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A trait of longevity: the microbiota of centenarians

Elena Biagi¹, Aurelia Santoro²

¹Microbiome Science and Biotechnology group, Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126, Bologna, Italy; elena.biagi@unibo.it

²Aging and Longevity group, Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Via San Giacomo, 12, Bologna, Italy; aurelia.santoro@unibo.it

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Abstract

Centenarians, as striking examples of individuals who reach the extreme limits of human lifespan, are a valuable model for studying how the microbiota-component can successfully maintain or re-establish a mutualistic relationship with the human host, along with the occurrence of age-related variations. Indeed, the gut microbiota of centenarians emerges as a peculiar ecosystem, different from that of younger elderly and adults, specifically adapted to an extremely aged host. The study of the centenarians' gut microbiota provided in the last decade a large amount of remarkable data, from different populations across the world, summarized in the present chapter. Comparison between data from different study populations pointed out that, while lifestyle, ethnicity and geography surely have an impact on such extreme microbiota adaptive variations, common signatures of longevity emerge among the studied populations. The possibilities to exploit such data for human health maintenance during aging are still being explored and interesting scenarios are being envisioned, from the

26 bioprospecting of age-specific probiotic bacteria to the possibility of using microbiota transplantation
27 to promote those features in the gut ecosystem that are known to be linked to longevity.

28

29

30 **Glossary**

31 **Aging:** the intricate and complex biological phenomenon represented by an age-dependent decline in
32 intrinsic physiological function and adaptive capacity leading to an increase in age-specific diseases
33 and mortality rate (i.e., a decrease in survival rate) (Franceschi et al., 2018b).

34 **Longevity:** represents the successful side of aging and is a complex, dynamic and multifactorial
35 phenomenon resulting from the peculiar reciprocal interaction between environment, genetics,
36 epigenetics and stochasticity (Franceschi et al., 2018b).

37 **Centenarians:** people who have reached the extreme limits of human life by successfully adapting
38 to a variety of stressors who were exposed lifelong and remodelling to increase robustness and thus
39 escape, postpone or survive to the major age-related diseases (Franceschi et al., 2000; Franceschi et
40 al., 2017).

41 **Lifespan:** The length of time for which an organism lives (from birth to death).

42 **Healthspan:** The length of time for which an organism lives without diseases.

43 **Immunosenescence:** the progressive decline in the functionality of the immune system that
44 accompany the advancement of age (Fulop et al., 2018).

45 **Inflammaging:** the peculiar, low-grade, chronic, and sterile inflammatory state that characterizes old
46 age and substantially contribute to the progression of the aging process and age-associated diseases
47 (Franceschi et al., 2018a).

48

1. The centenarians as model: why do we study the microbiota of the long lived?

A relatively small portion of the human population shows the capability to reach and cross the extreme limits of the species lifespan, a phenotypical trait that is called longevity (van den Berg et al., 2017). Few other mammals have been studied in this perspective and decades of observations allowed to define longevity as a complex phenomenon resulting from the intricate network of gene-environment (G×E) interactions, as well as other stochastic factors (Giuliani et al., 2018). Such complexity is even intensified by the fact that the human nuclear genome is not the only one residing within the human organism. Indeed, the mitochondrial DNA (mtDNA) and the cumulative genome of our microbial counterpart (microbiome) actively impact on our health along our whole life and during the aging process, consequently affecting our chances to attain longevity. The different genetic components interact among themselves, as well as with the external environment (Goodrich et al., 2017; Giuliani et al., 2018; Han et al., 2019). The microbiota has been described as an entity being “at the interface between the environment and our internal world”, and having a marked ability to adapt itself (i.e. its functionality, as well as its temporary composition in terms of bacterial species) to a changing environment. This huge adaptive potential contributes to the maintenance of an optimal relationship between our body and the external world (Flandroy et al., 2018).

In this scenario, variations in the microbiota have been proposed to be part of a dynamic, adaptive process of the human “superorganism” (i.e. the sum of the human and all microbes that inhabits its body niches, also called “metaorganism”) to both physiological and pathological unavoidable changes that occur lifelong. In other words, the gut microbiota is an element of higher plasticity, with respect to the human genetic asset, that helps in guaranteeing the superorganism adaptation to age-specific demands and thus to health and fitness (Santoro et al., 2020). According to this ecological and evolutionary view, the centenarians, as striking example of individuals who “successfully” get to the extreme limits of human lifespan, surely represent an incredibly valuable study model. Indeed, in this perspective, the centenarians microbiota somehow managed to efficiently and progressively re-

75 establish a mutualistic relationship with the host, along with the occurrence of progressive, age-
76 related internal (physiological and pathological) and environmental (lifestyle and diet among others)
77 variations (Biagi et al., 2017).

