Journal of Microbiological Methods 92 (2013) 387–397

Contents lists available at SciVerse ScienceDirect

Journal of Microbiological Methods

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

High-throughput analysis of the impact of antibiotics on the human intestinal microbiota composition

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ARTICLE INFO

Article history:
Received 12 July 2012
Received in revised form 12 December 2012
Accepted 14 December 2012
Available online 22 December 2012

Keywords:
Antibiotics
Intestinal microbiota
In-vitro fermentation screening-platform
Phylogenetic microarray

ABSTRACT

Antibiotic treatments can lead to a disruption of the human microbiota. In this *in-vitro* study, the impact of antibiotics on adult intestinal microbiota was monitored in a new high-throughput approach: a fermentation screening-platform was coupled with a phylogenetic microarray analysis (Intestinal-chip). Fecal inoculum from healthy adults was exposed in a fermentation screening-platform to seven widely-used antibiotics during 24 h *in-vitro* fermentation and the microbiota composition was subsequently determined with the Intestinal-chip.
Phylogenetic microarray analysis was first verified to be reliable with respect to variations in the total number of bacteria and presence of dead (or inactive) cells. Intestinal-chip analysis was then used to identify and compare shifts in the intestinal microbial composition after exposure to low and high dose (1 μg ml^{-1} and 10 μg ml^{-1}) antibiotics. Observed shifts on family, genus and species level were both antibiotic and dose dependent. Stronger changes in microbiota composition were observed with higher doses. Shifts mainly concerned the bacterial groups *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterobacteriaceae*, and *Lactobacillus*. Within bacterial groups, specific antibiotics were shown to differentially impact related species.
The combination of the *in-vitro* fermentation screening platform with the phylogenetic microarray read-outs has shown to be reliable to simultaneously analyze the effects of several antibiotics on intestinal microbiota.

1. Introduction

The human intestinal microbiota, a complex ecosystem mainly dominated by anaerobic bacteria, plays an important role in the health of its host (Guarner and Malagelada, 2003). Under normal conditions, the composition of the microbiota is relatively stable for long periods of time (Zoetendal et al., 1998), but this can change due to external factors, such as antibiotic treatments.

Antibiotics are used to treat specific bacterial infections. However, these agents are also known to kill or inhibit bacteria which are not primarily targeted, such as resident commensal gut microbiota (Brötz-Oesterhelt and Brunner, 2008). The extent of the impact on the non-targeted microbial populations depends on the spectrum of action, the mode of administration (oral vs. intravenous), the dose and the absorption rate (Sullivan, 2001). A disruption of the microbiota due to antibiotic treatments can lead in 5–35% of the cases to Antibiotic-Associated-Diarrhea (AAD), with Amoxicillin and Clindamycin having the highest impact (McFarland, 2008).

Increasingly, changes in the microbiota composition are correlated with health disorders (De La Cochetière et al., 2010; Kang et al., 2010; Zoetendal et al., 2008). Such correlations between microbiota composition and health disorders can be detected from *in-vivo* studies. These studies, however, are rather expensive and have a low-throughput. Moreover, comparison of the outcomes of different studies is difficult since many parameters in the study designs differ. In this perspective, *in-vitro* screening-platforms are considered useful tools to perform multiple fermentations in a high-throughput. Furthermore, to establish correlations between microbiota composition and human gut diseases, it has been suggested to use new high-throughput analytical tools like phylogenetic microarrays (Zoetendal et al., 2008). Such phylogenetic microarray have been developed and validated for e.g. oral and intestinal microbiota (Crielaard et al., 2011; Rajilić-Stojanović et al., 2009). Combining the use of *in-vitro* screening-platforms with intestinal microarray analysis appear to be promising to increase fermentation-throughput and compare straightforwardly the resulting bacterial fingerprints obtained under similar conditions.

Phylogenetic DNA microarrays enable quick determination of microbiota composition. However, the DNA array read-outs might be influenced by factors induced by the antibiotic treatment, such as variation in the total cell number (Bartosch et al., 2004) and presence of nucleic acids derived from dead (or inhibited) cells (Nocker et al.,

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http://dx.doi.org/10.1016/j.mimet.2012.12.011

2009). Reliability of the microarray read-outs still has to be tested on complex human microbiota in case of antibiotic treatments.

In this study, we addressed the use of a phylogenetic microarray to monitor the changes in the human intestinal microbiota after antibiotic treatment during 24 h *in-vitro* fermentation in screening-platforms. First, the reliability of the I-chip readouts was addressed with respect to the variation in cell numbers and presence of dead cells. Secondly, an overview of the impact of seven widely-used antibiotics on the microbiota composition was shown under comparable conditions.

2. Materials and methods

2.1. Antibiotics

Amoxicillin (AMX) (≥90%), Azithromycin (AZM) (≥98% HPLC), Cefadroxil (CFR), Ciprofloxacin (CIP) (≥98% HPLC), Clindamycin hydrochloride (CLI) (≤2 mol/mol EtOH), Doxycycline (DOX) (≥98% TLC), and Erythromycin (ERY) (≥85%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Their characteristics are presented in Table 1.

2.2. Microarray construction and validation of the intestinal microbiota representing microarray

The intestinal microbiota representing microarray was constructed as described for the oral microbiota microarray by Crielaard et al. (2011). Instead of primers for oral bacterial species, primers for intestinal bacterial species were selected based on scientific literature, sequence databases and 454 sequencing of fecal material, resulting in a DNA based microarray enabling the detection of more than 400 bacterial targets from the human large intestinal microbiota. The selected targets included primers that are specific at family, genus and species level. Several groups of bacteria, e.g. *Bacteroides*, *Bifidobacteria*, *Enterobacteriaceae*, *Clostridia* and *Lactobacillus* are targeted. These groups are known to be the main bacterial groups in the human intestinal microbiota (Zoetendal et al., 2006). The intestinal microarray (I-chip) performance was validated for the same criteria as mentioned by Crielaard et al. (2011).

2.3. Experimental set up and sampling

Intestinal microbiota were cultured by *in-vitro* fermentations in microtiterplate (96 wells—1.5 ml volume per well). The culture medium was based on the modified standard ileal efflux medium (SIEM) composition (Minekus et al., 1999) and modified as follow (g l^{-1}): pectin (0.047), xylan (0.047), arabinogalactan (0.047), amylopectin (0.047), starch (0.392), casein (24.0), Tween 80 (17.0), bactopepton (24.0), ox-bile (0.4) and cysteine (0.2). All medium components were provided by Tritium Microbiology (Veldhoven, The Netherlands). The pH was adjusted to 5.8.

A standardized pool of adult fecal inoculum was prepared as validated by Minekus et al. (1999). This pool approach was especially relevant in our study since it limited inter-individual variations and increased the probability to have a larger representation of potential bacterial species in the human colon. The fecal samples used to produce the standardized inoculum were from eight healthy European adults (25–45 years old) who neither received antibiotic treatments in the 2 months before donation nor consumed prebiotics or probiotics the week before donation. After storage at −80 °C in 12% glycerol, the standardized fecal inoculum was incubated in the adapted SIEM under anaerobic conditions overnight (37 °C; 300 rpm) in order to activate the bacteria. This pre-culture step was found not to significantly modify the microbiota composition and activity as determined by I-chip analysis (data not shown).

For antibiotic exposure experiments, SIEM, antibiotics (1 μg ml^{-1} or 10 μg $ml^{-1)}$ and pre-cultured inoculum (0.1% v/v) were mixed in each well. The fermentation was conducted under anaerobic conditions at 37° for 24 h. Inoculated SIEM without antibiotic was used as a blank. Each specific fermentation condition was performed 5 times. After 24 h of fermentation, collected samples were split in three parts. One part was directly stored at −20 °C for DNA isolation. A second part was immediately treated with propidium monoazide (PMA) (Biotium, Hayward, CA, USA) as described by Nocker et al. (2009) with a final concentration of 50 μM, and stored at −20 °C. The third part was directly stored at −80 °C for RNA isolation.

