
210 baseline CDAD risk⁵³. Another meta-analysis concluded that of the various probiotic strains,
211 only *S. boulardii* was effective against *C. difficile*⁵⁴, though a different meta-analysis relating
212 specifically to *S. boulardii* found that it reduced CDAD risk in children, but not in adults⁵⁵, with
213 low quality of evidence noted⁵⁶.

214 In taking a closer look on the individual studies forming the basis of these meta-analyses, *C.*
215 *difficile* incidence was non-existent (8 trials, **Table 1**) or low in the majority of the trials
216 regardless of treatment group, while the vast majority of trials included in meta-analyses (34
217 trials, **Table 1**) did not demonstrate a significant effect for probiotics of different strains on
218 CDAD or *C. difficile* infection. While this may be related to insufficient power of these studies to
219 demonstrate an effect in the context of low incidence of *C. difficile*, two RCTs featuring
220 populations with a high incidence of *C. difficile*, including the largest trial of probiotics for this
221 indication to date, did not find a difference between the treatment and placebo groups^{44,57}. Thus,
222 the effects observed in meta-analyses are mostly contributed by a minority of works
223 demonstrating a significant effect^{18,42,58-61}, of which two are non-peer-reviewed conference
224 abstracts^{62,63}. While *C. difficile* incidence in the placebo group was very high^{18,42,62} in most works
225 showing a beneficial effect^{18,42,58,59,61}, other works, in which CDAD was uncommon pointed
226 towards a lower level of evidence with respect to probiotics efficacy in preventing CDAD^{33,64}.
227 Together, variable baseline risk of CDAD among cohorts may potentially explain the differences

228 in outcomes between studies, as well as the fact that the majority of meta-analyses aggregated
229 studies testing a variety of probiotic strains, both fungal and bacterial⁶⁵.

230

231 ***Irritable Bowel Syndrome (IBS) and digestive complains.*** IBS is a common and clinically
232 variable disorder of unclear etiology. Trials assessing interventions to alleviate IBS are often
233 limited by the fact that this condition is defined by subjective criteria. As such, it is of paramount
234 importance to ensure that symptoms alleviation by probiotics is not equal or inferior to that of a
235 placebo effect⁶⁶. One recent meta-analysis has suggested that probiotics may be efficacious in
236 treating symptoms of IBS⁶⁷, although it should be noted that none of the single strain
237 preparations was proven effective for abdominal pain alleviation or for treatment of bloating,
238 flatulence and urgency. Even within probiotic combinations some were found effective in
239 reducing symptom persistence and abdominal pain scores, while others were not, emphasizing
240 the importance of informed strain selection on disease outcome. Correspondingly, a systematic
241 review of 9 systematic reviews and 35 RCTs did not find evidence for various probiotic strains
242 efficacy in IBS⁶⁸.

243

244 ***Neonatal sepsis.*** A promising indication for the efficacy of probiotics is the prevention of
245 neonatal late-onset sepsis and/or necrotizing enterocolitis (NEC), a gastrointestinal disease
246 typically affecting premature newborns^{69,70}. The protective mechanism against NEC may involve
247 anti-pathogen mucosal protection, coupled with induction of maturation of innate immunity and
248 intestinal epithelial cells by some probiotic strains (such as *LGG*), which prompt an attenuated
249 inflammatory response^{71,72}. Furthermore, a recent large-scale RCT strengthened these findings
250 by showing that rural Indian infants who received a combination of oral preparation of *L.*
251 *plantarum* PP 11-217 and fructooligosaccharide were protected from neonatal sepsis and
252 death¹⁴. It still remains debated whether probiotics reduces the risk for late-onset sepsis in
253 extremely low birth weight neonates⁷³⁻⁷⁵, and whether milk-fed preterm infants feature a better
254 response to this intervention as compared to formula-fed or infants kept on mixed feeding⁷⁶.
255 Importantly, the long-term consequences of probiotics on the development of the indigenous
256 gut microbiome and their effect on gut immune, metabolic, and anatomical development⁷⁷
257 warrants further studies.

258

259 ***Acute respiratory infection.*** Some systematic reviews and meta-analyses of studies empirically
260 testing probiotic strains, suggest that they may be effective in reducing the severity, duration or
261 incidence of common cold, respiratory infections and influenza-like symptoms in children,
262 adults, the elderly, and even in athletes^{78,79}, however quality of evidence was stated as low to
263 very-low and heterogeneity between studies was deemed significant. A meta-analysis
264 encompassing both children and adult studies proposed that probiotics might reduce the

265 severity and duration of respiratory tract infections, but not their incidence⁸⁰. These
266 discrepancies may stem, at times, from reliance on subjective or indirect measures to assess
267 infection, such as self-reporting⁸¹⁻⁸⁴, or antibiotic treatment and days of absence from
268 work/daycare^{78,85}. Discrepancies may also result from unadjusted results when treatment
269 groups were different at baseline⁸⁵, subsampling with no clear clinical or biological
270 justification^{86,87}, unexplained exclusion of trials from meta-analyses⁷⁸, and attributing an effect
271 to treatment despite a counter-intuitive dose-response relationship⁸⁷. On a causal level, there is
272 a great need of a data-driven explanation of mechanisms by which gastrointestinal-localized
273 probiotics would impact a disease involving a remote organ.

