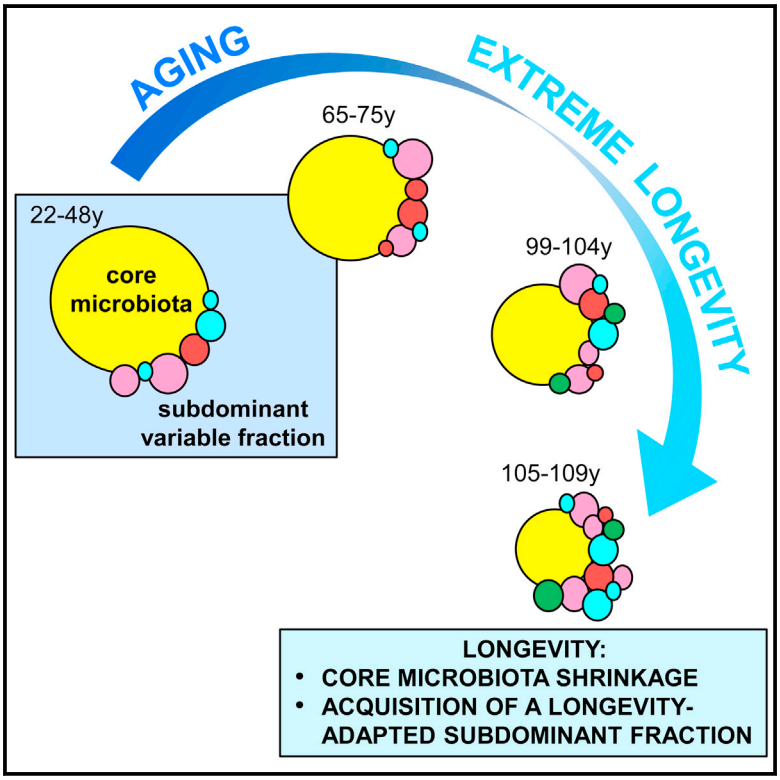
Report

Current Biology

Gut Microbiota and Extreme Longevity

Graphical Abstract



Highlights

- A core microbiota accompanies human life, decreasing in abundance along with aging
- In longevity, the age-related enrichment of subdominant taxa is boosted
- The microbiota of longevous hosts accommodates allochthonous bacteria
- "Longevity adaptation" seems to involve enrichment in health-associated gut bacteria

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In Brief

Biagi et al. reconstructed the longest available human microbiota trajectory by analyzing persons >105 years old, compared to adults, elderly, and centenarians. In longevity, the age-related increase of subdominant species is boosted, accommodating, along with pro-inflammatory species, also health-associated taxa that might support extreme aging.

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Current Biology
Report

Gut Microbiota and Extreme Longevity

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SUMMARY

The study of the extreme limits of human lifespan may allow a better understanding of how human beings can escape, delay, or survive the most frequent age-related causes of morbidity, a peculiarity shown by long-living individuals. Longevity is a complex trait in which genetics, environment, and stochasticity concur to determine the chance to reach 100 or more years of age [1]. Because of its impact on human metabolism and immunology, the gut microbiome has been proposed as a possible determinant of healthy aging [2, 3]. Indeed, the preservation of host-microbes homeostasis can counteract inflammaging [4], intestinal permeability [5], and decline in bone and cognitive health [6, 7]. Aiming at deepening our knowledge on the relationship between the gut microbiota and a long-living host, we provide for the first time the phylogenetic microbiota analysis of semi-supercentenarians, i.e., 105–109 years old, in comparison to adults, elderly, and centenarians, thus reconstructing the longest available human microbiota trajectory along aging. We highlighted the presence of a core microbiota of highly occurring, symbiotic bacterial taxa (mostly belonging to the dominant *Ruminococcaceae*, *Lachnospiraceae*, and *Bacteroidaceae* families), with a cumulative abundance decreasing along with age. Aging is characterized by an increasing abundance of subdominant species, as well as a rearrangement in their co-occurrence network. These features are maintained in longevity and extreme longevity, but peculiarities emerged, especially in semi-supercentenarians, describing changes that, even accommodating opportunistic and allochthonous bacteria, might possibly support health maintenance during aging, such as an enrichment and/or higher prevalence of health-associated groups (e.g., *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae*).

RESULTS AND DISCUSSION

Twenty-four semi-supercentenarians (105+; group S), i.e., 105–109 years old (18 females and 6 males; mean age 106.2), were enrolled for this study in Emilia Romagna and surrounding area, Italy. Fifteen young adults (group Y; eight females and seven males; aged 22–48 years; average age 30.5) were enrolled in the same geographic area. The study protocol was approved by the Ethical Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy) as EM/26/2014/U (with reference to 22/2007/U/Tess). Feces were collected, and total bacterial DNA was extracted from all samples (see the Supplemental Experimental Procedures).

To complete a human aging trajectory, we included extracted fecal DNA, stored at −80°C, from 15 centenarians (group C; 14 females and 1 male; aged 99–104 years; mean age 100.4) and 15 younger elderly (group E; seven females and eight males; aged 65–75 years; mean age 72.5; see also Table S2) enrolled in the same geographic area (Emilia Romagna, Italy), obtained by Biagi et al. [4], in the present study.

For detailed information on physical and cognitive status of the subjects enrolled and a summary of the reported dietary habits, see the Supplemental Experimental Procedures and Tables S1 and S2; in brief, young adults were healthy and medication-free, whereas the physical and cognitive health status of 105+ (as well as that of the centenarians enrolled in the previous study) [4], assessed by ADL (activities of daily living) scale [8] and standardized mini-mental state examination test (SMMSE) [9], mirrored that of the majority of Italian centenarians, as previously characterized by Franceschi et al. [10].

