BOOK OF ABSTRACTS

THE BARCELONA DEBATES
ON THE HUMAN MICROBIOME
››› 2024

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Title	Text	Authors	Author Affiliations
Longitudinal analysis of the gut microbiome in adolescent patients with anorexia nervosa: microbiome-related factors associated with clinical outcome	There is mounting evidence regarding the role of gut microbiota in anorexia nervosa (AN). Previous studies have reported that patients with AN show dysbiosis compared to healthy controls (HCs); however, the underlying mechanisms are unclear, and data on influencing factors and longitudinal course of microbiome changes are scarce. Here, we present longitudinal data of 57 adolescent inpatients diagnosed with AN at up to nine time points (including a 1-year follow-up examination) and compare these to up to six time points in 34 HCs. 16S rRNA gene sequencing was used to investigate the microbiome composition of fecal samples, and data on food intake, weight change, hormonal recovery (leptin levels), and clinical outcomes were recorded. Differences in microbiome composition compared to HCs were greatest during acute starvation and in the low-weight group, while diminishing with weight gain and especially weight recovery at the 1-year follow-up. Illness duration and prior weight loss were strongly associated with microbiome composition at hospital admission, whereas microbial changes during treatment were associated with kilocalories consumed, weight gain, and hormonal recovery. The microbiome at admission was prognostic for hospital readmission, and a higher abundance of Sutterella was associated with a higher body weight at the 1-year follow-up. Identifying these clinically important factors further underlines the potential relevance of gut microbial changes and may help elucidate the underlying pathophysiology of gut-brain interactions in AN. The characterization of prognostically relevant taxa could be useful to stratify patients at admission and to potentially identify candidate taxa for future supplementation studies aimed at improving AN treatment.	Nadia Andrea Andreani a,b*, Arunabh Sharma c*, Brigitte Dahmend, Hannah E. Spechtd, Nina Mannigd, Vanessa Ruand, Lara Kellerd, John F. Baines a,b, Beate Herpertz-Dahlmann d, Astrid Dempfle c, and Jochen Seitz d	a Section of Evolutionary Medicine, Max Planck Institute for Evolutionary Biology, Plön, Germany; b Section of Evolutionary Medicine, Institute for Experimental Medicine, Kiel University, Kiel, Germany; c Institute of Medical Informatics and Statistics, Kiel University, Kiel, Germany; d Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital, RWTH Aachen University, Aachen, Germany

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The Vaginal Microbiome: What's Cooking in Middle-Aged Women's Gardens?	Background The vaginal microbiome is the microbial community inhabiting the vagina. Changes related to women's life-history traits as well as cyclical perturbations render the characterization of the vaginal microbiome a challenge. Objectives Longitudinal analysis of diversity, composition, and changes of the vaginal microbiome as well as the study of the determinants (sociodemography, anthropometry, lifestyle, sexual and reproductive health, and menopause status) associated with this microbiome in middle-aged women. Methods Participants: 50 middle-aged women (41-58 years old) living in Valencia (Spain). Vaginal microbiome samples and potential determinants were collected three times over a year. Ecological dynamics were assessed through estimation of α-diversity (richness), β-diversity (composition), and taxonomic composition (β-diversity classified in three categories: Lactobacillus inners, other Lactobacillus, and other anaerobic bacteria). Clustering (Ward hierarchical clustering), and ordering methods (Principal Coordinate Analysis [PCoA] and Non-Metric Multidimensional Scaling [nMDS]) were implemented. Finally, descriptive and multivariate analyses were conducted. Results Indexes α and β were stable over time (p-value=0.894 and 0.112, respectively) while taxonomic composition did change (p-value=0.029). Increases in the average of α and β diversities were associated to menopause at baseline (α: β[95%IC]= perimenopause: 0,513[1,025,-0,001] and non-menopause -0,503[-0,885,-0,121]; β: perimenopause: -0.285[-0.586,0.015] and non-menopause: -0.206[-0.435,0.023] compared to menopause). Menopause at baseline presented microbiome more likely to belong into the anaerobic bacteria category (perimenopause β[95%IC]=-3.491[-5.520,-1.463], and non-menopause: -4.337[-5.754,-2.920]). To a lesser extent, higher diversities were associated with obesity, older age at first sexual intercourse, lower alcohol intake and no use of oral contraceptives. Conclusions. Menopause could be a risk factor of altered vaginal microbiome diversity and composition. Further studies with a larger sample size are warranted. Funding: GVA (INVEST/2022/310, INVEST/2023/219, CIACIF/2023/268, GV/2021/71, AICO/2021/182), ISCIII (ISCIII CD23/00090 - Co-financied by European Union), Grant CNS2023-145286 funded by MICIU/AEI /10.13039/501100011033 and by European Union NextGenerationEU/PRTR, and CIGE/2021/071.	Raul Beneyto, Maria-Jose Lopez-Espinosa, María Montegud, Camen Iñiguez, Reem Abumallouh, Natalia Marin, Oihane Alvarez, Nuria Jiménez-Hernández, M.Pilar Francino, Ignacio G. Bravo, Blanca Sarzo	Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Valencia, Spain. Epidemiology and Environmental Health Joint Research Unit, FISABIO-University Jaume I-University of Valencia, Valencia, Spain. Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain. Faculty of Nursing and Chiropody, University of Valencia, Valencia, Spain. Faculty of Mathematics, University of Valencia, Valencia, Spain Laboratory MIVEGEC (CNRS, IRD, Univ. Montpellier), French National Center for Scientific Research, Montpellier (France)

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Pre- and perinatal factors shaping gut microbiota diversity and composition in adolescence	Recent studies have stressed the importance of gut microbiota for human health. Little research exists on the factors shaping it in adolescents. The objective was to study the possible pre- and perinatal determinants of gut microbiota composition and diversity in adolescents Prospective study on 398 adolescents from 3 INMA cohorts (Valencia, Sabadell, Gipuzkoa) between 13-16 years old, whose mothers were recruited at week 12 of pregnancy (2003-2008). A fecal sample was taken and analyzed using 16S gene amplicon sequencing. Three indices of alpha-diversity were calculated: taxon richness (CHAO1), entropy (Shannon) and phylogenetic diversity (Faith). We calculated beta-diversity using the Bray-Curtis (BC) and the unweighted UniFrac (UF) indices. Analyses were performed at genus level. Sociodemographic, lifestyle, diet, anthropometric and clinical data were obtained during pregnancy/at birth. Determinants of alpha- and beta-diversity were studied using linear models and permutational analysis of variances (PERMANOVA), respectively. Finally, we employed the determinants selected by PERMANOVA and analyzed their effects on single taxa using Analysis of Compositions of Microbiomes with Bias Correction 2 (ANCOMBC2). All analyses were adjusted for type of stool at sampling, frequency of bowel movements and cohort Vegetable intake in pregnancy (per 100 g) was associated with the 3 alpha-diversity indices (CHAO1 β[95%CI]: 1.84[0.10,3.58]; Shannon: 0.05[0.01,0.09]; Faith: 0.23[-0.01,0.47]), while medium parental social class (SC) was associated with the Shannon index (-0.08[-0.16,-0.01]). Beta-diversity analysis with the BC index revealed that child's sex (PERMANOVA p-value:0.003) and maternal diet in pregnancy (meat p:0.028; cereals and pasta p:0.029; vegetables p:0.047) were associated with global composition of gut microbiota. With the UF index, sex (p:0.008), SC (p:0.022) and age (p:0.051) were the main drivers. In the single taxa analyses, sex was associated with the genera Haemophilus (log fold change[se]: -0.78[0.13]), Eisenbergiella (-0.48[0.11]), Megamonas (1.15[0.10]), Prevotella (1.18[0.29]) and Marvinbryantia (0.30[0.07]). Lower SC was associated with greater amounts of Desulfovibrio (0.87[0.20]) and Lactobacillus (0.65[0.20]), while cereal and pasta intake (per 100 g) during pregnancy was positively associated with Christensenella (0.48[0.10]) Gut microbiota could be affected by maternal diet and parental SC during pregnancy, and child's age and sex	Raul Beneyto, Blanca Sarzo, M Pilar Francino, Nuria Jiménez-Hernández, Jorge Vallejo-Ortega, Oihane Alvarez, Mariona Bustamante, Amaia Irizar, Sandra González-Palacios, Léa Maitre, Ziortza Barroeta, Sabrina Llop, Maria-Jose Lopez-Espinosa	Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO Public Health. Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP). Barcelona Institute for Global Health (ISGlobal). Universitat Pompeu Fabra (UPF). Bioinformatics and Genomics Program, Center for Genomic Regulation (CRG). Biodonostia Health Research Institute, Environmental Epidemiology and Child Development Group. Department of Preventive Medicine and Public Health, University of the Basque Country (UPV-EHU). Institute for Health and Biomedical Research of Alicante (ISABIAL). Miguel Hernandez University. Epidemiology and Environmental Health Joint Research Unit, FISABIO-University Jaume I-University of Valencia. Faculty of Nursing and Chiropody, University of Valencia

Title	Text	Authors	Author Affiliations
Bacterial gut microbiota and invasive enterococcal disease: prospective case-control study	Background Enterococcus faecalis/faecium are microorganisms highly resistant to extreme conditions mainly transmitted by ingestion of contaminated food. Despite their limited pathogenic potential in the immunocompetent host, in recent decades they have become increasingly relevant, causing Invasive Enterococcal Disease (IED) in elderly and immunosuppressed patients. We wondered if IED is related with a specific gut microbiota signature. Long-read sequencing enables full-length 16S rRNA gene analysis, enhancing bacterial species identification. This case-control study aims to characterize the bacterial gut microbiota of patients affected by IED by full-length 16S rRNA gene sequencing. Methods IED cases were prospectively included along with paired hospital (H) and primary care (PC) controls of similar age and Charlson comorbity index. Paired faeces samples collected at inclusion were processed in parallel as follows. DNA was extracted using the Quick-DNA Fecal/Soil Microbe Microprep (Zymo Research). In each extraction, in addition to a negative control (reagent only), the Zymobiomics Fecal Reference was included and used as a control for a healthy gut microbiota. The entire 16S rRNA gene was amplified and libraries were indexed using the 16S Barcoding Kit (Oxford Nanopore Technologies; ONT) in a single step. Pooled libraries were sequenced on a MinION Mk1C and reads were classified using the EPI2ME 16S workflow (ONT). Statistical analysis was performed after obtaining TMM-normalized OTU matrix and run in phyloseq in R. Results 18 participants were included (6 IED, 6 H and 6 PC). On average, 40k reads/sample were obtained, of which 71.8% were taxonomically classified under strict parameters. 454 different species were identified in samples (identity >90%, ≥10 reads), while no reads were detected in negative controls. PCA revealed significant differentiation among groups (Fig. 1), associated with a lower bacterial diversity for IED cases (Shannon=2.9±0.3) compared to control groups (3.6±0.2). In IED cases, the genus Enterococcus was significantly more abundant (~20%) compared to controls (~1%), whereas it was not detected in the healthy gut microbiota controls. Conclusions Preliminary data shows faecal enrichment in E. faecalis/faecium linked to dysbiosis suggesting a gut microbiota signature for IED. A larger study is ongoing. If confirmed, this data would open the door to prophylactic interventions in these patients.	Rosa Benitez1,2, Verónica Saludes3,4,5, Núria Mauri3, Antoni E. Bordoy3, Marta González6, Laia Soler3, Alexia Paris de León3, Oriol Pérez3, Vadim Leonov3, Mª Dolores Quesada3, Anna Sales1, Miquel de Homdedeu3, Carmen Bracke1,2, Ana Peris1,2, Alba Romero1,2, Roger Paredes1,2, Mª Lluïsa Pedro-Botet1,2,4*, Pere-Joan Cardona3,4,7*.	1. Department of Infectious Diseases, Germans Trias i Pujol University Hospital (HUGTiP), Badalona, Spain. 2. Fundació Lluïta Contra les Infeccions, Badalona, Spain. 3. Microbiology Department, Laboratori Clínic Metropolitana Nord, HUGTiP, Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain. 4. Universitat Autonoma de Barcelona, Bellaterra, Spain. 5. CIBER in Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain. 6. Llefià Primary Care Center, Badalona, Spain. 7. CIBER in Respiratory Diseases (CIBERES), Instituto de Salud Carlos III, Madrid, Spain. *corresponding authors: mlpbotet.germanstrias@gencat.cat; pcardonai.germanstrias@gencat.cat