Table 1
Characteristics of antibiotics used including class, type, mode of action and impact on Antibiotic-Associated-Diarrhea (AAD).

Class	*Name*	*Type*	*Mode of action*	*AAD[a] frequency*
Penicillin	Amoxicillin	Bacteriolytic	Inhibition peptidoglycan biosynthesis[c]	High[d]
Tetracycline	Doxycycline	Bacteriostatic	Translation inhibition[c]	Low[d]
Macrolide-lincosamide	Erythromycin	Bacteriostatic	Translation inhibition[c]	Low[b]
	Azithromycin	Bacteriostatic	Translation inhibition[c]	Low[b]
	Clindamycin	Bacteriostatic	Translation inhibition[c]	High[d]
Fluroquinolone	Ciprofloxacin	Bacteriolytic	Inhibition of peptidoglycan biosynthesis[e]	Medium/ High[d]

[a] AAD: Antibiotic-Associated-Diarrhea.
[b] Bartlett (2002).
[c] Brötz-Oesterhelt and Brunner (2008).
[d] McFarland (2008).
[e] Weil et al. (1995).

2.4. DNA isolation

Total fecal DNA from collected samples was isolated as described by Crielaard et al. (2011) with some minor adjustments: The samples were initially mixed with 250 μl lysis buffer (Agowa, Berlin, Germany), 250 μl zirconium beads (0.1 mm), and 200 μl phenol, before being introduced to a BeadBeater (BioSpec Products, Bartlesville, OK, USA) for twice 2 min.

2.5. Fecal RNA isolation and cDNA synthesis.

Next to DNA isolation, RNA isolation was necessary to investigate the activity of bacteria present in the samples. Isolation of RNA and cDNA synthesis was carried out for one replicate out of 5. RNA isolation through bead beating in phenol/chloroform extractions was performed following the protocol described by Kort et al. (2008). Isolated RNA was purified from DNA using the Invitrogen Kit (Invitrogen, Breda, The Netherlands) with a modified buffer (100 mM Tris/HCl pH7.5, 25 mM $MgCl_2$, 2.5 mM $CaCl_2$). RNA purity and concentration were determined on a 1.5% (w/v) agarose gel stained with ethidium-bromide (Sigma-Aldrich).

DNA copies (cDNA) were synthetized by incubating 2.5 μl RNA for 5 min at 60 °C with 10 μl anneal mix containing 5 μl dNTPs [2 mM] (Invitrogen), 0.1 μl RNAsin (Promega, Leiden, The Netherlands), 23 nl 1061-R primer (TCA CGR CAC GAG CTG ACG AC), 0.15 μl 0.1 M DTT (Invitrogen) and RNAse free water. After cooling the samples on ice, 8 μl RT mix containing 1 μl RNAsin (10 Uμl^{-1}, Promega), 4 μl First Strand Buffer 5× (Invitrogen), 2 μl 0.1 M DTT (Invitrogen) and 0.5 μl Superscript II enzyme (200 Uμl^{-1}, Invitrogen) were added. The reverse transcription took place at 42 °C during 2 h. Samples were inactivated at 70 °C for 10 min and stored at −20 °C. cDNA was amplified and labeled with PCR as described further.

2.6. PCR amplification and PCR product purification

Significant amounts of DNA or cDNA are needed for analysis on the microarray. A multiplex PCR was therefore performed on each sample (DNAs and cDNAs) with a 25 μl reaction mixture containing 12.5 μl 2× Multiplex PCR mix (Qiagen GmbH, Hilden, Germany), 0.5 μl of 16s-8-F/unibifi [25–2.5 pmol μl^{-1}] (AGA GTT TGA TCH TGG YTC AG/ TGG CTC AGG ATG AAC GCT G), 1 μl 16s-1061-R [25 pmol μl^{-1}] (TCA CGR CAC GAG CTG ACG AC), 0.25 μl Entero(Hsp60)-F-[25 pmol μl^{-1}] (GGT AGA AGA AGG CGT GGT TGC), 0.5 μl Entero(Hsp60)-R-[25 pmol μl^{-1}] (ATG CAT TCG GTG GTG ATC ATC AG), 5 μl of isolated DNA and 5.25 μl milli-Q water. The forward primers contained a 5′ phospho modification while the reverse primers contained a 5′–C6 Cy3 modification. Only 16s-8-F/unibifi and 16s-1061-R were used to amplify cDNAs. The program used for amplification was as follows: 94 °C for 15 min, 30 cycles of 94 °C for 30 s, 50 °C for 90 s, 72 °C for 80 s, 1 cycle of 72 °C for 2 min and cooled to 4 °C. The PCR products were analyzed on a 1.2% agarose gel (100 V; 45 min) and stained with Serva-G (SERVA Electrophoresis, Heidelberg, Germany).

The PCR products from DNA were purified using a SigmaSpin Post-Reaction Clean-up plate (Sigma-Aldrich) while the PCR products from cDNA were purified using autoseq G50 columns (GE Healthcare), as described by the manufacturers. The samples obtained were dried by vacuum centrifugation at 60 °C. A mixture of 0.5 μl lambda exonuclease (BioLabs inc. Frankfurt, Germany), 2 μL lambda exonuclease buffer and 17.5 μL water was added. Incubation took place for 30 min at 37 °C, and inactivation during 10 min at 75 °C. DNA was purified again with a SigmaSpin-2-Post-Reaction Clean-up plate (Sigma-Aldrich) and dried. The single-stranded products were analyzed on a 1.2% agarose gel (100 V; 45 min) and stained with Serva-G (SERVA Electrophoresis).

2.7. Hybridization

Hybridization of the PCR products on the I-chip was performed as described by Crielaard et al. (2011) with minor changes. Dried single-strand DNA was suspended in 45 μl DIG Easyhyb (Roche, Basel, Switzerland) for 20 min at 37 °C and denatured for 2 min at 95 °C before being placed on the pre-warmed microarray. Hybridization, cleaning steps and scanning were carried out according to the described procedure.

2.8. Data analysis

Imagene 5.6 software (BioDiscovery, Marina del Rey, CA, USA) was used to analyze the results. Signals were quantified by calculating the mean of all pixel values of each spot and calculating the local background around each spot. For each spot a signal to background ratio (S/B), namely signal intensity, was calculated and used for further analysis. Only the spots with a S/B ratio larger than two were used for further analysis. This cut-off was selected based on the observation that negative control spots never resulted in signals above this cut-off (data not shown). When comparing data from all experiments, the minimal number of observations higher than three times above its local background for each spot was set to 10. This criterion was mainly used to discard data resulting from technical noise. The data matrix (116 targets out of 400) was analyzed with Significant Analysis Microarray (SAM) to identify markers significantly different between predefined groups programs (TM4 software) and with a hierarchical clustering based on Euclidian distances. A Principal Component Analysis (PCA) was performed using the TM4 software to investigate the correlation among the bacterial fingerprints (Saeed et al., 2003).

2.9. Quantitative PCR

Quantitative PCR was performed to investigate the variation in the total amount of bacteria after different antibiotic exposures. Quantification of the total amount of DNA present in the samples was performed using the universal primers 16S-uni-II-F [10 pmol μl^{-1}] (TCCTACGG GAGGCAGCAGT) and 16S-uni-II-R [10 pmol μl^{-1}] (GGACTACCAGGGT ATCTAATCCTGTT), and probe 16S-uni-II [5 μM] (6FAM-CGTATTAC CGCGGCTGCTGGCAC-TAMRA) (Applied Biosystems, Bleiswijk, The Netherlands) The amplification was performed with 5 μl DNA sample and 25 μl q-PCR mixture that contained 15 μl 2× FastStart Universal Probe Mastermix (Roche, Mannheim, Germany), 1 μl of each primers and probe, and 7 μl MilliQ water. Total microbial fecal DNAs were diluted 1:10 before use in the q-PCR assay.

