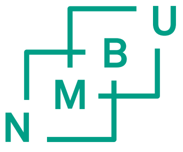

Preface and Acknowledgements

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Cecum samples and mice data for the experiment has been provided by Anne Graupner and Norwegian Institute of Public Health, Oslo via Dag Anders Brede.

I want to thank my supervisors Dzung Bao Diep and Dag Anders Brede for answering all my questions, discussing with me and learning me a lot.

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Thanks to everyone at LMG for always being smiling and helpful.

Anne Kristin Kjernlie

May 2014

Abstract

Selenium (Se) level in diet has been seen to affect gastrointestinal microbiota in mice, and microbiota has been seen to affect the uptake and expression of Se and selenoproteins. In this study, cecum from mice fed with low Se diet and normal Se diet has been examined, and their microbiota community composition compared. DNA from 16 cecum samples from each diet was extracted and 16S Metagenomic Sequencing was done with the Illiumina MiSeq Sequencer. Output was processed with Qiime pipeline for alpha and beta diversity analyses. Significant difference in abundance was found in two genera, *Akkermansia* and *Oscillospira.*

Sammendrag

Selen (Se) I fôr påvirker gastrointestinal mikrobiota I mus, og motsatt påvirker mikrobiota opptak og uttrykk av Se og selenoprotein. I dette studiet har cecum fra mus fôret med lavt Se fôr og normalt Se fôr blitt undersøkt, og sammensetning av mikrobiota har blitt sammenlignet. DNA fra 16 cecumprøver fra hver type fôringsregimeble ekstrahert og 16S Metagenomisk sekvensering ble utført med Illumina MiSeq Sequencer. Resultatene ble bearbeidet med Qiime pipeline og alfa- og betadiversitet ble undersøkt. I to genera ble det funnet signifikante forskjeller i mendge bakterier, genera *Akkermansia* og *Oscillospira.*

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1. Introduction

1.1 Selenium

The 34th chemical element, selenium (Se), is a trace mineral that is essential to animals, including humans, and is important in the matter of immunology and fertility (Hefnawy & Tortora-Perez 2010; Rayman 2000). Too low levels of Se can lead to deficiency diseases like Keshan disease (Chen 2012), while too high levels are toxic(Lee & Jeong 2012). For humans in Nordic countries the recommended level of Se intake is 50 µg/day and 60 µg/day for women and men, respectively (*Nordic nutrition recommendations 2012: integrating nutrition and physical activity* 2014). In its natural state Se is found in soil as selanate and selenite and is absorbed by plants which use these to biosynthesize mainly selenomethionine (SeMe) and selenocysteine (SeCys). These can further be absorbed by animals, or animals synthesize SeCys on their own (Rayman et al. 2008). The main source for human dietary Se is meat. The Se level in Nordic soil is low; hence the level of Se in Nordic people is low. Se in form of selenate or selenite is recommended as dietary supplement.

1.2 Selenoprotein

The essentiality of Se to mammals is due to its participation in selenoproteins. Se is incorporated into the 21th amino acid, selenocysteine, which further is incorporated into selenoproteins: glutathione peroxidases (GPx), thioredoxin reductases (Txnrd), deiodinases, selenophosphate-synthetase 2 and several other unrelated selenoprotein sequences (Reeves & Hoffmann 2009). These selenoproteins have been found to be important when it comes to mitigating oxidative stress, slow down tumor-cell growth, producing thyroid hormone and providing sperm motility and viability among other things (Rayman 2012).

In prokaryotes, selenoproteins similar to the mammalian ones are found, e.g. formate dehydrogenase, selenophosphate syntethase, glycine reductase and selenium-

dependent peroxiredoxin, in addition to a group of proteins where Se is bound to molybdenum, nickel or tungsten (Hrdina et al. 2009). Not all prokaryotes have selenoproteins, but they are found in 20 % of known bacteria(Kryukov & Gladyshev 2004).

1.3 Cecum and bacterial composition

Cecum is a part of the intestinal system and is in mice unlike in human fully functional. Here the host gets help from the microbiota to digest food and get access to different minerals and metabolites (e.g short chain fatty acids). Cecum is a part of the large intestine and is located between the ileum and colon(Snipes 1981). Gastrointestinal microbiota is present in abundance up to 10^{11} in the host(Xu & Gordon 2003). Microbiota community in mice intestine is a stable after 3 weeks, and is a result of factors like feeding, environment and heritage.

1.4 Metagenomics and Qiime

High throughput sequencing demands suitable software for further metagenomics analyzes of the output. Quantitative Insights Into Microbial Ecology (Qiime) is a pipeline that is used to compare and analyze the microbial communities using high-throughput amplicon sequencing data. It deals with raw data output from sequencer and processes it further to demultiplex and denoise the reads, pick operational taxanomic units (OTU) and assign taxanomies. It also construct phylogenetic trees from OTUs and do downstream statistical analysis (e.g. alpha diversity analysis, beta diversity analysis), generating plots and graphs.

1.5 Aim

Microbiota composition affects the Se uptake in mice intestine, and it also affects the selenoprotein expression in the host. This indicates a competition between the host and microbiota for the Se (Hrdina et al. 2009). Microbiota diversity is increasing with increasing Se level in diet(Kasaikina et al. 2011). In this study, microbiota compositions in cecum of mice fed with different diets were examined. Cecum samples of 32 mice,

16 of which were fed with low selenium diet and 16 fed a normal selenium diet, were used.

2. Materials and methods

2.1 Feed and living conditions

Sample intestines received for the experiment were from mice that were bred and fed at Norwegian Institute of Public Health, Oslo. Mice had been kept together in cages of two, one wild type individual and one knock out individual for a cell repair gene. Some cages had contained siblings (Table A1, Appendix). Low Se diet, containing 0.0198 mg Se/kg diet had been given to mice in 8 of the cages, while mice in the remaining 8 cages had been given the normal Se diet, containing 0.234 mg Se/kg diet. Mice was killed at age 11-20 weeks.

