Having older siblings is associated with gut microbiota development during early childhood

Evidence suggests that early life infections, presence of older siblings and furred pets in the household affect the risk of developing allergic diseases through altered microbial exposure. Recently, low gut microbial diversity during infancy has also been linked with later development of allergies. We investigated whether presence of older siblings, furred pets and early life infections affected gut microbial communities at 9 and 18 months of age and whether these differences were associated with the cumulative prevalence of atopic symptoms of eczema and asthmatic bronchitis at 3 years of age.

Bacterial compositions and diversity indices were determined in fecal samples collected from 114 infants in the SKOT I cohort at age 9 and 18 months by 16S rRNA gene sequencing. These were compared to the presence of older siblings, furred pets and early life infections and the cumulative prevalence of diagnosed asthmatic bronchitis and self-reported eczema at 3 years of age. The number of older siblings correlated positively with bacterial diversity (p=0.030), diversity of the phyla Firmicutes (p=0.013) and Bacteroidetes (p=0.004) and bacterial richness (p=0.006) at 18 months. Further, having older siblings was associated with increased relative abundance of several bacterial taxa at both 9 and 18 months of age. Compared to the effect of having siblings, presence of household furred pets and early life infections had less pronounced effects on the gut microbiota. Gut microbiota characteristics were not significantly associated with cumulative occurrence of eczema and asthmatic bronchitis during the first 3 years of life. Presence of older siblings is associated with increased gut microbial diversity and richness during early childhood, which could contribute to the substantiation of the hygiene hypothesis. However, no associations were found between gut microbiota and atopic symptoms of eczema and asthmatic bronchitis during early childhood and thus further studies are required to elucidate whether sibling-associated gut microbial changes influence development of allergies later in childhood.
Neonatal microbial colonization in mice promotes prolonged dominance of CD11b+Gr-1+ cells and accelerated establishment of the CD4+T cell population in the spleen

To assess the microbial influence on postnatal hematopoiesis, we examined the role of early life microbial colonization on the composition of leukocyte subsets in the neonatal spleen. A high number of CD11b+Gr-1+ splenocytes present perinatally was sustained for a longer period in conventionally colonized (CONV) mice than in mono-colonized (MC) and germfree (GF) mice, and the CD4+ T cell population established faster in CONV mice. At the day of birth, compared to GF mice, the expression of Cxcl2 was up-regulated and Arg1 down-regulated in livers of CONV mice. This coincided with lower abundance of polylobed cells in the liver of CONV mice. An earlier peak in the expression of the genes Tjp1, Cdh1, and JamA in intestinal epithelial cells of CONV mice indicated an accelerated closure of the epithelial barrier. In conclusion, we have identified an important microbiota-dependent neonatal hematopoietic event, which we suggest
impacts the subsequent development of the T cell population in the murine spleen.

**General information**

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Organisations: National Food Institute, Research Group for Gut Microbiology and Immunology, University of Copenhagen, Novo Nordisk A/S
Authors: Kristensen, M. B. (Intern), Metzdorff, S. B. (Ekstern), Bergström, A. (Intern), Damlund, D. S. M. (Ekstern), Fink, L. N. (Ekstern), Licht, T. R. (Intern), Frokiaer, H. (Ekstern)
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Older Siblings Affect Gut Microbiota Development in Early Childhood

Background: Evidence suggests that early life infections, presence of older siblings and furred pets in the household affect the risk of developing allergic diseases through altered microbial exposure. Recently, low gut microbial diversity during infancy has also been linked with later development of allergies.

Methods: We investigated whether presence of older siblings, furred pets and early life infections affected gut microbial communities at 9 and 18 months of age and whether these differences were associated with the cumulative prevalence of atopic symptoms of eczema and asthmatic bronchitis at three years of age. Bacterial compositions and diversity indices were determined in fecal samples collected from 114 infants in the SKOT cohort at age 9 and 18 months by 16S rRNA gene sequencing. These were compared to the presence of older siblings, furred pets and early life infections and the cumulative prevalence of diagnosed asthmatic bronchitis and self-reported eczema at three years of age.

Results: The number of older siblings correlated positively with bacterial diversity (p = 0.030), diversity of the phyla Firmicutes (p = 0.014) and Bacteroidetes (p = 0.004) and bacterial richness (p = 0.006) at 18 months. Further, having older siblings was associated with increased relative abundance of several bacterial taxa at both 9 and 18 months of age. Compared to the effect of having siblings, presence of household furred pets and early life infections had less pronounced effects on the gut microbiota. Gut microbiota characteristics were not significantly associated with cumulative occurrence of eczema and asthmatic bronchitis during the first three years of life.

Conclusions: Presence of older siblings is associated with increased gut microbial diversity and richness during early childhood, which could contribute to the substantiation of the hygiene hypothesis. However, no associations were found between gut microbiota and atopic symptoms of eczema and asthmatic bronchitis during early childhood and thus further studies are required to elucidate whether sibling-associated gut microbial changes influence development of allergies later in childhood.

The work has recently (July 2015) been accepted for publication in BMC Microbiology
Establishment of Intestinal Microbiota during Early Life: a Longitudinal, Explorative Study of a Large Cohort of Danish Infants

Fecal samples were obtained from a cohort of 330 healthy Danish infants at 9, 18, and 36 months after birth, enabling characterization of interbacterial relationships by use of quantitative PCR targeting 31 selected bacterial 16S rRNA gene targets representing different phylogenetic levels. Nutritional parameters and measures of growth and body composition were determined and investigated in relation to the observed development in microbiota composition. We found that significant changes in the gut microbiota occurred, particularly from age 9 to 18 months, when cessation of breastfeeding and introduction of a complementary feeding induce replacement of a microbiota characterized by lactobacilli, bifidobacteria, and Enterobacteriaceae with a microbiota dominated by Clostridium spp. and Bacteroides spp. Classification of samples by a proxy enterotype based on the relative levels of Bacteroides spp. and Prevotella spp. showed that enterotype establishment occurs between 9 and 36 months. Thirty percent of the individuals shifted enterotype between 18 and 36 months. The composition of the microbiota was most pronouncedly influenced by the time of cessation of breastfeeding. From 9 to 18 months, a positive correlation was observed between the increase in body mass index and the increase of the short-chain-fatty-acid-producing clostridia, the Clostridium leptum group, and Eubacterium hallii. Considering previously established positive associations between rapid infant weight gain, early breastfeeding discontinuation, and later-life obesity, the corresponding microbial findings seen here warrant attention.
Older siblings, pets and early life infections: impact on gut microbiota and allergy prevalence during the first three years of life

Background: Early life infections and presence of older siblings or pets in the household are factors known to affect the risk of developing allergic diseases, and this effect is suggested to be mediated by interactions between microbes and the immune system. However, very limited research has been done on the effect of these factors on the developing gut microbiota in infants. Thus, we aimed to elucidate associations between older siblings, pets and early life infections, the microbial gut communities at 9 and 18 months of age and the prevalence of allergies in three year old children.

Methods: Bacterial DNA was extracted from a total of 228 fecal samples obtained from 114 infants at both 9 and 18 months of age, belonging to the SKOT cohort. High throughput 16S rRNA gene sequencing was performed and the bacterial community composition of each sample was determined. Information on prevalence of respiratory allergy, eczema and presence of older siblings, pets and early life infections, previously collected through interviews with parents, were compared to the obtained data on bacterial taxonomy.

Results: Early life infections were positively associated with the risk of developing respiratory allergy (p = 0.044), while having siblings tended to decrease the risk of developing eczema (p = 0.105) before the age of three years. Having siblings correlated positively with the relative abundance of several gut microbial genera at both ages. At 18 months of
age, microbial alpha diversity (p = 0.045) and richness (p = 0.009) were significantly higher in individuals with siblings, whereas in children with registered early life infections, a lower alpha diversity (p = 0.067) and richness (p = 0.023) was found at 18 months of age. However, gut microbiota composition, diversity and richness in children with allergies did not differ substantially from that in children without symptoms.

Conclusions: Early life infections might precede childhood respiratory allergy and are associated with low microbial diversity/richness during late infancy. The presence of older siblings affects the gut microbiota composition, diversity and richness during late infancy and the risk of developing eczema during early childhood. However, gut microbiota in late infancy was not associated with eczema or respiratory allergy in early childhood. Further studies are warranted to assess whether the profound sibling effect on the gut microbiota has implication for development of allergies later in life.