78 Long-living people are considered the paradigm of “healthy aging”, being able to escape or survive
79 most of the age-accompanying co-morbidities (Cevenini et al., 2008). Centenarians are indeed
80 characterized by an uncommonly healthy phenotype with low blood pressure, optimal metabolic and
81 endocrine markers, low number of diseases, and very wholesome nutritional and lifestyle habits
82 (Franceschi et al., 2018b). The exceptional phenotype of centenarians has been described as
83 extremely complex, context-dependent and very dynamic, uniquely merging the aspects of adaptive
84 robustness and accumulating frailty, with the centenarian’s metaorganism manifesting the capability
85 to respond/adapt to both internal and external damaging stimuli (Santoro et al., 2018). For these
86 reasons, it has been a natural course for the study of human aging and longevity, to direct the attention
87 towards the microbiota of exceptionally old people, with the aim to provide insights on how the gut
88 microbiota successfully adapts and contribute to the maintenance of health and promotion of survival
89 beyond the human average life expectancy. Given the increasing importance that the scientific
90 community is devoting to the lifelong relationship between human and microbes for our health
91 (Knight et al., 2017), the questions that studies on centenarians’ microbiomes are attempting to
92 answer are the following:

- 93 a) what happens when this lifelong relationship is prolonged beyond the average human life
94 duration?
- 95 b) does it happen in all human population or it depends on lifestyle, geography, ethnicity and so
96 on?
- 97 c) what are the consequences for human physiology?
- 98 d) which is the contribution to longevity?
- 99 e) and, finally, can such knowledge be exploited in promoting healthy aging?

100

101 **2. The state of the art for microbiota and longevity: what has been done (how, where and**
102 **when).**

103

104 In order to answer to the first question (what happens when the microbiome-host relationship is
105 prolonged beyond the average life) researchers had to face the fact that longitudinal studies on human
106 aging and longevity are, up to date, unfeasible: such studies would need a timeframe that is longer
107 than the life of the researchers themselves. Moreover, studies should focus on a cohort impressively
108 large to be sure to “obtain”, at the end, an appreciable number of centenarians. However, centenarians
109 are a model of extreme phenotype, a sort of highly informative “supercontrol” that is supposed to
110 maximise the results when the aim is to study age-related and longevity-related traits, increasing the
111 research power. For this reason, many clinical studies have demonstrated that the study of
112 centenarians themselves, even without the possibility to obtain measurements or samples from their
113 past, provides priceless information about human aging, including the last 20–30 years of human life
114 that are usually neglected (Santoro et al., 2018).

115 Thus, first and foremost, you need to get centenarian’s samples. In particular, since the gut microbiota
116 is the most extensively studied and the one offering the wider expertise and comparative datasets
117 worldwide, you often need to obtain fecal samples.

118 Human life expectancy has increased with an impressive rate during the last couple of centuries,
119 making it ever less exceptional to encounter people counting more than 100 years (Partridge et al.,
120 2018), even if they continue to represent a very small percentage of the total population. Demographic
121 projections estimated to reach 3.7 million centenarians worldwide in 2050, with the higher
122 concentration located in China, followed by Japan, the United States, Italy and India (Department of
123 Economic and Social Affairs, 2015). In fact, the distribution of the longevity population is
124 geographically clustered, defining what is known as longevity regions (also called “blue zones”, i.e.
125 where the percentage of people reaching the age of 100 is several times greater than in other areas).

126 The populations living in the blue zones have been thoroughly studied for what concern their lifestyle,
127 genetics, environment and cultural aspects (Buettner & Skemp, 2016).

128 Thanks to the higher prevalence of centenarians and the availability of metadata, some longevity
129 regions have been chosen to study the relationship between gut microbiota and longevity during the
130 last decade. Indeed, the available studies on the gut microbiota of centenarians have been carried on
131 by enrolling subjects in Italy (Biagi et al., 2010; Biagi et al., 2016; Wu et al., 2019; Rampelli et al.,
132 2020; Wu et al., 2020), China (Wang F et al., 2015a, 2015b; Kong et al., 2016; Wang et al., 2019;
133 Luan et al., 2020), Japan (Odamaki et al., 2016), India (Tuikhar et al., 2019) and, only lately, Russia
134 (Kashtanova et al., 2020) (Fig. 1).

