

The Elderly Intestinal Microbiota: Opportunities for Probiotics

Forssten SD^{1*} and Ibrahim F²

¹Health & Nutrition, Danisco Bioactives, Kantvik, Finland

²Functional Foods Forum, University of Turku, Turku, Finland

Abstract

The composition and activity of the intestinal microbiota appears to change upon ageing as a result of changes in gut physiology, diet, as well as immunological changes. The elucidation of the age-related changes in the composition of the intestinal microbiota is essential for finding proper dietary interventions for maintaining health and prophylaxis of diseases in elderly. Therefore, we reviewed the changes in the intestinal microbiota in elderly compared to adults and the potential use of probiotics to restore the microbial balance in elderly. Studies regarding the changes in the elderly microbiota compared to adults so far are limited and have showed inconsistent findings. The inconsistency could be attributed in part by the use of conventional culture techniques vs. molecular methods for the identification of fecal microbiota, but there still exists some inconsistency even within studies that applied only molecular methods. However, there is some evidence that a decrease in bifidobacterial diversity accompanied with an increase in bacteroides diversity is a consequence of old age. Although, there is still a great deal of research to be conducted to systematically uncover the changes of elderly microbiota and the factors driving this change, the research exploring the opportunities of probiotics in elderly disease is already ongoing, and the recent work in this area will be reviewed.

Keywords: Elderly; Microbiota; Probiotics; Bifidobacteria; Bacteroides

Introduction

Demographic studies have shown that an increasing fraction of human population will be over 65 years of age, and EU has projected that the share of people aged ≥ 65 y in the total population will increase from 17% to 30% in 2060. Likewise, the number of people aged ≥ 80 y is estimated to almost triple from 21.8 million in 2008 to 61.4 million in 2060. There is also a constant increase in the percentage of the elderly population globally with different rates in various communities [1]. Although seniors can remain healthy for a large part of their life, ageing unavoidably leads sooner or later to a reduced performance of various physiological functions. Commonly suggested interventions to combat the deleterious effects of ageing are specific vaccination against viral disease, administration of anti-inflammatory and immunostimulatory drugs, nutrition interventions, and exercise. Pre- and probiotic have been introduced as potential tools to restore the immune functions in the elderly [2]. Probiotics provide an opportunity to modulate the functionality of the intestinal microbiota for the health benefit of the host. Therefore, this review will focus on age-related changes in the intestinal microbiota and the potential use of probiotics to restore the microbial balance in elderly.

Functional intestinal microbiota

The gastrointestinal tract serves as one of the biggest interfaces for interaction between the host and the external environment, and it has a complex community of microbes that vastly outnumber the host cells [3]. Over 1000 species can be identified within the intestinal microbiota [4] and the colonic population may attain levels as high as 10^{12} /g [5].

Although people without a gut microbiota can survive [6], a functional intestinal microbiota affects immunological and pathological processes and hence the health of the host is influenced. The microbiota is responsible for functions such as fermentation of undigested substrates, preventing growth or colonization of harmful species and training the immune system. In addition, syntheses of some vitamins and hormone production are also mediated by the microbiota [7]. The knowledge and understanding of the composition of the

intestinal microbiota is still limited but growing with the evolvement of molecular, culture-independent techniques [8].

Intestinal microbiota in elderly

In elderly, the composition of the intestinal microbiota was considered to be relatively stable [9], but it is now known that the composition changes with decreased species diversity and possibly reduced levels of beneficial bacteria [10,11]. A human individual will to some extent have a fixed microbiota, but changes in nutrient availability along the gastrointestinal tract are reflected in regional differences in bacterial concentrations and community. The individuality of the host has the largest impact on the composition of the microbial community [11,12], but it is also affected by physiological, dietary, and environmental factors. For instance, changes in the intestinal mobility and nutrients availability occurring with age will have an impact in the composition of the intestinal microbiota. A decreased degree of mobility together with small bowel motor pattern changes in elderly may influence the motility of the bowel, having a negative effect on digestion, causing constipation, and may hence alter the gut microbiota. In addition, psychosocial stress factors may also contribute to changes in the immune system that may affect the composition of the gut microbiota in elderly [13]. Korshunov and colleagues compared the diversity and the amount of intestinal microbiota in three age groups (25-36, 55-68, 88-94 yrs) living in Switzerland, and it was observed that the highest values of both diversity and the total microbial count occurred in the group aged 55-68 yrs, and the microbiota of the advanced aged group