The experiment was performed using the 7500 Fast Real Time PCR system (Applied Biosystems) at the following settings: 1 step of 2 min at 50 °C, 1 step of 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Dilution of the control microbiota was used as quantitative standards (5 fg μl^{-1} to 5 ng μl^{-1}).

2.10. Corrections of the I-chip readout

To address the reliability of the I-chip readouts with respect to the variation in cell numbers, the signal intensity (S/B) obtained directly from the I-chip needed to be corrected based on the variation of total cells in the samples. According to literature, the relative signal intensity of each target [1] (S/B of one target: total S/B) is directly proportional to the relative quantitative changes of the target (Rajilić-Stojanović et al., 2009). Because of this correlation, a correction factor [2] was calculated in our experiment on the basis of the total number of bacteria measured with qPCR (total number of bacteria in one sample: total number of bacteria in the control) and applied to the relative signal intensity of each target to calculate the corrected relative signal [3]:

$$\begin{aligned}\text{Corrected relative signal intensity [3]} &= \text{Relative signal intensity[1]} \times \text{Correction factor[2]} \\ &= \frac{\text{Signal intensity of one target}}{\text{Total signal intensity}} \times \frac{\text{Total number of bacteria in one sample}}{\text{Total number of bacteria in the control}}\end{aligned}$$

This correction factor enabled comparison of the samples in absolute abundance per target.

2.11. Statistical analysis

To evaluate whether two qPCR data sets were significantly different, a Student t-test was performed. P-values were calculated assuming equal variance and two-tailed distribution. Correlations were considered significant at a P-value lower than 0.001.

3. Results & discussion

In order to investigate the impact of antibiotics on a healthy adult intestinal microbiota, *in-vitro* fermentations were performed using a screening-platform, allowing up to 96 experimental variations at once in 1.5 ml volumes. Downscaling the fermentation did not influence the outcomes regarding the impact of antibiotics on the microbiota as similar results were observed in fermentation flasks of 120 ml (data not shown).

Seven antibiotics widely-used in The Netherlands (NETHMAP, 2009) and in Europe (ESAC Yearbook, 2009) were selected based on their classification and their mode of action (Table 1). A low dose (1 μg ml^{-1}) and a high dose (10 μg ml^{-1}) antibiotic were selected on the basis of a dose-series test performed on the screening platform. The low dose influenced either gram positive or gram negative bacteria, while the high dose mostly influenced both gram positive and gram negative bacteria (data not shown). This high dose, however, did not suppress all bacteria allowing a recovery of the non-affected bacteria during the fermentation time. The actual antibiotic concentrations used in the experiments were below the concentrations that could reach the colon considering an adult receiving 0.5 g to 1 g antibiotic per day and an absorption rate of 70 % to 90% (50 mg ml^{-1} to 300 mg ml^{-1}). The two selected doses were, however, in the range of

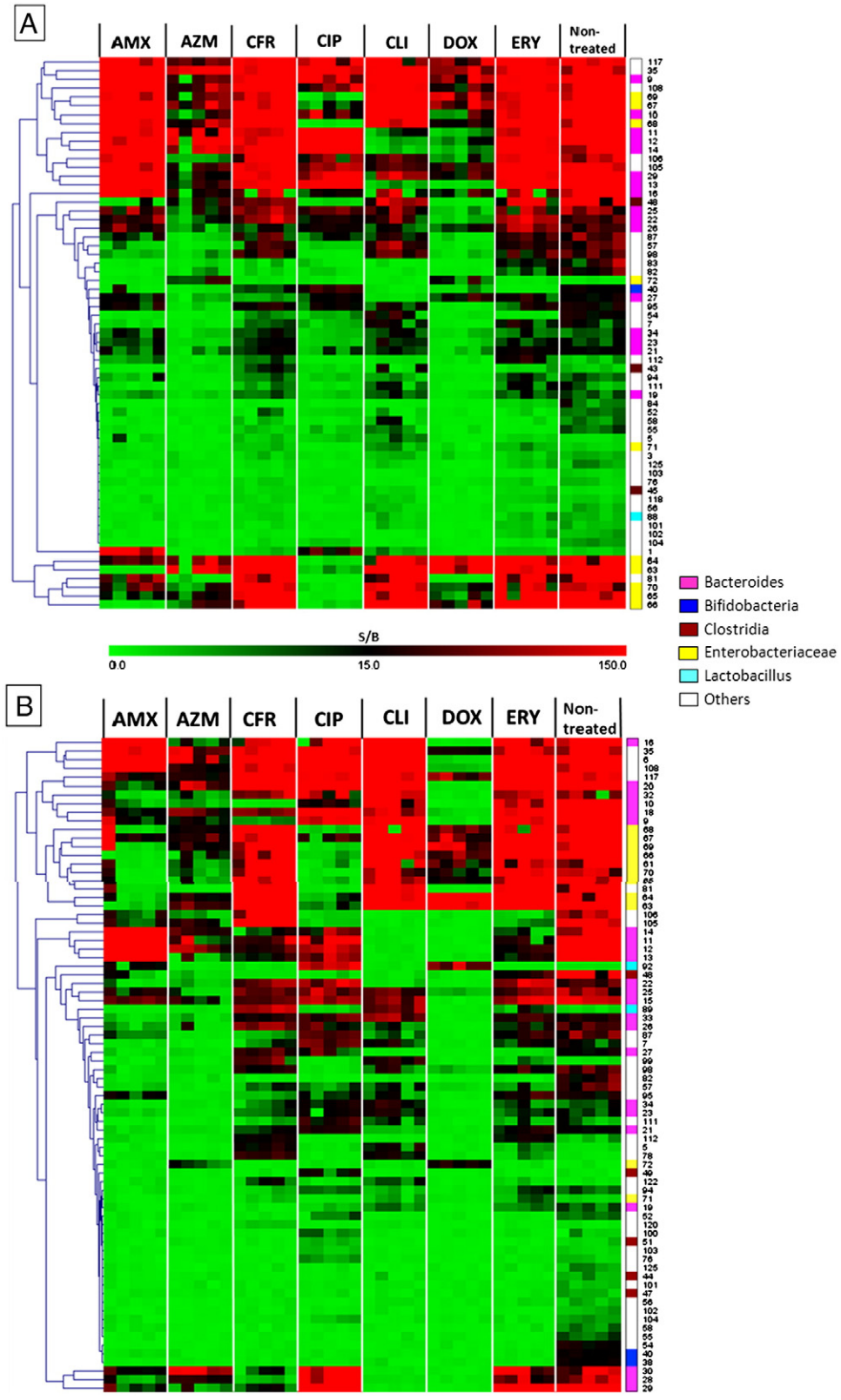

Fig. 1. Bacterial fingerprints of the non-treated and antibiotic-treated adult inoculum obtained with the I-Chip after 24 h *in-vitro* fermentation. Seven antibiotics were used in concentrations of 1 μg ml^{-1} (A.) and 10 μg ml^{-1} (B). Targets presented in the figures have intensities which were found to be significantly different among sets of samples (sets based on treatment) by SAM analysis (TM4 software). Main group of bacteria are highlighted in color and full name of targets can be found in Table 2 based on their numbering. Signal compared to the background (S/B): Green: below detectable level, Black: medium abundance, Red: high abundance.