135 The first study published on the microbiota of centenarians was the one from Biagi et al. (2010). The
136 authors chose to compare a group of Italian centenarians, with elderly people (65 years old
137 approximately) and young adults from the same, small geographic area, the Bologna surrounding area
138 in Italy. This area, even if it is not traditionally considered a “blue zone” like Sardinia island (Buettner
139 & Skemp, 2016) showed one of the highest centenarian’s prevalence (i.e. number of centenarians per
140 100,000 inhabitants) in Europe (Istat, 2015, <http://demo.istat.it/>; Biagi et al., 2016). The choice of
141 using elderly and young adults from the same geographic area and belonging to the same ethnicity as
142 control groups was aimed at reducing the lifestyle and environment confounding effects in exploring
143 the association between microbiota features and longevity. Such design has been replicated in many
144 of the following studies on longevity and microbiota (Biagi et al., 2016; Kong et al., 2016; Tuikhar
145 et al., 2019; Wu et al., 2019; Wu et al., 2020), even in full awareness that it is not free of possible
146 biases: for instance, this design does not allow to understand if the peculiarities of the gut microbiota
147 found in long-living individuals were already present at a younger age or, on the contrary, they were
148 (re)acquired later on (Biagi et al., 2017), with considerable impact on the interpretability of the results.

149 The problem is that, when human longevity is concerned, longitudinal studies are far from being
150 feasible, leading the scientific community to currently accept this type of comparative studies as the
151 best possible approximation of gut microbiota trajectory along the human life.

152 The first study of Biagi et al. (2010) was performed using a microarray-based technique, which is
153 hardly comparable with the following studies based on 16S rRNA gene sequencing technologies.
154 However, the study allowed to highlight that centenarians were to be considered a separate group
155 from adults and younger elderly in terms of gut microbiota composition, with their own compositional
156 peculiarities. The same cohort was reanalysed few years later using Illumina sequencing technique
157 based on 16S rRNA gene, which was considered the golden standard for human gut microbiome
158 analysis (Biagi et al., 2016). An even more exceptional group of subjects was added to the cohort, *i.e.*
159 a group of semi-supercentenarians, namely people >105 years old, enrolled from the same restricted
160 geographic area. The elongation of the covered lifespan, and the updated microbiota characterization
161 technique, allowed the Authors to describe a trajectory of the microbiota compositional modifications
162 along with aging and extreme aging. In this work, the Authors reported that exceptional survivors,
163 like centenarians and supercentenarians, showed all the modifications that are known to be associated
164 with aging itself (Biagi et al., 2012, 2013), namely the reduction in the abundance of known health-
165 promoting bacteria belonging to the genera *Faecalibacterium*, *Roseburia*, *Coprococcus*, associated
166 to an increase in the proportion of subdominant species, including putative pro-inflammatory bacteria,
167 such as those belonging to the *Enterobacteriaceae* and *Desulfovibrionaceae* families. At the same
168 time, the gut microbiota of centenarians and, especially, semi-supercentenarians showed some
169 peculiarities that, based on the available literature, might be able to contribute somehow to the
170 maintenance of health during the extreme phases of aging. In fact, the gut microbiota of the extremely
171 old presented higher prevalence of *Bifidobacterium*, a long time known probiotic group of bacteria,
172 as well as higher abundances of subdominant members of the human gut ecosystem that have been
173 explored only recently, namely *Akkermansia* and uncultured members of the family
174 *Christensenellaceae*. *Akkermansia muciniphila* is a mucin-degrading bacterium of the phylum
175 Verrucomicrobia, whose abundance in the human gut has been inversely correlated to several disease
176 states, especially related to the host's metabolic homeostasis (Everard et al., 2013; Greer et al., 2016;
177 Derrien et al., 2017; Geerlings et al., 2018). *Christensenellaceae* are a less known family of the human

178 gut that, like *Akkermansia*, has been associated to a state of metabolic health for the host, for instance
179 being correlated to a lean phenotype and a reduced visceral adipose tissue even if the biological
180 mechanisms of such correlation are yet to be discovered (Oki et al., 2016; Tavella et al., 2021).
181 Curiously, *Christensenella* also resulted one of the mostly heritable taxa in the human gut microbiota
182 (Goodrich et al., 2014, 2017) offering an interesting parallel with the fact that human longevity has a
183 recognized genetic component (Schoenmaker et al., 2006; Sebastiani & Perls, 2012). The mechanism
184 by which such bacteria could have been involved in health preservation during aging is currently
185 under exploration, even if the studies need to rely mostly on animal models. For instance, in a recent
186 work the microbiota of old mice was transferred in young germ-free mice: the microbiota of old mice
187 induced inflammation in the young one, but inflammation levels were negatively correlated with the
188 abundance of *Akkermansia* in the original microbiota (Fransen et al., 2017). The counteraction of
189 systemic inflammation is indeed one of the most hypothesized methods by which the microbiota is
190 supposed to contribute to healthy aging, *i.e.* by dampening the progression of the low-grade chronic
191 inflammatory states that characterizes the elderly, called “inflammaging” (Biagi et al., 2013;
192 Franceschi et al., 2018a). *Akkermansia* and *Christensenella* are indeed listed among the so-called
193 “next generation probiotics”, *i.e.* bacteria other than the traditional *Lactobacillus* and
194 *Bifidobacterium*, which could become part of more modern and targeted probiotic strategies (O’Toole
195 et al., 2017; Chang et al., 2019).

