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The Effects from DNA Extraction Methods on the Evaluation of Microbial Diversity Associated with Human Colonic Tissue

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Abstract Potentially valuable sources of DNA have been extracted from human colonic tissues and are retained in biobanks throughout the world, and might be re-examined to better understand host–microbe interactions in health and disease. However, the published protocols for DNA extraction typically used by gastroenterologists have not been systematically compared in terms of their recovery of the microbial fraction associated with colonic tissue. For this reason, we examined how three different tissue DNA extraction methods (the QIAGEN AllPrep DNA/RNA kit, salting out and high molecular weight (HMW) methods of DNA extraction) employed in past clinical trials, and the repeated bead beating and column (RBB+C) method might impact the recovery of microbial DNA from colonic tissue, using a custom designed phylogenetic microarray for gut bacteria and archaea. All four methods produced very similar profiles of the microbial diversity, but there were some differences in probe signal intensities, with the HMW method producing stronger probe intensities for a subset of the Firmicutes probes including *Clostridium* and *Streptococcus* spp. Real-time PCR analysis revealed that the HMW and RBB+C extracted DNA contained significantly more DNA of Firmicutes origin and that the different DNA extraction methods also gave variable results in terms of host DNA recovery. All of the methods tested recovered DNA from the archaeal community although there were some differences in probe signal intensity. Based on these findings, we conclude that while all four methods are efficacious at releasing microbial DNA from biopsy tissue samples, the HMW and RBB+C methods of DNA extraction may release more DNA from some of the Firmicutes bacteria associated with colonic tissue. Thus, DNA archived in biobanks could be suitable for retrospective profiling analyses, provided the caveats with respect to the DNA extraction method(s) used are taken into account.

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Introduction

The microbiome resident in the human large intestine is now widely recognised to provide a variety of physiological and ecological functions relevant to host nutrition and well-being [2, 18]. Furthermore, the gut microbiome has been shown to undergo some dramatic structural changes in obese and overweight subjects [54, 55], as well as in persons diagnosed with inflammatory bowel diseases [6, 7, 57] or colorectal cancer [18, 49]. Much still remains to be learned about the cause–effect relationships of microbiome alteration and disease, as well as whether such information

can be used for risk stratification and/or clinical management of disease.

Microbial diversity in environmental samples has traditionally been assessed by low throughput polymerase chain reaction (PCR)-based profiling methods such as the analysis of terminal restriction fragment length polymorphisms (T-RFLP), denaturing gradient gel electrophoresis (DGGE), or by the analysis of rRNA (*rrs*) gene clone libraries. Despite method-specific and PCR-based biases (e.g. primer selection and PCR amplification bias) these methodologies have been widely applied due to their relative simplicity and robustness. Of these methods, the analysis of *rrs* clone libraries has been considered the "gold standard"; however, this method becomes increasingly laborious when complex communities or multiple samples are processed. This is mainly due to the time-consuming nature of the technique and the costly method of Sanger DNA sequencing needed to provide precise taxonomic assignment and coverage of the microbial community. Conversely, both phylogenetic microarrays and next-generation sequencing technologies now offer an efficient, high throughput and affordable alternative to characterise microbial diversity. These methods however are also prone to PCR-based biases in addition to having their own specific limitations. Phylogenetic microarrays require prior information on community composition to facilitate probe design and microarrays are consequently incapable of identifying members of the community unless the appropriate probes are present. Moreover, poor probe design and/or non-specific hybridisation(s) can lead to erroneous determinations of microbial diversity. In contrast, next-generation sequencing is susceptible to overestimating microbial diversity due to inherent difficulties in accurately sequencing DNA homopolymer repeat regions [23, 46]. This and the volume of sequences produced necessitate the use of specialist software to process and analyse the resultant data (e.g. [8, 45, 51]). Nonetheless, both technologies continue to be widely adopted due to their ability to rapidly and sensitively profile microbial communities in large numbers of complex samples.