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Probiotic Effect on Gastrointestinal Symptoms and their Relation with Behavioral Changes in the ASD	Background: Autism spectrum disorder (ASD) is a behavioral syndrome that affects neurodevelopment and characterizes with alterations in communication and sociability, repetitive behavior, and resistance to change. There is an increase in the number cases of children diagnosed with autism, 1 case for each 88 children recently (1). Different phenotypes of ASD have been linked to gastrointestinal problems. The gut microbiome is central to the etiology of gastrointestinal diseases when it is out of balance (dysbiosis) (2). Some alternatives for treating gastrointestinal problems in these patients are the consumption of pre/pro and postbiotics, which improve gastrointestinal function and reduction of autistic symptoms (3,4). Methodology: 31 patients with ASD diagnosis, aged 5-10, were enrolled. The probiotic administered for 6 months was pediatric Lactipan®. The spectrum classification was made using the Childhood Autism Scale (CARS). Gastrointestinal symptoms were analyzed using the Rome IV Criteria questionnaires (https://theromefoundation.org/rome-iv/). Diet analysis was performed using Metabolic Pro software (Genetic Metabolic Dietitians International (GMDI), Hillsborough, Carolina del Norte, https://metabolicpro.org/index.php). Diagnosis of nutritional status was performed using the WHO Anthro Survey Analyzer software (https://www.who.int/tools/child-growth-standards/software). Additionally, bacterial abundance in faecal samples is in progress and will be determined by analysing the 16s rRNA marker gene sequences using next -generation sequencing. Results: 7 of 31 patients already finished treatment. Our results showed that after the intervention with probiotics for 6 months, gastrointestinal symptoms, such as pain and constipation, decreased. The CARS instrument allows us to assess behavioral changes linked to improvements in gastrointestinal symptoms before and after probiotic treatment. Improvement in gastrointestinal symptoms is related to changes in the visual response scales and verbal and non-verbal communication. Each patient served as their own control since they underwent an evaluation before the consumption of probiotics and at the end of the treatment. Rome IV gastrointestinal assessment and CARS results allowed us to observe changes before and after ingestion of probiotics. Results on faecal microbial diversity and abundance as well as functionality are in progress to complete this study. The patient nutritional status was reflected by their diet. Patients who had less diversity in their diet had macro and micronutrient deficiencies. In this study, no type of dietary intervention was performed. Conclusions: The consumption of probiotics for 6 months was shown to have positive effects on gastrointestinal symptoms and changes in autism spectrum severity. Future probiotic intervention research with a larger population is needed to help clarify the role of microbiome modulation in the microbiome-gut-brain axis over changes on severity in ASD.	DE SALES-MILLÁN, Amapola	Division of Biological and Health Sciences, Metropolitan Autonomous University-Lerma, State of Mexico 52006 Lerma, Mexico

Title	Text	Authors	Author Affiliations
Pre- and postnatal exposure to air pollution and green spaces influence infant's gut microbiota: results from the MAMI birth cohort study	Early childhood is a critical period for gut microbiota assembly, with long-lasting impacts on health programming. However, epidemiological studies focusing on the effects of air pollution and natural environments exposure in early childhood are still scarce. Here, we explored the effects of pre- and postnatal short- and long-term exposure to air pollution and green spaces on babies' gut microbiota composition during the first year of life. Using data from the MAMI birth cohort (València, Spain, N = 162), we estimated the levels of four air pollutants (NO2, O3, black carbon –BC–, and particulate matter with a diameter of 2.5µm or less –PM2.5–) and two greenspace indicators (NDVI and m2 of landscaped area, at 300, 500, and 1000m buffers) at the residential address for different pre- and postnatal periods. Infant gut microbiota profile was obtained by 16S rRNA gene sequencing of faecal samples collected longitudinally, at 7 days, and 1, 6 and 12 months of life. To study associations between exposures and diversity indicators, linear regression (cross-sectional analyses) and mixed models, including individuals as random effects (longitudinal analyses) were used. Differential abundance analyses were conducted at the genus level using the ANCOM-BC package with a log count transformation. Short-term exposure in the first week of life and long-term postnatal exposure to NO2 resulted in a reduced microbial alpha diversity, but the effects of green space exposure were not statistically significant. Short- and long-term (both pre- and postnatal) NO2 exposure were associated with increases in the relative abundance of taxa belonging to the Haemophilus, Raoultella, Eggerthella and Tyzzerella genera, while PM2.5 and BC were associated with Akkermansia and Alistipes, and Eggerthella and Haemophilus, respectively. Green spaces exposure was associated with increased Anaerococcus, and decreased Lachnoclostridium and Tyzzerella relative abundances. Decreased diversity and altered gut microbial composition were observed after air pollution exposure, and green space exposure was also related to specific shifts in the microbiome. Yet, further research is necessary, particularly focusing on paediatric population and with longitudinal design.	Pol Jimenez-Arenas a,b,c, Adrià Cruells a,b,c, Raúl Cabrera-Rubio d, Mariona Bustamante a,b,c, Dolors Pelegrí a,b,c, Marta Cirach a,b,c, Anna Samarra d, Cecilia Martínez-Costa e, María Carmen Collado d, Mireia Gascon a,b,c	a Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain b Universitat Pompeu Fabra (UPF), Barcelona, Spain c CIBER Epidemiología y Salud Pública (CIBERESP) d Institute of Agrochemistry and Food Technology-National Research Council (IATA-CSIC), Valencia, Spain e Department of Pediatrics, University of Valencia. INCLIVA Biomedical Research Institute, Valencia, Spain.

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Faecal metabolites as a readout of habitual diet that mediate dietary interactions with the gut microbiome.	Diet influences health, but the accurate assessment of intake is challenging. Food Frequency Questionnaires (FFQs) are commonly used but prone to bias. Metabolomics provides new avenues for dietary assessment, which can validate diet data with biomarkers of intake. Faecal metabolites are a readout of the gut microbiome, which can report on the diet-gut microbiome interplay. Here we explored the faecal metabolome as a novel approach for the assessment of habitual diet and gut microbiome interactions. We included 1,842 individuals from the TwinsUK cohort with faecal metabolites (n=522), gut microbiome composition from shotgun metagenomics, and dietary data from an adapted 131-item EPIC FFQ. Random forest models used faecal metabolites to predict adherence to dietary patterns defined by seven indices (aMED, DASH, PDI, hPDI, uPDI and total plant or meat percentage of diet) and intakes of 20 food groups. Linear mixed effects models were applied to identify biomarkers of consumption and dietary associated microbial species. Finally, we conducted mediation analysis for the metabolites and species that acted as mediators. Faecal metabolites accurately predicted healthy and unhealthy dietary patterns, achieving the highest AUC scores (>0.8) for the DASH, hPDI, and total meat % indices. Food groups were well predicted, specifically alcohol, meat, and tea & coffee (AUC>0.8). Intakes of 18 food groups were significantly associated with 222 metabolites, including 130 novel biomarkers uniquely associated with one group. Dietary-associated metabolites significantly correlated with microbial diversity and the abundance of 492 species, with some of these species mediating (or mediated by) the impact of diet on faecal metabolite levels. We demonstrate that the faecal metabolome is highly associated with diet and the gut microbiome, providing a precise approach to explore diet-gut microbiome interactions. Faecal metabolites accurately predicted adherence to dietary patterns relevant to health outcomes, and we identified faecal biomarkers of intake which can be used to validate self-reported diet data. We show species associated with metabolic disease acting as mediators in response to diet, such as Ruminococcus torques, Dorea sp. AF36 15AT and Anaerobutyricum hallii on faecal secondary bile acids in response to meat consumption. Our results enhance understanding of the gut microbiome's role in diet-related disease risk and provide a foundation for developing interventions.	Robert Pope 1, Xinyuan Zhang 1, Panayiotis Louca 1,2, Alessia Visconti 1,3, Francesco Asnicar 4, Kari Wong 5, Gregory Michelotti 5, Nicola Segata 4, Tim D. Spector 1,6, Emily R. Leeming 1, Rachel Gibson 7, Cristina Menni 1*, Mario Falchi 1*	1 Department of Twin Research & Genetic Epidemiology, King's College London, London, SE1 7EH, UK. 2 Department of Nutrition, University of Newcastle, Newcastle, UK. 3 Centre for Biostatistics, Epidemiology, and Public Health, Department of Clinical and Biological Sciences, University of Turin, Turin, Italy. 4 Department CIBIO, University of Trento, Trento, Italy. 5 Metabolon, Research Triangle Park, Morrisville, NC, USA. 6 Zoe Limited, London, UK. 7 Department of Nutritional Sciences, King's College London, London, UK. * Equal contribution

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Title	Text	Authors	Author Affiliations
Disease-specific bacterial signatures are associated to general population health status	There is growing evidence for the interaction between the gut microbiome composition and human health and disease. Two principal hypotheses inspire this research line: first, a certain degree of dysbiosis might already be present before the manifestation of disease; second, diseases might share altered physiological processes associated to similar profiles of gut microbiome. To test these hypotheses, we characterized the distribution of disease-specific bacterial signatures in the general population and investigated whether they are associated with general health. The characterization of diseases from curatedMetagenomicData data using coda4microbiome algorithm (Calle et al., 2023, BMC Bioinformatics), an innovative Compositional Data Analysis method, allowed to identify a total of 16 disease-specific bacterial signatures (i.e., combination of bacteria that best predicts a given outcome). These microbial signatures, which represent different disease-specific gut microbial dysbiosis, were replicated in a general population cohort, the LifeLines-DMP cohort (Gacesa et al., 2022, Nature), and tested for association with general health status. We found similar association trends between all signatures and health status: higher microbial disease scores were associated with poorer health overall. Basal characteristics (e.g., age, sex and BMI) predicted well the healthy phenotype, although the addition of the microbiome signature improved the model performance significantly. Some of the signatures also showed significant association with health factors and specific diseases. These findings indicate that some disease-specific bacterial signatures are informative of the health status and well-being. The identification of these different gut microbial dysbiosis also in the general population supports our initial hypothesis that certain degree of dysbiosis might already be present before the manifestation of illnesses. This study is a good start point for the development of tools focused on assessing gut microbial health and promoting preventive health care.	Meritxell Pujolassos Tanyà (1), Alexander Kurilshikov (3), Sasha Zhernakova (3), M.Luz Calle Rosingana (1,2)	(1) Bioscience Department, Faculty of Sciences, Technology and Engineering, University of Vic – Central University of Catalunya, Vic, 08500, Spain (2) Institut de Recerca i Innovació en Ciències de la Vida i de la Salut a la Catalunya Central (IRIS-CC), Vic, 08500, Spain (3) Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ, The Netherlands

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Multi-omics microbiome dynamics in IBD	Inflammatory Bowel Disease (IBD) is the term used to describe two of the most common chronic inflammatory diseases of the gastrointestinal (GI) tract: Crohn's Disease (CD) and Ulcerative Colitis (UC). Both IBD subtypes are characterized by the alternation of periods of clinical remission and relapse. Although the factors that trigger CD and UC development are still unknown, several studies demonstrated that gut microbiota alterations are associated with IBD, with an increase of Proteobacteria and depletion of Firmicutes in CD-affected individuals and a decrease of butyrate-producing bacteria in UC. However, environmental factors such as diet, smoking habits, antibiotic usage, stress or sleeping schedule have also been linked to the development of IBD. It is believed that the increase of pathogenic bacteria in the GI tract alters gut permeability, causing a disbalance in the microbial community known as dysbiosis, and an alteration of the metabolite composition in the GI tract, which ultimately leads to gut inflammation. To address this knowledge gap, we analyzed a total of 421 unique measurements generated by metagenomics, metatranscriptomics or metabolomics techniques from 67 IBD patients and 67 healthy controls. Our results revealed novel microbial signature species in CD, as well as shifts on the expression of key pathways in the gut microbial ecosystem and the alteration of the concentration of several metabolites in the GI tract. Finally, integrative analysis gave insight into the interaction of these factors contributing to dysbiosis in CD.	Gerard Serrano-Gómez, Zaida Soler, Marc Pons, Franzisca Yañez, Luis Mayorga, Chaysavanh Manichanh	1. Gut Microbiome Group, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain 2. MMedicine Department, Autonomous University of Barcelona (UAB), 08193 Cerdanyola del Vallès, Spain 3. Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Spain.