The fecal microbiota of the 69 samples was characterized by Illumina sequencing of the V3–V4 region of the bacterial 16S rRNA gene (see the Supplemental Experimental Procedures; sequences are available at the following MG-Rast link: http://metagenomics.anl.gov/linkin.cgi?project=17761). A total of 1,246,682 high-quality reads were obtained with a mean of 18,068 reads per subject. Reads were clustered in 11,587 operational taxonomic units at 97% of identity.

The four age groups showed a good separation on a principal coordinates analysis (PCoA) based on the unweighted UniFrac distance (Figure 1); indeed, corrected p values obtained by permutation test were <0.05 for all possible comparisons with the exception of groups C versus S. PCo1 separated young subjects

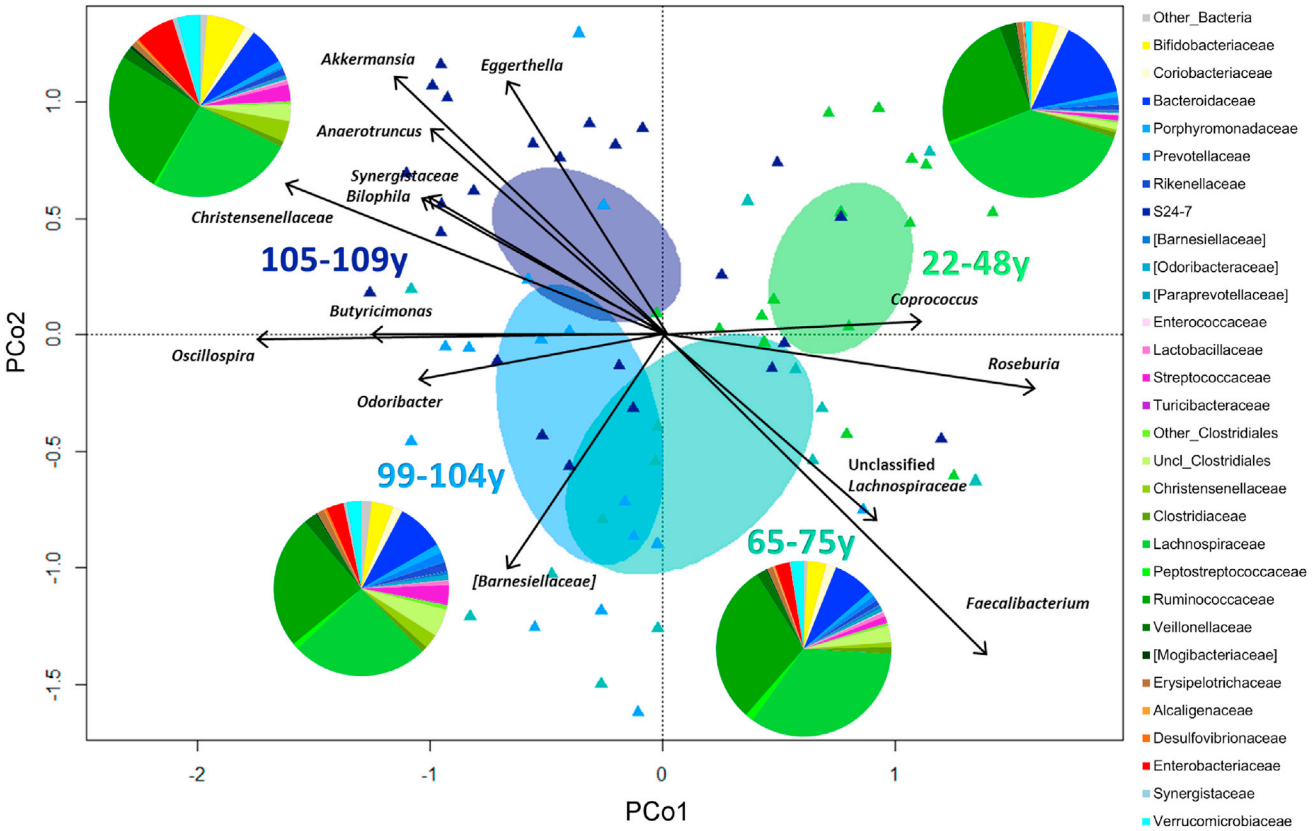

Figure 1. Gut Microbiota Variations across Different Age Groups

PCoA based on unweighted UniFrac distances of the fecal microbiota of the enrolled young adults (green), elderly (turquoise), centenarians (light blue), and semi-supercentenarians (dark blue). SEM-based ellipse around the centroid is plotted. Samples are identified by filled triangles. The first and second principal components (PCo1 and PCo2) are shown, explaining 6.6% and 4.0% of the variance in the dataset, respectively. For each group of subjects, a pie chart based on the average relative abundance at family level is shown; colors for each family are reported in the legend. The biplot of the average bacterial coordinates weighted by the corresponding bacterial abundance per sample was superimposed on the PCoA plot to identify the bacterial genera or families contributing to the ordination space (black arrows). Only the bacterial groups showing a highly significant correlation with the sample separation ($p < 0.005$) were considered. See also Figure S1 and Table S1.

(Y) from elderly (E) and long-living individuals (groups C and S; Pearson's $r = -0.61$; $p < 0.001$). As noticeable in the pie charts in Figure 1, the fecal microbiota in all age groups was dominated by just three families: *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, but their cumulative relative abundance decreased along with aging (77.8% ± 8.5% in Y; 71.1% ± 12.3% in E; 58.7% ± 11.8% in C; 57.7% ± 15.0% in S), highlighting an age-dependent increasing contribution of subdominant families. Seventy-year-old people (group E) showed similarities with young adults, such as the cumulative abundance of *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, but started to show also some of the age-associated features observed in centenarians, as demonstrated by the partial overlapping of the samples of the two groups in the PCoA. Centenarians and 105+ showed very similar relative abundance of *Bacteroidaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, as well as overlapping coordinate values on PCo1 (average PCo1 coordinates −0.36 and −0.33 for group C and S, respectively), but they significantly separated on PCo2 (average PCo2 coordinates −0.38 and 0.38 for groups C and S, respectively; pseudo-F-ratio permutational test; $p < 0.05$), hinting that differences were present between the microbiota structures of these two groups even if the age gap was very small, i.e., 6 years only in average.

