BOOK OF ABSTRACTS

THE BARCELONA DEBATES
ON THE HUMAN MICROBIOME
»» 2023

Title	Text	Authors	Author Affiliations
New insights into the role of Cutibacterium acnes-derived extracellular vesicles in inflammatory skin disorders	Background: Cutibacterium acnes (C. acnes) is one of the most prevalent bacteria that form the human skin microbiota and, depending on multifactorial conditions it can help to maintain the skin homeostasis. Actually, different phylotypes of C. acnes have been associated with different degrees of acne vulgaris development, while others, such as the H1 subtype, have been detected in patients with non-acneic skin. However, due to the physiology of the skin, the skin microbiota neither has direct access to the skin's sebaceous glands nor to the main immune cells, as they are protected by a sebum layer. Therefore, the inter-kingdom communication relies on secreted factors and bacterial extracellular vesicles (EVs). In this context, the purpose of this project was to study the role of EVs secreted by three different phylotypes of C. acnes (A1 as pathogenic, H1 as beneficial and H2 as commensal). Results: Main findings showed that the proteomic profile of the cargo embodied in the EVs reflects unique characteristics of the different C. acnes phylotypes in terms of lifestyle, survival and virulence. Moreover, in vitro skin models showed an extended pro-inflammatory modulation of A1 EVs, while H1 EVs displayed a high sebum-reducing potential. Conclusions: This study has highlighted the role of C. acnes EVs as key modulators during skin alterations, specially H1 EVs as an alternative based-natural treatment to fight acne vulgaris symptomatology.	Maria Pol Cros(1), Júlia Mir-Pedrol(1)(2), Lorena Toloza(1), Nastassia Knödlseder(1), Marc Güell(1), Julien Maruotti(3), Christos C. Zouboulis(4) and Maria-José Fábrega Fernández(1).	(1) Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona, ES (2) Universitaet Tuebingen Fachbibliothek Mathematik und Physik Tubingen, Baden-Württemberg, DE. (3) Phenocell, Grasse, FR. (4) Hochschulklinik für Dermatologie, Venerologie und Allergologie, Immunologisches Zentrum. Städtisches Klinikum Dessau. Medizinische Hochschule Brandenburg Theodor Fontane und Fakaltät für Gesundheitswissenschaften Brandenburg. Auenweg.

Title	Text	Authors	Author Affiliations
Understanding the peroxidase activity of Glutathione Peroxidase	The biological effects of selenium are largely mediated by selenium-containing proteins (selenoproteins) [1]. All these selenoproteins are broadly divided into three families such as Glutathione peroxidases (GPXs), Thioredoxin reductases (TRs) and Iodothyronine deiodinases (DIOs) [2] [3]. Different isoforms of Glutathione Peroxidase (GPX) have been labeled as potential targets to control or cause the oxidative stress during cancer evolution. [4] [5] In particular, eight different cysteine and selenocysteine containing isoforms of Glutathione Peroxidase (GPX1-8) isoforms have been identified in humans.[6] Mammalian GPX1, GPX2, GPX3, and GPX4 have shown to be selenium containing enzymes, whereas GPX6 is a selenoprotein in humans with cysteine containing homologous in rodents. GPX5, GPX7 and GPX8 contain cysteine.It is known that glutathione peroxidase (GPX) oxidizes thiols to disulfides with an active site that contains a Sec/Cys residue [7]. Methods The relationships between the mechanism and the structure is not completely known, which has led us to propose a combination of QM/MM methods and structural bioinformatics tools to unravel sequence-structure-function relationships. Results There exists strong structural homology among the different isoforms (see FIG 1, in PDF). Preliminary results show that catalytic activity of several reconstructed ancestral structures of GPX6 recover their peroxidase activity when the active site is mutated from Cys to Sec keeping the binding of glutathione in all cases. Conclusion We will focus on understanding both phylogeny and enzymatic mechanism in GPX6 (Sec- or Cys-containing), and our final goal is to run free energy calculations using the Empirical Valence Bond method to understand the effect of mutations in GPX6 and its orthologs.	Nayanika Das	Research Group on Bioinformatics and Bioimaging (BI2); Facultat de Ciències, Tecnologia i Enginyeries; Universitat de Vic - Universitat Central de Catalunya, Vic, Barcelona

Title	Text	Authors	Author Affiliations
Identification of a human anaerobic gut bacterium that increases muscular strength in the absence of exercise training	Background: Low muscular strength is associated with a 35% higher risk of premature mortality due to any cause, and lack of strength in muscles is a common cause of falls in the elderly population. So far, no pharmacological therapy has improved muscular strength beyond exercise training. However, exercise may not be accessible for everyone, emphasizing the need for alternative strategies to improve muscular strength. Thus, this study aims to investigate whether gut bacteria can enhance muscular strength in the absence of exercise training. Methodology: 16sRNA-seq was performed in fecal samples collected in independent cohorts of young sedentary adults (n=92; 22 y.o) and elderly sedentary adults (n=33; 67 y.o.). In these cohorts, muscular strength was measured with a handgrip dynamometer, and maximum volume oxygen capacity was measured in response to a maximal effort test. Three different bacterial strains from the same genus were purchased (DSMZ). Four groups of male C57Bl/6J mice were treated with antibiotics and orally administered a different bacterial strain to each group, three days per week, for eight weeks. Forelimb grip strength was measured, targeted metabolomic analysis was performed in the caecum, and proteomic analyses were performed in the plasma and muscle. Results: The relative abundance of a particular genus was positively related to higher muscular strength, VO2 max capacity and lean mass in young adults (n=92), confirmed in another cohort of elderly individuals (n=33). Detailed analyses revealed that particular bacterial species were driving these associations. Since the cross-sectional nature of this human study precluded establishing the direction of these relationships, we next treated a group of mice with the bacterium X. In contrast, other groups of mice were treated with other bacteria from the same genus as controls. Bacterium X increased the forelimb grip strength in mice compared to the other groups without exercise training (+30%; P<0.001). Metabolomic analysis in the caecum revealed that bacterium X induced massive changes in the composition of amino acids, not in the levels of short-chain fatty acids. Proteomic analysis in plasma also indicated a huge change of different amino acids, whereas muscle purine metabolism was mainly affected (patent application filed). Conclusions: Oral supplementation of mice with the anaerobic bacterium X increases muscular strength by 30%, supporting the further development of a probiotic	Borja Martinez-Tellez 1,2,3, Milena Schönke1,2, Arty Kovynev1,2, Lourdes Ortiz Alvarez3, David Jimenez Pavon4, Wiep-Klaas Smits5, Jonatan R. Ruiz3, Patrick CN Rensen1,2	1 Division of Endocrinology, Department of Medicine, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands (b.martinez-tellez@lumc.nl) 2 Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands 3 Department of Physical Education and Sports, Faculty of Sports Science, Sport and Health University Research Institute (iMUDS), University of Granada, Spain; Instituto de Investigación Biosanitaria, ibs.Granada, Spain; CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Spain 4 Biomedical Research and Innovation Institute of Cádiz (INiBICA), Cádiz, Spain 5 Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands; Center for Microbiome Analyses and Therapeutics, Leiden University Medical Center, Leiden, the Netherlands; Centre for Microbial Cell Biology, Leiden, the Netherlands.