2.2 Cell dissociation

Cecum was cut out of the rest of the intestine, cut open and rehydrated by adding 200 µl of sterile water. Cecum content was transferred to a 15 ml cell star tube on ice. Cecum was washed with 2.5 ml + 1 ml dissociation buffer, and the solution was added to the cell star tube. Tubes were centrifuged at 4 °C at 1500 rpm for 5 minutes, and the supernatant transferred to a new cell star tube. This step was done twice. Bacterial cell pellet was collected by centrifuging at 4 °C at 10 000 rpm for 10 minutes, and discarding the supernatant. Pellet was resuspended in 1ml cell wash buffer, centrifuged at 1170 rpm for 30 seconds, and the supernatant transferred to 2 ml fresh tube. Cell pellet was collected by centrifuging at 14 000 rpm for 5 minutes, and the supernatant discarded.

2.3 DNA extraction

Bacterial cell pellet was resuspended in 1 ml RBB+C buffer. For each ml solution in sample, 30 µl lysozyme (40 mg/ml) and 25 µl mutanolysin (5000 U/ml) was added and the tubes incubated at 37 °C for 30 minutes. 20 % SDS was added to reach 4 % and the

tubes incubated at 70 °C for 20 minutes, while mixing them every 5 minutes. For each sample the solution was split into two 2 ml tubes, and 5 M NaCl was added to reach 0.7 M. Preheated (55 °C) CTAB buffer was added, 1/10 of the solution volume, and incubated at 70 °C for 10 minutes. In fume hood one volume chloroform was added, mixed with the sample by inversion, and the tube centrifuged at 14 000 rpm for 10 minutes. Upper phase of the solution was transferred to new 2 ml tube, the rest discarded. CTAB and chloroform steps were repeated until the upper phase became clear. One volume phenol:chloroform:isoamylalcohol (25:24:1) was added, mixed with sample and centrifuged at 14 000 rpm for 10 minutes. Upper phase was transferred to new 2 ml tube, the rest discarded. The step was repeated, but upper phase transferred to 1.5 ml eppendorf tube. One volume cold isopropanol was added and mixed with sample by several thoroughly inversions of tube. DNA pellet was collected by centrifuging at 14 000 rpm for 20 minutes. All isopropanol was discarded and pellet washed by adding 500 µl 70 % ethanol and centrifuging at 14 000 rpm for 10 minutes. All ethanol was removed and the tube left open for drying for 30 minutes. One pellet from each sample was resuspended in 50 µl MQ-water, and solution transferred to second sample tube. DNA concentration and quality was measured at nanodrop. Samples were kept at -20 °C. DNA quality was additionally checked by running samples on 0.7 % agarose gel. 4 µl of sample was dyed with 1 µl of Gel Red, loaded with 1 µl loading buffer (6X) and run on gel at 70 V for 30 minutes. Sample bands were visualized by UV light. DNA samples remaining in sample tube were cleaned up by adding 1 µl RNAse (10 µl/ml) to each tube and incubating at 37 °C for 10 minutes. This step was done twice.

2.4 Amplicon preparation

2.4.1 16S Amplicon PCR and clean up

Samples were quantified with Qubit Broad Range, diluted to reach 5-10 ng/µl and quantified with Qubit High Spesificity. 2.5 µl of each sample was transferred to a 96-

well 0.2 ml PCR plate, 5 µl Amplicon PCR Forward Primer (1 µM), 5 µl Amplicon PCR Reverse Primer (1 µM) and 12.5 µl iProof buffer was added to every sample. Plate was sealed with microseal film and 16S Amplicon PCR preformed with following program:

- 98 °C for 3 minutes
- 25 cycles of
    - 98 °C for 30 second
    - 55 °C for 30 seconds
    - 72 °C for 30 seconds
- 72 °C for 5 minutes
- 4 °C until PCR clean up

PCR plate was centrifuged at 1000 x g at 20 °C for 1 minute, and seal removed. Cold AMPure XP beads were made evenly dispersed by vortexing for 30 seconds, and 1 ml of beads put in eppendorf tube to reach room temperature. 20 µl of AMPure XP beads were added to each sample and mixed by pipetting up and down 10 times. Samples were incubated for 5 minutes in room temperature before PCR plate was put on magnetic stand for a few minutes until the supernatant became clear. With PCR plate still on magnetic stand supernatant was discarded and samples washed with 200 µl freshly prepared 80% ethanol that was added, left to incubate for 30 seconds and discarded. Washing step was done twice. Beads were left to air dry for 10 minutes. PCR plate was removed from stand, 52.5 µl 10 mM Tris pH 8.5 was added to each sample and mixed by pipetting up and down 10 times. Samples were incubated in room temperature for 2 minutes and placed on magnetic stand for a few minutes until the supernatant became clear. With PCR plate still on magnetic stand 50 µl of supernatant was transferred to new 96-well PCR plate. PCR product quality was examined by running samples on gel with same procedure as for genomic DNA (2.3), but with 100 bp ladder.

2.4.2 Index PCR and clean up

5 µl of the cleaned 16S Amplicon PCR product of each sample was transferred to a new 96-well PCR plate and the plate arranged in TruSeq index Plate Fixture together with

Nextera XT Index 1 primers horizontally and Nextera XT Index 2 primers vertically in the rack (Figure 2-1). 5 µl each of Index 1 and Index 2 primers according to the Index Primer Sheet(Table A, Appendix), 10 µl MQ-water and 25 µl iProof buffer was added to each sample and mixed by pipetting up and down 10 times. Plate was covered with microseal and centrifuged at 1000 x g at 20 °C for 1 minute. Index PCR was preformed with following program:

- 98 °C for 3 minutes
- 8 cycles of
    - 98 °C for 30 seconds
    - 55 °C for 30 seconds
    - 72 °C for 30 seconds
- 72 °C for 5 minutes
- 4 °C until PCR clean up

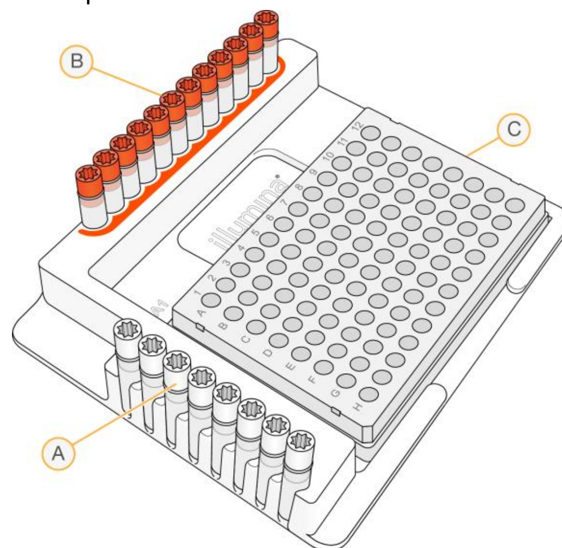


Figure 2-1 TruSeq Index Plate Fixture (Illumina MiSeq 16S Metagenomic Sequencing Library Preparation Protocol).

