Canadian Science Publishing
NRC Research Press journals | Publishing Services

Canadian Journal of Microbiology
Revue canadienne de de microbiologie

Microbiota on stainless steel coupons and correlation to the sink surface in domestic kitchens

Journal:	*Canadian Journal of Microbiology*
Manuscript ID	Draft
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Moen, Birgitte; Nofima, Norwegian Institute of Food, Fisheries and Aquaculture Research, Food safety and quality Røssvoll, Elin; Nofima, Norwegian institute of Food, Fisheries and Aquaculture Research; Animalia - Norwegian Meat and Poultry Research Center Måge, Ingrid; Nofima, Norwegian institute of Food, Fisheries and Aquaculture Research Møretrø, Trond; Nofima, Norwegian institute of Food, Fisheries and Aquaculture Research Langsrud, Solveig; Nofima, Norwegian institute of Food, Fisheries and Aquaculture Research
Keyword:	Microbiota, Stainless steel coupons, Sink surface, Domestic kitchens

Microbiota on stainless steel coupons and correlation to the sink surface in domestic kitchens

Running title: ***Microbiota on stainless steel coupons and correlation to the sink surface***

Birgitte Moen[1], Elin Røssvoll[1,2], Ingrid Måge[1], Trond Møretrø[1] and Solveig Langsrud[1]

[1] Nofima, Norwegian Institute of Food, Fisheries and Aquaculture Research, Osloveien 1, N-1430 Aas, Norway

[2] Animalia, Norwegian Meat and Poultry Research Center, P.O.Box 396 Økern, 0513 Oslo, Norway

Corresponding author: Birgitte Moen (e-mail: birgitte.moen@nofima.no)

ABSTRACT

Stainless steel coupons are readily used in biofilm studies in the laboratory and attached to different surfaces to create a "natural" biofilm to be studied further in laboratory trials. Little is however done to investigate how well the microbiota on such coupons represents the surrounding environment. The microbiota of coupons attached for three months and sink wall surfaces in eight domestic kitchen sinks was investigated by next generation sequencing (MiSeq) of the 16S rRNA gene derived from DNA and RNA (cDNA), and by cultivation/identification of colonies. The average number of colony forming units was about tenfold higher for coupons than sink surfaces and more variation in bacterial counts between kitchens was seen on sink surfaces than coupons. The microbiota in the majority of biofilms was dominated by *Moraxellaceae* (genus *Moraxella*/*Enhydrobacter*) and *Micrococcaceae* (genus *Kocuria*). Most of the variation (38.2%) in the microbiota was due to differences between kitchens, 10.8% was due to the nucleic acid template (DNA vs RNA) and 5.1% of the variation was a result of differences between coupons and sink surfaces. The variation between sink surfaces and coupons was smaller for samples derived from RNA than for DNA. Overall, our results suggest that stainless steel coupons are suited to model the dominating part of the natural microbiota of the surrounding environment, and furthermore suitable for different downstream studies.

Key words: Microbiota, stainless steel coupons, sink surface, domestic kitchens

INTRODUCTION

Studying microorganisms directly *in situ* is challenging for a number of reasons: it is not possible to perform certain analyses directly or transport the surfaces to a laboratory for further analysis, it is not safe to introduce pathogens outside the laboratory and it is difficult to compare different treatments, conditions or surfaces in a systematic and standardized way. Therefore most studies on biofilms are conducted using bacteria collected from environmental biofilms or laboratory strains in laboratory models. Biofilms produced at the laboratory are more or less relevant for the environments they are meant to mimic, with respect to a range of factors such as materials, microbiota, temperatures, nutrients, sanitation regimes and the dynamics for all these factors. To achieve a more realistic biofilm, some studies use the addition of food residues or organic soiling in the biofilm formation (Chaitiemwong, Hazeleger et al. 2014, Kuda, Shibata et al. 2015). Another approach to make the models more realistic is to place coupons at the site to be studied and allow for a natural biofilm to evolve. The biofilm or attached bacteria can then be investigated in different downstream studies. This will ideally allow studies on biofilms that are more relevant than those produced using laboratory models. The approach has been used to compare hygienic properties of different materials (Guobjornsdottir, Einarsson et al. 2005), identification of microbiota in food production factories (Hood and Zottola 1997, Mettler and Carpentier 1998, Gunduz and Tuncel 2006), detection of biofilm formation (Holah, Betts et al. 1989, Gibson 1995) and recently we used this approach to study the effect of kitchen cleaning methods (Rossvoll, Langsrud et al. 2015). Little is however done to evaluate how well the microbiota developed by this approach reflects the microbiota developed *in situ*.

Next generation sequencing (NGS) technology using the 16S rRNA gene as a taxonomic marker is often used to study complex microbial communities. NGS generates enormous

amounts of data helping to reveal a more complete picture of the microbiota compared to traditional cultivation based analyses, which is dependent on cultivation conditions such as nutrients, atmosphere and temperature. One drawback of DNA based microbiota analysis is however that it does not discriminate between dead and viable bacteria, and this may limit the applicability when studying matrixes with a high proportion of dead bacteria. In many studies the relative amount of dead bacteria is considered insignificant but when working with biofilms subjected to different environmental stress this may not be the case. DNA will give a good overview of the complete microbiota of the biofilm (dead and active) whilst the use of RNA in principle will estimate the current in situ activity of a community, because cellular rRNA concentration is generally well correlated with growth rate and activity (Poulsen, Ballard et al. 1993, Bremer 1996). rRNA are also thought to degrade only under certain stress conditions or when an RNA molecule is defective (Deutscher 2003).

In this study we aimed to investigate how the microbiota developing on stainless steel coupons placed in domestic kitchen sinks (stainless steel) for three months compared to the natural microbiota of the kitchen sink surfaces using samples from a previously published study (Rossvoll, Langsrud et al. 2015), where the effects of different hygiene procedures in reducing bacterial contamination was studied. Domestic sinks were chosen as a suitable environment since the material (stainless steel) was comparable to the coupons, and as these are heavily exposed to and colonized by bacteria (e.g. from raw produce, water and skin microbes). We compared the microbiota between coupons and sink surfaces derived from both DNA (live and dead bacteria) and RNA (potentially active bacteria). I addition we used traditional cultivation followed by 16S rRNA gene sequencing of isolates to see how well the RNA derived microbiota reflected what could be cultivated.

MATERIALS AND METHODS

Experimental design

The experimental setup of the steel coupons in the domestic kitchen sinks (stainless steel) has previously been described (Rossvoll, Langsrud et al. 2015). Briefly, nine volunteers (all having microbiological experience) attached three stainless steel coupons in their kitchen sinks (AISI 304, 2B; Norsk Stål AS, Nesbru, Norway) in January 2013. The surface of the stainless steel coupon was 2 x 6 cm. The volunteers were instructed to use their kitchen sinks as normal, but to avoid direct scrubbing of the coupons. The coupons were left in the kitchen sinks for three months. In April 2013 the volunteers were instructed to sample an area of the size of a coupon (2 x 6 cm) beside each of three different coupons in their kitchen sink. All volunteers were provided with equipment and detailed instructions of how to swab the specific areas in their kitchen sink surface (Hedin, Rynback et al. 2010).They were also instructed on how to remove the coupons in their sinks with gloved hands to avoid contamination, and place each coupon in a prelabeled 50 ml tube for transportation to the laboratory. The swabs and the coupons were sampled in the morning by the volunteers, brought to the laboratory and analysed within an hour. Of the nine kitchens, one volunteer (kitchen no 1) unfortunately sampled erroneously with only one instead of two swabs and was therefore not included in the analysis.

