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Original article

Microbial Community Dysbiosis and Functional Perturbations in Colorectal Cancer Patients

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Abstract

Colorectal cancer (CRC) has a very close association with Dysbiosis, a phenomenon causing alterations in the microbiome of gut. Some of the recent research implicates that gut microbiome plays a key role in colorectal carcinogenesis, where CRC patients often displays an imbalance in gut bacteria (dysbiosis) marked by depletion of beneficial microbes and proliferation of pro-inflammatory microbial species. The aim of our study was to conduct gut microbiome profiling of six CRC patients, alongside six publicly accessible datasets from healthy individuals with key parameters similar to those of the CRC patients (adult stool origin, V3–V4 16S rRNA gene region, Illumina MiSeq 2, >20,000 merged reads per sample), to examine changes in microbial diversity, taxonomic shifts, and functional perturbations. 16S rRNA gene sequencing of fecal samples was performed using primers specific to the V3-V4 region, assessing alpha and beta diversity, followed by PICRUSt2 to predict microbial metabolic pathways and metabolites. The CRC gut microbiome showed a significant reduction in alpha diversity, indicating an anticipated loss of microbial richness. Taxonomic profiling revealed depletion of health-associated commensals, notably short-chain fatty acid (SCFA) producers such as *Faecalibacterium*, alongside an enrichment of opportunistic and potentially pathogenic genera. Functional prediction analysis indicated corresponding losses in microbial metabolic capacity: CRC samples were deficient in pathways for SCFA biosynthesis (e.g., butyrate production) and B-vitamin biosynthesis (e.g., folate, cobalamin), among other nutrient metabolism pathways, suggesting a collapse of beneficial functions. Altogether, dysbiosis linked to CRC was evidently clear in both community structure and in its function, with almost half of microbial pathways significantly altered. These findings emphasize the relevance of gut microbiome disturbances with CRC. The depletion of beneficial microbes and their metabolites in CRC patients has potential clinical implications, from using microbial signatures as non-invasive diagnostic markers to restoring microbial balance as a therapeutic strategy.

Keywords: dysbiosis, gut microbiome, colorectal cancer, 16s rrna sequencing, ncbi, functional analysis
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Introduction

Identified as one of the most diagnosed malignancies, colorectal cancer (CRC) is also a leading cause of mortality. In 2020, about 1.9 million new CRC cases and ~1 million deaths on account of CRC were recorded worldwide (Morgan et al., 2023). CRC case numbers were found to be high in developed countries and are rising in growing populations, partly due to the adoption of westernized diets and lifestyles. While genetic disorders drive a small fraction of CRCs, the vast majority (~90%) of cases are believed to be imputable to environmental and lifestyle factors (Walker et al., 2024). Epidemiological research studies indicate diet as major contributor to CRC, specifically food with low fibre and highly processed meats amplify its risk. In the contrary, high-fibre diets are prophylactic, seemingly through impacts on the gut microbiome and its metabolites (Yang & Yu, 2018). This connection has channelled attention to the gut microbiota as a crucial intermediary in colorectal carcinogenesis (Kang et al., 2023).

Host physiological functions like metabolism, immune regulation, and gut health is profoundly influenced by an enormous microbial community harbouring in the human gut (Kim & Kim, 2018). Disequilibrium in the regulation of this intestinal microbiome has been linked with the onset and progression of CRC. Frequent reduction in overall gut microbial diversity and a compositional alteration has been seen in CRC patients compared to healthy individuals (Zou et al., 2018). Chief characteristics of CRC-associated dysbiosis is the loss of beneficial, symbiotic bacteria, elevating potentially harmful bacteria. For instance, butyrate-producing commensals such as *Faecalibacterium*, *Roseburia*, *Prevotella*, and *Bifidobacterium* are often found at depleted levels in CRC patients (Fong et al., 2020), which are found to be producing short-chain fatty acids (SCFAs), such as butyric acid, from dietary fibers, which provides energy to colonic epithelial cells and has anti-inflammatory and tumor-suppressive effects in the colon. A reduction of such SCFA producers can lead to lower intraluminal butyrate concentrations in CRC, weakening the intestinal barrier and immune homeostasis. Conversely, multiple pro-carcinogenic microbes tend to be enriched in CRC (Thulasinathan et al., 2025). Notably, *Fusobacterium nucleatum* (an invasive, pro-inflammatory bacterium) is frequently overrepresented in CRC patients and even within tumors, where it can promote colorectal tumorigenesis (Sun et al., 2019). Similarly, species like *Streptococcus bovis/gallolyticus* and enterotoxigenic *Bacteroides fragilis* (ETBF) have been associated with CRC due to their production of virulence factors and toxins that drive DNA damage or chronic inflammation. This microbiome–host crosstalk plays a pivotal role in CRC: microbi-

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al metabolites and cell components (e.g., lipopolysaccharide from Gram-negative bacteria) can trigger inflammatory pathways, modulate cell proliferation, and influence genetic stability in colonic cells (Périchon et al., 2022). In addition to compositional changes, functional alterations in the gut microbiome are increasingly recognized in CRC. Dysbiosis can lead to broad shifts in microbial metabolism – for example, an imbalance in bile acid-transforming bacteria or amino acid metabolism may create a more carcinogenic biochemical environment (Niekamp & Kim, 2023). Recent metabolomics-focused studies have noted that CRC patients often exhibit a distinct faecal metabolite profile, with reduced levels of protective compounds like SCFAs and indoles (tryptophan metabolites) and elevated levels of potentially harmful metabolites, for example, polyamines, secondary bile acids, and others. These metabolites can destroy the intestinal gut epithelial barrier, promoting oxidative DNA damage, along with bias immune responses toward a tumor-progression (Chen et al., 2021).

As there has been a significant influence of gut microbes in colorectal carcinogenesis, there is growing interest among researchers in leveraging the microbiome for clinical benefits. Unlike the human genome, the gut microbiota is a modifiable entity, making it a promising target for intervention (Fong et al., 2020). Alterations in specific microbial taxa or pathways could serve as biomarkers for early CRC detection, and strategies to restore a healthy gut microbiome through dietary changes, prebiotics or probiotics, or fecal microbiota transplantation could potentially complement CRC therapy (Villéger et al., 2018). On this basis, we conducted a pilot study using 16S rRNA gene sequencing on the gut microbiomes of CRC patients and Healthy controls. We not only characterized microbial community structure (diversity and taxonomy) but also employed in silico metagenomic prediction to assess functional pathways and metabolites disrupted in CRC. By amalgamation of these approaches, our study provides a comprehensive view of how both microbial communities shift and the loss of beneficial microbial functions result in CRC. This work is among the first from an Indian cohort to integrate taxonomic and functional microbiome analyses in CRC, addressing a gap in the literature for this population. We hypothesize that colorectal patients have characteristic dysbiosis and functional deficiencies in the gut microbiome. The findings from this study could deepen our understanding of CRC-associated microbial shifts and facilitate the way toward discovering microbiome-derived diagnostic markers and therapeutic strategies for CRC aid. In our study, we employed a 16S rRNA sequencing approach comprising comparison of gut microbiomes of CRC patients with healthy datasets from the European Nucleotide Archive (ENA), carefully selected with inclusion criteria comparable to the CRC patient profiles. We aimed to recognize distinctive microbial features and functional anomalies by studying its microbial diversity, taxonomic composition, and functional pathway alterations associated with CRC. Comprehending these microbial alterations will provide valuable perception into CRC pathogenesis and may enable the development of microbiome-based biomarkers and therapeutic approaches for CRC prevention and treatment. As this is a pilot investigation, our principal objective was to establish workflows and generate preliminary researched validation of dysbiosis in CRC. While detailed clinical covariates such as tumor stage, site, and dietary stratification were not uniformly available, this study was conceived as a pilot, community-level analysis focusing on global dysbiosis and functional perturbations in CRC. Notably, preceding metabolite profiling (supplementary file) was consistent with predicted functional losses, particularly short-chain fatty acid and vitamin biosynthesis pathways. Comprehensive metabolomic integration will be pursued in a forthcoming shotgun metagenomic study.