Title	Text	Authors	Author Affiliations
Prognostic significance of the microbiome–adipose tissue axis in rectal cancer: protocol of a prospective observational study	Colorectal cancer is the second-leading oncological cause of death worldwide1. The influence of the tumour microenvironment in rectal cancer progression is gaining significant attention. Investigations into the role of peritumoral fatty tissue in the pathogenesis of rectal, oesophageal, or breast cancer have shown tissue remodelling2. Another component of the tumour microenvironment analysed in rectal cancer is the gut microbiome. Previous studies have described rectal mucosa dysbiosis and the association of microorganisms with the progression of carcinogenesis3. These findings suggest there might be an interaction between microbiome and adipose tissue due to disruption of the intestinal barrier by pathogenic micro-organisms4,5. To understand how the tumour microenvironment could modulate short- and long-term outcomes, it is essential explore the complex relationship between the microbiome and adipose tissue dysfunction. The aim of this study is to analyse the microbiome– adipose tissue axis in patients with rectal cancer and determine the impact of the microbiome present in adipose tissue for predicting tumour progression and surgical outcomes. METHODS The BIORECTUM study is a prospective observational study conducted at a high-volume tertiary referral colorectal surgical unit at a university hospital (NCT04804956)(Fig.1). The study aims to enrol at least 100 consecutive patients with rectal cancer per year, from April 2021 to April 2023. To evaluate the microbiome–adipose tissue axis, the following samples will be collected: subcutaneous, visceral, and mesorectal adipose tissue; and faecal and rectal mucosa specimens. The microbiome adipose tissue will be characterized with 16s rDNA metagenomic analysis and shot-gun metagenomics. Adipose tissue dysfunctionality will be assessed through analyses of inflammation and angiogenesis. In addition to characterizing the microbiome-adipose tissue axis in patients with rectal cancer, we will analyze its prognostic value for 3- and 5- years local recurrence and for disease-free and overall survival, as well as its possible impact on surgical and pathological outcomes. DISCUSSION To our knowledge, this is the first prospective study to investigate the prognostic value of the microbiome-adipose tissue axis in rectal cancer patients. We hope to gain a better understanding of how the tumor microenvironment could affect rectal cancer progression that could help improve develop therapeutic strategies.	Pere Planellas1,2,3, Lídia Cornejo2, Ramon Farrés1,2,3, Anna Pigem1,2, Ander Timoteo1,2, Nuria Ortega1,2, Gianluca Pellino4 , José-Ignacio Rodríguez-Hermosa5, Eugeni López-Bonet6, José Manuel Fernández-Real2,3,7 and Antoni Codina-Cazador1,2,3	1Colorectal Surgery Unit, Department of General and Digestive Surgery, University Hospital of Girona Dr. Josep Trueta, Girona, Spain 2Girona Biomedical Research Institute (IDIBGI), Girona, Spain 3Department of Medical Sciences, Faculty of Medicine, University of Girona, Girona, Spain 4Colorectal Surgery, Vall d'Hebron University Hospital, Barcelona, Spain 5Endocrine Surgery Unit, Department of General and Digestive Surgery, University Hospital of Girona Dr. Josep Trueta, Girona, Spain 6Department of Anatomical Pathology, University Hospital of Girona Dr. Josep Trueta, Girona, Spain 7Department of Endocrinology, Diabetes and Nutrition, University Hospital of Girona Dr. Josep Trueta, Girona, Spain

Title	Text	Authors	Author Affiliations
Can profiling of the lower airways microbiota aid diagnostics of chronic lung disease?	Background: Whereas chronic obstructive pulmonary disease (COPD) is relatively easy to diagnose based on history and lung function measurements, diagnosis of interstitial lung diseases (ILDs) are difficult. Diagnosis relies on lung function measurements, medical history, high resolution computer tomography scans, and bronchoalveolar lavage (BAL) and/or lung biopsies. ILDs includes many diseases sometimes hard to differentiate, and diagnosis requires multidisciplinary discussions. We hypothesized that profiling of the lower airways microbiome could be used to aid diagnostics of ILDs. Methods: We used data from the MicroCOPD and MicroILD studies, collected 2012-2016, at Haukeland University Hospital, Bergen, Norway. We performed bronchoscopy with BAL on 103 healthy controls, 130 COPD patients, 35 sarcoidosis patients, 12 idiopathic pulmonary fibrosis (IPF) patients, and 11 patients with unspecific ILD. Whole genome sequencing was performed with a NovaSeq 6000 in paired-end 150 bp mode. Raw reads were analyzed with GAIA (v 2.02) to obtain operational taxonomic unit tables at different taxonomic levels. Differentially abundant (DA) species between each disease and healthy controls were identified with DESeq2 and ANCOM-BC, and based on these species, a dysbiosis index was calculated for IPF, sarcoidosis and COPD. Results: Alpha-diversity differed between sarcoidosis and healthy controls, but not between any other groups. With ANCOM BC, we identified 190 DA species between IPF and controls, and 276 between sarcoidosis and controls and 2 between COPD and controls. With DESeq2, the corresponding numbers of DA species were 31, 92, and 43 respectively. To calculate the dysbiosis indexes, ANCOM BC provided the best fit for IPF, DESeq2 for sarcoidosis and COPD. For IPF the dysbiosis index gave a sensitivity of 0.92 and specificity of 0.82 for correct classification of a given subject, for sarcoidosis the sensitivity was 0.79 and specificity 0.86, and for COPD 0.59 and 0.68. Conclusion: Dysbiosis index could be a diagnostic aid in selected diseases like ILDs, where there are distinct differences in the microbiomes between health and disease.	Eagan, Tomas Mikal 1; Knudsen, Kristel Svalland 1; Husebø, Gunnar Reksten 1; Nielsen, Rune 1; Roda, Xenia 2; Sanseverino Walter 2; Paytuvi, Andreu	1 Dept. of Thoracic Medicine, Haukeland University Hospital, Bergen, Norway 2 Sequentia Biotech, Barcelona, Spain

Title	Text	Authors	Author Affiliations
Revealing processes that shape the ecosystem of Prevotella-dominated gut communities	Healthy human gut microbiomes are commonly dominated by the Bacteroidota phylum. Studies of members of the genus Bacteroides, which is highly prevalent in industrialized microbiomes, have provided critical insights into the molecular understanding of gut commensals and their role in human health. However, why members of Prevotella, another genus in this phylum, are prevalent in the microbiomes of agrarian populations and how they affect human health remains underexplored. In order to explore niche interactions between members of the Prevotella and Bacteroides in the context of the human microbiome, we designed a defined community of human gut commensal isolates. We demonstrate that this reproducible and robust gut community model supports a substantial niche for Prevotella copri human isolates in vitro and in vivo, including co-existence of multiple P. copri strains. We found that despite a larger genetic capacity of Bacteroides strains in utilizing complex polysaccharides, P. copri strains displace Bacteroides in the presence of specific plant-derived complex polysaccharides. We show that these competition dynamics are dependent on the presence of commensals from other phyla within the community. Through analyzing genomic context, transcriptional and metabolic output of Prevotella and Bacteroides strains within defined communities, we aim to characterize community- and substrate-dependent mechanisms of competition between these prominent commensals.	Caroline Tawk, Till Strowig	Department of Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig, Germany.

Title	Text	Authors	Author Affiliations
Prevotella copri RNA landscape in the mouse gut drives identification of an abundant regulatory sRNA in vivo	Alterations in the human microbiome have been associated with a plethora of human diseases. A prominent example is the abundant human gut commensal Prevotella copri, which has been associated with both health and disease traits. A current limitation in the field is the inability to explore functionally the associations between microbiota-health-disease. The growing interest in Prevotella copri biology contrasts with a lack of knowledge about its basic gene expression features and physiological responses both in vitro and in vivo. In here, we used differential RNA-seq to characterize the primary transcriptome of P. copri DSM18205 model strain as well as genetically tractable P. copri HDD04 strain under laboratory conditions. We have generated a single-nucleotide resolution map of P. copri transcriptome that allowed the identification of nearly 1000 primary transcription start sites, consensus promoter sequences that drive P. copri gene expression and the presence of non-coding RNAs. Furthermore, we interrogated the new P. copri RNA map via transcriptomics analysis of P. copri HDD04 colonized mice and of the human donor stool from which it was isolated. We have identified intergenic small RNA PcnR276 to be highly induced in both the mouse gut and in the human donor suggesting a key function in vivo.	Youssef El Mouali1, Falk Ponath2, Jörg Vogel2,3, Till Strowig1	1. Helmholtz Centre for Infection Research (HZI), 38124, Braunschweig, Germany 2. Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), 97080, Würzburg, Germany. 3. Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, Germany

