Interindividual Differences in Microbial Counts and Biochemical-Associated Variables in the Feces of Healthy Spanish Adults

SUSANA DELGADO, MSc,* PATRICIA RUAS-MADIEDO, PhD,* ADOLFO SUÁREZ, PhD,†
and BALTASAR MAYO, PhD*

The aim of this study was to examine, over a period of 1 year, interindividual variations in the most prominent and representative of the cultivatable microbial populations in the feces of eight healthy Spanish persons. A number of biochemical variables (enzyme activities and ammonium and short-chain fatty acid [SCFA] concentrations) thought to be influenced by the GIT microbiota were also analyzed. Total cultivatable microbial counts ranged from 10^{10} to 10^{11} cfu/g of feces. The largest populations were obligate anaerobes belonging to the *Clostridium* clusters, followed by species of bifidobacteria and bacteroides. Coliforms and lactobacilli were found at a more intermediate level (10^5 – 10^9 cfu/g). The predominant anaerobe populations remained quite constant over time, but all other microbial groups showed significant interindividual differences. Enzyme profiles were individual-dependent, but within subjects, moderate to high intersample variations over time were recorded for some activities. Fecal ammonium concentration was the most unpredictable variable; this fluctuated widely between individuals and samples. Acetic acid was the most abundant SCFA in the feces, followed by butyric and propionic acids. SCFA concentrations also varied according to the individual; some subjects showed specific profiles in terms of SCFA composition or concentration. The fecal microbial and biochemical parameters studied seemed to be individual-dependent. Most variables were rather stable over time, while others (e.g., ammonium concentration) varied widely.

KEY WORDS: gastrointestinal microbiology; enzyme activity in feces; short-chain fatty acids; ammonium in feces; probiotics.

The human gastrointestinal tract (GIT) is colonized soon after birth by a complex and diverse collection of microbial species (1, 2). This microbiota influences several biochemical, physiological, and immunological characteristics of the host (3–5). Due to host-specific differences (age, sex, physiological state, etc.) or dietary habits, the types and numbers of microbial populations may be different in different human groups and even between individuals (6–8).

Current evidence suggests that these microorganisms may have important metabolic roles (fermentation of nondigested dietary and endogenous materials, production of vitamins, absorption of ions), trophic functions (control of cell proliferation and differentiation, development and homeostasis of the immune system), and protective effects (the barrier effect) (3, 9). Moreover, GIT microbiota show a series of important enzyme activities that can transform food and host-derived components into different metabolites that could be either beneficial or detrimental to the host (5, 10). Ascertaining the components of the GIT microbiota and their individual contribution to host health is therefore very important, since it might be plausible to promote or inhibit particular populations (and their activities) through the use of probiotics and/or

Manuscript accepted February 23, 2005; accepted March 24, 2005.

From *Instituto de Productos Lácteos de Asturias (CSIC), 33300-Villaviciosa, and †Servicio de Digestivo, Hospital de Cabueñes, 33394-Gijón, Spain.

Address for reprint requests: Baltasar Mayo, PhD, Instituto de Productos Lácteos de Asturias, Carretera de Infiesto s/n, 33300-Villaviciosa, Asturias, Spain; baltasar.mayo@ipla.csic.es.

prebiotics (11). Lactic acid bacteria (including bifidobacteria) are thought to have positive and protective effects on the GIT, thus contributing to the host health (3, 12).

Short-chain fatty acids (SCFAs) are considered among the most beneficial metabolites produced by the GIT microbiota. SCFAs (primarily acetic, butyric, and propionic acids) are organic anions formed in the large intestine through the fermentation of carbohydrates (9). These could be used as a source of energy for other microorganisms or even for the host (13). SCFAs stimulate the proliferation and differentiation of epithelial cells in the large and small intestine *in vivo* (14, 15), inhibiting in some instances the proliferation of neoplastic cells, which might contribute to cancer prevention (5, 10, 16). SCFA concentrations affect the microbial composition of the lumen by lowering the pH. A low pH may also help prevent colon cancer through the inhibition of certain bacterial enzymes (e.g., bacterial 7- α -dehydroxylases and β -glucuronidases) (5).