DNA extraction is a key factor affecting any approach for analysing microbial diversity. Numerous methods have been described for the isolation of microbial DNA from human stool samples, and like other fields of microbial ecology, mechanical lysis by bead beating tends to be favoured as such methods have been shown to be most effective at capturing the microbial diversity (e.g. [13, 28, 37, 48, 59]). In that context, the repeated bead-beating and column purification method (RBB+C) first described by Yu and Morrison [58] has recently been shown by Salonen et al. [48] to produce superior results in terms of DNA yield and recovery of phylogenetic diversity from human stool samples. In contrast, there does not yet appear to have been a systematic assessment of how different DNA extraction methods might influence the appraisal of the microbiome adherent to the colonic mucosa. This remains an important issue because several studies have shown that the mucosal and faecal associated microbiota differ (e.g. [14, 27, 41, 60]). These differences might be relevant to better understanding the role of the gut microbiota in human health and disease because in contrast to faecal samples, the colonic tissue and its associated microbial microenvironment can be sampled from where disease is manifestly apparent.

There are many clinical biobanks that contain DNA samples prepared from healthy and diseased colonic tissues that may prove to be extremely valuable for microbiological profiling. Unfortunately, the DNA extraction methods used by clinicians do vary, both from clinic-to-clinic, as well as from those used typically by environmental microbiologists and microbial ecologists. In particular, the nucleic acid extraction methods that have traditionally been employed by clinicians with colonic tissue: viz. the high molecular weight and salting out methods described by Marmur [30] and Miller et al. [35], respectively; and more recently, the QIAGEN AllPrep DNA/RNA kit for nucleic acid extraction, do not employ a bead-beating step. These methods were/are primarily used to recover host DNA/RNA and often without the anticipation that the same samples might also be analysed with respect to gut microbiome structure–function relationships. In particular, these DNA samples could facilitate powerful cross-sectional and inception-based studies of microbiome structure that might add to the host-based measurements already collected and published; if it can first be confirmed that microbial DNA was also effectively released by the DNA extraction methods typically (or historically) used with colonic tissue samples.

With this background, the aim of this study was to evaluate how well the DNA extraction methods routinely used by gastroenterologists compare with the RBB+C protocol in revealing the microbial diversity associated with colonic tissue samples. Similar to the studies described by Salonen et al. [48], we have used a custom-designed gut microbiome specific phylogenetic microarray (the Aus-HIT chip) recently shown to produce a rapid and accurate assessment of gut microbial diversity [21, 36].

Materials and Methods

Human Subject and Tissue Sampling

A colonic tissue sample was obtained from a 73-year-old male subject being examined for colorectal cancer at the

Queensland Institute for Medical Research, Australia. The colorectum was prepared with 4 L of Colonlytely PEG-based preparation solution taken orally. The site of tissue resection was clean of any overlying macroscopic adherent fluid or luminal fluid and the tissue was immediately examined post-operatively; with an area of the visually normal mucosa placed in a sterile specimen container and snap frozen in liquid nitrogen. The subject provided informed consent and the use of the tissue was approved by the QIMR ethical board.

Tissue DNA Extraction and Analysis

The tissue was thawed on ice and then cut into sections (≤30 mg tissue) with a sterile scalpel, to resemble the size of punch biopsy samples. These tissue samples were subject to one of three methods of DNA extraction typically or historically used by research gastroenterologists with up to seven subsamples processed per method. With the exception of the RBB+C samples, the tissue subsections were homogenised using an Ultra Thurax SP4 homogeniser at speed setting 4 until homogenous or for a maximum of 20 s. The QIAGEN AllPrep DNA/RNA Mini Preparation Kit was used as described by the manufacturer's instruction (QIAGEN) with the tissue samples homogenised in 600 µl of QIAGEN RLT buffer. The salting out method of DNA extraction followed the methods described by Miller et al. [35] except that a miniprep adapted protocol was followed. A miniprep protocol adapted from the high molecular weight DNA extraction method (HMW method) described by Marmur [30] was also used. Briefly, the tissue subsample was homogenised in 600 µl of cell lysis buffer (6 mM Tris–HCl pH 8, 100 mM EDTA, 1 M NaCl) and incubated at 75°C for 10 min to inactivate nucleases. Following cooling to ambient temperature, 15 µl of lysozyme (200 mg/ml stock) and 1.5 µl of mutanolysin (20 U/µl) were added and the sample was then incubated at 37°C overnight for maximum lysis. Following the incubation, 30 µl of 10% sodium laurylsarcosine and 7.5 µl of proteinase K (20 mg/ml) were added and the sample was incubated at 55°C for 30 min. The sample was extracted with phenol/chloroform/isoamylalcohol (25:24:1) and the residual contaminating phenol/chloroform was subsequently removed by a chloroform/isoamylalcohol (24:1) extraction. The aqueous phase was removed to a fresh microfuge tube and the DNA was precipitated with 3 M sodium acetate (pH 5.2) and isopropanol. The DNA pellet was washed with 70% ethanol and the DNA was air dried and resuspended in TE buffer. The fourth method employed was the RBB+C method as described by Yu and Morrison [58]. All of the DNA samples were washed using a Microcon column (Millipore) to remove PCR inhibitors and quantified using a NanoDrop ND-1000. The integrity of the DNA was determined by electrophoresis using a 0.7% *w*/*v* agarose gel followed by post-staining using SYBR® Safe DNA gel stain according to the manufacturer's instructions (Invitrogen).