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An update on the relationship between duodenal microbiota and celiac disease	Background The histological abnormalities in celiac disease (CD) patients are assessed through endoscopic sampling which implies four to six staged biopsies of the bulb and/or the second duodenum. A recent study showed that the microbial populations of the small intestine are greater than previously thought and differ significantly from those in the stool. Considering the surface area, the length, and its role in human nutrition and immune function, analysis of the small bowel microbiome may have a greater impact on our understanding of human disease than analysis of stool. The main objective of this review is to discuss several aspects of the duodenal microbiota on the development of CD since the early years of childhood. Methods Embase, Web of Science,PubMed and Scopus databases were searched for articles related to the duodenal microbiota and celiac disease. Keywords used for the search were; “celiac disease ”; “duodenal microbiota” AND “Celiac disease”; “children microbiota” AND “duodenal”; “adults microbiota” AND “celiac disease”. Studies considering all the gut microbiota, not specifically focused on duodenal microbiota were excluded. Results 22 studies were selected. A recent review evaluating the histological changes induced by gluten in the duodenal mucosa of patients from 9 contributing countries, identified mucosal alterations associated with Celiac Disease at early stages and concluded that architectural distortion starts at the Marsh 0 stage Studies of adult duodenal microbiota reported a modification of the duodenal mucosa microbiota where the Celiac Disease inflammation takes place with complete dominance of Enterobacteriaceae cluster, which is a potential pathobiont and subdominant of Bacteroidetes and Streptococcus. Studies of children’s duodenal microbiota showed that there was a significant association between the severity of histopathological changes of duodenal mucosa and high antibody titers Conclusions Current evidence into the composition of the intestinal microbiome and its role as a causative agent for CD is highly contradictory. Recent studies found lesions limited to the duodenal bulb in approximately 10% of the included patients. The studies considering duodenal biopsy as a method to diagnose celiac disease in children conducted so far have a few limitations; small cohort of patients, studies done on adult patients	Irsida Mehmeti	Faculty of Pharmacy, Cathoic University "Our Lady of Good Counsel", Tirana, Albania

Title	Text	Authors	Author Affiliations
Can shallow shotgun-based profiling of the gut microbiome improve the detection of host genetic effects?	One of the main questions in microbiome studies is to what extend microbiomes affect host phenotypes. Despite strong associations have been described, demonstrating causality is still difficult due to the presence of many confounders. To solve this problem, genetic epidemiology has recently become a promising approach as it uses host genetic variants as anchors to limit reverse causation and confounders in causal inference analyses. 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 among taxa; secondly, because most studies have relied on 16S data to characterise the microbiome, lacking species level resolution. So far, we evaluated shallow shotgun sequencing as an alternative to define taxonomic profiles of ceacal microbiomes from 1,041 outbred laboratory rats, using the Genome Taxonomy Database as a reference catalogue, given that the low depth provided by shallow shotgun sequencing requires a catalogue to assign taxa to reads without the assembling step normally applied for deep shotgun data. Then, we quantified aggregate host genetic effects (i.e., heritability) on the mapped taxa, ran a Genome Wide Association Studies (GWAS) analysis and compared the results with the ones obtained with 16S data from the same samples. More than 500 taxa (from species to order) were found as significant for shallow shotgun data, compared to only 1 family and 2 genera for 16S (FRD>10%). GWAS also revealed more hits with lower p values for shallow shotgun data with the highest hit found for both methods (-log10P>12) corresponding to the same position in the host genome and the same bacteria (Paraprevotella). This shed light on the potential of shallow shotgun as a new methodological perspective for detecting host genetic effects and the subsequent dissection of causal relationships between gut microbiomes and host phenotypes.	Felipe Morillo Sanz Dias	Centre for Genomic Regulation (CRG)

Title	Text	Authors	Author Affiliations
Microbiome profiling from Fecal Immunochemical Test reveals microbial signatures with potential for Colorectal Cancer screening	Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer deaths worldwide. Early diagnosis of CRC, which saves lives and enables better outcomes, is generally implemented through a two-step population screening approach based on the use of Fecal Immunochemical Test (FIT) followed by colonoscopy if the test is positive. However, the FIT step has a high false positive rate, and there is a need for new predictive biomarkers to better prioritize cases for colonoscopy. Here we used 16S rRNA metabarcoding from FIT positive samples to uncover microbial taxa, taxon co-occurrence and metabolic features significantly associated with different colonoscopy outcomes, underscoring a predictive potential and revealing changes along the path from healthy tissue to carcinoma. Finally, we used machine learning to develop a two-phase classifier which reduces the current false positive rate while maximizing the inclusion of CRC and clinically relevant samples.	Olfat Khannous-Lleiffe[1,2] Jesse R. Willis[1,2] Ester Saus [1,2] CRIPREV Consortium[&], Victor Moreno[3], Sergi Castellví-Bel[4], Toni Gabaldón[1,2,5,6]* .	1) Barcelona Supercomputing Center (BSC-CNS). Carrer de Jordi Girona, 29, 31, 08034 Barcelona 2) Institute for Research in Biomedicine (IRB). Carrer de Baldiri Reixac, 10, 08028 Barcelona 3) Catalan Institute of Oncology, Bellvitge Biomedical Research Institute (IDIBELL), Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP) and University of Barcelona, 08908 Barcelona, Spain. 4) Gastroenterology Department, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Hospital Clínic, University of Barcelona, Barcelona, Spain 5) Institució Catalana de Recerca i Estudis Avançats (ICREA). Pg. Lluís Companys 23, 08010 Barcelona, Spain. 6) Centro Investigación Biomédica En Red de Enfermedades Infecciosas (CIBERINFEC), Barcelona, Spain.

Title	Text	Authors	Author Affiliations
Periodontal Pathogens Consortia May Trigger gut dysbiosis in colorectal cancer patients	Background: Colorectal cancer (CRC) incidence is increasing worldwide. Therefore, research on new non-invasive CCR biomarkers is needed for CRC early diagnosis. High throughput sequencing studies demonstrated that oral and gut microbiotas of CRC patients are unbalanced. Microbiome dysbiosis can be triggered by translocation of oral pathobionts from periodontal pockets to the gut. Material and Methods: Different-nature samples (saliva, gingival crevicular fluid, feces, non-neoplastic and tumor tissues) of 128 individuals were analyzed using 16S rRNA metabarcoding procedures. Results: Parvimonas, Fusobacterium and Bacteroides fragilis were more abundant in feces of CRC patients than in healthy individuals. Besides, Faecalibacterium and Blautia, more abundant in the healthy control group, were significantly reduced in most CRC feces. Moreover, CRC samples were enriched in groups of periodontal anaerobes, including Fusobacterium, Parvimonas, Peptostreptococcus, Porphyromonas and Prevotella. Oral bacteria co-ocurrence patterns were observed in both subgingival pocket and tumor samples of CRC patients. Conclusions: We provide new evidence that oral pathobionts, normally located in subgingival pockets, translocate to the colon probably forming a synergistic consortium. This periodontal microbes association allows survival and proliferation, enhancing pro-inflammatory responses, that induces tumorigenesis initiation and/or progression. We propose the group Fusobacterium-Parvimonas-Bacteroides fragilis-Peptostreptococcus-Porphyromonas-Prevotella as an excellent biomarker for CRC early diagnosis.	Trigo-Tasende, Noelia1*; Conde-Pérez, Kelly1*; Aja-Macaya, Pablo1; Buetas, Elena2; Nasser-Ali, Mohammed1; Rumbo-Feal, Soraya1; Martín-De Arribas, Elsa3; Estévez, Lara S.4; Otero-Alén, Begoña4; Noguera, José F.5; Concha, Ángel4; Pardiñas-López, Simón6; Carda-Diéguez, Miguel2; Gómez-Randulfe, Igor7; Martínez-Lago, Nieves7; Ladra, Susana3; Aparicio, Luis M.A.7†;Bou, Germán1; Mira, Álex2; Vallejo, Juan A.1; and Poza, Margarita1,8	1 meiGAbiome, Microbiology Research Group, Institute of Biomedical Research (INIBIC) – University Hospital of A Coruña (HUAC) – Interdisciplinary Center for Chemistry and Biology (CICA) – University of A Coruña (UDC) – Spanish Network for Infectious Diseases (CIBERINFEC-ISCIII). Servicio de Microbiología, 3º planta, Edificio Sur, Hospital Universitario, As Xubias, 15006, A Coruña, Spain 2 Genomic and Health Department, FISABIO Foundation, Center for Advanced Research in Public Health. Avda Cataluña 21, 46020, Valencia, Spain. 3 Database Laboratory, Research Center for Information and Communication Technologies (CITIC), University of A Coruña (UDC). Campus de Elviña, 15008, A Coruña, Spain. 4 Pathological Anatomy Service and Biobank, University Hospital of A Coruña (HUAC) – Institute of Biomedical Research (INIBIC). 3º planta, Hospital Universitario, As Xubias, 15006, A Coruña, Spain. 5 General and Digestive Surgery Service, University Hospital of A Coruña (HUAC). Hospital Universitario, As Xubias, 15006, A Coruña, Spain. 6 Periodontology and Oral Surgery, Pardiñas Medical Dental Clinic – Cell Therapy and Regenerative Medicine Group, Institute of Biomedical Research (INIBIC). Rúa Real 66, 3ª planta, 15003, A Coruña, Spain. 7 Medical Oncology Department, University Hospital of A Coruña (HUAC). Maternal and Child Hospital. As Xubias, 15006, A Coruña, Spain. † R.I.P. 8 Microbiome and Health Group, Faculty of Sciences, University of A Coruña (UDC),