*Corresponding author: Forssten SD, Sokeritehtaantie 20, FI-02460 Kantvik, Finland, Tel: +35405367810; E-mail: sofia.forssten@danisco.com

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was the least diverse and had lowest total count [14]. These findings seem to be in agreement with that of Hopkin and colleagues [15] where lower viable counts of total anaerobes, bifidobacteria, *Bacteroides-Porphyrromonas-Prevotella*, and lactobacilli were observed in healthy elderly compared to young adults (67-88 vs 21-34 y). In addition, the elderly group harbored higher viable count of *Enterobacteriaceae* and clostridia. The same studies included a sub-group of geriatric patients (68-73 y) with *Clostridium difficile*-associated diarrhea, and the above-mentioned alterations in the intestinal microbiota was much more pronounced indicating that age itself may not have much influence, but the disease status may be the main inducer of commonly observed microbial shift. Close results were also reported by the same authors [16] where bacteroides species diversity increase in the faeces of elderly individuals, and bifidobacterial species diversity dramatically decreases with age.

The anaerobes *Bifidobacteria*, *Bacteroides*, *Clostridium*, *Eubacterium*, and *Ruminococcus* represent the dominant species in an adult intestinal microbiota. Bifidobacteria are considered as beneficial colonic species, and the bifidobacterial genus in adults may contain up to five different species. In elderly, a reduction of bifidobacterial species diversity occurs and one or two dominant organisms are present. It has been shown that *Bifidobacterium adolescentis* or the phenotypically similar *B. angulatum*, *B. bifidum* and *B. longum* are the dominant species [17-19] in elderly. The decrease in bifidobacterial species diversity may be due to reduced adhesion capacity to the intestinal mucosa though it is not clear if this is due to microbial changes or physiochemical changes in the structure of colonic mucosa [20,21]. A decreasing tendency of bifidobacteria counts were reported by culture based studies where bifidobacteria have been isolated from 50-80% of stool samples. Meanwhile, studies based on molecular techniques have identified bifidobacteria from 100% of the faecal samples from elderly groups [12,22]. A decrease in the *Bacteroides* species diversity with an increasing age was reported [12,18], but *Bacteroides thetaiotaomicron* were found in all individuals [23]. However, the levels of *Bacteroides*, *Enterococci*, *Enterobacteriaceae* and *Clostridium* were not different from adults while an increase of *Lactobacillus* and *Enterococci* was detected in a study by Mitsouka [24]. Higher quantities of *Ruminococcus* but lower quantities of *Eubacterium* and *Bacteroides* were found in elderly when compared to younger adults [20]. In contrast, higher level of enterobacteria was observed in the microbiota of elderly than among the microbiota of adults [25,26].

The *Clostridium* genus consists of a heterogeneous assemblage species with highly diverse nutritional requirements and habitats. In elderly, clostridia have been found to be prevalent in significantly higher numbers compared to younger subjects. *Clostridium* cluster IV has been showed to dominate in elderly [11], and *C. difficile* is frequently isolated from elderly, particularly at long-term-care facilities [27]. On the other hand, it was shown that levels of *Clostridium* cluster XIVa seems to decrease in elderly compared to younger persons [28]. Ljungberg and co-authors detected higher number of clostridia in feces from elderly subjects than young adults no matter whether or not were taken a ciprofloxacin regimen [29].

An increased diversity of eubacteria was reported in elderly population [16], and some members of the *Eubacterium* genus that are phylogenetically related to the clostridia were also found to increase with age [30]. Hopkins and coauthors [31] reported lower levels of *Bifidobacterium* and *Lactobacillus* in elderly subjects as compared to healthy adults. Some lactobacilli species like *Lactobacillus gasseri*,

Lactobacillus reuteri, and *Lactobacillus ruminis* may be present as indigenous species all the time while the alterations of levels of others species within the *Lactobacillus* genus may originate from food derived transient species, erroneously indicating an altered microbiota. This is not surprising as the consumption of probiotics at a daily dose of 10^9 - 10^{10} CFU evidently affect the composition and activity of the intestinal microbiota. Therefore, dietary intakes should be carefully controlled to obtain valid results. The diversity and the total counts of microbiota were compared among two elderly groups averaged 68 and 84 y group in a study by Benno and coauthors [10]. They found the total anaerobic bacteria, *Clostridium*, and *Bacteroides*, and the isolation rate of *Bacillus subtilis* to be lower in 84 y than 68 y, but bifidobacteria and fusobacteria were higher in the 84 y than 68 y. The findings of this study could be noticeably confounded by fiber intake.