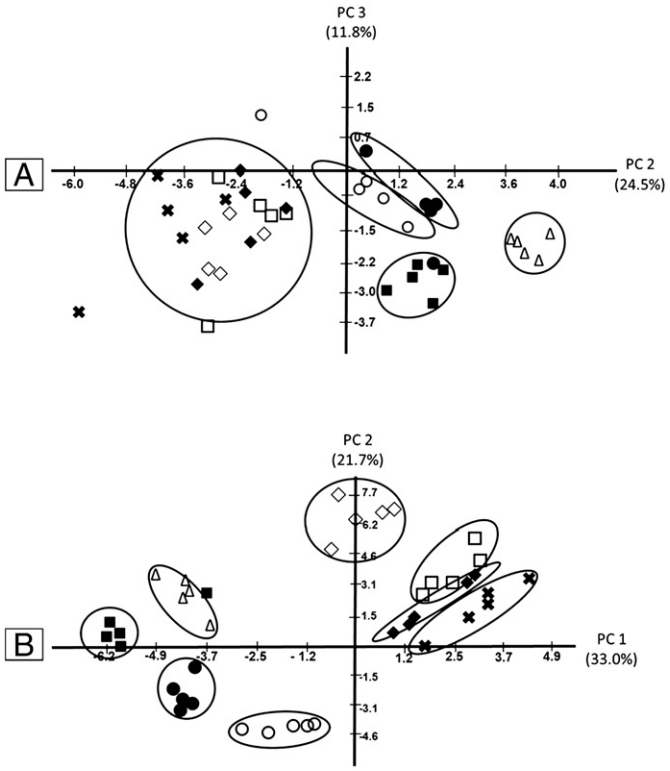

Fig. 2. Representation of the non-treated or antibiotic-treated samples in the plane defined by two principal components resulting from a PCA of bacterial fingerprints obtained with the I-Chip after 24 h *in-vitro* fermentation using adult inoculum. Seven antibiotics were used in a concentration of 1 μg ml^{-1} (A) or 10 μg ml^{-1} (B) : AMX (■), AZM (●), CFR (♦), CIP (Δ), CLI (✖), DOX (○), ERY (□), non-treated (◊).

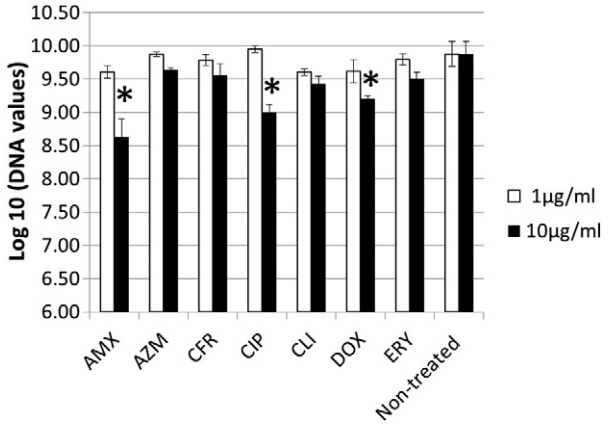

Fig. 3. Total amount of DNA in non-treated and antibiotic-treated inoculum from healthy adults after 24 h *in-vitro* fermentation measured with qPCR. Antibiotics were applied in concentration of 1 μg ml^{-1} □ or 10 μg ml^{-1} ■. Standard deviation (n = 5) is shown with the error bars. * Significant difference versus the non-treated microbiota ($p<0.001$).

MIC-values of currently used antibiotics (EUCAST breakpoints) and relevant for *in-vitro* fermentations with 0.1% fecal inoculum.

After 24 h fermentation, the changes in the microbiota composition due to 1 μg ml^{-1} and 10 μg ml^{-1} antibiotic treatments were monitored with a phylogenetic microarray, the I-chip (Fig. 1). The reproducibility of the impact of antibiotics (n = 5), as determined by Pearson's correlation (r), was good (r = 0.8) to very good (r = 0.98). The level of impact of the antibiotics was shown by a principal component analysis (Fig. 2). For the 1 μg ml^{-1} treatments, data for cefadroxil, clindamycin and erythromycin predominantly clustered with the non-treated samples, indicating minor changes in the bacterial fingerprints. Data for amoxicillin, azithromycin, ciprofloxacin and doxycycline clustered separately from the non-treated samples, hence a stronger impact on the microbiota was present. For the 10 μg ml^{-1} treatments, 8 separate clusters could be distinguished, with each antibiotic treatment resulting in a separate cluster and all antibiotics clearly differing from the control situation. These results thus show the strong impact of these antibiotics on the microbiota composition.

A more detailed description of the impact of each individual antibiotic on microbiota composition is presented later, but first the influence of a potential experimental bias due to variations in total bacterial cell numbers and the influence of nucleic acids derived from dead (or inhibited) cells present in the antibiotic treated samples are addressed.

3.1. Reliability of the I-chip readouts when using antibiotics

3.1.1. Influence of the variations in total cell numbers

In order to investigate the influence of variations in total bacterial cell numbers on the I-chip readout, the total number of bacteria after 24 h fermentation with antibiotics was determined with qPCR. Fig. 3 shows that the total number of cells was significantly reduced in case of 10 μg ml^{-1} of amoxicillin, ciprofloxacin and doxycycline ($p<0.001$). In these cases, relative comparisons between I-chip readouts from different treated-samples might lead to misinterpretations and, as a result, conclusions about changes in abundance might be incorrect.

Table 2 deals with the hypothesis that relative values from a microarray might not be comparable in case of antibiotic treatment as the total number of bacteria may differ per sample. Based on the qPCR measurements of the total bacteria numbers, the signal intensity (S/B) obtained directly from the I-chip was corrected as described in the Material and method section. Treatment with 10 μg ml^{-1} doxycycline was taken as an example. The three obtained readouts (S/B, relative S/B [1] and corrected relative S/B [3]) showed similar trends of changes in the microbiota composition. Therefore, modification of the microarray data is concluded not to be necessary. Similar conclusions were drawn for the 10 μg ml^{-1} amoxicillin and ciprofloxacin treatments (data not shown).

3.1.2. Influence of nucleic acids derived from dead or inactive cells

I-chip results were based on isolated DNA. DNA derived from both dead and inactive cells was potentially present after antibiotic-treatments and this could result in false positive results in our experiments. Hence, discrimination between dead and viable cells and between active and inactive cells was supportive in drawing reliable conclusions.

To discriminate dead from viable cells, a treatment with propidium monoazide (PMA) was applied on replicates of the collected samples. PMA can bind to DNA in case the bacterial membrane is permeable (indicative for dead cells), and thereby inhibiting PCR amplification (Nocker et al., 2009). The viability of cells was not checked on culturing plate since 50% to 90% of anaerobic bacteria from fecal sample are not cultivable. Culture independent methods are preferred for this type of analysis (Zoetendal et al., 1998). Results from PMA-treated samples were compared to the corresponding non-PMA-treated samples. Differences between the two sets of samples were detected neither with qPCR on the total cell numbers (Fig. 3) nor with I-chip on the microbiota composition (fingerprints similar to the ones observed in Fig. 1). Therefore, potentially present dead cells after antibiotic-treatments did not influence microarray measurements after 24 h *in-vitro* fermentation. This conclusion is in contrast with results from previous studies (Kobayashi et al., 2010; Nocker et al., 2009). However, Kobayashi et al. (2010) focused on specific species, which do not recover from the antibiotic treatment, while Nocker et al. (2009) performed measurements shortly after antibiotic addition and not after 24 h fermentation.

Table 2
Impact of doxycycline (10 μg ml^{-1}) after 24 h *in-vitro* fermentation on bacterial groups of an adult inoculum as evaluated with the signal intensity, the relative signal intensity and the corrected relative intensity.