To identify the bacterial genera or families with the most significant contribution (permutational correlation test; $p < 0.005$) to the sample ordination, we superimposed the genus/family abundance on the PCoA plot, identifying spatial correlations between samples and bacterial groups (Figure 1). The bacterial genera or families plotted in Figure 1 showed an interesting age-related ascending or descending trajectory (Figure 2). In particular, the abundance of *Coprococcus*, *Roseburia*, and *Faecalibacterium*, belonging to the *Lachnospiraceae* and *Ruminococcaceae* families, was negatively associated with age. The trend observed for *Coprococcus* and *Faecalibacterium* was already reported in Chinese centenarians [11], suggesting that they can be part of the aging process itself, regardless of lifestyle and dietary habits. On the contrary, *Oscillospira* was positively correlated with age, as well as two subdominant members of the Bacteroidales order (*Odoribacter* and *Butyricimonas*). Interestingly, a group of subdominant genera and families (*Eggerthella*, *Akkermansia*, *Anaerotruncus*, *Synergistaceae*, *Bilophila*, and *Christensenellaceae*) described a steeper, increasing trajectory along with aging

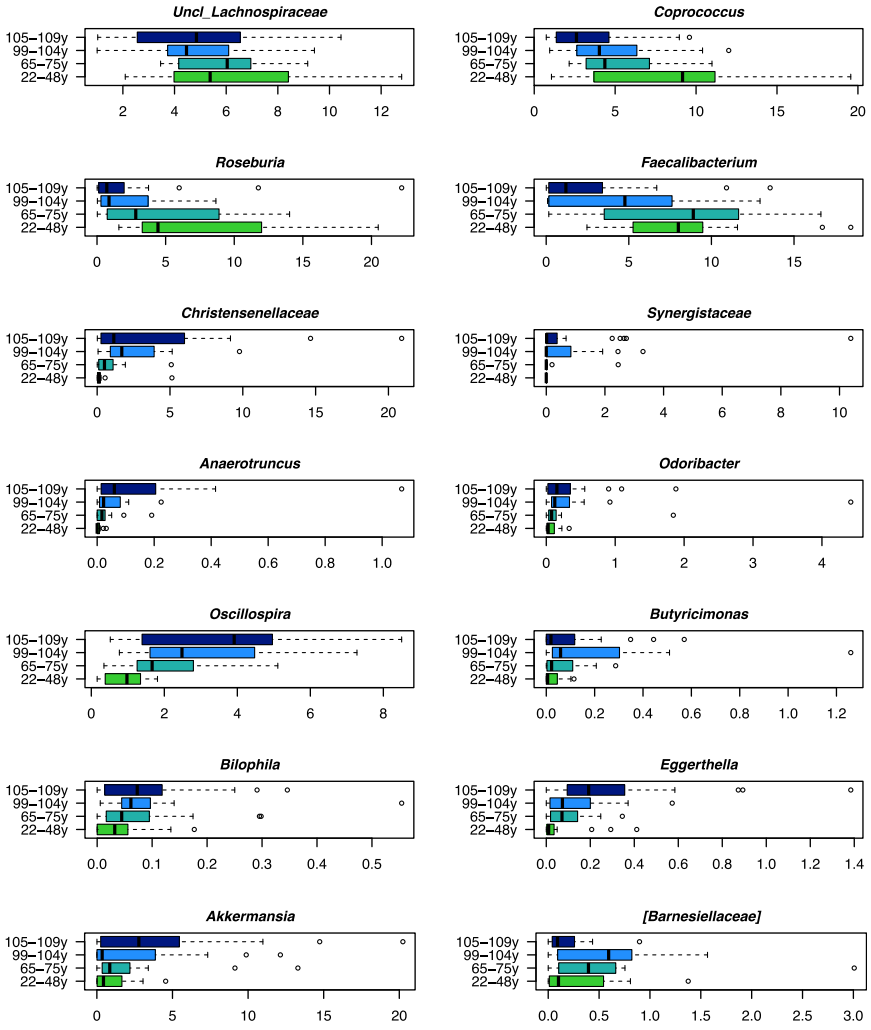

Figure 2. Aging-Related Trajectories of Selected Gut Microbiota Genera and Families

Box-and-whisker plot of the relative abundance distributions of the bacterial genera and families identified in the biplot in Figure 1. The distribution for each age group is shown (young adults, green; elderly, turquoise; centenarians, light blue; semi-supercentenarians, dark blue). Bacterial abundance is given as percentage of total sequences obtained for each sample. The central box of each dataset represents the distance between the 25th and the 75th percentiles. The median between them is marked with a black line. Whiskers identify the 10th and the 90th percentiles.

with a more prominent increase in the 105+ group, being responsible for the separation of the S group in the top-left portion of the plot (Figures 1 and 2).