Harmful compounds produced by the GIT microbiota may include ammonia and products of putrefaction such as phenol, *p*-cresol, indol, skatol, and other such compounds (5). Some of these have been related to a number of gastrointestinal malignancies, including colon cancer (17). Putrefactive substances are formed from amino acids, and while they do not appear to be carcinogenic by themselves, they could act as cocarcinogens or cancer promoters (18). Ammonia is mainly produced by the deamination of amino acids. This compound has been found to enhance cell proliferation and is a promoter of colon cancer in rats (19).

This paper reports the variations in the size of total and indicator microbial populations and the changes in some biochemical variables in the feces of eight healthy subjects over a period of 1 year. The work was aimed to an initial characterization of the GIT system from Spanish people. Variations between and within individuals were examined and correlations sought with enzyme activities and concentrations of ammonium and SCFAs. Finally, the results were compared to those in the literature from other human communities.

MATERIALS AND METHODS

Sampling and Sample Processing

The selection of donors and sampling was performed as recommended by the Regional Ethics Committee of the Principality of Asturias, Spain. The study subjects were four male and four female volunteers (mean age, 45 years; range, 39 to 56 years). At approximately monthly intervals fecal samples were collected from each individual in sterile containers. These were transported to the laboratory in anaerobic jars containing Anaerocult A (reducing agent; Merck, Darmstadt, Germany). All samples were processed within 2 hr after deposition in an anaerobic chamber

(Mac500; Down Whitley Scientific, West Yorkshire, UK) containing a 10% H₂, 10% CO₂, and 80% N₂ atmosphere.

Cultured Microbial Counts

Fecal samples were homogenized in a reducing medium containing Brain Heart Infusion (BHI) broth (Merck), 0.5% glucose, 0.5% yeast extract (Merck), 0.25% cysteine (Merck), 10 μ g vitamin K₁ L⁻¹ (Merck), and 0.02 g hemin L⁻¹ (Sigma Chemical Co., St. Louis, MO, USA). They were then serially diluted and plated in duplicate in agar media as follows.

Total Bacterial Counts. These were determined on BHI agar with 0.5% glucose, 0.5% yeast extract, 0.25% cysteine, vitamin K₁, and hemin. Plates were incubated anaerobically at 37°C for up to 5 days.

Clostridia. Clostridia were enumerated on Reinforced Clostridium Agar (RCA) (Merck) with 20 μ g polymyxin B ml⁻¹ (Sigma) after incubation under anaerobic conditions at 37°C for 48 hr.

Bacteroides. The bacteroides group was enumerated on Esculine Bile Agar (EBA) (Merck) with 100 μ g/ml kanamycin (Sigma) and 7.5 μ g vancomycin ml⁻¹ (Sigma). Incubations were performed in anaerobiosis at 37°C for 48 hr.

Lactobacilli and Bifidobacteria. Bifidobacteria and lactobacilli counts were made on de Man, Rogosa, and Sharpe (MRS) agar (Merck) with 0.25% cysteine after anaerobic incubation at 37°C for 72 hr. Distinction between lactobacilli and bifidobacteria was done by microscopic observation.

Enterobacteria and Coliforms. Violet Red Bile Glucose (VRBG) and Violet Red Bile Lactose (VRBL) agar (Merck) were used to enumerate enterobacteria and coliforms, respectively, following aerobic incubation for 24 hr at 37°C.

Enterococci. Enterococci were scored after 24–48 hr of aerobic incubation at 44°C in Slanetz and Bartley (SB) agar (Merck), a medium containing 10 g triphenyltetrazolium chloride (TTC) per liter.

Staphylococci. Dilutions were plated on Baird–Parker (BP) agar (Merck) with 10% tellurite egg yolk solution (Merck) and aerobically incubated for 24 hr at 37°C. Black colonies with and without egg yolk clearing were recorded.

Yeasts and Molds. Sample dilutions were plated on Chlorenchenicol Glucose Agar (CGA) (Merck) and incubated aerobically for 3–5 days at 25°C.