Title	Text	Authors	Author Affiliations
Parvimonas micra translocates from subgingival pockets to tumors in colorectal cancer patients	Background: Cancer is a multifactorial process involving both individual and environmental factors. In Spain, colorectal cancer (CRC) is the third most frequent type of cancer worldwide and caused 11,021 deaths over the last 2021 year, according to several epidemiological studies. Previous studies showed the microbiota of tumors (oncobiome) can influence the progress/development of CRC. Gut dysbiosis promotes inflammation, which can exacerbate the colorectal carcinogenesis process . In CRC patients, gut microbiota is unbalanced and enriched in oral pathobionts. The present work suggests a possible origin of the oral pathogen Parvimonas micra in colorectal tumors and adenomas. Methodology: A cohort of 93 CRC diagnosed individuals and 30 healthy volunteers were analyzed using metabarcoding approaches. P. micra isolates were obtained from gingival and tumor samples of CRC patients and their genomes were sequenced using two different platforms (Illumina and Oxford Nanopore). The whole bacteriome of one CRC patient was analyzed using different-nature samples by metabarcoding and metatranscriptomic approaches. Results: Our study confirmed that P. micra was over-enriched in feces of CRC patients whereas it was absent in healthy people. The comparison of oral and tumor P. micra genomes identified a pair of clones that shared >99.2% of identity, suggesting that the same clone translocated from oral to distant locations. Data indicated that P. micra cohabits with other periodontal pathogens, such as Fusobacterium at the gut, liver and periodontal pocket. We support the hypothesis that bacterial translocations could be more efficient when microorganisms form consortia and promote a transient bacteremia in the host. Moreover, RNA-seq analysis confirmed that oral microbes were more active at tumor tissues than in healthy tissues. Conclusions: P. micra translocates from the oral cavity to the gut, possibly aggregated with other pathobionts. This microbe can be considered as a CRC non-invasive biomarker due to its overabundance in CRC fecal samples. To conclude, we believe that gingivitis and periodontitis dissease could represent a high risk factor for gut cancer initiation, so we also propose that early oral treatments could contribute to reducing the incidence and prevalence of cancer development.	Kelly, Conde-Pérez1, Elena, Buetas2, Pablo, Aja-Macaya1, Elsa, Martin-De Arribas3, Iago, Iglesias-Corrás3, Noelia, Trigo-Tasende1, Mohammed, Nasser-Ali1, Lara S., Estévez4, Soraya, Rumbo-Feal1, Begoña, Otero-Alén4, Jose F., Noguera5, Ángel, Concha4, Simón, Pardiñas-López6, Miguel, Carda-Diéguez2, Igor, Gómez-Randulfe7, Nieves, Martínez-Lago7, Susana, Ladra3, Luis M.A., Aparicio7,†, Germán, Bou1, Álex, Mira2, Juan A., Vallejo1 and Margarita, Poza1,8	1meiGAbiome, Microbiology Research Group, Institute of Biomedical Research (INIBIC) – University Hospital of A Coruña (HUAC) – Interdisciplinary Center for Chemistry and Biology (CICA) – University of A Coruña (UDC) – Spanish Network for Infectious Diseases (CIBERINFEC-ISCIII). Servicio de Microbiología, 3º planta, Edificio Sur, Hospital Universitario, As Xubias, 15006, A Coruña, Spain. 2Genomic and Health Department, FISABIO Foundation, Center for Advanced Research in Public Health. Avda Cataluña 21, 46020, Valencia, Spain. 3Database Laboratory, Research Center for Information and Communication Technologies (CITIC), University of A Coruña (UDC). Campus de Elviña, 15008, A Coruña, Spain. 4Pathological Anatomy Service and Biobank, University Hospital of A Coruña (HUAC) – Institute of Biomedical Research (INIBIC). 3º planta, Hospital Universitario, As Xubias, 15006, A Coruña, Spain. 5General and Digestive Surgery Service, University Hospital of A Coruña (HUAC). Hospital Universitario, As Xubias, 15006, A Coruña, Spain. 6Periodontology and Oral Surgery, Pardiñas Medical Dental Clinic – Cell Therapy and Regenerative Medicine Group, Institute of Biomedical Research (INIBIC). Rúa Real 66, 3ª planta, 15003, A Coruña, Spain. 7Medical Oncology Department, University Hospital of A Coruña (HUAC). Maternal and Child Hospital. As Xubias, 15006, A Coruña, Spain. † R.I.P. 8Microbiome and Health Group, Faculty of Sciences, University of A Coruña (UDC). Campus da Zapateira, 15008, A Coruña, Spain.

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Comparison between 16S rRNA and shotgun sequencing data from colon cancer, adenoma, and healthy human gut microbiota	BThe gut microbiome is one active player that influences colorectal carcinogenesis. Broadly, taxonomic data is primarily obtained by metataxonomics (16S rRNA gene sequencing (16S)) and metagenomics (whole shotgun metagenomic sequencing (SGS)).The present study compares taxonomic results obtained by 16S, and SGS approaches to investigate their reliability for bacteria profiling, studying stool samples withdrawn from healthy, adenoma, and colon cancer patients. The DNA from 156 stool samples from subjects participating in a colorectal cancer screening was extracted using the NucleoSpin Soil kit. After different quality control checks, the samples were sequenced by both technologies. We compared the composition and diversity distributions obtained with the two sequencing strategies and tested their capability to distinguish the sample conditions. The 16S data were analyzed using the DADA2 workflow with SILVA v.138.1 as the taxonomical database, while the Kraken2 and Bracken2 tools were applied for SGS data using UHGG v2.0 database. The lineage abundance tables were filtered and normalized before statistical analysis. The filtered reads were rarefied (checked their rarefaction efficiency index) to calculate α-diversity through its richness (Chao1 index) and evenness (Shannon index) and compared using the Wilcoxon test. For β-diversity, the raw data were transformed using a centered-log-ratio before performing the exploratory analysis and the PERMANOVA. Moreover, co-inertia analysis was performed to measure the similarity between 16S and SGS abundance tables. Lastly, kernel-based analysis was applied to compare the subject distances calculated from each technique. All the analyses tackled from family to species rank. The results showed that 16S detects only part of the gut bacterial community revealed by SGS. In fact, the number of specific detected species was 1005 and 317 for SGS and 16S, respectively. Analogously, α-diversity was higher for SGS, given the higher resolution. Nevertheless, more than 50% of species in 16S were present in SGS, reaching up to 90% at the phylum level. The most common species detected by both techniques was F. prausnitzii, the Blautia genus and Lachnospiracaeae/Oscillospiraceae families. The strength of the relationship between 16S and SGS was statistically significant as assessed by the co-inertia and kernel analysis. Regarding β-diversity on the sample conditions, no cluster has been observed in the PCA.	David Bars-Cortina¥, Blanca Rius-Sansalvador¥, Elies Ramon¥, Ainhoa García-Serrano, Lourdes Criado-Mesas, Ferran Moratalla-Navarro, Lois Riobó-Mayo, Núria Moragas, Núria Mach, Gemma Ibáñez-Sanz, Lorena Rodríguez-Alonso, Núria Mulet-Margalef, Alfredo Mata, Ana García-Rodríguez, Silvia Murcia, Carmen Atencia, Elisabet Guinó, Toni Gabaldón, Ville Nikolai Pimenoff, Mireia Obón-Santacana*, Victor Moreno*.	