In order to explore the evolution of the gut microbiota network along with human aging, we performed an analysis of co-occurrence, meaning the frequency of concomitant detection of two bacterial groups. Co-occurrence associations between genera were obtained as detailed in the Supplemental Experimental Procedures, and genera were clustered into four co-occurrence groups (COGs) ($p < 0.01$; permutational multivariate ANOVA; see also Figure S1) according to the co-occurrence pattern. Co-occurrence network plots for all samples together (Figure 3A) and for the four age groups (Figures 3B–3E) were obtained, using the prevalence of bacterial genera in the microbiota of all samples or each age group (i.e., the percentage of subjects in each group in which a genus was present) as factor proportional to the dimension of the spots. Plotted genera showed relative abundance >0.5% in at least 30% of subjects in the considered group. The four COGs were named *Bacteroides* (yellow), *Roseburia* (pink), *Lachnospira* (red), and *Dialister* (cyan) COG. The *Bacteroides* COG defined a sort of core microbiome including highly co-occurring genera, almost always present, with high prevalence, in all age groups. These genera together represented the majority of the intestinal ecosystem in terms of relative abundance, accounting for 68.6% in average in all samples, but with a coverage decreasing along with age: 75.3% in group Y; 70.9% in group E; 65.7% in group C; and 64.9% in group S (see also Figure S1). Genera in this COG did not show sensible variations in prevalence across age groups, with the exception of *Faecalibacterium*, for which a marked decrease in prevalence was found (100% in Y and E, 93% in C, and 79% in S), and *Bifidobacterium* that showed a decreasing trend in E and C (80% and 87%, respectively) compared to Y (93%) to go up again in S (92%). On the contrary, aging involved a reassembly of the other three COGs, which emerged as ancillary, mutually exclusive groups of genera poorly co-occurring among themselves. The *Dialister* COG was the most variable in terms of present genera and co-occurrence network in the different age groups: in group Y, only three genera were present; in group E, more genera appeared; whereas in group C, some of those disappeared, leaving space for unclassified members of the *Mogibacteriaceae* family. Semi-supercentenarians' co-occurrence and prevalence network shared some features with the centenarians' one (presence of unclassified *Christensenellaceae* in the *Roseburia* COG; presence of unclassified *Enterobacteriaceae* in the *Lachnospira* COG) but showed also some peculiarities, such as the high prevalence of *Akkermansia* in the *Roseburia* COG and *Phascolarctobacterium* in the *Lachnospira* COG and the mono-genus arrangement of the *Dialister* COG, in which only the unclassified *Mogibacteriaceae* are present with a very high prevalence (92%).

According to these observations, extreme longevity seems to involve an invasion of the gut ecosystem by micro-organisms typical from other niches, such as *Mogibacteriaceae* and *Synergistaceae*, known to be abundant in the periodontal environment [12–14]. However, extremely long-living people seem to experience a parallel increase in several health-associated taxa. In particular, the family *Christensenellaceae*, which increased in

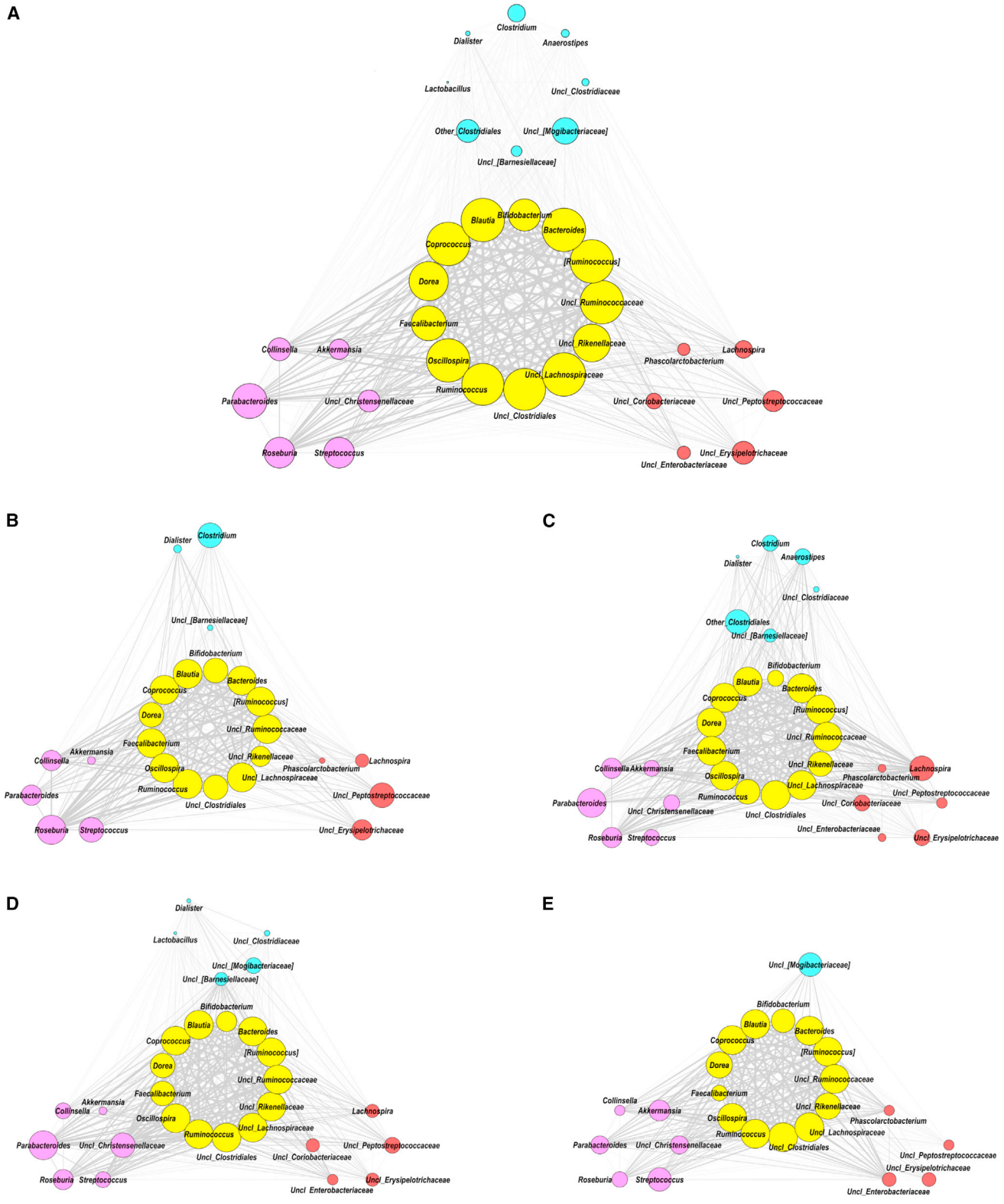

Figure 3. Co-occurrence Network and Prevalence of Genera among Age Groups

Network plots describing co-occurrence and prevalence of bacterial genera in the gut microbiota of all samples (A), young adults (B), elderly (C), centenarians (D), and semi-supercentenarians (E). Bacterial genera with at least 0.5% of relative abundance in at least 30% of the samples in each group were plotted, with the

