Alpha Diversity Analysis of Microbiota Dysbiosis in Normal and Colorectal Cancer of Mice Feces

Muhammad Iqbal[1,*], M. Iqbal Rivai[2], Rini Suswita[2], Irwan[1] and Avit Suchitra[1]

[1]*Anatomy Department, Faculty of Medicine, Andalas University, Indonesia*

[2]*Division of Digestive Surgery, Surgery Department, Faculty of Medicine, Andalas University, Indonesia*

Abstract: *Background*: Colorectal cancer development is influenced by both environmental and genetic factors, with the gut microbiota playing a significant role. This research investigates how alterations in gut microbiota are associated with the incidence, progression, prognosis, and early detection of CRC.

Methods: An experimental laboratory study was carried out using Sprague Dawley rats that were induced with azoxymethane (AOM) and Dextran Sodium Sulfate (DSS). The thirty rats were divided into three groups: normal, cancer-induced, and treatment. The fecal microbiota profiles were examined through Next Generation Sequencing (NGS), and the data were analyzed for alpha diversity, highlighting the dynamics of the microbial community.

Results: The cancer-induced group (K2 Plus) exhibited the highest microbial diversity across Shannon, Simpson, Chao1, and PD Whole Tree indices, while the treatment group (P2 Plus) demonstrated the lowest.

Conclusion: These findings suggest that the increase in diversity observed in cancer-induced mice reflects disruption of community stability and blooming of pathobionts. Conversely, treatment with *Lactococcus lactis D4* reduced diversity, potentially by selectively suppressing pro-inflammatory or pathogenic taxa, indicating a beneficial probiotic effect in mitigating dysbiosis associated with colorectal cancer.

Keywords: Colorectal cancer, gut microbiota, Next Generation Sequencing, dysbiosis, alpha diversity.

INTRODUCTION

Colorectal cancer (CRC) is a prevalent form of cancer that affects individuals globally and represents a major public health issue. The rates of CRC incidence and mortality show significant variation across different countries and regions, closely linked to the socioeconomic status of those areas. Regions with higher income levels typically report a greater number of new cases and fatalities, whereas lower-income areas tend to have fewer instances of this disease [1].

Environmental and genetic factors are the main drivers of CRC, leading to tumor formation in the epithelial cells of the colon and rectum. Among the environmental risk factors, the gut microbiota has been identified as a contributor to the development of CRC. Growing evidence indicates that gut microbiota significantly influences the onset, progression, and spread of CRC. Numerous studies have established a link between pathogenic bacteria and CRC, with *Streptococcus* infection being noted as a potential indicator of increased risk for CRC [2]. Furthermore, whole-genome sequencing has revealed elevated levels of *Fusobacteria* sequences in tissues affected by colorectal carcinoma. Other research has detected the presence of *Enterotoxigenic Bacteroides fragilis* and *Fusobacterium nucleatum* in CRC tissues, with the latter being linked to increased microsatellite instability [3, 4]. In addition, research has shown that *E. coli* from phylogroup B2 is more frequently present in colorectal cancer tissues, producing cyclomodulin, which is crucial for mutations in the epithelial cells of the large intestine. Moreover, Zhao *et al*. analyzed fecal samples from patients with CRC and found elevated levels of *Bacteroides fragilis, Enterococcus, Escherichia/ Shigella, Klebsiella, Streptococcus,* and *Peptostreptococcus* [5, 6].

Colorectal cancer is a multifaceted disease influenced by multiple factors, with its development involving several mechanisms, such as dysplasia and inflammation. The progression of CRC linked to inflammatory bowel disease (IBD-CRC) follows a well-established pathway referred to as the 'inflammation-dysplasia-carcinoma' sequence [7]. This sequence describes the transition from persistent inflammation to dysplasia and eventually to carcinoma. In relation to IBD, growing evidence indicates that IBD-CRC may arise and progress through a unique tumorigenic pathway, differing from that of sporadic colorectal cancer [8]. Persistent inflammation, a defining characteristic of IBD, is associated with the onset of CRC via several proinflammatory pathways. The inflammatory environment present in IBD fosters conditions that facilitate tumor development by activating these pathways, which are crucial for the initiation and advancement of CRC. Additionally, the

*Address correspondence to this author at the Anatomy Department, Faculty of Medicine, Andalas University, Indonesia; E-mail: dr.m.iqbal92@gmail.com

altered immune responses and immunological processes within the inflamed colonic mucosa significantly contribute to the carcinogenesis linked to inflammation [9].