Materials and methods

Sample Collection

The faecal samples were collected from patients diagnosed with colorectal cancer in sterile stool containers (N-6) at Gujarat Gastro & Vascular Hospital, Surat, to avoid environmental contamination and immediately subjected to low temperatures. All the samples were processed for DNA isolation and 16S ribosomal RNA sequencing (V3-V4 region). The patients included individuals ranging from 25 and 70 years of age. The inclusion conditions for CRC patients in this study comprised patients with no gastrointestinal disorders, no antibiotic consumption during the last 3 months, and patients who had been recently diagnosed and had not yet started treatment, independent of their diet. Healthy controls were obtained from the ENA, carefully selected to match Indian origin, V3–V4 16S region, and sequencing depth, thereby minimizing demographic or technical mismatches. Although combining datasets can introduce batch variability, we processed both CRC and control samples using a unified QIIME2 pipeline with harmonized parameters to reduce potential biases. Gut microbiome sequencing data from six colorectal cancer patients (samples CRC4–CRC9) and six publicly available healthy adult stool samples (European Nucleotide Archive Accession IDs ERR9830949, ERR9830964, ERR9830961, ERR9830908, ERR9830929, ERR9830934) were processed together using a unified 16S rRNA analysis pipeline on the Nephele platform (NIAID), which employs QIIME2-based tools for quality control and analysis. Healthy control datasets were selected to match the patient samples in key parameters (adult stool origin, V3–V4 16S rRNA gene region, Illumina MiSeq 2, >20,000 merged reads per sample). QIIME2 was used for processing of raw sequencing reads for quality control, feature selection, taxonomic abundance, and predictive functional analysis (Table 1).

Table 1. Sample Metadata included in the study

Sample ID	Group	Age (years)	Sex	Diet	Antibiotics (Past 3 Months)	Origin
CRC4	CRC	52	Female	Non-Veg	No	India
CRC5	CRC	55	Female	Non-Veg	No	India
CRC6	CRC	25	Male	Veg	No	India
CRC7	CRC	78	Male	Veg	No	India
CRC8	CRC	57	Female	Veg	No	India
CRC9	CRC	55	Female	Non-Veg	No	India
ERR9830908	Healthy	70	Male	Non-Veg	No	India
ERR9830929	Healthy	54	Female	Non-Veg	No	India
ERR9830934	Healthy	71	Female	Veg	No	India
ERR9830949	Healthy	23	Female	Veg	No	India
ERR9830961	Healthy	45	Male	Non-Veg	No	India
ERR9830964	Healthy	54	Male	Non-Veg	No	India

DNA Extraction and Sequencing

All the stool samples were subjected to DNA extraction through the Qiagen Stool DNA isolation kit. The quality of extracted DNA was assessed through NanoDrop and GEL Check, followed by its amplification. PCR amplification of the Variable V3-V4 region 16S rRNA gene was carried out through high-fidelity DNA polymerase, 0.5 mM dNTPs, 3.2 mM $MgCl_2$, and PCR buffer. Extracted DNA of a total 40 ng was amplified using 10 pM of each primer (V13F: 5' AGAGTTTGATGMTGGCTCAG3', V13R: 5' TTAC-

quantification using the Qubit dsDNA High Sensitivity assay kit. The Illumina MiSeq was used with a 2×300 PE V3 sequencing kit for sequencing.

Bioinformatics Analysis

Raw paired-end reads from all samples underwent quality filtering and adapter trimming with Cutadapt, followed by denoising into amplicon sequence variants (ASVs) using the DADA2 algorithm. The ASVs obtained underwent taxonomic classification against the SILVA-138 reference database to obtain microbial community profiles. Suspected contaminants were identified and removed using the decontam package, and low-prevalence or low-abundance ASVs were filtered out before downstream analysis. Alpha and Beta diversity metrics were computed to determine microbial richness and community shifts. To assess microbial richness (count of unique taxa) and evenness (taxonomic distribution), various alpha diversity indices, such as the Shannon diversity index, Simpson's index, and Observed OTUs, were evaluated. To investigate variation among individuals' microbial communities, beta diversity analysis was carried out using Bray-Curtis dissimilarity and UniFrac distances. Principal Coordinate Analysis (PCoA) plots were used for the visualization of these metrics, revealing distinct clustering patterns in comparison between CRC patients and healthy datasets. To assess functional potential, PICRUSt2 was used to infer metagenomic functions from the 16S data: ASV abundances were normalized by predicted 16S rRNA gene copy number, and the pipeline generated predicted enzyme commission (EC) numbers and MetaCyc metabolic pathways for each sample, which were summarized into pathway abundance tables. These predictions provide an approximation of functional potential and should be con-

CGCGGCMGCSGGCAC3') by setting conditions of 25 cycles of initial denaturation at 95°C for 15 seconds, succeeded by annealing at 60°C for 15 seconds, elongation at 72°C for 2 minutes, and extension at 72°C for 10 minutes. Ampure beads were utilized to ensure the removal of unused primers, eventually purifying 16S amplicons obtained from each sample, which later on were further subjected to gel electrophoresis and a Nanodrop Quality check. Libraries were generated by undergoing an additional eight cycles of PCR using Illumina barcoded adapters and purified with Ampure beads, followed by sidered indicative rather than experimentally validated. Finally, both the ASV abundance tables and the predicted functional profiles were exported for statistical comparison of microbiome differences between colorectal cancer and healthy control groups. All samples were processed uniformly with harmonized methods and sequenced over the same V3–V4 region, ensuring technical comparability between datasets. Potential batch variation between locally generated and publicly available datasets was evaluated using Principal Coordinate Analysis (PCoA), which revealed no dataset-driven clustering, indicating minimal batch effects.

Results

The study was conducted on a small sample set (n=6 per group) as a pilot investigation to generate preliminary insights and establish workflows before scaling to a larger cohort. While statistical power is limited, the findings provide a strong rationale for expanding the study. Before proceeding for sequencing, all genomic DNA samples were subjected to 16S rRNA library preparation and quality control to ensure suitability for amplicon-based metagenomic analysis. As shown in Figure 1, gel electrophoresis confirmed the successful amplification of the V3 and V4 regions of the 16S rRNA gene in six colorectal cancer (CRC) samples. Clear, sharp bands were observed for each sample at the expected size of approximately 575 base pairs, indicating the production of high-quality amplicons suitable for sequencing. The QC report further validated that all libraries passed quality criteria and exceeded the minimum concentration threshold (>50 ng/µL).

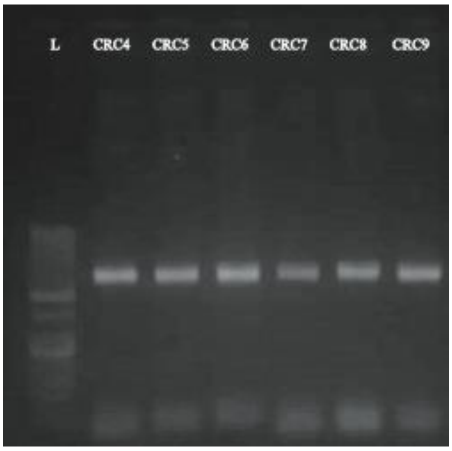

Fig. 1 Agarose gel electrophoresis of amplified V3–V4 regions of the 16S rRNA gene from six colorectal cancer (CRC) fecal DNA samples (CRC4–CRC9 and 1kb Ladder (L). Clear bands of ~575 bp indicate successful amplification and high-quality amplicons suitable for sequencing.

Diversity Analysis

Alpha Diversity

Remarkable reduction was exhibited in gut microbiome alpha diversity compared to healthy controls. The mean number of observed OTUs was significantly lower in the CRC group (59.5 ± 12.0) than in controls (362.0 ± 98.9, $p<0.01$), and a similar trend was observed with the Chao1 richness estimator (Figure 2). Shannon diversity indices were also decreased in CRC (mean 3.30 vs 3.97 in healthy, $p<0.01$), indicating a loss of within-sample diversity in the CRC microbiota. In contrast, Simpson index (evenness) did not differ significantly between CRC and healthy groups (median ~0.94 in both, $p>0.05$), suggesting that the evenness of the community remained comparable despite the reduced richness. Overall, these results demonstrate a significantly diminished gut microbiome diversity in CRC patients (Figure 2), consistent with a dysbiosis characterized by lower species richness.