274

275 **Gut colonization**

276 An unresolved issue associated with probiotics mechanisms of action relates to the
277 administered microorganisms capacity to stably or even transiently colonize the host
278 gastrointestinal mucosal surface, and whether their colonization is necessary to exert beneficial
279 impacts on the host. The proximity of probiotic strains to the host lining epithelial layer may be
280 mechanistically crucial, as mucosal adhesion or even presence at low titers, may provide the
281 micrometer distance of probiotics strains to the host gastrointestinal epithelium, which is a
282 prerequisite for many activities including contact-dependent immune modulation^{88,89},
283 metabolite secretion in effective concentrations⁹⁰, and mucus layer modification⁹¹. This
284 decades-long debate is comprised of two inherently distinct colonization-related questions,
285 which have often been confusingly intermingled with each other, in the absence of concrete
286 experimental data:

287 **Question 1: Do probiotics colonize the gut mucosa during consumption?** Surprisingly, this
288 critically important topic has not been directly explored in a comprehensive manner in humans
289 until recently. Most probiotics colonization claims have been extrapolated from assessment of
290 their abundance in stool, without directly examining whether this actually reflects their
291 colonization capacity, or merely a passage of non-engaging microbes across the GI tract and
292 their excretion into stool⁹². Like stool assessment, probiotics adherence to human
293 gastrointestinal cells *in vitro*^{93,94} may be a poor indicator of *in vivo* colonization due to a myriad
294 of host and microbiome factors that are absent in the *in vitro* setting.

295 Direct quantification of mucosal probiotics colonization was determined by endoscopies in a
296 handful of trials, with some studies in humans⁹⁵⁻⁹⁸ and pigs^{99,100} suggesting that probiotic
297 bacteria could be universally isolated from various gastrointestinal organs during or even after
298 supplementation, while others showing a highly limited and variable colonization patterns,
299 observed in only a minority of tested individuals¹⁰¹⁻¹⁰⁴. Noteworthy, the universal utilization of
300 culturing or 16S rDNA techniques in these studies considerably limits the ability to distinguish
301 between the administered probiotic strain and endogenous closely related commensals of the

302 same species/genus (see **Box 1**). A species- and strain-sensitive metagenomic assessment of
303 human participants evaluated by colonoscopy and gastroscopy before and after consumption of
304 11 probiotic strains belonging to the four most widely used probiotic genera (or placebo)⁹²
305 featured a significant expansion of the mucosa-associated probiotics in 60% of the
306 supplemented individuals, and a near-total colonization resistance in the other 40%, even when
307 measured by ultra-sensitive quantitative PCR. The degree of mucosal association was unrelated
308 to the bloom of probiotic strains in stool, and could be predicted by a combination of baseline
309 host and microbiome factors, highlighting a potential future prospect of probiotics tailoring to
310 the individual. Interestingly, transplantation of fecal microbiome from ‘resistant’ or ‘permissive’
311 individuals into germ-free (GF) mice recapitulated the donor susceptibility to probiotics
312 colonization, indicating a dominant microbiome-mediated colonization resistance mechanism⁹².
313 Other postulated non-colonization-dependent probiotics effects on the host, such as impacts on
314 food digestion merit evidence-based experimental proof. With this respect, in the above study⁹²
315 probiotic strains in ‘resistant’ individuals were not detected even in the gut lumen during active
316 consumption (**Gut Microbes, in press**), suggesting that temporarily/persistently colonizing
317 mucosa-associated probiotics may serve as an important reservoir for luminal bacteria.

318

319 **Question 2: Do probiotics persistently colonize the gut mucosa, even after cessation of**
320 **consumption?** Even in ‘permissive’ individuals, it remains unclear whether probiotic
321 colonization is maintained after supplementation ceases. In rats fed a fermented milk product
322 (FMP) containing 5 probiotic strains, all strains were shed during feeding, but only a subset of
323 rats continued to shed one of the five probiotics strains (*L. lactis* CNCM I-1631) two days
324 following supplementation. Transferring the distinct microbiomes of ‘permissive’ or ‘resistant’
325 rats to GF rats replicated colonization permissiveness of the donors¹⁰⁵. In humans, detectable
326 shedding of probiotics in stool samples during supplementation that diminishes following
327 cessation has been described for *Bifidobacterium* strains *infantis* 35624¹⁰⁶, *animalis* sbsp. *lactis*
328 Bb-12¹⁰⁷, *Lactobacillus* strains *acidophilus* R52¹⁰⁸, *casei* DN-114 001¹⁰⁹, *johnsonii* La1^{104,110},
329 *plantarum* 299v¹¹¹, *reuteri* DSM17938^{112,113}, *rhamnosus* (LGG, R11, 19070-2)^{103,108,113}, and
330 *salivarius* CECT5713¹¹⁴ among others¹¹⁵. However, follow-up periods were limited to 1-2 weeks
331 after cessation of consumption in most studies. Patterns emerging from longer follow-ups
332 suggest both strain- and person-specific persistence variability. Two months following
333 supplementation cessation, *L. rhamnosus* was detected only in 1/10 individuals¹¹⁶, whereas one-
334 third of *B. longum* AH1206 consumers continued to shed the probiotic species in stool up to 6
335 months after discontinuation¹¹⁷. Subject- and strain-specific post-cessation shedding were also
336 noted in humans supplemented with the aforementioned 5-strains mix FMP, in which only *L.*
337 *lactis* CNCM I-1631 was shed in stool samples five weeks following cessation, and only by a
338 subset of individuals characterized by a distinct microbiome composition¹⁰⁵.