				Signal to Background (S/B)		Relative S/B [1]		Corrected relative S/B [3]	
								Correction factor [2]	0.91
	Family	Genus	Species	Average	StDev	Average	StDev	Average	StDev
1	Alcaligenaceae	unclassified_ Burkholderiales		1.2	0.3	0.1	0.0	0.1	0.0
2	Archaea_domain			2.9	0.6	0.2	0.0	0.2	0.0
3	Archea			2.2	0.4	0.2	0.0	0.2	0.0
4	Archea			1.4	0.2	0.1	0.1	0.1	0.0
5	Bacillaceae	Bacillus	group	1.2	0.2	0.1	0.0	0.1	0.0
6	Bacteriales_order			2.3	0.6	0.2	0.0	0.2	0.0
7	Bacteriales_order			1.3	0.2	0.1	0.0	0.1	0.0
8	Bacteriales_order			1.2	0.1	0.1	0.0	0.1	0.0
9	Bacteroidaceae	Bacteroides	dorei	1.6	0.5	0.1	0.0	0.1	0.0
10	Bacteroidaceae	Bacteroides	dorei	1.1	0.2	0.1	0.0	0.1	0.0
11	Bacteroidaceae	Bacteroides	fragilis	1.2	0.2	0.1	0.0	0.1	0.0
12	Bacteroidaceae	Bacteroides	fragilis	1.1	0.2	0.1	0.0	0.1	0.0
13	Bacteroidaceae	Bacteroides	fragilis	1.4	0.3	0.1	0.0	0.1	0.0
14	Bacteroidaceae	Bacteroides	fragilis/uncultured	1.1	0.1	0.1	0.0	0.1	0.0
15	Bacteroidaceae	Bacteroides	group	1.1	0.2	0.1	0.0	0.1	0.0
16	Bacteroidaceae	Bacteroides	group	1.3	0.2	0.1	0.0	0.1	0.0
17	Bacteroidaceae	Bacteroides	massiliensis	1.3	0.2	0.1	0.0	0.1	0.0
18	Bacteroidaceae	Bacteroides	ovatus	2.3	0.7	0.2	0.0	0.2	0.0
19	Bacteroidaceae	Bacteroides	stercoris	1.1	0.1	0.1	0.0	0.1	0.0
20	Bacteroidaceae	Bacteroides	thetaiotaomicron	1.6	0.2	0.1	0.0	0.1	0.0
21	Bacteroidaceae	Bacteroides	thetaiotaomicron	1.4	0.2	0.1	0.1	0.1	0.0
22	Bacteroidaceae	Bacteroides	thetaiotaomicron	1.2	0.2	0.1	0.0	0.1	0.0
23	Bacteroidaceae	Bacteroides	thetaiotaomicron	1.3	0.2	0.1	0.0	0.1	0.0
24	Bacteroidaceae	Bacteroides	uncult	1.2	0.3	0.1	0.0	0.1	0.0
25	Bacteroidaceae	Bacteroides	uncultured	4.0	0.9	0.3	0.1	0.3	0.1
26	Bacteroidaceae	Bacteroides	uncultured	1.1	0.1	0.1	0.0	0.1	0.0
27	Bacteroidaceae	Bacteroides	uncultured	1.2	0.0	0.1	0.0	0.1	0.0
28	Bacteroidaceae	Bacteroides	uniformis	1.4	0.3	0.1	0.0	0.1	0.0
29	Bacteroidaceae	Bacteroides	uniformis	1.2	0.2	0.1	0.0	0.1	0.0
30	Bacteroidaceae	Bacteroides	uniformis	1.3	0.2	0.1	0.0	0.1	0.0
31	Bacteroidaceae	Bacteroides	uniformis	1.4	0.3	0.1	0.0	0.1	0.0
32	Bacteroidaceae	Bacteroides	xylanisolvens	1.4	0.4	0.1	0.1	0.1	0.1
33	Bacteroidaceae	Bacteroides	xylanisolvens/ finegoldii	1.2	0.1	0.1	0.0	0.1	0.0
34	Bacteroidaceae	Bacteroides	xylanisolvens/finegoldii	1.1	0.2	0.1	0.0	0.1	0.0
35	Bacteroidetes_phylum			25.5	3.5	2.2	0.5	2.1	0.4
36	Bifidobacteriaceae	Bifidobacterium	catenulatum / angulatum/ pseudocatenulatum	1.2	0.1	0.1	0.0	0.1	0.0
37	Bifidobacteriaceae	Bifidobacterium	gallinarum	3.9	6.1	0.3	0.5	0.3	0.4
38	Bifidobacteriaceae	Bifidobacterium	longum	1.3	0.2	0.1	0.0	0.1	0.0
39	Bifidobacteriaceae	Bifidobacterium	longum	1.1	0.1	0.1	0.0	0.1	0.0
40	Bifidobacteriaceae	Bifidobacterium	species	1.2	0.2	0.1	0.0	0.1	0.0
41	Carnobacteriaceae	Carnobacterium	divergens	1.1	0.1	0.1	0.0	0.1	0.0
42	class "Clostridia"			1.2	0.2	0.1	0.0	0.1	0.0
43	Clostridiaceae	Clostridium	carnis	1.3	0.5	0.1	0.1	0.1	0.1
44	Clostridiaceae	Clostridium	carnis/ tertium/ sardiniense/ vincentii	1.2	0.1	0.1	0.0	0.1	0.0
45	Clostridiaceae	Clostridium	disporicum	1.2	0.1	0.1	0.0	0.1	0.0
46	Clostridiaceae	Clostridium	disporicum	1.1	0.1	0.1	0.0	0.1	0.0
47	Clostridiaceae	Clostridium	group	1.3	0.3	0.1	0.0	0.1	0.0
48	Clostridiaceae	Clostridium	group (7)	1.3	0.5	0.1	0.0	0.1	0.0
49	Clostridiaceae	Clostridium	group 2	1.2	0.1	0.1	0.0	0.1	0.0
50	Clostridiaceae	Clostridium	paraputrificum	1.5	0.2	0.1	0.1	0.1	0.1
51	Clostridiaceae	Clostridium	uncultured	1.1	0.1	0.1	0.0	0.1	0.0
52	Clostridiales_order			1.2	0.4	0.1	0.0	0.1	0.0
53	Clostridiales_order			1.2	0.0	0.1	0.0	0.1	0.0
54	Coriobacteriaceae	Collinsella	aerofaciens	1.3	0.4	0.1	0.0	0.1	0.0
55	Coriobacteriaceae	Collinsella	aerofaciens	1.2	0.3	0.1	0.0	0.1	0.0
56	Coriobacteriaceae	Olsenella	profusa	1.2	0.2	0.1	0.0	0.1	0.0
57	Coriobacteriaceae			2.1	0.5	0.2	0.0	0.2	0.0
58	Coriobacteriaceae			1.1	0.1	0.1	0.0	0.1	0.0
59	Desulfovibrionaceae	Desulfovibrio		1.2	0.2	0.1	0.0	0.1	0.0
60	Enterobacteriaceae	Cronobacter	sakazakii/ turicensis	1.3	0.2	0.1	0.0	0.1	0.0
61	Enterobacteriaceae	E.coli/ Shigella		48.9	28.9	3.6	1.9	3.4	1.8
62	Enterobacteriaceae	Enterobacter	cloacae/ asburiae	1.1	0.2	0.1	0.0	0.1	0.0
63	Enterobacteriaceae	Escherichia /shigella	E.coli/shigella	245.0	13.3	18.9	2.4	18.1	2.7

below detectable level	<2	<0,1 %	<0,1 %
low abundance	2< <4	0,1% < < 0,2%	0,1% < < 0,2%
medium abundance	4 < < 50	0,2% < < 4%	0,2% < < 4%
high abundance	>50	>4%	>4%

Table 2 (*continued*)