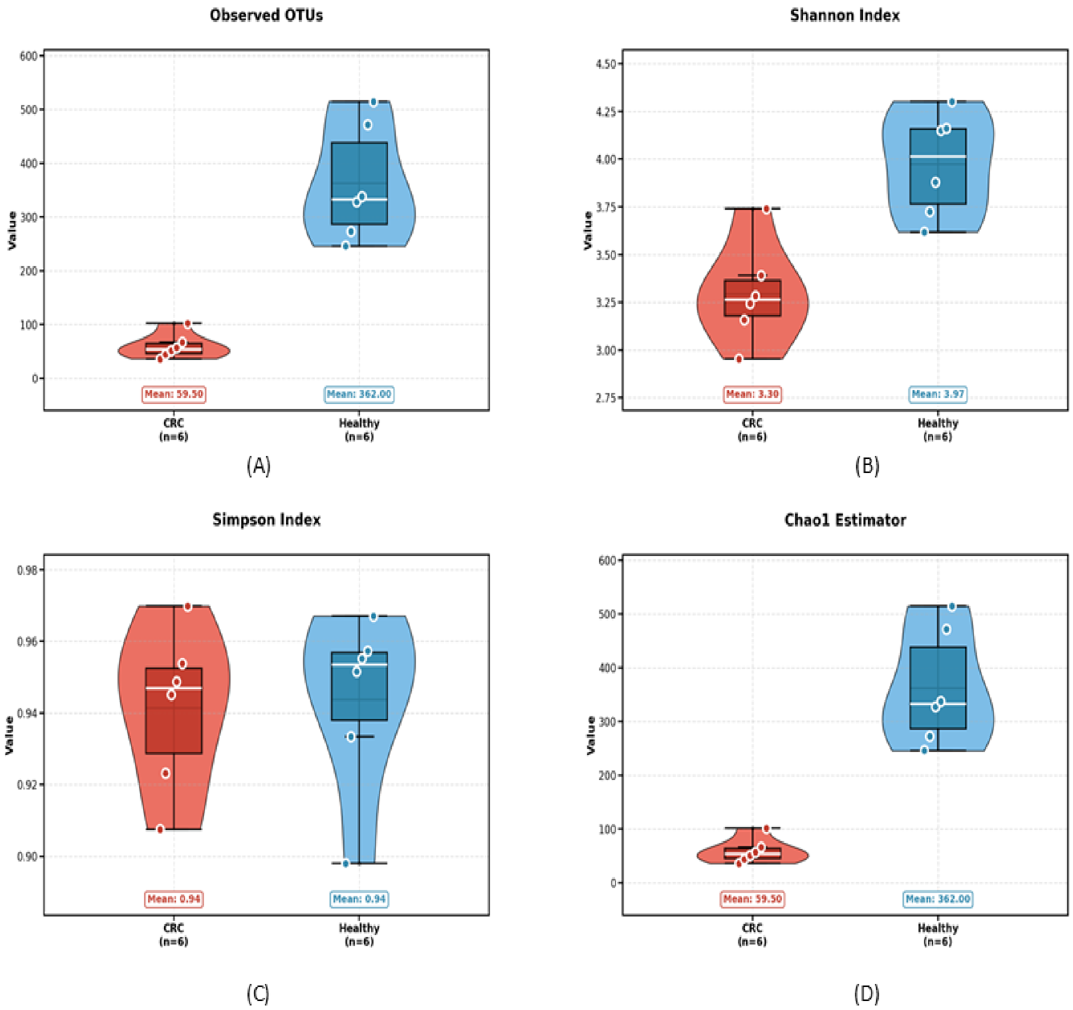

Fig. 2. Comparison of alpha diversity between colorectal cancer (CRC, n=6) and healthy controls (n=6). Violin plots show (A) Observed OTUs, (B) Shannon index, (C) Simpson index, and (D) Chao1 richness estimator. CRC samples exhibit significantly reduced richness and diversity ($p<0.01$, Welch's t-test) compared to healthy controls, indicating loss of microbial complexity.

Beta Diversity

Beta diversity analyses uncovered a distinctly clustered microbial communities amongst healthy datasets and those from patients with CRC. Principal Coordinates Analysis (PCoA) plots based on both taxonomic and phylogenetic distance metrics showed a clear separation between CRC and control samples (Figure 3). For example, unweighted UniFrac PCoA ordination highlighted

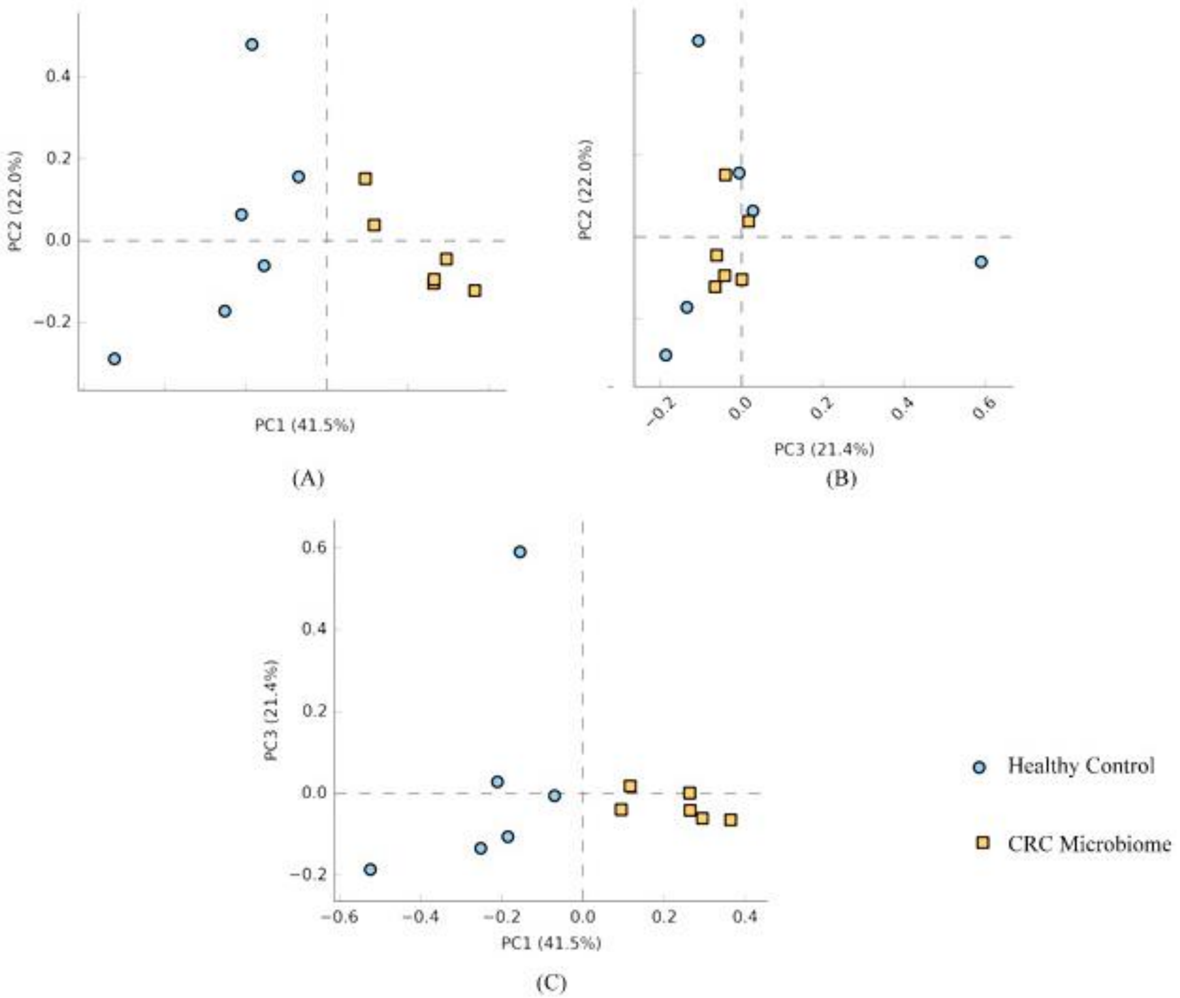

Fig.3. Principal Coordinates Analysis (PCoA) of beta diversity in CRC (n=6) and healthy controls (n=6). Ordinations based on Bray–Curtis dissimilarity demonstrate distinct clustering of CRC (orange squares) and healthy (blue circles) microbiomes. PC1 (41.5%) and PC2 (22.0%) together explain 63.5% of total variance. Group separation was statistically significant (PERMANOVA, $p<0.01$). The ordination also confirmed the absence of batch variation between datasets.

non-overlapping clusters for CRC vs. healthy microbiomes (Figure 2), reflecting significant differences in community membership. Similarly, Bray–Curtis and Jaccard distance-based PCoA demonstrated that CRC fecal samples cluster apart from healthy controls along the principal axes (Figure 3). These patterns suggest a significant alteration in overall constitution of the gut microbiome in CRC patients relative to healthy guts. Permutational ANOVA (PERMANOVA) confirmed that diagnosis (CRC vs. healthy) explained a noteworthy portion of the change in microbial composition for all beta diversity metrics (Bray–Curtis, Jaccard, unweighted and weighted UniFrac; $p<0.01$ for each), verifying that community structure in CRC is significantly different from that of healthy individuals.