(legend continued on next page)

terms of both relative abundance and prevalence in centenarians and 105+, is a recently reported health-associated bacterial taxon that has been inversely correlated to BMI [15] and positively associated to improved renal function [16]. Together with *Akkermansia* and *Bifidobacterium*, well-known health-associated genera whose abundance and/or prevalence interestingly increased in semi-supercentenarians, known to promote immunomodulation, protect against inflammation, and promote a healthy metabolic homeostasis [17, 18], *Christensenellaceae* might represent a signature of the ecosystem of extremely longevous people. Moreover, the family *Christensenellaceae* has recently emerged as the gut microbiota component whose abundance is the most significantly influenced by host genetics [15], suggesting an interesting possible link to the heritable component of human longevity [19].

In conclusion, we presented the longest available trajectory of the human gut microbiota along aging, with a focus on longevity and extreme longevity, represented by a group of 105+, a demographically very selected group of subjects, as the ratio between centenarians and 105+ is 21.7 (one 105+ every 21 100+ subjects). Confirming the known features of an aging microbiota, we highlighted the presence of a core microbiota of highly occurring, symbiotic bacterial groups, which remains approximately constant during aging but varies in the cumulative relative abundance of its members. The aging-associated microbiota is characterized by an increasing contribution of subdominant species, as well as a rearrangement in their co-occurrence network. These features are maintained in longevity and extreme longevity, but peculiarities emerged, especially in semi-supercentenarians. The microbial ecosystem found in extremely old people, even accommodating opportunistic and allochthonous bacteria, is enriched in health-associated *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae*. It is not possible to know whether these health-associated features were already present at a younger age in these exceptional individuals, and/or they are somewhat related to the past lifestyle, due to the cross-sectional nature of the study; indeed, only longitudinal studies, which would be very difficult to apply to the field of human longevity, could explain whether these gut bacteria are always lost during aging and reacquired by the subjects who get to live longer or whether they are maintained across aging and longevity only by long-living subjects. However, it is tempting to hypothesize that these particular bacterial taxa might be involved in the establishment of a new homeostasis with the aging host, thus contributing to reach the extreme limits of human life.

ACCESSION NUMBERS

The accession number for the sequences reported in this paper is MG-Rast: 17761.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, one figure, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.04.016.

AUTHOR CONTRIBUTIONS

Conceptualization, C.F., P.B., and M. Candela; Investigation, E.B., C.C., and S.Q.; Formal Analysis, E.B., S.R., and M. Severgnini; Writing – Original Draft, E.B., S.R., and M. Candela; Writing – Review & Editing, E.B., P.B., and S.T.; Resources and Data Curation, R.O. and M. Scurti; Funding Acquisition, C.F., D.M., M. Capri, and P.B. All authors discussed the results and commented on the manuscript.

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exception of the network involving all samples (A) for which the genera present in at least one of the other networks were plotted. Co-occurrence groups (COGs) are named after the included genera with the highest relative abundance and are color coded as follows: *Bacteroides* COG (yellow); *Roseburia* COG (pink); *Lachnospira* COG (red); and *Dialister* COG (cyan). Circle size represented the prevalence, i.e., the percentage of subjects in each group in which a genus was present at 0.1% of relative abundance. The thickness of connection between nodes represented the co-occurrence. See also Figure S1.