196 Such interesting findings were paralleled by the publication of analogous studies focused on the
197 microbiota of centenarians living in other longevity spots, in particular in rural or isolated areas of
198 China and Japan (Kong et al., 2016; Odamaki et al., 2016). These long living populations have a very
199 different nutritional habits, lifestyle pattern, as well as genetic background, from the Italian
200 population analyzed by Biagi et al. (2010, 2016). In such scenario, an important question arose: does
201 a universal longevity signature exist in the human gut microbiota? Or is it masked by the impact of
202 confounding variables, *i.e.* lifestyle, geography, and genetics, on microbiota composition? The
203 comparison between all the long-living subjects analyzed by the international scientific community

204 is offering interesting answers, which will become even more reliable with the increasing amount of
205 data produced by microbiome studies worldwide. Indeed, next-generation sequencing and
206 bioinformatics, thanks to approaches able to correct for possible study biases, allow for analyzing
207 data coming from different studies and, possibly, for providing new, interesting conclusions.

208 Kong et al. (2016) provided the comparison between the study performed on Italian centenarians and
209 the data obtained from Sichuan province, in China. The microbiota of both Italian and Chinese
210 centenarians showed higher biodiversity with respect to the young controls from the same country
211 and, most interestingly, in both cohorts centenarians showed higher abundance of sequences assigned
212 to *Akkermansia*, and to uncultured bacteria belonging to the families *Christensenellaceae* and
213 *Ruminococcaceae*.

214 The comparison between Italians and Japanese was concomitantly provided by Biagi et al. (2017),
215 using the data published by Biagi et al. (2016) and Odamaki et al. (2016). A common trajectory was
216 indeed found by this analysis, that confirmed that the reduction of *Faecalibacterium* could be a shared
217 aging signature and that the oldest old people seems to be enriched in unclassified members of the
218 *Ruminococcaceae* family, finding in this family a common ground with the signature provided by
219 Kong et al. (2016) in Chinese and Italian centenarians.

220 Later on, a study involving centenarians from a rural area of India was published (Tuikhar et al.,
221 2019) and the results were compared to all those available at the moment: Italian (Biagi et al., 2016),
222 Chinese (Kong et al., 2016) and Japanese centenarians (Odamaki et al., 2016). The Indian study
223 population showed its own peculiarity, with an unprecedented enrichment in *Erysipelotrichaceae*,
224 *Enterobacteriaceae* and *Lactobacillaceae*, traits possibly due to the different lifestyle and nutritional
225 habits. In spite of this distinctiveness, the Authors confirmed that centenarians showed higher gut
226 biodiversity than younger people (as previously reported by Kong et al., 2016, 2019) and highlighted
227 that the internal biodiversity within the family *Ruminococcaceae* was most strikingly higher in
228 centenarians from all considered countries. Moreover, offering a parallel with the observation of
229 Kong et al. (2016) and Biagi et al. (2017), a high abundance of an unclassified *Ruminococcaceae*

230 species, previously reported as putative major butyrate producer, was found being a common
231 longevity signature across four populations that are very different in terms of ethnicity, genetics,
232 lifestyle, diet and culture. As stated by the Authors, this increased biodiversity within such a
233 metabolically relevant family of the human gut ecosystem, might point to a high metabolic plasticity
234 and versatility of the microbiome of long-living individuals.

235 Even more recently, Russian centenarians were taken into account (Kashtanova et al., 2020). They
236 showed more similarity to the Italian centenarians' cohort (Biagi et al., 2016) than to the Japanese
237 centenarians (Odamaki et al., 2016) and were reported to be enriched in *Ruminococcaceae* and
238 *Christensenellaceae* with respect to younger elderly from the same geographic area.

239 In conclusion, looking at all the data and comparisons available to date for the centenarians gut
240 microbiota composition, we could summarize that, even if the microbiota signature of longevity
241 seems to be as context-dependent as the genetics of human longevity (Giuliani et al., 2018), common
242 aspects can be found among populations that are very far from each other in terms of geography,
243 culture and genetics. However, the population living in the longevity spots that have been taken into
244 account so far represent only a minor portion of the human population (Fig. 1). Since ethnicity and
245 geography were reported to strongly impact the gut microbiota composition (Deschasaux et al., 2018;
246 He et al., 2018), long living groups of people from other parts of the world (especially African and
247 American continents) will hopefully be analyzed in future, progressively allowing for the definition
248 of an ever more global microbiota longevity signature.