Surface sampling and cultivation methods

The swabbing and cultivation was as described previously (Rossvoll, Langsrud et al. 2015). Briefly, two swabs were used for each coupon/sink area and both swabs were put in the same tube with 3 ml D/E (Dey/Engley) Neutralizing Broth (BD Difco™, New Jersey, USA) and

serial 10-fold dilutions were prepared in PBS and spiral plated on Tryptic soy agar (TSA; Oxoid, Basingstoke, UK). The plates were incubated at 25°C for 3 days before determination of cfu and isolating single colonies. A total number of 20 colonies (or less at low cell numbers) were picked at random from plates from each kitchen resulting in up to 60 colonies picked per kitchen. The colonies were restreaked on TSA, incubated at 25°C for three days before preparation for sequencing.

DNA and RNA extraction and cDNA synthesis

The leftover material used for cultivation (approx. 2 ml per coupon/sink surface) was used to extract DNA and RNA. The Neutralizing broth originating from the swabs from three coupons per kitchen were mixed and then split into two samples; one for DNA extraction and one for RNA extraction. The same were done for the three sink surface areas. For DNA extraction the samples was centrifuged at 13000 *g* for 5 min and then frozen at -20°C for one/two weeks before extraction using the QiaAmp Stool Kit (Qiagen, Valencia, CA). Briefly, the bacterial pellet was resuspended in 500 µl ASL buffer (stool lysis buffer, Qiagen), transferred to Lysis Matrix E (MP Biomedicals, Solon, USA) tubes, and lysed in a FastPrep bead beater (MP Biomedicals) for 40 s at 6 m/s. The samples were centrifuged briefly before adding additional 500 µl ASL buffer and vortexed. The samples were thereafter incubated at 70°C for 5 min, centrifuged at 14 000 *g* for 5 min before transferring to new tubes, adding 400 µl ASL buffer and following the manufacturer's protocol.

The samples for RNA extraction were added to tubes containing RNA Protect (Qiagen), vortexed for 5 s, incubated 5 min at room temperature, centrifuged at 5000 *g* for 10 min, the supernatant was decanted and the pellets was kept at -20°C/-80°C until extraction using the RNeasy mini kit (Qiagen) and an on-column DNase digestion (Qiagen). Briefly, 700 µl buffer

RTL (lysis buffer, Qiagen) (with 40 µl 1M DTT/ml RTL) was added to the pellet, vortexed 5-10 s and then transferred to Lysis Matrix E (MP Biomedicals) tubes, and lysed as described above. The samples were centrifuged at 14000 ×*g* for 5 min before adding ethanol and following the Qiagen protocol from this point. The RNA was measured using nanodrop (NanoDrop Technologies, Inc., Wilmington, USA) and stored at -80°C until cDNA synthesis. The cDNA synthesis was performed using SuperScript™ III reverse transcriptase (Invitrogen, Life Technologies Ltd, Paisley, UK) as recommended by the manufacturer, with and without enzyme (negative control).

PCR and sequencing of colonies

PCR and sequencing was performed as described previously (Rossvoll, Langsrud et al. 2015) using universal primers (Nadkarni, Martin et al. 2002) for 16S rRNA gene amplification (V3-V4) and sequencing. The taxonomy was identified using the RDP (Ribosomal Database Project) SeqMatch (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp). The thresholds used in the RDP search was: both type and none type strains; both uncultured and isolates; only good sequences >1200nt and KNN=1.

Biofilm microbiota study (NGS)

DNA and RNA (cDNA) from sink surface and coupon samples (described above) were used as template for the NGS (MiSeq, Illumina Inc., San Diego, USA) analysis. A portion of the 16S rRNA gene spanning the variable region 4 (V4) was amplified using the barcoded, universal primer set (515F/806R) (Caporaso, Lauber et al. 2012). PCR mixture and thermal cycling conditions were the same as described by Caporaso et al. (Caporaso, Lauber et al.

2012). In addition to the experimental samples, the MiSeq run also contained a control library made from phiX Control v3, which in this run accounted for 50 % of reads. The library quantification and sequencing was performed by the Norwegian Sequencing Centre (http://www.sequencing.uio.no/). The sample pool was quantified using the Invitrogen Qubit, diluted to 2nM, and the MiSeq Protocol provided by Illumina was then followed.

The total number of sequences was 18,162,924. The forward and reverse reads were joined using the QIIME toolkit (Caporaso, Kuczynski et al. 2010) (version 1.7.0) and the barcodes corresponding to the reads that failed to assemble were removed. The total number of sequences that joined were 10,517,341 with an average join length of 49.18. The sequences were then demultiplexed in QIIME allowing zero barcode errors and a quality score of 30 (Q30) resulting in 6,187,913 sequences with a median sequence length of 253 bp. The average number of sequence per sample was 193,372 (min 160,167; max 226,801). Reads were assigned to their respective bacterial id using two-step open-reference operational taxonomic unit (OTU) picking workflow (Rideout, He et al. 2014). Briefly, after sequences were demultiplexed and quality filtered, reads were first clustered with a reference database (the Greengenes database (gg_13_5)) pre-clustered at 97% identity. Second, reads that did not group with any sequences in the reference collection were clustered *de novo*. Clustering at 97% identity was carried out using the UCLUST algorithm (Edgar 2010). Reads that did not match a reference sequence were discarded. Representative sequences were chosen for each OTU (cluster centroids) and aligned against the Greengenes core set with PyNAST (Caporaso, Bittinger et al. 2010). Chimeric sequences were removed in QIIME using ChimeraSlayer. Singeltons were removed resulting in 5,955,225 sequences. In total 5661 OTUs passed the filter. Of these, 48% were ‘novel’ (i.e. not found in the Greengenes database (gg_13_5).

178 **Statistical analyses**

179 The alpha diversity (observed species) in all kitchens was calculated in QIIME by 100.000
180 rarefactions, and differences between groups were tested using paired t-tests (Minitab®
181 (Minitab 16.1.1, 2010 (Minitab Ltd., Coventry, UK)). The differences between average
182 bacterial counts were also tested using paired t-tests (Minitab®).

183 The differences in microbiota were analyzed by principal component analysis (PCA) and 50-
184 50-MANOVA (Langsrud 2002). 50-50-MANOVA is a method for multivariate analysis of
185 variance with a high number of collinear responses and was used to focus on partitioning the
186 variation due to differences between kitchens, sink surface vs coupon and DNA vs RNA, and
187 on identifying the bacterial groups that are significantly different. All analyses were
188 performed at the genus level (level 6 table from QIIME). 50-50-MANOVA was calculated in
189 MATLAB (Release 2013b, The MathWorks, Inc., Natick, Massachusetts, USA) and the taxa
190 were scaled to unit variance in order to remove abundance effects from the analysis.

191

192 **RESULTS**

193 **Total bacterial counts of coupons and sink surface swabs**

194 The average bacterial counts were significantly ($p<0.05$) higher for coupons than
195 corresponding samples taken from the sink surface, with an average cfu of log 6.2 and log 5.4,
196 respectively (Figure 1). The bacterial counts on the coupons from all kitchens were similar
197 (not significantly different between kitchens). The bacterial counts on the sink surface on the
198 other hand was significantly different between the different kitchens, and kitchen no 2 had the
199 highest CFU count. Figure 1 shows the average cfu (log_{10}) for coupons and sink surface (both
200 12 cm^2) for all kitchens.