Beta diversity analysis exhibited distinct microbial community profiles between patients with colorectal cancer (CRC) and healthy controls. Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarity revealed clear separation of the two groups (Figure 3). The first principal coordinate (PC1) explained 41.5% of the variance, while PC2 explained 22.0%, together accounting for the total variation of 63.5%. This separation was further supported by PERMANOVA ($p < 0.05$), confirming statistically significant differences in overall microbial structure. This ordination also served to evaluate potential batch variation between locally generated and publicly sourced datasets. The absence of distinct clustering by dataset origin indicated that batch effects were minimal. This ordination also served to evaluate potential batch variation between locally generated and publicly sourced datasets. The absence of distinct clustering by dataset origin indicated that batch effects were minimal.

Hierarchical clustering analysis further confirmed community-level segregation between CRC and healthy samples. CRC samples were characterized by a higher relative abundance of *Lactobacillales*, *Enterobacterales*, and *Peptostreptococcales-Tissierellales*, taxa often associated with pro-inflammatory and opportunistic behaviors. In contrast, healthy samples were enriched in *Oscillospirales*, *Bifidobacteriales*, *Caulobacterales*, and *Clostridiales*, which comprise beneficial commensals and producers of short-chain fatty acids (SCFAs). These clustered patterns confirm the distinctly observed differences in community structure, as revealed by beta diversity analysis. STAMP extended error bar plot analysis (Figure 4) revealed significant taxonomic shifts between CRC patients and healthy controls. Genera significantly enriched in healthy controls included *Prevotella_9* (q = 0.014), *Prevotella copri* (q = 0.015), *Roseburia inulinivorans* (q = 0.022), *Faecalibacterium prausnitzii* (q = 0.030), *Agathobacter* (q = 0.035), *Subdoligranulum* (q = 0.034), and *Lachnospira* (q = 0.042). These organisms are key producers of short-chain fatty acids (SCFAs) and are generally regarded as protective commensals in maintaining gut health.

In contrast, CRC-enriched genera included *Enterococcus* (q = 0.070), *Clostridium sensu stricto 11* (q = 0.071), *Klebsiella* (q = 0.086), *Escherichia-Shigella* (q = 0.248), and *Peptostreptococcus* (q = 0.467). Although some did not reach strong statistical significance after FDR correction, their consistent elevation in CRC suggests their potential as disease-associated taxa. Majority of these, particularly *Fusobacterium* (not mentioned in this subset) and *Peptostreptococcus*, have been responsi-

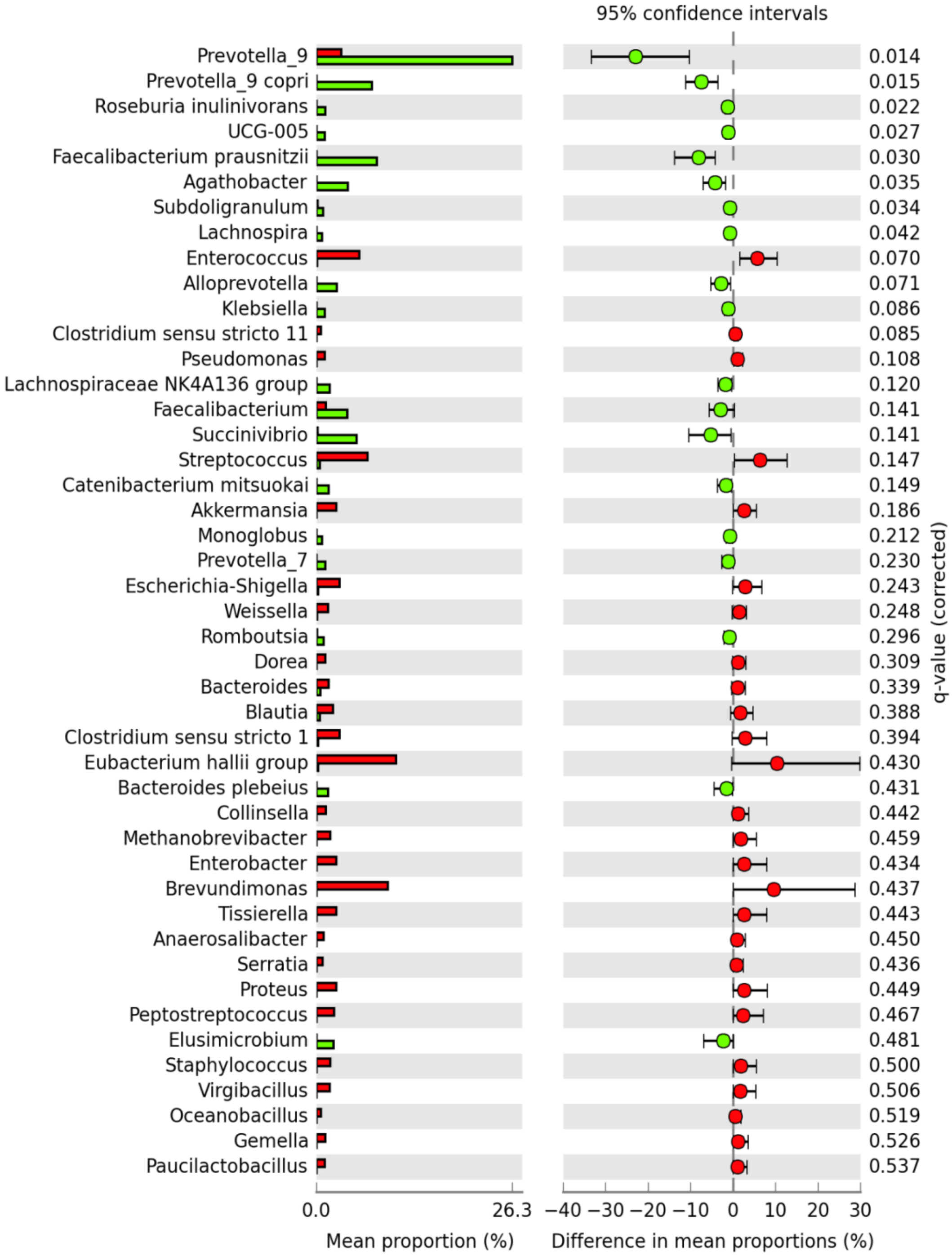

Fig.4. Differentially abundant taxa between CRC (n=6) and healthy controls (n=6) identified by STAMP extended error bar plot. Green bars indicate enrichment in healthy controls, red bars indicate enrichment in CRC. Several short-chain fatty acid (SCFA) producers (e.g., Faecalibacterium prausnitzii, Roseburia inulinivorans, Agathobacter) were significantly depleted in CRC (FDR-adjusted q<0.05), while opportunistic taxa (e.g., Enterococcus, Peptostreptococcus) were relatively enriched.

ble for CRC through their pro-inflammatory activity, toxin production, and disruption of the epithelial barrier. Additionally, *Eubacterium hallii* (q = 0.430), another essential butyrate producer, was notably reduced in CRC and enriched in healthy controls, consistent with its protective role in colon health. Other taxa, such as *Romboutsia*, *Collinsella*, and *Bacteroides*, showed minor but non-significance trends. Overall, the STAMP analysis reveals a dysbiotic signature characterized by the depletion of protective SCFA-producing taxa in CRC and the enrichment of opportunistic/pathogenic organisms, supporting the concept of disrupted microbial homeostasis in colorectal cancer.