339

340 **Mechanism of activity**

341 Beneficial effects of probiotics have been postulated to occur through diverse mechanisms,
342 including induction of immunomodulation, protection against physiological stress, suppression
343 of pathogens, microbiome modulation and improvement of gut epithelium barrier function (**Fig.**
344 **2**). These mechanistic probiotics studies often suffer from several major limitations, including
345 heavy reliance on utilization of cell culture systems that do not account for the myriad of crucial
346 physiological cues that dictate microbe-microbe and microbe-host interactions within the
347 complex GI mucosa microenvironment, and are thus often not replicated in *in vivo* trials. Other
348 limitations stem from the poor colonization capacity of exogenous ‘human compatible’
349 probiotics in the murine GI mucosa, compared to that noted in humans^{92,118}. Host discordance
350 may be functionally significant, as administration of human commensals to mice can result in a
351 markedly distinct effect on the immune system^{119,120} or host metabolome¹²¹ compared to mice
352 harboring a murine microbiome. Importantly, some probiotic traits may represent class effects
353 and be uniformly present between different members of the species or even the genus, for
354 example both *Bifidobacterium spp.* and *Lactobacillus spp.* produce the enzyme beta-
355 galactosidase, which may compensate in lactase insufficiency^{122,123}, while other traits may be
356 species-¹²⁴ or even strain-specific¹²⁵, or require interaction between probiotic strains¹²⁶, as
357 further discussed. Several major mechanisms have been suggested to be involved in probiotics
358 effector functions:

359

360 **Immunomodulation.** Many probiotics studies suggested *in vitro* effects on expression of
361 immune-related genes, inflammatory pathways activity and immune marker levels, including
362 modulation of intestinal epithelial cell (IEC) NFκB, mitogen-activated protein kinase (MAPK),
363 Akt / phosphoinositide 3-kinase (PI3K), peroxisome proliferator-activated receptor γ activity,
364 CRP, IL-6, IL-8, tumor necrosis factor (TNF)-α, IL-1β, and interferon γ, through multiple, mostly
365 contact-dependent mechanisms (reviewed in¹²⁷). Interestingly in some studies, live and dead
366 bacteria featured a differential effect on gene expression, suggesting that both cell surface and
367 actively secreted molecules may affect intestinal transcriptome¹²⁸. Additional examples of
368 suggested immune impacts include *Lactobacillus-mediated* TLR2-dependent stimulation of TNF-
369 α secretion through lipoteichoic acid (LTA)¹²⁹, *B. longum-mediated* contact-dependent IL-10
370 secretion¹³⁰, sortase-dependent pili in *Bifidobacterium* evoking a TNF-α response⁹³, cell surface
371 exopolysaccharide (sEPS) in *B. longum* 36524 modulating proinflammatory cytokines and Th17
372 responses in the gut and the lung¹³¹, and immuno-stimulatory cell surface appendages termed
373 SpaCBA in *LGG*, mediating (*in vitro*) both binding to human intestinal mucus and TLR2-
374 dependant modulation of TNF-α, IL-6, IL-10, and IL-12¹³².

375 Additional examples of suggested *in vivo* mechanisms include *LGG* inducing the generation of
376 reactive oxygen species and consequently inhibiting TNF- α -induced intestinal NF κ B activation
377 through SpaC-mediated adhesion to intestinal epithelium¹³³; Peptidoglycan from *L. salivarius*
378 Ls33, but not *L. acidophilus* NCFM, protecting mice from chemically induced colitis in a
379 nucleotide-binding oligomerization domain-containing protein 2 (NOD2)-IL-10-dependent
380 manner¹²⁴; *L. acidophilus* L-92 binding to microfold ('M') cells mediated immune modulation by
381 its surface layer protein A (SlpA)¹³⁴; *B. infantis* 35624 inducing TLR2-dependent T regulatory
382 cells in humans¹³⁵; and *B. animalis* sbsp. *lactis* Bb-12 inducing IgA secretion^{136,137}. Collectively,
383 most of the above examples point to a requirement of physical contact or proximity between
384 host cells and probiotics to potentially induce both pro- and anti-inflammatory responses,
385 highlighting the importance of the context in which they are administered. The clinical outcome
386 of such changes observed in colonized individuals, whether beneficial or not, merits further
387 human studies.