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Characterization of the infant gut microbiota in a cohort of 330 Danish children at 9, 18 and 36 months by quantitative PCR array (GULDA) analysis
We have developed a qPCR-based array (GUT Low Density Array, GULDA), which simultaneously determine the relative abundance of >30 different bacterial 16S rRNA gene targets in a given DNA-sample covering selected phylogenetic levels. GULDA was applied to fecal DNA from 330 healthy Danish infants (the so called SKOT-cohort), sampled at 9, 18 and 36 months after birth. The resulting data together with previously determined nutritional and anthropometrical parameters were used as input for multivariate statistics. We found significant changes in the composition of the gut microbiota between 9 and 18 months, corresponding to dietary changes during weaning; changes were far less pronounced between 18 and 36 months. Few studies have undertaken similar longitudinal and multiparametric analysis for such numerous participants. GULDA was seen to constitute a sensitive, cost-effective tool for microbial community characterization, which here provides new insights into the interactions between the gut microbiota, diet and physiology in infants.

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Dietary Xylooligosaccharide Downregulates IFN-γ and the Low-Grade Inflammatory Cytokine IL-1β Systemically in Mice
Dietary carbohydrates improve growth conditions for distinct populations of bacteria that may affect mucosal and systemic immunity. In this study, we fed in a parallel experiment a 10% xylooligosaccharide (XOS)–supplemented diet or a control diet to 2 groups of male C57BL/6NTac mice for 10 wk from weaning. We found that the XOS diet significantly increased Bifidobacterium throughout the intestine compared with control-fed mice, with the highest proportions found in the ileum after XOS feeding (P <0.001). In the intestinal epithelium, most innate immune-related genes were unaffected by XOS feeding, whereas expression of interleukin 1β (IL1β) (P <0.01) and interferon γ (Ifnγ) (P <0.05) was significantly less in blood from XOS-fed mice than from control-fed mice. In vitro treatment of blood with propionate significantly decreased IL1β (P <0.01), Ifnγ (P <0.01), and interleukin 18 (Il18) (P <0.001) expression, supporting our hypothesis that increased production of short-chain fatty acids (SCFAs) in the gut, which are transported across the intestine and into the systemic
compartments, results in downregulation of low-grade inflammatory cytokines. The defensin regenerating islet-derived protein 3γ (RegIIIγ) was significantly more highly expressed in the small intestine (P < 0.01) in XOS-fed mice compared with control-fed mice, suggesting only minor contact between bifidobacteria and epithelial cells. In support of this, the SCFA-induced sodium/hydrogen exchanger isoform 3 expression tended to be greater in the XOS group than in the control group (P = 0.06), indicating an indirect SCFA-mediated antiinflammatory effect of XOS. In conclusion, XOS feeding decreases systemic inflammation, and this effect is most likely caused by higher SCFA concentrations as a result of an increased bifidobacterial saccharolytic fermentation in the entire gut and not only in the large intestine.

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Scopus rating (2016): CiteScore 3.93 SJR 1.956 SNIP 1.335
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.271 SNIP 1.505 CiteScore 4.08
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.089 SNIP 1.596 CiteScore 4.13
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.172 SNIP 1.614 CiteScore 4.6
ISI indexed (2013): ISI indexed yes
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BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.919 SNIP 1.671 CiteScore 4.45
ISI indexed (2012): ISI indexed yes
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BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.838 SNIP 1.603 CiteScore 4.32
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.7 SNIP 1.575
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.559 SNIP 1.545
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 1.575 SNIP 1.42
Web of Science (2008): Indexed yes
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Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.706 SNIP 1.562
Intake of whole apples or clear apple juice has contrasting effects on plasma lipids in healthy volunteers

PURPOSE:
Fruit consumption is associated with a decreased risk of CVD in cohort studies and is therefore endorsed by health authorities as part of the '5 or more a day' campaigns. A glass of fruit juice is generally counted as one serving. Fruit may cause protection by affecting common risk factors of CVD.

METHODS:
Apples are among the most commonly consumed fruits and were chosen for a comprehensive 5 × 4 weeks dietary crossover study to assess the effects of whole apples (550 g/day), apple pomace (22 g/day), clear and cloudy apple juices (500 ml/day), or no supplement on lipoproteins and blood pressure in a group of 23 healthy volunteers.

RESULTS:
The intervention significantly affected serum total and LDL-cholesterol. Trends towards a lower serum LDL-concentration were observed after whole apple (6.7 %), pomace (7.9 %) and cloudy juice (2.2 %) intake. On the other hand, LDL-cholesterol concentrations increased by 6.9 % with clear juice compared to whole apples and pomace. There was no effect on HDL-cholesterol, TAG, weight, waist-to-hip ratio, blood pressure, inflammation (hs-CRP), composition of the gut microbiota or markers of glucose metabolism (insulin, IGF1 and IGFBP3).

CONCLUSIONS:
Apples are rich in polyphenols and pectin, two potentially bioactive constituents; however, these constituents segregate differently during processing into juice products and clear juice is free of pectin and other cell wall components. We conclude that the fibre component is necessary for the cholesterol-lowering effect of apples in healthy humans and that clear apple juice may not be a suitable surrogate for the whole fruit in nutritional recommendations.

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Freezing fecal samples prior to DNA extraction affects the Firmicutes to Bacteroidetes ratio determined by downstream quantitative PCR analysis

Freezing stool samples prior to DNA extraction and downstream analysis is widely used in metagenomic studies of the human microbiota but may affect the inferred community composition. In this study, DNA was extracted either directly or following freeze storage of three homogenized human fecal samples using three different extraction methods. No consistent differences were observed in DNA yields between extractions on fresh and frozen samples; however, differences were observed between extraction methods. Quantitative PCR analysis was subsequently performed on all DNA samples using six different primer pairs targeting 16S rRNA genes of significant bacterial groups, and the community composition was evaluated by comparing specific ratios of the calculated...
abundances. In seven of nine cases, the Firmicutes to Bacteroidetes 16S rRNA gene ratio was significantly higher in fecal samples that had been frozen compared to identical samples that had not. This effect was further supported by qPCR analysis of bacterial groups within these two phyla. The results demonstrate that storage conditions of fecal samples may adversely affect the determined Firmicutes to Bacteroidetes ratio, which is a frequently used biomarker in gut microbiology.

General information
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Organisations: National Food Institute, Division of Microbiology and Risk Assessment
Authors: Bahl, M. I. (Intern), Bergström, A. (Intern), Licht, T. R. (Intern)
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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
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Web of Science (2014): Indexed yes
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BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.069 SNIP 0.817 CiteScore 2.25
ISI indexed (2012): ISI indexed yes
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BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.096 SNIP 0.761 CiteScore 2.26
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BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.07 SNIP 0.756
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.111 SNIP 0.835
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
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Scopus rating (2007): SJR 1.095 SNIP 0.859
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.091 SNIP 0.851
Web of Science (2006): Indexed yes
Freezing fecal samples prior to DNA extraction affects the Firmicutes to Bacteroidetes ratio determined by downstream quantitative PCR analysis

Freezing stool samples prior to DNA extraction and downstream analysis is widely used in metagenomic studies of the human microbiota but may affect the inferred community composition. In this study DNA was extracted either directly or following freeze storage of three homogenized human fecal samples using three different extraction methods. No consistent differences were observed in DNA yields between extractions on fresh and frozen samples, however differences were observed between extraction methods. Quantitative PCR analysis was subsequently performed on all DNA samples using six different primer pairs targeting 16S rRNA genes of significant bacterial groups and the community composition was evaluated by comparing specific ratios of the calculated abundances. In seven out of nine cases the Firmicutes to Bacteroidetes 16S rRNA gene ratio was significantly higher in fecal-samples that had been frozen compared to identical samples that had not. This effect was further supported by qPCR analysis of bacterial groups within these two phyla. The results demonstrate that storage conditions of fecal samples may adversely affect the determined Firmicutes to Bacteroidetes ratio, which is a frequently used biomarker in gut microbiology.