Research on colorectal cancer has been conducted using various cancer cell lines and multiple animal models to explore its molecular mechanisms, as well as potential preventive and treatment strategies. Colitis-associated CRC is a thoroughly examined condition characterized by the emergence of colon tumors in individuals with ulcerative colitis. To replicate this disease in animal studies, scientists often utilize a combination of azoxymethane (AOM) and dextran sodium sulfate (DSS) to provoke significant colonic inflammation that leads to tumor development. The process of administering AOM followed by DSS results in colonic inflammation and the formation of several tumors, mirroring the progression observed in human cases [10]. ALOX15, known for its role in suppressing inflammation and cancer, has been shown to impede the rapid progression of colorectal tumor development induced by AOM in the context of DSS-induced colitis when it is expressed in the epithelial cells of the colon [11]. Moreover, research indicates that the AOM/DSS model is extensively utilized to study colorectal carcinogenesis associated with inflammation, where AOM induces tumors in the context of an inflammatory trigger provided by DSS [12]. In addition, the AOM/DSS model has played a crucial role in assessing the protective effects of different substances against inflammation and tumor development in CRC [13].

The AOM/DSS model has been essential for elucidating the mechanisms involved in colitis-associated colon cancer. This approach entails administering AOM followed by multiple doses of DSS, yielding important information about the regulation of this type of cancer [14]. Additionally, the AOM/DSS model has been employed to investigate the impact of various interventions, including dietary supplements and pharmaceutical agents, on the progression of colitis-associated cancer [15, 16]. Investigators have examined the functions of different proteins and signaling pathways, including lipin-1 and the Nrf2/ Keap1 pathway, in relation to colorectal cancer induced by the AOM/DSS model [17, 18]. Furthermore, the AOM/DSS model has been utilized to assess how various dietary elements, including foxtail millet and cocoa, influence colitis-associated colorectal cancer [15, 18].

The relationship between inflammation and dysplasia in the development of CRC is highlighted by the role of the gut microbiome. Research indicates that the microbiome can modify the inflammatory environment in the gut, which in turn influences the onset and advancement of CRC linked to colitis [19].

Considering the link between gut microbiota and overall health, it is important to examine how alterations in gut microbiota relate to the onset, progression, prognosis, and early identification of diseases. Most research on gut microbiota relies on fecal samples, which are non-invasively collected and thought to represent variations in the colon's microbiota. Several studies have identified shifts in the fecal microbiome of CRC patients. Consequently, fecal analysis can serve as a screening tool for assessing CRC risk, facilitating timely interventions and improving clinical outcomes. Reliable screening biomarkers that enable early detection can significantly decrease CRC-related mortality. Alongside conventional invasive endoscopic methods, various noninvasive tools for early CRC screening are available, including commonly used fecal immunochemical tests, which have proven effective in lowering CRC rates and mortality. Nonetheless, this method remains controversial because of its comparatively low sensitivity. As a result, there is a pressing need for effective, safe, cost-effective, and highly sensitive non-invasive screening options for CRC [20]. Some studies have shown the potential to combine fecal microbiota data with fecal immunochemistry tests to improve CRC detection [21].

This study aims to analyze the alpha diversity of gut microbiota in relation to the occurrence, development, and prognosis of CRC, as well as to explore its potential for early detection in colitis-associated CRC.

MATERIALS AND METHODS

This research was conducted as a laboratory experimental study employing a Randomized Control Group Posttest-Only Design, targeting Sprague Dawley rats aged 6 to 7 weeks. The experiment took place at Ina Lab Laboratory between May and October 2023. The inclusion criteria specified male Sprague Dawley rats weighing between 170 and 220 grams that displayed signs of colitis in the colon. Rats were excluded from the study if they experienced substantial weight loss, showed visible signs of illness or death, or had any anatomical irregularities.