Microbiota (NGS)

Overall bacterial composition

The microbiota across all samples (DNA and RNA, coupons and sink surfaces) was dominated by phylum *Proteobacteria* (average 54 %), followed by *Actinobacteria* (34 %), *Firmicutes* (8 %), *Bacteroidetes* (2 %), *Cyanobacteria* (1 %) and *Fusobacteria* (0.2 %). Most *Proteobacteria* belonged to the class *Gammaproteobacteria* (44 %). Figure 2 shows the average relative abundances (percent) of the dominant bacterial taxa (phylum/family level) for DNA (coupon and sink surface) and RNA (coupon and sink surface).

Overall the biofilm samples were dominated by two families; *Moraxellaceae* (genus *Moraxella/Enhydrobacter*) and *Micrococcaceae* (genus *Kocuria*). There were however variation between the kitchens and kitchen no 8 and 9 had a different dominating bacterial population. The sink surface in kitchen no 8 had a more diverse microbiota than the other samples and had high relative values of *Bacilli* (*Staphylococcaceae* and *Streptococcaceae*) in addition to *Moraxellaceae* and *Micrococcaceae,* while the sink surface in kitchen no 9 (DNA) was dominated by *Enterobacteriaceae*. Table 1 shows the distribution of taxa down to family level (represented above 5 % in one or more samples) for all samples.

Bacterial diversity within samples

To investigate the bacterial diversity within the different samples an alpha diversity analysis was performed (QIIME). This analysis revealed a tendency (not significant at 5% level) of higher diversity (observed species) in samples derived from DNA than from RNA, and in sink samples compared to coupons.

Bacterial diversity between samples

To investigate the variation in bacterial composition between the samples, a beta diversity analysis (weighed- and unweighed unifrac) was performed (QIIME). This analysis revealed that many of the low abundant bacteria differed between the experimental variables (kitchens, RNA/DNA, coupons/sink surface) and that the dominating microbiota was similar for most biofilms. Further statistical analysis was therefore performed on standardized variables (in order to give equal weight to all OTUs regardless of abundance). This analysis revealed some significant differences in the bacterial composition between the experimental variables (kitchen (A); sink surface/coupon (B) and DNA/RNA (C) (see Table 2).

The differences between kitchens accounted for the largest variation in the data, both with regard to main effect (38.2%) and interaction with sink surface/coupon (21.8%). This means that there was a significant difference between sink surface and coupon but that this difference was not systematic. The variation due to differences between coupons/sink surface (5%) and DNA/RNA (11%) were small in comparison. This indicates that the coupon was quite representative for the sink surface, and that the main results were similar based on analyses for both RNA and DNA. The differences, however small, are illustrated in principal component (PC) plots in Figure 3. From the scores plot (A) it is clear that there was a separation between samples derived from RNA (green) and DNA (blue) along PC1 (explains 17% of the variance). Note also that the variation in microbiota in sink surfaces (outlined area) was larger than in coupons (filled area), and that this variation was larger in samples derived from DNA than RNA. This indicates that there was a systematic difference between sink surface and coupon for DNA, but not for RNA. The loadings plot (B) shows the significant bacteria (determined from 50-50 MANOVA) as filled circles, and the circle size is proportional to abundance. The taxa of the bacteria significantly different in one or more sample categories are listed in the table in Figure 3. From this we can see that relative proportions of *Acinetobacter*, *Dermacoccus*, *Dermabacteriaceae*, *Chryseobacterium*,

Streptophyta, *Actinomycetales* and *Comamonadaceae* were significantly different in the microbiota derived from DNA and RNA, where the order *Actinomycetales* (including *Dermacoccus* and *Dermabacteriaceae*) had a higher abundance in RNA derived samples. There were three bacterial taxa significantly different between coupons and sink surfaces (*Streptococcus*, *Chryseobacterium* and *Exiguobacterium*), where *Streptococcus* had a higher abundance in sink surface samples.

Bacterial taxa from isolates cultured from coupons

To get a more comprehensive overview of the viable population of the microbiota on the coupons, the identity of randomly selected isolates were determined (Table 3).

Results from NGS analysis derived from RNA was used for comparisons with the microbiota determined from identification of cultivated bacteria as the former should in principle reflect the active part of the population. Both methods resulted in the same dominating families/genera; *Micrococcaceae* (genus *Kocuria*) and *Moraxellaceae* (genus *Moraxella/Enhydrobacter*). Bacteria belonging to the genera *Rhodococcus* (f *Nocardiaceae*), *Microbacterium* (f *Micrococcaceae*) and *Brevundimonas* (f *Caulobacteraceae*) were isolated from some coupons, but these genera were not found using NGS. NGS detected *Dermacoccaceae* (0.1-12.4 %) and *Rhodobacteriaceae* 0.1-15.9 %) from most coupons, but these families were not represented among the cultivated isolates.

DISCUSSION

In the present study we investigated how the microbiota developing on stainless steel coupons placed in domestic kitchen sinks (stainless steel) compared to the natural microbiota of the

kitchen sink surfaces. In addition we compared the microbiota derived from DNA and RNA to get a picture of the total (live and dead) microbiota and the potentially active microbiota, and last to see how well the RNA derived microbiota reflected what could be cultivated.

The results showed that the bacterial composition of coupons correlated well with the sink surface, with the best correlation resulting from microbiota derived from RNA samples. The cultivation results showed higher bacterial counts on coupons than sink swabs. For some coupons, a visible fouling was observed at the lower parts, and that water attached to the fouling. This could produce a more humid environment with higher survival and growth of bacteria compared to the sink surface. The biofilm on the coupons were also younger (three months) compared to the biofilm on the sink walls and one cannot exclude the possibility that in the quantitative analysis a higher proportion of cells were detached from the coupons than the sink surfaces, as it is known that mature biofilms are difficult to remove, and require increased mechanical force e.g. brushing rather than wiping. Further studies are needed to find the optimum attachment time and sampling method. Also, the chemical composition of the biofilm was not assessed and structural and chemical differences between biofilms of the sink surface and coupons cannot be excluded. However, the selective pressure, for example long periods of drying, was still quite similar for coupons and the sink surface as the dominating microbiota was not systematically different.

Overall, the majority of the biofilms were dominated by *Moraxellaceae* (genus *Moraxella/Enhydrobacter*) and *Micrococcaceae* (genus *Kocuria*). This is in accordance with what have been found by others, although there are variations between studies. The microbiota in domestic kitchen sinks have been studied in some detail by Flores et al., (Flores, Bates et al. 2013) where sink samples from four kitchens were investigated together with over 80 other kitchen surfaces. Compared to the other surfaces they found the least diverse

296 communities associated with metallic surfaces in and around sinks, which were dominated by
297 biofilm-forming Gram-negative bacteria, including known biofilm-formation organisms like
298 *Sphingomonadaceae*. They found *Moraxellaceae* to be the dominating family in sink basin
299 and sink backsplash. In another study on common household surfaces, *Kocuria* spp. were
300 found to be among the most frequent recovered isolates and the most frequent recovered
301 isolate from kitchen sinks (Saha, Wheeler et al. 2014), and Stellato et al. (Stellato, La Storia et
302 al. 2015) found *Kocuria* in all sink samples belonging to the pre-processing zones in a
303 cooking center for hospital foodservice.