A: Nextera Index 2 Primers, B: Nextera Index 1 Primers, C: PCR Sample Plate.

PCR plate was centrifuged at 280 x g at 20 °C for 1 minute and seal removed. Cold AMPure XP beads we made evenly dispersed by vortexing for 30 seconds, and 2 ml of beads put in eppendorf to reach room temperature. 56 µl AMPure XP beads was added to each sample and mixed by pipetting up and down 10 times. Samples was

incubated, supernatant discarded, beads washed with ethanol and air dried as in PCR Clean Up 1(2.4.1). PCR plate was removed from magnetic stand, 27.5 µl 10 mM Tris pH 8.5 added to each sample and mixed by pipetting up and down 10 times. Samples were incubated in room temperature for 2 minutes and placed on magnetic stand for a few minutes until the supernatant became clear. With PCR plate still on magnetic stand 25 µl of supernatant was transferred to new 96-well PCR plate. PCR product quality was examined by running samples on gel with same procedure as after PCR Clean Up 1(2.4.1).

2.4.3 Library pooling

DNA concentration in ng/µl was measured with Qubit High Specificity and DNA concentration in nM was calculated using formula (1) in Illumina MiSeq 16S Metagenomic Sequencing Library Preparation Protocol where 630 bp is the average library size.

$$(1) \qquad Concentration\ i\ nM = \frac{concentration\ i\ ng/\mu l}{660\frac{g}{mol} \times 630bp} \times 10^6$$

Samples were diluted with MQ-water to reach a concentration of 4 nM each and 5 µl from every sample were pooled together in 1.5 ml eppendorf tube and mixed by pipetting up and down.

2.4.4 Library denaturing and MiSeq loading

Library denaturing and MiSeq sample loading was performed using MiSeq v3 reagent kit. 5 µl from pooled library was transferred to 1.5 ml eppendorf tube and 5 µl freshly diluted 0.2 N NaOH was added. In another 1.5 ml eppendorf tube control 4 nM PhiX library was prepared by adding 2 ul 10 nM PhiX library together with 3 µl Resuspension Buffer. Further, 5 µl freshly diluted 0.2 N NaOH was added to the tube. Both the

pooled library tube and the PhiX library tube were briefly vortexed and incubated for 5 minutes at room temperature. 990 µl of cold hybridization buffer, HT1, was added to each tube to reach concentration 20 pM for both libraries. In this sequencing, 8pM loading concentration was used, to get this concentration 240 µl of each library was transferred to new tubes and 360 µl cold HT1 was added to both tubes. 30 µl 8 pM PhiX library was transferred to new 1.5 eppendorf tube and 570 µl 8 pM amplicon library was added. Immediately before loading onto MiSeq v3 reagent cartridge tube was incubated at 96 °C for 2 minutes, mixed by inverting 2 times and placed in ice-water bath for 5 minutes. 16S amplicon sequencing was performed by Illumina MiSeq Sequencer.

2.5 Statistics

Illumina MiSeq output in fastq format was processed by Qiime online software. Qiime was further used for several statistical analyses; alpha diversity was examined by drawing rarefaction curves; Microbial community compositions of samples were summarized by plotting bar graphs of taxonomies from phylum to genus level; Beta diversity was examined by running Principal Coordinate Analyses (PCoAs). Tests were applied with concern to the Se diet parameter, but also cage, siblings, genotype, age and weight. In addition, OTU table obtained from Qiime was modified in format to be used in Calypso v3 for ANOVA test from phylum to genus level.

3. Results

3.1 Alpha diversity

Two of the samples, 2 and 38, had reads that ended around 100 000 sequences per sample in the rarefaction curve (Figure 3-1), while the rest of the samples had a rarefaction curve that ended around 200 000 sequences. Most of the samples had enough observed species to be used for further analyzes. Comparing the curves at a

given number of sequences showed some variation in observed species between samples, but this was not different between the different diets.