Biochemical Analysis of Feces

Enzymatic Activities. Enzyme activities were assessed by the semiquantitative API ZYM method (bioMérieux, Montalieu-Vercieu, France). Fresh fecal samples were suspended 1/500 in distilled water and 65 μ l of this suspension was deposited in each well. All measurements were made according to the manufacturer's instructions after 4 hr of anaerobic incubation.

Ammonium Concentration. Fecal samples were diluted 1/10 in distilled water and centrifuged for 10 min at 4500 rpm. The ammonium concentration was then measured using an ion-selective probe (Crison Instruments SA, Barcelona, Spain).

Identification and Quantification of Short-Chain Fatty Acids. To identify and quantify SCFAs in the samples, 0.01 g of feces was supplemented with 0.1 ml of 2-ethyl butyric acid as an internal standard and acidified with 0.1 ml of 20% formic acid (v/v). The acidic solution was then extracted with 1 ml of methanol. The separation and quantification of SCFAs in the supernatants were accomplished by gas chromatography in a

PE 8600 Gas Chromatograph (Perkin Elmer, Wellesley, MA, USA) using an HP-FFAP column (Hewlett-Packard, Palo Alto, CA, USA). SCFAs were separated over a temperature range of 100–220°C and detected by flame ionization.

Statistical Analysis

Statistical analysis was performed using SPSS 11.0 software for Windows (SPSS Inc., Chicago, IL, USA). Bacterial counts, organic acid concentrations, and enzyme activity data were subjected to one-way ANOVA using “individual” as a factor with eight categories. The least-significant difference test (LSD test) was used to compare means and significance was set at $P < 0.05$.

RESULTS

Microbial Counts

Table 1 shows the average concentrations and standard deviations of the microbial populations in the feces of the eight volunteers, as well as a summary of the statistical analysis. Total cultivatable bacterial counts were $>10^{10}$ cfu g⁻¹ in most samples. Representative colonies from the BHI agar plates used for total counts were genetically classified by sequencing of part of their 16S rRNA gene and the sequences compared to those on the databases (data not shown). The largest populations were composed of strict anaerobes, Gram-positive spore-forming species, thought to belong to the *Clostridium* clusters, followed at a short distance by bifidobacteria and bacteroides species (Table 1). The most numerous populations of facultative anaerobes and aerobes were those of lactobacilli, coliforms, enterococci, staphylococci, and yeasts and molds (in decreasing numerical order). Some subjects consistently showed a greater number of coliforms ($\sim 10^8$ cfu g⁻¹) than lactobacilli ($<10^7$ cfu g⁻¹). Furthermore, in two individuals (L and M), lactobacilli numbers were below the detection limit ($\sim 10^5$ cfu g⁻¹).

The size of the numerically predominant anaerobe populations (clostridia, bifidobacteria, and bacteroides) showed less variation between individuals than did those of the facultative anaerobe populations (coliforms and lactobacilli) (Table 1). This suggests that the dominant populations are more stable, although intersample variations were recorded. One-way ANOVA indicated the differences between individuals in most populations to be statistically significant, except for the bacteroides.

Biochemical Analysis

Ammonium Concentration. Fecal ammonium concentration varied widely between subjects and between samples. The means and standard deviations for the ammonium concentrations in the different individuals (mmol kg⁻¹ wet feces) were as follows: Individual A, 9.5 ± 1.3 ;

Individual C, 22.3 ± 1.25 ; Individual E, 14.3 ± 0.7 ; Individual F, 5.8 ± 0.34 ; Individual G, 14.3 ± 0.74 ; Individual H, 6.5 ± 0.57 ; Individual L, 12.8 ± 0.67 ; and Individual M, 9.0 ± 0.53 . The ammonium concentration on the samples and the dispersion of the values are presented in Figure 1.