304 The genus *Enhydrobacter* has been found in widely diverse environments like athletic
305 equipment (Wood, Gibbons et al. 2015), skin (buttocks) (Zeeuwen, Boekhorst et al. 2012),
306 toilet samples (Jeon, Chun et al. 2013) and a beer bottling plant (Timke, Wang-Lieu et al.
307 2005). A search in BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed a 100% match to
308 both *Moraxella osloensis* and *Enhydrobacter aerosaccus* for the OTU/isolates representing
309 genus *Enhydrobacter* in our study. Near full length 16S rRNA gene sequences of one random
310 isolate (classified as genus *Enhydrobacter*) confirmed that our isolates was most similar to
311 these two species (99% identity, data not shown). Both *Moraxella osloensis* and
312 *Enhydrobacter aerosaccus* have been found in skin microbiota (Gao, Tseng et al. 2007, Jeon,
313 Chun et al. 2013). *Moraxella osloensis* has also been found in the biofilm of various pipe
314 materials in drinking water distribution systems (Zhu, Wu et al. 2014) and is the bacterium
315 responsible for the locker-room smell or shower-curtain odor (Kubota, Mitani et al. 2012).
316 *Moraxella* spp. was also identified as a part of the microbial population on stainless steel
317 coupons placed in fish and shrimp factories for a three month period (Guobjornsdottir,
318 Einarsson et al. 2005), but not as the dominant genus. *Moraxella* is neither associated with
319 food borne infections or spoilage. The best sequence match for the OTU/isolates representing
320 genus *Kocuria* was *K. rhizophila* (confirmed by near full length 16S rRNA gene sequencing

of a few random isolates). Members of the genus *Kocuria* have been isolated from a wide variety of natural sources, including mammalian skin, soil, the rhizosphere, fermented foods, clinical specimens, freshwater, and marine sediments. The genus has also been isolated from other food production environments (Carpentier and Chassaing 2004, Moretro, Hoiby-Pettersen et al. 2011, Møretrø 2013). Survival in these environments can be explained by resistance to desiccation, biofilm forming abilities and tolerance to chlorine (Leriche, Briandet et al. 2003, Møretrø 2013). Others have shown that *K. rhizophila* can survive on dry surfaces for several days as well as being tolerant to high salt concentrations in growth medium (Kovacs, Burghardt et al. 1999, Kim, Nedashkovskaya et al. 2004). *Kocuria* spp. is not considered to be pathogenic, but in a study on bacteria surviving cleaning and disinfection in food processing plants, a *Kocuria varians* strain increased biofilm production in *Listeria monocytogenes* (Carpentier and Chassaing 2004). Further analyses are however needed if one wants to determine if our isolates represents a threat for safety.

As expected, most of the variation in the microbiota was related to different kitchens and not the sampling site (coupon/sink surface). This variance is likely to be associated with specific selective characteristics such as physical and chemical cleaning regimes, food preparation regimes and water availability. In a kitchen sink environment high loads of organic particulate matter such as fats and proteinecous material represent a source of nutrients for attached and/or transient microorganisms. The different kitchens would also have been exposed to different sources of bacteria from raw produce, different microbiota of the residents' skin as well as difference in the faucet water (five of the eight kitchens had different water sources; kitchen no 2 and 5 had the same source and kitchen no 6, 7 and 9 had the same source). Flores et al., (Flores, Bates et al. 2013) identified three indicator taxa from raw produce (*Enterobacteriaceae*, *Microbacteriaceae* and *Bacillales*), four from the human skin (*Propionibacteriaceae*, *Corynebacteriaceae*, *Staphylococcaceae* and *Streptococcaceae*) and

three from the faucet water samples (*Sphingomonadaceae*, *Methylobacteriaceae* and *Gallionellaceae*). Two of the indicator taxa from the human skin (*Staphylococcaceae* and *Streptococcaceae*) were major taxa in one of the kitchens (no 8) in our study. Further studies are needed to demonstrate the effect of differential usage of the sink.

More differences were found between microbiota on coupons and sink surfaces when using results derived from DNA compared to RNA, indicating differences in the dead population of cells. This was not surprising since the dead cell population will reflect the part of the population not selected for survival and this may be different for a surface exposed to bacteria for years compared to coupons that had been placed in the sink for a three month period. RNA was chosen to illustrate the active taxa since cellular rRNA concentration is generally well correlated with growth rate and activity (Poulsen, Ballard et al. 1993, Bremer 1996). There are several studies that have used rRNA to characterize the growing or active microbes, and Blazewicz et al. (Blazewicz, Barnard et al. 2013)found >100 studies that used rRNA for these purposes, including recent studies using rRNA to identify currently active microbes (e.g. (Gentile, Giuliano et al. 2006, DeAngelis, Silver et al. 2010, Jones and Lennon 2010, Gaidos, Rusch et al. 2011, Lanzen, Jorgensen et al. 2011, Wust, Horn et al. 2011, Brettar, Christen et al. 2012, Mannisto, Kurhela et al. 2013)). Blazewicz et al. however argued that there are conflicting patterns between rRNA content and growth rate indicating that rRNA is not a reliable metric for growth or activity and rather suggested employing rRNA abundance data as an index of potential activity that provides basis for further investigations (Blazewicz, Barnard et al. 2013). Recognizing that the RNA derived microbiota reflects past, current and future activities in addition to different life strategies we cannot conclude that the RNA observed microbiota reflect the true viable, active bacteria. However, the fact that the microbiota on coupons and sink surfaces correlated better when derived from RNA comparted to DNA, and that systematic differences between coupons and sink surfaces were not found in

samples derived from RNA, indicate that RNA gave the best picture of the dominating, active microbiota in our study. To investigate this further, we also identified a random selection of isolates cultivated from the coupons. The cultivation results showed a high number of cultivable bacteria and, although some differences in the microbiota were observed, the dominating taxa correlated well with the NGS result. One must have in mind that only a few isolates were analyzed compared to the high throughput results from the NGS analysis. The cultivation results are also likely to be influenced by the use of a single culture medium which is unlikely to meet the nutritional requirements necessary to maximize the recovery of all the bacteria present. The NGS results could also have been influenced by the choice of PCR primers and PCR conditions. For example *Microbacterium* (f *Micrococcaceae*) was only detected by cultivation as previously also reported by Brightwell et al. (Brightwell, Boerema et al. 2006). Our study clearly shows that both culture-independent and culture-dependent techniques are important to give the best representations of the microbiota in domestic kitchen sinks.

The results presented show that stainless steel coupons are suited to model the active and dominating part of the domestic kitchen sink surface. Such coupons are therefore suited for further studies of, e.g. effects of hygienic procedures (Rossvoll, Langsrud et al. 2015). The methodology could also be developed for use in other environments and could potentially be used to study the ability of pathogens to attach to a biofilm produced *in situ*, an experiment that would not be feasible to perform in e.g. food processing environments or in the domestic environment. Sampling of the surrounding surfaces should, however always be performed as a control. We have also shown that the choice of nucleic acid template will influence the results, and that care should be taken with respect to interpretation of bacterial activity.

ACKNOWLEDGEMENTS

The authors wish to thank The Norwegian Sequencing Centre for their assistance. We thank Janina Berg, Signe Marie Drømtorp, Merete Rusås Jensen and Anette Wold Åsli for excellent technical assistance. This work was supported by the Norwegian Research Council (grant no. 185143/I10), the Foundation for Research Levy on Agricultural products and research funds from the Norwegian Agricultural Authority.

REFERENCES

Blazewicz, S. J., R. L. Barnard, R. A. Daly and M. K. Firestone (2013). "Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses." Isme Journal **7**(11): 2061-2068.

Bremer, H., Dennis, PP. (1996). "Modulation of chemical composition and other parameters of the cell by growth rate." Neidhardt FC, editor. Escherichia coli and Salmonella. ASM Press; Washington D.C.: pp. 1553-1569.

Brettar, I., R. Christen and M. G. Hofle (2012). "Analysis of bacterial core communities in the central Baltic by comparative RNA-DNA-based fingerprinting provides links to structure-function relationships." Isme Journal **6**(1): 195-212.

Brightwell, G., J. Boerema, J. Mills, E. Mowat and D. Pulford (2006). "Identifying the bacterial community on the surface of Intralox belting in a meat boning room by culture-dependent and culture-independent 16S rDNA sequence analysis." Int J Food Microbiol **109**(1-2): 47-53.

Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen and R. Knight (2010). "PyNAST: a flexible tool for aligning sequences to a template alignment." Bioinformatics **26**(2): 266-267.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Tumbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight (2010). "QIIME allows analysis of high-throughput community sequencing data." Nature Methods **7**(5): 335-336.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J. A. Gilbert, G. Smith and R. Knight (2012). "Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms." Isme Journal **6**(8): 1621-1624.

Carpentier, B. and D. Chassaing (2004). "Interactions in biofilms between Listeria monocytogenes and resident microorganisms from food industry premises." International Journal of Food Microbiology **97**(2): 111-122.

Chaitiemwong, N., W. C. Hazeleger and R. R. Beumer (2014). "Inactivation of Listeria monocytogenes by Disinfectants and Bacteriophages in Suspension and Stainless Steel Carrier Tests." Journal of Food Protection **77**(12): 2012-2020.

DeAngelis, K. M., W. L. Silver, A. W. Thompson and M. K. Firestone (2010). "Microbial communities acclimate to recurring changes in soil redox potential status." Environmental Microbiology **12**(12): 3137-3149.

Deutscher, M. P. (2003). "Degradation of stable RNA in bacteria." Journal of Biological Chemistry **278**(46): 45041-45044.

Edgar, R. C. (2010). "Search and clustering orders of magnitude faster than BLAST." Bioinformatics **26**(19): 2460-2461.

Flores, G. E., S. T. Bates, J. G. Caporaso, C. L. Lauber, J. W. Leff, R. Knight and N. Fierer (2013). "Diversity, distribution and sources of bacteria in residential kitchens." Environmental Microbiology **15**(2): 588-596.

Gaidos, E., A. Rusch and M. Ilardo (2011). "Ribosomal tag pyrosequencing of DNA and RNA from benthic coral reef microbiota: community spatial structure, rare members and nitrogen-cycling guilds." Environmental Microbiology **13**(5): 1138-1152.

Gao, Z., C. H. Tseng, Z. H. Pei and M. J. Blaser (2007). "Molecular analysis of human forearm superficial skin bacterial biota." Proceedings of the National Academy of Sciences of the United States of America **104**(8): 2927-2932.

Gentile, G., L. Giuliano, G. D'Auria, F. Smedile, M. Azzaro, M. De Domenico and M. M. Yakimov (2006). "Study of bacterial communities in Antarctic coastal waters by a combination of 16S rRNA and 16S rDNA sequencing." Environmental Microbiology **8**(12): 2150-2161.

Gibson, H., Taylor, JH., Hall, KH., Holah, JT. (1995). Biofilms and their detection in the food industry. R&D report No. 1. Chipping Campden, Gloucesterchire, UK.
Gunduz, G. T. and G. Tuncel (2006). "Biofilm formation in an ice cream plant." Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **89**(3-4): 329-336.
Guobjornsdottir, B., H. Einarsson and G. Thorkelsson (2005). "Microbial adhesion to processing lines for fish fillets and cooked shrimp: Influence of stainless steel surface finish and presence of gram-negative bacteria on the attachment of Listeria monocytogenes." Food Technology and Biotechnology **43**(1): 55-61.
Hedin, G., J. Rynback and B. Lore (2010). "New technique to take samples from environmental surfaces using flocked nylon swabs." Journal of Hospital Infection **75**(4): 314-317.
Holah, J. T., R. P. Betts and R. H. Thorpe (1989). "The Use of Epifluorescence Microscopy to Determine Surface Hygiene." International Biodeterioration **25**(1-3): 147-153.
Hood, S. K. and E. A. Zottola (1997). "Isolation and identification of adherent gram-negative microorganisms from four meat-processing facilities." Journal of Food Protection **60**(9): 1135-1138.
Jeon, Y. S., J. Chun and B. S. Kim (2013). "Identification of Household Bacterial Community and Analysis of Species Shared with Human Microbiome." Current Microbiology **67**(5): 557-563.
Jones, S. E. and J. T. Lennon (2010). "Dormancy contributes to the maintenance of microbial diversity." Proceedings of the National Academy of Sciences of the United States of America **107**(13): 5881-5886.
Kim, S. B., O. I. Nedashkovskaya, V. V. Mikhailov, S. K. Han, K. O. Kim, M. S. Rhee and K. S. Bae (2004). "Kocuria marina sp. nov., a novel actinobacterium isolated from marine sediment." Int J Syst Evol Microbiol **54**: 1617-1620.
Kovacs, G., J. Burghardt, S. Pradella, P. Schumann, E. Stackebrandt and K. Marialigeti (1999). "Kocuria palustris sp. nov. and Kocuria rhizophila sp. nov., isolated from the rhizoplane of the narrow-leaved cattail (Typha angustifolia)." International Journal of Systematic Bacteriology **49**: 167-173.
Kubota, H., A. Mitani, Y. Niwano, K. Takeuchi, A. Tanaka, N. Yamaguchi, Y. Kawamura and J. Hitomi (2012). "Moraxella species are primarily responsible for generating malodor in laundry." Appl Environ Microbiol **78**(9): 3317-3324.
Kuda, T., G. Shibata, H. Takahashi and B. Kimura (2015). "Effect of quantity of food residues on resistance to desiccation of food-related pathogens adhered to a stainless steel surface." Food Microbiology **46**: 234-238.
Langsrud, Ø. (2002). "50-50 multivariate analysis of variance for collinear responses." Journal of the Royal Statistical Society Series D-the Statistician **51**: 305-317.
Lanzen, A., S. L. Jorgensen, M. M. Bengtsson, I. Jonassen, L. Ovreas and T. Urich (2011). "Exploring the composition and diversity of microbial communities at the Jan Mayen hydrothermal vent field using RNA and DNA." Fems Microbiology Ecology **77**(3): 577-589.
Leriche, V., R. Briandet and B. Carpentier (2003). "Ecology of mixed biofilms subjected daily to a chlorinated alkaline solution: spatial distribution of bacterial species suggests a protective effect of one species to another." Environmental Microbiology **5**(1): 64-71.
Mannisto, M. K., E. Kurhela, M. Tiirola and M. M. Haggblom (2013). "Acidobacteria dominate the active bacterial communities of Arctic tundra with widely divergent winter-time snow accumulation and soil temperatures." Fems Microbiology Ecology **84**(1): 47-59.
Mettler, E. and B. Carpentier (1998). "Variations over time of microbial load and physicochemical properties of floor materials after cleaning in food industry premises." Journal of Food Protection **61**(1): 57-65.
Moretro, T., G. S. Hoiby-Pettersen, O. Habimana, E. Heir and S. Langsrud (2011). "Assessment of the antibacterial activity of a triclosan-containing cutting board." International Journal of Food Microbiology **146**(2): 157-162.
Møretrø, T., Langsrud, S., Heir, E. (2013). "Bacteria on meat abattoir process surfaces after sanitation: characterisation of survival properties of Listeria monocytogenes and the commensal bacterial flora." Advances in Microbiology **3**: 255-264