Predicted Functional Pathways and Metabolic Changes

Predictive metagenomic functional analysis (PICRUSt-based) revealed that the microbiome of CRC patients not only differs taxonomically but also exhibits functional disruptions, particularly in nutrient transport and metabolism pathways (Figure 5). These predictions provide preliminary insights but require validation with shotgun metagenomics or metabolomics. The top predicted microbial functions in both groups were dominated by core metabolic and transport processes; however, CRC-associated microbiomes showed reduced capacity in several key transport functions.

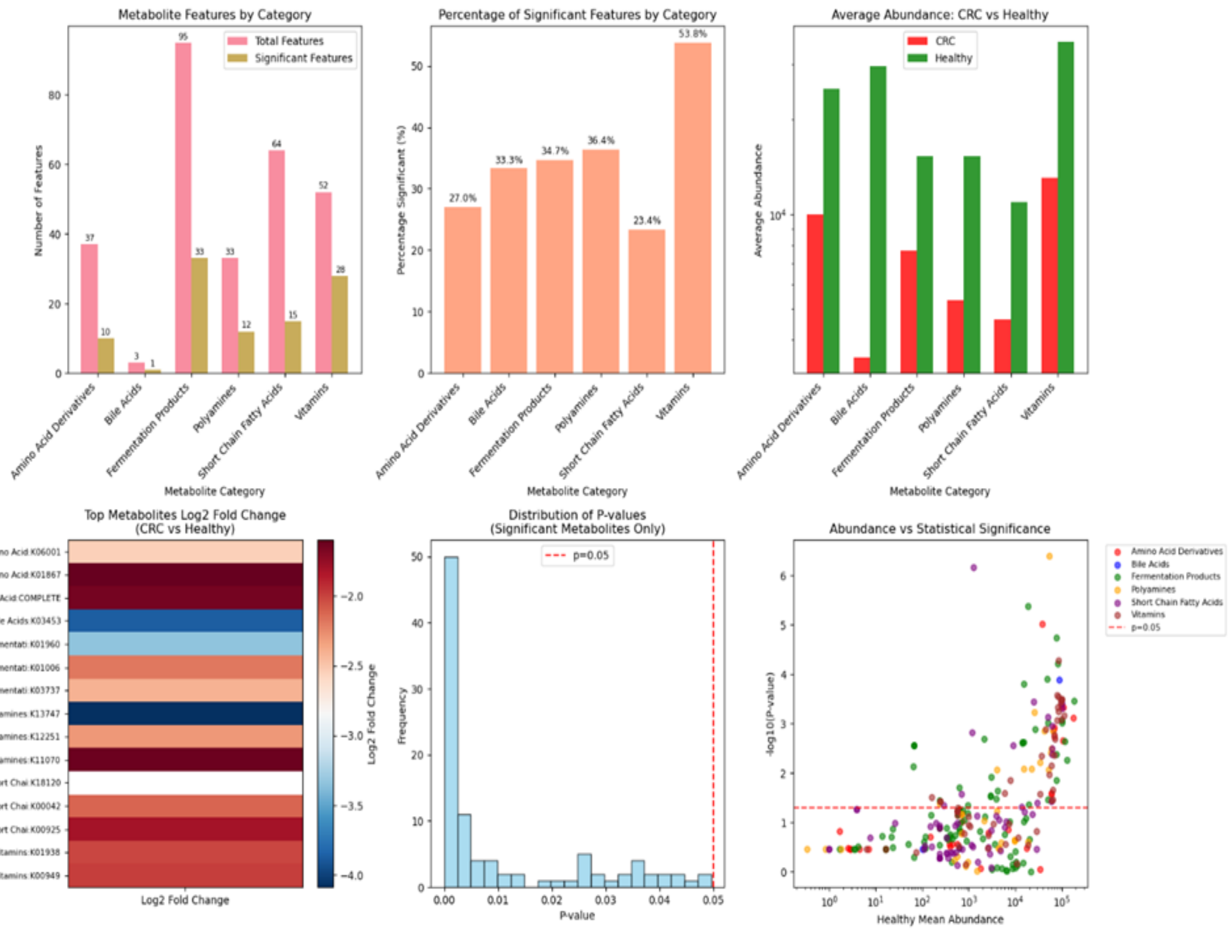

Fig. 5. Differentially abundant taxa between CRC (n=6) and healthy controls (n=6) identified by STAMP extended error bar plot. Green bars indicate enrichment in healthy controls, red bars indicate enrichment in CRC. Several short-chain fatty acid (SCFA) producers (e.g., Faecalibacterium prausnitzii, Roseburia inulinivorans, Agathobacter) were significantly depleted in CRC (FDR-adjusted q<0.05), while opportunistic taxa (e.g., Enterococcus, Peptostreptococcus) were relatively enriched.

Remarkably, multiple genes encoding components of ATP-binding cassette (ABC) transporters were depleted in CRC to a great extent relative to healthy controls. For instance, the gene K02003 (annotated as a putative ABC transport system ATP-binding protein, part of an ABC.CD sugar transporter) was among the most reduced functions in CRC samples (CRC mean ~1.44×10^5 vs. healthy ~5.57×10^5; Figure 5). Likewise, other ABC transporter subunits – including permeases and ATP-binding proteins (e.g., K01990, K01992 corresponding to ABC-2 type transporter components) – had lower predicted abundances in CRC patients. In parallel, microbial iron acquisition pathways were diminished in the CRC microbiome: genes for iron complex transport systems (siderophore uptake), such as the substrate-binding protein K02016 and ATP-binding protein K02013 of ferric iron transporters, were less abundant in CRC as compared to healthy guts (Figure 6). These declines in ABC transporters and iron transport-related functions suggest a loss of metabolic versatility and nutrient-scavenging ability in CRC-associated microbial communities.