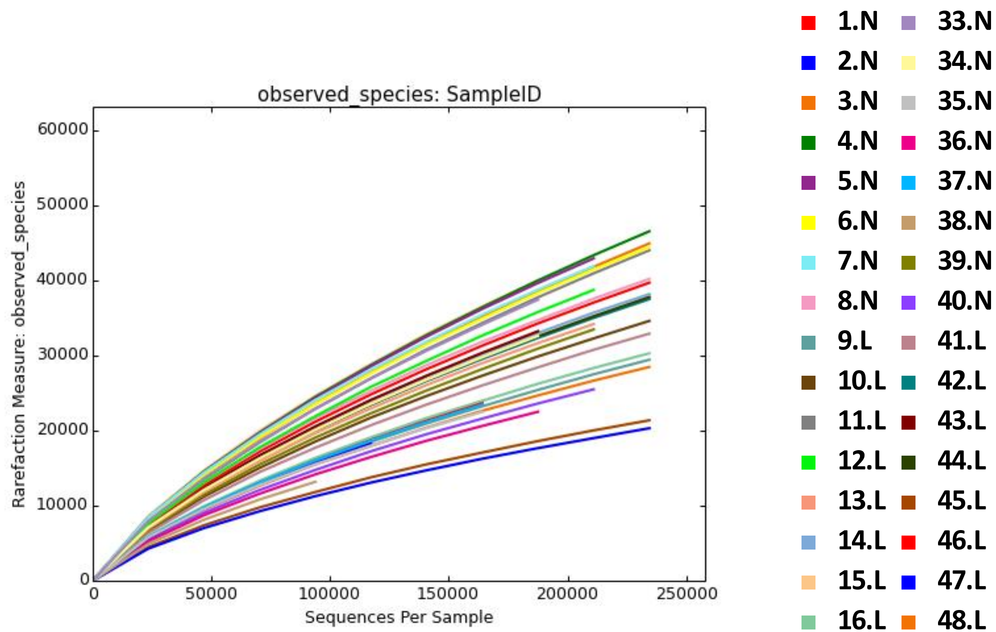
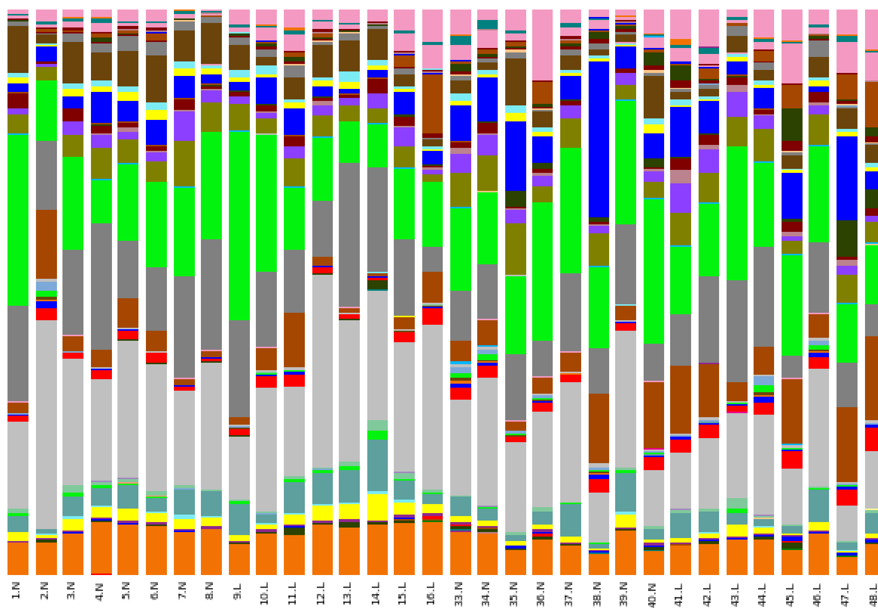


Figure 3-1 Rarefaction analyses of cecum samples. The curves show observed species as function of sequences per sample

Further looking at the Taxa Summary for all samples from phylum to genus level (genus, Figure 3-2), the bar charts showed the bacterial composition in each sample. In general the same taxa were predominant in most samples at every level. At phylum level the predominant groups were Firmicutes and Bacteriodetes, while at class level Clostridia and Bacteriodia were most represented. Further, at order level, the Clostridales and Bacteriodales were the predominant groups, and at family level it was S24-7, Lachnospiracea, Ruminicoccaceae and unclassified families from order Clostridales that were predominant. Finally, at genera level the far more predominant



a)

Legend	Taxonomy(genera)	Legend	Taxonomy(genera)	Legend	Taxonomy(genera)
	Unclassified		Macrococcus		Thermacetogenium
	Methanoculleus		Staphylococcus		Erysipelotrichaceae(family)
	Bacteria(kingdom)		Gemellales(order)		Erysipelotrichaceae(family)
	Actinobacteria(phylum)		Gemellales(order)		Allobaculum
	Actinobacteria(class)		Gemellaceae(family)		Bulleidia
	Actinomycetales(order)		Gemella		Coprobacillus
	Corynebacterium		Lactobacillales(order)		Eubacterium
	Geodermatophilaceae(family)		Aerococcaceae(family)		OPB54(class)
	Kineococcus		Aerococcaceae(family)		OPB46(class)
	Microbacteriaceae(family)		Alkalibacterium		OPB72(order)
	Microbacteriaceae(family)		Carnobacterium		TIBD11(family)
	Microbacterium		Enterococcaceae(family)		Proteobacteria(phylum)
	Rathayibacter		Enterococcaceae(family)		Alphaproteobacteria(class)
	Micrococcaceae(family)		Enterococcus		Alphaproteobacteria(class)
	Citricoccus		Vagococcus		Brevundimonas
	Nesterenkonia		Lactobacillaceae(family)		RF32(order)
	Rothia		Lactobacillus		Rhizobiales(order)
	Mycobacterium		Leuconostocaceae(family)		Martelella
	Nakamurellaceae(family)		Leuconostocaceae(family)		Afipia
	Rhodococcus		Leuconostoc		Bradyrhizobium
	Propionibacterium		Weissella		Brucellaceae(family)
	Saccharopolyspora		Streptococcaceae(family)		Devosia
	Sanguibacter		Lactococcus		Methylobacterium
	Williamsia		Streptococcus		Rhizobiaceae(family)
	Yaniellaceae(family)		Clostridia(class)		Agrobacterium
	Yaniella		Clostridia(class)		Rhizobium
	Bifidobacteriaceae(family)		BSA2B-08(order)		mitochondria(family)
	Bifidobacterium		Clostridiales(order)		Sphingomonas
	Coriobacteriaceae(family)		Clostridiales(order)		Betaproteobacteria(class)
	Coriobacteriaceae(family)		Caldicoprobacter		Burkholderiales(order)
	Adlercreutzia		Christensenellaceae(family)		Sutterella
	Atopobium		Christensenellaceae(family)		Burkholderia
	Eggerthella		Clostridiaceae(family)		Tepidimonas
	Bacteroidetes(phylum)		Alkaliphilus		Ralstonia
	Bacteroidales(order)		Candidatus Arthromitus		Deltaproteobacteria(class)
	Bacteroidales(order)		Clostridium		Desulfobulbus
	Bacteroidaceae(family)		Dehalobacteriaceae(family)		Desulfovibrionales(order)
	Bacteroides		Dehalobacterium		Desulfovibrionaceae(family)
	Porphyromonadaceae(family)		Pseudoramibacter Eubacterium		Desulfovibrionaceae(family)
	Porphyromonadaceae(family)		Lachnospiraceae(family)		Bilophila
	Parabacteroides		Lachnospiraceae(family)		Desulfovibrio
	Prevotellaceae(family)		Anaerostipes		Gammaproteobacteria(class)
	Prevotella		Blautia		Enterobacteriaceae(family)
	Rikenellaceae(family)		Coprococcus		Enterobacteriaceae(family)
	Rikenellaceae(family)		Dorea		Erwinia
	AF12		Moryella		Serratia
	Rikenella		Roseburia		Pasteurellales(order)
	S24-7(family)		Ruminococcus		Pasteurellaceae(family)
	Odoribacter		Peptococcaceae(family)		Actinobacillus
	Paraprevotellaceae(family)		Peptococcaceae(family)		Bibersteinia
	Prevotella		rc4-4		Mannheimia
	Cytophagales(order)		Ruminococcaceae(family)		Pseudomonadaceae(family)
	Cloacibacterium		Ruminococcaceae(family)		Pseudomonas
	Pedobacter		Anaerotruncus		Anaerobaculum
	Cyanobacteria(phylum)		Faecalibacterium		Aminobacterium
	4C0d-2(class)		Oscillospira		TM7(phylum)
	YS2(order)		Ruminococcus		TM7-3(class)
	Streptophyta(order)		Syntrophomonas		CW040(order)
	Firmicutes(phylum)		Veillonella		CW040(order)
	Bacilli(class)		Mogibacteriaceae(family)		EW055(order)
	Bacillales(order)		Mogibacteriaceae(family)		Rs-045(family)
	Bacillaceae(family)		Tissierellaceae(family)		Tenericutes(phylum)
	Bacillaceae(family)		Tissierellaceae(family)		Mollicutes(class)
	Bacillus		Sporanaerobacter		Anaeroplasma
	Planococcaceae(family)		Tepidimicrobium		RF39(order)
	Planococcaceae(family)		MBA08(order)		S1
	Sporosarcina		Natranaerobiales(order)		Verrucomicrobia(phylum)
	Staphylococcaceae(family)		ML1228J-1(family)		LD1-PB3(order)
	Staphylococcaceae(family)		SHA-98(order)		Verrucomicrobiaceae(family)
	Jeotgalicoccus		D2(family)		Akkermansia
			Thermoanaerobacteraceae(family)		WCHB1-15(order)