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Organisations: National Food Institute, Division of Microbiology and Risk Assessment
Authors: Bahl, M. I. (Intern), Bergström, A. (Intern), Licht, T. R. (Intern)
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Main Research Area: Technical/natural sciences
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Introducing GUt Low-Density Array (GULDA) - a validated approach for qPCR-based intestinal microbial community analysis

Alterations in the human gut microbiota caused, for example, by diet, functional foods, antibiotics, or occurring as a function of age are now known to be of relevance for host health. Therefore, there is a strong need for methods to detect such alterations in a rapid and comprehensive manner. In the present study, we developed and validated a high-throughput real-time quantitative PCR-based analysis platform, termed ‘GUt Low-Density Array’ (GULDA). The platform was designed for simultaneous analysis of the change in the abundance of 31 different microbial 16S rRNA gene targets in fecal samples obtained from individuals at various points in time. The target genes represent important phyla, genera, species, or other taxonomic groups within the five predominant bacterial phyla of the gut, Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia and also Euryarchaeota. To demonstrate the applicability of GULDA, analysis of fecal samples obtained from six healthy infants at both 9 and 18 months of age was performed and showed a significant increase over time of the relative abundance of bacteria belonging to Clostridial cluster IV (Clostridia leptum group) and Bifidobacterium bifidum and concurrent decrease in the abundance of Clostridium butyricum and a tendency for decrease in Enterobacteriaceae over the 9-month period.
Nature of bacterial colonization influences transcription of mucin genes in mice during the first week of life

In summary, our data show that development of the expression of genes encoding secreted (Muc2/Tff3) and membrane-bound (Muc1/Muc3/Muc4) mucus regulatory proteins, respectively, is distinct and that the onset of this development may be accelerated by specific groups of bacteria present or absent at the mucosal site.

General information
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Organisations: National Food Institute, Division of Food Microbiology, Center for Biological Sequence Analysis, University of Copenhagen
Authors: Bergström, A. (Intern), Kristensen, M. B. (Intern), Bahl, M. I. (Intern), Metzdorff, S. B. (Ekstern), Fink, L. N. (Intern), Frøkiær, H. (Ekstern), Licht, T. R. (Intern)
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Subacute oral toxicity investigation of nanoparticulate and ionic silver in rats

Subacute toxicity of 14 nm nanoparticulate silver (Ag-NP) stabilised with polyvinylpyrrolidone and ionic silver in the form of silver acetate (Ag-acetate) was investigated in four-week-old Wistar rats. Animals received orally by gavage the following: vehicle control (10 $, 6 #); Ag-NP at doses: 2.25 (8 $), 4.5 (8 $) or 9 mg/kg bw/day (10 $, 6 #); or Ag-acetate 9 mg silver/kg bw/day (8 $) for 28 days. Clinical, haematological and biochemical parameters, organ weights, macro- and microscopic pathological changes were investigated. Caecal bacterial phyla and their silver resistance genes were quantified. For the Ag-NP groups, no toxicological effects were recorded. For Ag-acetate, lower body weight gain (day 4–7, 11–14, 14–16, P<0.05; overall, day 1–28, P<0.01), increased plasma alkaline phosphatase (P<0.05), decreased plasma urea (P<0.05) and lower absolute (P<0.01) and relative (P<0.05) thymus weight were recorded. In conclusion, these findings indicate toxicity of 9 mg/kg bw/day ionic silver but not of an equimolar Ag- NP dose. This is in accordance with previously reported data showing that oral Ag-acetate, in comparison with an equimolar dose of Ag-NP, resulted in higher silver plasma and organ concentrations.
Validation of GUt Low Density Array (GULDA), a novel qPCR approach to the study of the intestinal microbial ecosystem

Causal relationships between the vast numbers of bacterial species present in the human intestines contain a lot of potential information on the regulation of the gut in the healthy as well as in diseased states. Based on the hypothesis that the human gut microbiota constitutes a dynamic ecosystem, interesting correlations between the presences of the given species should exist at any time. In order to analyze this, we have developed GULDA, a cheap, flexible, reliable and high throughput qPCR-based gut low-density array (GULDA), which simultaneously gives the quantities of approximately 40 different selected bacterial 16S rRNA targets on all relevant phylogenetic levels in a given sample of DNA. In comparison to other strategies e.g. metagenomic sequencing and microarrays, GULDA focuses on selected targets only and requires only little complex bioinformatical post-processing.
Given the setup, where one standard qPCR program is used for ~40 primer sets, validation is important. We present here strategies involved in verification of GULDA as a valid tool for analysis of the human gut microbiota.

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Organisations: National Food Institute, Division of Microbiology and Risk Assessment
Authors: Bergström, A. (Intern), Licht, T. R. (Intern), Bahl, M. I. (Intern)
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Validation of Gut Low Density Array (GULDA), a novel qPCR approach to the study of the intestinal microbial ecosystem
Causal relationships between the vast numbers of bacterial species present in the human intestines contain a lot of potential information on the regulation of the gut in the healthy as well as in diseased states. Based on the hypothesis that the human gut microbiota constitutes a dynamic ecosystem, interesting correlations between the presences of the given species should exist at any time. In order to analyze this, we have developed GULDA, a cheap, flexible, reliable and high throughput qPCR-based gut low-density array (GULDA), which simultaneously gives the quantities of approximately 40 different selected bacterial 16S rRNA targets on all relevant phylogenetic levels in a given sample of DNA. In comparison to other strategies e.g. metagenomic sequencing and microarrays, GULDA focuses on selected targets only and requires only little complex bioinformatical post-processing.
Given the setup, where one standard qPCR program is used for ~40 primer sets, validation is important. We present here strategies involved in verification of GULDA as a valid tool for analysis of the human gut microbiota.

**General information**
State: Published
Organisations: National Food Institute, Division of Microbiology and Risk Assessment
Authors: Bergström, A. (Intern), Licht, T. R. (Intern), Bahl, M. I. (Intern)
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Xylo-oligosaccharides inhibit pathogen adhesion to enterocytes in vitro
We previously reported that the non-digestible carbohydrates inulin and apple pectin promoted Listeria monocytogenes infection in guinea pigs, whereas xylo- and galacto-oligosaccharides (XOS and GOS), prevented infection by this pathogen. In the present study, mechanisms that could explain the previous in vivo observations were explored. Mixing bacterial cultures with XOS significantly (P < 0.05) decreased the ability of two out of three strains of L. monocytogenes to adhere to Caco-2 cells. Additionally, 2 h incubation with XOS followed by washing of the bacteria significantly (P < 0.05) decreased the ability of all three strains to adhere to Caco-2 cells. Consistently, expression of the adhesion-relevant genes inlA and lap was reduced by the presence of XOS. The observation that XOS inhibit the adhesion of Listeria to the intestinal epithelium in vitro may explain the reported preventive effect of XOS on Listeria infection in guinea pigs in vivo, while the preventive effect of GOS was not explicable by the assays chosen here.

**General information**
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Organisations: National Food Institute, Division of Microbiology and Risk Assessment, University of Nebraska
Authors: Ebersbach, T. (Intern), Andersen, J. B. (Intern), Bergström, A. (Intern), Hutkins, R. W. (Ekstern), Licht, T. R. (Intern)
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Publication information
Gut Low Density Array (GULDA), a novel qPCR approach to the study of the intestinal microbial ecosystem
Causal relationships between the vast numbers of bacterial species present in the human intestines contain a lot of potential information on the regulation of the gut in the healthy as well as in diseased states. Based on the hypothesis that the human gut microbiota constitutes a dynamic ecosystem, interesting correlations between the presences of the given species should exist at any time. However, due to technical restrictions, it has not previously been possible to analyze such intrinsic bacterial patterns and correlations rapidly for a sufficiently large number of samples. To this purpose, we developed GULDA; a qPCR low-density array with particular focus on bacteria of relevance to the human gut microbiota. The output is given as arbitrary bacterial quantities, which for large sample numbers allow for further characterization of the gut microbiota by uni- and multivariate statistical methods.