249 During the last few years, the study of the human microbiome progressively entered the so-called
250 "metagenomics era", in which, beside the composition of the gut ecosystem, the functions performed
251 by the microorganisms are explored, bringing the researchers potentially closer at answering others
252 of the above listed questions about microbiota and longevity: what are the consequences for the
253 human physiology? how can it contribute to longevity? On the long term, when additional
254 metagenomics studies will be available on long-living individuals from different places across the

255 globe, functional common signature of longevity might reveal how bacteria, even if belonging to
256 different species, can contribute with their metabolism to the longevity phenotype.

257 Up to date, only Italian centenarians have been explored in this perspective, from two different
258 cohorts: the previously discussed Bologna cohort (Rampelli et al., 2020) and a cohort from Sardinia
259 island, a well-known blue-zone in the Mediterranean Sea (Wu et al., 2019). Both the Italian cohorts
260 were explored also by 16S sequencing and proved to have similarities in the age-dependent trajectory
261 of gut microbiota composition and in the presence of age-associated, as well as longevity associated,
262 signatures (Wu et al., 2020). The work performed by Wu et al (2019) on the Sardinian elderly and
263 centenarians was mostly focused on variations on metabolic pathways in the gut metagenome. They
264 highlighted that the gut metagenome of centenarians showed potential health-promoting signatures,
265 most importantly an augmented capability for glycolysis and short-chain fatty acids (SCFA)
266 production, the latter being health-promoting fermentation products of gut microbes, able to
267 positively influence immune system homeostasis and inflammatory status of the host (Koh et al.,
268 2016). In the opinion of the Authors, such features of microbial metabolisms might be involved in
269 boosting the chances of the host to maintain and prolong its metabolic and immunological health,
270 ultimately favoring longevity. Indeed, such variation would be balancing other pro-inflammatory
271 traits, such as a shrinkage in the abundance of genes involved in the degradation of carbohydrates,
272 that were still found in centenarians and could be considered “maladaptive” for the extremely aged
273 human host (Wu et al., 2019). The work of Rampelli et al. (2020) on the Bologna cohort, partly
274 confirming the decrease in carbohydrate metabolism in centenarians highlighted by Wu et al (2019),
275 also pointed out that long living individuals showed a gut metagenome enriched in functions related
276 to the degradation of xenobiotics, such as ethylbenzene, chlorobenzene, toluene and other pervasive
277 environmental contaminants. Such molecules are generated during the processing of petroleum
278 products, i.e. plastics, and are known to be more concentrated in indoor environments. The Authors
279 postulates that the reduced mobility of centenarians, which bring them to a mostly indoor lifestyle,
280 together with the long history of exposure to xenobiotic stressors, derived from their long life, might

281 have concurred in progressively selecting for a gut microbiome enriched in bacteria able to degrade
282 such chemicals. This observation offer an interesting parallel with the results reported by Tuikhar et
283 al (2019) from their analysis of gut metabolome of Indian centenarians: the intestinal environment of
284 long living individuals showed indeed a lower load of some environmental chemical contaminants,
285 and the Authors hypothesized that the gut microbiota of centenarians could provide enzymes to
286 degrade such compounds.

287 Metagenomics was very recently used on the gut microbiome of centenarians to explore a different
288 aspect, by changing, for the first time, the study design. Luan and colleagues (2020) proposed the
289 first longitudinal sampling of gut microbiota of centenarians along more than one year, without
290 comparing them with people of different age group, but stratifying them retrospectively based on the
291 date of death. The Authors showed that significant changes in the gut microbiota composition become
292 detectable from 7 months prior to death, with significant decrease of the abundance of several species,
293 including the already associated to healthy aging *Akkermansia muciniphila*. The Authors speculated
294 that such changes might occur before the clinical symptoms of deterioration in the health status of the
295 enrolled long living individuals, pointing at the gut metagenome as a possible alarm bell for the health
296 decline in aged people. It is still hard to place the findings coming from this unique work in a larger
297 picture, but it is undeniable that this change in the approach can pave the way to future studies that
298 tackle the mystery of longevity from a new perspective.

299 Concluding, it is quite clear that, from the functional point of view, many pieces are missing from the
300 puzzle and it is hard to understand what the complete picture will be, i.e. how microbiome is
301 connected to all the other aspects (i.e. genetics and environment) that define and influence the
302 longevity phenotype. In particular, the progresses in computational science will have a strong impact
303 on this aspect of aging sciences, since the more studies from across the world are published, the more
304 variables are thrown on the plate together with the actual data. In this scenario, it is worth reminding
305 that the gut microbiota is only part, even if the most thoroughly explored, of the total human
306 microbiome, and that some other human body ecosystems (such as the skin, and the genito-urinary

307 tract) are now starting to be included in microbiome studies on long-living individuals (Wu et al.,
308 2020). However, our ability to deal with big and complex data is increasing every day with an
309 unprecedented speed, meaning that the day in which such knowledge could be sorted with and finally
310 put into use, i.e. be exploited for actual health maintenance strategies, might not be so far.