The ethical considerations for this study received approval from the Health Research Ethics Committee at the Faculty of Medicine, University of Andalas, under approval number 79/UN.162.KEP-FK/2023.

Animals

The rats were divided into three groups: the normal rat group (K0), which included rats that were neither induced nor treated; the cancer rat group (K2 Plus), consisting of rats that were induced with AOM and DSS until cancer developed; and the treatment rat group (P2 Plus), which comprised cancer-affected rats that received treatment with *Lactococcus lactis* D4.

Preparation of *Lactococcus lactis* D4

Lactococcus lactis D4 was obtained from Dadih, a type of fermented buffalo milk stored in bamboo tubes, as noted by Sukma (2017), and was later produced at the Faculty of Animal Science, Universitas Andalas. The bacteria were cultivated using the streak quadrant technique on MRS agar plates. To reduce the risk of contamination, the cultivation plates were tightly sealed before being incubated at 30°C for 48 hours. After the incubation period, the bacteria were prepared for inoculation by using an aseptic needle to collect a bacterial suspension from the agar, which was then added to 10 ml of MRS Broth and incubated at 30°C for an additional 24 hours.

After completing the inoculation process, the L. lactis D4 culture was placed into microtubes, each holding 1 ml of the culture. Following this, centrifugation was performed to separate the supernatant from the pellet, and the supernatant was carefully discarded.

Sample Collection

A total of 30 Sprague Dawley rats, each weighing between 170 and 220 grams, were acclimatized in the laboratory for one week. Out of these, 20 rats were induced with intraperitoneal AOM at a dosage of 10 mg/kgBW and received 2.5% DSS for five days during the first week of the study. At the start of the third week, two rats were euthanized using ether anesthesia, and their colon tissues were prepared histopathologically by a qualified anatomical pathologist to check for cancer presence. If cancer was confirmed, fecal samples were collected from the positive control group. Following this, ten rats from the treatment group were given *Lactococcus lactis* D4 rectally at a concentration of 8×10^9 CFU/mL for 14 days, after which fecal samples were collected from this group as well. Fecal samples of 5 mg each were then gathered from all groups for microbiota analysis of the intestinal contents using Next Generation Sequencing (NGS).

Statistical Analysis

Sequencing data were processed using the Novogene 16S Amplicon QIIME1 pipeline. Raw paired-end Illumina reads (PE250) were merged and filtered to obtain Clean Tags. Chimeric sequences were identified and removed to produce Effective Tags for downstream analysis. Operational Taxonomic Units (OTUs) were clustered at 97% sequence identity using the UPARSE algorithm, and representative sequences for each OTU were taxonomically annotated against reference databases (QIIME v1.9.1). Alpha diversity was assessed using multiple indices, including Observed Species, Shannon, Simpson, Chao1, ACE (Abundance-based Coverage Estimator), Goods Coverage, and PD Whole Tree. Biodiversity curves (rarefaction and rank abundance curves) were also generated to evaluate sequencing depth and richness.

Statistical significance of differences in microbial community structures among groups was evaluated using ANOSIM, MRPP, and related non-parametric tests as implemented in QIIME. Environmental factor correlations were further assessed using Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA) where appropriate.

RESULTS

The findings of this study are displayed through NGS amplicon metagenomic sequencing outcomes, specifically focusing on the sequencing results and data processing. The data from the amplicon metagenomic sequencing is analyzed in terms of alpha diversity.