Enzyme Activities. Table 2 summarizes the modal enzyme values measured in the feces by the API ZYM system. Five of the 19 activities for which assays were performed were never detected (lipase C14, cysteine arylamidase, trypsin, α -quimiotrypsin, and α -mannosidase). Some individuals showed high intersample fluctuations, which may be related to the semiquantitative nature of the results provided by the API-ZYM system. However, it could also be due to variations in activity over the sampling period (although no tendency was observed). Therefore one-way ANOVA was not possible.

Personal profiles could be envisioned for at least some of these subjects. Individual E, for instance, showed regularly reduced acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, and β -glucuronidase activities but strong β -galactosidase activity. Individual F had Leu- and Val-aminopeptidase activities clearly above the mean. In contrast, individual H showed lower than average β -galactosidase and α -glucosidase activities.

SCFA Profiles and Concentrations. Table 3 shows the average concentrations and standard deviations for fecal SCFAs. Approximately 50 to 60 mmol of acetic acid was found per kilogram of wet feces. In most individuals butyric acid was the second most common SCFA ($5\text{--}22$ mmol kg⁻¹), followed by propionic acid ($5\text{--}20$ mmol kg⁻¹). Traces of valeric and isovaleric acids were seen in all samples, whereas caproic and isobutyric acids were only detected in samples from certain individuals, although they were never observed together. Some SCFA profiles seemed to be rather personal, such as that of individual G (characterized by a high caproic acid concentration) and subject L (in whom butyric and propionic acid levels were ~ 10 -fold below that of acetic acid). One-way ANOVA (when it could be performed) indicated that the values for most SCFAs (except isovaleric acid) were significantly different between individuals.

DISCUSSION

The general microbial picture obtained by culturing on selective and differential media was one of feces dominated by anaerobic species coexisting with smaller populations of facultative organisms. This agrees well with previous culture-based results from experiments performed on other human communities (6–8). It is also well established that facultative anaerobic and aerobic microorganisms

TABLE 1. MEAN AND STANDARD DEVIATION OF COUNTS OF THE DIFFERENT MICROBIAL POPULATIONS STUDIED IN THIS WORK: RESULTS OF ONE WAY-ANOVA USING "INDIVIDUAL" AS A FACTOR AND MULTIPLE MEANS COMPARISON BY THE LEAST-SIGNIFICANT DIFFERENCE TEST ($P < 0.05$) ARE ALSO INDICATED

Individual	Total counts	Microbial counts (log cfu/g wet feces)							
		Clostridia	Bifidobacteria	Bacteroides	Coliforms*	Lactobacilli	Enterococci	Staphylococci	Yeasts and molds
A	10.78 ± 0.32 ^b	9.93 ± 0.56 ^{ab}	9.37 ± 1.05 ^{ab}	9.15 ± 1.05	6.23 ± 0.91 ^a	8.56 ± 0.37 ^c	5.94 ± 0.55 ^{ab}	3.80 ± 0.50 ^{ab}	2.77 ± 0.76 ^{ab}
C	10.32 ± 0.47 ^{ab}	10.30 ± 0.26 ^{ab}	9.24 ± 0.71 ^a	8.79 ± 0.20	6.94 ± 1.17 ^{ab}	6.24 ± 0.61 ^{ab}	5.51 ± 0.84 ^a	4.23 ± 1.81 ^{abc}	3.02 ± 1.33 ^{abc}
E	10.24 ± 0.38 ^{ab}	10.31 ± 0.49 ^b	9.13 ± 0.50 ^a	8.98 ± 0.09	7.50 ± 0.76 ^b	7.04 ± 0.20 ^{ab}	6.48 ± 0.81 ^b	5.02 ± 1.23 ^c	2.39 ± 0.42 ^a
F	10.76 ± 0.44 ^b	9.97 ± 0.30 ^{ab}	9.14 ± 0.71 ^a	8.94 ± 0.71	6.62 ± 0.95 ^a	7.10 ± 1.07 ^b	5.87 ± 0.51 ^{ab}	3.60 ± 1.07 ^a	3.21 ± 1.14 ^{abc}
G	10.27 ± 0.63 ^{ab}	10.47 ± 0.08 ^b	10.34 ± 0.26 ^b	8.66 ± 0.62	8.27 ± 0.59 ^c	5.95 ± 0.58 ^a	5.54 ± 1.06 ^a	4.35 ± 0.68 ^{abc}	3.97 ± 0.81 ^c
H	10.11 ± 0.27 ^a	9.69 ± 0.37 ^a	8.68 ± 0.89 ^a	8.80 ± 0.44	6.51 ± 0.67 ^a	6.58 ± 0.50 ^{ab}	5.95 ± 1.28 ^{ab}	4.84 ± 1.29 ^{bc}	3.33 ± 0.81 ^{bc}
L	9.89 ± 0.34 ^a	9.66 ± 0.40 ^a	9.30 ± 0.41 ^a	8.28 ± 0.99	7.76 ± 0.83 ^{bc}	nd†	5.84 ± 0.76 ^{ab}	5.12 ± 0.96 ^c	2.50 ± 0.44 ^{ab}
M	9.84 ± 0.47 ^a	9.83 ± 0.49 ^{ab}	9.19 ± 0.44 ^a	8.65 ± 0.07	7.88 ± 0.80 ^{bc}	nd	4.92 ± 0.68 ^a	4.77 ± 0.45 ^{abc}	3.79 ± 0.44 ^c
ANOVA	*	§	§	NS	***	***	§	§	**

