

Technical University of Denmark



Faecalibacterium Gut Colonization Is Accelerated by Presence of Older Siblings

Laursen, Martin Frederik; Laursen, Rikke Pilmann; Larnkjær, Anni; Mølgaard, Christian; Michaelsen, Kim F.; Frøkiær, Hanne; Bahl, Martin Iain; Licht, Tine Rask

Published in:
Eukaryotic Cell

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Laursen, M. F., Laursen, R. P., Larnkjær, A., Mølgaard, C., Michaelsen, K. F., Frøkiær, H., ... Licht, T. R. (2017). Faecalibacterium Gut Colonization Is Accelerated by Presence of Older Siblings. Eukaryotic Cell, 2(6).

DTU Library

Technical Information Center of Denmark

General rights



Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



Faecalibacterium Gut Colonization Is Accelerated by Presence of Older Siblings

 Martin Frederik Laursen,^a Rikke Pilmann Laursen,^b Anni Larnkjær,^b Christian Mølgaard,^b Kim F. Michaelsen,^b Hanne Frøkiær,^c Martin Iain Bahl,^a  Tine Rask Licht^a

National Food Institute, Technical University of Denmark, Lyngby, Denmark^a; Department of Nutrition, Exercise and Sports, University of Copenhagen, Frederiksberg, Denmark^b; Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Copenhagen, Denmark^c

ABSTRACT *Faecalibacterium prausnitzii* is a highly abundant human gut microbe in healthy individuals, but it is present at reduced levels in individuals with gastrointestinal inflammatory diseases. It has therefore been suggested to constitute a marker of a healthy gut and is associated with anti-inflammatory properties. However, factors affecting the colonization of *F. prausnitzii* in the human gut during early life are very poorly understood. By analysis of 16S rRNA amplicon sequencing data from three separate infant study populations, we determined the colonization dynamics of *Faecalibacterium* and factors affecting its establishment in the gut. We found that in particular, the presence of older siblings was consistently associated with *Faecalibacterium* gut colonization during late infancy and conclude that acquisition of *Faecalibacterium* is very likely to be accelerated through transfer between siblings.

IMPORTANCE *Faecalibacterium prausnitzii* has been suggested to constitute a key marker of a healthy gut, yet the factors shaping the colonization of this highly oxygen-sensitive, non-spore-forming species in the intestinal environment remain poorly understood. Here, we provide evidence from three separate infant study populations that *F. prausnitzii* colonization in the gut happens during late infancy and is affected by the number of older siblings in the family. We conclude that *Faecalibacterium* acquisition is highly likely to be accelerated by contact between siblings. Bearing in mind the immunoregulatory properties of *F. prausnitzii* and the well-established protective effects against allergic disorders related to the presence of older siblings, early colonization of this species may have profound consequences for child health.

KEYWORDS *Faecalibacterium*, infancy, siblings

Starting around birth, the infant gut is colonized by a bacterial community, which gradually increases in richness and diversity (1, 2). A number of factors, including mode of delivery, breastfeeding, and introduction of complementary feeding affect this colonization. After approximately 3 years, the gut microbiota of the child is comparable to that of an adult (3).

Faecalibacterium prausnitzii, which is the sole species within the genus *Faecalibacterium*, is one of the most prevalent and abundant human gut microbes (4), and it has been suggested that it constitutes a marker of gut health due to its lower abundance in patients suffering from inflammatory bowel diseases compared to healthy controls and to its immunoregulatory properties (5, 6). Currently, very little is known about factors that impact the colonization of *F. prausnitzii* during early life and the putative contribution of this species to immune system development (7). Animal studies have shown that prior colonization with other microbes is necessary to obtain robust establishment of *F. prausnitzii* in germfree rodents (8, 9), and it has been suggested that

Received 28 September 2017 Accepted 9 November 2017 Published 29 November 2017

Citation Laursen MF, Laursen RP, Larnkjær A, Mølgaard C, Michaelsen KF, Frøkiær H, Bahl MI, Licht TR. 2017. *Faecalibacterium* gut colonization is accelerated by presence of older siblings. *mSphere* 2:e00448-17. <https://doi.org/10.1128/mSphere.00448-17>.