b)

Figure 3-2 Summary of taxonomy for cecum samples. a) Bar chart of all samples at genus level. b) List of bacteria in barchart a).

groups were the same as at family level, without any specificity to genera. Individual samples showed variation compared to most of the other samples in the bar chart.

3.2 Beta diversity

Samples compared according to diet did not show clear grouping in the PCoA plot at genus level (Figure 3-3). When comparing according to the other parameters in the experiment it showed that most of the samples from the mice that lived in same cage grouped together in the PCoA plot, having more similar microbiota composition compared to others, but there was not a clear grouping for genotype or weight factors (Figure 3-4, 3-5, 3-6). Mice living in same cage were of same age, so they grouped in the same way as seen for cage. Sibling mice in a cage did not group more together than non sibling mice in a cage.

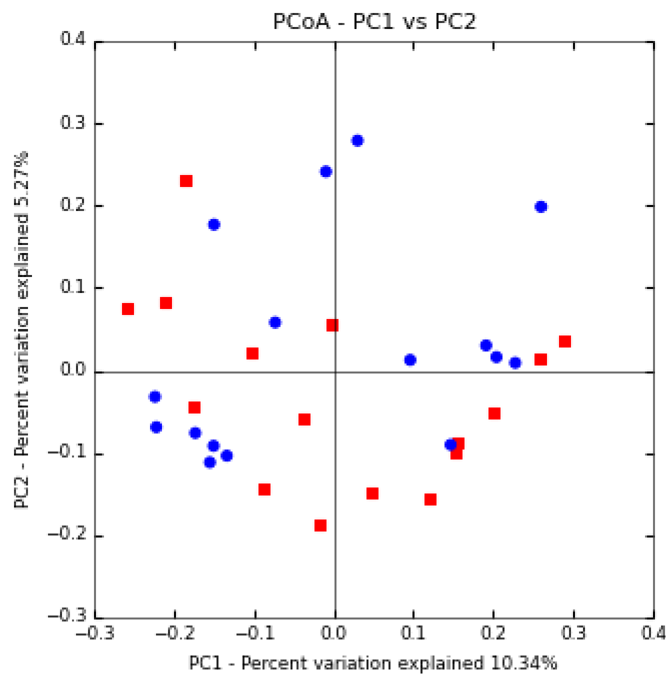


Figure 3-3 Principle Coordinate Analyses, diet plot. Blue circle: Normal Se diet sample. Red square: Low Se diet sample.

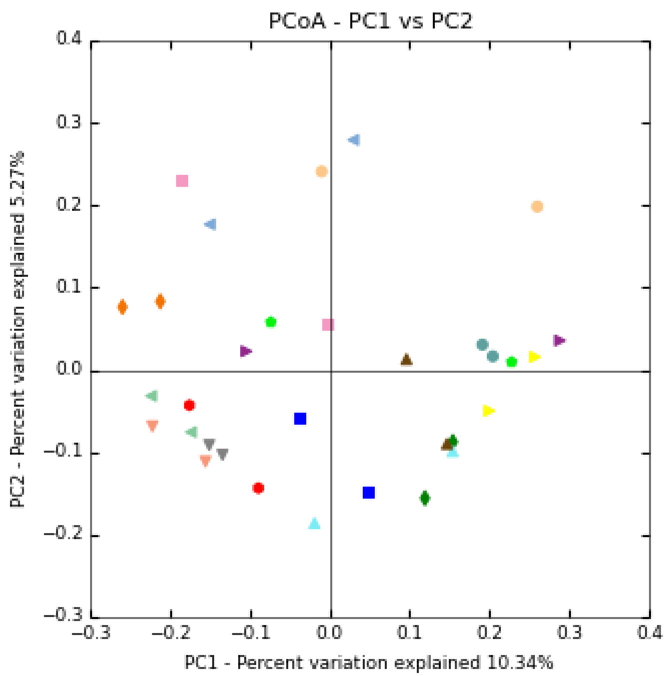


Figure 3-4 Principle Coordinate Analyses, cage plot. Blue triangle: Normal15. Pink triangle: Normal14. Grey triangle: Normal8. Green triangle: Normal21. Pink square: Low27. Red circle: Low6. Orange diamond: Low8. Turquoise triangle: Low21.Brown triangle: Normal7. Grey circle: Normal6. Pink circle: Normal20. Green circle: Normal9. Green diamond: Low10. Blue square: Low7. Purple triangle: Low18. Yellow triangle: Low 20.