Note. ANOVA: NS, no significant differences; § $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Means in the same column not followed by the same superscript letter differ significantly ($P < 0.05$). *Coliforms counts usually matched enterobacteria counts, therefore only coliforms are related. †nd, not detected.

Fig 1. Ammonium concentration in the feces of the different individuals and distribution of the data throughout the samples.

appear in much lower numbers in feces than do strict anaerobes (2, 3, 9). Although less numerous in feces, these populations could be dominant in other parts of the GIT (2, 3). Culture-based analyses have recently been complemented by several culture-independent molecular techniques (20–23) which gave very similar results—at least with respect to the bacterial groups compared.

All populations showed less variation over time (inter-sample variation) than between individuals (interindividual variation). These long-term intersample and interindividual microbial variations were quite in accordance with those described elsewhere in a short-term follow-up

period (24). The greater stability of the numerically predominant anaerobe populations (clostridia, bifidobacteria, and bacteroides) compared to the facultative anaerobes is in agreement with the results of other authors (8, 25, 26).

The most striking difference between the present and previously published results are the consistently low bacteroides counts. It was first believed that excessive selectivity of the EBA medium might account for this, however, similar results were obtained when using a culture-independent technique based on sequence and analysis of 16S rDNA libraries using universal bacterial primers from two selected fecal samples (Delgado and Mayo,

TABLE 2. MODAL VALUES OF ENZYMIC ACTIVITIES IN FECAL SAMPLES MEASURED WITH THE API-ZYM SYSTEM. UNITS OF ACTIVITY ARE EXPRESSED AS MICROMOLES OF SUBSTRATE HYDROLYZED PER HOUR PER GRAM WET WEIGHT OF FECES

Enzymatic activity	Individual							
	A	C	E	F	G	H	L	M
Esterase-lipase (C8)	10	10	10	19	29	29	5	14
Leu-arylamidase	10	5	38	77	10	38	19	31†
Val-arylamidase	10	nd	nd	38	nd	nd	nd	nd
Acid phosphatase	58	29	10	38	29	38	19	29
Naphthol-AS-BI-phosphohydrolase	19	19	10	29	19	19	19	29
α -Fucosidase	10	10	10	10	nd	5	5	8*
Alkaline phosphatase	77	77	77	77	77	77	77	77
Esterase C4	19	10	19	29	38	19	19	19
α -Galactosidase	48	38	19	48	29	29	38	38
β -Galactosidase	77	48	77	38	38	29	58	58
β -Glucuronidase	38	77	19	38	58	38	19	34*
α -Glucosidase	58	67	58	38	29	19	48	58
β -Glucosidase	38	38	38	38	77	48	38	29
N-Acetyl- β -glucosaminidase	77	58	38	58	58	48	58	58

Note. nd, not detected. Lipase C14, cysteine arylamidase, trypsin, α -quimiotrypsin, and α -mannosidase activities were never detected in fecal samples.

