Bacterial Taxa Associated with High Adherence to Mediterranean Diet in a Spanish Population [†]

Carles Rosés [1],*, Amanda Cuevas-Sierra [2], Salvador Quintana [3], José I. Riezu-Boj [2,4], J. Alfredo Martínez [2,4,5], Fermín I. Milagro [2,4,5] and Anna Barceló [1]

[1] Servei de Genòmica i Bioinformàtica, Universitat Autònoma de Barcelona, Barcelona, Spain
[2] Centre for Nutrition Research; Department of Nutrition, Food Sciences and Physiology, University of Navarra, Pamplona, Spain
[3] Unaffiliated
[4] Navarra Institute for Health Research (IdISNA), Pamplona, Spain
[5] Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain
* Correspondence: crosespol@gmail.com
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Abstract: The Mediterranean diet (MD) is recognized as one of the healthiest diets worldwide and is associated with the prevention of cardiovascular and metabolic diseases, among others. Dietary habits are considered one of the strongest modulators of the gut microbiota, which seems to play a significant role in the health and disease of the host. The purpose of the present study was to evaluate interactive associations between gut microbiota composition and habitual dietary intake in 360 Spanish adults of the Obekit cohort (normal weight, overweight and obese subjects). Dietary intake and adherence to the MD tests together with fecal samples were collected from each subject. Fecal 16S rRNA sequencing was performed and checked against the dietary habits. MetagenomeSeq was the statistical tool applied to analyse at species taxonomic level. Results from this study confirm that a strong adherence to the MD increases the population of some beneficial bacteria improving microbiota status towards a healthier pattern. *Bifidobacterium animalis* is the species with the strongest association with the MD. One of the highlights is the positive association between several SCFA-producing bacteria and high adherence to MD. In conclusion, this study shows that MD, fibre, legumes, vegetables, fruit and nuts intake are associated with an increase in butyrate-producing taxa like *Roseburia faecis*, *Ruminococcus bromii* and *Oscillospira (Flavonifractor) plautii.*

Keywords: *Bifidobacterium animalis*; gut microbiota; short-chain fatty acids; obesity; butyrate

1. Introduction

The gut microbiota status has a direct impact on health and disease of the host [1]. Dietary habits are considered one of the strongest modulators of the gut microbiota. Serious conditions can show-up due to sedentarism and bad dietary habits: hypertrophied adipocytes release inflammatory molecules (i.e., interleukins and tumour necrosis factor) which over enough time can favour the development of several inflammation-related disorders such as metabolic syndrome, cardiovascular disease, colorectal cancer, neurodegenerative diseases [2,3] and autoimmune disorders like Crohn's disease, ulcerative colitis and allergies [4]. In this context, the Mediterranean diet (MD) is recognized as one of the healthiest diets worldwide. Therefore, we would expect a modulation of the gut microbiota as one of the positive health effects of the MD [5]. The main objective of the present work relays on the bacteria that are more closely associated with a high adherence to the MD.

2. Material and Methods

2.1. Subjects

This cross-sectional study enrolled 360 Spanish adults (251 females and 109 males) ranged 45.0 ± 10.5 years old. Major exclusion criteria included a history of diabetes mellitus, cardiovascular disease and hypertension, pregnant or lactating women, and current use of lipid-lowering drugs.

2.2. Anthropometric and Biochemical Measurements

The volunteers were classified as normal weight when BMI: 18.5–24.9 kg/m^2 (n = 64), overweight when BMI: 25.0–29.9 kg/m^2 (n = 115), and obesity when BMI > 30.0 kg/m^2 (n = 181). Blood biochemistry (glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides) was analysed. Insulin resistance índex (HOMA-IR) was calculated.

2.3. Dietary Estimation

Habitual dietary intake at baseline was collected with a validated food frequency questionnaire [6]. A 14-item questionnaire, PREDIMED validated test, was also used in this study adherence of participants to the MD [7].

2.4. Faecal Sample Collection and DNA Extraction

2.4.1. Metagenomic Data: Library Preparation

Metagenomics studies were performed by analysing the variable regions V3–V4 of the prokaryotic 16S ribosomal RNA gene (16S rRNA), which gives 460 bp amplicons in a two rounds PCR protocol. Finally, paired-end sequencing was performed in a MiSeq platform (Illumina).

2.4.2. Metagenomics Data: Analysis and Processing

16S rRNA sequences obtained were filtered following quality criteria of the OTU (Operational Taxonomic Units) processing pipeline LotuS (release 1.58) [8]. Taxonomy was assigned using HITdb achieving up to species-level sensitivity. BLAST was used when HITdb failed to reach an homology higher than 97% [9,10]. Global normalization was performed using the library size as a correcting factor and log2 data transformation [11].

2.5. Statistical Analysis

Microbiome Analyst tool [12] was used for statistical differences in microbiota profiles between groups (tertiles) through Zero-inflated Gaussian approach of MetagenomeSeq and using the cumulative sum scaling (CSS) normalization.