Title	Text	Authors	Author Affiliations
Maternal breastmilk selectively seeds the infant gut microbiota and preterm birth impacts the transfer of breastmilk microbes to the infant gut	Background: Breastmilk contains diverse microbiota that have been shown to vary from early to late lactation and by gestational age, and delivery mode. Currently, there is a lack of studies that have examined the breast milk microbiota from mothers with preterm birth (PTB). This research aims to offer essential insights into the initial colonization and development of the human microbiota and study the impact of pregnancy-related complications. Methodology: Longitudinal breastmilk(colostrum, transitional and mature) and infant fecal(meconium, 1/2 month, 2/3 month, 3/4 and 5/6 months) samples were collected mother-infant dyads from the Molecular signatures in pregnancy(MSP) cohort. The cohort was classified into Preterm (n=18, gestational age<37 weeks), Term(n=30) deliveries. Microbial composition was analyzed by sequencing the 16S rRNA gene. Downstream analysis was carried out using QIIME pipeline and R software. Results: Significant differences were noted in the overall composition of controls BM samples compared to that of the preterm (p-value <0.001). Streptococcus, Veillonella, and Bifidobacterium were among the most abundant genera common to both the breast milk and infant gut sample. Interestingly, while Streptococcus and Bifidobacterium seemed to transfer from breastmilk to infant gut in a consistent and proportional manner across all time points, the abundance of Veillonella increases progressively in both the Breastmilk and infant gut. We also found that the transmission rate of microbiota is dependent on the lactation stage as well as gestational age. Colostrum breastmilk samples demonstrated the highest sharing with infant stool sample collected at birth, whereas the transitional and the mature milk samples contributed to a higher degree to the later time points(1/2 month, 2/3 month, 3/4 and 5/6 months respectively). The breastmilk samples from the preterm mother demonstrated greater sharing of common gut commensal (such as Enterococcus, Ruminococcus, Feacalibacterium, Bacteroides, and Dialister) with their infants as compared term. Conclusion: Breastmilk seeds the infant gut microbiota in a lactation stage-dependent manner and preterm mothers, on average, shared a greater proportion of their breastmilk microbiota with their infants and demonstrate highly specific microbial transfer likely tailored to meet the unique needs of preterm infants who face unique challenges at birth.	Parul Singh a,b*, Nadhir Djekidel b, Selvasankar Murugesan b, Noora Almohannadi b, Fajr Almarzooqi b, Basirudeen Syed Ahamed Kabeer b , Annalisa Terranegrab, Arun Prasath Laxmananb, Shaikha Al Abduljabbarb, Sidra Aftabb, Alexandra Katharina Marrb, Tomoshige Kinob, Tobias Brummaierc,,d,e, Rose McGreadyc,d, François Nostenc,d, Damien Chaussabelg, Souhaila Al Khodorb#	a. College of Health & Life Sciences, Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), 24404, Doha, Qatar b. Research Department, Sidra Medicine, Doha, Qatar c. Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand d. Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom e. Swiss Tropical and Public Health Institute, Basel, Switzerland f. University of Basel, Basel, Switzerland g. The Jackson Laboratory for Genomic Medicine, Farmington CT, USA *. Presenting author #. Corresponding autor

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Differences in microbiome diversity between self- and physician-collected HPV positive and negative cervicovaginal samples.	Introduction: Growing evidence suggests that the cervicovaginal microbiota could play a significant role in the HPV life cycle. Cervical cancer screening samples could be tested to determine the microbiome composition and help identify women with an increased risk of developing high-grade lesions or cancer. We aim to analyze how two validated sampling methods may affect the microbiome composition, comparing liquid-based cytology (LBC) physician-collected samples and Evalyn brush self-collected specimens (SCS). Methods: We analyzed the microbiome composition of 22 women using leftover samples from cervical cancer screening. For each woman, two samples were collected using two methods: LBC and SCS (n = 44). First, DNA was isolated and tested for HPV using COBAS. Then, the remaining DNA was amplified with 16S rRNA primers and barcoded for further sequencing. The amplicons were sequenced in 12-plex groups using a MinION device (Oxford Nanopore Technologies). Samples with fewer than 10,000 reads, a Q-score lower than 16, and non-paired samples were discarded. Finally, taxonomy was assigned to 8 HPV+ and 6 HPV- samples (n = 28) at the genus level using Kraken2 software. Results: HPV-associated microbiome features are detectable using both LBC and SCS methods. Alpha diversity analysis indicated comparable diversity regardless of how sample was collected, however it showed a higher diversity in HPV positive samples (Figure 1). Notably, beta diversity analysis revealed closer grouping of paired samples, suggesting sampling method insignificance in microbiome composition (Figure 2). We observed a high abundance of Lactobacillus genus in cervicovaginal samples, consistent with previous studies. However, we also noted an increase in the relative abundance of several genera in HPV-positive samples compared to HPV-negative samples. These genera included Atopobium, Parvimonas, Mageeibacillus, Sneathia, Megasphaera, Peptoniphilus, and Dialister. Conclusions: The results indicate that the self-sampling method is as effective as samples collected by professionals, as the microbiome composition remains consistent within each patient. The methodology used in this study enables the rapid determination of a woman's microbiome using the residual volume of both LBC physician-collected and Evalyn brush self-collected specimens, all within 24 hours.	Laura Asensio-Puig1,2*, Álvaro de Andres-Pablo1,2*, Olfat Khannous-Lleiffe3,4, Raquel Ibañez 1,7, Amelia Acera8, Silvia de Sanjosé7,9, Toni Gabaldón3,4,5,6, Laia Alemany,1,7 Laia Bruni1,7, Miquel Àngel Pavón1,7	1. Cancer Epidemiology Research Programme, Catalan Institute of Oncology, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain. 2. Programa de Doctorat en Biomedicina, Universitat de Barcelona (UB), Barcelona, Spain. 3. Barcelona Supercomputing Center (BSC-CNS), Barcelona, Spain.

Title	Text	Authors	Author Affiliations
Enhancing Robustness in Differential Abundance Testing for Microbiome Data Analysis through Consensus-Based Approach	Introduction: The task of Differential Abundance (DA) testing in microbiome data poses significant challenges for both parametric and non-parametric statistical methods due to the data's sparsity, high variability, and compositional nature. Microbiome-specific statistical methods often resort to classical distribution models or consider compositional specifics. However, these approaches yield results that fluctuate within the specificity versus sensitivity space, making it difficult to accurately determine type I and type II errors in real microbiome data when a single method is employed. Results: To enhance the robustness and reproducibility of DA testing in microbiome data, we have developed the 'dar' R package, available at GitHub. The 'dar' package facilitates automatic statistical testing under various data distribution assumptions and theoretical frameworks in a fully customizable manner, using state-of-the-art methods such as ANCOM, DESeq2, and Lefse. The 'dar' package can generate consensus results under a majority-vote mechanism, which can be tailored by the user by assigning weights to each method in the consensus. Additionally, the package provides the functionality to export and import the parameters for each test and the characteristics of the consensus strategy into structured text files, enhancing the shareability and reproducibility of results. The 'dar' package was evaluated on the metaHIV dataset using all available methods with default parameters, showing a total of 24 differentially abundant microbial features. Notably, 'dar' successfully reproduced results validated by the original article by detecting species of the Bacteroides and Prevotella genus, which play a crucial role in the stratification of men who have sex with men (MSM) and non-MSM. Conclusion: The 'dar' package, designed for differential abundance testing in microbiome data, enhances the robustness and reproducibility of results by facilitating automatic statistical testing under various distribution assumptions and theoretical frameworks. It employs state-of-the-art methods and can generate consensus results through a user-customizable majority-vote mechanism.	Francesc Català-Moll, Judit Farré-Badia, Marc Noguera-Julian and Roger Paredes	IrsiCaixa AIDS Research Institute, Hospital Universitari Germans Trias i Pujol, Campus Can Ruti, Badalona, Spain

Title	Text	Authors	Author Affiliations
Taxonomic classification of metagenome-assembled genomes with kMetaShot	The human microbiota represents the ensemble of the microbial community colonizing specific body niches such as skin, gut, vagina or airways which includes Prokaryotes, Fungi, Protista and Viruses. Microbiota is essential for supplying metabolic pathways missing in humans, training the immune system and overall plays an essential role in host health (Lazar et al. 2018). The investigation about crosstalk among host and its microbiota is critical to unveil its functional role. Metagenomics is essential in microbiota profiling both at level of taxonomic composition and for assessing its genetic potential reconstructing the component genomes as Metagenome-Assembled Genomes (MAG) (Quince et al. 2017). To this aim, we developed kMetaShot, an accurate tool for prokaryotic MAGs taxonomic classification. kMetaShot relies on kmer/minimizer counting: its reference is obtained by counting minimizers from RefSeq Bacterial and Archaea RNAs and storing relevant minimizers. The minimizers are considered as relevant when are exclusively shared among strains of the same genus and not redundant among genera. Reference minimizers are stored in a dedicated reference matrix. The classification module processes MAGs, performs the minimizer counting and queries the reference matrix to achieve the taxonomic classification relying on a prevalence-based approach. kMetaShot has been benchmarked against two of the most used comparable tools, i.e. GTDBtk (Chaumeil et al. 2020) and CAMITAX (Bremges, Fritz, and McHardy 2020) by classifying MAGs obtained by 20 in silico generated samples of the CAMI2 (Meyer et al. 2022) contest (i.e. simulated communities for human Gastro-Intestinal and Airways niches). The results show kMetaShot outperformed both GTDBtk and CAMITAX in term of overall precision (100%, 85%, 72%, respectively) and sensitivity (85.2%, 76.4%, 71.4%, respectively) at species-genus levels. Differently from other tools, kMetaShot is also able to perform classification at strain level with a Balanced-Accuracy at 81.3%. Notably, kMetaShot is also the fastest tool, being the most convenient in term of CPU and RAM consumption and with the smallest reference object (i.e. about 17Gb). kMetaShot is to our knowledge the most user-friendly, computationally effective and accurate tool for MAGs taxonomic classification up to the strain level. It is released as Conda package and Docker container and is available and documented at https://github.com/gdefazio/kMetaShot.	Giuseppe Defazio (1), Marco Antonio Tangaro (2), Graziano Pesole (1,2,3), Bruno Fosso (1)	(1) Department of Biosciences, Biotechnology and Environment, University of Bari Aldo Moro, 70126 Bari, Italy (2) Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, Consiglio Nazionale delle Ricerche, 70126 Bari, Italy, (3) Consorzio Interuniversitario Biotecnologie, 34148, Trieste, Italy.

Title	Text	Authors	Author Affiliations
Harnessing Microbiome Big Data with MADAME: a User-friendly Tool for Sequencing Data and Metadata Retrieval	Microbiomes' multi-layered nature has prompted research in this field to extend the analysis of the single studies further, leading to the urge for multiple datasets' integration. Aggregated datasets have emerged as pivotal for statistical power increase and overcoming the lack of methodological standardization, revealing both generalizable trends' and specific shifts' detection. However, retrieving and downloading the required resources from public repositories proved arduous for the research community, resulting in the current bottleneck for microbiome data reuse and the consequent potential loss of valuable information held within. To tackle the challenge of addressing this significant barrier to microbiome progress, we developed MADAME (MetADAta MicrobiomE). This open-source bioinformatic tool streamlines data and metadata retrieval and download from the European Nucleotide Archive (ENA). In the efforts to extend data and metadata access, including to those researchers without specific bioinformatic skills, MADAME's user-friendly interface guides users through its four modules. To offer users a rapid metadata overview in addition to data and metadata download, MADAME allows users to retrieve the associated publications and assess the suitability of downloaded metadata by generating comprehensive reports, including plots and statistics. To showcase MADAME's functionality, we conducted a test case by formulating a text query concerning the human skin microbiome ("((((((human skin) AND face) OR cheek) OR forehead) OR glabella) OR eyelid) AND microbiome"). MADAME efficiently retrieved 21 amplicon-based and shotgun sequencing projects comprising 8,501 samples and a cumulative data size of 263 GB. Analysis of related publications and the generated report revealed notable heterogeneity among the retrieved samples. Reflecting the lack of metadata standardization in public repositories, our samples required filtering before downloading to exclude unsuitable data. Our results emphasize the crucial need for an easily accessible bioinformatic tool that provides comprehensive control to evaluate metadata suitability before data retrieval, ultimately conserving time and resources, and promoting efficient data reuse. Aligned with FAIR principles (Findability, Accessibility, Interoperability, and Reusability), MADAME strives to enable researchers to fully leverage existing resources, thereby aiming to contribute to the advancements of microbiome research.	Sara Fumagalli 1, Giulia Soletta 1, Giulia Agostinetto 1, Manuel Striani 2, Massimo Labra 1, Mireia Valles-Colomer 3, Maurizio Casiraghi 1, Antonia Bruno 1	1 Biotechnology and Biosciences Department, University of Milano-Bicocca, Milan, Italy 2 Computer Science Institute DISIT, University of Piemonte Orientale, Alessandria, Italy 3 Medicine and Life Sciences Department, University Pompeu Fabra, Barcelona, Spain