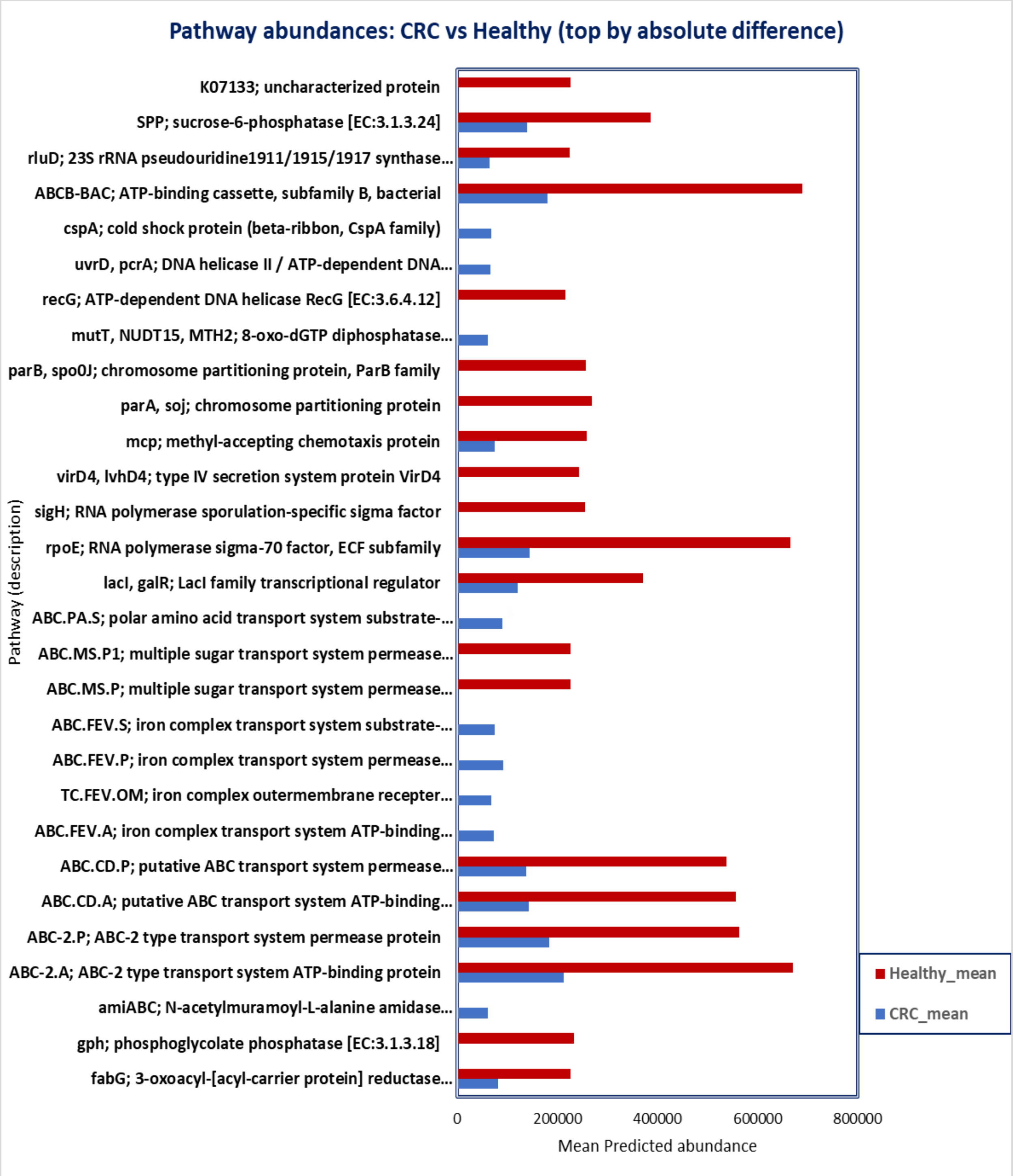

Fig. 6. Functional pathway differences between CRC (n=6) and healthy controls (n=6). Predicted abundances of ABC transporters, sugar and iron uptake systems, and amino acid metabolism pathways were significantly reduced in CRC. Conversely, stress response and chemotaxis pathways were relatively enriched, suggesting a shift toward opportunistic, inflammation-associated microbial functions.

Conversely, relatively elevated (though not always significant statistically) CRC patient microbiomes by a few functional. Patients with CRC showed more prominent stress response and motility functions, consistent with an enrichment of opportunistic bacteria. For example, genes encoding a bacterial cold-shock protein (cspA, KO K03704) and a methyl-accepting chemotaxis protein (mcp, KO K03406) were detected at higher relative levels in CRC metagenomes (Figure 5), possibly reflecting an adaptation of the CRC microbiota to a more inflammatory or nutrient-stressed gut environment. These values represent inferred microbial functional potential rather than direct metabolite measurements. In addition, comparative pathway analysis revealed significant differences in comprehensive metabolic pathways between the two groups. Inferred pathways for arginine and proline metabolism, aminoacyl-tRNA biosynthesis, and amino sugar and nucleotide sugar metabolism were all altered in abundance between healthy and CRC microbiomes (each $p<0.05$, data not shown), indicating a global functional shift accompanying the taxonomic dysbiosis. Taken together, these results highlight that the CRC-associated gut microbiome is functionally impaired in crucial nutrient transport and metabolic processes (such as ABC transporter-mediated uptake of sugars, iron sequestration systems, and amino acid metabolism) while potentially harboring microbes with greater stress response and chemotactic capabilities. This functional dysbiosis may contribute to the colorectal cancer disease environment, either by reducing beneficial metabolite production (e.g., butyrate) or by favoring microbial activities that promote inflammation and tumor progression. A total of 672 metabolites were obtained from microbial sources, of which 239 (35.6%) were markedly altered in CRC patients in compared to healthy datasets (Welch's *t*-test, $p < 0.05$, Figure 1). Short-chain fatty acids (SCFAs) and vitamin-related pathways were among the most obstructed. Particularly, 4-hydroxybutyrate dehydrogenase (a key SCFA enzyme) was completely lacking in CRC samples. Vitamin biosynthesis, particularly B-vitamin pathways, also showed extensive depletion, with 28 out of 52 vitamin-related features significantly reduced. Polyamine biosynthesis was impaired as well, with carboxynorspermidine decarboxylase downregulated ~17-fold. Altogether, these results demonstrates that CRC microbiomes exhibit a speculated loss of chief metabolic functions essential for intestinal health.

Parallel analyses of taxonomic, pathway, and functional gene profiles revealed widespread disruptions. Among 1,470 taxa, 128 (8.7%) differed significantly in abundance (Welch's *t*-test). In contrast, 172 of 376 pathways (45.7%) and 1,741 of 6,465 predicted microbial functions (26.9%) were significantly altered. Most of these changes involved depletions in CRC rather than enrichments. Volcano plots confirmed a strong trend toward reduced abundance of beneficial functions—such as SCFA production, vitamin biosynthesis, and stress response pathways—in CRC microbiomes.

These multi-level findings indicate that CRC is associated with a substantial functional breakdown of the gut microbiome, marked by the loss of beneficial taxa, critical metabolic pathways, and protective microbial metabolites (Figure 7).

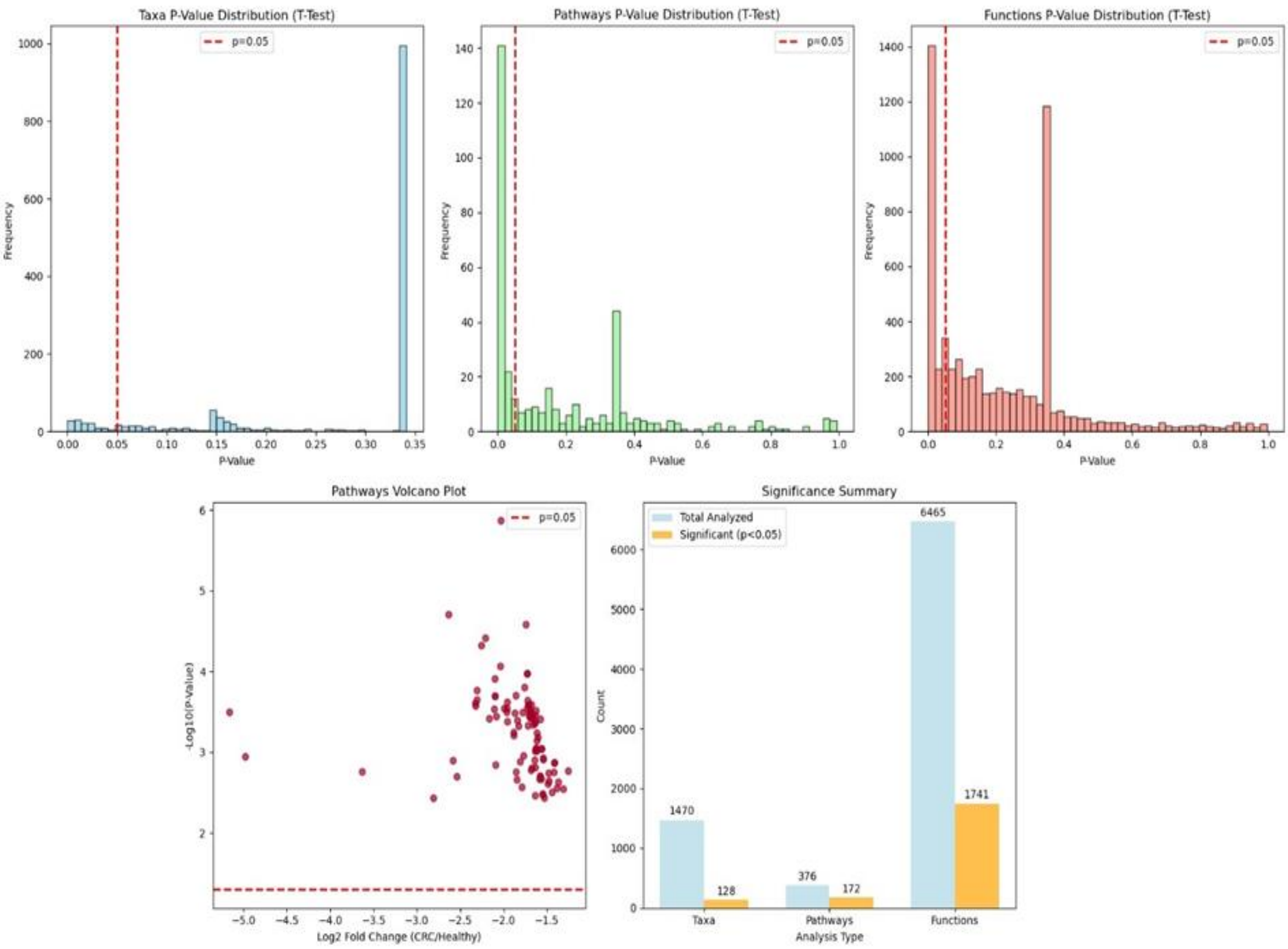

Fig. 4. Multi-level statistical comparison of microbial taxa, pathways, and predicted functions between CRC (n=6) and healthy controls (n=6). (Top row) Histograms of p-value distributions for taxonomic (left), pathway (center), and function (right) comparisons. (Bottom row) Volcano plots of significantly altered pathway (left) and a summary bar plot (right) showing proportions of features significantly changed. Functional features (26.9%) and pathways (45.7%) were more extensively disrupted than taxa (8.7%), emphasizing that CRC is associated with broad functional breakdown of the gut microbiome.