503 Nadkarni, M. A., F. E. Martin, N. A. Jacques and N. Hunter (2002). "Determination of bacterial load by
504 real-time PCR using a broad-range (universal) probe and primers set." Microbiology **148**(Pt 1): 257-
505 266.
506 Poulsen, L. K., G. Ballard and D. A. Stahl (1993). "Use of rRNA fluorescence in situ hybridization for
507 measuring the activity of single cells in young and established biofilms." Appl Environ Microbiol **59**(5):
508 1354-1360.
509 Rideout, J. R., Y. He, J. A. Navas-Molina, W. A. Walters, L. K. Ursell, S. M. Gibbons, J. Chase, D.
510 McDonald, A. Gonzalez, A. Robbins-Pianka, J. C. Clemente, J. A. Gilbert, S. M. Huse, H. W. Zhou, R.
511 Knight and J. G. Caporaso (2014). "Subsampled open-reference clustering creates consistent,
512 comprehensive OTU definitions and scales to billions of sequences." Peerj **2**.
513 Rossvoll, E., S. Langsrud, S. Bloomfield, B. Moen, E. Heir and T. Moretro (2015). "The effects of
514 different hygiene procedures in reducing bacterial contamination in a model domestic kitchen."
515 Journal of Applied Microbiology **119**(2): 582-593.
516 Saha, R., S. Wheeler, L. Bestevelt and R. Donofrio (2014) "Microbial hotspots and diversity on
517 common houshold surfaces." Charles River Laboratories International, Inc. **Technical Sheet**.
518 Stellato, G., A. La Storia, T. Cirillo and D. Ercolini (2015). "Bacterial biogeographical patterns in a
519 cooking center for hospital foodservice." International Journal of Food Microbiology **193**: 99-108.
520 Timke, M., N. Q. Wang-Lieu, K. Altendorf and A. Lipski (2005). "Community structure and diversity of
521 biofilms from a beer bottling plant as revealed using 16S rRNA gene clone libraries." Applied and
522 Environmental Microbiology **71**(10): 6446-6452.
523 Wood, M., S. M. Gibbons, S. Lax, T. W. Eshoo-Anton, S. M. Owens, S. Kennedy, J. A. Gilbert and J. T.
524 Hampton-Marcell (2015). "Athletic equipment microbiota are shaped by interactions with human
525 skin." Microbiome **3**: 25.
526 Wust, P. K., M. A. Horn and H. L. Drake (2011). "Clostridiaceae and Enterobacteriaceae as active
527 fermenters in earthworm gut content." Isme Journal **5**(1): 92-106.
528 Zeeuwen, P. L., J. Boekhorst, E. H. van den Bogaard, H. D. de Koning, P. M. van de Kerkhof, D. M.
529 Saulnier, S. van, II, S. A. van Hijum, M. Kleerebezem, J. Schalkwijk and H. M. Timmerman (2012).
530 "Microbiome dynamics of human epidermis following skin barrier disruption." Genome Biol **13**(11):
531 R101.
532 Zhu, Z., C. Wu, D. Zhong, Y. Yuan, L. Shan and J. Zhang (2014). "Effects of Pipe Materials on Chlorine-
533 resistant Biofilm Formation Under Long-term High Chlorine Level." Appl Biochem Biotechnol.

534

535

536 **TABLES**

537 **Table 1** Relative abundances (percent) of the dominant bacterial taxa (family level) across all samples (all taxa represented above 5 % in one or

538 more samples). The two overall dominating families are highlighted in gray.

539

			Actinobacteria				***Bacteroidetes***	***Cyanobacteria***	***Firmicutes***				***Proteobacteria***								***Other***
			Actinobacteria				*Flavobacteria*	*Chloroplast*	*Bacilli*				*Alphaproteobacteria*					*Gammaproteobacteria*			
			Actinomycetales				*Flavobacteriales*	*Streptophyta*	*Bacillales*	*Exiguobacterales*	*Lactobacillales*		*Rhizobiales*		*Rhodobacterales*	*Rhodospirillales*	*Sphingomonadales*	*Enterobacteriales*	*Other*	*Pseudomonadales*	
	Kitchen no		*Dermabacteraceae*	*Dermacoccaceae*	*Micrococcaceae*	*Promicromonosporaceae*	*Flavobacteriaceae*		*Staphylococcaceae*	*Exiguobacteraceae*	*Leuconostocaceae*	*Streptococcaceae*	*Methylobacteriaceae*	*Rhizobiaceae*	*Rhodobacteraceae*	*Acetobacteraceae*	*Sphingomonadaceae*	*Enterobacteriaceae*	*Other*	*Moraxellaceae*	
DNA	**Sink**	**2**	0.3	3.1	33.6		1.8	0.4		2.8		3.7		0.1	0.1	0.1	0.3	0.3		51.2	2.0
		3	0.1	0.1	3.1		4.2	4.3	0.7	0.4	0.5	3.5	0.3	0.3	0.1	0.4	3.5	12.6	1.0	54.6	10.3
		4	3.5	1.0	9.8	0.1	1.0	0.1			0.3	4.7			0.6	0.3	0.5			73.2	4.9
		5	0.1	11.9	5.8	6.7	6.4	7.2	0.3		0.5	2.9	0.1	2.1	0.4	0.4	1.1	1.1	0.3	33.3	19.5
		6	0.2	0.3	17.3		2.3	5.5	1.3		0.2	4.6	2.1	3.1	5.5	2.7	3.8	0.7		39.0	11.4
		7	1.7	0.5	12.6		0.6	8.0	1.0			12.8	0.1		0.1	0.7	0.1	0.4		50.6	10.8
		8	0.1	0.3	10.1		0.8	1.0	14.3		0.2	24.3	0.5	0.8	0.1	0.1	0.7	4.4		5.8	36.4
		9	0.5	2.9	19.9		0.4	0.7	0.5		1.5	2.4				0.1	0.2	52.4	0.1	5.6	12.7
	Coupon	**2**	1.1	3.4	8.7		4.7	0.2		5.9		3.3		1.1	0.6	0.2	0.9	0.7		62.9	6.4
		3	1.2		10.7		9.3	0.6		3.4		0.4	0.2	1.3	0.5	0.3	2.6	0.3	0.6	60.4	7.8
		4	4.4	3.5	25.7		0.4	0.1	0.1			0.1	0.2		1.3	0.7	0.5	1.0		59.2	2.7
		5	0.5	5.3	15.3		5.7	2.8	0.1		0.1	2.0		1.4	0.2	0.8	1.2	1.0		58.5	4.9
		6	0.1	0.4	23.5		2.2	2.4	0.3		0.1	1.1	0.3	0.5	4.0	1.1	1.1	0.1		58.7	4.0
		7	4.6	1.0	45.0		0.9	1.0	0.3			2.9	0.3	0.4	0.3	2.8	1.2	0.1		34.7	4.4
		8	0.7	0.5	27.6		0.9	0.1	1.0			4.1	0.1	1.6			3.7	0.4	0.2	54.6	4.4
		9	3.2	4.4	39.2		3.2	0.1			0.3	0.5	0.2	0.8	2.4	0.7	1.8	3.8	0.1	34.7	4.7
RNA	**Sink**	**2**	1.3	8.0	56.8		0.2			1.2		0.7		0.1	0.4	0.6	0.2	0.1		28.1	2.2
		3	1.2	0.2	17.2		0.2	0.2	2.3	0.4	0.4	0.6	0.6	0.1	0.6	8.4	10.1	0.4	8.5	44.5	4.2
		4	18.1	2.1	18.2		0.2		0.1			0.1	0.1		3.4	9.4	0.5	0.1	0.1	46.7	0.9

