Anaerobe 30 (2014) 169–177

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Clinical microbiology

Biosynthesis and cellular content of folate in bifidobacteria across host species with different diets

Maria R. D'Aimmo [a], Monica Modesto [b], Paola Mattarelli [b, *], Bruno Biavati [b], Thomas Andlid [a]

[a] Department of Chemical and Biological Engineering/Food Science, Chalmers University of Technology, Gothenburg, Sweden
[b] Department of Agricultural Sciences, Bologna University, Bologna, Italy

ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form
10 September 2014
Accepted 24 September 2014
Available online 12 October 2014

Keywords:
Folate biosynthesis
Bifidobacterium
Orangutan
Chimpanzee
Primates
Diet

ABSTRACT

Background: Bifidobacteria, one of the most common bacteria of the intestinal tract, help establish balance in the gut microbiota and confer health benefits to the host. One beneficial property is folate biosynthesis, which is dependent on species and strains. It is unclear whether the diversity in folate biosynthesis is due to the adaptation of the bifidobacteria to the host diet or whether it is related to the phylogeny of the animal host. To date, folate production has been studied in the bifidobacteria of omnivorous, and a few herbivorous, non-primate hosts and humans, but not in carnivores, non-human primates and insects. In our study we screened folate content and composition in bifidobacteria isolated from carnivores (dog and cheetah), *Hominoidea* omnivorous non-human primates (chimpanzee and orangutan) and nectarivorous insects (honey bee).

Results: *Bifidobacterium pseudolongum* subsp. *globosum*, a species typically found in non-primates, was isolated from dog and cheetah, and *Bifidobacterium adolescentis* and *Bifidobacterium dentium*, species typically found in humans, were respectively obtained from orangutan and chimpanzee. Evidence of folate biosynthesis was found in bifidobacteria isolated from non-human primates, but not from the bifidobacteria of carnivores and honey-bee. On comparing species from different hosts, such as poultry and herbivorous/omnivorous non-primates, it would appear that folate production is characteristic of primate (human and non-human) bifidobacteria but not of non-primate.

Isolates from orangutan and chimpanzee had a high total folate content, the mean values being 7792 μg/100 g dry matter (DM) for chimpanzee and 8368 μg/100 g DM for orangutan. The tetrahydrofolate (H_4folate) and 5-methyl-tetrahydrofolate (5-CH_3–H_4folate) distribution varied in the bifidobacteria of the different animal species, but remained similar in the strains of the same species: *B. dentium* CHZ9 contained the least 5-CH_3–H_4folate (3749 μ/100 g DM), while *B. adolescentis* ORG10 contained the most (8210 μg/100 g DM).

Conclusion: Our data suggest a correlation between phylogenetic lineage and capacity of folate production by bifidobacteria, rather than with dietary type of the host.

1. Background

Human and non-human animal microbiota are intimately associated with certain tissues and organs with which they share a long evolutionary history [1]. The gastrointestinal tract represents the most complex of these ecosystems, with numerous microbes interacting with each other and with the host cells. The gut microbiota can be viewed as an active organ with its own functions in the body [2]. Bifidobacteria play an important role in gut function and typically comprise up to 90% of infant microbiota, remaining present in large numbers also in the gut of adults [3]. Bifidobacteria appear to be beneficial by stimulating and maintaining normal immune function, displacing pathogens, helping food digestion and producing vitamins such as folate [4,5]. The ecology of bifidobacteria is specific, and is normally restricted to the gastrointestinal tract of animals (comprising humans) but there can also be extra-body habitats, like sewage, through faecal contamination. To date 41 species of bifidobacteria have been described: 10 species are from humans, 6 from nonhuman primates and the remaining 25

* Corresponding author.
E-mail addresses: mdaimmo@gmail.com (M.R. D'Aimmo), monica.modesto@unibo.it (M. Modesto), paola.mattarelli@unibo.it (P. Mattarelli), bruno.biavati@unibo.it (B. Biavati), andlid@chalmers.se (T. Andlid).

http://dx.doi.org/10.1016/j.anaerobe.2014.09.018
1075-9964/

from other animals such as cow, rabbit, chicken, and social insects such as honey bees and bumblebees etc. [6–8]. Note that *Bifidobacterium animalis*, *Bifidobacterium longum*, *Bifidobacterium pseudolongum* and *Bifidobacterium thermacidophilum* have been further subdivided into subspecies (Table 1S).

Folates, natural, chemically reduced forms of folic acid (vitamin B9), are co-factors in essential metabolic pathways such as DNA synthesis and methylation pathways [9]. Low folate levels increase the risk for neural tube defects and megaloblastic anemia and may increase the risk of certain cancer forms, cardiovascular disease and Alzheimer's disease [10,11]. Mammals are auxotroph for folate, and depend on dietary sources such as leafy green vegetables, yeast extracts, liver, beans and citrus fruits. Folate can also be derived from indigenous folate-synthesizing bacteria in the gut [12–14]. However, the magnitude and importance of this bacterial source of folate for host nutrition is still not understood.

Our earlier research showed that bifidobacteria isolated from humans can produce different folate quantities, depending on species and strains, whereas bifidobacteria isolated from non-primates did not produce folate [15]. To date, it is still unclear whether this capacity of bifidobacteria to synthesize folate is the result of natural bacterial selection in the host animal's gut, due to diet (*e.g.* carnivores *versus* herbivores/omnivores), or whether it is the result of the evolutionary relatedness of different host species (primates *versus* non-primates). Thus, in order to discern between these hypotheses, we decided to measure folate production by bifidobacteria isolated from other animal species across a wider range of diet.