3. Results

Microbiota Composition: MD Adherence

MD tertiles 1 and 3 have been compared through metagenomeSeq analysis. Significant differences appeared when comparing both tertiles (FDR < 0.05). Species shown in Figure 1 are strongly influenced by the MD-score. Subjects with a higher adherence to the MD are represented in the 3rd tertile while those who are far from the MD model are in the 1st tertile. This work focus on the high adherence species and their distribution, with box plots (Figure 2). All box plots represent those species with significant differences between high and low adherence tertiles.

High adherence (3rd tertile)		Low adherence (1st tertile)	
Species	**FDR**	**Species**	**FDR**
Bifidobacterium animalis	1.21E-07	*OTU100|NN=Eubacterium saphenum GU427005|D=91*	4.44E-05
Bacteroides cellulosilyticus	4.47E-07	*OTU375|NN=Succinivibrio dextrinosolvens Y17600|D=97*	0.0001
OTU946|NN=Paraprevotella clara AB331896|D=86.8	1.72E-05	*OTU759|NN=Gordonibacter pamelaeae AB566419|D=87.6*	0.0005
OTU1682|NN=Oscillibacter valericigenes AB238598|D=91.1	3.42E-05	*OTU11|NN=Butyricicoccus pullicaecorum EU410376|D=89.5*	0.0002
OTU1065|NN=Oscillospira (Flavonifractor) plautii Y18187|D=86.6	3.42E-05	*Christensenella minuta*	0.0020
OTU1173|NN=Roseburia faecis AY804149|D=94.9	0.0008	*Parabacteroides goldsteinii*	0.0073
OTU1517|NN=Catabacter hongkongensis AB671763|D=87	0.0008	*OTU1625|NN=Anaerotruncus colihominis DQ002932|D=89.9*	0.0120
OTU1296|NN=Ruminococcus bromii DQ882649|D=92.3	0.0120	*Alistipes timonensis*	0.0155
Erysipelatoclostridium ramosum	0.0176	*Prevotella corporis*	0.0192
OTU521|NN=Papillibacter cinnamivorans AF167711|D=89	0.0463		

Figure 1. Bacterial species with a significant relation with adnerence to the MD (FDR < 0.05) by metagenomeSeq test.
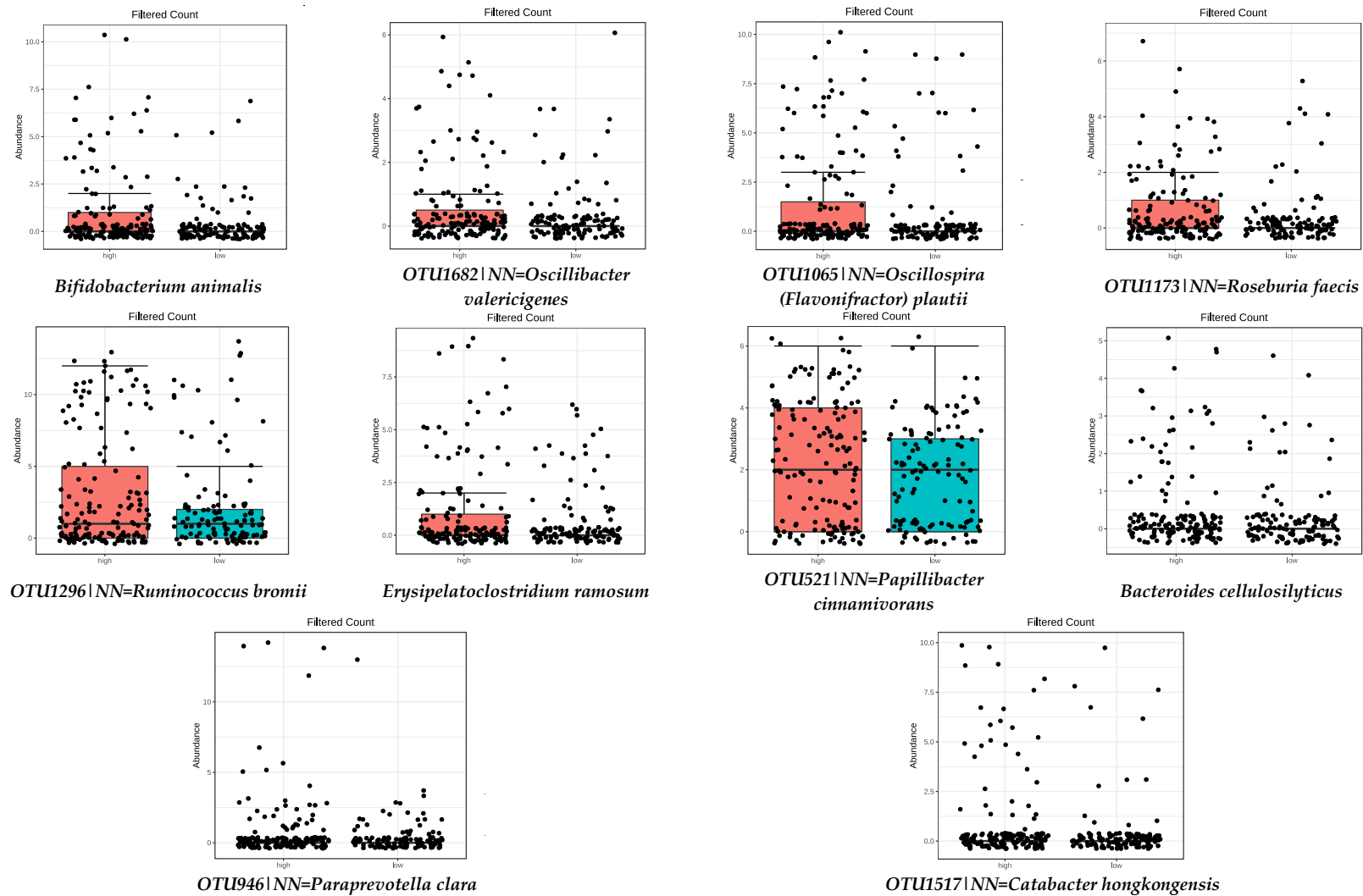
Figure 2. Bacterial species that were significantly more abundant in the group with high adherence to MD (FDR < 0.05) by metagenomeSeq test. Red boxes represent subjects with a higher adherence to the MD and blue boxes low adherence.