		5	1.1	24.1	15.2		1.9		0.8		1.1	4.4	0.5	5.2	4.5	2.2	1.2		0.4	31.1	6.2
		6	0.1	0.5	33.6		0.1		1.9			1.8	5.3	0.5	17.7	7.8	0.9		0.1	27.7	2.0
		7	2.0	0.5	3		0.3		2.4		0.1	2.9	0.1		0.1	1.6	0.1	0.1		58.3	1.5
		8	0.8	1.0	26.8				41.8		0.2	14.7	0.3		0.1		1.2			4.2	8.7
		9	1.1	6.4	49.5		0.1		1.8		9.2	2.1	0.1		0.5	0.2	0.3	6.9		15.9	5.9
	Coupon	**2**	4.6	12.4	15.2		1.5			5.2		0.8		1.2	3.0	1.3	0.8	0.2		47.6	6.0
		3	7.0	0.1	27.5		2.2		0.1	2.0		0.1	1.0	1.1	5.4	1.4	4.0	0.1	0.9	40.4	6.8
		4	13.9	5.0	31.6										8.4	5.1	0.5	0.1	0.1	34.2	1.1
		5	2.2	11.2	30.4		3.2		0.1		0.1	1.6	0.1	1.5	0.8	3.5	0.4	0.1	0.1	41.9	2.8
		6	1.2	0.7	27.9		0.7		0.4			0.4	0.8	0.3	10.7	3.3	0.5			51.2	1.7
		7	7.4	1.6	51.0		0.3		0.6			0.5	0.3	0.5	1.1	8.5	0.8			24.4	3.0
		8	2.6	1.1	49.4				0.5			1.2	0.6	0.7	0.1	0.2	7.6		0.2	34.9	0.9
		9	10.4	9.4	27.5		1.4				0.2	0.1	0.7	0.7	15.9	2.7	0.9	0.1	0.1	27.2	2.6

540

541

TABLE 2. Explained variance due to the different experimental variables (50-50 MANOVA). The analysis is done on the 35 most abundant bacteria on genus level, and variables were standardized to remove abundance effects prior to the analysis.

Source	d.f.	Explained variance (%)	No. of significant bacteria
Kitchen (A)	7	38.2***	9
Sink surface/Coupon (B)	1	5.1***	3
DNA/RNA (C)	1	10.8***	7
A×B	7	21.8***	5
A×C	7	13.5 (ns)	0
B×C	1	2.3 (ns)	0
Error	7	8.2	

*** p<0.001; ns= not significant at 5% level.

Table 3. Relative abundance (percentage) of the different bacterial taxa (genus level) characterized from isolates cultured from coupons (partial 16S rRNA gene). Total number of sequenced isolated was 113. The two overall dominating families/genera are highlighted in gray. The "n" is the number of isolates/sequences per coupon.

		Actinobacteria				***Bacteroidetes***		***Proteobacteria***							
		Actinobacteria				*Flavobacteria*	*Sphingobacteria*	*Alphaproteobacteria*			*Gammaproteobacteria*				
		Actinomycetales				*Flavobacteriales*	*Sphingobacteriales*	*Caulobacterales*	*Rhizobiales*	*Sphingomonadales*	*Enterobacteriales*		*Pseudomonadales*		
		Nocardiaceae	*Dermabacteraceae*	*Microbacteriaceae*	*Micrococcaceae*	*Flavobacteriaceae*	*Sphingobacteriaceae*	*Caulobacteraceae*	*Rhizobiaceae*	*Sphingomonadaceae*	*Enterobacteriaceae*		*Moraxellaceae*		*Pseudomonadaceae*
Kitchen no	**Coupon no**	*Rhodococcus*	*Brachybacterium*	*Microbacterium*	*Kocuria*	*Chryseobacterium*	*Sphingobacterium*	*Brevundimonas*	*Rhizobium*	*Sphingomonas*	*Enterobacter*	*unclassified_ Enterobacteriaceae*	*Acinetobacter*	*Moraxella/ Enhydrobacter*	*Pseudomonas*
2	3 (n=6)	17		17	17			50							
	6 (n=6)	17		67	17										
3	2 (n=7)		14		29									57	
	4 (n=4)		50									25		25	
	5 (n=6)			33	50									17	
4	2 (n=7)				43					14				43	
	4 (n=7)				14	14			14	14				43	
	6 (n=4)			25	50	25									
5	2 (n=5)				40			20						40	
	3 (n=5)				20			20						60	
	5 (n=6)	17					17						50		17
6	2 (n=7)				29			14						57	
	3 (n=5)				20									80	
	6 (n=4)				50									50	
7	2 (n=5)		40	20	20			20							
	4 (n=4)				100										
	6 (n=4)		50		25									25	
8	2 (n=3)				67				33						
	4 (n=6)				83									17	
	6 (n=3)									67				33	
9	2 (n=2)		50								50				
	6 (n=7)		29		71										

559

560

561 **FIGURE CAPTIONS**

562

563 **Figure 1.** Bar chart showing the average cfu (log_{10}) for coupons and sink surface samples
564 (both 12 cm^2) for eight kitchens (no 2 - no 9). Each bar represents three replicates per kitchen
565 (only two replicates for kitchen no 2 and no 9) and the different shades of grey represent the
566 different kitchens, starting from left with kitchen no 2. The error bars are SEM (standard error
567 of mean).

568

569 **Figure 2.** Average relative abundances (percent) of the dominant bacterial taxa
570 (phylum/family level) for DNA (sink surface and coupon) and RNA (sink surface and
571 coupon). Taxa represented above 5 % in one or more samples is shown. Blue shades:
572 *Proteobacteria*; green shades: *Actinobacteria*; purple shades: *Firmicutes*; apricot shade:
573 *Bacteroidetes*; pink shade: *Cyanobacteria* and grey shade: Other.

574

575 **Figure 3.** Overview of results from PCA and 50-50 MANOVA. The scores plot (A) shows
576 the distribution of samples, where labels S/C corresponds to sink surface (outlined area) and
577 coupon (filled area), and colors correspond to DNA (blue) and RNA (green). The loadings
578 plot (B) shows the bacteria significantly different between one or more sample categories
579 (determined from 50-50 MANOVA) as filled circles, and the circle size is proportional to
580 relative abundance. The corresponding table show which bacteria that were significantly
581 different ($p<0.05$) between the sample categories; kitchens (A), sink/coupons (B), DNA/RNA
582 (C) and interaction between A*B. One of the dominating OTU (affiliated with
583 *Enhydrobacter*) had a statistically significantly interaction between sink surface/coupon and
584 kitchen. This means that there was a significant difference in the relative amount of this

585 bacteria between sink surfaces and coupons but that this difference was not systematic, that is
586 the relative amount was sometimes higher in sink surface than coupon and vice versa.