Analysis of human microbiota of fecal samples from ten individuals (five young and five elderly adults) using a phylogenetic microarray, the human intestinal tract chip (HITChip), revealed contrasting results with the other studies with different methodologies either conventional or molecular methods [32]. The marked differences are that members of *actinobacteria* and *bacilli* were highly abundant in elderly compared to young while members of *clostridium* and *bacteroides* were highly abundant in adults. A more recent study with the same methodology found that the microbial composition and diversity of the gut ecosystem of young adults and seventy-year old people is highly similar but they differ significantly from that of the centenarians [33]. In a study with 230 healthy subjects at four European locations (France, Germany, Italy, and Sweden) categorized into two age groups (20 - 50 y and >60 y), the fecal microbiota was analyzed using a set of 14 group- and species-specific 16S rRNA-targeted oligonucleotide probes by fluorescence in situ hybridization coupled with flow cytometry [34]. Marked age-related differences in the microbiota composition were observed, but the result cannot be generalized as the differences were not independent of the location [34]. For example the group identified as *Bacteroides-Prevotella* (Bac 303) was significantly higher in elderly compared to adults in Germany but the opposite was true in Sweden and Italy. Similar age-regional comparison conducted in China using reference strains for target groups and 16S rRNA real-time PCR primers showed similar findings to the European study regarding the age-region interactions [35] as independent age or regional effects did not contribute to the observed changes.

Enck and colleagues analyzed fecal flora by conventional microbiological methods using stool samples from a very large number of subjects (35-292) expanding wide range of age (18 to 96 y) from young adult to elderly in advanced age [36]. Based on the results from this study, the total bacterial counts can be considered constant with no age-related differences. However, some species increased with age (*E. coli* and *Enterococci* spp.), others decreased with age (*Bacteroides* spp.), and others stay stable throughout life (lactobacilli, bifidobacteria). In another study [37] where qPCR was used to assess of human fecal microbiota from three age-groups (infants, adults and the elderly), the most pronounced difference was that a dramatic reduction in the ratio of *Firmicutes* to *Bacteroidetes* from 10.9 in adults to 0.6 in elderly. Similar results were reported in other studies [38].

The age-related alterations in the composition of the gut microbiota will also be translated into changes in the metabolic activity which in turn has various consequences on the health of the host. The gut microbiota can positively contribute to the intestinal epithelial cell differentiation and proliferation through the production of short-

chain fatty acids (SCFA). A decrease in the SCFA production with an increasing age is primarily associated with antibiotic treatment [30], but it is also associated with a decline in the levels of butyrate-producing bacteria, such as *Eubacterium* and *Faecalibacteria* [39]. On the other hand, fusobacteria ferment amino acids, resulting in production of several harmful end-products such as indoles and ammonia [40]. Similar activities are reported for *Propionibacterium acnes*, and an increase of these bacteria has been reported in elderly populations [18,30,31] leading to unfavorable putrefactive microbial metabolism [26]. The microbial activity in the colon may also be influenced by an increased transit time in elderly as prolonged transit time reduces the availability of nutrients, shifting the balance towards putrefaction. This shift was observed in a study where the activities of nine enzymes and the concentrations of 11 metabolites in fecal samples from 21 elderly persons (69 to 89 y), 15 adults (30-46 y), and 14 children (3-15 y), the enzyme activities were not significantly different between groups, nor the major carbohydrate fermentation metabolites, e.g., acetate, propionate, butyrate, and L-lactate [41]. However, the elderly had higher D/L lactate ratio and higher concentrations of protein fermentation metabolites (ammonia, valerate, iso-butyrate and iso-valerate).