DIAGNOSTICS / METHODS

Title	Text	Authors	Author Affiliations
Metabolic modulation of Bacteroides fragilis toxicity in colorectal cancer	Background: Bacteroides fragilis is a ubiquitous commensal of human gut microbiota, which, also behaves as an opportunistic pathogen, by producing a proteolytic enterotoxin (BFT). Clinical evidence has already demonstrated the driving role of B. fragilis enterotoxigenic strain (ETBF) in colorectal cancer (CRC). However, the exact mechanism behind ETBF overwhelming over a non-toxicogenic strain (NTBF) in this context is still unknown. We thus hypothesize that a specific metabolic environment in the tumor tissue from CRC patients is influencing B. fragilis toxicity and metabolism. Methods A global targeted metabolomic approach was adopted to characterize the metabolic landscape in the mucosal scraps of tumor tissue in a cohort of CRC patients. Anaerobic culturing methods were adopted to grow NTBF and ETBF strains in multiple conditions. A targeted metabolomics approach was used to quantify the polyamine content in bacterial biomass and secretomes. We performed an RNAseq analysis on bacterial pellets to study the bacterial transcriptome. The functional validation assays were performed on a gut-on-a-chip model. Results: A metabolomics approach to a CRC human cohort identified the polyamines pathway as one of the main metabolic signatures of tumor tissue. The analysis of both the growth and metabolic status of NTBF versus ETBF strains in the presence of different concentrations of polyamines and precursors demonstrated a clear dose-dependent effect on B. fragilis toxicity. RNAseq analysis on bacteria grown in supplemented media with spermidine or arginine showed the activation of pathways related to oxidative stress resistance and response regulatory systems. In parallel, quantitative expression of genes related to ROS formation and scavenging revealed a major H2O2 scavenging activity of the ETBF grown in higher spermidine concentrations, as well as higher ROS formation as compared to NTBF. The effect of the bacterial secretomes was also tested in a gut-on-a-chip model, where the ETBF strains disrupted the epithelial integrity and caused the activation of genes related to the endoplasmic reticulum (ER) stress. Conclusions: In this work, we propose a potential mechanism behind the predominance of ETBF over NTBF in the metabolic context of CRC, characterized by the adaptive growth selection of ETBF strains in a high-spermidine tumoral microenvironment, where this pathobiont takes advantage of polyamines to develop a selective pressure toward the NTBF stra	Serena Galiè	1Department of Experimental Oncology, IRCCS European Institute of Oncology, Milano, Italy 2Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France 3MiMic Lab, Dipartimento di Elettronica Informazione e Bioingegneria,Politecnico di Milano

Title	Text	Authors	Author Affiliations
Mapping the neuroactive potential of over a million microbial genomes with GBM2	Metabolites produced by gut bacteria impact neurotransmission, immune response, and even behavior through the gut-brain axis. Characterizing this metabolism is crucial for understanding its impact on mental health. However, in the rapidly evolving microbiome field, current methods display limited performance in comprehensively characterizing the neuroactive potential of gut microbial genomes. We introduce GBM2 (Gut-Brain Modules 2), a computational pipeline including over 200 curated metabolic pathways of well-known neuroactive and immunomodulatory metabolites like short-chain fatty acids (SCFAs), and derivatives from tryptophan, tyrosine, arginine, and glutamate metabolism, as well as emerging compounds such as those from vitamin and bile acid metabolism. Besides the number of pathways, GBM2 incorporates several key innovations as compared to its previous version [1], including Enzyme Commission (EC) and Uniref-based Annotations that allow it to account for enzymatic redundancies within microbial communities, overcoming limitations of proprietary systems like KEGG Orthology. After extensive validation using experimental data on known metabolite-producing strains to ensure prediction accuracy, we applied GBM2 to a comprehensive microbial genome (MG) database[2]. We mapped the distribution of neuroactive potential metabolism in the pan-genomes of 116.913 microbial species. As a case in point, for the neurotransmitter GABA, its synthesis from glutamate (GABA shunt pathway) is the most prevalent pathway (9.3% MGs, N=105.092), being the only one found in Bacteroidota. Other less prevalent pathways are GABA synthesis from arginine (1.6% MGs, N=19016) directly; through putrescine (0.4%, N=4897), and from nicotine (9e-3% MGs, N=108). We also observe co-exclusion among GABA synthesis pathways, found in 13 of the 47 phyla examined, even in some Archaea (8,1%, N=97). Among these, Actinobacteria is the phyla with the highest overall prevalence (55.6%, N=7015) followed by Bacteroidota (22.4%, N=1468). GBM2 provides a more comprehensive assessment of the gut-brain axis by considering enzymatic redundancies, utilizing open-source databases, and accounting for moonlighting activities. This work paves the way for identifying microbiome-based therapeutic strategies for neurological disorders. 1 Valles-Colomer, M. et al. Nat Microbiol 4, 623–632 (2019) 2 Blanco-Míguez, A. et al. Nat. Biotechnol. 41, 1633–1644 (2023)	Albert Garcia-Valiente1,4, Gwen Falony2, Sara Vieira-Silva2,3, Nicola Segata4, Mireia Valles-Colomer1,4	1. MELIS Department, Pompeu Fabra University, Barcelona, Spain 2. Institute of Medical Microbiology and Hygiene and Research Center for Immunotherapy (FZI), University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany 3. Institute of Molecular Biology (IMB), Mainz, Germany 4. Department CIBIO, University of Trento, Trento, Italy

Title	Text	Authors	Author Affiliations
Applying single-cell omics technologies to unravel the impact of E. coli on Crohn's disease immunopathology	Crohn's disease (CD) is a chronic and relapsing inflammatory condition of the gastrointestinal tract characterized by exacerbated immune responses to the gut microbiota in genetically predisposed individuals. Although a causative microbe has not been identified, Escherichia coli, particularly the adherent-invasive E. coli (AIEC) pathotype, has been proposed to contribute to CD pathogenesis. Yet, it remains unclear whether the presence of E. coli plays a role in disease heterogeneity by triggering distinct adaptive and innate immune responses in the gut mucosa. It is also unknown how the presence of specific microbiome profiles in CD associates with a unique mucosal antibody repertoire. To address these important questions, we initially analyzed the composition of intestinal mucus-embedded microbiota through 16S rRNA sequencing in both non-IBD controls and CD patients. Our preliminary findings confirmed a reduction in mucosal bacterial alpha diversity in CD, with a notable decrease in several beneficial bacterial species, such as F. prausnitzii, alongside a significant increase in mucus-embedded E. coli among a subset of CD patients. Building upon these results, we selected an exploratory cohort comprising non-IBD controls, and CD patients with or without the expanded E. coli population for single-cell RNA and V(D)J sequencing (n=10) to map the gut mucosal immune responses. Our analysis revealed a differential proportion of mucosal immune cell subsets and distinct cellular modules driving inflammation in patients with expanded E. coli. Moreover, these patients display an increased frequency of IgG-expressing plasma cells and extensive B cell clonal expansion. Altogether, our preliminary findings suggest that the presence of E. coli might be promoting a unique inflammatory signature in CD and could potentially trigger mucosal humoral immunity.	Núria Gumà-Vique1,4; Leticia Suárez-García1; Sonia Tejedor Vaquero1; Pau Berenguer-Molins1; Christian Romero1; Julia Perera-Bel1; Mar Iglesias2; Andrea Cerutti1; Lucía Márquez-Mosquera3; Giuliana Magri4	1Hospital del Mar Research Institute (IMIM), Barcelona, Spain; 2Pathology Department, Hospital del Mar, Barcelona, Spain; 3Department of Gastroenterology, Hospital del Mar, Barcelona, Spain; 4Immunology Unit, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain

Title	Text	Authors	Author Affiliations
Assessment of different microbiome profiling approaches for host genetic effects analyses	Knowing how host genetics affect the gut microbiome composition is crucial for interventions in the gut microbial community for precision medicine purposes. However, to this date very few variants have been robustly associated with microbiome profiles. We hypothesize this happens for two reasons: firstly, because most studies have focused on taxonomy rather than function, even though we know that microbial functions can be redundant between species; secondly, because most studies have relied on 16S data to characterise the microbiome, which lacks species resolution among other limitations that affect precision. Thus, we compared the aggregate host genetic effects (i.e., heritability) on the gut microbiome using taxonomic profiles built with shallow shotgun (SS) and 16S sequencing data from the same caecal samples of outbred laboratory rats. Then, we also used SS data to run the same analyses on gene-based functional profiles and guild profiles defined by species co-abundances. As a result, SS not only revealed more taxa with significant heritability than 16S (FDR<10% and Bonferroni<5%) but also higher heritability values for the set of taxa mapped by both methods. Besides, even though the gene-centric functional approach did not reveal functions with significant heritability values, when species were grouped in co-abundance guilds the heritability values were improved, which makes this a good taxonomy-free approach to study host genetic effects on the gut microbiome, possibly revealing shared functional aspects that are important for the interaction with the host.	Felipe M S Dias	Centre for Genomic Regulation (CRG)

Title	Text	Authors	Author Affiliations
Unveiling allo-coprophagy from metagenomics data of laboratory rats	Commensal microorganisms can be transferred between humans who come in contact and/or who share a living space. The transmission of these commensal microorganisms could have important health consequences. It is therefore important to understand the processes that facilitate microbiome transfer. Allo-coprophagy in rodents can be used as a model to study the transmission of commensal microorganisms. Allo-coprophagy is difficult to detect via direct observation and/or through video recording. This study aimed to detect and quantify allo-coprophagy from deep shotgun data in outbred laboratory rats, which is the first step towards understanding the transfer of microorganisms that occurs through allocoprophagy. The study relied on the hypothesis that the cells and DNA present in a rat faeces, which originate from gut epithelial cell shedding, get ingested during allo-coprophagy. As a result, both their own DNA and that of the cage mate can be found in the gut of a rat that ingested the faeces and detected by sequencing. In this study, we extracted DNA from the gut of 2540 "heterogeneous stock" (HS) rats housed in pairs and groups of 3 using deep shotgun sequencing and utilized only the reads that aligned to the rat reference genome. Pre-existing statistical methodologies [1] were employed to detect and quantify instances of DNA mixtures. An additional statistical analysis was conducted to ascertain if the observed mixture was a result of contamination and/or allo-coprophagy. This study highlights the potential of using deep shotgun sequencing of the gut content to quantify a behaviour that is important for microbial transfer between individuals. This provided valuable information in understanding the process of microbial transfer.	Kauthar Omar1,2, Amelie Baud1.	1. Centre for Genomic Regulation (CRG) 2. Universitat Pompeu Fabra (UPF)

Title	Text	Authors	Author Affiliations
Synthetic biology applications for Cutibacterium acnes: advances in skin microbiome engineering	The skin microbiota plays a crucial role in maintaining skin homeostasis and regulating the inflammatory response in chronic skin disorders. Cutibacterium acnes (C. acnes), a bacterial species that inhabits the hair follicle, is one of the most abundant bacteria that make up the human skin microbiota, and is becoming a target of interest for the development of novel and promising therapies. Synthetic biology has the potential to generate new smart therapies through microbiome engineering. This discipline allows significant genetic modification of bacterial agents of interest, making it a promising strategy for treating dermatological disorders. Here we present novel tools for the engineering of C. acnes toward a therapeutic use. Specifically, we devised a CRISPR-based approach that allows us to introduce small (5 bp) edits in the C. acnes genome without any selection marker, which paves the way for the future development of strains that comply with regulatory requirements. We also devised an intercellular signalling system that allows for E. coli to C. acnes communication, a first step toward engineering inter-microbiome communication in the gut-skin axis. These results highlight the potential of synthetic biology for designing novel interventions to improve dermatological health and offer new perspectives for therapeutic development through genetic modification of C. acnes from a synthetic perspective.	Cristóbal Parra-Cid, Javier Santos Moreno, Marc Güell	Department of Medicine and Life sciences, University Pompeu Fabra, Barcelona, Spain