587

588

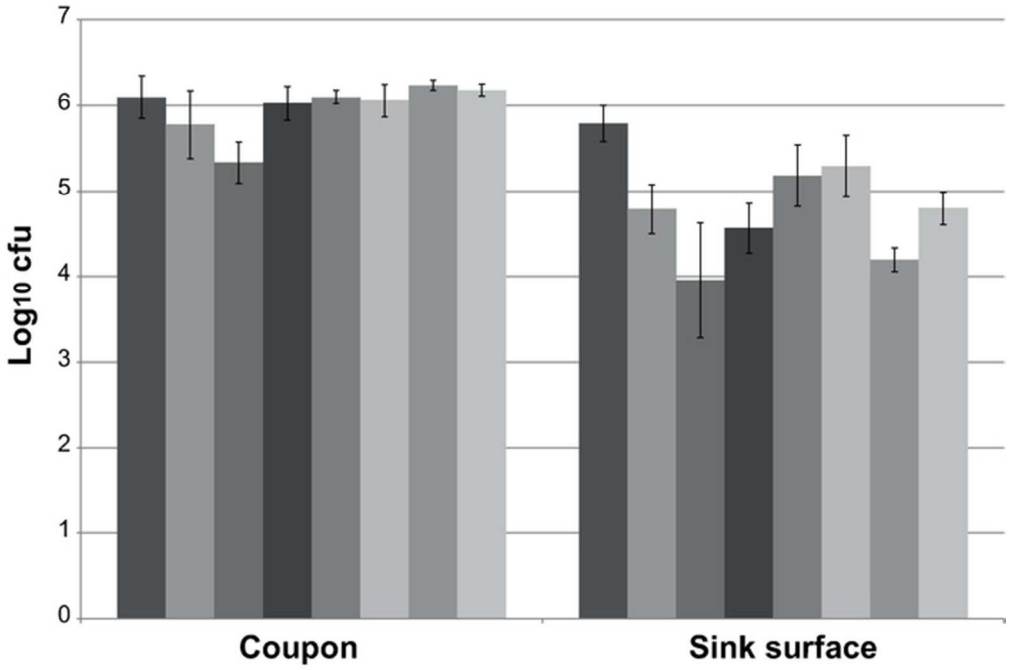
589

590

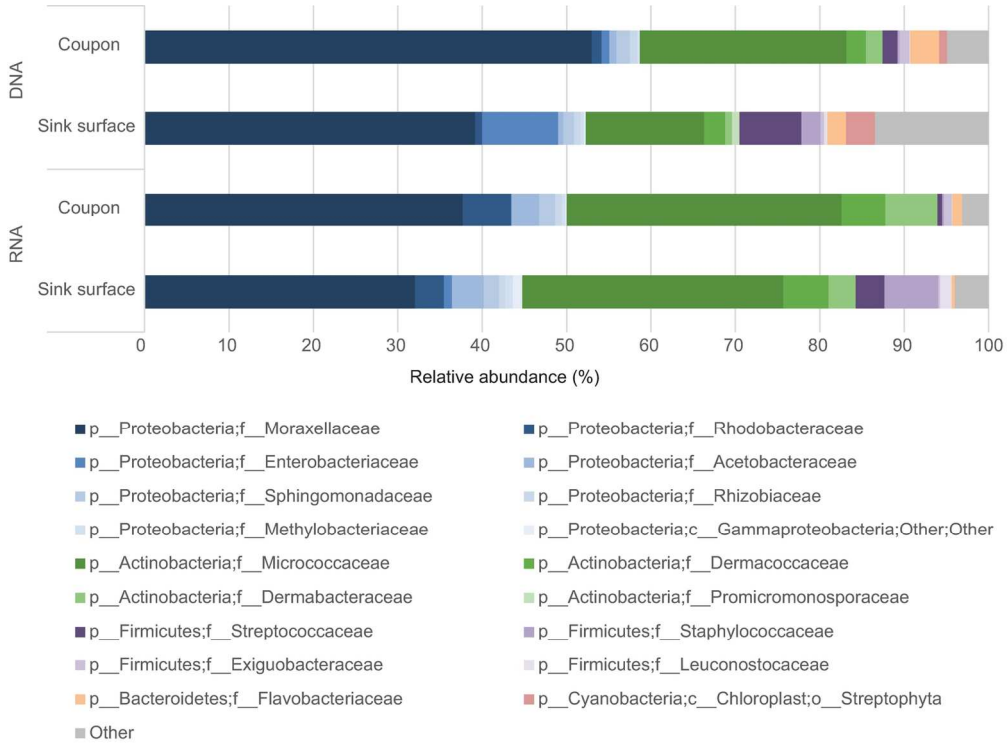
591

592

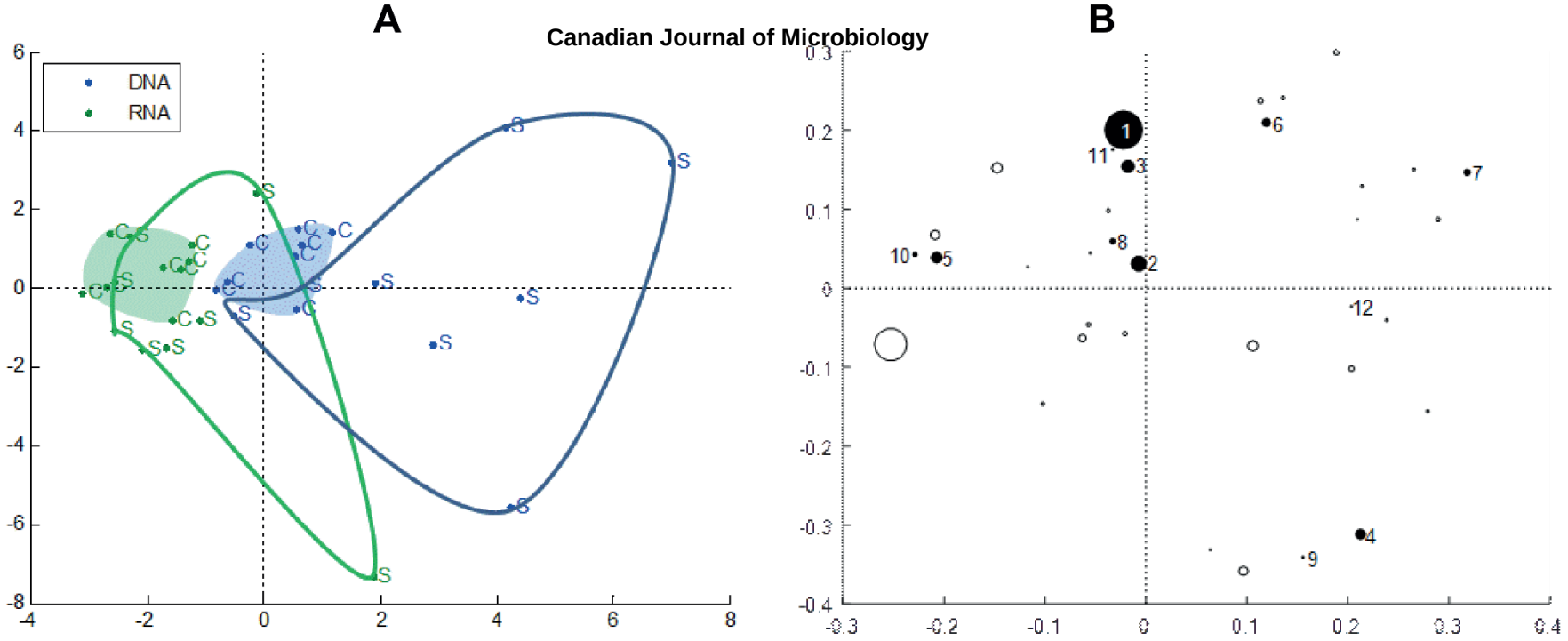
593



59x39mm (300 x 300 DPI)



146x107mm (300 x 300 DPI)



	Bacterial taxa	Kitchen (A)	Sink/Coupon (B)	DNA/RNA (C)	A×B
1	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Enhydrobacter	*	ns	ns	*
2	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Acinetobacter	***	ns	*	ns
3	Actinobacteria;Actinobacteria;Actinomycetales;Dermacoccaceae;Dermacoccus	*	ns	*	ns
4	Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus	**	**	ns	**
5	Actinobacteria;Actinobacteria;Actinomycetales;Dermabacteraceae;Other	*	ns	*	ns
6	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Chryseobacterium	**	*	**	ns
7	Cyanobacteria;Chloroplast;Streptophyta;;	ns	ns	*	ns
8	Firmicutes;Bacilli;Exiguobacterales;Exiguobacteraceae;Exiguobacterium	***	*	ns	*
9	Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus	**	ns	ns	*
10	Actinobacteria;Actinobacteria;Actinomycetales;Other;Other	*	ns	***	ns
11	Proteobacteria;Alphaproteobacteria;Rhizobiales;Other;Other	ns	ns	ns	**
12	Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Other	ns	ns	**	ns