Sequencing Results and Data Processing

The sequencing results for the three groups of rats—P2 Plus, K2 Plus, and K0—demonstrate a comprehensive analysis of microbial diversity. In the P2 Plus group, a total of 2,050,092 raw paired-end sequences were generated, leading to 200,022 combined sequences and 194,959 qualified sequences, ultimately resulting in 183,258 effective sequences. The average length of these sequences was approximately 42,226 base pairs, with a GC content of 52.91%. The quality metrics were impressive, with Q20 and Q30 values at 98.10% and 94.01%, respectively, yielding an effective percentage of 89.35%. For the K2 Plus group, 118,111 raw sequences were recorded, which were processed to yield 103,079 effective sequences. This group had an

average sequence length of 41,660 base pairs and a GC content of 53.37%, with quality scores of 98.27% for Q20 and 94.39% for Q30, resulting in an effective percentage of 87.27%. The K0 group exhibited 202,161 raw sequences, leading to 174,420 effective sequences, with an average length of 41,977 base pairs and a GC content of 53.79%. The quality scores for this group were 98.18% for Q20 and 94.26% for Q30, resulting in an effective percentage of 86.28%. Overall, the results indicate a robust sequencing performance across all groups, showcasing varying levels of effective sequences and quality metrics.

Alpha Diversity

The Alpha Diversity results reveal significant differences in microbial community diversity among the three samples: P2 Plus, K2 Plus, and K0. The K2 Plus sample exhibited the highest observed species count at 675, indicating greater microbial richness compared to P2 Plus (465 species) and K0 (598 species). This trend is further supported by the Shannon index, where K2 Plus scored 6.959, reflecting the highest diversity in terms of species richness and evenness. The Simpson index also favored K2 Plus with a value of 0.982, suggesting a lower likelihood of dominance by any single species. Both the Chao1 and ACE estimators indicated that K2 Plus has the highest estimated total species richness, with values of 675.865 and 677.861, respectively. All samples demonstrated high Goods Coverage, indicating sufficient sampling to capture the majority of species present. Additionally, K2 Plus showed the highest phylogenetic diversity with a PD