388

389 **Protection against pathogens.** Probiotics have been suggested to inhibit pathogen colonization
390 by attaching to epithelial cells and physically blocking the pathogen ability to adhere. This has
391 been shown in culture¹³⁸ and indirectly in mice for *Salmonella* and *L. acidophilus* LAP5 or *L.*
392 *fermentum* LF33¹³⁹. *L. acidophilus* A4 can also antagonize adhesion of *E. coli* O157:H7 to IEC
393 through up-regulation of MUC2, IL-8, IL-1 β , and TNF- α ¹⁴⁰. Several *Bifidobacterium spp.* have
394 been shown to produce acetate *in vivo*, consequently inhibiting Shiga toxin-producing *E. coli*
395 (STEC) O157:H7 through acidity-related mechanisms^{141,142}. Several lactic acid bacteria can
396 produce bacteriocins, compounds that demonstrate antimicrobial activity¹⁴³. For example,
397 production of Abp118 bacteriocin by *L. salivarius* UCC118 protects mice from infection with *L.*
398 *monocytogenes*¹⁴⁴. Other mechanisms may involve the disruption of quorum sensing (QS), for
399 instance *L. acidophilus* La-5 inhibited autoinducer-2 (AI-2) and reduced the expression of some
400 virulence factors of *E. coli* O157:H7 *in vitro*¹⁴⁵; *L. acidophilus* GP1B inhibited AI-2 activity for *C.*
401 *difficile* *in vitro* and its administration to mice with *C. difficile* infection improved their
402 survival¹⁴⁶; and *L. reuteri* RC-14 produced mediators to interfere with *S. aureus* QS and thus
403 repressed its virulence, including the expression of toxic shock syndrome toxin-1¹⁴⁷.
404 Importantly, production and response to QS signals is a trait shared between pathogens and
405 commensals¹⁴⁸, thus the complexity of QS signals and abundance of responders *in vivo* may
406 differ from that of *in vitro* experiments¹⁴⁹, and QS manipulation *in vivo* can even result in
407 inhibition of commensal bacteria¹⁵⁰.

408

409 **Improved barrier function.** Several underlying mechanisms have been suggested for probiotics
410 stabilization of gut barrier function, and are reviewed elsewhere¹⁵¹, including up-regulation of
411 tight-junction (TJ) proteins (Claudin-1, Occludin, and ZO-1) and improved transepithelial

412 resistance, promotion of mucus secretion (by up-regulating MUC2, MUC3 and MUC1), elevation
413 of butyrate levels, as well as microbiome modulation. These effects may be mediated by locally
414 secreted metabolites, for example *L. plantarum* produces hydroxy-cis-12-octadecenoic acid
415 (HYA), which has been demonstrated to suppress TJ permeability and the down-regulation of
416 occludin, ZO-1, and claudin-1 induced by IFN- γ and TNF- α in culture, by regulating TNFR2
417 expression via the G protein-coupled receptor (GPR) 40/mitogen-activated protein kinase
418 (MEK)/extracellular-signal-regulated kinase (ERK) pathway¹⁵². In mice, HYA decreased skin
419 TNF- α and increased claudin-1 in a model of atopic dermatitis¹⁵³, and ameliorated pathogen-
420 induced gingival epithelial barrier disruption in a GPR40-dependent manner¹⁵⁴. Two secreted
421 proteins purified from *LGG* (termed p40 and p75) have been suggested to promote intestinal
422 epithelial homeostasis by inhibiting cytokine-induced epithelial cell apoptosis¹⁵⁵. Other effects
423 may require direct mucosal adherence, as demonstrated for MUC3 mucin expression induced by
424 *Lactobacillus* strains in HT29 cells¹⁵⁶, as well as MUC2 and *L. casei* GG in Caco-2 cells⁹¹. The
425 requirement for adhesion may explain why VSL#3 supplementation *in vivo* results in conflicting
426 findings regarding the ability to increase mucin secretion^{157,158}. Importantly, when attempting to
427 validate these findings in clinical trials the results were inconclusive, with probiotics-associated
428 improvement observed in some trials¹⁵⁹⁻¹⁶¹, but not in others¹⁶²⁻¹⁶⁵, across multiple underlying
429 conditions. Whether these discrepancies represent the result of variable probiotics colonization
430 not appreciated by early studies remains to be established.

431

432 **Additional suggested mechanisms.** Resistance to bile inhibition is one of the prerequisites for
433 commercial probiotics. For example, *Lactobacillus* and *Bifidobacterium spp.* feature bile
434 resistance by the production of bile salt hydrolases (BSH), which deconjugate glycine or taurine
435 from the steroid core¹⁶⁶. BSH activity has been associated with systemic beneficial metabolic
436 effects, including reduction in mouse weight gain, plasma cholesterol, and liver triglycerides¹⁶⁷,
437 as well as cholesterol lowering in humans¹⁶⁸. Nonetheless, deconjugation of bile acids may lead
438 to impaired digestion of dietary lipids and the formation of gallstones¹⁶⁶, as well as impaired
439 glucose tolerance¹⁶⁹.