Discussion

Our findings from this pilot work exhibit that colorectal cancer (CRC) is associated with a markedly perturbed gut microbiome characterized by reduced diversity within-sample and an altered community structure. We observed significantly lower alpha diversity in CRC patients compared to healthy controls, indicating a loss of microbial richness and evenness associated with the disease. This reduction is consistent with CRC-associated dysbiosis reported in other populations, although some studies note variability depending on diet and region. In our cohort, the sharp drop in alpha diversity – with some CRC samples harbouring only a few dozen detectable taxa – suggests a collapse of the usually complex gut ecosystem (Wirbel et al., 2019). Such loss of diversity may impair key microbiome functions (e.g., nutrient fermentation, colonisation resistance, and immune modulation) and likely reflects the overgrowth of opportunistic species that thrive in the tumour-altered gut environment (Kim et al., 2023). Beta diversity analysis further showed that CRC and control microbiomes clustered distinctly, underscoring a significant community-level divergence. Indeed, unsupervised ordination cleanly separated CRC cases from controls in our data, echoing larger studies that have shown microbiome profiles can distinguish CRC status with moderate accuracy. This segregation highlights a robust dysbiosis signal in CRC, which could potentially serve as a non-invasive biomarker for disease detection (B. Yuan et al., 2021).

A hallmark of CRC-associated dysbiosis is a shift in taxonomic composition, characterised by the enrichment of pro-inflammatory, potentially pro-tumorigenic microbes alongside the depletion of beneficial commensals. Many of the overrepresented genera in CRC stools – including *Fusobacterium, Bacteroides, Streptococcus, Peptostreptococcus*, *Enterococcus*, and other Enterobacteriaceae – are known pathobionts implicated in colorectal carcinogenesis (Vipperla & O'Keefe, 2016). For instance, *Fusobacterium nucleatum* is widely associated with CRC as it adhere to and invades the colonic mucosa, promotes tumor progression, and subvert immune surveillance. For instance, *Fusobacterium nucleatum* is widely associated with CRC, as it adheres to and invades the colonic mucosa, promotes tumour progression, and subverts immune surveillance. Although *F. nucleatum* was not dominant in our faecal samples (likely because it colonises tumour tissue more than stool), many studies have found higher faecal *F. nucleatum* in CRC patients than in healthy individuals, correlating with advanced disease and poor prognosis (Yu et al., 2022). We also observed an overgrowth of Bacteroides, consistent with reports that high-fat, Western diets foster *Bacteroides*-dominated microbiotas, which can promote carcinogenesis via inflammatory toxin production and enhanced bile acid metabolism. In particular, toxin-producing strains of *Bacteroides fragilis* can induce chronic colonic inflammation and DNA damage via a metalloprotease toxin, creating a pro-tumorigenic environment in the colon (Jin et al., 2020).

Another noteworthy dysbiotic change in CRC is the bloom of oral-derived anaerobes such as *Streptococcus* and *Peptostreptococcus*. *S. gallolyticus* (formerly *S. bovis*) has a well-established epidemiologic link to CRC – its presence in blood or endocarditis is considered an indication for colonoscopy. Mechanistically, *streptococcal* components can induce mucosal inflammation, and *S. gallolyticus* has been found to enhance colon tumor cell proliferation and COX-2 expression. Similarly, *Peptostreptococcus anaerobius*, another oral bacterium enriched in CRC, produces metabolites (e.g., polyamines, hydrogen sulfide) that may damage DNA or alter epithelial signalling, thus potentiating carcinogenesis (Pardo et al., 2020). We also noted an increased representation of *Enterobacteriaceae* (e.g., *Escherichia coli*, *Klebsiella*) and Enterococcus in CRC samples, taxa that are often absent in healthy guts. . Certain strains of *E. coli* produce the genotoxin colibactin, causing DNA double-strand breaks in host cells. These bacteria thrive in the tumour's inflammatory, high-redox environment and exacerbate it by releasing endotoxins and other virulence factors (Feng et al., 2015). Notably, many of the bacteria mentioned above (including *F. nucleatum, B. fragilis, S. gallolyticus*, *P. anaerobius, E. coli*, and others) have been identified across studies as CRC-associated microbial markers, and several appear in panels proposed for CRC diagnosis. (Périchon et al., 2022) This pro-tumorigenic consortium – rich in inflammation-associated, proteolytic, and biofilm-forming species – likely both results from and reinforces the tumour-promoting inflammatory milieu. It thereby creates a self-perpetuating cycle (Vega et al., 2022).

In contrast, a significant depletion of health-associated commensals was observed in CRC-microbiome, notably those producing short-chain fatty acids (SCFAs) and other beneficial metabolites. A remarkable example is the complete absence of *Faecalibacterium prausnitzii* in all CRC patients (Dikeocha et al., 2022). *F. prausnitzii,* an abundant butyrate producer in the healthy colon, is frequently regarded as a sentinel of gut health. Butyrate nourishes colonic epithelial cells, strengthens the intestinal barrier, and induces apoptosis in cancer cells while modulating immune responses; thus, it exhibits potent anti-inflammatory and anti-cancer effects. Reduced levels of *F. prausnitzii* are a consistent finding not only in CRC but also in other inflammatory conditions, such as ulcerative colitis (Lenoir et al., 2016). Loss of this keystone organism deprives the host of a crucial SCFA (butyrate) at the tumour site, contributing to a pro-inflammatory, tumour-permissive environment. Indeed, diminished *F. prausnitzii* and other butyrate producers have been linked to worse clinical outcomes in CRC (Štofilová et al., 2022). In our study, several other butyrate-producing genera from the Ruminococcaceae and Lachnospiraceae families (e.g., *Roseburia*, *Eubacterium*, *Blautia*) were absent or significantly reduced in CRC stools. This aligns with the consensus that CRC microbiomes harbour fewer fibre-fermenting, SCFA-producing bacteria and are skewed toward protein- and mucin-degrading taxa. Consequently, the colonic milieu in CRC is likely to contain lower levels of butyrate and other SCFAs, along with higher concentrations of potentially harmful fermentation products (Yuan, 2024).

For instance, our healthy control microbiomes showed higher *Prevotella* (a genus adept at fermenting dietary fiber into SCFAs), whereas *Prevotella* was nearly absent in CRC patients. *Prevotella* thrives on complex plant polysaccharides and is associated with fiber-rich diets, so its scarcity in CRC may reflect lower fiber intake or an inhospitable, inflammation-dominated gut environment in those patients (Niccolai et al., 2021). Likewise, *Bifidobacterium*, a beneficial genus known for producing acetate, lactate, and B-vitamins, were detected in healthy individuals but were virtually depleted in CRC stools in our study. *Bifidobacterium* are essential for maintaining gut homeostasis and mucosal integrity; their loss in CRC is consistent with prior findings of reduced *Bifidobacterium* in colorectal tumor cases (Gagnière, 2016). Together, the depletion of SCFA pro (like) signifies the loss of critical microbial functions that usually protect the colonic epithelium. To sum up, elimination of critical microbial functions, which usually are responsible for protecting colonic epithelium, is signified by the depleting SCFA producers (like *Roseburia, Bifidobacterium, Faecalibacterium, Prevotella*, and others). Accordingly, the CRC-associated microbiome shifts from a mutualistic, health-promoting community to a disruptive, pathogenic ensemble (Jia et al., 2024).