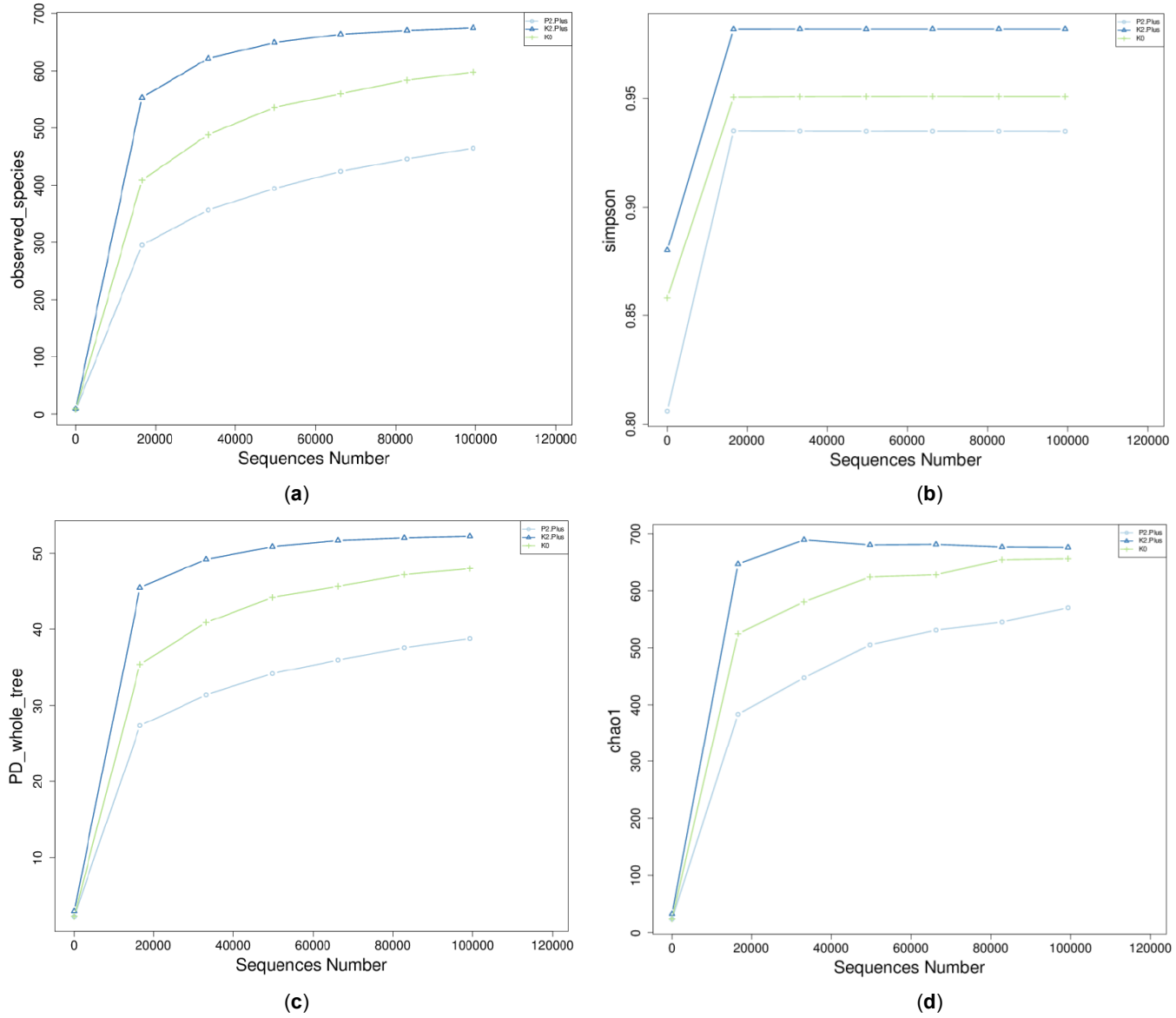

Figure 1: Biodiversity curves.

Whole Tree value of 52.185, suggesting a more varied evolutionary history among its microbial species. In summary, K2 Plus stands out as the most diverse sample, while P2 Plus shows the least diversity across multiple metrics.

Various alpha diversity indices are closely related to biodiversity curves (Figure **1**) that visually represent these diversity metrics. The indices quantify different aspects of alpha diversity, including species richness, evenness, dominance, and phylogenetic breadth. The observed species curve corresponds to richness, while the Simpson index reflects evenness and dominance, and the Chao1 index estimates total species richness, including those not observed.

DISCUSSION

Sequencing Results and Data Processing

This research utilized NGS amplicon metagenomic sequencing, an advanced technique that significantly enriches our comprehension of microbiota across diverse biological and clinical scenarios. The outcomes of the sequencing were articulated through the lens of alpha diversity. NGS amplicon metagenomic sequencing has markedly propelled microbiota research by facilitating the extensive characterization of microbial communities [22]. The careful choice of primers for amplicon sequencing, along with the employment of NGS technologies that produce short reads, can significantly influence the characterization of microbiota [23]. Targeted amplicon sequencing has been instrumental in elucidating microbial communities residing in diverse anatomical sites, investigating microbiota in relation to various pathological states, and examining the impact of environmental variables, including pesticides [24, 25]. The juxtaposition of amplicon sequencing with shallow metagenomic sequencing has yielded comprehensive understanding regarding the diversity and composition of microbiota [26, 27].

The findings derived from NGS amplicon metagenomic sequencing illuminate the microbial diversity, particularly when analyzing samples P2, K2, and K0. The examination of alpha diversity within these samples indicates unique microbiota dynamics. The effective integration and sequencing quality, encompassing parameters such as GC content, Q20, and Q30, are crucial for comprehending the reliability and precision of the acquired data. The comparative analysis of these samples yields significant insights into variations in microbial diversity, elucidating information regarding microbial composition and potential ecological interactions present within the samples [28-30].

Moreover, the ramifications of these discoveries within a research framework are considerable. The insights derived from NGS amplicon metagenomic sequencing can inform subsequent research trajectories, encompassing the examination of the functional capabilities of the microbiota, the exploration of microbial interrelations, and the comprehension of microbiota dynamics as they respond to environmental alterations. These results also facilitate the implementation of longitudinal studies aimed at evaluating the temporal fluctuations of microbial diversity, as well as intervention studies designed to clarify the influence of specific environmental variables on microbiota structure and functionality [31, 32].