4. Discussion

The gut microbiota co-develops with the host, and its bacterial proportions are modified by the action of the diet and other extrinsic stressors [13].

MD High Adherence Species

High MD adherence has many beneficial outputs to human health. It is a great resource to manage obesity-related comorbidities, such as cardiovascular diseases, type 2 diabetes and pro-inflammatory conditions [14–16]. Table 2 shows those species that are more associated with the adherence to MD.

Bifidobacterium animalis belongs to the pylum Bacteroides, associated with obesity-related alterations in bacterial gut microbiota and the genus *Bifidobacterium* might have a critical role in weight regulation [17]. *B. animalis s*ubsp. *lactis* GCL2508 is a probiotic strain with an anti-metabolic syndrome effect [18], capable of proliferating and producing SCFA in the gut. These compounds have a regulatory effect on inflammatory conditions [19]. *Bacteroides cellulosilyticus* degrade cellulose [20], with an unprecedent number of carbohydrate active enzymes providing a versatile carbohydrate utilization [21]. *Paraprevotella clara,* a common member of the human intestinal microbiota [22], is closely related to carbohydrate-active enzymes known to degrade insoluble fibre [23]. Indeed, *P. clara* is known to produce acetic acid [22]. *Oscillibacter valericigenes* produces valeric acid, a SCFA [24]. Valeric acid has been reported to have an inhibitory effect on histone deacetylase (HDAC) isoforms implicated in a variety of pathologies such as cancer, colitis, and cardiovascular and neurodegenerative diseases [25]. High levels of *Oscillospira* (*Flavonifractor*) *plautii* have been strongly correlated with a high production of SCFA, especially propionate and butyrate [26]. This species correlate with a lean host phenotype [27]. Furthermore, *Oscillospira* genus has been correlated with the production of secondary bile acids known to prevent *Clostridium difficile*-associated infectious disease in humans [28]. *Roseburia faecis* is a butyrate producer whose abundance has been directly related with weight loss and a reduced glucose intolerance in mice [29]. *Catabacter hongkongensis,* regular in the human intestinal microbiota [30] *Ruminococcus bromii* has been related with diets rich in fibres and resistant starch, and greatly contributes to butyrate production in the colon [31].

It is important to highlight some beneficial effects of butyric acid as it has been reported to improve the intestinal barrier integrity [32], regulate cell apoptosis [33], stimulate production of anaerobic hormones [34] and, by inducing differentiation of colonic regulatory T cells, suppress inflammatory and allergic responses [35]. On the other hand, many conditions have been associated with low levels of butyrate, such as colon cancer or obesity [31]; so increased butyrate production in colon may be beneficial to human health.

Erysipelatoclostridium ramosum is a member of the Erysipelotrichaceae family known to interfere in various ways with the enterohepatic circulation and excretion of bilirubin, transforming it into urobilin [36]. *Papillibacter cinnamivorans* is not well known but has been found in lower amount in centenaries than in any other age [37].

5. Conclusions

Our results indicate that the well-known beneficial factors of the MD may be triggered by changes in intestinal microbiota due to diet habits. A high adherence to MD seems to increase the abundance of some species associated with health: *Bifidobacterium animalis*, *Oscillibacter valericigenes*, *Oscillospira (Flavonifractor) plautii*, *Roseburia faecis*, *Ruminococcus bromii*, *Butyricicoccus pullicaecorum* and *Papillibacter cinnamivorans.* This study strongly suggests that MD increase butyrate production from *R. faecis*, *R. Bromi* and *Oscillospira (Flavonifractor) plautii. Erysipelatoclostridium ramosum* is the only bacteria from this study that does not show a clear beneficial effect on health, although this identification should be taken with caution. A deeper taxonomy is required to put some light into it.

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