Generation and Fluorescent Labelling of cRNA

The Bacteria and Archaea *rrs* genes were PCR amplified using 100 ng of a single DNA sample per extraction method and primers 4Fa, 27F and 1492-T7R (Table 1). The PCR reactions were performed in triplicate and pooled to reduce the effect of amplification biases. The PCR master mix included 200 nM of each primer, 200 µM of each deoxyribonucleotide, 1× PCR buffer, 3 mM $MgCl_2$ and 0.5 U Native *Taq* DNA polymerase (Invitrogen). The PCR reaction was initiated by incubating the mixtures at 95°C for 5 min, and followed by 30 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 90 s and 1 cycle of 72°C for 5 min. To eliminate any aberrant amplicons that can be produced by primers 27F and 1492R during PCR, as shown by Osborne et al. [40], all of the PCR reactions were subjected to agarose gel electrophoresis and the *rrs* amplicons (~1.5 kb) were gel extracted using a QIAGEN Minelute kit. As an internal standard, the mitochondrial *rrs* gene was PCR amplified using human DNA as the template and primers MitoF and MitoR-T7 (Table 1). The PCR conditions were the same as those described above except that the elongation step was performed at 72°C for 30 s. Where necessary, the samples were concentrated using Pellet Paint Co-Precipitant (Novagen) as directed by the manufacturer.

The gel extracted *rrs* amplicons were quantified using a NanoDrop ND-1000, and then 500 ng aliquots of the DNA were used as the template for the in vitro synthesis of single-stranded RNA (cRNA). We chose to produce cRNA for hybridization to the microarray because Palmer et al. [43] had previously reported that cRNA allowed significantly enhanced hybridisation specificity, in comparison to the use of dsDNA. The cRNA was produced using the MEGAScript T7 *in vitro* transcription kit (Ambion) and purified using the MEGAclear kit (Ambion) as described by Kang et al. [21].

cRNA Labelling, Microarray Hybridization and Image Capture

The Aus-HIT chip and associated methods are described in detail by Kang et al. [21] and the relevant microarray probe and other methodological details, together with the hybridisation results, are also accessible at the GEO database (http://www.ncbi.nlm.nih.gov/geo/; GPL9353 and GSE18420 respectively). In brief detail, 500 ng of the *rrs* cRNA sample was mixed with 7 ng of mitochondrial cRNA (as an internal standard) and fragmented and labelled using