Title	Text	Authors	Author Affiliations
The Hologenome 2.0	Genetics is traditionally understood as the study of how an individual's phenotype is affected by its own genotype. However, in recent years it has become clear that the genotypes of the individual's social partners (relatives and peers) and the genetic makeup of the individual's microbiota are also important. Furthermore, commensal microbes can be exchanged between socially interacting conspecifics. Therefore, we believe the individual should be studied not in isolation but, instead, together with its social partners and microbiota, which we refer to as the "Hologenome 2.0". I will discuss the expected evolutionary consequences of the Hologenome 2.0 and present the empirical strategies we are developing to study it, using genetically diverse (outbred) and deeply phenotyped laboratory rodents as study system. We have already uncovered widespread and, in some cases, strong genetic effects arising from social partners (cage mates), and pinpointed specific genes involved. We are now developing an approach to gain insights into the traits of conspecifics that are most influential, leveraging the phenome-wide data available in our rodent cohorts to do so. We have also studied host/microbiome interactions in thousands of outbred rats, and have started developing methods to quantify and characterise microbial transmission between cage mates.	Amelie Baud, Felipe Morillo, Kauthar Omar, the NIDA P50 Center for GWAS in Outbred Rats, Rob Knight, Abraham Palmer	Centre for Genomic Regulation, Barcelona, Spain Center for Microbiome Innovation, San Diego, CA, USA University of California San Diego, CA, USA

Title	Text	Authors	Author Affiliations
Genetic Control of Human Gut Commensals: A Gateway to Microbiome Intervention Specificity	A longstanding goal in microbiome research has been to functionally understand the role of human gut commensals in health and disease, aiming to intervene by either promoting the colonization of beneficial members or specifically removing undesired gut residents. However, a significant challenge persists due to the limited biological understanding of most human gut residents beyond their genome sequences. A prominent example is Segatella copri (former Prevotella copri), a prevalent and abundant member of the human gut microbiota associated with both health and disease traits. Despite increasing interest in the biology of S. copri, there remains a significant gap in understanding the fundamental gene expression regulation and physiological responses crucial for effective gut colonization, which hinders informed intervention efforts. Here, by mapping the primary transcriptome of S. copri and examining gene expression in human-derived samples, we uncovered an essential regulator of gut colonization: the small regulatory RNA (sRNA) named SrcF. Identified as the most abundant sRNA in the human gut, deletion of SrcF rendered S. copri unable to colonize the gut in a mouse model. We demonstrate that human microbiome composition and breakdown of dietary components by cohabitating commensals impact SrcF expression, suggesting that complex carbohydrate breakdown not only serves as an energy source but also mediates inter-species signaling among commensals to regulate colonization. Importantly, our results highlight the relevance of non-coding sRNAs in the biology of a prevalent gut commensal and underscore the necessity to extend beyond the bacterial coding genome to fully capture commensal interactions in the human gut microbiome. This study emphasizes non-coding intergenic sRNAs as key regulators of gut colonization and ideal targets for specific microbiome intervention.	Youssef El Mouali1, Caroline Tawk1, Kun D. Huang1, Lena Amend1, Till Robin Lesker1, Falk Ponath2, Jörg Vogel2,3 and Till Strowig1	1Department of Microbial Immune Regulation, Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany 2Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany 3Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, Germany.

Title	Text	Authors	Author Affiliations
TREX2 influence in the skin and gut microbiome	In contrast to the influence of gut microbiome on health, the importance of the skin microbiome in some inflammatory skin diseases such as Atopic Dermatitis and Psoriasis have been discovered only in the recent years. Here, we aim to unravel the relation between the skin microbiome and TREX2, , a exonuclease that shows restricted expression in keratinocytes, where it facilitates nuclear DNA degradation in stressed keratinocytes, promoting cell death and inflammation (Manils et al., 2016, 2017, 2022). Here, using inbred Trex2 deficient mice, we sampled skin, tongue and stool. Using 16S rRNA sequencing, we have shown how the absence of TREX2 alone affect the alpha diversity, the beta diversity and the species differential abundances in the skin and gut. Although further research is needed, this is the only study to our knowledge to explore the influence of a DNA exonucleases on the skin microbiome. Studies under pathological skin conditions are the next step to gather more information on the nature and impact of this influence.	Alejandra Gadea1,$, Serena G Piticchio1,2,$, Ingrid Filgaira1, Daniel López-Ramajo1, Maria Martin1, Sonia-Vanina Forcales1, Juan José Rojas1, Joan Manils1, Corinna Bang2, Philipp Rausch2 and Concepció Soler1	Immunology Unit, Department of Pathology and Experimental Therapy, School of Medicine, Universitat de Barcelona – UB; Immunity, inflammation and Cancer Group, Oncobell Program, Institut d'Investigació Biomèdica de Bellvitge – IDIBELL, L'Hospitalet de Llobregat, Spain. 2Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany.

Title	Text	Authors	Author Affiliations
Multiomics Profiling for a Comprehensive Understanding of Immune and Microbial Signatures in Pediatric Inflammatory Bowel Disease	Background: Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder with a complex pathophysiology. Emerging studies suggest that an abnormal immune response to environmental factors disrupts intestinal homeostasis, leading to inflammation. To identify predictive biomarkers of relapse, we developed a strategy integrating blood immune responses to innate stimuli at the transcriptional level with microbial profiling during disease flare, remission in children with IBD and compare those profiles to healthy controls. Methodology: In this prospective longitudinal study, we implemented a serial collection of samples including blood, saliva, and stool samples from IBD patients over a least five visits to Sidra Medicine IBD clinic. Samples were collected during disease flare and remission. Blood samples were processed using a multidimensional stimulation assay and immune signatures were assessed both at the transcriptome and proteome levels. In parallel, salivary and stool samples were analyzed for microbiome composition and diversity. Matching demographic, clinical, and phenotypic data were collected from both patients and controls. Different omics were then integrated to identify potential biomarkers for disease progression and response to treatment. Results: Our study identified unique gene expression and microbial signatures for each IBD subtype, with clear distinctions during disease flare compared to remission and healthy controls. Proteomics and immune transcriptome data showed specific correlations for symptomatic episodes, relapse, and remission. This let to creation of a machine learning (ML) classifier to predict markers for each disease stage. Conclusion: Taken together, our results have underscored the presence of distinct immune patterns and dysbiotic microbiota during disease relapse in contrast to remission. We believe that this comprehensive understanding of the dysfunctional immune system's role in IBD pathogenesis has the potential to reveal novel mechanisms and, ultimately, facilitate the discovery of innovative therapeutic targets. Our findings, coupled with the ML classifier, have the potential to complement existing treatments for individuals affected by IBD.	Manoj Kumar1^, Mohamed Nadhir Djekidel1^, Marwa Saadaoui1#, Duaa Ahmed Elhag1#, Mohamed H. Rahal2, Nazira Ibrahim3, Khaled Abouhazima3, Fatma Al Mudhaka3, Anthony Akobeng3, Mamoun Elawad3* and Souhaila Al Khodor1*	1Research Branch, Sidra Medicine, Doha, Qatar. 2 Department of Pediatrics, Sidra Medicine, Doha, Qatar. 3 Department of Pediatric Gastroenterology, Hepatology and Nutrition, Sidra Medicine, Doha, Qatar.

Title	Text	Authors	Author Affiliations
Newly described microbiome diversity in the Indri indri microbiome reveals extensive transmission within social groups	Background: The indri (Indri indri) is a lemur endemic to Madagascar rainforests, which feeds on a folivorous diet and frequently engages in soil-eating behaviours (geophagy). It is classified as “critically endangered” by the IUCN red list and can only survive in the wild. Characterising the microbial communities in the indri's gut could enhance our understanding of their health determinants, dietary habits, and behaviour, and potentially guide conservation efforts (Correa et al., Frontiers in Microbiology., 2021). Methods: We assessed gut and soil microbiome composition by shotgun metagenomic profiling of Indri faecal samples from 5 different social groups (N=18 indris), geophagic and non-geophagic soil (N=4, N=7). We performed metagenomic assembly to expand reference databases, and then examined taxonomic (MetaPhlAn) and functional (HUMAnN) microbiome composition and social transmission patterns by strain-level profiling (StrainPhlAn) (Valles-Colomer et al., Nature, 2023). Results: The great majority of the microbial diversity in the Indri indri microbiome has not been cultivated to date (0.007% relative abundance of cultivated species). Through metagenome assembly, we successfully reconstructed 256 high-quality MAGs (metagenome assembled genomes), belonging to 41 species, which after integrating them on an updated reference database, substantially improved the mappability of the indri samples (average mappable fraction of metagenomic reads of 85%). The most represented phyla in the indris gut microbiome were Bacteroidota (43.53% ± 14.42%), Proteobacteria (32.75% ± 10.03%), Actinobacteria (10.89% ± 11.51%) and Synergistetes (8.61% ± 4.68%). Female indris displayed a significantly higher species alpha-diversity (Simpson) and evenness (Pielou) compared to males. Social group belonging emerged as the main determinant of microbiome composition (beta-diversity). We also found extensive bacterial strain sharing across indris from the same social group, without significant differences by type of relationship. The resulting microbiome transmission networks recapitulated the geographic distribution of the groups. In contrast, no evidence for microbiome acquisition through geophagy was detected. Conclusions: The Indri indri gut microbiome differs from that of other non-human primates, and is extensively exchanged within social groups.	Francisca Labisa-Morais, Aitor Blanco-Miguez, Michal Puncochar, Paolo Manghi, Albert Garcia-Valiente, Daria Valente, Istoni Daluz-Santana, Luigimaria Borruso, Paola Mattarelli, Camillo Sandri, Caterina Spiezio, Nicola Segata, Mireia Valles-Colomer	MELIS Department, Universitat Pompeu Fabra, Barcelona, Spain

Title	Text	Authors	Author Affiliations
Biological Sex and Microbiota Drive DNA Methylation Patterns in the Mouse Liver	How the gut microbiota and its host communicate and react together to environmental stimuli is essential to understand a mammalian holobiont. In a biomedical context, such communication might be a reason for staying healthy, if it is balanced, or associated with diseases when the communication is disrupted. Microbiota and host produce metabolites that can regulate and limit epigenetic enzymes, controlling epigenetic marks in the host. Using a germ-free mouse model, including males and females, we investigated how biological sex and microbiota status influenced the "microbiota-nutrient metabolism-host epigenetic" axis of communication. We studied microbes, metabolism, epigenetic marks and gene expression in the host, focusing on the liver as target tissue, through a multi-omics and correlational analysis approach. Our results show the interaction of biological sex and microbiota in controlling the levels of DNA methylation in an additive manner. Further, we have observed the additive effect of sex and microbiota in the levels of DNA methylation, where males are hypomethylated compared to females, and where the presence of microbiota accentuates this pattern in both sexes, being more pronounced in males. Males without microbiota present a feminised pattern. Additionally, we have observed a strong positive correlation between the levels of host DNA methylation in genes involved in testosterone degradation, and the Ruminococcaceae family, more abundant in males than females. Altogether, we propose Ruminococcaceae as a key ecological player in the holobiont ecosystem, influencing host gene regulation in a sex-dependent manner, through the possible modulation of methylation levels of testosterone degradation genes.	Joan Miro-Blanch1,2, Jordi Rofes1,2, Jordi Capellades1,2, Alexandra Junza1,2, Christian M Heyer3,10, Ignasi Forné4, Magdalini Serefidou4, Luisa Santus5, Belen Carbonetto6, Tamara García-Barrera7, Teresa Rubio8, Salvador Casani-Galdon8, Aurélie Balvay9, Claire Maudet9, Sylvie Rabot9, Pedro González-Torres6, Ana Conesa8, Axel Imhof4, Marta Melé5, Matthias Schlesner3 and Oscar Yanes.1,2	1 Universitat Rovira i Virgili, Department of Electronic Engineering, IISPV, Tarragona, Spain 2 CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III, Madrid, Spain 3 Biomedical Informatics, Data Mining and Data Analytics, Faculty of Applied Computer Science and Medical Faculty, University of Augsburg, Augsburg, Germany 4 Protein Analysis Unit, BioMedical Center, Faculty of Medicine, Ludwig-Maximilians-University, Martinsried, Germany 5 Life Sciences Department, Barcelona Supercomputing Center, Barcelona, Spain 6 Microomics Systems S.L., IIB Sant Pau, Barcelona, Spain 7 Research Center on Health and The Environment (RENSMA), Department of Chemistry "Prof. J.C. Vílchez Martín", University of Huelva, Huelva, Spain 8 Institute for Integrative Systems Biology, Spanish National Research Council, Paterna, Spain 9 Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, Jouy-en-Josas, France 10Faculty of Biosciences, Heidelberg University, Germany