Alpha diversity indices such as Shannon, Simpson, Chao1, and PD whole tree are essential for assessing biodiversity within ecological communities, measuring aspects like species richness, evenness, dominance, and phylogenetic breadth. These indices help researchers understand community structure, stability, and the presence of rare species, offering insights into ecosystem dynamics. For example, the Chao1 index estimates unseen species to provide a fuller picture of species richness [33], while the Simpson index measures species evenness and dominance [34]. Phylogenetic diversity goes a step further by considering evolutionary relationships, enriching our understanding of functional diversity within communities [35].

These indices play a critical role in evaluating ecological stability and resilience, with higher biodiversity often correlating with greater ecosystem stability [36]. They also inform conservation strategies, highlighting areas with high biodiversity that may need protection [34]. However, the uncritical use of these indices can lead to misinterpretations, underscoring the need for careful consideration of ecological context, history, and human impacts to ensure effective landscape planning and conservation efforts [37].

This study has several limitations. First, the relatively small sample size may limit statistical power. Second, only one animal model (AOM/DSS-induced CRC) was used, which may not fully replicate human CRC-associated dysbiosis. Third, the cross-sectional sampling design precluded evaluation of longitudinal

microbial dynamics. Future studies with larger cohorts, multiple models, and integration of host immune and metabolic parameters are warranted to validate and expand these findings.

CONCLUSION

Cancer-induced mice in this study displayed unexpectedly higher microbial diversity, likely reflecting community disruption and blooming of pathobionts, while treatment with *Lactococcus lactis D4* reduced diversity, potentially through selective suppression of pro-inflammatory or pathogenic species. These findings highlight the complexity of interpreting diversity metrics in colorectal cancer and suggest that *Lactococcus lactis D4* may beneficially modulate dysbiosis. Further work is needed to explore the underlying mechanisms and evaluate translational relevance in human CRC.

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Not Applicable.

CONFLICT OF INTERESTS

No potential conflict of interest was reported by the author(s).

AUTHOR CONTRIBUTIONS

Conceptualization: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Data curation: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Funding acquisition: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Investigation: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Methodology: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Project administration: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Resources: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Supervision: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Validation: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Visualization: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Writing – original draft: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

Writing – review & editing: Muhammad Iqbal, Rini Suswita, M. Iqbal Rivai, Irwan, Avit Suchitra

REFERENCES

[1] Hossain MS, Karuniawati H, Jairoun AA, *et al.* Colorectal Cancer: A Review of Carcinogenesis, Global Epidemiology, Current Challenges, Risk Factors, Preventive and Treatment Strategies. Cancers (Basel) 2022; 14. https://doi.org/10.3390/cancers14071732

[2] Gupta A, Madani R, Mukhtar H. Streptococcus bovis endocarditis, a silent sign for colonic tumour. Colorectal Dis 2010; 12: 164-71. https://doi.org/10.1111/j.1463-1318.2009.01814.x

[3] Kostic AD, Gevers D, Pedamallu CS, *et al.* Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome Res 2012; 22: 292-8. https://doi.org/10.1101/gr.126573.111

[4] Viljoen KS, Dakshinamurthy A, Goldberg P, *et al.* Quantitative profiling of colorectal cancer-associated bacteria reveals associations between fusobacterium spp, enterotoxigenic Bacteroides fragilis (ETBF) and clinicopathological features of colorectal cancer. PLoS One 2015. 10: p. e0119462. https://doi.org/10.1371/journal.pone.0119462

[5] Buc E, Dubois D, Sauvanet P, *et al.* High prevalence of mucosa-associated E. coli producing cyclomodulin and genotoxin in colon cancer. PLoS One 2013; 8: e56964. https://doi.org/10.1371/journal.pone.0056964

[6] Wang T, Cai G, Qiu Y, *et al.* Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. ISME J 2012; 6: 320-9. https://doi.org/10.1038/ismej.2011.109