Editor Garret Suen, University of Wisconsin—Madison

Copyright © 2017 Laursen et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Tine Rask Licht, trli@food.dtu.dk.

depletion of oxygen in the gut or putatively another type of conditioning of the gut environment by other microbes is required for *F. prausnitzii* to colonize the gut (7). The sensitivity of *F. prausnitzii* to low pH and bile salts explains the observation that it predominantly colonizes the distal gastrointestinal tract of humans (6), but it remains to be established how this highly oxygen-sensitive, non-spore-forming bacterium is transmitted between individuals and which factors affect its colonization process. We therefore analyzed 16S rRNA amplicon sequencing data from fecal samples originating from three previous studies of Danish infants entitled SKOT I (10), SKOT II (11), and ProbiComp (12), respectively. Comparison of *Faecalibacterium* abundance to a number of selected variables allowed identification of factors influencing colonization of *Faecalibacterium*.

F. prausnitzii is not detected or found at very low levels during the first 4 to 6 months of life, but it increases rapidly during the first years (13–15). By investigation of three independent study populations, SKOT I ($n = 114$), SKOT II ($n = 113$), and ProbiComp ($n = 255$), we found that the relative abundance and prevalence of *Faecalibacterium* increased significantly during the period from late infancy (8 to 12 months old) to toddlerhood (14 to 19 months old) (Fig. 1A to F). In contrast to the SKOT infants, which were sampled as close as possible to 9 and 18 months of age, the infants in the ProbiComp study were sampled over a larger age span (Fig. 1G). This enabled correlation analysis between age and *Faecalibacterium* abundance and revealed a significant positive correlation at late infancy, but no such correlation at toddlerhood (Fig. 1H), suggesting that *Faecalibacterium* colonization reaches a plateau (Fig. 1I). To investigate how external factors and host variables may affect the relative abundance of *Faecalibacterium* during late infancy, we analyzed the association of its abundance with (i) gender, (ii) gestational age at birth, (iii) Caesarean section, (iv) breastfeeding, (v) pets, (vi) older siblings, and (vii) anthropometrics in all three infant study populations. Among these factors, only the presence of older siblings in the home was consistently correlated with the relative abundance of *Faecalibacterium* across the three study populations (Fig. 2A). Both the relative abundance and the prevalence of *Faecalibacterium* were higher during late infancy in infants with older siblings than in infants with no older siblings in the three populations (see Fig. S1 in the supplemental material). These differences were no longer present in toddlerhood, and *Faecalibacterium* was detected in almost all individuals (Fig. S1). Combination of the three data sets ($n = 482$) and stratification of individuals with no ($n = 270$), one ($n = 156$), or two or more ($n = 56$) older siblings revealed a stepwise increase in relative abundance ($P < 0.0001$ by the Kruskal-Wallis test) and prevalence (P for the trend [P_{fortrend}] of 0.0002 by the chi-square test) of *Faecalibacterium* with increasing number of older siblings during late infancy (Fig. 2B). Moreover, age-adjusted partial correlation analysis between relative abundance of *Faecalibacterium* during infancy and the number of older siblings revealed that this association was highly significant ($\rho = 0.23$; $P = 6.4 \times 10^{-7}$; $n = 482$). The association was no longer present when the children became toddlers, suggesting that at this age, children will have acquired *Faecalibacterium* from other encounters. As infants with older siblings are putatively introduced earlier to solid foods, we investigated diet as a potential confounder, since we have previously found that diet influences the microbiota (11). By analysis of detailed dietary records obtained for the SKOT cohorts (11, 16), we found that transition to family foods ($\rho = 0.16$; $P = 0.018$; $n = 217$) and dietary intake of rye bread ($\rho = 0.20$; $P = 0.003$; P value for the corrected false-discovery rate [$P_{\text{FDRcorrected}}$] of 0.07; $n = 217$), but not other dietary parameters, were associated with *Faecalibacterium* (Fig. S2). However, the association between relative abundance of *Faecalibacterium* and the number of older siblings persisted after adjustment for both transition to family foods ($\rho = 0.26$; $P = 0.0001$; $n = 217$) and intake of rye bread ($\rho = 0.25$; $P = 0.0001$; $n = 217$). Together, this shows that rather than gender, mode of delivery, breastfeeding, complementary diet, exposure to pets or anthropometric measures, it is the exposure to older siblings that predominantly affects *Faecalibacterium* acquisition during late infancy.