Title	Text	Authors	Author Affiliations
The inflammasome sensor NLPR3 is at the crossroads of microbiota-gut-lung crosstalk during pneumococcal pneumonia.	Streptococcus pneumoniae causes 30-50% of community-acquired pneumonia cases, killing <1.6 million people/year. NLRP3, an inflammasome sensor which mediates activation and secretion of the proinflammatory cytokines IL-1β and IL-18, is engaged by pneumococci. However, its role in pneumococcal pneumonia is unclear as it may contribute to protection or pathology. Among the factors influencing resistance to pneumonia, high fiber diet improves lung function and decreases inflammation, conferring resistance to respiratory pathogens. The diet directly shapes the intestinal microbiota, the largest and most diverse microbial bodily ecosystem with remarkable biosynthetic and metabolic capabilities. Compelling links between intestinal dysbiosis, microbiota's metabolism and respiratory diseases revealed the existence of the gut-lung axis of immune regulation which influences pneumococcal pneumonia outcomes. Among gut microbial metabolites, short-chain fatty acids (SCFAs) derived from the saccharolytic fermentation of fiber are needed for intestinal and lung homeostasis. Independent studies showed that gut dysbiosis is detrimental for invasive pneumococcal pneumonia, while SCFA can protect. Also, NLPR3 is involved in maintenance of intestinal and lung homeostasis through inflammasome-dependent and independent mechanisms, being a key factor for maintaining eubiosis. However, how the interplay between NLRP3, intestinal dysbiosis and SCFA controls susceptibility to invasive pneumococcal pneumonia has not been established. Using a model of invasive pneumococcal pneumonia, we show that NLRP3 KO mice are more susceptible to invasive disease than WT mice. Analysis of fecal microbiome revealed loss of intestinal butyrate-producing bacteria in NLRP3 KO and lower levels of the fecal SCFAs acetate, propionate and butyrate. Mechanistically, dysbiosis in NLRP3 KO is the result of lower expression of Aicda which encodes the enzyme necessary for IgA class-switching, leading to IgA deficiency and imbalanced gut microbial populations. Co-housing of NLRP3 KO with WT or supplementation with butyrate, restored fecal IgA levels and resistance to invasive pneumococcal pneumonia. Supplementation with retinoic acid, which acts downstream butyrate to induce intestinal IgA class-switching, also restored IgA in NLRP3 KO. Our results underscore the importance of NLPR3 and SCFA in intestinal eubiosis though IgA and their role in the gut-lung axis of immune modulation during pneumococcal pneumonia.	Natalia Muñoz-Wolf1,2*, Craig P. McEntee1*, Kate Roche 1,2, Ross W. Ward1, Hugh Harris3, Paul O'Toole3 and Ed C. Lavelle1	Affiliations 1. Adjuvant Research Group, School of Biochemistry and Immunology, Trinity College Dublin 2. Translational & Respiratory Immunology Group, Clinical Medicine Tallaght, School of Medicine, Trinity College Dublin 3. APC Microbiome Institute, and School of Microbiology, Food Sciences & Technology Building, University College Cork, Ireland * Equally contributed

Title	Text	Authors	Author Affiliations
Accessing impact of Nicotine on Salivary Microbiome: A multi-omics, ex-situ approach	The oral microbiome is the second most complex microbial community in humans after the gut microbiome. Recent work has shown that the oral microbiome can influence health and disease, not only in the oral cavity but also in other parts of our body. In this regard, it is clear that diet, genetic and environmental factors can shape the oral microbiome but it is difficult to pinpoint the effect of each specific condition. This is because most studies are observational, which makes it hard to disentangle causation and correlation, or based on animal models, which rely on extrapolation. We applied this method to assess the effect of nicotine, one of the major components in smoking products and also a potent antimicrobial agent. Our preliminary results show high viability and relative stability of the oral microbiome for several hours after collection, albeit with a measurable decline of anaerobic species. Controlling for this effect we can assess the direct impact of the presence of nicotine, which differentially alters the relative and absolute abundances of distinct groups of microbes. In addition, metabolomics measurements pre- and post-treatment show evidence of downstream derivatives of nicotine, presumably due to microbial degradation. Finally, we observe different responses in different individual microbiomes, which suggest a complex relationship between the endogenous microbiome composition and the impact of exogenous factors.	Mrinalini Parmar	IRB Barcelona

Title	Text	Authors	Author Affiliations
Exploring the Influence of Faecal Microbiota from a Chronic Stress Porcine Model on Axenic Mice	Chronic stress is a key risk factor for major depressive disorder, emphasizing the need for a better understanding of the underlying mechanisms driving depressive behaviours. In this regard, the similarity of pigs in physiology and anatomical size to humans enhances their value as biomedical models. The current study aimed to investigate the impact of faecal microbiota transfer from a porcine model of chronic stress on axenic mice. A total of 60 Duroc pigs, were randomly assigned during the growing finish-period to stressed (SP) and control (CP) groups, each containing 30 animals. The stress challenge was based on mixing animals from the stress cohort twice during the growing period. The available space in CP was 50% greater than in SP. Faecal metabolomics profile was explored at the end of the experiment (190 days). 60 six-week-old mice were used to orally administrate 200 µl faecal matter from either CP or SP pigs. Mice faecal samples were collected weekly, and animals were euthanised at three and five weeks. The 16S rRNA gene was sequenced using Illumina MiSeq. Sequences were analysed with Qiime2, and functional prediction was done using Picrust2. Plasma and gut tissues were collected during the euthanasia to assess immune response, antioxidant status, and mucosal integrity. Compared to SP group, the CP animals weighted on average 7.09% more and had an additional average daily gain of 6.32%. Analysis of the faecal metabolome showed shifts in composition, notably indicating reduced biogenic amine synthesis and transport of neurotransmitter in stress-exposed pigs. Likewise, significant differences were observed in mice. The genus Erysipelatoclostridium was found to be the most abundant in the microbiota of mice fed with fecal matter of SP, whereas Prevotella and Bacteroides were characteristics of the control condition. Compared to controls, the predicted functional profiles in mice showed elevated trimethylamine biosynthesis and L-histidine degradation pathways, alongside decreased abundance of oxaloacetate, propionate, vitamins B1, and B6. Additionally, mice fed with fecal content from SP showed elevated levels of IgA and IgG, coupled with a reduction in total antioxidant capacity, and mucus-producing goblet cell counts. Our results confirm a negative impact of chronic stress on pig performance and faecal metabolomic profile. Additionally, the transfer of pig faecal matter to mice reveals changes affecting taxonomic, functional, and immune profiles.	Raquel Río-López 1, Adrià Clavell-Sansalvador2, Rebeca Martín-Rosique3, Sead Chadi3, Alberto Valdés4, Olga González-Rodríguez2, Aurelie Balvay3, Anne Foussier3, Gustavo Zigovski de Paula5, Maria Ballester2, Raquel Quintanilla2, Xavier Xifró6, Jesús García-Gil7, Antoni Dalmau1, Yuliaxis Ramayo-Caldas2	1 Animal Welfare Subprogram, Institute of Agrifood Research and Technology (IRTA), 17121, Monells, Girona, Spain 2 Department of Genetics and Microbiology, Faculty of Bioscience, Autonomous University of Barcelona, 08193, Bellaterrra (Cerdanyola del Vallès), Barcelona, Spain. 3 Animal Breeding and Genetics Program, Institute of Agrifood Research and Technology (IRTA), 08140, Torre Marimon, Caldes de Montbui, Barcelona, Spain 4 Department of Biology, Faculty of Science, University of Girona, 17003, Campus Montilivi, Girona, Spain. 5 INRAE, AgroParisTech, Micalis Institut,, Paris-Saclay University, 78350, Jouy-en-Josas, France 6 Department of Bioactivity and Food Analysis, Institute of Food Science Research (CIAL), Spanish National Research Council (CSIC), 28049, Madrid, Madrid, Spain. 7 Graduate Program in Animal Science. Pontifical Catholic University of Paraná (PUCPR), 82590-300, Prado Velho, Curitiba, Paraná, Brazil. 8 New Therapeutic Targets Group, Department of Medical Science, Faculty of Medicine, University of Girona, Girona, Spain. 9 Digestive diseases and microbiota group. Biomedical Research Institute of Girona (IDIBGI). Girona, Spain.

Title	Text	Authors	Author Affiliations
Understanding Modern and Ancient Gut Microbiome Assemblies	The gut microbiota of healthy individuals is commonly dominated by the Bacteroidota phylum. Within this phylum, members of the Bacteroidaceae and Prevotellaceae family tend to exclusively predominate the microbiomes of populations with modern and traditional lifestyles, respectively. These predominance patterns are mainly attributed to distinct diets and are associated with ambiguous health outcomes. Prevotellaceae predominance in the microbiota has been associated with healthy lifestyles and improved health outcomes, whereas Bacteroidaceae predominance is associated with diets high in protein and animal fat. Interrogating experimentally how such microbiota compositional patterns are shaped beyond association studies is critical for elucidating microbiome health biomarkers. Through synthetic community design and metagenomics analysis, we elucidate the principles that govern such major microbiota signatures in human populations. Experimentally, we explore niche interactions between members of Bacteroidaceae and Prevotellaceae, and we assess the effect of ~100 dietary components on the interaction between members of these two families. We find that Segatella copri (former Prevotella copri) can displace Bacteroidaceae strains in the presence of specific dietary polysaccharides in a process that requires the presence of other key commensals. These results suggest that a combination of nutrient competition and specific microbe-microbe relationships determine major microbiota signatures. These results bring us closer to answering the long-standing question of what drives major human microbiota signatures.	Caroline Tawk1, Youssef El Mouali1, Kun D. Huang1, Johanna Rapp2, Hannes Link2, Athanasios Typas3, Till Strowig1	1Department of Microbial Immune Regulation, Helmholtz Centre for Infection Research (HZI), Inhoffenstraße 7, Braunschweig 38124, Germany.

Title	Text	Authors	Author Affiliations
Exploring Determinants and Host-Performance Implications of Pig Gut Microbial Enterosignatures	Background: The pig gut microbiota is a complex ecosystem composed of microbial guilds that until recently were unknown. Studies in human have reported the existence of microbial signatures of commonly co-occurring bacterial guilds, defined as enterosignatures (ES). Compared to the complementary enterotype approach, the ES concept considers the intrinsic ecosystem information, returning the proportional representation of bacterial assemblages rather than a categorical classification for each sample. Methodology: We implemented the non-negative matrix factorization approach to decompose the pig faecal microbiota of 648 healthy pigs during transition (n=400) and growing-finishing (n=248) periods in bacterial communities defined as pig enterosignatures (PES). We investigated PES composition, identifying key drivers and determining whether they exhibited stable or dynamic fluctuations across time. In addition, we explored their prevalence and association to different host and environmental determinants. Results: Pig faecal microbiota can accurately be described by combinations of at least six PES, driven by Prevotella (PES-Prev), Treponema (PES-Trep), Lactobacillus (PES-Lact), Clostridium (PES-Clost), Streptococcus (PES-Strep), and UBA2810 (PES-UBA2). PES assembly changed dynamically with the age of the host, where PES-Prev, PES-Strep, and PES-Lact seemed to be core components, while the remaining played age-specific roles. Furthermore, our findings indicated that stress can impact PES assembly, decreasing PES-Lact prevalence while increasing the abundance of PES-Strep during the growing-finish period. We noted a positive relationship between PES-Prev and growth at 60-days, whereas during the growing period a negative link to feed efficiency was observed. Remarkably, a negative association between the abundance of PES-Lact and levels of hair cortisol (as indicator of chronic stress) was also found during this period. Conclusions: Overall, our findings offer novel insights into the pig gut microbiota assembly, showing that PES are influenced by the age of the host, genetic, and chronic stress. Additionally, we show their associations with pig performance and physiological traits. Finally, our results underscore the relevance of customizing microbial consortia based on the nutritional and health requirements at each stage of the porcine production chain.	Ioanna-Theoni Vourlaki, Raquel Rio-Lopez, Adria Clavell-Sansalvador, Lino C. Ramírez-Ayala, Maria Ballester, Juan Pablo Sanchez, Miriam Piles, Raquel Quintanilla, Angela Cristina da Fonseca de Oliveira, Leandro Batista Costa, Antoni Dalmau, Yuliaxis Ramayo-Caldas	Animal Breeding and Genetics Program, IRTA, Torre Marimón, 08140 Caldes de Montbui, Barcelona, Spain Animal Welfare Subprogram, IRTA, 17121 Monells, Girona, Spain Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brasil, 80215-901