The aims of this work were the isolation and the identification of bifidobacteria from carnivores (dog and cheetah) and omnivorous Hominidae non-human primates (chimpanzee and orangutan), sources not available in the public Collection of Microorganisms. Those isolates, together with bifidobacteria from nectarivorous insects (honey bee), were further screened for folate content and composition.

2. Methods

2.1. Sample collection

A total of 42 isolates of *Bifidobacterium* spp. from different sources was studied (Table 1). Thirty six are new isolates obtained in the present study and 6 strains were obtained from the Bologna University Scardovi Collection of Bifidobacteria (BUSCOB).

Thirty six *Bifidobacterium* spp. strains were isolated from fresh faecal samples collected from 4 different animal species: 14 isolates from chimpanzee (*Pan troglodytes*), 10 isolates from orangutan (*Pongo pygmaeus*), 9 isolates from dog (*Canis lupus familiaris*) and 3 isolates from cheetah (*Acinonyx jubatus*). All the animals, except for the dog, were housed at Borås Djurpark, a zoo in southern Sweden (Alidelundsgatan 11, 506 31 Borås, Sweden). Based on their diet, the cheetah and dog were classified as carnivores while the orangutan and chimpanzee were classified as omnivores. All the animals were adult and free of intestinal infection, confirmed by routine two-monthly checks for intestinal parasites, and their general health status was monitored constantly. None had been administered antibiotics, probiotics, or prebiotics for at least two months prior to sample collection. The faecal samples, taken by their keepers as no animals were kept or maintained for the purpose of this study, were collected aseptically immediately after excretion; 2.0 g of the samples were suspended in 18 ml of glycerol broth [16] to avoid qualitative and quantitative modification of the microbiota. Samples were stored at −80 °C until bacteriological examination. Six additional strains were obtained from BUSCOB: 4 strains from Eastern honey bee (*Apis cerana*) and 2 from Western honey bee (*Apis mellifera*).

Table 1
Species, origin and host diet of 42 *Bifidobacterium* strains[a] screened for their folate content.

Strain	Species	Origin	Host diet
D1 (DSMZ28533), D2, D4, D5, D7, D8, D9 (DSMZ 28534), D10	*B. pseudolongum* subsp. *globosum*	Dog faeces	Carnivore
D3	*B. indicum*		
ORG1, ORG2, ORG3, ORG4 (DSMZ28529), ORG5, ORG6, ORG7, ORG8 (DSMZ 28530), ORG9, ORG10	*B. adolescentis*	Orangutan faeces	Omnivore
CHZ1, CHZ3, CHZ4, CHZ5, CHZ6, CHZ7 (DSMZ 28535), CHZ8, CHZ9, CHZ10, CHZ11, CHZ12, CHZ13, CHZ14, CHZ15 (DSMZ 28536)	*B. dentium*	Chimpanzee faeces	Omnivore
G1 (DSMZ 28531), G3 (DSMZ 28532), G4	*B. pseudolongum* subsp. *globosum*	Cheetah faeces	Carnivore
[a]C410, [a]C550, [a]C580	*B. indicum*	Eastern honey bee	Herbivore
[a]C567	*B. asteroides*		
[a]C51 (ATCC 25911)	*B. asteroides*	Western honey bee	
[a]C339	*B. coryneforme*		

[a] Strains from Bologna University Scardovi Collection of Bifidobacteria.

2.2. Recovery of bifidobacteria on selective medium, purification and DNA isolation

Serial dilutions from frozen stored faecal samples were plated in "Trypticase-Phytone-Yeast Extract" agar (TPY) [6] modified (mTPY) by adding mupirocin (100 mg/L), glacial acetic acid (1 ml/L) and colistin (25 mg/L) (Applichem GmbH, Darmstadt, Germany) for selective outgrowth of bifidobacteria [17,18]. Agar plates were incubated anaerobically (Anaerocult A, Merck, Darmstadt, Germany), at 37 °C for 72 h.

Colonies selected from mTPY agar plates consisting of cells of irregular shapes (knob, branching, swelling, etc.) were picked and transferred as stab cultures in 0.5% agar TPY and grown anaerobically at 37 °C for 24 h. The isolates were further subcultured in TPY liquid medium under the same incubation conditions. After centrifugation (10,000 × g, 4 °C, 15 min) and addition of cryoprotective solutions (skim milk 20%, lactose 0.3%, yeast extract 0.3%), the cells were maintained both frozen at − 80 °C and freeze-dried in order to be available for different identification assays (fructose-6-phosphate-phosphoketolase (F6PPK) assay, enterobacterial repetitive intergenic consensus sequences (ERIC), phylogenetic analysis).

Genomic DNA was extracted from the isolates according to the method of Rossi et al. [19] with slight modifications. Briefly, the cells of overnight cultures were harvested by centrifugation and washed with TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6). The pellets were resuspended in 1 ml of TE containing 50 mg lysozyme ml^{-1}, and then incubated overnight at 37 °C. All subsequent steps were carried out as described by Rossi et al. [19].

2.3. F6PPK assay

The presence in cell-free extracts of fructose-6-phosphate phosphoketolase (F6PPK), the key enzyme considered a