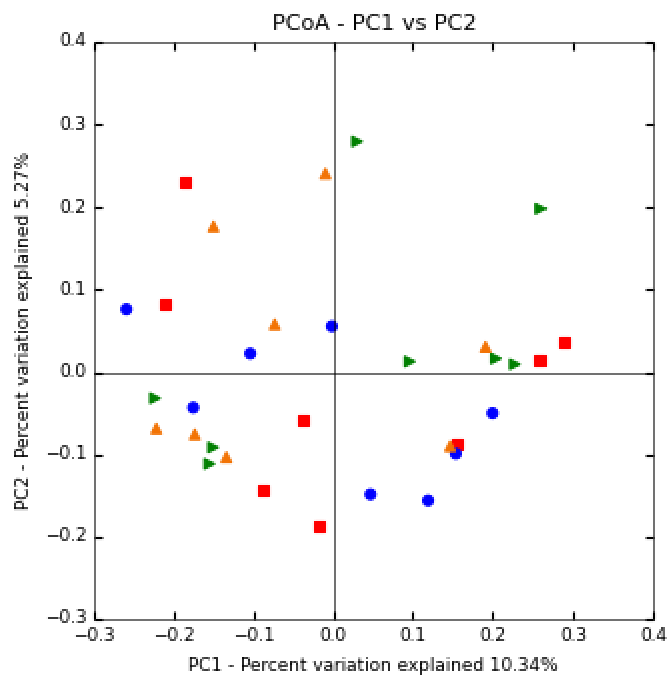


Figure 3-5 Principle Coordinate Analyses, genotype plot. Orange triangle: Normal Se, wild type mice. Green triangle: Normal Se, knock out mice. Red square: Low Se, wild type mice. Blue circle: Low Se, knock out mice.

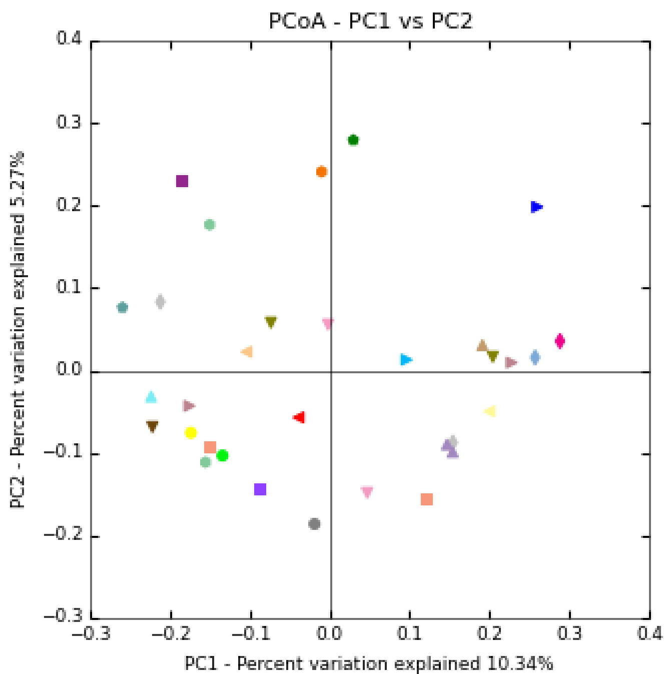


Figure 3-6 Principle Coordinate Analyses, weight plot.

3.3 Significant differences in community

Taxa summaries grouped in order of diet did not show clear differences between the groups in any level. Yet ANOVA showed significant difference for two genera, *Akkermansia* and *Oscillospira* (Figure 3-7). The abundance of *Akkermansia* was increased with the low Se diet, whereas the abundance of *Oscillospira* was decreased. For the other levels, ANOVA showed significant difference for Verrucomicroba, Verrucomicrobiae, Verrucobicrobiales and Verrucomicrobiaceae for phylum, class, order and family, respectively. The abundance of these increased in the low Se diet.

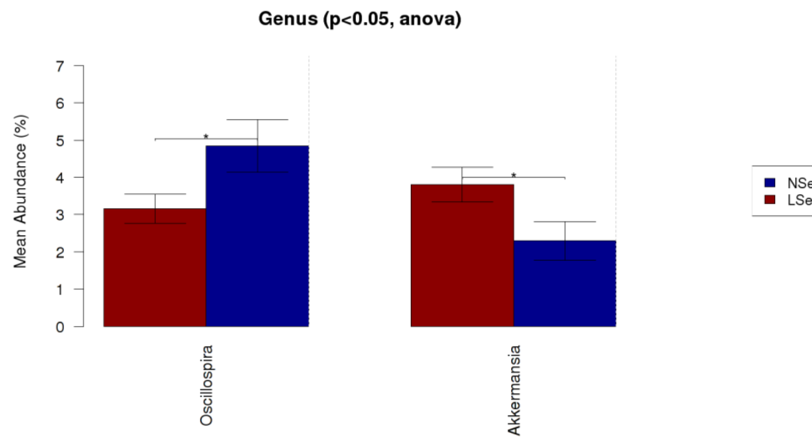


Figure 3-7 ANOVA test for diet, significant difference in abundance of genera.

There were more genera that changed significantly in abundance when grouping the samples according to cage (Figure 3-8).*Akkermansia* was affected in this grouping as well, in addition to *Desulfovibrio*, *Allobaculum* and *Dorea*. A significant difference was also seen in family Lachnospiraces, orders Clostridiales and Bacteriodales, phylum Firmicutes and unclassified bacteria, neither of these with classified genera.