Title	Text	Authors	Author Affiliations
Exploring how butyrate modulates monocyte responses in pneumococcal pneumonia: Carrying a message beyond the gut	Understanding the intricate dynamics between host immune cells and pathogens is crucial for devising effective therapeutic strategies against infectious diseases. Lower respiratory infections are the 4th cause of death worldwide. Community-acquired pneumonia (CAP) is a leading cause of hospitalisation and mortality. Streptococcus pneumoniae accounts for 30-50% of CAP cases. Alongside invasive pneumonia, pneumococcus causes other life-threatening manifestations such as invasive pneumococcal disease (IPD), which affects children <5 and adults >65, causing ~1.6 million deaths/year. Despite available vaccines, challenges persist due to serotype replacement and increasing antibiotic resistance. Thus, understanding the mechanisms underlying susceptibility to IPD is crucial. Risk factors for IPD are multifactorial, including age, immunodeficiencies, underlying conditions, environmental pollution, and genetic predispositions. Additionally, lifestyle factors such as smoking, excessive alcohol consumption and type of dietary habits play significant roles in determining susceptibility to pneumococcal pneumonia and IPD. Many epidemiological studies have associated high fibre diets with improved lung function and resistance to respiratory infections, indicating an interplay between the gut and the lungs, a concept known as the gut-lung axis of immune regulation. Mouse studies have demonstrated that antibiotic-induced gut dysbiosis increases susceptibility to pneumococci, resulting in compromised ability of alveolar macrophages to kill the bacteria. This emphasises a key role of the intestinal microbiota in providing resistance against invasive pneumococcal pneumonia however the underlying mechanisms remain unclear. Here, we propose that short-chain fatty acids (SCFAs), derived from microbial fermentation of dietary fibres, help balance local responses in the lungs by modulating the phenotype of patrolling monocytes which are essential for combating pneumococcal infections. By using a murine model, we demonstrate that supplementation with butyrate protects against lethal pneumococcal pneumonia. This supplementation decreases IL6 plasma levels and promotes alveolar infiltration of Ly6C+ and CD115+ monocytes, while downregulating interstitial macrophages and Ly6C- monocytes. Using THP1 monocytic cells, we showed that butyrate promotes bacterial uptake and killing while simultaneously modulating inflammatory cytokine production in a concentration-dependent manner.	Melanie Fidel	Translational & Respiratory Immunology Lab, Department of Clinical Medicine Tallaght, School of Medicine – Trinity College Dublin, D02 R590, Ireland. School of Biochemistry & Immunology - Trinity College Dublin, D02 R590, Ireland. Adjuvant Research Group, School of Biochemistry & Immunology - Trinity College Dublin, D02 R590, Ireland.

Title	Text	Authors	Author Affiliations
Bread diet impact on gut microbiota dysbiosis and IBS-like quiascent UC. A pilot study	Human gut microbiota is rich, diverse and host-specific [1-4] protecting the host from pathogens throughout competition for ecological niches and fostering host immunity's development [5, 6]. The gut microbiota impacts human health and a wide range of pathologies (e.g., Crohn's disease and ulcerative colitis (UC)). However, no evidences exist for those patients suffering of UC in remission with irritable bowel syndrome (IBS)-like symptoms. Bread is a global source of carbohydrates, vitamins, minerals (P, Mg, Ca), vegetable proteins and dietary fibre [7]. Accordingly bread might exert a role as microbiota modulator [7, 8], even differences might exist with respect to traditional or modern industrial production processes [9, 10]. Accordingly and in order to assess gut microbiota shifts and symptomatology relief of IBS-like symptoms in patients with quiescent UC, we followed-up 31 UC patients in remission with IBS-like symptoms which were randomly assigned to a dietary intervention with 200 g/d of bread (either treatment or control) for 8 weeks. Changes in faecal microbiota composition were assessed by 16S rRNA gene sequencing (v3-v4 region). Clinical symptomatology was tested using questionnaires and inflammatory parameters (e.g., calprotectin, C-reactive protein, ...). Results suggested that treatment bread reduced the Firmicutes/Bacteroidetes (F/B) ratio (p-value = 0.058) which seems to be associated with improving IBS-like symptoms (p-value = 0.059). No statistically significant differential abundances were found at any microbial taxonomic level. Also, a decrease in IBS-like symptomatology was observed after both the treatment (p-value = 0.003) and control bread (p-value < 0.001) interventions. All in all, the intake of a traditionally ellaborated bread decreased the F/B ratio, which seemed to be associated with improving IBS-like symptoms in quiescent UC patients. These findings suggest that the traditional bread elaboration has a potential prebiotic effect improving gut health. This project was funded by the Spanish Ministry of Economy under RETOS program (RTC-2017-6467-2). Refs: 1, Halfvarson et al. 2017. Nat Microbiol; 2, Lloyd-Price et al. 2017. Nature; 3, Lloyd-Price et al. 2019. Nature; 4, Lv et al. 2019. World J Gastroenterol; 5, Bäckhed et al. 2005.Science; 6, Odenwald et al. 2023. Nat. Microbiol; 7, Arias et al. 2017 J Funt Foods; 8, Costabile et al 2014 PloS ONE; 9, Costabile et al 2008 Br J Nutr; 10, Saa et al 2017 LWT-Food Sci Technol	Aleix Lluansí1, Marc Llirós2, Robert Carreras-Torres1 , Anna Bahí1 , Montserrat Capdevila1 , Anna Feliu1 , Laura Vilà-Quintana1 , Núria Elias-Masiques3 , Emilio Cuevas3 , Laia Peries4 , Leyanira Torrealba4 , Josep Oriol Miquel Cusachs4 , Míriam Sàbat5 , David Busquets Casals4 , Carmen López Núñez4 , Sílvia Delgado-Aros6 , Jesús L Garcia-Gil7 , Isidre Elias3 , Xavier Aldeguer1	1 Institut d'Investigació Biomèdica de Girona Dr Josep Trueta (IDIBGI), Digestive Diseases and Microbiota Group, Girona, Spain, 2Universitat de Vic–Universitat Central de Catalunya, Vic, Spain, Bioinformatics and Bioimaging (BI-SQUARED) research group, Biosciences Department, Faculty of Sciences, Technology and Engineerings, Vic, Spain, 3Elias-Boulanger S.L., Vilassar de Mar, Spain, 4Hospital Universitari de Girona Dr. Josep Trueta, Department of Gastroenterology, Girona, Spain, 5Hospital de Santa Caterina, Department of Gastroenterology, Girona, Spain, 6Gastroenterology Scientific advisor to Elias-Boulanger S.L, Vilassar de Mar, Spain, 7Universitat de Girona, Deparment of Biology, Girona, Spain

Title	Text	Authors	Author Affiliations
The antihypertensive effect of protein hydrolysates from agro-food industry by-products is mediated, at least in part, by gut microbiota	Background: In recent years, the intestinal microbiota has been shown to play an important role in the development of hypertension, one of the most critical and costly public health problems. Thus, modulation of the microbiota has received considerable interest. Protein is an important macronutrient that can affect these bacteria by serving as a source of nitrogen, affecting the production of postbiotics, beneficial metabolites. Reciprocally, these bacteria can metabolize proteins releasing peptides with different activities including antihypertensive effects. Hence, the goal of this study was to investigate the antihypertensive capacity of different protein hydrolysates obtained from agri-food by-products and previously selected for their ability to modulate the intestinal microbiota and evaluate the influence of these bacteria on their activity. Methodology: In previous studies by the group, six hydrolysates were selected for their high inhibitory activity of the angiotensin-converting enzyme (ACEI) and their ability to modulate the intestinal microbiota of prehypertensive patients in vitro. Antihypertensive activity was determined in spontaneously hypertensive rats (SHR) at a dose of 55 mg/kg by telemetry. Water and captopril were used as controls. Subsequently, the experimental design was repeated in SHR rats, which had been administered a cocktail of antibiotics (ABX) for 1 week. Best hydrolysates were selected and administered to SHR for 4 weeks. Results: Two of the hydrolysates showed antihypertensive activity and this effect was lost after ABX treatment. In the chronic experiment, the antihypertensive effect was observed only during the first weeks of administration. Significant effects in gut microbiota composition were found, specially increased firmicutes abundance. Conclusions: The intestinal microbiota may play a critical role in the antihypertensive effects of protein hydrolysates.	Rafael A. López-Villalba1,2, Fabiola Garcia-Reyes1,2, Ana B. Gutiérrez-Reyes1, Manuel Suárez1,2,3, Francisca Isabel Bravo1,2,3, Cristina Torres-Fuentes1,2,3	1Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Tarragona, Spain 2Universitat Rovira i Virgili, Institut d'Investigació Sanitària Pere Virgili, Nutrigenomics Research Group, Tarragona, Spain 3Universitat Rovira i Virgili, Center of Environmental, Food and Toxicological Technology (TecnATox), Nutrigenomics Research Group, Tarragona, Spain.

Title	Text	Authors	Author Affiliations
Developing microbiome and dietary strategies based on Chrononutrition for targeting ADHD: Hypothesis, research aims and project design	Background and hypotheses: Mental illness is alarmingly rising, and circadian disruptions linked to a modern lifestyle may largely explain this trend. Evidence indicates that circadian disruption negatively affects brain development and function and impaired circadian rhythms, including mistimed and irregular eating patterns, are widely associated with ADHD. Furthermore, the high efficiency of chronotherapy approaches for improving ADHD points out the circadian system as a promising therapeutic target. The gut microbiota exhibits diurnal rhythmicity, as largely governed by meal timing, which regulates the host's circadian rhythms. In this regard, the temporal circadian regulation of feeding has emerged as a chronotherapeutic strategy to prevent and/or help with the treatment of mental illnesses, mainly through the modulation of gut microbiota. Taking all this into account, we firmly believe that resynchronization of circadian rhythms by aligning the habitual eating schedule with the circadian rhythms through Circadian Fasting (CF) will improve the clinical symptoms in ADHD patients and/or their QoL via modulation of the gut microbiota and intestinal ecology. Purpose: To assess, for the first time, in the framework of the CronoNutriBiome project, the impact of CF on cognitive and behavioural alterations, circadian disruption, serum, and fecal biomarkers, and quality of life linked to ADHD, as well as the biological mechanisms, including the role of gut microbiota and intestinal ecology, in mediating the potential therapeutic CF effects on ADHD. Project design: First, by conducting and coupling the results from a case-control study and a 12-week CF clinical trial 14:10 in human adults, we will investigate the chrono-disruption markers linked to adult ADHD, including alterations in saliva microbiota diurnal oscillations, and evaluate the effect of CF on ADHD rhythmicity alignment and quality of life, cognition, behaviour, circadian health, inflammation, and gut and salivary microbiota. Additionally, we will seek associations between clinical CF-induced changes and structural and functional microbiota signatures, as well as for lifestyle factors linked to microbiome composition that could influence adherence and/or clinical response to intervention. Second, we will use in vivo rat models of ADHD and germ-free mice to further deepen our understanding of the molecular mechanisms linking circadian fasting intervention to gut microbiota changes and behavioural out	Jose Martinez-Raga1*, Fernando Gámiz2*, Vicent Balanzá-Martínez3, Pablo Cervera1, Alberto Real1, Nuria López-Villaplana 1, Marta Arroyo4*, María Carmen Cenit4*	1 Department of Psychiatry and Clinical Psychology, Hospital Universitario Doctor Peset, University of Valencia, 46017 Valencia, Spain. 2 Department of Psychobiology, Institute of Neurosciences (CIBM), University of Granada, Spain. 3 Teaching Unit of Psychiatry and Psychological Medicine, Department of Medicine, University of Valencia, CIBERSAM, INCLIVA, Valencia, Spain. 4 Institute of Agrochemistry and Food Technology (IATA), Spanish Research Council (CSIC), Carrer del Catedràtic Agustín Escardino Benlloch, 7, 46980 Valencia, Spain.