311

312

313 **3. Towards exploitation: how this knowledge might become of use?**

314

315 In order to answer to this last, but not least, question (how the knowledge gained in the last decade
316 about the gut microbiota of centenarians can become useful) we need to go back again to why
317 exploring the gut microbiome of long living, successfully-aged people is of particular interest.
318 Microbiome of aged people is featured by traits that strongly correlate with age-associated
319 phenomena, such as immunosenescence, the progressive decline of the immune system functionality,
320 and inflammaging which are both necessary to extend longevity (Fulop et al., 2018). This aspect is
321 thoroughly explored in several review articles published in the last few years (such as Biagi et al.,
322 2012, 2013; O'Toole and Jeffery, 2015; Partridge et al., 2018; Bana and Cabreiro, 2019; Badal et al.,
323 2020; Santoro et al., 2020). Such traits can be briefly, even if not exhaustively, summarized in the
324 reduction of biodiversity of the gut microbiota, a decrease in known health-promoting bacteria able
325 to produce SCFA and positively influence the immunological homeostasis of the host, accompanied
326 by an increase in species able to thrive in an inflamed environment and contribute to the overall
327 inflammation, such as enterobacteria and other LPS-producing groups. Such variations occur in
328 association to the physiological changes of the aging gut, supporting inflammation in turn, in a sort
329 of self-sustaining loop that makes the resulting “age-associated” microbiota structure hardly
330 modifiable (Biagi et al., 2013; DeJong et al., 2020). The hope that drove the research in the field of
331 microbiota and longevity was to find how the gut microbiota can successfully adapt to such

332 compromised situation, and acquire or preserve – hard to tell which one, due to the unfeasibility of
333 longitudinal studies – other, different traits that could sustain healthy aging and promote longevity.
334 The state of the art summarized in the previous paragraph show that this might indeed be the case:
335 the centenarians gut microbiome has peculiarities that could prove to be health promoting and that
336 are not found in younger elderly, the most recurring of which is the presence of a higher biodiversity,
337 regarding the whole gut microbiota (Kong et al., 2016, 2018) or only a bacterial subpopulation, as
338 highlighted by (Tuikhar et al., 2019). The biodiversity of a bacterial ecosystem is strictly related to
339 its resilience, i.e. its resistance against disruptive, disbiotic changes (Sommer et al., 2017) and
340 tendency to return to a previous, balanced structure after a stressing event. Resilience is a relevant
341 concept when we are trying to define health not as merely the absence of disease, but as the capacity
342 of a living being to respond and recover after relevant stresses. Indeed, it has been proposed that
343 successfully aged individuals do not escape physiological decline and age-related diseases, but they
344 are characterized by a higher enough resilience to effectively slow down these processes (Borras et
345 al., 2020). Indeed, the phenotype of old people is very dynamic because it is characterized by a high
346 capacity to respond and adapt to internal and external detrimental stimuli that an individual is exposed
347 lifelong. This phenomenon has been known as “remodelling” (Franceschi et al., 1995).

348 The resilience of the gut bacterial ecosystem and the resilience of the aging human hosts might be
349 interconnected. In other words, the biodiversity of the gut microbiota, by guaranteeing a good enough
350 level of resilience of the gut ecosystem, could be part of the complex process of maintaining the
351 resilience, and the health, of the human being. As a consequence, strategies to maintain health as long
352 as possible for the elderly should surely include means for maintaining elevated levels of biodiversity
353 in the gut microbiota.

354 In this perspective, it has been repeatedly proposed that interventions based on diet and supplements
355 of pro/prebiotics could be useful to maintain microbiota diversity, it is a very general concept and
356 there are still few evidences regarding the pervasiveness of such effects on the long term (Leeming
357 et al., 2019). Indeed, the hope in studying centenarians was to obtain more specific hints regarding

358 aging, such as identifying commensal microbes strongly related to the maintenance of health during
359 aging that could be isolated, studied and reintroduced (DeJong et al., 2020).

360 *Akkermansia* is among those bacteria that were found enriched in centenarians (Biagi et al., 2016;
361 Kong et al., 2016) and its decrease was recently pointed out as a marker for health decline (Luan et
362 al., 2020) in extremely aged people. In addition, several studies on animal models seems to confirm
363 the ability of *Akkermansia* to provide benefits to the host, marking this bacterium as a promising
364 candidate for the so-called next generation probiotics (Gomez-Gallago et al., 2016; Cani & de Vos,
365 2017; Sanders et al., 2019). Taken together, the data available point at *Akkermansia* as an excellent
366 candidate for “aging-specific probiotics” and, surely, studies performed on other species, such as
367 *Christensenella*, will, in due time, add candidates to such list.