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Supplemental Information

Gut Microbiota and Extreme Longevity

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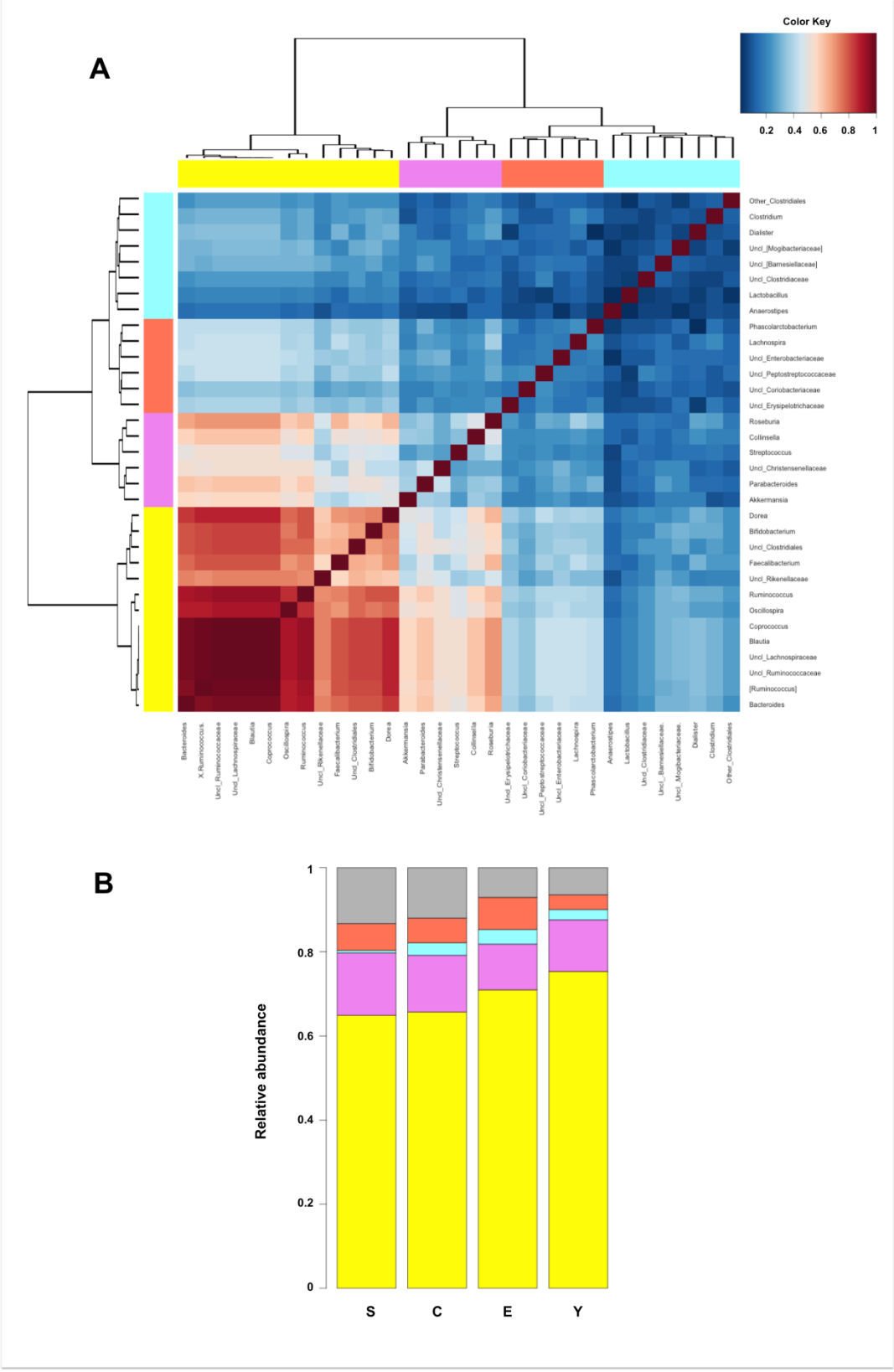

<u>Figure S1</u>. Related to Figure 1.

Assignments of co-occurrence groups (COGs) of bacterial genera (A) and average cumulative abundance of the genera in each COG for the four age groups (B). COG assignment relied on heat plot (A) showing co-occurrence between genera, clustered by the Spearman correlation coefficient and Ward-linkage hierarchical clustering. Colors are indicative of the four identified COGs: *Bacteroides* COG (yellow), *Roseburia* COG (pink), *Lachnospira* COG (red), and *Dialister* COG (cyan). Permutational MANOVA was performed to determine if the COGs were significantly different from each other. All four COGs displayed significantly different inter-relationships from each other (yellow vs pink $P<0.0001$, yellow vs red $P=0.01$, yellow vs cyan $P<0.0001$, pink vs red $P=0.008$, pink vs cyan $P<0.0001$, red vs cyan $P=0.01$). The average, cumulative relative abundance in the microbiota of the subjects belonging to each age group (Y, young adults; E, elderly; C, centenarians; S, semi-supercentenarians) is also plotted (B). In grey, the portion of genera not considered because of the filtering in the COG assignment is highlighted.

Table S1. **Related to Figure 1. Summary of the samples included in the present study.**

Subject	Group	Sex	Age	Enrollment	Geographical region
S010	S	F	107	this study	Emilia Romagna and surrounding
S020	S	F	105	this study	Emilia Romagna and surrounding
S030	S	M	105	this study	Emilia Romagna and surrounding
S050	S	F	109	this study	Emilia Romagna and surrounding
S080	S	F	109	this study	Emilia Romagna and surrounding
S100	S	F	107	this study	Emilia Romagna and surrounding
S110	S	M	105	this study	Emilia Romagna and surrounding
S120	S	F	107	this study	Emilia Romagna and surrounding
S130	S	F	106	this study	Emilia Romagna and surrounding
S140	S	M	106	this study	Emilia Romagna and surrounding
S150	S	M	105	this study	Emilia Romagna and surrounding
S180	S	M	106	this study	Emilia Romagna and surrounding
S190	S	F	105	this study	Emilia Romagna and surrounding
S200	S	F	105	this study	Emilia Romagna and surrounding
S210	S	F	107	this study	Emilia Romagna and surrounding
S220	S	F	108	this study	Emilia Romagna and surrounding
S230	S	F	107	this study	Emilia Romagna and surrounding
S240	S	M	105	this study	Emilia Romagna and surrounding
S260	S	F	105	this study	Emilia Romagna and surrounding
S280	S	F	105	this study	Emilia Romagna and surrounding
S290	S	F	104	this study	Emilia Romagna and surrounding
S300	S	F	106	this study	Emilia Romagna and surrounding
S320	S	F	105	this study	Emilia Romagna and surrounding
S330	S	F	109	this study	Emilia Romagna and surrounding
C018	C	F	100	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C017	C	F	101	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C011	C	F	99	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C004	C	F	99	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C024	C	F	100	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C010	C	F	101	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C012	C	F	101	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C052	C	M	104	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C054	C	F	100	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C021	C	F	101	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C003	C	F	99	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C048	C	F	99	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C007	C	F	100	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C042	C	F	102	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
C002	C	F	100	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K300	E	F	75	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K304	E	M	76	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K113	E	M	65	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K306	E	F	76	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K125	E	F	70	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K303	E	F	76	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K309	E	F	70	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K100	E	M	75	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K106	E	M	70	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K301	E	F	70	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K105	E	M	76	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K124	E	F	71	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K108	E	M	76	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K316	E	M	75	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
K119	E	M	67	Biagi et al, 2010 [5]	Emilia Romagna and surrounding
Y1	Y	M	37	this study	Emilia Romagna and surrounding
Y2	Y	M	48	this study	Emilia Romagna and surrounding
Y3	Y	F	25	this study	Emilia Romagna and surrounding
Y4	Y	F	32	this study	Emilia Romagna and surrounding
Y5	Y	F	30	this study	Emilia Romagna and surrounding
Y6	Y	M	32	this study	Emilia Romagna and surrounding
Y7	Y	F	46	this study	Emilia Romagna and surrounding
Y8	Y	M	26	this study	Emilia Romagna and surrounding
Y9	Y	F	24	this study	Emilia Romagna and surrounding
Y10	Y	F	25	this study	Emilia Romagna and surrounding
Y11	Y	M	24	this study	Emilia Romagna and surrounding
Y12	Y	F	22	this study	Emilia Romagna and surrounding
Y13	Y	M	26	this study	Emilia Romagna and surrounding
Y14	Y	M	33	this study	Emilia Romagna and surrounding
Y15	Y	F	28	this study	Emilia Romagna and surrounding