Opportunities for probiotics

The age-related changes in the composition and activity of the intestinal microbiota seem to correlate with a greater vulnerability to inflammatory and viral disease, and, therefore, restoring the intestinal balance may have a significant health impact on the elderly population. Probiotic intervention is a potential tool for attaining such balance. Although the alteration in the elderly microbiota still not fully elucidated, several studies aimed at the use of specific probiotic strains in elderly disease prevention and/or treatment have already been conducted in various countries, and the main findings will be presented. Specific probiotic strains have been shown to exert beneficial effects or health improvements on elderly such as modulation of immune system functions and composition of the gut microbiota, as well as the different aspects of gut health and function. In respect to the changes in the intestinal microbiota, a number of strains have been shown to increase the faecal levels of endogenous bifidobacteria and/or lactobacilli [42]. This does not necessarily equal to a clinical health benefit, but may serve as a marker for improved gut environment.

Recently, various elderly populations have been targeted for interventions with different probiotics strains, strain combinations, or symbiotic preparations [2,43]. Two studies showed that specific probiotic strains might reduce the risk of catching common cold or influenza in elderly [44,45] whereas in another study the rate of infection was not affected but the duration of respiratory tract infection and rhinopharyngitis was shortened [46]. The consumption of *Lactobacillus casei* DN-114 001 for elderly population (76 yrs) has led to the reduction in both the episode and the cumulative duration of respiratory tract infections and rhinopharyngitis but it did not affect the severity, fever, pathogen's occurrence, nor some immune blood parameters [46]. The consumption of this strain had also led to a change in the intestinal microbiota as the level of *L. casei* species in the stools increased during the time of the consumption. Another study showed that the same strain, *L. casei* DN-114001, did reduce the duration of the infection but not the incidence of winter infection [47]. A mixture of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* increased the defecation frequency in elderly population but it did not reduce the laxative use [48].

Strain	Immune markers	References
<i>Bifidobacterium longum</i> BB536	Influenza vaccine antibody titre, NK cells and bactericidal activity of neutrophils	[45]
<i>Bifidobacterium lactis</i> HN019	Phagocytic activity, NK cells, relative proportions of leukocyte subsets	[49]
<i>Bifidobacterium lactis</i> Bb-12	TNF-alpha and IL-10.	[19]
<i>Lactobacillus rhamnosus</i> HN001	NK cells and phagocytic activity	[50]
<i>Lactobacillus johnsonii</i> La1	Percentage of days with infections, TNF-alpha, phagocytic activity	[51]
<i>Lactobacillus casei</i> DN-114 001	Resistance to common infectious disease, influenza-vaccine antibody titre	[46,52]

Table 1: Commercial probiotics shown to enhance immune system in elderly.

Various immune markers have showed improvement by the intake of probiotics by in elderly (Table 1). Additionally, a study by Schiffrin and others showed that the intake of probiotics might help in reducing the low-grade chronic inflammation in elderly [53].

A few studies have directly addressed the effect of probiotic intakes on the composition of the intestinal microbiota. In some studies, institutionalized elderly (n = 17, 78-94 y) had less diversity of total bacterial diversity and bifidobacteria than the young adults (n = 17, 18-31 y) but higher numbers of *Bacteroides* cells [54]. It was possible to modify the intestinal microbiota even by the intake of a single strain, *B. lactis* HN019, in a dose range from 6.5×10^7 to 5×10^9 CFU/day. This led to an increase of the number of resident *Bifidobacteria*, *Lactobacilli*, *Enterocci*, but reduced the *Enterobacteria* counts [55]. However, synbiotics (prebiotic and probiotic) might be more effective in changing the microbiota composition, affecting in particular *Bifidobacteria* [56].

Conclusions

Studies regarding the changes in the elderly microbiota compared to adults so far are limited and have showed inconsistent findings. The inconsistency could be attributed to different methodology, approach, setting, and individual variations. However, there is some evidence that a decrease in bifidobacterial diversity accompanied with an increase in bacteroides diversity is a consequence of old age. Several studies indicated that the use of probiotics bacteria hold promise in the prevention of some elderly disease, but the use of probiotic should be more focused on restoring the alterations in original composition of the intestinal microbiota. Therefore, it is important to improve our knowledge of the age-related changes in the composition and activity of the intestinal microbiota before probiotics interventions strategies can be accurately recommended as prophylaxis for elderly diseases.

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