				Signal to Background (S/B)		Relative S/B [1]		Corrected relative S/B [3]	
								Correction factor [2]	0.91
	Family	Genus	Species	Average	StDev	Average	StDev	Average	StDev
64	Enterobacteriaceae	Escheriachia /shigella	E.coli/shigella	246.9	13.8	21.5	5.4	20.1	4.6
65	Enterobacteriaceae	Escheriachia /shigella		21.9	6.1	1.8	0.3	1.7	0.3
66	Enterobacteriaceae	Escherichia /shigella	E.coli/ Shigella	49.2	9.4	4.6	1.7	4.3	1.5
67	Enterobacteriaceae	Escherichia /shigella	E.coli/shigella	48.3	24.5	4.0	1.2	3.7	1.2
68	Enterobacteriaceae			65.6	24.7	5.9	3.2	5.5	2.8
69	Enterobacteriaceae			136.1	90.3	10.4	5.6	9.9	5.4
70	Enterobacteriaceae			20.0	13.2	1.7	1.0	1.6	0.9
71	Enterobacteriaceae			1.5	0.2	0.1	0.0	0.1	0.0
72	Enterobacteriaceae			25.2	8.1	2.2	1.0	2.1	0.9
73	Enterobacteriaceae			1.3	0.3	0.1	0.0	0.1	0.0
74	Erysipelotrichaceae	Coprobacillus		1.3	0.2	0.1	0.0	0.1	0.0
75	Erysipelotrichaceae	Turicibacter		1.4	0.3	0.1	0.0	0.1	0.0
76	Erysipelotrichaceae			1.1	0.1	0.1	0.0	0.1	0.0
77	Erysipelotrichaceae			1.6	0.1	0.1	0.0	0.1	0.0
78	Fibrobacteraceae	Fibrobacter	succinogenes	1.2	0.3	0.1	0.0	0.1	0.0
79	Fusobacteriaceae	Fusobacterium	group	1.3	0.1	0.1	0.0	0.1	0.0
80	Fusobacteriaceae	Fusobacterium		7.1	5.9	0.5	0.4	0.5	0.4
81	Gammaprotein_class			1.2	0.2	0.1	0.0	0.1	0.0
82	Incertae Sedis XI	Peptoniphilus	harei	0.9	0.3	0.1	0.0	0.1	0.0
83	Incertae Sedis XI	Peptoniphilus	harei	1.2	0.1	0.1	0.0	0.1	0.0
84	Incertae Sedis XI	Peptoniphilus	harei	1.1	0.1	0.1	0.0	0.1	0.0
85	Lachnospiraceae	Incertae Sedis	xylanophilum	2.1	1.9	0.2	0.1	0.2	0.1
86	Lachnospiraceae	Roseburia	cecicola/intestinalis	1.1	0.1	0.1	0.0	0.1	0.0
87	Lachnospiraceae	unclassified		1.2	0.1	0.1	0.0	0.1	0.0
88	Lactobacillaceae	Lactobacillus	acidophilus	1.3	0.3	0.1	0.1	0.1	0.1
89	Lactobacillaceae	Lactobacillus	brevis / hammesii/ parabrevis	1.2	0.2	0.1	0.0	0.1	0.0
90	Lactobacillaceae	Lactobacillus	group	1.2	0.1	0.1	0.0	0.1	0.0
91	Lactobacillaceae	Lactobacillus	group 6	1.2	0.1	0.1	0.0	0.1	0.0
92	Lactobacillaceae	Lactobacillus	johnsonii / gasseri / taiwanensis	79.3	36.0	6.6	2.2	6.2	2.1
93	Lactobacillaceae	Lactobacillus	plantarum/ paraplantarum/ coryniformis/ pentosus	30.5	13.6	2.6	1.0	2.5	1.0
94	Leuconostocaceae	Leuconostoc	group 2	1.3	0.1	0.1	0.0	0.1	0.0
95	Leuconostocaceae			1.3	0.2	0.1	0.0	0.1	0.0
96	Micrococcaceae	Arthrobacter	globiformis	1.5	0.2	0.1	0.0	0.1	0.0
97	Moraxellaceae	Acinetobacter	group	2.3	1.3	0.2	0.1	0.2	0.1
98	Nitrospiraceae			3.8	1.2	0.3	0.1	0.3	0.1
99	order "Lactobacillales"			1.1	0.2	0.1	0.0	0.1	0.0
100	order Bacillale			1.2	0.2	0.1	0.0	0.1	0.0
101	order Clostridiale			1.3	0.3	0.1	0.0	0.1	0.0
102	Peptococcaceae	Peptococcus	uncultured	1.2	0.1	0.1	0.0	0.1	0.0
103	Peptostreptococcaceae	Sporacetigenium		1.1	0.1	0.1	0.0	0.1	0.0
104	Peptostreptococcaceae			1.4	0.3	0.1	0.0	0.1	0.0
105	Porphyromonadaceae	Parabacteroides	distasonis	1.2	0.3	0.1	0.0	0.1	0.0
106	Porphyromonadaceae	Parabacteroides	uncultured/ distasonis	1.2	0.4	0.1	0.0	0.1	0.0
107	Porphyromonadaceae	Parabacteroides	uncultured/ distasonis	1.2	0.1	0.1	0.0	0.1	0.0
108	Prevotellaceae	Prevotella	group	3.7	0.6	0.3	0.1	0.3	0.1
109	Prevotellaceae	Prevotella	group 3	1.1	0.2	0.1	0.0	0.1	0.0
110	Prevotellaceae	Prevotella	group 5	1.2	0.1	0.1	0.0	0.1	0.0
111	Prevotellaceae	Prevotella	group 7	1.2	0.1	0.1	0.0	0.1	0.0
112	Rikenellaceae	Alistipes	onderdonkii/shahii	1.2	0.3	0.1	0.0	0.1	0.0
113	Ruminococcaceae	group		1.0	0.3	0.1	0.0	0.1	0.0
114	Ruminococcaceae	Ruminococcus	albus	1.2	0.2	0.1	0.0	0.1	0.0
115	Ruminococcaceae	Ruminococcus	flavefaciens/ callidus	1.4	0.5	0.1	0.0	0.1	0.0
116	Ruminococcaceae	unclassified		1.3	0.2	0.1	0.1	0.1	0.0
117	Sphingobacteriaceae			74.8	39.1	6.1	2.6	5.7	2.6
118	Sphingobacteriales	Chitinophaga		1.3	0.3	0.1	0.0	0.1	0.0
119	Staphylococcaceae	Staphylococcus	caprae	1.3	0.2	0.1	0.0	0.1	0.0
120	Staphylococcaceae	Staphylococcus	group	1.1	0.1	0.1	0.0	0.1	0.0
121	Streptococcaceae	Streptococcus	agalactiae/ equi	1.3	0.1	0.1	0.0	0.1	0.0
122	Streptococcaceae	Streptococcus	group	1.2	0.1	0.1	0.0	0.1	0.0
123	Streptococcaceae	Streptococcus	group	1.2	0.2	0.1	0.0	0.1	0.0
124	Streptococcaceae	Streptococcus	oligofermentas/ infantarius	1.5	0.5	0.1	0.0	0.1	0.0
125	Streptococcaceae	Streptococcus	thermophilus	1.2	0.4	0.1	0.0	0.1	0.0
126	Veillonellaceae	Phascolarctobacterium	faecium	1.7	0.9	0.1	0.1	0.1	0.1
127	Verrucomicrobiaceae	Akkermansia	muciniphila	1.5	0.4	0.1	0.1	0.1	0.1
128	Yeast	Galactomyces	geotrichum	1.2	0.1	0.1	0.0	0.1	0.0

below detectable level	<2	<0,1 %	<0,1 %
low abundance	2< <4	0,1% < < 0,2%	0,1% < < 0,2%
medium abundance	4 < < 50	0,2% < < 4%	0,2% < < 4%
high abundance	>50	>4%	>4%

In our experimental set-up, the non-affected bacteria grow during 24 h fermentation time and the error due to the presence of dead cells apparently becomes negligible.

To discriminate actively growing cells from inactive cells, I-chip hybridizations were performed for both DNA and RNA isolated from the same sample (Fig. 4). The differences between bacterial fingerprints based on DNA versus RNA especially concerned the bacteria present in low abundances. The signal intensities based on RNA were most often higher than the ones based on DNA. These differences indicate that these bacteria are active albeit present in low numbers, which is in line with literature reporting that more copies of RNA are present in one cell than copies of DNA (Klappenbach et al., 2001). In some occasions, it was observed that the signal intensity based on RNA was lower than the one based on DNA e.g. 1 μg ml^{-1} amoxicillin and 1 μg ml^{-1} clindamycin. Low RNA signals as compared to DNA signals are the differences to be discriminated as the bacteria are present but not active. These differences are not dependent on the mode of action of the antibiotic: Inhibition of activity was also observed with amoxicillin, which is known as a bacteriolytic antibiotic (Table 1). Apparently, antibiotics may be bacteriolytic for the bacteria they target for, but may be bacteriostatic for other bacteria, as also reported earlier (Brötz-Oesterhelt and Brunner, 2008). Overall, bacterial fingerprints based on DNA differ from the ones based on RNA. However these differences especially concern a low proportion of the total microbiota. Hence, in our study aiming at screening for major differences in the microbiota composition due to antibiotic treatments, no major misinterpretations are made when ignoring the presence of inhibited cells for any antibiotic mode of actions.