440 Probiotics were also suggested to affect signaling to the enteric and central nervous systems,
441 and conduce anxiolytic, antidepressant, and ant nociceptive effects on the host¹⁷⁰. Mice fed with
442 *L. rhamnosus* JB-1 experience specific regional changes in mRNA for γ -aminobutyric acid
443 (GABA)-A and -B receptor in the brain, associated with attenuation of the corticosterone
444 response to stress and an anxiolytic phenotype, which was not observed in vagotomized
445 animals¹⁷¹. Nonetheless, the same strain failed to modulate stress or cognitive performance in
446 humans¹⁷². In mice, maternal high-fat diet results in dysbiosis of both the dam and the offspring,
447 which has a causal role (as demonstrated by transplantations to GF mice) in impairing social
448 behavior in the offspring. Treatment with *L. reuteri* ATCC PTA 6475, but not *L. johnsonii* ATCC

449 33200, restored oxytocin levels in the paraventricular nuclei that were reduced by maternal
450 HFD, and improved social behavior¹⁷³. *L. reuteri* DSM 17938 may also present an ant nociceptive
451 effect in rats in a transient receptor potential vanilloid 1 (TRPV1)-dependent manner¹⁷⁴. *L.*
452 *acidophilus* NCFM induced expression of μ -opioid and cannabinoid receptors in intestinal
453 epithelial cells, and had an analgesic effect in rats¹⁷⁵. With the potential of beneficially
454 influencing the gut-brain axis by probiotics notwithstanding, key molecular players are still
455 unknown and will be critical for proper translation of findings in animal models to human-
456 relevant therapies.

457

458 **Interactions with the indigenous microbiome**

459 While probiotics impact on the host may not necessarily relate to their interactions with the
460 indigenous microbiome, their use is often associated with claims related to 'beneficial
461 modulation of the microbiota' and 'normalization of perturbed microbiota', either as favorable
462 outcomes on their own or as a mechanism by which probiotics protect the host against disease¹.
463 Nonetheless, the extent, if any, by which probiotics modulate the intestinal microbiota in
464 healthy individuals remains highly debated, as highlighted by a 2015 systematic review that
465 reported lack of evidence for probiotics effect on the microbiota in 6/7 analyzed studies¹⁷⁶, as
466 well as an earlier systematic review analyzing different trials, of which only 21% resulted in
467 microbiome alterations¹⁷⁷. Presumable effects on the microbiome may stem from analytical
468 biases (**Box 1**). In all, the majority of studies on probiotics in healthy adults, children, and
469 elderly individuals reported no effect of probiotics on the fecal microbiota composition,
470 regardless of the supplemented strains, dose, duration or microbiome analysis method (**Table**
471 **2**). Importantly, there is a paucity of trials characterizing the effect of probiotics on the
472 gastrointestinal microbiome *in situ* (**Box 2**).

473 One important determinant that may affect the ability of probiotics to modulate the microbiome
474 is the pre-exposure assembly, which may differ between individuals. Antibiotics significantly
475 perturb the microbiome¹⁷⁸, thus relieving colonization resistance to probiotics¹¹⁸, but also to
476 pathogens¹⁷⁹. In this context, probiotics are postulated to serve as placeholders in the cleared
477 niche, preventing pathogen colonization and antibiotic-associated diarrhea³⁸, or as means of
478 correcting antibiotic-associated dysbiosis¹, but evidence to support an ability of probiotics to
479 facilitate reconstitution of the gut microbiome following antibiotics perturbation is often based
480 on bacterial cultures or specific FISH or qPCR probes, which represent only a minimal fraction
481 of the perturbed microbiome, and even using this methodology, the restoration reported may be
482 partial^{180,181} or minimal¹⁸² and is highly debated¹⁷⁷. Overall, the majority of studies do not
483 support a role for probiotics in compositional or functional microbiome modulation, other than
484 transient presence of the probiotic strains themselves during the consumption period (**Table**
485 **2**)^{176,177}. Among the studies that report probiotics-associated microbiome alterations, it is

486 difficult to point towards commonly altered microbial patterns of change (**Box 1**). While some
487 works reported microbiome alterations to co-occur with health promoting effects, none
488 demonstrated causality, and it is thus far impossible to *a priori* claim that such microbiome
489 alterations are beneficial.

490

491 **Safety**

492 While the efficacy of probiotics in treating or preventing disease constitutes a decades-long
493 ongoing debate, human supplementation with probiotic microorganisms is generally considered
494 safe, and is recognized as such for most probiotic strains by regulatory authorities¹⁸³. This safety
495 profile is mainly based on history of safe use in foods, and on observations noted in clinical
496 trials assessing probiotics efficacy, rather than safety as the major readout⁴. While probiotics
497 may be safe in healthy adults, their use has been associated with higher risk for infections
498 and/or morbidity in young infants¹⁸⁴ and very low birth weight neonates¹⁸⁵, critically ill adult
499 and infant patients in intensive care units, and postoperative, hospitalized or immuno-
500 compromised patients, in part due to bacteremia and fungemia^{38,186-188}. Of note, two large-scale
501 systematic reviews of hundreds of probiotics trials concluded that adverse events and safety
502 issues are poorly reported^{189,190}, calling for the performance of non-industry sponsored
503 independent, high quality, multi-centered controlled trials assessing both efficacy and adverse
504 effects in the above at-risk populations, preferentially coupled with regulatory body
505 assessment¹⁹¹.