368 However, this is not the only possible path towards the development of probiotic strategies. Indeed,
369 instead of focusing on known species and strains that studies reported as associated to the longest
370 living individuals, a possibility is to directly isolate strains from samples taken from healthy
371 centenarians. This approach is not novel: strains of *Bifidobacterium* and *Lactobacillus* have been
372 isolated from centenarians fecal samples by different research group, and they were brought to the
373 public attention as having increased health promoting functionalities of being able to perform specific
374 metabolic functions (Hao et al., 2011; Shen et al., 2011; An et al., 2014; Liu et al., 2014, 2015; Sun
375 et al., 2015; Nicola et al., 2016; Jiang et al., 2019; Zhang et al., 2019; Dong et al., 2020; Huang et al.,
376 2020; Jin et al., 2020). For instance, the strains of *Bifidobacterium* isolated from centenarians by Shen
377 and colleagues (2011) and by Huang et al (2020) have been observed to possess marked antioxidant
378 activity both in vitro and in vivo. Other *Bifidobacterium* strains isolated from centenarians have been
379 studied for their peculiar ability to stimulate immune system (Nicola et al., 2016), for their high acid
380 resistance ability (Sun et al., 2015) or for their bile stress response (An et al., 2014). Such strains
381 could prove to be specifically adapted to the aging host and could be able to provide specific benefits,
382 in framework of their specific inflammatory status, metabolic balance and peculiar lifestyle. The

383 translation of such strains can end up in proposing innovate solutions in the context of targeted
384 prevention and personalization of medical care.

385 On the other hand, currently, the most promising strategies for geroprotection tends to include the
386 possibility of transferring the whole microbiome, instead of trying to modulating it by means of diet
387 or probiotics. Recently, data have been provided showing effectiveness and safety of fecal microbiota
388 transplantation (FMT) in the prevention and treatment of age-related pathological conditions (i.e.
389 atherosclerosis, type 2 diabetes and Parkinson's disease) (Vaiserman et al., 2017). It has been shown
390 that by transplanting the gut microbiota of long-living individual into mouse models improved their
391 general health, reduces aging-related indices and transfers beneficial bacteria (Chen et al., 2020).
392 Studies on mice and other animals have also been performed in order to understand if fecal
393 transplantation might also impact on promoting longevity, by increasing lifespan (Callaway, 2017).
394 For instance, by transplanting wild-type, normal microbiota into mouse model of accelerated aging
395 (progeroid mice) Barcena and colleagues (2019) demonstrated that healthspan and lifespan of the
396 progeroid individuals increased, and that similar results could be obtained also by transferring
397 *Akkermansia* only, a species reported to be associated to longevity and one of the most interesting
398 putative next-generation probiotics. Such interesting results seems to point at a possible future in
399 which fecal transplantation or other innovative microbiome-modulating strategies (e.g. isolation and
400 transplantation of reduced communities from healthy donors, artificial evolution of microbial
401 communities, separation and administration of beneficial and modulatory metabolites from feces, etc)
402 might become used for improving and maintaining health during aging, as well as enlarging human
403 health span and lifespan. However, despite the great therapeutic potential of the FMT procedure, its
404 implementation in clinical practice, especially in aging people, is still limited by several concerns,
405 including donor screening, limited viability of fresh stool samples, fears about potential pathogen
406 transmission, lack of a standardized treatment regimen, and patients not consenting to be treated (Choi
407 & Cho, 2016).

408

409 **4. Conclusions and perspectives**

410

411 The first three paragraphs of this chapter were aimed at summarizing the actual knowledge about the
412 gut microbiota of the oldest people, as well as give a comprehensive view of the reasons for the
413 interests that the scientific community showed for this particular research field and the possible
414 applications of the knowledge that this relatively young research field is gaining.

415 Before concluding, we would like to focus also on possible misinterpretation of the results obtained
416 by the studies presented above, using hypothetical questions.

417 1) The fact that the microbiota of centenarians shows potentially health-promoting peculiarities
418 makes it somehow “younger” than the microbiota of “normal elderly”? The answer is no: the
419 microbiota of centenarians seems to retain all the modifications commonly induced by the age
420 advancement, it is equally compromised by the age-related phenomena as the microbiota of
421 people 70 or 80 years old with comparable health status. Also, the health promoting
422 peculiarities detected by the studies performed up to date could be partially population-
423 specific and be linked to the specific context of the enrolled long-living individuals.

424 2) Is the microbiome of centenarians somehow “better” than the microbiota of people of different
425 age or with poor health status? Again, the answer is no. The gut microbiota of centenarians
426 could be considered “better adapted” to the physiological changes that accompany the aging
427 process. In other words, centenarians’ microbiome might “find alternative solution” (i.e.
428 provide alternative metabolic functions) to obtain an improved performance than that of not-
429 long-lived elderly.