Table S2. Main anagraphical, anthropometric, health-related and dietary characteristics of enrolled semi-supercentenarians (group S).

			Health status						Diet information (collected where possible) (3)													
Subject	Sex	Age	Past cancer (age)	Type of cancer (still present)	Dementia (age)	BMI (1)	Smoke	Eating ability (help) (2)	Meals per day	Whole cereals	Fruits	Vegetables	Beans	Olive oil	Fish	Red meat	Poultry	Eggs	Cheese	Milk/ Yoghurt/ Soft cheese	Wine	Mediterranean diet score (4)
S010	F	107	no		no	30.2	no	no or little	3	n	s	d	n	s	s	s	n	s	s	d	n	5
S020	F	105	no		yes (102)		no	yes														
S030	M	105	no		no		ex	yes	4	n	n	s	n	d	n	s	n	s	d	d	s	3
S050	F	109	no		yes (106)	20.0	no	yes														
S080	F	109	no		no		no	no or little	3	n	s	d	n	s	n	s	s	s	s	d	n	4
S100	F	107	no		yes (102)		no	no or little	3	n	s	d	s	d	s	s	s	n	s	d	d	7
S110	M	105	no		no	16.3	ex	yes														
S120	F	107	no		no		ex	no or little	4	n	d	s	s	d	n	n	s	n	d	s	s	6
S130	F	106	yes (106)	breast (yes)	yes (103)		no	yes	3	n	d	s	n	d	n	s	s	s	d	d	n	5
S140	M	106	yes (84)	prostate (yes)	no	20.8	no	no or little														
S150	M	105	no		no	21.1	ex	no or little	3	n	d	d	n	d	s	n	s	n	d	d	n	7
S180	M	106	no		no	25.0	ex	no or little														
S190	F	105	no		no		no	no or little														
S200	F	105	no		yes (96)		no	yes														
S210	F	107	no		no		no	no or little	3	n	n	n	n	d	n	s	n	n	d	d	n	2
S220	F	108	no		yes (NA)	16.4	no	yes														
S230	F	107	no		no		no	no or little	4	n	d	d	n	d	n	s	s	n	s	d	d	6
S240	M	105	no		no	28.3	ex	no or little	4	s	d	d	n	d	s	s	s	s	d	d	d	8
S260	F	105	no		yes (103)	20.8	no	yes														
S280	F	105	yes (90)	epithelioma (yes)	yes (104)		no	no or little	4	n	d	d	n	d	n	s	s	n	n	d	n	6
S290	F	104	yes (52)	breast (no)	no	26.0	no	no or little	4	n	d	d	s	d	n	d	s	n	s	d	n	7
S300	F	106	yes (103)	colon (no)	yes (105)	24.1	no	no or little	4	n	d	d	n	d	s	s	s	n	d	n	n	7
S320	F	105	no		no	28.2	no	no or little	6	n	d	d	n	d	n	s	s	n	d	d	d	6
S330	F	109	no		no	24.4	no	no or little	4	s	d	d	s	d	s	n	s	n	d	d	d	9

(1) height and weight were measured if possible.

(2) "no or little help" identifies subjects who could need help in cutting or spreading food.

(3) Food consumption code: n, never; d, daily; s, sometimes.

(4) Mediterranean diet score was calculated by adding 1 for "sometimes" consumption, and 2 for "daily" consumption, of whole cereals, fruits, vegetables, beans, olive oil and fish.