General information
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Organisations: Division of Microbiology and Risk Assessment, National Food Institute
Authors: Bergström, A. (Intern), Andersen, J. B. (Intern), Licht, T. R. (Intern)
Publication date: 2011
Event: Abstract from Keystone symposium on Microbial Communities as Drivers of Ecosystem Complexity, Colorado, Denver, USA.
Main Research Area: Technical/natural sciences
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Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2011

The complexity of the murine microbiota influences the important recruitment of immune cells in early life

General information
State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Department of Systems Biology
Authors: Kristensen, M. B. (Intern), Frøkiær, H. (Intern), Bergström, A. (Intern), Licht, T. R. (Intern)
Publication date: 2011
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Main Research Area: Technical/natural sciences
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Analysis of the intestinal microbiota of oligo-saccharide fed mice exhibiting reduced resistance to Salmonella infection
Certain indigestible carbohydrates, known as prebiotics, are claimed to be beneficial for gut health through a selective stimulation of certain gut microbes including bifidobacteria. However, stimulation of such microbes does not necessarily imply a preventive effect against pathogen infection. We recently demonstrated a reduced resistance to Salmonella infection in mice fed diets containing fructo-oligosaccharides (FOS) or xylo-oligosaccharides (XOS). In the present study, faecal and caecal samples from the same mice were analysed in order to study microbial changes potentially explaining the observed effects on the pathogenesis of Salmonella. Denaturing gradient gel electrophoresis revealed that the microbiota in faecal samples from mice fed FOS or XOS were different from faecal samples collected before the feeding trial as well as from faecal profiles generated from control animals. This difference was not seen for caecal profiles.
Further analysis of faecal samples by real-time PCR demonstrated a significant increase in the Bacteroidetes phylum, the Bacteroides fragilis group and in Bifidobacterium spp. in mice fed FOS or XOS. The observed bifidogenic effect was more pronounced for XOS than for FOS. The Firmicutes phylum and the Clostridium coccoides group were reduced by both FOS and XOS. Surprisingly, no significant differences were detected between faecal samples collected before and after pathogen challenge in any of the groups. Furthermore, no effect of diets on caecal concentrations of short-chain fatty acids was recorded. In conclusion, diets supplemented with FOS or XOS induced a number of microbial changes in the faecal microbiota of mice. The observed effects of XOS were qualitatively similar to those of FOS, but the most prominent bifidogenic effect was seen for XOS. An increased level of bifidobacteria is thus not in itself preventive against Salmonella infection, since the same XOS or FOS-fed mice were previously reported to be more severely affected by Salmonella than control animals.

Effects of apples and specific apple components on the cecal environment of conventional rats: Role of apple pectin

Background: Our study was part of the large European project ISAFRUIT aiming to reveal the biological explanations for the epidemiologically well-established health effects of fruits. The objective was to identify effects of apple and apple product consumption on the composition of the cecal microbial community in rats, as well as on a number of cecal parameters, which may be influenced by a changed microbiota. Results: Principal Component Analysis (PCA) of cecal microbiota profiles obtained by PCR-DGGE targeting bacterial 16S rRNA genes showed an effect of whole apples in a long-term feeding study (14 weeks), while no effects of apple juice, puree or pomace on microbial composition in cecum were observed. Administration of either 0.33 or 3.3% apple pectin in the diet resulted in considerable changes in the DGGE profiles. A 2-fold increase in the activity of beta-glucuronidase was observed in animals fed with pectin (7% in the diet) for four weeks, as compared to control animals (P <0.01). Additionally, the level of butyrate measured in these pectin-fed animal was more than double of the corresponding level in control animals (P <0.01). Sequencing revealed that DGGE bands, which were suppressed in pectin-fed rats, represented Gram-negative anaerobic rods belonging to the phylum Bacteroidetes, whereas bands that became more prominent represented mainly Gram-positive anaerobic rods belonging to the phylum Firmicutes, and specific species belonging to the Clostridium Cluster XIVa. Quantitative real-time PCR confirmed a lower amount of given Bacteroidetes species in the pectin-fed animals as well as in the apple-fed rats in the four-week study (P <0.05). Additionally, a more than four-fold increase in the amount of Clostridium coccoides (belonging to Cluster XIVa), as well as of genes encoding butyryl-coenzyme A CoA transferase, which is involved in butyrate production, was detected by quantitative PCR in fecal samples from the pectin-fed animals. Conclusions: Our findings show that consumption of apple pectin (7% in the diet) increases the population of butyrate- and beta-glucuronidase producing
Clostridiales, and decreases the population of specific species within the Bacteroidetes group in the rat gut. Similar changes were not caused by consumption of whole apples, apple juice, puree or pomace.
Influence of the gut microbiota on transcriptional regulation of genes involved in early life development of the intestinal mucus layer

The interplay between the gut microbiota and the intestinal mucus layer is important both in the maintenance of the epithelial barrier as part of the innate immune defense, and in the conservation of gut homeostasis. Little is known about how the microbiota regulates mucin proteins, which protect the mucosal surfaces of all epithelial linings by physical hindrance or specific binding of pathogenic agents including virus and bacteria. It has been shown that the presence and composition of the microbiota is directly involved in the regulation of gene transcription in the intestinal epithelium. The intestinal mucus layer of germ free mice has been shown to display a distinctly different composition and structure compared to mucus from conventionally bred animals in vitro and in vivo. This points towards an important role of the microbiota in the regulation of mucin production. To which extent expression of all mucin genes are dependent on the presence of microorganisms and whether specific bacteria are capable of regulating mucus production in early life remains, however, to be established. The very first period after birth is believed to be vulnerable for establishment of the gut microbiota and consequently for the health and integrity of the epithelium throughout life. In this period, a development regulated by endogeneous factors such as hormones, in parallel with gene regulation caused by the microorganisms present in the gut, takes place. Although the microflora undoubtedly plays a regulatory role in the regulation of production of mucin, the importance of endogenous regulation as opposed to gut microbiota has not been investigated. Four groups of mouse pups (n=8 in each group) from differently colonized dams were analyzed with respect to expression of genes involved in mucin production (muc1-4, tff3) in ileal segments isolated on Day 1 and Day 6 after birth. Additionally, the presence of Lactobacillus and E. coli in the ileal samples was assessed by 16S rRNA gene quantification. The pups in the groups were born from dams that were either: 1) germ free (GF), 2) conventional specific pathogen free (SPF), 3) monoclonized with Lactobacillus acidophilus NCFM (Lb NCFM), or 4) monoclonized with E. coli Nissle (E. coli). All data was found by quantitative real-time PCR (qPCR) on Applied Biosystems platforms. Results from these studies showed interesting differences between the four tested animal groups and the two different days tested, which will be presented at the meeting. This is the first study to examine effects of different colonizing bacteria on mucin related gene expression levels in new born mice. These results may thus improve our understanding of the complex interplay between the gut microbiota and epithelial development in the very early life phases.

Bibliographical note
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Organisations: National Food Institute, Division of Microbiology and Risk Assessment, Department of Systems Biology
Authors: Bergström, A. (Intern), Kristensen, M. B. (Intern), Metzdorff, S. B. (Ekstern), Fink, L. N. (Intern), Frøkiær, H. (Ekstern), Licht, T. R. (Intern)
Publication date: 2010

Publication information
Original language: English
Main Research Area: Technical/natural sciences
Mucin genes, Intestinal integrity in the newborn, Probiotic gut monoclonization, Germ Free animals, Gut microbiota

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Source: orbit
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Publication: Research › Sound/Visual production (digital) – Annual report year: 2010
Influence of the gut microbiota on transcriptional regulation of genes involved in early life development of the intestinal mucus layer

The interplay between the gut microbiota and the intestinal mucus layer is important both in the maintenance of the epithelial barrier as part of the innate immune defense, and in the conservation of gut homeostasis. Little is known about how the microbiota regulates mucin proteins, which protect the mucosal surfaces of all epithelial linings by physical hindrance or specific binding of pathogenic agents including virus and bacteria. It has been shown that the presence and composition of the microbiota is directly involved in the regulation of gene transcription in the intestinal epithelium. The intestinal mucus layer of germ free mice has been shown to display a distinctly different composition and structure compared to mucus from conventionally bred animals in vitro and in vivo. This points towards an important role of the microbiota in the regulation of mucin production. To which extent expression of all mucin genes are dependent on the presence of microorganisms and whether specific bacteria are capable of regulating mucin production in early life remains, however, to be established. The very first period after birth is believed to be vulnerable for establishment of the gut microbiota and consequently for the health and integrity of the epithelium throughout life. In this period, a development regulated by endogeneous factors such as hormones, in parallel with gene regulation caused by the microorganisms present in the gut, takes place. Although the microflora undoubtedly plays a regulatory role in the regulation of production of mucin, the importance of endogenous regulation as opposed to gut microbiota has not been investigated. Four groups of mouse pups (n=8 in each group) from differently colonized dams were analyzed with respect to expression of genes involved in mucin production (muc1-4, tff3) in ileal segments isolated on Day 1 and Day 6 after birth. Additionally, the presence of Lactobacillus and E. coli in the ileal samples was assessed by 16S rRNA gene quantification. The pups in the groups were born from dams that were either: 1) germ free (GF), 2) conventional specific pathogen free (SPF), 3) monoclonized with Lactobacillus acidophilus NCFM (Lb NCFM), or 4) monoclonized with E. coli Nissle (E. coli). All data was found by quantitative real-time PCR (qPCR) on Applied Biosystems platforms. Results from these studies showed interesting differences between the four tested animal groups and the two different days tested, which will be presented at the meeting. This is the first study to examine effects of different colonizing bacteria on mucus related gene expression levels in new born mice. These results may thus improve our understanding of the complex interplay between the gut microbiota and epithelial development in the very early life phases.