506 Interestingly, following antibiotics treatment, enhanced probiotics colonic colonization was
507 associated with a persistent long-term probiotics-induced dysbiosis¹¹⁸, which significantly
508 delayed the reconstitution of both the fecal and the GI mucosal microbiome compared to no
509 post-antibiotics intervention. Soluble factors secreted from the administered *Lactobacillus*
510 species were suggested (at least *ex-vivo*) to directly inhibit human microbiome growth¹¹⁸. In
511 agreement, two additional trials demonstrated post-antibiotics probiotics administration to be
512 associated with a lower number of observed species compared to no probiotic treatment^{192,193}.
513 Importantly, inhibiting reconstitution of the microbiome quantity and diversity towards its pre-
514 antibiotic configuration may result in significant long-term health effects. Such persistent
515 dysbiosis hampers the colonization resistance to pathogens conferred by the microbiome,
516 which may potentially explain several associations made between probiotics use after
517 antibiotics and increased risk of communicable^{38,185,187,194,195,196}, and non-communicable disease
518 such as type 1 and type 2 diabetes, obesity, idiopathic arthritis, asthma and allergies, and IBD¹⁷⁹.
519 Given these observations, it is crucial, in our view, to better assess probiotics long-term safety in
520 this context in future clinical trials, and in particular in children, immunosuppressed
521 individuals, and the critically ill.

522

523 **Future directions**

524 The probiotics field is one of the most opinionated and polarized disciplines in biomedical
525 sciences. Data, personal beliefs, solid proof, intuition and commercial interests, coupled with
526 lack of medical regulation, are often intermingled in ways making objective interpretation close
527 to impossible. With this unfortunate situation notwithstanding, we envision that recent
528 discoveries in the microbiome field and the introduction of novel high-throughput sequencing
529 and experimental techniques may allow to revisit some elementary notions about probiotics
530 and focus on biologically relevant questions to facilitate the transition from empirical into
531 target-, disease- and patient-oriented therapeutics (**Fig. 3**). Instead of a 'black-box' *modus*
532 *operandi*, that is, haphazardly administering one member or more of a limited array of bacteria
533 with the intent to elicit health-promoting effects, a mechanism-oriented approach should be
534 adopted, in which probiotic preparations are devised *ad hoc*, following a set of meticulously
535 established criteria. These may include careful consideration of the population to be treated and
536 the medical indication to be targeted. The aim of microbial therapy should be similarly carefully
537 determined: is the effect on the host mediated remotely or indirectly through secretion of
538 molecules by allochthonous bacteria, by modulation of the indigenous microbiome, or by other
539 putative contact-dependent mechanisms inter-linking these bacteria to the intestinal
540 epithelium? Are the intended probiotic effects strain-specific or represent a class effect? Could a
541 nonfood-grade strain be suited to address a particular medical indication? For example, *A.*
542 *muciniphila* supplementation in mice prevents diet-induced metabolic syndrome and protects
543 against chemically induced colitis¹¹. *Faecalibacterium prausnitzii* is inversely correlated with
544 Crohn's disease activity, IBS, and colorectal cancer, and suggested to protect mice from
545 chemically-induced colitis¹¹. As with currently available commercial probiotics, it would be
546 important to deepen our understanding of the interactions between these novel potential
547 microorganisms, the host and its resident microbiome, when administered exogenously.

548 Development of means of tackling colonization resistance may be necessary in many instances,
549 and should require careful patient-subset selection¹⁹⁷, development of predictive algorithms
550 assessing colonization potential based on baseline host and microbiome features^{92,105,117,118},
551 rational co-administration of "prebiotics"¹⁴, colonization modifying agents¹⁹⁸, or those tailored
552 to support an administered strain¹⁹⁹, generation of defined consortia fitting individualized
553 patterns, and counteracting commensal-generated inhibitory mechanisms. The adverse effects
554 of probiotics on post-antibiotic host and indigenous microbiome reconstitution need to be
555 comprehensively assessed with more antibiotic regimens, probiotic strain combinations, and
556 modeled using human microbiome transfers into GF mice, allowing for the assessment of the
557 potential long-term clinical consequences of probiotics-induced dysbiosis. However, the very
558 same potentially negative impact of probiotics-associated dysbiosis noted in the post-antibiotic
559 setting, may be harnessed as positive therapeutic means in other clinical contexts. As such, the

560 apparent improved colonization of probiotics following ‘niche freeing’ induced by antibiotics
561 may be utilized as means of potentiating probiotics function, by allowing their colonization in a
562 variety of microbiome-associated multi-factorial disorders. Such shift from the empiric “one-
563 size-fits-all” scheme into a person- and condition-tailored approach would inherently
564 necessitate a better understanding of the forces shaping exogenous bacterial colonization and
565 resistance to colonization along the human gut interface. However, it may hold promise in
566 generating more robust and reproducible results in relation to specific strains utilization, in
567 specific human subpopulations, in specific clinical contexts, while accounting for consumer
568 safety.