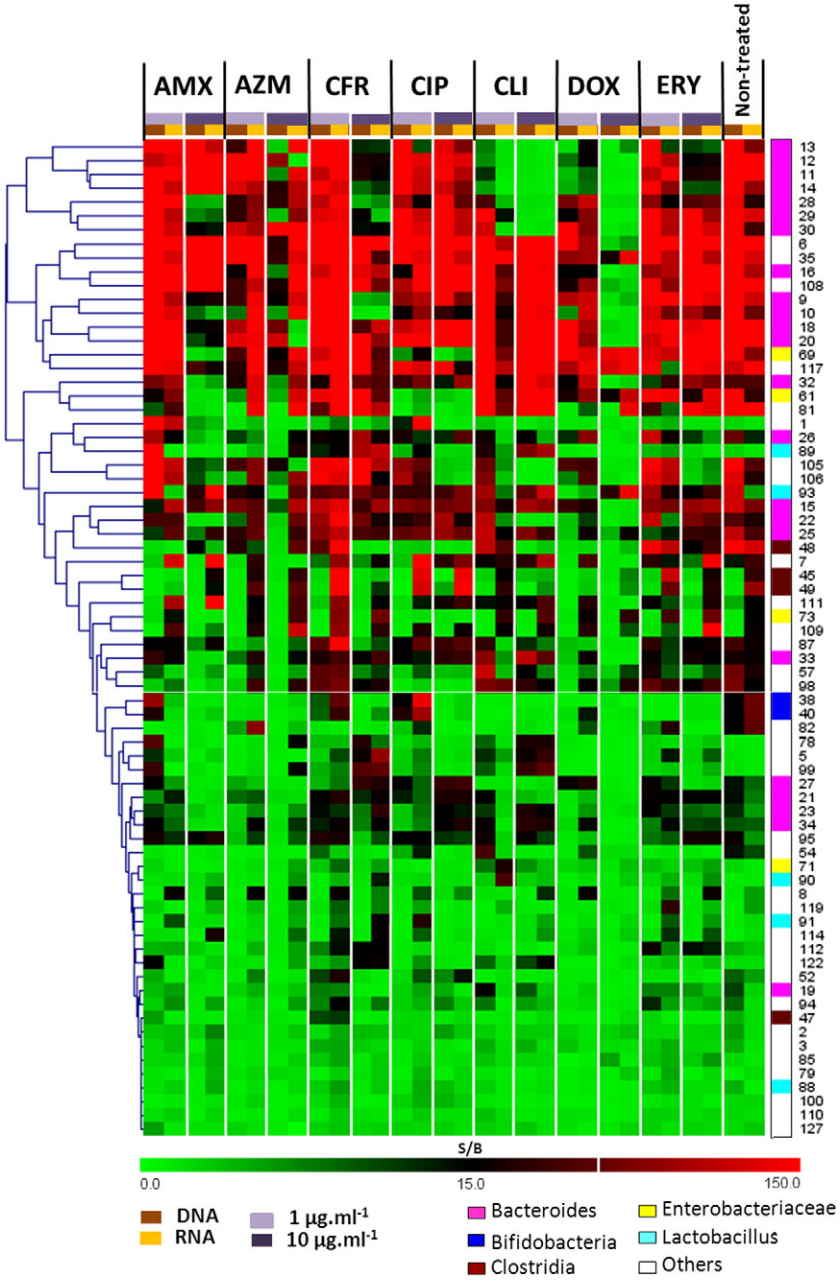

Fig. 4. Bacterial fingerprints of non-treated and antibiotic-treated adult inoculum obtained with the I-chip based on DNA vs. RNA after 24 h *in-vitro* fermentation. Seven antibiotics were tested in concentrations of 1 μg ml^{-1} and 10 μg ml^{-1}. Main group of bacteria are highlighted in color and full name of targets can be found in Table 2 based on their numbering. Signal to the background (S/B): Green: below detectable level, Black: medium abundance, Red: high abundance.

In conclusion, the I-chip readout is not influenced by the variations in the total number of bacteria nor the presence of dead or inactive cells after 24 h *in-vitro* fermentation. Hence the impact of antibiotics on the adult microbiota can be made with this microarray and conclusions on biological effects can be drawn from Fig. 1.

3.2. Impact of different antibiotics on the intestinal microbiota of healthy adults

Seven antibiotics were tested at low dose (1 μg ml^{-1}) and high dose (10 μg ml^{-1}). The impact of the antibiotics on microbiota as compared to the non-treated microbiota was determined with the I-chip 24 h after starting the exposure.

3.2.1. Amoxicillin

(Fig. 1 A/B—AMX): At 1 μg ml^{-1} antibiotic concentration, the abundance of *Bacteroides* remained stable, that of *Enterobacteriaceae* slightly decreased (*Escherichia coli* mainly) while those of *Bifidobacteria*, *Lactobacillus* and *Clostridia* decreased to an undetectable level. At 10 μg ml^{-1}, the abundances of most *Bacteroides* species (except *B. fragilis*) and *Enterobacteriaceae* decreased to undetectable levels, although one outlier did not show significant reduction in the last group of bacteria. The abundance of *Lactobacillus gasseri* slightly increased. These results are in agreement with literature where amoxicillin is described as a broad spectrum antibiotic with an increasing suppression of both anaerobic and aerobic bacteria with increasing doses, although levels of *Enterobacteriaceae* are reported to increase but also to decrease (Sullivan, 2001).

3.2.2. Azithromycin

(Fig. 1 A/B—AZM): At 1 μg ml^{-1}, the abundance of a few *Bacteroides* species (mostly *B. uniformis* and *B. vulgatus*) slightly decreased while *Bifidobacteria* abundance was reduced to undetectable levels. The abundances of the other groups of bacteria remained stable. At 10 μg ml^{-1}, the abundances of *Bacteroides* and *Enterobacteriaceae* were slightly lowered whereas that of *Clostridia* was reduced to an undetectable level. The abundance of *Lactobacillus* remained stable. These results fit with literature indicating a strong activity for AZM against gram positive bacteria and an low activity against aerobic gram negative rods (Drew and Gallis, 1992).

3.2.3. Cefadroxil

(Fig. 1 A/B—CFR): At 1 μg ml^{-1}, the abundances of *Bifidobacteria*, *Clostridia* and *Lactobacillus* slightly decreased, while those of the other groups remained stable. At 10 μg ml^{-1}, the abundances of *B. uniformis*, *B. dorei*, *B. fragilis*, *Bifidobacteria*, and *Clostridia* decreased to undetectable levels. The abundance of *Enterobacteriaceae* remained stable, while that of *Lactobacillus brevis* increased. No major effects of cefadroxil (1 g, 10 days) on the microbiota is reported in literature (Sullivan, 2001), indicating that the absorption rate of cefadroxil *in-vivo* might be greater than 90% as assumed in the present study.

3.2.4. Ciprofloxacin

(Fig. 1 A/B—CIP): At 1 μg ml^{-1}, the abundances of *Enterobacteriaceae* and *Clostridia* were dramatically reduced and became undetectable. At this low dose, the abundance of *Bifidobacteria* slightly increased and that of *Lactobacillus* remained stable. At 10 μg ml^{-1}, the abundance of *Bifidobacteria* decreased to undetectable levels and that of *L. gasseri*

Table 3
Overview of the impact of antibiotics on the main bacterial groups of adult inoculum measured with the I-Chip after 24 h *in-vitro* fermentation as compared to non-treated microbiota.