Beyond compositional shifts, our functional analyses indicate that the CRC microbiome is profoundly reprogrammed in its metabolic capacity. Predictive metagenomic profiling (PICRUSt2) and pathway enrichment analysis revealed broad functional disruptions that mirror the taxonomic changes (Yachida et al., 2019), however, these predictions should be interpreted cautiously as they are not experimentally validated. These represent predicted functional differences based on 16S-derived inference, which should be interpreted cautiously since PICRUSt2 predictions may not fully align with shotgun metagenomic validation (Douglas et al., 2020). Noticeably, predicted genes and pathways participating in fermentative metabolism and SCFA biosynthesis were putatively underrepresented in CRC with reference to controls. For example, several key enzymes for butyrate production, glycolysis, and pyruvate fermentation showed marked depletion in CRC (often dropping to near-zero levels in our cohort). This suggests a diminished capacity of the CRC microbiota to ferment dietary fibers into beneficial SCFAs, congruent with the loss of butyrate-producing taxa discussed above (Wang et al., 2019). We also predicted a reduction in microbial pathways for vitamin biosynthesis (e.g., B-vitamins and vitamin K/E) in CRC samples. Commensal bacteria commonly synthesize vitamins that supplement the host diet; their depletion could lead to local micronutrient deficiencies in the colonic mucosa (Bellerba et al., 2022).

In addition to short-chain fatty acid pathways, our predictive analysis also revealed notable alterations in microbial vitamin and polyamine metabolism. Several predicted B-vitamin biosynthesis routes (including those for folate, biotin, and riboflavin) were markedly reduced in CRC samples, suggesting a potential deficiency in microbial vitamin supply to the host. These vitamins play critical roles in DNA synthesis, redox balance, and epigenetic regulation, and their depletion has been associated with tumor-promoting metabolic stress in the colonic mucosa (Hillege et al., 2025). Similar findings have shown that gut bacteria serve as essential vitamin suppliers to the host, and disruption of these microbial biosynthetic routes can compromise mucosal metabolic balance (LeBlanc et al., 2013).

Moreover, polyamine biosynthesis pathways were predicted to be downregulated, whereas polyamine catabolism and transport showed relative enrichment, indicating a possible imbalance in luminal polyamine homeostasis. Polyamines such as spermidine and putrescine have been linked to epithelial proliferation and tumor progression, and microbial dysregulation of these pathways may further contribute to CRC pathophysiology (Lu et al., 2023).

Conversely, pathways for protein degradation and amino acid catabolism were overrepresented, indicating a shift toward proteolytic fermentation. Accordingly, CRC microbiomes likely generate more pro-carcinogenic byproducts such as ammonia, phenols, indoles, and polyamines (Moinard et. al., 2005; Soda, 2011). In particular, tryptophan catabolism was elevated, potentially leading to the accumulation of immunomodulatory metabolites that favour tumour immune evasion (Hanus et al., 2021). Bile acid metabolism was also perturbed: CRC samples showed reduced gene abundance for primary bile acid deconjugation and transformation, alongside increases in those modulating bile acid pools. These changes are expected to increase levels of secondary bile acids, such as deoxycholic acid, which can damage DNA and promote colorectal tumorigenesis (Ocvirk & O'Keefe, 2017). In summary, the CRC gut microbiome is predicted to exhibit reduced capacity beneficial functions (SCFA production, vitamin synthesis, antioxidant pathways) while gaining pathways that produce inflammatory and genotoxic compounds (Tang et al., 2020). This metabolic reprogramming likely reflects a shift in the balance of microbial metabolites—from protective products such as SCFAs and vitamins toward carcinogenic intermediates including secondary bile acids and polyamines—linking diet, microbial metabolism, and tumorigenesis (O'Keefe, 2016). Importantly, these functional shifts are associative rather than necessarily causal – microbiome changes might result from the tumour microenvironment – so longitudinal studies are needed to determine causality.

While metadata such as tumor stage, location, and detailed dietary habits were not uniformly available, the harmonized pipeline and batch evaluation ensured reliable group-level comparisons. These microbiome alterations carry significant clinical implications. Most importantly, the distinct microbial signatures associated with CRC hold promise as non-invasive diagnostic biomarkers. As our analysis (Table 1) and other studies demonstrate, a combination of microbial taxa can accurately distinguish between CRC patients and healthy individuals. Several taxa highlighted in our study – including *Fusobacterium, B. fragilis, Peptostreptococcus, Streptococcus, Enterococcus*, along with the absence of key SCFA-producing bacteria – have been proposed as components of faecal microbiome-based panels for early CRC detection.

Beyond diagnosis, the gut microbiome offers opportunities for therapeutic intervention in CRC, with two broad strategies: suppressing deleterious microbes and restoring beneficial ones (Z. Yuan et al., 2021). For example, preclinical studies have shown that eliminating *F. nucleatum* from CRC-bearing mice slows tumour growth, underscoring this bacterium as a potential therapeutic target. Conversely, efforts are underway to replenish the depleted beneficial taxa in CRC patients. Clinical trials are exploring high-fibre diets, prebiotics, and probiotic cocktails (including butyrate-producing *Clostridia* and *Bifidobacterium* strains) to rebalance the gut ecosystem (Zhu et al., 2021). The goal of such interventions is to boost the production of anti-carcinogenic metabolites, such as SCFAs, and re-establish colonisation resistance against tumour-promoting microbes.

Looking forward, incorporating microbial biomarkers into CRC screening (e.g., stool microbiome assays alongside occult blood tests) could improve early detection. Moreover, tailoring therapy based on an individual's microbiome profile might enhance treatment efficacy, as emerging evidence suggests that specific microbiota compositions influence responses to chemotherapy and immunotherapy.

In concise, our study adds to the growing evidence that gut microbiome dysbiosis is intricately linked with colorectal cancer, particularly in Indian populations. The apparent depletion of protective, metabolically vital microbes and the enrichment of pathogenic, pro-inflammatory species, predicted functional genes and pathways in CRC not only shed light on tumour biology but also open new possibilities for clinical management. Restoring a healthy microbiome – or at least counteracting tumour-promoting microbial functions – may become a valuable strategy for CRC prevention and therapy. Ultimately, deciphering the complex interplay between the gut microbiota and colorectal carcinogenesis will be crucial to harnessing the microbiome for enhanced patient outcomes.

Conclusion

This pilot investigation provides preliminary evidence that colorectal cancer is associated with distinct alterations in the gut microbiome. Our results demonstrated a significant decline in microbial diversity among CRC patients compared to healthy controls, accompanied by the depletion of protective commensals such as short-chain fatty acid (SCFA) producers and Bifidobacteria, alongside the enrichment of opportunistic and pro-inflammatory taxa. Functional predictions suggested potential disruptions in metabolic pathways essential for intestinal health, specifically those related to SCFA and vitamin biosynthesis. There is a significant transition from a balanced, health-promoting gut flora to a dysbiotic condition that may lead to a tumor-favouring microenvironment which has been clearly suggested by microbial and functional shifts.

At the same time, certain limitations must be acknowledged. The small sample size hampers the statistical robustness, and the use of publicly available datasets for healthy controls, although carefully matched to our cohort, may introduce residual variableness. Additionally, functional interpretations are based on predictive tools (PICRUSt2) and should be considered indicative rather than definitive. Nevertheless, the consistency of the observed taxonomic and functional patterns underscores their biological relevance and provides a strong rationale for extending this work.

Altogether, these findings emphasize the potential of gut microbial signatures as non-invasive biomarkers and highlight the clinical importance of restoring microbial balance in CRC patients. Future scope of research with larger, statistically matched cohorts through shotgun metagenomics and metabolomics profiling will be crucial to validate these preliminary observations and further elucidate microbiome's role in colorectal cancer risk, progression, and management.

Institutional Review Board Statement

The study involved six colorectal-cancer patients who voluntarily provided stool samples for microbiome profiling under medical supervision. Written informed consent was obtained from all participants. The study protocol was reviewed and approved by the Institutional Research Advisory Committee (RAC), Veer Narmad South Gujarat University, and deemed minimal-risk observational research consistent with the ICMR (2017) ethical guidelines and the Declaration of Helsinki.

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