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State: Published
Organisations: National Food Institute, Division of Microbiology and Risk Assessment, Center for Biological Sequence Analysis, Department of Systems Biology
Authors: Bergström, A. (Intern), Kristensen, M. B. (Intern), Metzdorff, S. B. (Ekstern), Fink, L. N. (Intern), Frøkiær, H. (Ekstern), Licht, T. R. (Intern)
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Source-ID: 271291
Publication: Research - peer-review > Conference abstract in proceedings – Annual report year: 2010

Influence of the intrinsic gut microbiota on transcriptional regulation of genes involved in the early life development of intestinal epithelial integrity

The interplay between the gut microbiota and the integrity of the intestinal mucus layer is important both in the maintenance of the epithelial barrier as part of the innate immune defense, and in the conservation of gut homeostasis. Interesting parameters are the mucins, which protect the mucosal surfaces of all epithelial linings by physical or specific hindrance of pathogenic species e.g. virus and bacteria. Moreover, the proteins constituting the tight junctions in the apical membrane of the epithelial cells are important as they take part in controlling, which substances can penetrate the barrier from the gut lumen to the blood circulation. Previously, it has been shown that the early life mucus layer in germ-free mice has a distinctly different composition than in conventionally colonized animals. In this study, four groups of differently colonized mice were used to analyze mRNA expression by real-time quantitative PCR of relevant mucin (Muc1-4) and tight junction genes (JAM-A, E-Cad, Tjp-1) on RNA purified from isolated ileum samples (n=8 in each group). The groups were: 1) Germ Free (GF), 2) Specific Pathogen Free (SPF) i.e. “conventional microbiota”, 3) NCFM (GF monoclonized with Lactobacillus NCFM), 4) E.coli (GF monoclonized with E.coli). Ileal samples were taken on day 1 and day 6 after birth in order to analyze early life developmental parameters. On the day 6 samples, mucin-related mRNA’s showed significantly higher expression levels in the GF animals compared to the SPF animals, possibly as part of protective mechanism. Monoclonization with Lactobacillus NCFM and E.coli seemed to decrease levels towards levels observed in the SPF animals (except for Muc-3 in E.coli). Two of the tight junction genes (JAM-A, E-Cad) showed similar tendencies, whereas Tjp-1 showed high levels in both GF and SPF. Comelli EM et al (2008) have shown very similar results on the
microarray gene chip technology was applied to cDNA created from RNA isolated from oxygen restricted and non-cell grown without oxygen restriction. In order to identify transcriptional differences contributing to different invasiveness, *in vitro* and *in vivo* data suggest that an oxygen-restricted *L. monocytogenes* cell represents a significantly higher risk than a present in a given food item, but that also the physiological condition of these bacteria is important for food safety. The in more invasive than similar cultures grown without oxygen restriction. This means that not only the number of *Listeria* ingestion are decisive for its *in vivo* infective potential in the gastrointestinal tract after passage of the gastric barrier. Infection of Caco-2 cells revealed that *Listeria* cultivated under oxygen-restricted conditions were approximately 100 fold more invasive than *similar* cultures grown without oxygen restriction. This means that not only the number of *Listeria* present in a given food item, but that also the physiological condition of these bacteria is important for food safety. The in vitro and in vivo data suggest that an oxygen-restricted *Listeria* cell represents a significantly higher risk than a cell grown without oxygen restriction. In order to identify transcriptional differences contributing to different invasiveness, microarray gene chip technology was applied to cDNA created from RNA isolated from oxygen restricted and non-

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State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, University of Copenhagen
Authors: Bergström, A. (Intern), Kristensen, M. B. (Intern), Frøkjær, H. (Ekstern), Licht, T. R. (Intern)
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Publication: Research › peer-review › Conference abstract in proceedings – Annual report year: 2010

**Influence of the intrinsic gut microbiota on transcriptional regulation of genes involved in the early life development of intestinal epithelial integrity**

The interplay between the gut microbiota and the integrity of the intestinal mucus layer is important both in the maintenance of the epithelial barrier as part of the innate immune defense, and in the conservation of gut homeostasis. Interesting parameters are the mucins, which protect the mucosal surfaces of all epithelial linings by physical or specific hindrance of pathogenic species e.g. virus and bacteria. Moreover, the proteins constituting the tight junctions in the apical membrane of the epithelial cells are important as they take part in controlling, which substances can penetrate the barrier from the gut lumen to the blood circulation. Previously, it has been shown that the early life mucus layer in germ-free mice has a distinctly different composition than in conventionally colonized animals. In this study, four groups of differently colonized mice were used to analyze mRNA expression by real-time quantitative PCR of relevant mucin (Muc1-4) and tight junction genes (JAM-A, E-Cad, Tjp-1) on RNA purified from isolated ileum samples (n=8 in each group). The groups were: 1) Germ Free (GF), 2) Specific Pathogen Free (SPF) i.e. “conventional microbiota”, 3) NCFM (GF monocolonized with *Lactobacillus NCFM*), 4) *E.coli* (GF monocolonized with *E.coli*). Ileal samples were taken on day 1 and day 6 after birth in order to analyze early life developmental parameters. On the day 6 samples, mucin-related mRNA's showed significantly higher expression levels in the GF animals compared to the SPF animals, possibly as part of protective mechanism. Monocolonization with *Lactobacillus NCFM* and *E.coli* seemed to decrease levels towards levels observed in the SPF animals (except for Muc-3 in E.coli). Two of the tight junction genes (JAM-A, E-Cad) showed similar tendencies, whereas Tjp-1 showed high levels in both GF and SPF. Comelli EM et al (2008) have shown very similar results on the mucin genes, when colonizing with human adult or baby "full" microbiota. This is the first study with monocolonization however. Finally, we observed inverse correlation between Muc-1 and *Lactobacillus 16S rRNA* expression. The analysis of the day 1 samples is ongoing and results will be presented at the meeting

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Organisations: Division of Microbiology and Risk Assessment, National Food Institute, University of Copenhagen
Authors: Bergström, A. (Intern), Kristensen, M. B. (Intern), Frøkjær, H. (Ekstern), Licht, T. R. (Intern)
Publication date: 2010

**Event**: Poster session presented at TNO Beneficial Microbes Conference : International conference on the health impact and future potential of beneficial microbes, Noordwijkerhout, the Netherlands, .

**Main Research Area**: Technical/natural sciences

**Source**: orbit

**Source-ID**: 259456

**Publication**: Research › peer-review › Conference abstract in proceedings – Annual report year: 2010

**Oxygen restriction increases the infection potential of *Listeria monocytogenes* - a transcriptional analysis.**

*Listeria monocytogenes* has been implicated in several food borne outbreaks as well as sporadic cases of disease during the last two decades. Increased understanding of the biology of this organism is important in the prevention of food borne listeriosis. This is highly relevant for safety assessment of this organism in food. We have previously shown (Andersen et al., BMC Microbiology, 2007, 7:55) that the environmental conditions to which *L. monocytogenes* is exposed prior to ingestion are decisive for its in vivo infective potential in the gastrointestinal tract after passage of the gastric barrier. Infection of Caco-2 cells revealed that *Listeria* cultivated under oxygen-restricted conditions were approximately 100 fold more invasive than similar cultures grown without oxygen restriction. This means that not only the number of *Listeria* present in a given food item, but that also the physiological condition of these bacteria is important for food safety. The in vitro and in vivo data suggest that an oxygen-restricted *Listeria monocytogenes* cell represents a significantly higher risk than a cell grown without oxygen restriction. In order to identify transcriptional differences contributing to different invasiveness, microarray gene chip technology was applied to cDNA created from RNA isolated from oxygen restricted and non-

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The text is provided as a natural representation of the content of the document. It includes key findings from the studies discussed, focusing on the impact of oxygen restriction on the infection potential of *Listeria monocytogenes* and the transcriptional changes associated with it. The text is structured to highlight the significance of these findings for food safety and future potential implications.
restricted cultures. The analysis confirmed several relevant genes to be differentially transcribed in the two environmental conditions e.g. genes related to virulence potential of Listeria monocytogenes.