	μg ml^{-1}	*Bacteroides*	*Bifidobacterium*	*Lactobacillus*	*Clostridia*	*Enterobacteriaceae*
AMX	1	=	↓↓	↓	↓↓	↓ *E. coli*
	10	↓↓ *B. dorei* *B. uniformis* *B. thetaiotaomicron*	↓↓	↑ *L. gasseri*	↓↓	↓↓
AZM	1	↓ *B. vulgatus* *B. uniformis*	↓↓	=	=	=
	10	↓	↓↓	=	↓↓	↓
CFR	1	=	↓	↓	↓	=
	10	↓↓ *B. dorei* *B. uniformis* *B. fragilis*	↓↓	↑↑ *L. brevis*	↓↓	=
CIP	1	=	↑	=	↓↓	↓↓
	10	=	↓↓	↑↑ *L. gasseri*	↓↓	↓↓
CLI	1	↓↓ *B. fragilis*	↓↓	↑ *L. brevis*	=	=
	10	↓↓ *B. fragilis* *B. uniformis*	↓↓	↑↑ *L. brevis*	↓↓	=
DOX	1	↓↓ *B. fragilis*	↓	↓↓	↓↓	↓
	10	↓↓	↓↓	↑↑ *L. gasseri*	↓↓	↓
ERY	1	=	↓↓	=	=	=
	10	↓↓ *B. fragilis*	↓↓	=	↓	=

↓↓: major decrease, ↓: mild decrease, =: constant, ↑: mild increase ↑↑: major increase.

increased. The abundance of *Bacteroides* remained stable for the two doses tested. Strong activity of 10 μg ml^{-1} treatment is well known against *Enterobacteriaceae* and *Clostridia* (Sullivan, 2001) but not the activity against other bacterial groups.

3.2.5. Clindamycin

(Fig. 1 A/B—CLI): At 1 μg ml^{-1}, the abundances of *B. fragilis* and *Bifidobacteria* decreased to an undetectable level, that of *L. brevis* slightly increased and those of the other groups remained stable. At 10 μg ml^{-1}, the abundances of *B. fragilis*, *B. uniformis and Clostridia* were reduced to undetectable levels, that of *Enterobacteriaceae* remained stable, and that of *L. brevis* increased. In line with the observed results, Clindamycin activity is reported to mainly be active against anaerobic bacteria. (Sullivan, 2001) Different impact per species of *Bacteroides* has been reported (Donskey et al., 2003), although our data do not show survival of *B. fragilis*.

3.2.6. Doxycycline

(Fig. 1 A/B—DOX): At 1 μg ml^{-1}, the abundances of *Bifidobacteria* and *Enterobacteriaceae* were slightly reduced while those of *B. fragilis*, *Clostridia* and *Lactobacillus* decreased to undetectable levels. At 10 μg ml^{-1}, the abundances of *Bacteroides* and *Bifidobacteria* were reduced to undetectable levels, that of *L. gasseri* increased, while that of *Enterobacteriaceae* did not further decrease as compared to the abundances observed with 1 μg/ml. The class of "Tetracycline" is not often studied (Sullivan, 2001). Only a decrease of *Bifidobacteria* and of the general microbiota diversity measured by PCR-DGGE have been reported by Saarela et al. (2007).

3.2.7. Erythromycin

(Fig. 1 A/B—ERY): At 1 μg ml^{-1}, the abundance of each group of bacteria remained stable except for the abundance of *Bifidobacteria*, which decreased to an undetectable level. At 10 μg ml^{-1}, the abundance of *B. fragilis* slightly decreased while the abundance of *Clostridia* decreased under the detectable level. The abundances of *Enterobacteriaceae* and *Lactobacillus* remained stable. These results are according to the trends reported in literature although a stronger impact of erythromycin towards *Enterobacteriaceae* and anaerobes has been reported (Sullivan, 2001).

3.3. Overview of the antibiotic impact on the human intestinal microbiota

The impact of seven antibiotics on the human microbiota composition was now monitored under similar conditions in *one* experimental set-up. Although the interaction between host and microbiota are not mimicked in *in-vitro* system, our findings for specific antibiotic were in general consistent with trends described in previous studies (De La Cochetière et al., 2005; Donskey et al., 2003; Jernberg et al., 2005; Mangin et al., 2010; Rafii et al., 2008; Sullivan, 2001). The outcomes of previous studies are, however, sometimes difficult to compare among each other or with current data due to the use of different conditions and analytical techniques. The advantage of our approach is illustrated in Table 3 where the straightforward comparison of the outcomes regarding antibiotic impact on *Bacteroides*, *Bifidobacteria*, *Clostridia*, *Enterobacteriaceae* and *Lactobacillus* is summarized.

In general, the impact on the microbiota is antibiotic and dose dependent, even if antibiotics belong to the same class, as reported in literature (Rafii et al., 2008). Details of the description are at family, genus but also species level when appropriate. Within a bacterial group, a specific antibiotic can have different impacts for different species (e.g. *Bacteroides*) and the dose of this antibiotic can influence specific species within a bacterial group (e.g. *Lactobacillus*). These differences at species level are not often considered in literature although predominance or absence of certain species might have an influence on the ecosystem and, therefore, on human health. For instance, *L. gasseri* that became predominant upon a treatment with 10 μg ml^{-1} amoxicillin, ciprofloxacin and doxycycline, has been shown to result in a significant reduction of inflammation in IL-10-deficient mice (Carroll et al., 2007).

For all the antibiotics except ciprofloxacin, the concentration of 1 μg ml^{-1} is below the MIC values for the pathogens targeted. It is interesting to notice in Table 3 that this low dose of antibiotic can already influence some bacterial groups of the microbiota. *Lactobacillus*, for instance, tends to survive antibiotic treatments. This survival of *Lactobacillus* can be seen as an ability to persist through antibiotic treatment and, therefore, accentuate their probiotic effects or as a means to spread the antibiotic resistance genes within the gut (Woodmansey, 2007). On the contrary, *Bifidobacteria* seem to be very sensitive microorganisms. All antibiotics reduced the abundance of this group at 1 μg ml^{-1} except for ciprofloxacin, which reduced it only at 10 μg ml^{-1} concentration. As (minor) changes in the microbiota composition are reported to have consequences for colonic health with respect to development of resistant bacteria (Gullberg et al., 2011) and may cause disturbance of colonic fermentation (Yap et al., 2008), sub-MIC dose of antibiotics reaching the colon should, therefore, not be under estimated with respect to undesired health effects.

4. Conclusion

To our knowledge, this study is the first to demonstrate the potential utility of coupling the high-throughput fermentation screening-platform to the I-chip analysis to monitor the effect of antibiotics on the microbiota in comparable conditions. The reliability of the microarray analysis was validated in case of antibiotic exposure. Errors due the variation in cell numbers and presence of dead cells were negligible after 24 h *in-vitro* fermentation. With this high-throughput approach, a detailed level of information at family, genus and species level was simultaneously obtained for all groups of bacteria whereas most previous studies focus on specific antibiotic or specific bacteria or group of bacteria. Although the interaction between host and microbiota cannot be mimicked completely in an *in-vitro* system, the detailed impact of seven antibiotics as obtained from *one* experimental set-up using a complex ecosystem allows comparison of the different antibiotics. Such detailed high-throughput evaluation could not be reached either in *in-vitro* studies using other molecular tools or in *in-vivo* studies.

Acknowledgments

This project is jointly financed by the European Union, European Regional Development Fund and The Ministry of Economic Affairs, Agriculture and Innovation, Peaks in the Delta, the Municipality of Groningen, the Province of Groningen as well as the Dutch Carbohydrate Competence Center (CCC-WP2), and by FrieslandCampina. The authors thank Hakim Rahaoui for the technical support on the I-chip.

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