430 3) Would the microbiome of centenarians be “good for everyone”? The answer is another “no”.
431 By transplanting the microbiota of centenarians into young people not only would not provide
432 any benefit, but we would probably damage the recipient. The microbiota found in long lived
433 individuals is specifically adapted to the extreme aging process

434 Regarding this last point, however, the gut microbiota of centenarians might become a good place to
435 look at for operating a sort of “bioprospecting”, i.e. the search for unknown microbial functions in
436 the metagenome. Indeed, centenarians, expanding the average duration of the symbiosis between
437 human and microbiome and providing a sort of “extreme environment” (i.e. extremely old, extremely
438 modified physiology) could allow for the emergence of microbial functions that are not detected in
439 the microbiome of younger people. Such functions could not just be useful to the centenarians but, if
440 thoroughly explored and correctly exploited, they could become relevant for promoting health in
441 different situations.

442 In conclusion, the questions that studies on centenarians’ microbiomes are attempting to tackle (see
443 first paragraph) have not been answered completely, yet. However, many steps forward have been
444 taken and this particular research field can now count on a small body of solid literature (see second
445 paragraph), the peculiarity of which is the common effort put by the different research group into
446 comparing each other results (Kong et al., 2016; Biagi et al., 2017; Tuikhar et al., 2019; Luan et al.,
447 2020). Such attempt is surely going to ensure that more studies in the field are correctly designed to
448 provide pieces of information that are still missing from the puzzle. The exploitation of the data
449 obtained by studies on centenarians microbiome is still in its infancy (see paragraph number three),
450 even if the progressively increasing age of the human population, as well as the public interest into
451 maintaining health as long as possible while people grow old, is prompting the research field to move
452 from simple observation to more focused studies, exploring for instance, the relationship between
453 microbiome variations in healthy centenarians and the type of medications they underwent, the
454 features of the environment in which they lived in the past, as well as in the present, and, most
455 importantly, their dietary habits.

456

457

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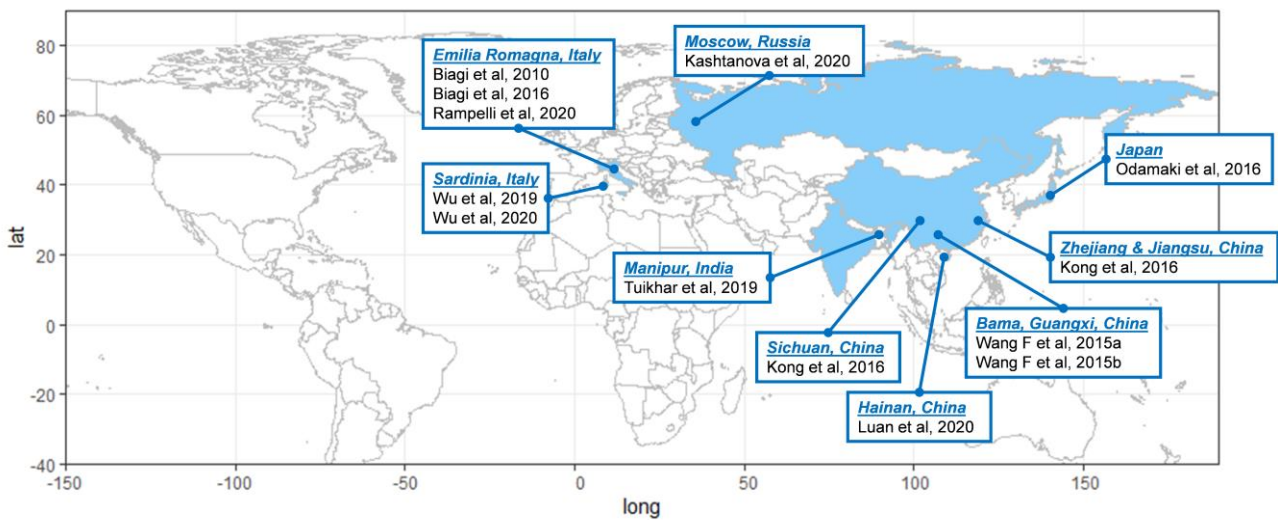
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716

717 Figures

718



719

720 **Figure 1.** Geographical distribution of studies focused on the microbiota features of centenarians
721 around the world. Represented countries are depicted in light blue. References and indication of the
722 geographical region in which centenarians were enrolled are provided. World map has been obtained
723 using the R statistical software (www.r-project.org), and the R packages ggplot2 (Wickham, 2016)
724 and maps (<https://cran.r-project.org/web/packages/maps/index.html>).