The Influence of Different Apple Based Supplements on the Intestinal Microbiota of Humans.
Background and objective: The present project is part of the large ISAFRUIT project, where one of the objectives is to identify effects of apple and apple product on parameters related to gut health. In a previous rat study we observed changes in the intestinal microbiota of rats fed whole apples, pomace or apple pectin ([1], and we were interested in finding out if the same effect can be observed in humans. Method: The study was conducted as a randomized, controlled 5 x 28 days cross-over study with 24 healthy persons of both genders. The persons were following a pectin- and polyphenol free restriction diet during the control period, and in the four other periods it was supplied with four different apple based supplements. Between the diets there was a 2-week wash-out period still on the restriction diet. The four apple based supplements were: 1) whole apples, 2) clear apple juice (pectin-free), 3) cloudy juice (apple juice with pulp), and 4) pomace (press cake from the cloudy juice production process). Fecal samples were taken before and after each diet period. After DNA extraction, Denaturing Gradient Gel Electrophoresis (DGGE) with universal primers and specific primers for bifidobacteria and Clostridium cluster XIVa was performed. Bands differing between the periods were sequenced, and qPCR was performed to verify the changes observed by DGGE. Results: Changes in the microbiota was observed by DGGE in persons consuming whole apples and pomace. In contrast, the two juice supplements did not show any effect on the microbiota by DGGE. Conclusion: Consumption of whole apples or pomace is able to modify the intestinal microbiota of humans.
intestinal microbiota of humans.

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Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Division of Toxicology and Risk Assessment, Research Institute of Pomology and Floriculture, University of Copenhagen
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Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 271172
Publication: Research - peer-review › Poster – Annual report year: 2010

**Does an onion-enriched diet beneficially affect the microbiota composition in healthy human subjects?**
Regular onion consumption may have many beneficial effects on human health due mainly to well documented probiotic and antioxidant effects. Health effects comprise e.g. anti-inflammatory, anti-tumorigenic, cardiovascular, and gastrointestinal properties. However little is known of the specific mechanisms involved. Onions are rich in fructooligosaccharides (FOS), which are well acknowledged prebiotic substances. FOS consumption have previously been associated with an increased level of fermenting bacterial genera e.g. Lactobacillus and Bifidobacterium. Generally, these groups of bacteria are considered to have beneficial effects on the intestinal environment. The aim of the present study was to analyze the effects of onion consumption on the gut microbiota profile. In this project, five male and five female subjects were randomized to two 14 days intervention periods including one onion enriched diet and one non-enriched supplemented diet in a double-blinded crossover design with a 25 days wash-out period in between. Six of the subjects delivered fecal samples on the last two days before starting on the diet and on the two last days of the 14 day diet. Total DNA was isolated from these samples using the Qiagen Stool Kit and subsequently quantitative PCR was performed with primers representing the Genera: Lactobacillus, Bifidobacterium, Bacteroidetes, and Clostridium to analyze effects of onion consumption on the gut microbial composition. Moreover, principal component analysis of profiles of the faecal microbiota obtained by denaturing gradient gel electrophoresis of PCR amplified universal bacterial 16S rRNA genes was done to analyze for differences in the phylogenetic profiles as a consequence of the onion consumption. Results from these experiments will be presented at the LMC symposium.

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Organisations: National Food Institute, Division of Microbiology and Risk Assessment, University of Copenhagen, Consejo Superior de Investigaciones Cientificas
Publication date: 2009
Event: Poster session presented at 7th Symposium of Food Microbiology, Helsingør, Denmark.
Main Research Area: Technical/natural sciences
Electronic versions:
LMC poster May 2009.pdf
Source: orbit
Source-ID: 246170
Publication: Research › Poster – Annual report year: 2009

**Effect of apple pectin on gut microbiota - qPCR in applied microbiology**
This study was part of the large European project ISAFRUIT aiming to reveal the biological explanations for the epidemiologically well-established health effects of fruits. The objective was to identify effects of apple and apple product consumption on the composition of the cecal microbial community in rats, as well as on a number of cecal parameters, which could be influenced by a changed microbiota. Principal Component Analysis (PCA) of cecal microbiota profiles obtained by PCR-DGGE targeting bacterial 16S rRNA genes showed an effect of whole apples in a long-term feeding study (14 weeks), while no effects of apple juice, purée or pomace on microbial composition in cecum were observed. Administration of pectin derived from apples resulted in considerable changes of these DGGE profiles. A 2-fold increase in the activity of beta-glucuronidase was observed in animals fed with pectin (7% in the diet) for four weeks, as compared to control animals (P

**General information**
State: Published
Organisations: National Food Institute, Division of Microbiology and Risk Assessment, Division of Toxicology and Risk Assessment, University of Copenhagen
Publication date: 2009
Effect of apple pectin on gut microbiota - qPCR in applied microbiology
This study was part of the large European project ISAFRUIT aiming to reveal the biological explanations for the epidemiologically well-established health effects of fruits. The objective was to identify effects of apple and apple product consumption on the composition of the cecal microbial community in rats, as well as on a number of cecal parameters, which could be influenced by a changed microbiota. Principal Component Analysis (PCA) of cecal microbiota profiles obtained by PCR-DGGE targeting bacterial 16S rRNA genes showed an effect of whole apples in a long-term feeding study (14 weeks), while no effects of apple juice, purée or pomace on microbial composition in cecum were observed. Administration of pectin derived from apples resulted in considerable changes of these DGGE profiles. A 2-fold increase in the activity of beta-glucuronidase was observed in animals fed with pectin (7% in the diet) for four weeks, as compared to control animals.

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Organisations: National Food Institute, Division of Microbiology and Risk Assessment, Division of Toxicology and Risk Assessment, University of Copenhagen
Publication date: 2009
Event: Poster session presented at 4th International qPCR Symposium & Industrial Exhibition & Application Workshop, Freising, Germany.
Main Research Area: Technical/natural sciences
Electronic versions:
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Publication: Research › Poster – Annual report year: 2009

Effect of onion consumption on the composition of the gut microbiota

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State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute
Authors: Bergström, A. (Intern)
Publication date: 2009

Publication information
Original language: English
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Effect of onion consumption on the composition of the gut microbiota

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Conference: 7th Symposium of Food Microbiology, Helsingør, Denmark, 13/05/2009 - 13/05/2009
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Effect of onion consumption on the composition of the gut microbiota

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Organisations: Division of Microbiology and Risk Assessment, National Food Institute, University of Copenhagen, Consejo Superior de Investigaciones Científicas
Publication date: 2009
Event: Poster session presented at 7th Symposium of Food Microbiology, Helsingør, Denmark.
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Publication-ID: 245346

Oxygen restriction and virulence of Listeria monocytogenes: A transcriptome analysis

General information
State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Authors: Andersen, J. B. (Intern), Bergström, A. (Intern), Hansen, T. B. (Intern), Roldgaard, B. (Intern), Christensen, B. B. (Intern), Boye, M. (Intern), Licht, T. R. (Intern)
Number of pages: 44
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Host publication information
Title of host publication: 7th Symposium on Food Microbiology: Abstracts
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Main Research Area: Technical/natural sciences
Conference: 7th Symposium of Food Microbiology, Helsingør, Denmark, 13/05/2009 - 13/05/2009
Source: orbit
Publication-ID: 245315

Oxygen restriction increases the infection potential of Listeria monocytogenes — verification of microarray chip data by quantitative real-time PCR

Listeria monocytogenes has been implicated in several food borne outbreaks as well as sporadic cases of disease during the last two decades. Increased understanding of the biology of this organism is important in the prevention of food born listeriosis. This is highly relevant for safety assessment of this organism in food. We have previously shown (Andersen et al., BMC Microbiology; 2007, 7:55) that the environmental conditions to which L. monocytogenes is exposed prior to ingestion are decisive for its in vivo infective potential in the gastrointestinal tract after passage of the gastric barrier. Infection of Caco-2 cells revealed that Listeria cultivated under oxygen-restricted conditions were approximately 100 fold more invasive than similar cultures grown without oxygen restriction. This means that not only the number of Listeria present in a given food item, but that also the physiological condition of these bacteria is important for food safety. The in vitro and in vivo data suggest that an oxygen-restricted L. monocytogenes cell represents a significantly higher risk than a cell grown without oxygen restriction. In order to identify transcriptional differences contributing to different invasiveness, microarray gene chip technology was applied to cDNA created from RNA isolated from oxygen restricted and non-restricted cultures. The analysis confirmed several relevant genes to be differentially transcribed in the two environmental conditions e.g. genes related to virulence potential of Listeria monocytogenes. Quantitative PCR was used to verify the quantitative differences identified with the microarray chip for a selection of relevant and differentially transcribed genes.