Table 1 Primers used in this study

Primer name	Primer target(s)	Primer sequence 5′-3′	Approximate amplicon size	Reference
4Fa	Universal *rrs* primer (archaea)	TCCCGGTTGATCCTGCCRG	–	[19]
27F	Universal *rrs* primer (bacteria)	AGAGTTTGATCMTGGCTCAG	–	[24]
1492-T7R[a]	Universal *rrs* primer (bacteria/archaea)	TC*TAATACGACTCACTATAGGG*GGYTACCTTGTTACGACTT	–	[24]
MitoF MitoR-T7[a]	Human mitochondrial specific *rrs* primer	TACTACCAGACAACCTTAGC TC*TAATACGACTCACTATAGGG*GTTTCGGGGGTCTTAGCTTT	– –	[21]
AllBac296f AllBac412r	*Bacteroides* spp. specific *rrs* primer	GAGAGGAAGGTCCCCCAC CGCTACTTGGCTGGTTCAG	106 bp[b]	[26]
PreGen4F PreGen4R	*Prevotella* spp. specific *rrs* primer	GGTTCTGAGAGGAAGGTCCCC TCCTGCACGCTACTTGGCTG	121 bp	[52]
StrepGenF StrepGenR	*Streptococcus* spp. specific *rrs* primer	CGACGATACATAGCCGACCTGAG TCCATTGCCGAAGATTCCCTACTG	102 bp	[9]
g-Ccoc-F g-Ccoc-R	*Clostridium coccoides* group specific *rrs* primer	AAATGACGGTACCTGACTAA CTTTGAGTTTCATTCTTGCGAA	440 bp	[31]
1114F 1221R	Universal *rrs* primer (bacteria)	CGGCAACGAGCGCAACCC CCATTGTAGCACGTGTGTAGCC	130 bp	[11]
β-act-F β-act-R	β-actin specific primer	CCTCGCCTTTGCCGA TGGTGCCTGGGGCG	171 bp	[3]

[a] The 1492R and Mito-R based primers were modified to include a T7 promoter sequence (italicised)

[b] Base pairs

the *Label* IT µArray Cy5 labelling kit (Mirus) following the manufacturer's specifications. Four replicate hybridisations were performed for each labelled cRNA sample, and the microarray slides were scanned using an Axon Genepix 4000A microarray scanner (Axon Instruments, Union City, CA, USA). The images obtained were analysed using GenePix Pro 6.0 software (Axon Instruments) and probe signal intensities were quantified as the difference between foreground and background intensities at 635 nm.

Data Processing and Analysis

The raw signal data was processed using the Genespring GX10 software (Agilent Technologies) and the three hybridisation profiles showing the most even intensity distributions for each extraction method were selected for normalisation. Intensity values were transformed to log2, normalised using the quantile normalisation method and probes not giving higher intensities than the negative controls in at least all replicates of one extraction method were discarded. The profiles obtained were then analysed with a multivariate method derived from numerical ecology: between group analysis (BGA) applied to correspondence analysis [10, 15], using the R package *ade4* [12]. A Monte Carlo permutation test was performed to assess the significance of the constraint being evaluated. Moreover, the stability of gene contributions to the modelled constraint was assessed using the *multistab* package [4]. Only stable probes ($p<0.05$) with a greater contribution to the models than the spiked controls were considered as being affected by extraction method.

Real-Time PCR Analysis

Quantitative real-time PCR reactions were performed using five independent DNA samples prepared using each of the four methods described above ($n=20$). A dilution series of the template DNA was constructed and used to identify non-specific amplification and to calculate amplification efficiency using the primer sets described in Table 1. Each PCR reaction mixture contained 10 ng of DNA template, 200 nM of each primer and 1X iQ SYBR Green Supermix (BioRad). Each reaction was dispensed, in quadruplicate, into 5 µl aliquots in a 384-well plate using a Biomek 2000 automated workstation (Beckman). The real-time PCR reactions were performed using a 7900HT sequence detection system (Applied Biosystems) and the following conditions: one cycle of 95°C for 10 min, 40 cycles of 95°C for 15 s and 58°C for 30 s followed by a dissociation curve cycle of 95°C for 15 s, 60°C for 15 s and 95°C for 15 s. Individual reactions showing aberrant amplification profiles were discarded from further analysis. The data obtained from each individual reaction and the amplification efficiencies derived from the dilution series were used to determine the relative quantification of the various DNA targets. Briefly, the data for each specific microbial group

was normalised by the Livak method [29] using Bacteria-specific *rrs* gene primers (Table 1) as the reference; and differences were expressed with reference to the data obtained using the DNA samples prepared with the QIAGEN AllPrep DNA/RNA kit. The recovery of host (human) DNA was assessed by quantitative real-time PCR targeting the β-actin gene using specific primers (Table 1).