569 Finally, diligently planned large-scale randomized and blinded clinical trials, preferentially
570 devoid of commercial interests, should be the mainstay of evidence-based policy formulation.
571 Endpoints should be objectively assessed and stratified to account for inter-individual
572 differences that might mask effect sizes or confound desirable or undesirable outcome. Adverse
573 reactions should be better studied, reported, and published. Unbiased risk and benefit
574 assessment by treating physicians and consumers alike should be encouraged, in improving
575 accurate data-driven decision-making at various clinical settings. Data should be made readily
576 accessible and shared to allow for a global collaborative effort to reproduce positive results
577 before guidelines are drafted or modified. In contrast to the unfortunate historical lack of
578 sufficient medical regulation for currently available probiotics, one cannot underscore the
579 critical importance of a formal regulatory approval process to be utilized with ‘next generation’
580 probiotics, similarly to any other human medical intervention.

581

582 **Figure legends**

583 **Figure 1. Precision aspects of probiotics.** Distinct initial host and microbiome conditions and
584 environmental exposures can result in different outcomes when supplemented with the same
585 probiotic preparation. Probiotic bacteria isolated from distinct host populations may present
586 with differential properties, such as adhesion, hydrophobicity and autoaggregation^{197,200}.
587 Underlying medical conditions, such as atopic dermatitis²⁰¹ or milk hypersensitivity²⁰², modified
588 the effects probiotics exerted on host immune cells. Features of the indigenous microbiome can
589 also account for different impacts of probiotics on the host, as microbiomes that allow
590 colonization were associated with ameliorated clinical responses in women with IBS²⁰³ and
591 murine models of colitis²⁰⁴ and depression²⁰⁵. These ‘permissive’ microbiomes were also more
592 prone to compositional and functional alterations in response to probiotics, and their hosts’ gut
593 epithelium exhibited enrichment in distinct pathways compared to ‘resistant’ microbiomes⁹².
594 Pre-supplementation butyrate levels were associated with a differential effect of probiotics on
595 the microbiome and butyrate²⁰⁶. Diet may also affect properties of probiotics, as dietary
596 polyunsaturated fatty acids (PUFA) modulated probiotics adhesion in vitro. Similarly, it may

597 affect clinical outcome, as preterm infants fed with human milk showed a reduced risk of late-
598 onset sepsis and a shorter time to achieve full enteral feeding, while formula-fed infants did
599 not⁷⁶.

600

601 **Figure 2. Mechanistic interactions of probiotics with the host and its microbiome.**

602 Probiotic may have several effects on the host, including metabolism of nutrients to improve
603 digestion (lactose) or produce systemic effects (bile salts), direct and indirect pathogen
604 antagonism (but potentially also promoting virulence), improved barrier function, altering the
605 microbiome, affecting signaling to the nervous system, and immunomodulation. These may be
606 contact-dependent and/or mediated by surface molecules (such as LTA, sEPS, SpaCBA, and
607 sortase-dependent pili), or by secreted molecules (such as SCFA, bacteriocins, p40 and p75).
608 Dashed lines represent putative mechanisms. BSH, bile salt hydrolase; B-gal, beta-galactosidase;
609 QS, quorum sensing; SlpA, S-layer protein A; sIgA, secreted immunoglobulin A; M-Cell, microfold
610 cell; DC, dendritic cell; MOR, mu-opioid receptor; GABA, Gamma-Aminobutyric Acid; PVN,
611 paraventricular nucleus of the hypothalamus; TGF- β , Transforming growth factor beta; LTA,
612 lipoteichoic acid; TLR, toll-like receptor; IFN γ , interferon gamma; TNF α , tumor necrosis factor
613 alpha; HYA, 10-Hydroxy-cis-12-octadecenoic acid; GPR40, G-protein-coupled receptor 40; Akt,
614 Protein kinase B; LPS, lipopolysaccharide; ROS, reactive oxygen species.

615

616 **Figure 3. Common limitations of the current approach to probiotic research and**

617 **proposed strategies to overcome them.** Translating the large body of probiotic research into
618 clinical guidelines can sometimes be challenging due to inconclusive or conflicting evidence
619 deriving from suboptimal study conduct and data analysis methodology. A novel perspective to
620 probiotics may include expanding the variety of administered strains and examining them
621 separately per-strain and per-individual according to personalized considerations, such as
622 baseline host and microbiome parameters, the medical condition to treat and the specific aim of
623 treatment. This will require a mechanism-based approach, implemented through meticulously
624 planned high-quality studies in humans, preferably regulated by health authorities, which
625 directly assess the organ of interest and do not overlook long-term safety.

626

627 **Table 1. Individual trials included in meta-analyses addressing a role for probiotics in *C.***
628 ***difficile* diarrhea, infection or recurrence.** Trials with more than one intervention arm appear

629 as separate rows with the difference indicated in the “probiotics intervention” column. Eight
630 trials had a significant effect, and 34 trials did not. P-values and confidence intervals (CI) are
631 taken from the published works, NA indicates that these values were not calculated as part of
632 the work. CDAD, *Clostridium difficile*-associated diarrhea.