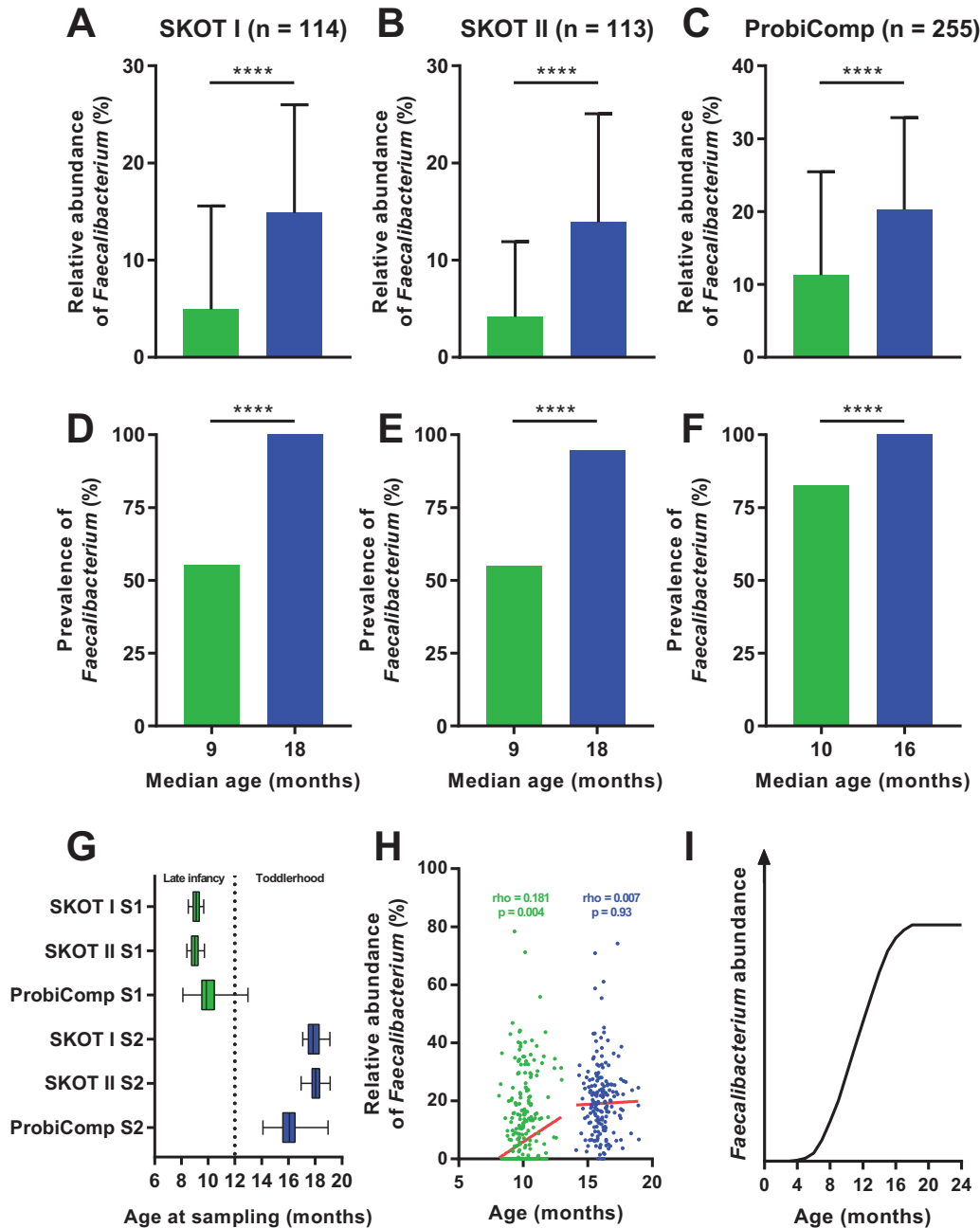


FIG 1 Colonization dynamics of *Faecalibacterium prausnitzii*. (A to C) Mean relative abundance of *Faecalibacterium* in SKOT I, SKOT II, and ProbiComp during late infancy (8 to 12 months old) (green) and toddlerhood (14 to 19 months old) (blue). Values are means plus standard deviations (SD) (error bars). Mean values that are significantly different ($P < 0.0001$) by Mann-Whitney test are indicated by a bar and four asterisks. (D to F) Prevalence of *Faecalibacterium* in SKOT I, SKOT II, and ProbiComp during late infancy (green) and toddlerhood (blue). Mean values that are significantly different ($P < 0.0001$) by Fisher exact test are indicated by a bar and four asterisks. (G) Variation in age at the two sampling points (sampling point 1 [S1], late infancy; S2, toddlerhood) for SKOT I, SKOT II, and ProbiComp. Boxes indicate 25th to 75th percentiles, with median values marked as a line and whiskers indicating minimum and maximum values. The dotted line indicates the age that distinguishes late infancy from toddlerhood. (H) Spearman's rank correlations of age versus relative abundance of *Faecalibacterium* during late infancy (green) and toddlerhood (blue) in ProbiComp. Red lines indicate robust nonlinear regression fit to the data points. (I) Proposed colonization dynamics of *Faecalibacterium* as a function of age based on data from this study and previous studies (13–15).