Results

Extraction of DNA from Human Colonic Tissue Samples

Three of the four DNA extraction methods produced similar yields of total DNA. The HMW method recovered the most DNA (1,578.0±573.6 ng DNA per mg tissue) followed by the salting out (1,258.5±570.7 ng DNA per mg tissue) and RBB+C (1,129.3±302.3 ng DNA per mg tissue). However in our hands, the QIAGEN AllPrep DNA/RNA method recovered much less total DNA (282.7±201.4 ng DNA per mg tissue). The A260/A280 ratio of the DNA extractions were all similar (HMW method 1.89±0.01; salting out method 1.83±0.03; RBB+C method 1.89±0.01; QIAGEN AllPrep DNA/RNA method 1.84±0.06) indicating that the preparations were comparably pure. Agarose gel electrophoresis revealed that the majority of the DNA was in a high molecular weight form (>10 kb) and with no significant shearing observed below 1.5 kb irrespective of the DNA extraction method used (Fig. 1). Taken together, the results suggest that all four methods provide high quality DNA suitable for downstream processing.

Aus-HIT Chip-Based Characterisation of the Extracted DNA Samples

We used the Aus-HIT chip to determine the ability of the individual DNA extraction methods to capture the microbial diversity associated with the colonic tissue samples. The hybridization profiles were essentially identical, except for a small percentage (<10%) of the probes that gave rise to statistically significant differences in signal intensity ($p < 0.05$). These findings suggest that while the efficacy of the four DNA extractions methods examined here are similar, and will produce qualitatively similar profiles of microbial diversity from mucosal DNA samples, there does appear to be some differences between the extraction methods in terms of DNA release.

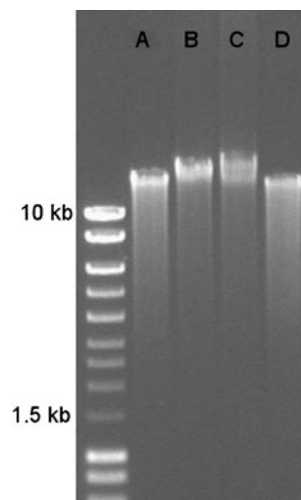

Figure 1 Characterisation of extracted DNA as determined by agarose gel (0.7%) electrophoresis. *a* QIAGEN AllPrep DNA/RNA Kit; *b* salting out method; *c* HMW method; *d* RBB+C method

The microarray hybridization data produced for each DNA extraction method were subjected to a BGA in order to better evaluate these preliminary findings and to further dissect the effect of the individual extraction methods on DNA recovery from different microbial groups. The principal axes of BGA are defined as the linear combination of probes that maximises the between-group variance allowing the identification of groups of probes that discriminate between classes of samples. The results of this analysis are illustrated in Fig. 2a and reveal that the main axis of variation was characterised by a general inverse relationships between several Bacteroidetes and Firmicutes group-specific probes. The analysis revealed that the QIAGEN AllPrep DNA/RNA and salting out methods produced profiles most similar to that of the RBB+C method; but when compared to the RBB+C method the probe signal intensities for some of the *Bacteroides* and *Prevotella* spp. probes were stronger; and weaker for some of the *Veillonella* and *Streptococcus* spp.-based probes (Fig. 2b). Interestingly, the profile arising from DNA extracted using the HMW method was separated from the others by the second principal axis, because it provided the strongest signals for a small number of *Clostridium*-specific probes, as well as *Streptococcus* spp.-based probes.

We also examined the ability of the individual DNA extraction methods to detect the archaeal diversity associated with the colonic tissue. There were no statistically significant differences in the hybridisation profiles for methanogenic archaea for all four DNA extraction methods tested ($p > 0.05$). Similar to previous studies [14, 42], we detected the presence of *Methanobrevibacter smithii* with colonic tissue but in addition we also detected the presence of *Methanosaeta* spp. and *Methanocaldococcus* spp. None of the DNA extracts produced a detectable signal for *Methanosphaera* spp. We also observed positive signals for some non-methanogenic archaea. The DNA extracts produced by the HMW method resulted in significantly stronger hybridization signals for probes targeting the euryarchaeote *Halobacterium* spp. in contrast to the other three methods ($p < 0.05$); and the salting out method resulted in a significantly stronger hybridization signals for the crenarchaeote *Sulfobus* spp. in contrast to the QIAGEN AllPrep DNA/RNA and RBB+C methods ($p < 0.05$).