633

634 **Table 2. Probiotics supplementation effect on fecal microbiome composition of healthy**
635 **individuals.** *, Significant taxonomic differences relative to the control group / baseline,
636 excluding the administered strains; N.D., not determined / no data; MZ, monozygotic; CFU,
637 colony forming units. S, Streptococcus; L, Lactobacillus; B, Bifidobacterium.

638

639 **Box 1: microbiome analysis strategies in probiotics research**

640 Advances in the field of microbiome research now offer implementing a finer resolution when
641 studying the interaction between probiotics and the resident microbial community, while
642 addressing previous methodological limitations and biases to potentially resolve contrasting
643 reports. A major contributor to this confusion is the lenient definition of “microbiome
644 alterations”. The majority of reports assessing probiotics-induced microbiota modulation utilize
645 16S rDNA relative abundances (RA) in stool samples. As supplemented probiotic bacteria are
646 excreted in stool, increase in their RA concomitantly leads to a spurious reduction in RA of other
647 community members, sometimes misleadingly interpreted as microbiota modification²⁰⁷. Thus,
648 an increase in the RA of the administered probiotic strain should not be interpreted as a *bone-*
649 *fide* effect on the microbiome²⁰⁸. Interestingly, even introduction of heat-killed bacteria was
650 suggested to result in supposed microbiome alterations²⁰⁹. Utilization of culture-based methods
651 or species-specific probes can overcome this caveat by describing probiotics-associated changes
652 in their absolute abundances²¹⁰, while accounting for viability²¹¹, but cannot describe global
653 shifts in microbiome configuration compared to pre-supplementation or placebo (beta
654 diversity) or alterations in species richness (alpha diversity). While shotgun metagenomic
655 sequencing may also result in conflicting reports^{212,213}, it offers the advantage of strain-level
656 resolution and characterizing potential probiotics effect on microbiome function. Interestingly,
657 several studies have reported probiotics-related effects on microbiota-encoded function or its
658 associated metabolites, despite no apparent effect on global composition, although these
659 functional microbiome alterations may represent genes contributed by the supplemented
660 probiotic strain, rather than global modulation^{117,214,215}. An additional limitation concerns the
661 definition of the sought “healthy microbiome” that probiotics presumably contribute to. Even
662 when assessing the studies that do suggest probiotics-associated microbiome modulation, no
663 consensus signature of such impacts can be reached (**Table 2**), and reports of microbiome
664 changes induced by probiotics are in many times conflicting, for example in the case of
665 *Clostridium perfringens*^{209,210,216} or *Escherichia*^{211,216,217}, and in various clinical contexts¹⁷⁷. For
666 example, a probiotics-associated fecal bloom of butyrate-producing bacteria (belonging mainly
667 to Clostridiales), and a reduction in *Bilophila wadsworthia* and *Parabacteroides distasonis*, was
668 noted in individuals with IBS (n=28)²¹², and mirrored (for *B. wadsworthia*) in a separate cohort
669 of individuals (n=107) in a subset of “responders”, which experienced alleviation of symptoms
670 following the intervention²⁰³ but was not reproduced by a third RCT (n=55)²¹⁸. Importantly,

671 even in cases in which probiotics administration was associated with microbiome changes,
672 these changes could be stemming from disease modulation rather than directly from exposure
673 to probiotics. To the best of our knowledge, no study to date has demonstrated a direct causal
674 role for probiotics-related microbiome modulations in improving a disease phenotype.

675

676 **Box 2: quantifying probiotics effect on the gastrointestinal microbiome *in situ*.**

677 While stool samples may not accurately represent the GI mucosa-adherent microbiome²¹⁹, only
678 a handful of studies have characterized the effect of probiotics on the intestinal microbiome *in*
679 *situ*. A culture-based study of *L. plantarum* 299v-supplemented individuals (n=29)
680 demonstrated an enrichment of Clostridia in fecal samples, but not in the rectal or ascending
681 colon mucosa¹⁰². Likewise, no significant alterations at the lower GI luminal or mucosal
682 microbiome was noted in probiotics-supplemented humans, compared either to their own
683 baseline or to placebo-administered individuals⁹². In rats, VSL#3 exacerbated the reduction in
684 luminal species diversity associated with the induction of chemically-induced colitis, but had no
685 effect on the mucosa-associated microbiome²²⁰. In contrast, in a mouse model of colitis-
686 associated colorectal cancer (azoxymethane-treated Il10-/- mice), VSL#3 supplementation
687 resulted in mucosal expansion of Proteobacteria, and reduction in *Verrucomicrobiaceae*,
688 *Porphyromonadaceae*, and *Clostridium*, changes that were associated with enhanced
689 tumorigenesis²²¹. Conflicting results regarding probiotics-related microbiome modulation were
690 also observed in patients with pouchitis^{159,222}, although the reported alterations may be merely
691 stemming from the introduction of the VSL#3 bacteria into the niche²²².

692

693

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714

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716 All authors have researched data for the article, made substantial contribution to discussion of
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718

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