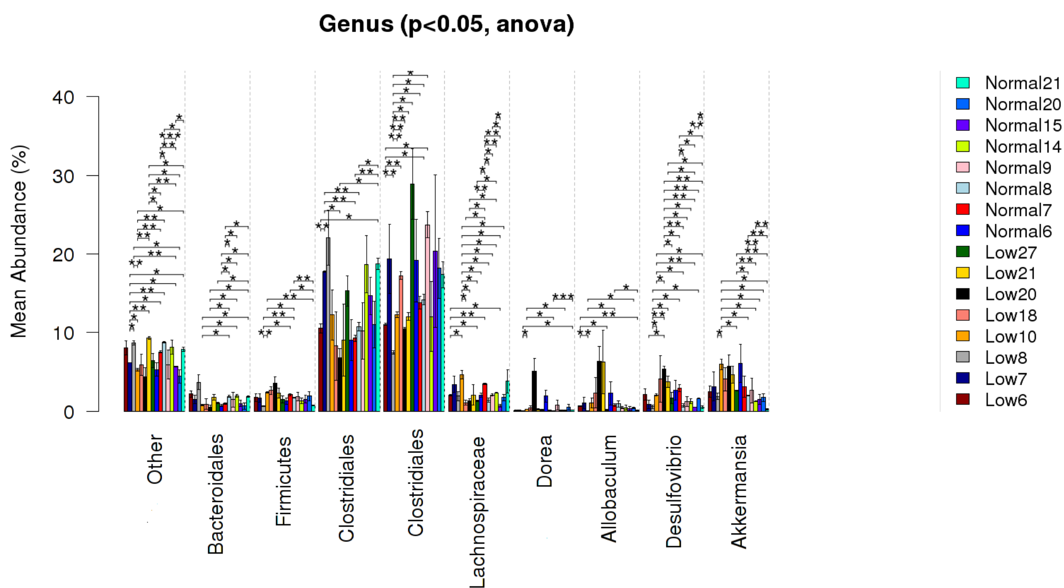


Figure 3-8 ANOVA test for cage, significant difference in abundance of genera.

4. Discussion

Se level in diet affects the microbiota composition, increasing the diversity with increasing levels of Se(Kasaikina et al. 2011). Hence the expectations to see difference in the microbiota composition with low and normal Se diets in this thesis.

Principal coordinate analyses did not show a general grouping of samples according to different diets (Figure 3-3), and this factor cannot be said to affect the general composition of microbiota. Still, the ANOVA test showed significant difference for the two genera *Akkermansia* and *Oscillospira*. Why these two show significant difference, but no other genera does can have several explanations. One could be that the microbiota wins the competition against the host about the Se, and at the Se level examined in this experiment the genera *Akkermansia* and *Oscillospira* are the only ones that are sensitive to changes. The type of Se in the diets may also affect the results. Se in experimental diets was mainly SeMet (Appendix), and bacteria might easier absorb and incorporate SeCys or other forms of Se.

Another important point from the results is that they show large individual variety of the bacterial composition in the taxonomical summaries (Figure 3-2a). Every individual has an initial microbiota composition based on several factors. This is quite stable, and will at some extend resist outer strain and changes(Rasmussen et al. 2009). Having this in mind a sample set of 32 might not be large enough to reveal any differences between the groups.

When looking at the other factors in the experiment, neither genotype, nor weight or pair of siblings seemed to effect the bacterial composition, but the cage factor did. Figure 3-4 showed that mice living in the same cage mostly grouped together in the plot, indicating similarity in bacteria community. Mice that are living in the same cage will eat each other's feces, and this might affect their microbiota composition to becoming more similar.

Additionally, even if the rarefaction curve gave enough species output to represent the samples in a good way, the light steepness of the curve indicates that there could be more species to investigate in the samples. The examined diet may have affected low abundance species that were not seen here.

5. Conclusions

Mice fed with low Se diet and normal Se diet showed significantly differences in abundance for the two genera, *Akkermansia* and *Oscillospira.* The rest of the microbiota community composition seemed to be unchanged.

References

Chen, J. S. (2012). An original discovery: selenium deficiency and Keshan disease (an endemic heart disease). *Asia Pacific Journal of Clinical Nutrition*, 21 (3): 320-326.

Hefnawy, A. E. & Tortora-Perez, J. L. (2010). The importance of selenium and the effects of its deficiency in animal health. *Small Ruminant Research*, 89 (2-3): 185-192.

Hrdina, J., Banning, A., Kipp, A., Loh, G., Blaut, M. & Brigelius-Flohe, R. (2009). The gastrointestinal microbiota affects the selenium status and selenoprotein expression in mice. *Journal of Nutritional Biochemistry*, 20 (8): 638-648.

Kasaikina, M. V., Kravtsova, M. A., Lee, B. C., Seravalli, J., Peterson, D. A., Walter, J., Legge, R., Benson, A. K., Hatfield, D. L. & Gladyshev, V. N. (2011). Dietary selenium affects host selenoproteome expression by influencing the gut microbiota. *Faseb Journal*, 25 (7): 2492-2499.

Kryukov, G. V. & Gladyshev, V. N. (2004). The prokaryotic selenoproteome. *Embo Reports*, 5 (5): 538-543.

Lee, K. H. & Jeong, D. (2012). Bimodal actions of selenium essential for antioxidant and toxic pro-oxidant activities: The selenium paradox (Review). *Molecular Medicine Reports*, 5 (2): 299-304.

Nordic nutrition recommendations 2012: integrating nutrition and physical activity. (2014). vol. 2014:002. [København]: Nordisk Ministerråd. 627 s. : fig. pp.

Rasmussen, H. E., Martinez, I., Lee, J. Y. & Walter, J. (2009). Alteration of the gastrointestinal microbiota of mice by edible blue-green algae. *Journal of Applied Microbiology*, 107 (4): 1108-1118.

Rayman, M. P. (2000). The importance of selenium to human health. *Lancet*, 356 (9225): 233-241.

Rayman, M. P., Infante, H. G. & Sargent, M. (2008). Food-chain selenium and human health: spotlight on speciation. *British Journal of Nutrition*, 100 (2): 238-253.

Rayman, M. P. (2012). Selenium and human health. *Lancet*, 379 (9822): 1256-1268.

Reeves, M. A. & Hoffmann, P. R. (2009). The human selenoproteome: recent insights into functions and regulation. *Cellular and Molecular Life Sciences*, 66 (15): 2457-2478.

Snipes, R. L. (1981). ANATOMY OF THE CECUM OF THE LABORATORY MOUSE AND RAT. *Anatomy and Embryology*, 162 (4): 455-474.