[7] Porter RJ, Arends MJ, Churchhouse AMD, *et al.* Inflammatory Bowel Disease-Associated Colorectal Cancer: Translational Risks from Mechanisms to Medicines. J Crohns Colitis 2021; 15: 2131-2141. https://doi.org/10.1093/ecco-jcc/jjab102

[8] Lucafò M, Curci D, Franzin M, *et al.* Inflammatory Bowel Disease and Risk of Colorectal Cancer: An Overview From Pathophysiology to Pharmacological Prevention. Front Pharmacol 2021; 12: 772101. https://doi.org/10.3389/fphar.2021.772101

[9] Nardone OM, Zammarchi I, Santacroce G, *et al.* Inflammation-Driven Colorectal Cancer Associated with Colitis: From Pathogenesis to Changing Therapy. Cancers (Basel) 2023; 15. https://doi.org/10.3390/cancers15082389

[10] Tanaka T, Kohno H, Suzuki R, *et al.* A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. Cancer Sci 2003; 94: 965-73. https://doi.org/10.1111/j.1349-7006.2003.tb01386.x

[11] Tian R, Zuo X, Jaoude JC, *et al.* ALOX15 as a Suppressor of Inflammation and Cancer: Lost in the Link. Prostaglandins & Other Lipid Mediators 2017. https://doi.org/10.1016/j.prostaglandins.2017.01.002

[12] Ohnishi S, Hiramoto K, Ma N, *et al.* Chemoprevention by Aspirin Against Inflammation-Related Colorectal Cancer in Mice. Journal of Clinical Biochemistry and Nutrition 2021. https://doi.org/10.3164/jcbn.20-189

[13] Yang X, Wang Q, Zhang X, *et al.* Purple Yam Polyphenol Extracts Exert Anticolitis and Anticolitis-Associated Colorectal Cancer Effects Through Inactivation of NF-κB/p65 and STAT3 Signaling Pathways. Journal of Agricultural and Food Chemistry 2023. https://doi.org/10.1021/acs.jafc.3c00346

[14] Stolfi C, Rizzo A, Franzè E, *et al.* Involvement of interleukin-21 in the regulation of colitis-associated colon cancer. Journal of Experimental Medicine 2011; 208: 2279-2290. https://doi.org/10.1084/jem.20111106

[15] Zhang B, Xu Y, Liu S, *et al.* Dietary Supplementation of Foxtail Millet Ameliorates Colitis-Associated Colorectal Cancer in Mice via Activation of Gut Receptors and Suppression of the STAT3 Pathway. Nutrients 2020; 12. https://doi.org/10.3390/nu12082367

[16] Ock CY, Kim E-H, Hong H, *et al.* Prevention of Colitis-Associated Colorectal Cancer with 8-Hydroxydeoxyguanosine. Cancer Prevention Research 2011; 4: 1507-1521. https://doi.org/10.1158/1940-6207.CAPR-11-0161

[17] Meana C, García-Rostán G, Peña L, *et al.* The phosphatidic acid phosphatase lipin-1 facilitates inflammation-driven colon carcinogenesis. JCI Insight 2018; 3. https://doi.org/10.1172/jci.insight.97506

[18] Pandurangan AK, Saadatdoust Z, Mohd NE, *et al.* Dietary cocoa protects against colitis-associated cancer by activating the Nrf2/Keap1 pathway. BioFactors 2015; 41: 1-14. https://doi.org/10.1002/biof.1195

[19] Khalyfa AA, Punatar S, Aslam R, *et al.* Exploring the Inflammatory Pathogenesis of Colorectal Cancer. Diseases 2021; 9. https://doi.org/10.3390/diseases9040079

[20] Rosenberg E, DeLong EF, Thompson F, *et al.* The prokaryotes: Human Microbiology 2013; 1-554. https://doi.org/10.1007/978-3-642-30144-5

[21] Cheng Y, Ling Z, Li L. The Intestinal Microbiota and Colorectal Cancer. Front Immunol 2020; 11: 615056. https://doi.org/10.3389/fimmu.2020.615056