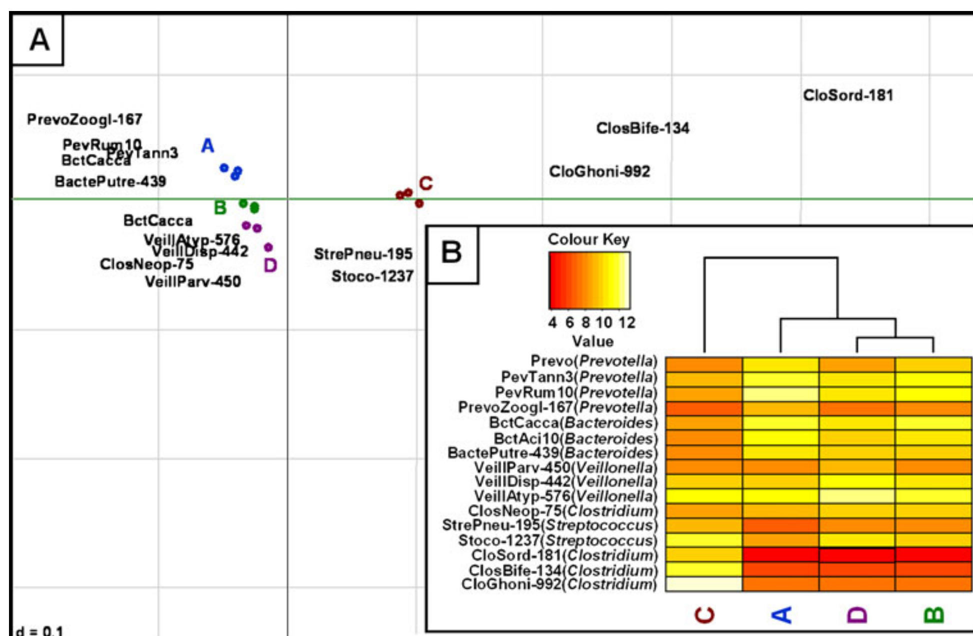

Figure 2 **a** Discrimination of the microarray profiles derived from the four different DNA extraction methods using BGA applied to correspondence analysis. The most discriminant bacterial probes occurring at least twice at the genus level are plotted and the relative positions of objects (probes and profiles) provide a measure of the strength of their association. The BGA analysis indicated that the different DNA extraction methods employed had a significant effect on the microbial community profiles generated, accounting for most of the variance found (65.9%, $p<0.05$). The axes represent 60.2% (*X*) and 5.7% (*Y*) of the variation. The three individual hybridisations per DNA extraction method are shown. **b** Heat map of the probes identified in **a** with hierarchical clustering based on DNA extraction method. **a** QIAGEN AllPrep DNA/RNA Kit, **b** salting out method, **c** HMW method, and **d** RBB+C method of DNA extraction. The probe names are defined as follows: BctAci10 (*Bacteroides acidofaciens*); BctCacca (*Bacteroides caccae*); BactePutre-439 (*Bacteroides putredinis*); ClosBife-134 (*Clostridium bifermentans*); CloGhoni-992 (*Clostridium ghoni); ClosNeop-75 (Clostridium neopropionicum); CloSord-181 (Clostridium sordellii); PevRum10 (Prevotella ruminicola); Prevo (Prevotella sp.); PevTann3 (Prevotella tannerae); PrevoZoogl-167 (Prevotella zoogleoformans); StrePneu-195 (Streptococcus pneumoniae); Stoco-1237 (Streptococcus sp.); VeillAtyp-576 (Veillonella atypical); VeillDisp-442 (Veillonella dispar); VeillParv-450 (Veillonella parvula)*

Real-Time PCR Analysis of Colonic Tissue-Extracted DNA

To further evaluate the microarray-based observations, several of the bacterial groups identified by the BGA analysis to be variant with respect to DNA extraction method were subjected to real-time PCR analysis. The real-time PCR analyses confirmed that DNA extracted using the QIAGEN AllPrep DNA/RNA kit and salting out methods do contain a greater relative amount of microbial DNA representing *Bacteroides* spp. and *Prevotella* spp. compared to DNA extracted using the HMW and RBB+C methods ($p<0.05$, Fig. 3). Similarly, the DNA extracted using the HMW and RBB+C methods were found to contain a greater amount of DNA representing *Streptococcus* spp. and the *Clostridium coccoides* group ($p<0.05$), although the difference between the HMW and salting out methods for *Streptococcus* spp. was marginal ($p<0.07$). These findings suggest that the variation in probe intensities observed for the microarray data were as a consequence of the DNA extraction method per se, rather than any bias introduced during the PCR amplification of the *rrs* genes or in the production of cRNA for the microarray analysis. In summation, we interpret these findings as showing that the DNA extraction method used with mucosal tissue does have a subtle impact on the recovery of microbial DNA; with the RBB+C and HMW methods of DNA extraction releasing more DNA from some Firmicutes bacteria.