*A modal value was not recorded in three samples, so the mean value is presented.

TABLE 3. MEAN AND STANDARD DEVIATION OF THE DIFFERENT SHORT-CHAIN FATTY ACIDS (SCFAs) DETECTED IN FECES: RESULTS OF ONE-WAY ANOVA USING "INDIVIDUAL" AS A FACTOR AND MULTIPLE MEANS COMPARISON BY THE LEAST-SIGNIFICANT DIFFERENCE TEST ($P < 0.05$) ARE ALSO INDICATED

Individual	SCFAs (mmol kg ⁻¹ wet feces)						
	Acetic	Butyric	Propionic	Isobutyric	Valeric	Isovaleric	Caproic
A	61.96 ± 4.7 ^{bc}	12.67 ± 1.6 ^b	20.48 ± 4.1 ^f	nd	1.50 ± 0.5 ^d	0.93 ± 0.5	1.30 ± 1.3
C	63.60 ± 4.8 ^{bc}	22.54 ± 4.3 ^c	8.80 ± 1.5 ^{ab}	0.22 ± 0.5	1.30 ± 0.1 ^{cd}	1.18 ± 0.3	nd
E	52.71 ± 9.1 ^a	13.02 ± 5.1 ^b	11.12 ± 4.1 ^{bc}	0.16 ± 0.3	0.81 ± 0.7 ^{ab}	1.17 ± 0.3	nd
F	55.65 ± 6.7 ^{ab}	20.16 ± 4.0 ^c	18.07 ± 3.8 ^{ef}	nd	1.30 ± 0.2 ^{cd}	1.14 ± 0.5	0.47 ± 0.8
G	69.80 ± 7.4 ^c	15.18 ± 3.4 ^b	13.58 ± 2.8 ^{cd}	nd	1.47 ± 0.4 ^d	0.98 ± 0.2	6.71 ± 2.3
H	58.34 ± 9.6 ^{ab}	15.53 ± 6.5 ^b	11.17 ± 4.6 ^{bc}	0.17 ± 0.9	0.95 ± 0.3 ^{ac}	1.17 ± 0.4	nd
L	51.07 ± 9.9 ^a	5.58 ± 2.9 ^a	5.85 ± 1.7 ^a	nd	0.62 ± 0.2 ^a	0.71 ± 0.1	nd
M	61.52 ± 6.5 ^{bc}	11.74 ± 1.6 ^b	15.72 ± 1.3 ^{de}	nd	1.26 ± 0.4 ^{bcd}	1.30 ± 0.9	nd
ANOVA	**	***	***	NP	**	NS	NP

Note. nd, not detected. ANOVA: NP, not performed (because of the wide dispersion of the data); NS, no significant difference; ** $P < 0.01$, *** $P < 0.001$. Means in the same column not followed by the same superscript letter differ significantly ($P < 0.05$).

unpublished). This suggests that low levels of *Bacteroides*-like bacteria in feces might be an intrinsic characteristic to our subject community. However, this conclusion should be taken with caution given the small number of individuals analyzed.

Ammonium is considered an undesirable bacterial metabolite associated with protein degradation with tumor-promoting action in animal models (5, 19). The literature contains little information of fecal ammonium concentrations (see Refs. 25–27). Moreover, differences between studies may simply reflect differences in the analytical methods used. But as this compound is strongly influenced by diet and feces retention time (28, 29), these two factors may account for the wide spread of the data, as suggested by Ikeda *et al.* (25).

A direct comparison of the enzyme activities reported in this work with others in the literature (25, 30–32) is difficult given the different methodologies used and the semi-quantitative nature of our results. High standard deviations for enzyme activities, however, are constantly seen for β -galactosidase, β -glucuronidase, or urease (25, 30–32). These activities are affected by dietary changes (31, 32), but whether they are also affected by changes in microbial populations is not yet known. The Pearson correlation coefficient calculated with the present data was unclear in this respect (data not shown).