Title	Text	Authors	Author Affiliations
MBK-01 freeze-dried liophilyzed oral capsules are superior to fidaxomicin in recurrent episodes of Clostridioides difficile infection	Background: Clostridioides difficile (CD) is the main pathogen responsible for antibiotic-associated diarrhea in developed countries. Fidaxomicin is an approved antibiotic against CD infections (CDI), while Fecal Microbiota Transplantation (FMT) has emerged as an effective and safe treatment for recurrent CDI. The aim was to assess the efficacy and safety of the experimental product MBK-01 compared to fidaxomicin in CDI cases 8 weeks after the start of the treatment, evaluating the absence of diarrhea recurrences. Methodology: A phase III, multicenter, controlled, open-label, parallel, randomized, prospective follow-up clinical trial was performed in a cohort of patients with CDI (either first or recurrent episodes). The experimental group received a single dose treatment of 4 oral capsules containing lyophilized heterologous fecal microbiota (MBK-01, ≥2.1-2.5 x10^11 microorganisms), while the control group received oral fidaxomicin, 200 mg/12 hours for 10 days. Patients who had received prior antibiotic treatment for CDI underwent a 24–48-hour washout period. Adults with confirmed CDI, an episode of diarrhea (≥3 stools/24 hours) and presence of CD toxin were eligible, excluding those with previous FMT. Results: 92 patients from 21 sites in Spain were randomly distributed: 45 patients were assigned to the MBK-01 group and 47 to fidaxomicin, with 23 and 25 patients completing the study, respectively. In the overall population, 89.19% of the MBK-01-treated patients had no recurrence of CDI-associated diarrhea, while for fidaxomicin it was 77.50%. MBK-01 proved non-inferiority than fidaxomicin in primary CDI cases and within patients with no previous antibiotic treatment ($p<0.01$), as MBK-01 was 100% effective versus 80% for fidaxomicin in this subgroup. For recurrent CDI cases there was a significant difference in proportions of 0.4279 ($p=0.014$), thus demonstrating superiority of MBK-01 than fidaxomicin. Diarrhea was the only treatment-related Adverse Event for both groups, with MBK-01 showing less events than fidaxomicin (17 vs 27). Conclusions: MBK-01 may provide a higher clinical benefit than fidaxomicin in cases of CDI. In primary events and when no antibiotic pre-treatment was used non-inferiority of MBK-01 was proven, potentially reducing the need of antibiotics, whereas for recurrent CDI there was a superior efficacy of MBK-01 than fidaxomicin. Altogether, MBK-01 is an effective, safe, easy to administer, accessible and low-risk treatment for CDI.	Morales C, del Rio P, Aurtenetxe O y Basterra J,	Mikrobiomik Healthcare S.L.

Title	Text	Authors	Author Affiliations
Unraveling the Interplay of Vitamin D, Salivary Microbiome, and Oral Health in Qatar: A Comprehensive Investigation	Background: The salivary microbiome (SM), comprising a diverse community of microorganisms in saliva, plays a pivotal role in oral and potentially overall health. Vitamin-D, essential for calcium absorption, bone health, and immune function, faces deficiency concerns in Qatar due to limited sunlight exposure and cultural practices. Despite limited research on the gut microbiome's connection to vitamin-D deficiency, the influence of SM and Vitamin-D in oral hygiene remains largely unexplored. This study aims to elucidate the potential connection between SM, vitamin-D levels, and oral diseases in the Qatari population. Methods: Saliva samples from 2974 participants were collected as part of the Qatari-Genome-Project (QGP) and sourced from the Qatar-Biobank (QBB). Participants were stratified based on their Vitamin-D levels (<20ng/mL:deficient, ≥20ng/mL:non-deficient) and responses to an oral hygiene questionnaire. High-throughput sequencing of 16S-rDNA libraries and subsequent analysis using the QIIME pipeline were employed to evaluate the composition of the SM. PICRUST was utilized to predict functional metabolic pathways associated with the SM. Results: Among all the subjects included, Firmicutes and Bacteroidetes were the predominant phyla. Differential abundance analysis revealed that pathogenic members of the salivary microbiome, including Peptostreptococcaceae, RF-39, Staphylococcus, and Wolinella, were significantly enriched in Vitamin-D-deficient participants with bleeding and painful gums, respectively. Conversely, non-deficient participants with bleeding and painful gums exhibited a significant increase in Campylobacter, Neisseria, and Propionivibrio. PICRUSt analysis unveiled significantly elevated metabolisms of Linoleic acid, Phenylalanine, and alanine in the Vitamin D-deficient and painful gum groups. Conclusions: This study pioneers the investigation of Vitamin-D's impact on the salivary microbiome in a large Qatari cohort, focusing on oral health. It identifies microbial changes in those with Vitamin-D deficiency and gum diseases, highlighting Staphylococcus and Peptostreptococcaceae as significant pathogens. Metabolic pathway analysis confirms altered metabolisms in deficient groups, underscoring reduced Vitamin-D bioavailability. Integration of multi-omics data promises further insights into this relationship.	Selvasankar Murugesan, Souhaila Al Khodor	Reproductive and Perinatal Health Division, Sidra Medicine, Doha, Qatar

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Intestinal-Derived Short Chain Fatty Acid Modulates Alveolar Macrophage Response to Streptococcus pneumoniae.	Background: Streptococcus pneumoniae is a major human respiratory pathogen responsible for 30-50% of community acquired pneumonia cases and over a million deaths every year. Alveolar macrophages are resident phagocytes in the lower airways with a key role against pneumococcal infection as they promote early bacterial clearance and co-ordinate downstream inflammatory responses. While many factors influence susceptibility to respiratory infections, the intestinal microbiota has emerged as a key regulator of respiratory immune processes. The immunological crosstalk between the intestinal and lung compartments is known as the gut-lung axis of immune regulation. The intestinal microbiota influences this crosstalk in different ways; one such mechanism is the production of immunomodulatory microbiota-derived metabolites, namely short chain fatty acids (SCFA). SCFAs are exclusively produced by the microbiota through the saccharolytic fermentation of dietary fibre in the gut. Millimolar concentrations of SCFAs are metabolised in the colon where they have potent immunomodulatory properties and reach the portal circulation at sub-millimolar levels. Intestinal dysbiosis and low SCFAs have been linked to macrophage dysfunction and susceptibility to pneumococcal pneumonia. The SCFA acetate (C2) and propionate (C3) protect against pneumococcal pneumonia by enhancing alveolar macrophage function. However, the role of the four carbon SCFA butyrate remains to be explored in this context. Methods: An in vivo model of acute pneumococcal pneumonia was used to investigate the ability of butyrate to modulate disease severity. Murine foetal liver derived alveolar macrophage (FLAM) and ex vivo alveolar macrophage (MexAM) were infected with S. pneumoniae or the synthetic TLR2 agonist Pam3CSK4. Results and Conclusions: In vivo butyrate supplementation protected mice against invasive pneumococcal pneumonia. In vitro, butyrate increased IL-1□ secretion in MexAM stimulated with Pam3CSK4. Preliminary experiments in FLAMs showed that butyrate enhanced the expression of Nlrp3 and Tnfa upon S. pneumoniae infection or stimulation with Pam3CSK4. Collectively this evidence points towards a role of butyrate in enhancing alveolar macrophage function and increasing resistance against pneumococcal pneumonia. Our data highlights the therapeutic potential of dietary interventions and modulation of the gut microbiota as means to increase resistance to invasive pneumonia.	Kate Roche	Roche K. [1], McEntee C. P. [2], Cloonan S. [3], Carpenter S. [4], Rial A. [5], Chabalgoity J. A. [5], Lavelle E. C. [2] and Muñoz-Wolf N. [1]. 1 Translational and Respiratory Immunology, Clinical Medicine Tallaght, School of Medicine, Trinity College Dublin, Ireland. 2 Adjuvant Research Group, School of Biochemistry and Immunology, Trinity College Dublin, Ireland. 3 Clinical Medicine Tallaght, School of Medicine, Trinity College Dublin, Ireland. 4 Molecular Cell and Developmental Biology Department, University of California Santa Cruz, United States. 5 Laboratory for Vaccine Research, Hygiene Institute, School of Medicine, Universidad de la República, Uruguay.

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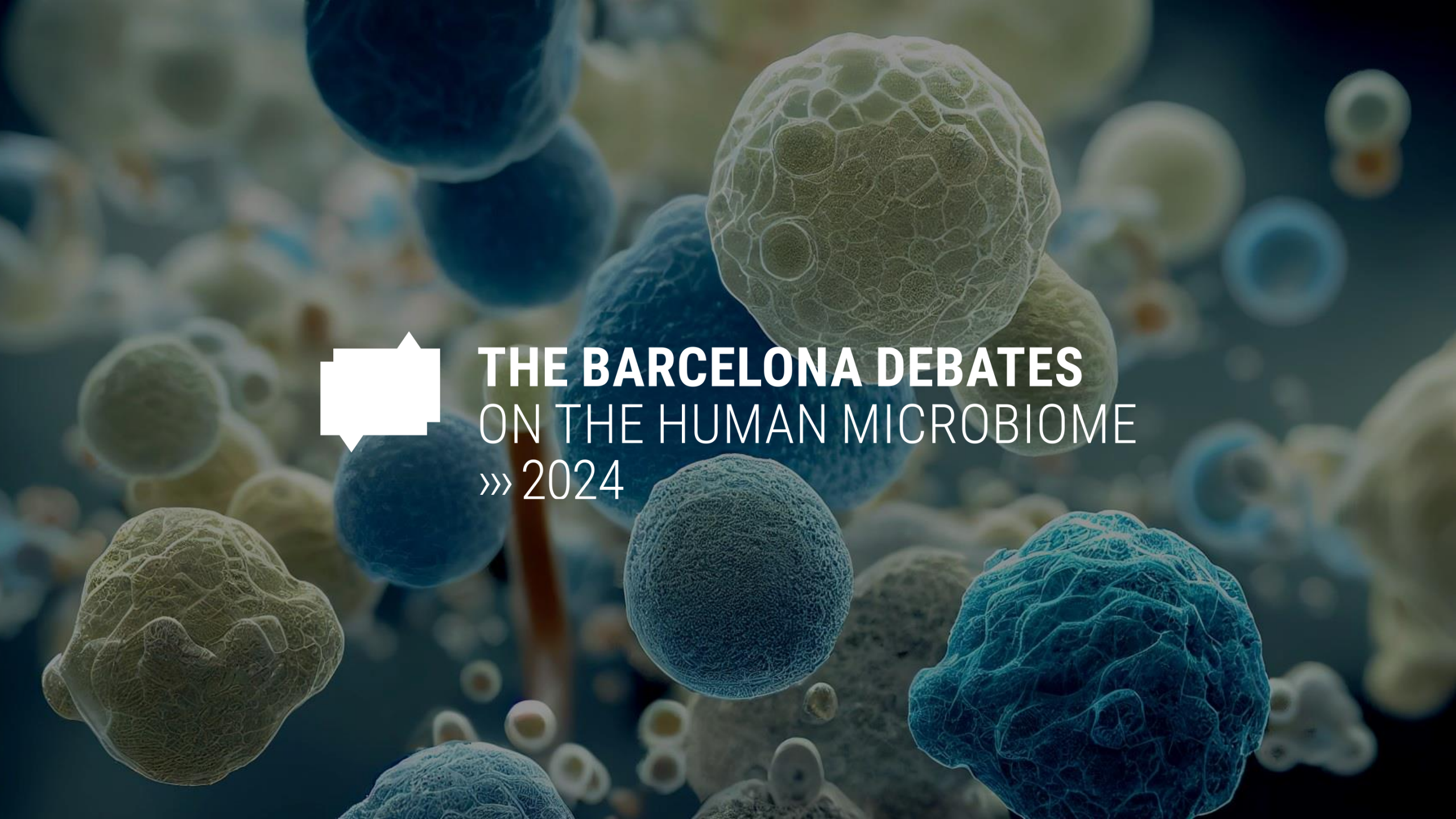
Engineering of the human skin bacterium Cutibacterium acnes for medical applications	A prominent goal of synthetic biology is the development of living medicines based on engineered cells. The genetic modification of the human microbiome presents fewer risks for the host than the editing of human cells, and bacterial therapeutics have been employed to treat metabolic disorders, to limit cancer progression, or to address microbiome dysbiosis. In contrast to the numerous attempts to program gut bacteria for therapeutics, the engineering of the skin microbiome has remained largely unexplored despite its enormous potential. Cutibacterium acnes, a bacterium that thrives within the sebaceous follicles, is the most abundant human skin microbe and has demonstrated promising engraftment properties when transplanted from a donor to a recipient individual. Despite being an ideal chassis for the development of living skin biotherapeutics, the engineering of C. acnes has long been hampered by the extremely low transformation efficiency and the lack of molecular biology tools available. Here we reverted this situation and rendered C. acnes an engineerable chassis for skin disorders. First, we increased the transformability of C. acnes by several orders of magnitude. This paved the way for the generation of an engineering toolbox for C. acnes that includes modular plasmids and cloning schemes, a promoter library, a library of fluorescent reporters, and gene expression control tools such as CRISPRi and inducible transcription factors. The use of these tools enables the construction of synthetic gene circuits to control C. acnes behaviour in a programmable manner, and the development of strains that produce and secrete molecules with therapeutic potential.	Javier Santos-Moreno, Guillermo Nevot, Lorena Toloza, Nastassia Knödlseder & Marc Güell.	Department of Medicine and Life sciences, University Pompeu Fabra, Barcelona, Spain.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