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State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute
Authors: Bergström, A. (Intern), Andersen, J. B. (Intern), Christensen, B. B. (Intern), Licht, T. R. (Intern)
Publication date: 2009
Event: Poster session presented at 4th International qPCR Symposium & Industrial Exhibition & Application Workshop, Freising, Germany.
Main Research Area: Technical/natural sciences
Oxygen restriction increases the infection potential of Listeria monocytogenes – verification of microarray chip data by quantitative real-time PCR

Listeria monocytogenes has been implicated in several food borne outbreaks as well as sporadic cases of disease during the last two decades. Increased understanding of the biology of this organism is important in the prevention of food borne listeriosis. This is highly relevant for safety assessment of this organism in food. We have previously shown (Andersen et al., BMC Microbiology, 2007, 7:55) that the environmental conditions to which L. monocytogenes is exposed prior to ingestion are decisive for its in vivo infective potential in the gastrointestinal tract after passage of the gastric barrier. Infection of Caco-2 cells revealed that Listeria cultivated under oxygen-restricted conditions were approximately 100 fold more invasive than similar cultures grown without oxygen restriction. This means that not only the number of Listeria present in a given food item, but that also the physiological condition of these bacteria is important for food safety. The in vitro and in vivo data suggest that an oxygen-restricted L. monocytogenes cell represents a significantly higher risk than a cell grown without oxygen restriction. In order to identify transcriptional differences contributing to different invasiveness, microarray gene chip technology was applied to cDNA created from RNA isolated from oxygen restricted and non-restricted cultures. The analysis confirmed several relevant genes to be differentially transcribed in the two environmental conditions e.g. genes related to virulence potential of Listeria monocytogenes. Quantitative PCR was used to verify the quantitative differences identified with the microarray chip for a selection of relevant and differentially transcribed genes.

Behavioral, proliferative and molecular corrections in the rat chronic mild stress model of depression

General information
State: Published
Organisations: Aarhus University Hospital
Authors: Wiborg, O. (Ekstern), Jayatissa, M. N. (Ekstern), Bisgaard, C. (Ekstern), Christensen, T. (Ekstern), Henningsen, K. (Ekstern), Bergström, A. (Intern)
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Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 2.005 SNIP 1.161 CiteScore 4.34
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.871 SNIP 1.2 CiteScore 4.18
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.186 SNIP 1.548 CiteScore 4.75
Stress sensitivity and resilience in the chronic mild stress rat model of depression. An in situ hybridization study

We used the validated chronic mild stress (CMS) paradigm to induce anhedonia, a core symptom of major depression, in rats. Thirty percent of animals exposed to CMS are resistant to the development of anhedonia, whereas the remaining are responsive, CMS resilient and CMS sensitive, respectively. We used in situ hybridization to elucidate the molecular mechanisms, which may be involved in the development of anhedonia during CMS. In the CA3 of the ventral hippocampus, we found upregulation of brain-derived neurotrophic factor (BDNF) mRNA in the CMS resilient group indicating protective role of BDNF in stress. Moreover, in the CA3 we found downregulation of vascular endothelial growth factor (VEGF) mRNA in the CMS sensitive group. Downregulation of VEGF suggests impaired hippocampal function, caused by loss of trophic factor neuroprotective support, as part of a previously uncharacterized mechanism for development of anhedonia. CMS induced anhedonia was not related to mRNA expression differences of the dopamine receptors D, and D-2, enkephalin, dynorphin, the NMDA receptor subtype NR2B in the ventral striatum. BDNF expression in the dentate gyrus, nor corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) in the paraventricular nucleus of the hypothalamus. In particular, HPA axis seems to be activated in the CMS resilient group suggesting other pathways protecting against stress sensitivity. We applied the restraint stress procedure to compare effects of a faster and simpler form of stress to CMS and found the latter to be more valid as rats probably easier adapt to restraint stress. Finally, we used the conditioned place preference model to demonstrate a clear tendency towards a distinct morphine induced behavioral difference between CMS resilient and CMS sensitive animals. (C) 2007 Elsevier B.V. All rights reserved.

General information
State: Published
Organisations: H. Lundbeck A/S, Aarhus University Hospital
Authors: Bergström, A. (Intern), Jayatissa, M. N. (Ekstern), Merk, A. (Ekstern), Wiborg, O. (Ekstern)
Pages: 41-52
Publication date: 2008
Main Research Area: Technical/natural sciences
Changes in 5HT4 receptor binding in animal models of depression related states

General information
State: Published
Organisations: Psychiatric Hospital in Aarhus
Authors: Licht, C. L. (Ekstern), Wegener, G. (Ekstern), Zueger, M. (Ekstern), Bergström, A. (Intern), Gass, P. (Ekstern), Wiborg, O. (Ekstern), Overstreet, D. H. (Ekstern), Aznar, S. (Ekstern), Knudsen, G. M. (Ekstern)
Publication date: 2007
Event: Poster session presented at Society for Neuroscience Meeting, San Diego, USA,
Molecular pathways associated with stress resilience and drug resistance in the chronic mild stress rat model of depression - a gene expression study

The current antidepressant drugs are ineffective in 30 to 40% of the treated patients; hence, the pathophysiology of the disease needs to be further elucidated. We used the chronic mild stress (CMS) paradigm to induce anhedonia, a core symptom of major depression, in rats. A fraction of the animals exposed to CMS is resistant to the development of anhedonia; they are CMS resilient. In the CMS-sensitive animals, the induced anhedonic state is reversed in 50% of the animals when treating with escitalopram, whereas the remaining animals are treatment resistant. We used the microarray and the real-time quantitative reverse transcription polymerase chain reaction technique, as well as the ingenuity pathway analysis software to identify the differential gene expression pathways, which are associated with the occurrence of the treatment resistance and the stress-resilient rats. In the hippocampus, we found a significant upregulation of apoptotic pathways in the treatment-resistant animals and significantly increased expression levels of genes involved in hippocampal signaling in the -CMS-resilient rats. We hypothesize that sensitivity to the stress-induced anhedonia in rats is correlated with the impairment of hippocampal neurogenesis.

General information
State: Published
Organisations: Psychiatric Hospital in Aarhus, Own Research ApS
Authors: Bergström, A. (Intern), Jayatissa, M. N. (Ekstern), Thykjær, T. (Ekstern), Wiborg, O. (Ekstern)
Pages: 201-215
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Molecular Neuroscience
Volume: 33
Issue number: 2
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.943 SNIP 0.631 CiteScore 2.17
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.989 SNIP 0.66 CiteScore 2.11
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.153 SNIP 0.738 CiteScore 2.28
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.24 SNIP 0.779 CiteScore 2.63
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.201 SNIP 0.766 CiteScore 2.59
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.134 SNIP 0.757 CiteScore 2.54
ISI indexed (2011): ISI indexed yes
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Scopus rating (2010): SJR 1.295 SNIP 0.735
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.148 SNIP 0.637
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.009 SNIP 0.492
Scopus rating (2007): SJR 0.982 SNIP 0.501
Stress and antidepressant resistance in the chronic mild stress animal model of depression

General information
State: Published
Organisations: Unknown
Authors: Bergström, A. (Intern), Mørk, A. (Ekstern), Wiborg, O. (Ekstern)
Publication date: 2007
Event: Poster session presented at PhD-dag 2007, Århus, Denmark.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 237591
Publication: Research › Poster – Annual report year: 2007

Stress resilience and antidepressant drug resistance in the Chronic Mild Stress animal model of depression

General information
State: Published
Organisations: Unknown
Authors: Bergström, A. (Intern), Wiborg, O. (Ekstern)
Publication date: 2007

Publication information
Original language: English
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 237585
Publication: Research › Ph.D. thesis – Annual report year: 2007

A new rat model for evaluation of efficacy and time point for onset of action for antidepressants

General information
State: Published
Organisations: Aarhus University Hospital
Authors: Jayatissa, M. N. (Ekstern), Bergström, A. (Intern), Christensen, T. (Ekstern), Bisgaard, C. (Ekstern), Wiborg, O. (Ekstern)
Pages: S351-S351
Publication date: 2006
Conference: The 19th Congress of the European-College-of-Neuropsychopharmacology, Paris, France, 01/01/2008
Main Research Area: Technical/natural sciences

Publication information
Journal: European Neuropsychopharmacology
Volume: 16
Issue number: Suppl. 4
ISSN (Print): 0924-977X
Ratings:
Stress and antidepressant resistance in the chronic mild stress animal model of depression.