Acetic and butyric acids are thought to be important in the physiology of the GIT, either as energy sources or in the prevention of colon cancer (3, 9, 10, 13). SCFAs are strongly influenced by dietary intake, but the types of microbial populations from the GIT that contribute to their concentrations are currently unknown. Fecal concentrations of acetic, propionic, and butyric acids in our study were within the ranges reported in the literature (9, 10, 25, 27, 33). Moreover, the molar ratios of the three predominant SCFAs obtained in this study were quite similar to

those on the referred reports. However, we could not estimate the total SCFAs produced per individual, because the amount of feces in the samples was not recorded. Furthermore, SCFAs excretion is a dual process involving production and intestinal absorption.

The statistical analysis of both the microbial and the biochemical variables showed the differences between individuals to be statistically significant in most cases. These data are consistent with those of previous reports indicating that each individual harbors unique microbial communities which determine characteristic and unique metabolic profiles (20–23, 25). Some of the variables studied in this work are necessarily related, but Pearson's correlation coefficient for within-individual analysis showed no clear relationships. This further reflects the complexity of the GIT ecosystem and the great number of factors that influence it, including microbial and biochemical variables, environmental variables, and diet- and host-related factors.

A general picture was produced of the composition and changes in the cultured microbial populations of the GIT from some healthy Spanish people. Microbial and biochemical profiles seemed to be individual-dependent. Some variables were rather stable over time, whereas others varied widely. The results of this work could ultimately be used in the development of probiotics, prebiotics, and synbiotics, (by using specific species) and in the study of their effect on the GIT microbial populations. The data could also be of help in the comparison of microbial and biochemical parameters of health and illness stages in Spanish individuals.

ACKNOWLEDGMENTS

This work was supported by a project funded by the Comisión Interministerial en Ciencia y Tecnología (CICYT) del Ministerio

de Ciencia y Tecnología (Reference AGL2000-1474). S. Delgado was the recipient of a fellowship from the Ministerio de Ciencia y Tecnología (Programa FPI). The skillful technical assistance of A. M. Hernández-Barranco in the analysis of short-chain fatty acids is gratefully acknowledged.