We also used real-time PCR-targeting the β-actin gene as a measure of the host DNA recovered by the different extraction methods. Interestingly, the salting out and HMW methods of DNA extraction recovered significantly more host DNA ($p<0.05$) than the QIAGEN AllPrep DNA/RNA and RBB+C methods (Fig. 3). The latter methods use nucleic acid binding columns and it may be that the smaller bacterial chromosomes and/or DNA fragments generated from the homogenisation or bead beating process are able to bind more efficiently to the columns, and are thus enriched. However, despite the greater amounts of "background" host DNA in these samples, the concordance between the real-time PCR and microarray results suggests that the amount of host DNA present did not adversely impact microbial DNA extraction or its amplification.

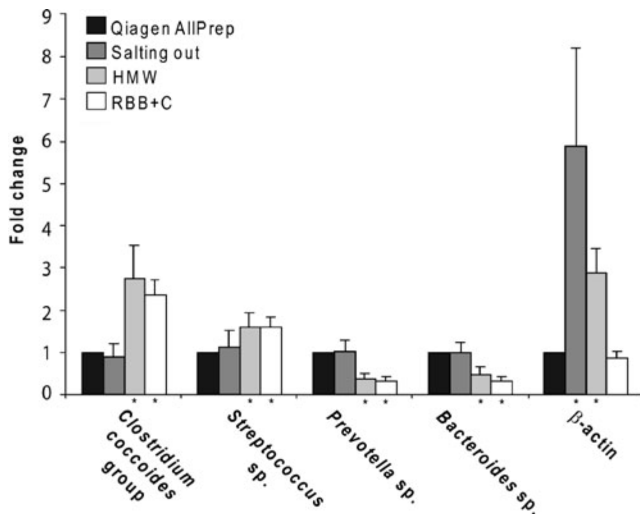

Figure 3 Real-time PCR analysis of resected colonic tissue extracted DNA. Data for each specific group was normalised by the Livak method [29] using Bacteria-specific *rrs* gene primers [11]; *bars* represent the average fold change in relation to the QIAGEN AllPrep DNA/RNA sample. Significant differences ($p<0.05$) between DNA extraction methods identified by real time PCR results targeting specific bacterial groups are annotated by an *asterisk* although the difference between the HMW and salting-out methods for *Streptococci* spp. is marginal ($p<0.07$)

Discussion

The goal of this study was to establish whether the method of DNA extraction used with mucosal tissue samples may impact the recovery and representation of the microbial diversity associated with that tissue. Previous studies have described the effects from various DNA extraction protocols on microbial diversity from stool samples as assessed by DGGE [58, 59], T-RFLP [37] and PCR [33]. Here, we applied the Aus-HIT chip to characterise the effects from different DNA extraction methods on the recoverable microbial diversity associated with colonic tissue.

Our results demonstrate that all four methods of DNA extraction are efficacious in releasing DNA from the microbial populations associated with the colonic tissue; and produce comparably similar profiles of community diversity. Nonetheless, a small percentage of probes were identified that gave statistically significant differences in signal intensity and based on the results presented here, these differences arise because the QIAGEN AllPrep DNA/RNA and salting out methods were not as efficient as the HMW or RBB+C methods for recovering DNA from some of the Firmicutes like *Veillonella*, *Streptococcus* and *Clostridium* spp. These differences in extraction efficiencies observed are likely due to the mechanism of lysis. Lysis by the QIAGEN AllPrep DNA/RNA or salting out methods are relatively gentle, especially when compared to the extensive enzymatic treatment and chemical based lysis used with the HMW method, and the mechanical- and chemical-based lysis used with the RBB+C method. These differences in lysis efficiency are most likely to be observed with bacteria that possess a cell wall ultrastructure that is especially difficult to lyse and/or mechanically disrupt. The differences between the HMW and RBB+C profiles for the Firmicutes-based probes are interesting and assuming that lysis by the RBB+C method is relatively non-discriminatory this suggests that the enzymatic based lysis inherent to the HMW method may be prone to bias.