Supplemental Experimental Procedures

Subjects and study groups. Twenty-four semi-supercentenarians (105+), i.e. 105-109 years old (18 females, 6 males, mean age 106.2), defined "group S", were enrolled for this study in Emilia Romagna and surrounding area, Italy. Fifteen young adults (group Y, 8 females, 7 males, aged 22 to 48 years, average age 30.5) were enrolled in the same geographic area. The study protocol was approved by the Ethical Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy) as EM/26/2014/U (with reference to 22/2007/U/Tess). To complete a human ageing trajectory, extracted fecal DNA, stored at -80°C, from 15 centenarians (group C, 14 females, 1 male, aged 99 to 104 years, mean age 100.4) and 15 younger elderly (group E, 7 females, 8 males, aged 65 to 75 years, mean age 72.5), all enrolled in Emilia Romagna, (Table S1) obtained during the study published in Biagi et al. [S1], was included. The reason for the choice of this geographic region lies in the high prevalence of long-living people in Italy, and in the surrounding area of Bologna city in particular, that is one of the highest in Europe. In Italy, the prevalence of 100+ subjects (i.e. number of centenarians per 100,000 inhabitants) is 31.4 (Istat, 2015, January 1st, i.e. 1:3,184; total number of centenarians, 19,094, http://demo.istat.it/). In Emilia Romagna, the prevalence is 31.9, but looking at the city of Bologna (the most important city of the region, in the surrounding area of which most of the 100+ and 105+ subjects involved in the study were enrolled) the prevalence rises to 64.0. The comparison with other countries is made difficult by the fact that national statistics are differently updated; however, for instance, in France 100+ have a prevalence of 32.1 (Insee-Institut National de la statistique et des ètudes èconomiques survey on 2016, January 1st, Population totale par sexe et âge au 1er janvier 2016, http://www.insee.fr/fr/themes/detail.asp?ref_id=bilan-demo&reg_id=0&page=donnees-detaillees/bilan-demo/pop_age2.htm); in Spain, 100+ had a prevalence of 23.6 in 2012 (Istituto National de Estadìstica, http://www.ine.es/jaxi/Datos.htm?path=/t20/e245/p04/a2012/l0/&file=0ccaa003.px); in UK, 100+ had a prevalence of 22.4 in 2014 (Office for National Statistics, https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/ageing/bulletins/estimatesoftheveryoldincludingcentenarians/2015-09-30); in Belgium, the prevalence of 100+ was 16.9 in 2015 (Statbel, Retrieved 27 Dec. 2015. http://statbel.fgov.be/fr/statistiques/chiffres/population/structure/agesexe/centenaires/); in Germany, 100+ had a prevalence of 16.4 in 2011 (https://www.destatis.de/DE/Methoden/Zensus_/Downloads/2F_BevoelkerungAlterGeschlecht.html).
After obtaining written informed consent, a standard questionnaire to collect information regarding the health status, dietary habits, drugs use, clinical anamnesis, and lifestyle was administrated. Subjects in group Y were non-institutionalized and living in their own household. They all showed good physical and cognitive health conditions. The physical and cognitive health status of 105+ in group S was assessed by ADL-Activities of Daily Living scale [S2] and Standardized Mini-Mental State Examination test (SMMSE) [S3] (Table S2). Of the 24 subjects, 7 resided in a nursing home; 19 were severely disabled, but only 7 were confined to bed. Only 5 subjects had been affected by cancer in the past (breast, prostate, colorectal, epithelioma), 3 of which were affected by cancer at the moment of the interview (breast, prostate, epithelioma). Sixteen 105+ needed no or very little assistance in eating. A 3-day dietary recall was possible for 15 of the 24 subjects in group S, and for all subjects in group Y. All subjects in group Y followed an approximate Mediterranean diet. Of the 15 105+ for which a dietary recall was available, 5 followed a semisolid diet (soft food), 13 consumed daily or frequently fruits, vegetables, olive oil, dairy products, red meat or poultry, while only few of them consumed frequently whole meal cereals (2 out of 15), legumes (4 out of 15) and fish (6 out of 15). Six out of 15 subjects had a moderate daily intake of wine (Table S2). Subjects in group Y were medication-free, whereas 105+ were assuming a variety of medications as it is common for very old people.

DNA extraction from feces. Feces were collected from each subject and stored at -80°C. DNA extractions were performed within 3 months. Total microbial DNA was extracted from feces of subjects in groups S and Y as previously reported by Biagi et al. [S1], using QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) introducing three 1-min FastPrep (MP Biomedicals, Irvine, CA) bead-beating steps at 5m/s speed, with 5-min incubations in ice between treatments [S4]. DNA recovery was evaluated using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

16S rDNA sequencing. The V3-V4 region of the 16S rRNA gene was PCR amplified in 50-µL volumes containing 25 ng of microbial DNA, 2X KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Resnova, Rome, Italy), and 200 nmol/L of S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 primers [S5] carrying Illumina overhang adapter sequences. Thermal cycle consisted of 3 min at 95°C, 25 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, and a final 5-min step at 72°C. The 460 bp amplicons were purified with a magnetic bead-based clean-up system (Agencourt AMPure XP; Beckman Coulter, Brea, CA) and sequenced on Illumina MiSeq platform using a 2×300 bp paired end protocol, according to the manufacturer's instructions (Illumina, San Diego, CA). Indexed libraries were pooled at equimolar concentrations, denatured and diluted to 6 pmol/L before loading onto the MiSeq flow cell.

Bioinformatics and statistics. Raw sequences were processed using a pipeline combining PANDAseq [S6] and QIIME [S7]. High-quality reads were binned into operational taxonomic units (OTUs) at a 0.97 similarity threshold using UCLUST [S8] and a "de novo" approach. Taxonomy was assigned using the RDP classifier against Greengenes database (May 2013 release). All singleton OTUs were removed in an attempt to discard the majority of chimera sequences. Alpha rarefaction was analyzed by

using the Faith's phylogenetic diversity, observed species, and Shannon index metrics. Beta diversity was estimated by computing weighted and unweighted UniFrac distances. Unweighted UniFrac distances were used for Principal Coordinates Analysis (PCoA) and plotted by the vegan package of R (R version 3.1.3). Data separation in the PCoA was tested using a permutation test with pseudo F ratios (function adonis in the vegan package). Bacterial groups with the largest contribution to the ordination space were found by using the function envfit of the R package vegan on the genus relative abundances and the unweighted UniFrac ordination.

Co-occurrence network analysis. The analysis was carried out using R (packages stats, made4 and vegan) and Cytoscape software [S9]. Bacterial genera were selected considering only those present at >0.5% of abundance in at least 30% of the samples in each group. The co-occurrence between each pair of genera was evaluated as percentage of subjects that showed the two specific genera at more than 0.5% of relative abundance, and displayed with hierarchical Ward-linkage clustering based on the Spearman correlation coefficients. The results obtained when we considered the entire pool of samples were used to define the co-occurrence groups (COGs). Permutational multivariate analysis of variance [S10] was used to determine whether COGs were significantly different from each other. Co-occurrence network plots were created as previously described [S11]. Circle size represented the prevalence, i.e. the percentage of subjects in each group in which a genus was present at >0.1% of relative abundance. The thickness of connection between nodes represented the co-occurrence.

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