Study populations and samplings. Data from three infant study populations were included in the present study. The study protocol for ProbiComp was approved by the Committees on Biomedical Research Ethics for the Capital Region of Denmark (H-4-2014-032), and the study was registered at clinicaltrials.org (NCT02180581). The study

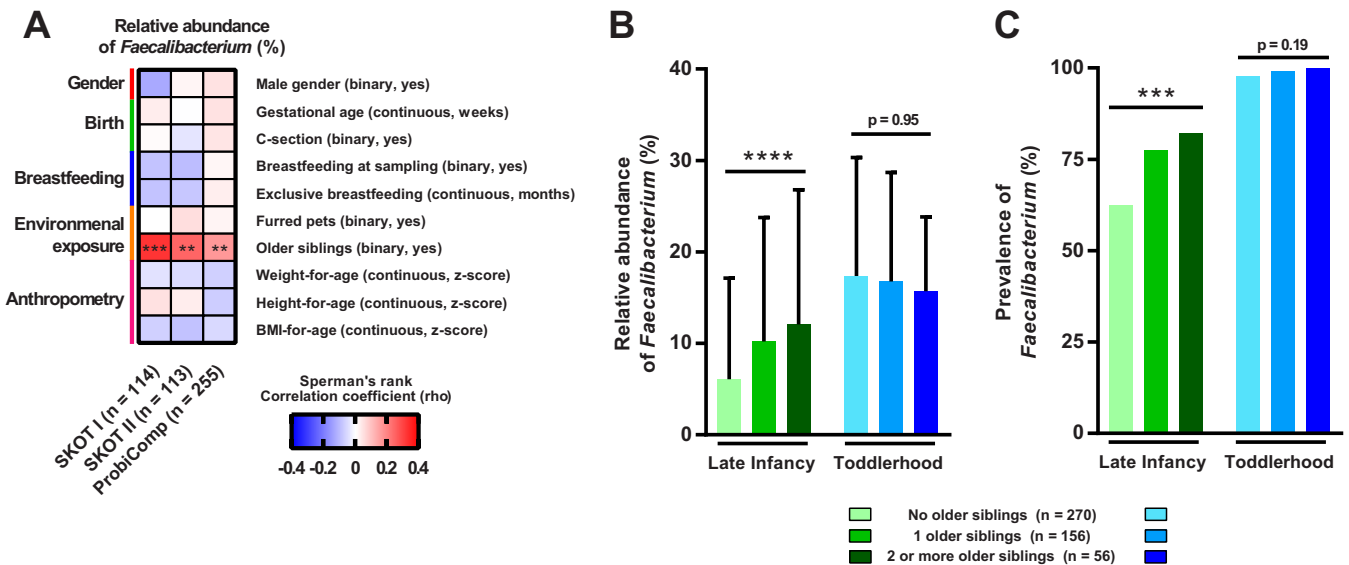


FIG 2 *Faecalibacterium* colonization during late infancy is impacted by older siblings. (A) Heatmap of Spearman's rank correlation coefficients between the relative abundance of *Faecalibacterium* and selected variables during late infancy in SKOT I, SKOT II, and ProbiComp. Statistical significance is indicated by asterisks as follows: **, $P = 0.01$; ***, $P < 0.001$. (B and C) Relative abundances (B) and prevalence of *Faecalibacterium* (C) in the combined data set ($n = 482$) during late infancy and toddlerhood stratified according to the number of older siblings. Mean values plus SD (error bars) are shown in panel B. Statistical significance is indicated by asterisks as follows: ****, $P < 0.0001$ by the Kruskal-Wallis test; ***, $P < 0.001$ by the chi-square test for trend.