Xu, J. & Gordon, J. I. (2003). Honor thy symbionts. *Proceedings of the National Academy of Sciences of the United States of America*, 100 (18): 10452-10459.

Appendix

Mice diet

70% wheat

0.2% DL-Methionine

13.5% Torula Yeast

5% corn oil, 3.5% mineral mix

1.1% $CaCO_3$, 1% Vitamin Mix

5.7% Sucrose with

0.234 mg Se/kg diet for sufficient Se diet and

0.0198 mg Se/kg for insufficient diet

Selenium content is mainly SeMet in the wheat, measured med ICP-MS at NMBU

Table A1 Mice Sample Sheet

ID	Cage name	Mice name	Born	Age when killed (weeks)	Genotype	Diet	Weight [g] 17.04. (#1-16) og 19.4. (#33-48)	Date of death
1	**Normal15**	NormalSe9.3M5	160113	13	He	normSe	28,1	17.04.2013
2	**Normal15**	NormalSe1.3M2	210113	12,3	KO	normSe	25,3	17.04.2013
3	**Normal14**	NormalSe10.2M4	210113	12,3	He	normSe	26,4	17.04.2013
4	**Normal14**	NormalSe10.2M1	210113	12,3	KO	normSe	28,1	17.04.2013
5	**Normal8**	NormalSe4.1M4	61212	18,9	He	normSe	27,2	17.04.2013
6	**Normal8**	NormalSe4.1M1	61212	18,9	KO	normSe	27,3	17.04.2013
7	**Normal21**	NormalSe7.4M4	270113	11,4	He	normSe	25,7	17.04.2013
8	**Normal21**	NormalSe6.5M7	250113	11,7	KO	normSe	25,9	17.04.2013
9	**Lav27**	LavSe7.4M1	120113	13,6	He	lowSe	25,5	17.04.2013
10	**Lav27**	LavSe7.4M4	120113	13,6	KO	lowSe	26	17.04.2013
11	**Lav6**	LavSe8.1M1	281112	20	He	lowSe	30,2	17.04.2013
12	**Lav6**	LavSe8.1M4	281112	20	KO	lowSe	31	17.04.2013
13	**Lav8**	LavSe7.1M2	301112	19,7	He	lowSe	29	17.04.2013
14	**Lav8**	LavSe7.1M1	301112	19,7	KO	lowSe	26,1	17.04.2013
15	**Lav21**	LavSe4.4M4	281212	15,7	He	lowSe	26,7	17.04.2013
16	**Lav21**	LavSe4.4M2	281212	15,7	KO	lowSe	28,2	17.04.2013
33	**Normal7**	NormalSe2.2M3	31212	19,6	He	normSe	28,2	19.04.2013
34	**Normal7**	NormalSe10.1M1	301112	20	KO	normSe	29,4	19.04.2013
35	**Normal6**	NormalSe3.1M3	281112	20,3	He	normSe	29,8	19.04.2013
36	**Normal6**	NormalSe3.1M1	281112	20,3	KO	normSe	30	19.04.2013
37	**Normal20**	NormalSe7.4M1	270113	11,7	He	normSe	25,2	19.04.2013
38	**Normal20**	NormalSe7.3M5	250113	12	KO	normSe	25	19.04.2013
39	**Normal9**	NormalSe8.2M2(6)	81212	18,9	He	normSe	30	19.04.2013
40	**Normal9**	NormalSe11.1M2	41212	19,4	KO	normSe	31	19.04.2013
41	**Lav10**	LavSe1.1M1	31212	19,6	He	lowSe	29	19.04.2013
42	**Lav10**	LavSe9.1M3	31212	19,6	KO	lowSe	27,3	19.04.2013
43	**Lav7**	LavSe6.1M1	271212	16,1	He	lowSe	24,3	19.04.2013
44	**Lav7**	LavSe8.1M7	281212	16	KO	lowSe	26	19.04.2013
45	**Lav18**	LavSe6.3M6	211212	17	He	lowSe	29,2	19.04.2013
46	**Lav18**	LavSe6.3M4	211212	17	KO	lowSe	28	19.04.2013
47	**Lav20**	LavSe9.3M2	261212	16,3	He	lowSe	27,6	19.04.2013
48	**Lav20**	LavSe9.3M4	261212	16,3	KO	lowSe	28,3	19.04.2013

	N701	N702	N703	N704	N705	N706	N707
S501	L1	L2	L3	L4	L5	1	2
S502	3	4	5	6	7	8	9
S503	10	11	12	13	14	15	16
S504	33	34	35	36	37	38	39
S505	40	41	42	43	44	45	46
S506	47	48	A1	A2	A3	A4	A5

0.7 % Agarose gel
2.8g ultra pure agarose in 400ml TAE-buffer
Microwave 10-15 minutes to dissolve
Keep on 55 °C water bath

Buffers

1M Tris(crystallized free base)
Tris(hydroxymethyl) aminomethane
FW = 121.4g/mol
6.057g dissolved in 50 ml MQ-water.
pH adjusted to 8 by 37 % HCl
Autoclaved

0.5M EDTA
Diaminoethane tetraacetic acid
FW = 372.2g/mol
9.3g in 50ml MQ-water
pH adjusted to 8.0 using NaOH pellets under vigorous stirring (EDTA not dissolved until pH 8.0)
Autoclaved

5M NaCl solution
FW = 58.44g/mol
29.22g dissolved in 100 ml MQ-water
Autoclaved

20% SDS
Sterilized by filtering

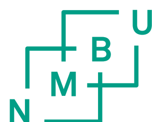
Dissociation buffer

1.5 ml methanol
150 µl Tween 80
1.5 ml 2-metyl-2-propanol
MQ-water added to 150 ml
pH adjusted to 2 with 37 % HCl
Sterilized by filtering

Cell wash buffer
1 ml 1M TrisHCl
20 ml 5M NaCl
MQ-water added to 100 ml

RBB+C lysis buffer
10 ml 5M NaCl
5 ml 1M TrisHCl
10 ml 0.5M EDTA
MQ-water added to 100 ml

CTAB buffer (10 % w/v CTAB, 0.7M NaCl)
3.5 ml 5M NaCl
2.5g CTAB
MQ-water added to 25 ml
Heated to 65 °C to dissolve CTAB
Sterilized by filtering



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