Title	Text	Authors	Author Affiliations
coda4microbiome: compositional data analysis for microbiome cross-sectional and longitudinal studies	One of the limitations of high throughput sequencing techniques for metagenomic studies is their sequencing capacity, which limits the total number of reads that can be revealed from the sample. Data constraint to a total sum is called compositional data, and such total sum restriction in metagenomic data implies a great dependence between observed taxa abundances. Specific Compositional Data Analysis (CoDA) methods allow addressing the compositional structure of microbiome data, whereas other methods that ignore its compositionality may lead to spurious results. In 1982, Aitchison introduced the so-called log-ratio approach, consisting of analysing logarithms of ratios between components instead of each component separately, which represents the basis of CoDA. We present coda4microbiome, a new algorithm for microbiome analysis within the CoDA framework for the identification of microbial signatures that best predict a given outcome. The algorithm is developed for cross-sectional and longitudinal analysis and implemented as an R Package. coda4microbiome algorithm is structured in three main steps: modelling, variable selection and reparameterization. A regression model with all pairwise log-ratios of microbial species is considered (modelling), followed by a variable selection step with elastic-net penalization that identifies those log-ratios more associated to the outcome; finally, the linear predictor of the log-ratio model is reparametrized to obtain a microbial signature written in terms of the selected bacteria, instead of pairs of bacteria (reparameterization). For longitudinal data, the modelling step is preceded by a summary of the log-ratio trajectories (the area under these trajectories), which are then introduced in the penalized regression. In this work, we illustrate the functionality of coda4microbiome for cross-sectional studies with data from a Crohn's disease (CD) study including 975 subjects (662 with CD and 313 controls) and bacterial abundance of 48 genera. The algorithm stablished the optimal penalized parameter by cross-validation and selected a total of 27 pairwise log-ratios. After reparameterization, the microbial signature contained 24 taxa, and it was expressed as a weighted balance between 11 taxa with positive coefficient (Roseburia, Bacteroides and Faecalibacterium, among others) and 13 with negative coefficient (including Dialister and Aggergatibacter). The model resulted in a mean cross-validation AUC of 0.84±0.008.	M. Luz Calle (1), Meritxell Pujolassos (1), Antoni Susin (2)	(1) Biosciences Department, Faculty of Sciences, Technology and Engineering, University of Vic – Central University of Catalonia, Carrer de La Laura, 13, 08500, Vic, Spain (2) Mathematical Department, UPC-Barcelona Tech, Barcelona, Spain

Title	Text	Authors	Author Affiliations
MycoDM: a comprehensive web resource for mycobiome database, biomarker discovery and inter-kingdom interaction	MycoDM (Mycobial Database and Markers), is a data downloading, marker discovery, and analysis web server that was constructed to provide a comprehensive, user-friendly resource for researchers in micro- and mycobiome field. MycoDM provides a tree-view download page for the FunOMICv2 database (https://manichanh.vhir.org/funomic/), which consists of more than 2 million fungal single-copy marker genes and more than 21 million fungal protein sequences. A collection of mycobial markers associated with bacterial species and related to diseases, including T1D, T2D, CD, UC, and ESRD, is another highlight feature of MycoDM. Furthermore, MycoDM displays fungal-bacterial inter-kingdom interactions from thousands of associations recovered from 1146 human shotgun metagenomes. The online analysis platform allows the users to perform basic statistical analysis and visualize the results using our built-in dataset or the user-uploaded datasets. Additionally, we also encourage potential collaborators to submit the accession of their shotgun sequencing data through the submit page on our web server. This initiative would help us improve the discovery of disease markers by constantly including new samples and increasing the cohort size.	Zixuan Xie 1,2, Xavier Martínez 3, Sara Vega-Abellaneda 1, Chaysavanh Manichanh 1,2	1 Microbiome Lab, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain 2 Departament de Medicina, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain 3 Departament d'Educació de la Generalitat de Catalunya, Via Augusta

Title	Text	Authors	Author Affiliations
Shifts in systemic inflammation after Fecal Microbiota Transplant in people with HIV	HIV can be considered a chronic inflammatory disease, driven mainly by the microbiome, that increases the risk of mortality. That long-lasting inflammation, that is present even when antiretroviral therapy (ART) is effective, contributes to increased risk of non-AIDS events. It is unclear if targeting the microbiome therapeutically can restore the original immune functionality in people with HIV (PWH) and, as a result, improve clinical outcomes. In a fecal microbiota transplant (FMT) trial in PWH, we found that Lachnospiraceae and Ruminococcaceae families were the taxa more robustly engrafted across time points. Here, we aimed to better characterize the FMT effects on systemic inflammatory pathways. METHODS. We randomized 30 PWH on ART to either weekly fecal microbiota capsules or placebo for eight weeks. Stool donors were selected based on anti-inflammatory microbiota profiles (high Faecalibacterium and butyrate). We measured the plasma expression of 368 inflammatory proteins at weeks 0, 1, 8, and 24 using the Proximity Extension Assay by Olink. We fitted mixed models to compare the protein trajectories and selected the most significant to explore their correlations with 14 bacterial taxa and 7 biomarkers of inflammation. We performed a functional analysis using Gene Set Enrichment Analysis. RESULTS. FMT resulted in a significant decrease of 54 proteins related to cytokines and leukocyte activation and increase of 2 proteins with anti-inflammatory activity compared to placebo. CCL22, CD22, FGF5, and JUN showed the most consistent pattern of decline after each FMT; CCL22, IL20, and JUN were those for which the effect lasted until week 24; and TNFRSF4 and AOC1 showed the most significant differences between groups (p=0.0003 and p=0.0004) (Figure 1A). Increases in Erysipelotrichaceae and the Bacteroides/Prevotella ratio correlated with increased CCL20 (p=1·10-7) (Figure 1B). Also, the biomarkers of inflammation sTNFR-II and IFABP have positive strong correlations with most differentially expressed plasma proteins analysed (Figure 1C). CONCLUSIONS. FMT from selected donors resulted in a shift towards lower inflammation through effect in proteins related to cytokines and leukocyte activation. CCL20 is a promising target to further explore its relationship with the microbiome and the inflammation processes. Our results support that the microbiome is a viable target to modulate systemic inflammation during treated HIV.	Claudio Díaz-García*1,2,3, Elena Moreno1,3, Alba Talavera,1,3, José A. Pérez-Molina1, Fernando Dronda1,3, María José Gosalbes1, Jorge Diaz1,3, Javier Martínez-Sanz1,3, Raquel Ron1,2,3, María Jesús Vivancos1,3, Santiago Moreno1,2,3, Sergio Serrano-Villar1,3	1 Department of Infectious Diseases, Hospital Universitario Ramón y Cajal, IRYCIS, 28034, Madrid, Spain 2 Universidad de Alcalá, Department of Medicine, 28871, Madrid, Spain 3 CIBERINFEC, Instituto de Salud Carlos III, 28029, Madrid, Spain *Presenting author

Title	Text	Authors	Author Affiliations
Comparison of the gut microbiota of Spanish schoolchildren carriers and non-carriers of Extended-Spectrum ß-Lactamases Producing Enterobacterales	Extended Spectrum ß-lactamases producing Enterobacterales (ESBL-E) have been identified by the WHO among the top pathogens of concern presenting resistance to antibiotics. ESBL-E, colonize the intestinal tract, and are able to cause infections or transfer resistance to other commensal microorganisms. The number of ESBL-E infections in children has increased in recent years but the impact of ESBL-E carriage on their intestinal microbiota remains unknown. The aim of this study was to analyze the composition of the gut bacterial community in children that are ESBL-E carriers and non-carriers and assess whether it is altered by the presence of this group of resistant bacteria. Methodology Fecal samples from 24 ESBL-E carriers and 24 non-carriers (3-11 years old) were collected. In all but one case the colonising strain was Escherichia coli. The bacterial community was analyzed by amplifying and sequencing the V3-V4 region of the 16S rRNA gene by Illumina MiSeq. Richness and diversity indices, relative abundance of taxa at different taxonomic levels of clr-transformed data were assessed by the Wilcoxon test, and generalized linear models were applied to identify differentially abundant species among ESBL-E carriers and non-carriers. Abundance of E. coli was assessed by quantitative PCR. Results Richness (Sobs = 21-55) and diversity (Shannon = 2.57-3.69, Simpson = 0.89-0.97) of the bacterial community were similar in ESBL-E carriers and non-carriers. Regarding composition, we found no significant differences in abundance at the phylum, class and order levels, but generalized linear models identified five species with a significantly different abundance between groups. Three of these species were more abundant in ESBL-E carriers, while two of them were more abundant in the non-carriers. E. coli abundance in children colonized by ESBL-E tend to be higher than in those not colonized, but without reaching statistical significance (p = 0.119). Conclusions This study revealed that ESBL-E carriage is not associated with a major shift in α-diversity nor in ß-diversity but a differential microbiological signature between ESBL-E carriers and non-carriers has been identified. Further longitudinal studies are required to establish if these changes predispose to or are consequence of ESBL-E carriage, and if modulation of the gut microbiota may prevent or eliminate ESBL-E colonization.	Martina Cardinali 1,2,3, Olfat Khannous-Lleiffe 1,2, Ester Saus 1,2,3, David Carmena3,4, Michael J. McConnell 5, Toni Gabaldón 1,2,3,6, Mireia López-Siles 7	1. Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain 2. Barcelona Supercomputing Centre (BSC-CNS), Institute for Research in Biomedicine (IRB), Barcelona, Spain 3. Centro de Investigación Biomédica En Red de Enfermedades Infecciosas (CIBERINFEC), Madrid, Spain. 4. Parasitology Reference and Research Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III (ISCIII), Madrid, Spain 5. Intrahospital Infections Unit, Reference and Research Laboratory in Resistance to Antibiotics and Infections related to Healthcare, National Centre for Microbiology, Instituto de Salud Carlos III (ISCIII), Madrid, Spain 6. ICREA, Barcelona, Spain. 7. Microbiology of Intestinal Diseases, Biology Department, Universitat de Girona, Girona, Spain