protocols for the SKOT cohorts were approved by the Committees on Biomedical Research Ethics for the Capital Region of Denmark (H-KF-2007-0003 and H-3-2010-122). All parents signed a consent form. The ProbiComp study ($n = 290$) was a double-blind randomized placebo-controlled study where infants, aged 8 to 13 months and starting daycare within 12 weeks after the start of the study were randomly assigned to receive a combination of the two probiotics, BB-12 (*Bifidobacterium animalis* subsp. *lactis* BB-12 strain) and LGG (*Lactobacillus rhamnosus* GG), or placebo during the 6-month study period (17). Inclusion criteria were single birth and starting daycare at 8 to 14 months of age. Exclusion criteria were low birth weight (<2,500 g), gestational age at birth of <36 weeks, chronic illness, regular medication, antibiotics within 4 weeks prior to the start of the study, and non-Danish speaking parents. Fecal samples were obtained before ($n = 265$; age 8 to 13 months) and after ($n = 210$; age 14 to 19 months) the study and were stored at -80°C until DNA extraction and microbiota profiling as described previously (12). The fecal microbiotas were profiled in 255 and 201 samples before and after the study, respectively, and the treatment had no impact on the fecal microbiota (12). The SKOT cohort studies monitored children during the first 3 years of life with the overall aim to investigate relationships between early diet, growth development, and later disease risks. In SKOT I (18), infants from a random sample of mothers were recruited ($n = 311$), whereas in SKOT II (19), infants of obese mothers (body mass index [BMI] of $>30 \text{ kg/m}^2$) were recruited ($n = 184$). Inclusion criteria for both cohorts were single birth, full-term delivery, age of 9 months ± 2 weeks at the first visit, and absence of chronic illness. Participants in both cohorts were examined at 9 (± 2 weeks), 18 (± 4 weeks), and 36 (± 12 weeks) months of age, and fecal samples were collected and stored at -80°C until DNA extraction. The fecal microbiota data from the first two sampling points in subsets of 114 (SKOT I) and 113 (SKOT II) infants have been published previously (11).

In all study populations, fecal samples were freshly delivered on the morning of the visit or had been stored in the participant's home, either in the freezer (-18°C) or in the fridge (4°C) for maximally 24 h before storage at -80°C . Information on gender, gestational age, Caesarean section, breastfeeding (months of breastfeeding only and the frequency of breastfeeding at sampling times), presence of older siblings and furred pets was collected from parental interviews at the sampling times in all three study

populations. Anthropometrics were obtained at examinations and Z-scores were calculated as described previously (11, 17). Food records were obtained and analyzed in the SKOT studies as described previously (11, 19).

DNA extraction and microbiota profiling. The procedures for DNA extraction, PCR amplification, and sequencing of the V3 region of the 16S rRNA gene have been described previously (11, 12). Briefly, DNA was extracted (PowerLyzer PowerSoil DNA isolation kit [catalog no. 12855-100; MoBio]), the V3 region of the 16S rRNA gene was amplified (Phusion High-Fidelity PCR kit [catalog no. F-553L; Thermo Fisher Scientific]) with sample-specific barcoded forward primers (PBU [5'-A-adapter-TCAG-barcode-CCT ACGGGAGGCAGCAG-3']) and the universal reverse primer (PBR [5'-trP1-adapter-ATTA CCGCGGCTGCTGG-3']) according to the following PCR program: (i) 30 s at 98°C; (ii) 24 cycles, with 1 cycle consisting of 15 s at 98°C and 30 s at 72°C; (iii) 5 min at 72°C. PCR products were purified (HighPrep PCR magnetic beads [catalog no. AC-60005; MAGBIO]), DNA quantities were measured (Qubit dsDNA [double-stranded DNA] HS [high-sensitivity] assay [catalog no. Q32851; Invitrogen]), and samples were pooled to obtain equimolar libraries, which were sequenced using the Ion OneTouch and Ion PGM (personal genome machine) platform with an Ion 318 Chip kit. Sequence data were analyzed as described previously for SKOT data (11) and ProbiComp data (12), and the levels of all sequences classified as *Faecalibacterium* by the RDP classifier, with a confidence threshold of 0.5 (20) against the Greengenes database v. 13.8 (21) were pooled within each sample and normalized to the total reads in that sample.