REFERENCES

- Conway PL: Microbial ecology of the human large intestine. *In* Human Colonic Bacteria: 4. Role in Nutrition, Physiology, and Pathology. GR Gibson, GT Macfarlane (eds). Boca Raton, FL, CRC Press, 1995, pp 1–24
- Tannock GW: A fresh look at the intestinal microflora. *In* Probiotics. A Critical Review. GW Tannock (ed). Norfolk, UK, Horizon Scientific Press, 1999, pp 5–14
- Guarner F, Malagelada JR: Gut flora in health and disease. *Lancet* 360:512–519, 2003
- Gill HS: Stimulation of the immune system. *Int Dairy J* 8:535–544, 1998
- Parodi PW: The role of intestinal bacteria in the causation and prevention of cancer: modulation by diet and probiotics. *Aust J Dairy Technol* 54:103–121, 1999
- Holdeman LV, Cato EP, Moore WEC: Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl Environ Microbiol* 32:359–375, 1976
- Koornhof HJ, Richardson NJ, Wall MD, Moore WE: Fecal bacteria in South African rural blacks and other population groups. *Isr J Med Sci* 15:335–340, 1976
- Moore WE, Moore LH: Intestinal flora of populations that have a high risk of colon cancer. *Appl Environ Microbiol* 61:3202–3207, 1995
- Cummings JH, Macfarlane GT: Role of intestinal bacteria in nutrient metabolism. *Clinical Nutr* 16: 3–11, 1997
- Scheppach W, Bartram HP, Richter F: Role of short-chain fatty acids in the prevention of colon cancer. *Eur J Cancer* 31A:1077–1080, 1995
- Ziemer CJ, Gibson GR: An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *Int Dairy J* 8:473–479, 1998
- Vaughan EE, de Vries MC, Zoetendal EG, Ben-Amor K, Akkermans ADL, de Vos WM: The intestinal LABs. *Antonie van Leeuwenhoek* 82:341–352, 2002
- Mortensen PB, Clausen MR: Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. *Scand J Gastroenterol* 216:S132–S148, 1996
- Frankel WL, Zhang W, Singh A, Klurfeld MD, Don S, Sakata T, Modlin I, Rombeau JL: Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. *Gastroenterology* 106:375–380, 1994
- Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JL: Molecular analysis of commensal host–microbial relationships in the intestine. *Science* 291:881–884, 2001
- Gibson PR, Moeller I, Kagelari O, Folino M, Young GP: Contrasting effects of butyrate on the expression of phenotypic markers of differentiation in neoplastic and non-neoplastic colonic epithelial cells in vivo. *J Gastroenterol Hepatol* 7:165–172, 1992
- Rowland IR: Toxicology of the colon. Role of intestinal microflora. *In* Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology. GR Gibson, GT Macfarlane (eds). Boca Raton, FL, CRC Press, 1995, pp 155–174
- Bingham SA: High-meat diets and cancer risk. *Proc Nutr Soc* 58:243–248, 1999
- Lin HC, Visek WJ: Large intestinal pH and ammonia in rats: dietary fat and protein interaction. *J Nutr* 121:832–843, 1991
- Wilson KH, Blitchington RB: Human colonic biota studied by ribosomal DNA sequence analysis. *Appl Environ Microbiol* 62:2273–2278, 1996
- Zoetendal EG, Akkermans ADL, de Vos WM: Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol* 64:3854–3863, 1998
- Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD, Doré J: Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl Environ Microbiol* 65:4799–4807, 1999
- Sghir A, Gramet G, Suau A, Rochet V, Pochart P, Doré J: Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. *Appl Environ Microbiol* 66:2263–2266, 2000
- Delgado S, Suárez A, Otero L, Mayo B: Variation of microbiological and biochemical parameters in the faeces of two healthy people over a 15 day period. *Eur J Nutr* 43:375–380, 2004
- Ikeda N, Saito Y, Shimizu J, Ochi A, Mizutani J, Watabe J: Variations in concentrations of bacterial metabolites, enzyme activities, moisture, pH and bacterial composition between and within individuals in faeces of seven healthy adults. *J Appl Bacteriol* 77:185–194, 1994
- Fujiwara S, Seto Y, Kimura A, Hashiba H: Intestinal transit of an orally administered streptomycin-riparfampicin-resistant variant of *Bifidobacterium longum* SBT2928: its long-term survival and effect on the intestinal microflora and metabolism. *J Appl Microbiol* 90:43–52, 2001
- Cummings JH: The Large Intestine in Nutrition and Disease. Brussels, Institute Danone, 1997
- Rowland IR, Mallet AK, Wise A: The effect of diet on the mammalian gut flora and its metabolic activities. *Crit Rev Toxicol* 16:13–103, 1985
- Stephen AM, Wiggins HS, Cummins JH: Effects of changing transit times on colonic microbial metabolism in man. *Gut* 28:601–609, 1987
- Mykkänen H, Laiho K, Salminen S: Variations in faecal bacterial enzyme activities and associations with bowel function and diet in elderly subjects. *J Appl Microbiol* 85:37–41, 1998
- Mallet AK, Rowland IR, Bearne CA, Flint JC, Fehilly BJ, Udeen YS, Farthing MJG: Effect of dietary supplements of apple pectin, wheat bran or fat on the enzyme activity of the human faecal flora. *Microbial Ecol Health Dis* 1:23–39, 1988
- Ling WH, Hänninen O, Mykkänen H, Heikura M, Salminen S, von Wright A: Colonization and fecal enzyme activities after oral *Lactobacillus* GG administration in elderly nursing home residents. *Ann Nutr Metabolism* 36:162–166, 1992
- van Nuenen MHMC., Venema K, van der Woude JCJ, Kuipers EJ: The metabolic activity of fecal microbiota from healthy individuals and patients with inflammatory bowel disease. *Dig Dis Sci* 49:485–491, 2005