Title	Text	Authors	Author Affiliations
Dysbiosis index from gut and vaginal microbiomes at recurrent urinary tract infection	Urinary tract infections (UTIs) are among the most commonly occurring infections. The uropathogens responsible for UTIs are known to originate from the gut microbiota and subsequently colonize the bladder through the vaginal tract. In approximately 10-20% of patients, UTIs can develop into recurrent urinary tract infections (rUTIs), which are characterized by at least two relapses per year. Unfortunately, the current diagnostic methods for rUTIs may take up to one or two years to confirm a case. Methodology This study aimed to compare the gut and vaginal microbiota of 71 women, 37 with rUTI (patients) and 34 without (controls), by using shotgun sequencing. The reads were classified into operational taxonomic units (OTUs) with GAIA software, a bioinformatics tool from Sequentia Biontech, and analyzed in R using the SELBAL package to assign a dysbiosis index. The samples were collected during a UTI-free period. Results The gut microbiome model was examined to identify significant taxa for calculating the dysbiosis index. Fiver such taxa were identified, and the dysbiosis index was calculated as the ratio of Candidatus Melainabacteria, Lachnospira and Coproccus in the numerator and Strepteococcus and Romboutsia in the denominator. The median dysbiosis index values were 2.92 for the patient group, vs. 0.47 for control group. Our model demonstrated potential as a discriminatory tool between the two groups, as evidenced by an area under the curve (AUC-ROC) of 0.908 (Fig1). The vaginal microbiome was also analyzed to identify for the dysbiosis index, with two taxa being the optimal number to differentiate between the two groups. The numerator was represented by Achromobacter and the denominator by Bacillus. The median dysbiosis index for the patient group was 1.8, while for the control group it was -4.3. Our model demonstrated high accuracy in classifying both groups, with an area under the curve (AUC-ROC) of 0.939 (Fig2). These results suggest that the dysbiosis index has the potential to serve as a useful biomarker for differentiating between rUTI patients and controls. Conclusions These results could represent a promising first step towards reducing the time required to diagnose rUTI, potentially decreasing the current diagnostic delay from 1-2 years to 1-2 weeks. Further studies are needed to confirm these findings and validate the use of the dysbiosis index as a reliable biomarker for rUTIs.	A. Cruells Gascon 1, M. Rubio Bueno 1, L. Mateu Arrom 2, C. Errando-Smet 3, A. Paytuví-Gallart 4, W. Sanseverino 4, N. Luqui Scarcelli 5, J. López-Contreras 6, C. Vanrell Barbat 5, C. Alonso-Tarrés 7, E. Miro Cardona 1, F. Navarro Risueño 1.	1Microbiology, Hospital de la Santa Creu i Sant Pau, Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona – Barcelona (Spain), 2Dermatology, Hospital de la Santa Creu i Sant Pau, Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona – Barcelona (Spain), 3Functional and Female Urology Unit, Puigvert Foundation – Barcelona (Spain), 4Sequentia Biotech SL – Barcelona (Spain), 5Gynaecology, Hospital de la Santa Creu i Sant Pau, Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona – Barcelona (Spain), 6Infectious Diseases Unit, Hospital de la Santa Creu i Sant Pau, Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona – Barcelona (Spain), 7Microbiology, Puigvert Foundation – Barcelona (Spain)

Title	Text	Authors	Author Affiliations
Genetic domestication of Cutibacterium acnes for enhanced skin probiotics	Recent advances in synthetic biology offer exciting opportunities to engineer the microbiome as a platform for developing novel smart living therapies that can selectively target and modulate the host response. While many studies focused on modifying the gut microbiome for targeting metabolic disorders or similar conditions, the engineering of the skin microbiome remains largely unexplored. However, chronic inflammatory skin diseases, which affect approximately 250 million people globally, have proven to be challenging to treat with current therapies often causing unwanted side effects. Hence, engineering the skin microbiome is a promising strategy that could allow for in situ production of therapies that are triggered only upon specific signals. Methodology Cutibacterium acnes, the most abundant skin commensal, is a promising candidate for skin probiotic engineering as it colonizes sebaceous pores and shows high engraftment upon topical application. Despite its suitability as chassis for genetic engineering, no genetic tools have been described for this organism. To solve this, we have developed a modular cloning library based in the popular Gibson assembly and performed RNA-seq to adapt the natural transcriptional response of C. acnes to develop both constitutive and inducible promoters among other genetic tools. Results We validated a new set of genetic tools for C. acnes, including 6 fluorescent reporters, 4 RBSs, and 10 constitutive promoters among others. Moreover, we repurposed natural genetic regulatory elements to create inducible promoters that detect oxidative stress and high temperatures. We validated the use of these tools in 3D human skin models, showing its applicability for microscopy imaging without affecting bacterial growth. Finally, we used these set of tools for the production and secretion of antioxidant molecules with therapeutic potential. Conclusion In this study, we enabled the use of C. acnes as a tool for skin microbiome research and, remarkably, as a platform for the development of enhanced skin probiotics with the potential to monitor and treat skin diseases.	Guillermo Nevot1, Javier Santos-Moreno1, Lorena Toloza1, María José Fábrega1, Nastassia Knödlseder1, Ellen van der Boogard2 and Marc Güell1	1Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain 2Department of Dermatology, Radboud University Medical Center (Radboudumc), Radboud Institute for Molecular Life Sciences (RIMLS), Nijmegen, The Netherlands

Title	Text	Authors	Author Affiliations
Effect of in vitro gastrointestinal digestion on the ACE inhibitory activity and the modulation of gut microbial populations of protein hydrolysates	It has been evidenced that gut microbiota is involved in the development of non-communicable diseases including hypertension. However, their relationship and underlying mechanisms remain largely unknown. The global incidence of this chronic disease is high and continuously increasing. Therefore, new preventive methods are required. In this regard, the development of new natural compounds, such as bioactive peptides, has emerged as a potential alternative, mainly those able to inhibit the angiotensin-converting enzyme (ACE). This study aimed to evaluate the ability of different protein hydrolysates to modulate in vitro microbial populations and ACE inhibitory (ACEi) activity, considering the influence of gastrointestinal digestion on their bioactivity. The antihypertensive effects of the hydrolysates were also studied. Twenty hydrolysates were obtained from three food industry byproducts using different food-grade enzymes and hydrolysis conditions. Hydrolysates were subjected to an in vitro simulation of gastrointestinal digestion using the Infogest method. ACEi activity was determined in the hydrolysates before and after gastrointestinal digestion. Both types of samples were also fermented with fecal human microbiota for 24 h, and the bacterial composition was analyzed by qPCR. The antihypertensive effects of the three best hydrolysates were evaluated in spontaneously hypertensive rats after acute administration (55 mg/kg). All the hydrolysates showed high ACEi activity (>90%), which was not affected by their in vitro gastrointestinal digestion. Moreover, the different hydrolysates presented a differential ability to modulate microbial populations, and gastrointestinal digestion changed this modulation in most cases, either increasing or decreasing, depending on the population analyzed. Three hydrolysates were selected according to their bioactivity to evaluate their antihypertensive effects. One of these stood out for its ability to reduce systolic blood pressure in hypertensive animals, mainly in the first 8h post-administration. In conclusion, in vitro gastrointestinal digestion affected the bioactivity of the hydrolysates, and this step should be considered when selecting bioactive peptides in vitro. Protein hydrolysates may be useful for modulating microbial populations and reducing blood pressure; however, further research is needed to fully understand the mechanisms involved in the interaction between the gut microbiota and hypertension development.	Rafael A. López-Villalba1,2,*, Adrià Martínez-García 1, Fabiola Garcia-Reyes1,2, Manuel Suárez1,2, Cristina Torres-Fuentes1,2 and Francisca Isabel Bravo1,2.	1Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Marcel.li Domingo 1, 43007, Tarragona, Spain. 2Nutrigenomics Research Group, Institut d'Investigació Sanitària Pere Virgili. C/ Marcel·lí Domingo s/n 43007 Tarragona, Spain