Statistics. Mann-Whitney, Kruskal-Wallis, Spearman's rank correlations, Fisher exact test, and chi-square tests were performed with the GraphPad Prims software (v. 7.0; GraphPad Software, Inc., La Jolla, CA). When indicated, *P* values were corrected for multiple testing using the false-discovery rate (FDR) (22). Partial Spearman's rank correlation analysis adjusted for age or dietary parameters was performed in R (version 3.1.0; R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) using the *ppcor* package.

Accession number(s). Sequencing data are deposited at NCBI Sequence Read Archive with the accession number [SRP100762](https://www.ncbi.nlm.nih.gov/seqread/submitseq/SRP100762) under BioProject [PRJNA360073](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA360073) for ProbiComp study and accession number [SRP052851](https://www.ncbi.nlm.nih.gov/seqread/submitseq/SRP052851) under BioProject [PRJNA273694](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA273694) for SKOT cohorts.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/mSphere.00448-17>.

FIG S1, EPS file, 0.02 MB.

FIG S2, EPS file, 0.1 MB.

ACKNOWLEDGMENTS

We thank the infants and their families participating in the SKOT and ProbiComp studies for their contributions. We thank Vivian Anker, Birgitte Hermansen, Julie Brønholm, and Inge Rasmussen for technical assistance.

The ProbiComp study was funded by Innovation Fund Denmark (grant 0603-00579b). SKOT studies were supported by the Danish Directorate for Food, Fisheries and Agribusiness (grant 3304-FSE-06-0503). The microbiota analysis was supported by the Gut, Grain and Greens (3G) Center, financed by the Danish Council for Strategic Research (grant 11-116163/0603-00487B).

We declare that we have no conflict of interest.

K.F.M., C.M., and A.L. designed the SKOT cohort studies and the ProbiComp intervention study. R.P.L. obtained data and collected fecal samples from ProbiComp participants and conducted data analyses on study participant characteristics. M.F.L. performed 16S rRNA amplicon library preparation. M.F.L., M.I.B., H.F., and T.R.L. analyzed and interpreted the data. M.F.L. wrote a draft of the paper. All authors read and approved the final manuscript.