Salonen et al. [48] also reported a higher proportion of Bacteroidetes and a lower proportion of Clostridium cluster XIV and Actinobacteria with DNA recovered from faeces using a QIAGEN Stool DNA Mini kit. The observations by Salonen et al. [48] are consistent with those of other studies (e.g. [14, 22]) and they hypothesised that the QIAGEN Stool DNA Mini kit was able to extract DNA more readily from the Gram-negative population of the human faecal community resulting in an apparent overestimation of their prevalence in the sample. In a separate study characterising the effectiveness of DNA recovery from faecal samples, Wang et al. [56] concluded that DNA was released from Gram-negative bacteria with much higher efficiency (80–100%) than from Gram-positive bacteria (1–20%). Other studies have also reported similar variations in DNA extraction efficiency (e.g. [33, 39]) and taken together this further confirms our observation with the Aus-HIT chip that the mechanism of DNA extraction can significantly impact the recovery of microbial diversity leading to differential representation of particular phylogenetic groups.

We found that all four DNA extraction methods were broadly comparable in detecting the archaeal diversity associated with the colonic tissue with only slight variations observed between the methods. The predominant human methanogenic gut archaea, *M. smithii* was detectable by all four extraction methods; however, in contrast *Methanosphaera* spp. was not detected. Recent studies have revealed that the human colonic methanogenic archaeal community may be more diverse than previously estimated [16, 34, 38, 50] and in addition to *M. smithii*, we also detected *Methanosaeta* spp. and *Methanocaldococcus* spp. in association with the tissue sample. *Methanosaeta* spp. has not previously been associated with human samples; however, it and the broader *Methanosaetacae* have been detected in anaerobic digesters containing anaerobic sewage sludge or a combination of anaerobic sewage sludge with bovine faecal material as an inoculum [17, 32]. Oxley et al. [42] also identified *Methanosaeta* spp. *rrs* gene sequences (≥99% similarity) in association with table salt suggesting a possible dietary source for this archaea. In contrast, the *Methanococcacae* have previously been detected in rumen gastrointestinal environments by probe-based approaches, but these observations remain to be conclusively confirmed

[20]. Nonetheless, putative Methanococcales and Methanosarcinales DNA sequences were identified in a metagenomic dataset produced from human faecal samples [16]. We also detected non-methanogenic archaea in association with the colonic tissue, in particular *Halobacterium* spp. and *Sulfolobus* spp. These and other phylogenetically similar archaea have previously been shown to be associated with human faecal and/or tissue samples [16, 38, 42, 47]. Taken together, this suggests that the archaeal diversity within the human colon may potentially have been significantly underestimated and a deep sequencing approach targeting the human colonic archaea may provide further insight into the phylogenetic diversity and ecophysiological role of this community.

Recent observations suggest that there is a microbial phylogenetic core that is associated with human faecal samples [44, 53]. In addition, a recent study revealed the presence of a faecal-associated core measurable microbiota in mice; the abundance of which is shaped by both environmental and host genetic factors [5]. The mucosal and faecal associated microbiota have been shown to differ and it remains to be determined if a similar or distinct mucosal associated phylogenetic core exists. It may be that in addition to genetic susceptibility, host-mediated perturbations in the mucosal microbiota also contribute to disease propensity. The Aus-HIT chip, as do the other HIT microarrays, offers a useful tool complementary to next generation sequencing methods for this type of diversity analysis.

Conclusion

Our results show that while all four methods are equally efficacious at releasing microbial DNA, the HMW and RBB+C methods of DNA extraction were more efficient in extracting DNA from some of the Firmicutes bacteria associated with colonic tissue. Importantly, these results also suggest that tissue DNA extracts held in archival biobanks may be amenable to retrospective microbial diversity analyses provided that comparisons are restricted to samples subjected to the same DNA extraction method. The purity of the archived DNA sample(s), the storage temperature and the effects of oxidative damage are also important considerations prior to commencing detailed analyses [1, 25].

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