General information
State: Published
Organisations: Unknown
Authors: Bergström, A. (Intern), Mørk, A. (Ekstern), Wiborg, O. (Ekstern)
Publication date: 2006
Event: Poster session presented at PhD-dag 2006, Århus, Denmark.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 237040

Stress and antidepressant resistance in the chronic mild stress animal model of depression.
Stress resilience and drug resistance in the chronic mild stress (CMS) rat model of depression

General information
State: Published
Organisations: Unknown
Authors: Bergström, A. (Intern), Mørk, A. (Ekstern), Wiborg, O. (Ekstern)
Publication date: 2004
Event: Poster session presented at Society for Neuroscience Meeting, San Diego, USA.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 237588
Publication: Research › Poster – Annual report year: 2004

Projects:

MICROBESE – A novel approach to the study of the intestinal microbial ecosystem and its putative role in obesity development
National Food Institute
Division of Food Microbiology
Department of Systems Biology
Period: 01/01/2011 → 20/09/2013
Number of participants: 5
Gut microbiota low density array, Early life obesity biomarkers, qPCR, Multivariate statistics, Gut community analysis
Acronym: MICROBESE
Project participant:
Licht, Tine Rask (Intern)
Bro, Rasmus (Intern)
Skov, Thomas Hjort (Intern)
Michaelsen, Kim Fleischer (Ekstern)
Project Manager, organisational:
Bergström, Anders (Intern)

Financing sources
Source: Public research council
Name of research programme: Det Fri Forskningsråd/Teknologi og Produktion
Amount: 2,181,600.00 Danish Kroner
Project

Nutritional Immunology
This project runs under the FoodDTU umbrella, and one of its purposes is to create new collaborations between different DTU institutes with ongoing research related to food science. The participating institutes are DTU-Food, DTU-Biosys and DTU-Aqua. The purpose is to elucidate the impact of specific dietary components including e.g. fish oil on the intestinal microbiota and thereby on the development of the immune system in early life. The results are expected to create a basis for better nutritional advice for pregnant women.

National Food Institute
Department of Systems Biology
University of Copenhagen
Number of participants: 14
Project participant:
Kristensen, Matilde Bylov (Intern)
Wilcks, Andrea (Intern)
Bergström, Anders (Intern)
Nellemann, Christine (Intern)
Nutritional Immunology
This project runs under the FoodDTU umbrella, and one of its purposes is to create new collaborations between different DTU institutes with ongoing research related to food science. The participating institutes are DTU-Food, DTU-Biosys and DTU-Aqua. The purpose is to elucidate the impact of specific dietary components including e.g. fish oil on the intestinal microbiota and thereby on the development of the immune system in early life. The results are expected to create a basis for better nutritional advice for pregnant women.

National Food Institute
Department of Systems Biology
Period: 01/08/2007 → 31/12/2011
Number of participants: 13
Project participant:
Kristensen, Matilde Bylov (Intern)
Wilcks, Andrea (Intern)
Bergström, Anders (Intern)
Andersen, Jens Bo (Intern)
Nellemann, Christine (Intern)
Kølln, Charlotte (Intern)
Jacobsen, Charlotte (Intern)
Nielsen, Nina Skall (Intern)
Horn, Anna Frisenfeldt (Intern)
Mathiassen, Jakob Hovalt (Intern)
Hellgren, Lars (Intern)
Fink, Lisbeth Nielsen (Intern)

Project Manager, organisational:
Licht, Tine Rask (Intern)

Nutritional Immunology
National Food Institute
Department of Systems Biology
National Institute of Aquatic Resources
Period: 04/01/2007 → 31/12/2011
Number of participants: 10
Project participant:
Wilcks, Andrea (Intern)
Bergström, Anders (Intern)
Andersen, Jens Bo (Intern)
Metzdorff, Stine Broeng (Intern)
Fink, Lisbeth Nielsen (Intern)
Nielsen, Nina Skall (Intern)

Project Manager, organisational:

Financing sources
Source: [Ordinær drift UK 10]
Name of research programme: [Ordinær drift UK 10]
Amount: 3,250,000.00 Danish Kroner

Effects of bacterial colonization on immune maturation
The Gut Ecology group at the National Food Institute, Technical University of Denmark investigates effects of bacterial colonization on the maturation of the immune system in early life.

We do this by use of germ-free and monocolonized mouse models.

The project is closely related to other projects in the Gut Ecology research group, where we analyze the intestinal microbiota in infants.

Project financing:
Globalization funds (through FoodDTU)
National Food Institute
Division of Food Microbiology
Communications and Management Secretariat
Period: 01/01/2007 → 01/01/2012
Number of participants: 7
Number of related Ph.D. students: 1
Project participant:
Bergström, Anders (Intern)
Nellemann, Christine (Intern)
Frøkiær, Hanne (Intern)
Metzdorff, Stine Broeng (Intern)
Fink, Lisbeth Nielsen (Intern)
Project Manager, organisational:
Licht, Tine Rask (Intern)
Phd Student:
Kristensen, Matilde Bylov (Intern)

Prebiotics for Prevention of Gastrointestinal Infections
National Food Institute
Department of Systems Biology
Period: 01/01/2007 → 01/09/2011
Number of participants: 7
Acronym: PreGI
Project participant:
Wilcks, Andrea (Intern)
Bergström, Anders (Intern)
Andersen, Jens Bo (Intern)
Poulsen, Morten (Intern)
Project Manager, organisational:
Licht, Tine Rask (Intern)
Frøkiær, Hanne (Intern)
Pedersen, Susanne Brix (Intern)

Financing sources
PreGI - Prebiotics for Prevention of Gut Infections

There is increasing evidence that (i) intestinal beneficial bacteria are selectively stimulated by ingestion of specific (prebiotic) carbohydrates, and that (ii) beneficial bacteria ingested as probiotics are capable of suppression of bacterial pathogens in the gut. The idea of this project is to utilize existing animal models to identify dietary (prebiotic) carbohydrates that inhibit infection with selected pathogenic bacterial challengers. Carbohydrates with the best potential for pathogen inhibition will then be further studied with respect to effects on beneficial gut bacteria, production of short-chain fatty acids (SCFAs), and immune modulation in the host animals. Visualization of pathogenic challengers as well as of prebiotic-stimulated beneficial species in the intestinal environment will reveal whether an observed inhibition of a given pathogen results e.g. from competition for adhesion sites. The results obtained will be analyzed in a multivariate approach, in order to determine which of the above-mentioned factors have important impact on the anti-pathogen effect of prebiotics.

Division of Microbiology and Risk Assessment

National Food Institute

Department of Systems Biology

University of Copenhagen

Danisco AS
Period: 01/01/2007 → 30/11/2010
Number of participants: 11
Project participant:
Poulsen, Morten (Intern)
Wilcks, Andrea (Intern)
Bergström, Anders (Intern)
Petersen, Anne (Intern)
Ebersbach, Tine (Intern)
Frøkjær, Hanne (Ekstern)
Pedersen, Susanne Brix (Intern)
Sørensen, Rikke Brandt (Intern)
Ouwehand, Arthur (Ekstern)
Lahtinen, Sampo (Ekstern)

Project Manager, organisational:
Licht, Tine Rask (Intern)

Influence of the gut microbiota on transcriptional regulation of genes involved in early life development of the intestinal mucus layer

Period: 9 Nov 2010 → 11 Nov 2010
Anders Bergström (Speaker)

National Food Institute

Division of Microbiology and Risk Assessment

Description
Place: GutMicroEcology international scientific conference, Kosice, Slovakiet

Related external organisation

Unknown external organisation
Activity: Talks and presentations › Conference presentations
Effect of onion consumption on the composition of the gut microbiota (LMC foodmicro); 7
Period: 13 May 2009
Anders Bergström (Speaker)
National Food Institute
Division of Microbiology and Risk Assessment

Description
Place: LO skolen, Helsingør

Related external organisation
Unknown external organisation
Activity: Talks and presentations › Conference presentations