REFERENCES

- Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, Watkins C, Dempsey E, Mattivi F, Tuohy K, Ross RP, Ryan CA, O' Toole PW, Stanton C. 2017. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome* 5:4. <https://doi.org/10.1186/s40168-016-0213-y>.
- Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE. 2011. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A* 108(Suppl 1):4578–4585. <https://doi.org/10.1073/pnas.1000081107>.
- Bergström A, Skov TH, Bahl MI, Roager HM, Christensen LB, Ejlerskov KT, Mølgaard C, Michaelsen KF, Licht TR. 2014. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. *Appl Environ Microbiol* 80:2889–2900. <https://doi.org/10.1128/AEM.00342-14>.
- Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Zhang X, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, MetaHIT Consortium, Bork P, Ehrlich SD. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464:59–65. <https://doi.org/10.1038/nature08821>.
- Miquel S, Martín R, Rossi O, Bermúdez-Humarán LG, Chatel JM, Sokol H, Thomas M, Wells JM, Langella P. 2013. *Faecalibacterium prausnitzii* and human intestinal health. *Curr Opin Microbiol* 16:255–261. <https://doi.org/10.1016/j.mib.2013.06.003>.
- Lopez-Siles M, Duncan SH, Garcia-Gil LJ, Martinez-Medina M. 2017. *Faecalibacterium prausnitzii*: from microbiology to diagnostics and prognostics. *ISME J* 11:841–852. <https://doi.org/10.1038/ismej.2016.176>.
- Miquel S, Martín R, Bridonneau C, Robert V, Sokol H, Bermúdez-Humarán LG, Thomas M, Langella P. 2014. Ecology and metabolism of the beneficial intestinal commensal bacterium *Faecalibacterium prausnitzii*. *Gut Microbes* 5:146–151. <https://doi.org/10.4161/gmic.27651>.
- Wrzosek L, Miquel S, Noordine M-L, Bouet S, Chevalier-Curt MJ, Robert V, Philippe C, Bridonneau C, Cherbuy C, Robbe-Masselot C, Langella P, Thomas M. 2013. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol* 11:61. <https://doi.org/10.1186/1741-7007-11-61>.
- Miquel S, Leclerc M, Martin R, Chain F, Lenoir M, Raguideau S, Hudault S, Bridonneau C, Northen T, Bowen B, Bermúdez-Humarán LG, Sokol H, Thomas M, Langella P. 2015. Identification of metabolic signatures linked to anti-inflammatory effects of *Faecalibacterium prausnitzii*. *mBio* 6:1–10. <https://doi.org/10.1128/mBio.00300-15>.
- Laursen MF, Zachariassen G, Bahl MI, Bergström A, Høst A, Michaelsen KF, Licht TR. 2015. Having older siblings is associated with gut microbiota development during early childhood. *BMC Microbiol* 15:154. <https://doi.org/10.1186/s12866-015-0477-6>.
- Laursen MF, Andersen LBB, Michaelsen KF, Mølgaard C, Trolle E, Bahl MI, Licht TR. 2016. Infant gut microbiota development is driven by transition to family foods independent of maternal obesity. *mSphere* 1:e00069-15. <https://doi.org/10.1128/mSphere.00069-15>.
- Laursen MF, Laursen RP, Larnkjær A, Michaelsen KF, Bahl MI, Licht TR. 2017. Administration of two probiotic strains during early childhood does not affect the endogenous gut microbiota composition despite probiotic proliferation. *BMC Microbiol* 17:175. <https://doi.org/10.1186/s12866-017-1090-7>.
- Hopkins MJ, Macfarlane GT, Furrie E, Fite A, Macfarlane S. 2005. Characterisation of intestinal bacteria in infant stools using real-time PCR and Northern hybridisation analyses. *FEMS Microbiol Ecol* 54:77–85. <https://doi.org/10.1016/j.femsec.2005.03.001>.
- Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J, Jun W. 2015. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* 17:690–703. <https://doi.org/10.1016/j.chom.2015.04.004>.
- Koga Y, Tokunaga S, Nagano J, Sato F, Konishi K, Tochio T, Murakami Y, Masumoto N, Tezuka JI, Sudo N, Kubo C, Shibata R. 2016. Age-associated effect of kefir on *Faecalibacterium prausnitzii* and symptoms in the atopic dermatitis infants. *Pediatr Res* 80:844–851. <https://doi.org/10.1038/pr.2016.167>.
- Laursen MF, Bahl MI, Michaelsen KF, Licht TR. 2017. First foods and gut microbes. *Front Microbiol* 8:356. <https://doi.org/10.3389/fmicb.2017.00356>.
- Laursen RP, Larnkjær A, Ritz C, Hauger H, Michaelsen KF, Mølgaard C. 2017. Probiotics and child care absence due to infections: a randomized controlled trial. *Pediatrics* 140:e20170735. <https://doi.org/10.1542/peds.2017-0735>.
- Madsen AL, Schack-Nielsen L, Larnkjær A, Mølgaard C, Michaelsen KF. 2010. Determinants of blood glucose and insulin in healthy 9-month-old term Danish infants; the SKOT cohort. *Diabet Med* 27:1350–1357. <https://doi.org/10.1111/j.1464-5491.2010.03134.x>.
- Andersen LBB, Pipper CB, Trolle E, Bro R, Larnkjær A, Carlsen EM, Mølgaard C, Michaelsen KF. 2015. Maternal obesity and offspring dietary patterns at 9 months of age. *Eur J Clin Nutr* 69:668–675. <https://doi.org/10.1038/ejcn.2014.258>.
- Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261–5267. <https://doi.org/10.1128/AEM.00062-07>.
- DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 72:5069–5072. <https://doi.org/10.1128/AEM.03006-05>.
- Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Methodol* 57:289–300.