[22] Rausch P, Rühlemann M, Hermes BM, *et al.* Comparative analysis of amplicon and metagenomic sequencing methods reveals key features in the evolution of animal metaorganisms. Microbiome 2019; 7: 133. https://doi.org/10.1186/s40168-019-0743-1

[23] Heidrich V, Inoue LT, Asprino PF, *et al.* Choice of 16S Ribosomal RNA Primers Impacts Male Urinary Microbiota Profiling. Front Cell Infect Microbiol 2022; 12: 862338. https://doi.org/10.3389/fcimb.2022.862338

[24] Hoffman C, Siddiqui NY, Fields I, *et al.* Species-Level Resolution of Female Bladder Microbiota from 16S rRNA Amplicon Sequencing. mSystems 2021; 6: e0051821. https://doi.org/10.1128/msystems.00518-21

[25] Höyhtyä M, Korpela K, Saqib S, *et al.* Quantitative Fecal Microbiota Profiles Relate to Therapy Response During Induction With Tumor Necrosis Factor α Antagonist Infliximab in Pediatric Inflammatory Bowel Disease. Inflamm Bowel Dis 2023; 29: 116-124. https://doi.org/10.1093/ibd/izac182

[26] Zhu Q, Huang S, Gonzalez A, *et al.* Phylogeny-Aware Analysis of Metagenome Community Ecology Based on Matched Reference Genomes while Bypassing Taxonomy. mSystems 2022; 7: e00167-22. https://doi.org/10.1128/msystems.00167-22

[27] Peterson D, Bonham KS, Rowland S, *et al.* Comparative Analysis of 16S rRNA Gene and Metagenome Sequencing in Pediatric Gut Microbiomes. Front Microbiol 2021; 12: 670336. https://doi.org/10.3389/fmicb.2021.670336

[28] Jovel J, Patterson J, Wang W, *et al.* Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics. Front Microbiol 2016; 7: 459. https://doi.org/10.3389/fmicb.2016.00459

[29] Poretsky R, Rodriguez RL, Luo C, *et al.* Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. PLoS One 2014; 9: e93827. https://doi.org/10.1371/journal.pone.0093827

[30] Rezasoltani S, Ahmadi Bashirzadeh D, Nazemalhosseini Mojarad E, *et al.* Signature of Gut Microbiome by Conventional and Advanced Analysis Techniques: Advantages and Disadvantages. Middle East J Dig Dis 2020; 12: 5-11. https://doi.org/10.15171/mejdd.2020.157

[31] Zheng X, Zhu Q, Qin M, *et al.* The Role of Feeding Characteristics in Shaping Gut Microbiota Composition and Function of Ensifera (Orthoptera). Insects 2022; 13. https://doi.org/10.3390/insects13080719

[32] Sharpton TJ. An introduction to the analysis of shotgun metagenomic data. Front Plant Sci 2014; 5: 209. https://doi.org/10.3389/fpls.2014.00209

[33] Willis AD. Rarefaction, Alpha Diversity, and Statistics. Front Microbiol 2019; 10: 2407. https://doi.org/10.3389/fmicb.2019.02407

[34] Moreno C, Barragan F, Pineda E, *et al.* Reanálisis de la diversidad alfa: alternativas para interpretar y comparar información sobre comunidades ecológicas. Revista Mexicana de Biodiversidad 2011; 82: 1249-1261. https://doi.org/10.22201/ib.20078706e.2011.4.745

[35] McCoy CO, Matsen FAT Abundance-weighted phylogenetic diversity measures distinguish microbial community states and are robust to sampling depth. Peer J 2013; 1: e157. https://doi.org/10.7717/peerj.157

[36] Walters KE, Martiny JBH. Alpha-, beta-, and gamma-diversity of bacteria varies across habitats. PLoS One 2020; 15: e0233872. https://doi.org/10.1371/journal.pone.0233872

[37] Contoli L. Diversity Indices as 'Magic' Tools in Landscape Planning: A Cautionary Note on their Uncritical Use. Landscape Research 2011; 36: 111-117. https://doi.org/10.1080/01426397.2010.535896

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