Title	Text	Authors	Author Affiliations
Lack of p38γ and p38δ MAPKs in myeloid cells controls gut microbiome composition and affects tumour development in a colitis-associated colorectal cancer mouse model	Colorectal cancer is the third most common cancer worldwide. Patients suffering from inflammatory bowel diseases, such as ulcerative colitis or Crohn's disease, are at major risk for developing colitis-associated colorectal cancer (CAC). Gut microbiome and its interaction with immune cells play a critical role in inflammation and tumour development regulation in CAC. The activity of these cells is mediated by components of inflammatory molecular pathways as the p38 mitogen-activated protein kinases (MAPK) members p38γ and p38δ. Genetically modified female mice lacking p38γ and p38δ in myeloid cells (LysMCre/+p38γ/δf/f) and their control littermates (p38γ/δf/f), where housed separately (single-housed; SH) or together (co-housed; CH) to facilitate microbiota transfer. After 21 days, mice were subjected to the azoxymethane (AOM)/dextran sodium sulphate (DSS) mouse model to induce CAC, which consists of an initial injection of the mutagen AOM (day 0) followed by 3 cycles of 2 % DSS in drinking water for 5 days, in order to induce chronic inflammation in the colon. Faecal samples were collected at day -1, 40 and 77 of the treatment and used for the analysis of the gut microbiome composition by 16S rRNA sequencing of the V3-V4 region. Mice were sacrificed at day 80 of the treatment and tumour number and volume were determined. Additionally, infiltration of inflammatory cells in the tumours was analysed by flow cytometry. Our results show that SH and CH LysMCre/+p38γ/δf/f mice have less tumour burden than SH p38γ/δf/f mice, while CH p38γ/δf/f mice have similar tumoral development compared to SH and CH LysMCre/+p38γ/δf/f mice. Gut microbiota populations analysis showed that SH LysMCre/+p38γ/δf/f mice have different gut microbiota composition compared to SH p38γ/δf/f mice, while CH LysMCre/+p38γ/δf/f and p38γ/δf/f animals had a unified pattern of gut microbiota composition, that is similar to the SH LysMCre/+p38γ/δf/f mice. Also, some bacteria taxa that are differentially distributed in CH p38γ/δf/f and SH and CH LysMCre/+p38γ/δf/f, in comparison with SH p38γ/δf/f mice, correlate with tumour burden. Thus, mice lacking p38γ/δ in myeloid cells (LysMCre/+p38γ/δf/f) have less tumour burden than p38γ/δf/f in the AOM/DSS murine model. This decrease in tumour development is linked to a differential gut microbiome composition in LysMCre/+p38γ/δf/f mice. This protective effect can be transferred to p38γ/δf/f mice thanks to cohousing-mediated horizontal microbiota tran	Daniel Mora-Diego; Pilar Fajardo; Ester Díaz-Mora; Juan J Sanz-Ezquerro; Ana Cuenda	Spanish National Center for Biotecnology (CNB)

Title	Text	Authors	Author Affiliations
GSR-DB: a manually curated and optimised taxonomical database for 16S rRNA amplicon analysis	Amplicon-based 16S ribosomal RNA sequencing remains the most widely used method to profile microbial communities, as a low-cost and low-complexity approach. Reference databases are a mainstay for taxonomic assignments, which typically rely on popular databases such as SILVA, Greengenes, GTDB, or RDP. However, the inconsistency of the nomenclature across databases, and the presence of shortcomings in the annotation of these databases are limiting the resolution of the analysis. To overcome these limitations, we created the GSR database (Greengenes, SILVA, and RDP database), an integrated and manually curated database for bacterial and archaeal 16S amplicon taxonomy analysis. Unlike previous integration approaches, this database creation pipeline includes a taxonomy unification step to ensure consistency in taxonomical annotations. The database was validated with three mock communities and two real datasets and compared with existing 16S databases such as Greengenes, GTDB, ITGDB, SILVA, RDP, and MetaSquare. Results showed that the GSR database enhances taxonomical annotations of 16S sequences, outperforming current 16S databases at the species level. The GSR database is available for full-length 16S sequences and the most commonly used hypervariable regions: V4, V1-V3, V3-V4, and V3-V5.	Leidy Alejandra G. Molano 1, Sara Vega-Abellaneda 1 , and Chaysavanh Manichanh 1,2	1 Microbiome Lab, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain 2 Medicine department, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

Title	Text	Authors	Author Affiliations
Exploring the Correlation between Gut Microbiome Composition and Mental Health Outcomes in a Spanish Population	The COVID-19 pandemic has resulted in a significant increase in the prevalence of anxiety and depression. Addressing these complex disorders requires the identification of all potential factors that contribute to their development and progression in order to identify novel treatment strategies. The aim of this study was to investigate the fecal microbial features associated with mental health outcomes in a Spanish cohort in the aftermath of the COVID-19 pandemic. Methods: We performed amplicon sequencing of the V3-4 region of the 16S ribosomal RNA gene on DNA extracted from fecal samples of our study participants. Subsequently, we analyzed microbial diversity and community structure, as well as relative taxonomic abundance Results: In our cohort of 198 individuals, the prevalence of anxiety symptoms was the highest, with 37.37% reporting state anxiety symptoms and 40.90% reporting trait anxiety symptoms. Depressive symptoms were reported by 17.17% of participants, while 8.08% reported symptoms of PTSD. There was a high level of comorbidity between these symptoms. Simpson's diversity index was found to be lower in individuals with trait anxiety. Moreover, individuals with comorbid symptoms of PTSD, depression, state, and trait anxiety showed lower levels of Fusicatenibacter saccharivorans. In contrast, those with depressive symptoms exhibited an expansion of Proteobacteria and depletion of Synergistetes phyla. Notably, childhood trauma was positively correlated with the abundance of Anaerostipes, while individuals who experienced life-threatening traumas had a higher abundance of Turicibacter sanguinis and lower levels of Lentisphaerae. Furthermore, the overall microbial composition was influenced by COVID-19 infection and vaccination and was associated with distinct relative taxonomic abundance profiles. Conclusion: These findings provide valuable insights into the potential microbial role players in symptoms of anxiety, depression, and PTSD and could serve as future therapeutic targets for improving mental health outcomes.	Stefanie Malan-Müller	Department of Pharmacology and Toxicology, Faculty of Medicine, University Complutense Madrid (UCM), Madrid, Spain; Biomedical Network Research Center of Mental Health (CIBERSAM), Institute of Health Carlos III, Madrid, Spain

Title	Text	Authors	Author Affiliations
Genetic epidemiology to study the gut-brain axis in outbred laboratory rats	Epidemiological studies suggest a gut-brain axis exists whereby gut and brain functions are interconnected and the gut microbiota influences behaviour. However, epidemiological studies are prone to confounding from environmental and genetic factors. In order to limit the extent of confounding and to leverage host genetic variants to infer causality, we investigated the gut-brain axis in 4,004 genetically heterogeneous rats that were extensively phenotyped, including many measures relevant to drug-abuse disorders. The population of rats we used is descended from eight inbred founder strains through more than eighty generations of outbreeding, hence their chromosomes are highly recombined and (host) genetic effects can be finely dissected. We characterised the microbiome of 4,004 rats using 16S sequencing. Over 400 measures relevant to drug-abuse were collected in each rat, as well as multiple other physiological and metabolic phenotypes, with the phenotyping occuring in three different phenotyping centers. Finally, all rats were genotyped by sequencing. Using the 16S data we characterised the taxonomic profiles of each microbiome. We quantified the heritability of the microbiome and compared with the heritability of the behavioural and non-behavioural phenotypes collected in the same rats. We then mapped all microbial traits and identified “robust” loci associated with the microbiome in more than one phenotyping center and loci associated with unrelated bacterial taxa. We estimated genetic correlations and carried out colocalisation and mediation analyses to investigate the causal relationship between the microbiome and host phenotypes. We found that up to 31% of microbial traits were heritable in this dataset. Heritability for microbial traits was generally lower than for non-behavioural and even behavioural phenotypes. We identified multiple “robust” loci affecting microbial traits, some of which encompass only a few genes in their 95% confidence interval. We found significant phenotypic and genetic correlations between microbial traits and organismal phenotypes of the host, as well as microbiome-associated loci co-localising with loci affecting organismal phenotypes. Mediation analysis, however, did not provide evidence of causal links for the loci studied so far, suggesting genetic effects may independently affect microbiome traits and host phenotypes.	Amelie Baud, Oksana Polesskaya, NIDA P50 Centre for GWAS in Outbred Rats, Rob Knight, Abraham Palmer	AB: Centre for Genomic Regulation, Barcelona, Spain OP, RK and AP: University of California San Diego, USA

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Multi-Omics Analyses Characterize the Gut Microbiome and Metabolome Signatures of the Paraquat-Induced Oxidative Stress Model in Male Wistar Rats	Oxidative stress disrupts homeostasis and can lead to major health conditions in humans. Current biomarkers detect oxidative stress but often lack the sensitivity to detect it before the onset of disease. By characterising the metabolic and microbial profile associated with oxidative stress, this imbalance can be monitored and detected earlier. To determine the metabolic effects of oxidative stress, male Wistar rats were subjected to a single intraperitoneal injection of paraquat (PQ) of 15 or 30 mg/kg. To confirm the validity and characterize the model, current biomarkers of oxidative stress were measured in plasma, urine and the liver at the end of the study. In addition, the omics' profile was assessed; GC-qTOF and UHPLC-qTOF were performed to evaluate the plasma metabolome; 1H-NMR to evaluate the urine metabolome; and shotgun metagenomics sequencing to analyse the gut microbiome. PQ induced an alteration of the redox mechanism characterised by an increase in glutathione reductase. Furthermore, isoprostane levels, a current biomarker of oxidative stress, correlated with several metabolites and microbiota. Within the metabolome 3-hydroxibutiric acid, sphingomyelins (SMs) and lysophospholipids (LPCs) were altered in a dose-dependent manner in all groups, presenting as potential alternative signature for monitoring oxidative stress. Thus, β-oxidation of fatty acids together with other related lipid pathways are important in this experimental approach. Additionally, disrupted tricarboxylic acid (TCA) cycle and one-carbon metabolism seems to play a key role in this metabolic profile. Analysis of the microbiota demonstrates a shift in microbial composition under oxidative stress conditions, resulting in dysbiosis and the loss of microbial species. When considering relative abundances, Bacteroidetes expanded at the expense of Proteobacteria and Firmicutes, under conditions of oxidative stress. Severe oxidative stress resulted in a complete composition change where Proteobacteria dominated, along with the expansion of Verrucomicrobia and the almost complete loss of Bacteroidetes. Species that are more tolerant to these conditions, such as Escherichia coli and Akkermansia muciniphila are able to persist. A. muciniphila may even produce advantages effects combating the disruption of homeostasis. These findings provide an overview of the metabolite and gut microbiota signature associated with oxidative stress.	Julia Hernandez-Baixauli [1,2], Harry Tracey [1,3,4], Antonio J Cortés-Espinar [5], Nerea Abasolo [6], Hector Palacios-Jordan [6], Elisabet Foguet-Romero [6], David Suñol [7], Mar Galofré [7], Josep M del Bas [8*], Miquel Mulero [5*]	[1] Eurecat, Centre Tecnològic de Catalunya, Unitat de Nutrició i Salut, Reus, 43204, Spain [2] Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, 43007 Tarragona, Spain [3] Department of Medical Sciences, School of Medicine, University of Girona, 17004 Girona, Spain [4] School of Science, RMIT University, Bundoora, VIC, Australia [5] Nutrigenomics Research Group, Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, 43007 Tarragona, Spain [6] Eurecat, Centre Tecnològic de Catalunya, Centre for Omic Sciences (COS), Joint Unit Universitat Rovira i Virgili-EURECAT, 43204 Reus, Spain [7] Eurecat, Centre Tecnològic de Catalunya, Digital Health, 08005 Barcelona, Spain [8] Eurecat, Centre Tecnològic de Catalunya, Àrea Biotecnologia, Reus, Spain

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Tumor-associated microbiome composition and response to neoadjuvant chemotherapy (NACT) in early triple negative breast cancer (TNBC)	Background: Breast cancer-associated microbiome and its role in treatment efficacy are poorly understood. We aimed to study tumor-associated microbiome composition in TNBC, its dynamics upon NACT, and its correlation to TNBC outcomes. Methods: Diagnostic biopsies from patients with TNBC and unpaired samples from surgery after NACT were selected. A 16S rRNA subunit gene analysis was performed to describe α-diversity using the Shannon Index and study taxonomic profiles at genus levels. To control for differences related to the type of sample, we also analyzed diagnostic biopsies and surgical samples from patients that had upfront surgery without NACT. Results: We analyzed 115 TNBC samples from: i) Diagnostic biopsies (n=87) either from patients that had subsequent NACT (n=48, mean age 57) or from patients that had upfront surgery (n=39, mean age 78); ii) Surgery samples (n=28) from patients with prior NACT (n=14, all unpaired; mean age 47) and from patients with upfront surgery (n=14, all paired with diagnostic biopsies; mean age 85). α-diversity was significantly lower in post-treatment surgery samples compared to pretreatment biopsy samples (p=0.03), with a significant reduction of Prevotella relative abudance (padj=0.03) increase of relative abundance of Paracoccus (padj=0.03). Abundance of Capnocytophaga were significantly higher among patients that did not achieve pCR (padj <0.001) whereas higher abundance of Lawsonella and Coprococcus was observed among patients with pCR (padj<0.001). In patients receiving NACT, increased microbiome diversity at baseline correlated to worse Event-Free Survival (EFS, Hazard Ratio [HR] = 1.064; 1.036 – 1.093, p=0.001) and Overall Survival (OS, p=0.014). At genus level, abundance of Prevotella at baseline, significantly correlated to EFS and Eusobacterium abundance related to improved EFS and OS. Abundance of Blautia and Rubellimicrobium and lower abundance of Acinetobacter was significantly higher among patients with relapse (padj<0.001). Biopsy samples correlated to an increased number of genera compared to surgery samples (p<0.05). Conclusions: Our results suggest that in TNBC, NACT has a significant impact in microbiome composition and some genera are correlated to outcomes. The type of sample (biopsy / surgical specimen) must be taken into account in breast cancer-associated microbiome studies. This work provides the rationale for expanding microbiome analysis in order to find novel putative biomarkers of response	Andri Papakonstantinou (1-3), Stefania Napoli (4), Laia Joval(5), Maria Butjosa-Espin (6), Alba Mas Malavila (7), María Borrell (1,8), Isabel Pimentel (1,8), Esther Zamora (1,8), Pedro Gonzales Torres (9), Nerea Carron Rodas (9), Cristina Saura (1,8), Jose Antonio Seoane (6), Lara Nonell (7), Paolo Nuciforo (4), Mafalda Oliveira (1,8**)**	1. Breast Cancer Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain 2. Department of Oncology-Pathology, Karolinska Institutet and University Hospital, Stockholm, Sweden 3. Division of Breast cancer, Endocrine tumors and Sarcoma, Karolinska University Hospital, Stockholm, Sweden 4. Molecular Oncology Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain 5. Oncology Data Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain 6. Cancer Computational Biology Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain 7. Bioinformatics Unit, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain 8. Medical Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain 9. Microomics Systems S.L. Barcelona, Spain


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