Comparison of the Human Gut Microbiota between Normal Control Subjects and Patients with Colonic Polyps and Colorectal Cancer

Kittipot Uppakarn  
Prince of Songkla University

Khotchawan Bangpanwimon  
Prince of Songkla University

Tipparat Hongpattarakere  
Prince of Songkla University

Worrawit Wanitsuwan (wworrawi@medicine.psu.ac.th)  
Prince of Songkla University

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Abstract

**Background:** The human gut microbiota has been related to numerous colonic diseases. To identify colorectal cancer (CRC)-associated microbiota, the gut microbiomes of patients with colonic polyps and CRC compared to normal controls were analyzed.

**Methods:** Between July and December 2020, forty-four stool samples were obtained from participants older than 50 years who were scheduled for elective colonoscopies at the Surgery Clinic, Songklanagarind Hospital. The samples were divided into 3 groups (17 normal control, 17 colonic polyps, and 10 CRC) and were collected for analysis with a 16s metagenomic sequencing library preparation with MiSeq Reporter software (MSR) following the protocol of the 16s metagenomics workflow. The microbiome data were analyzed with Kruskal–Wallis test with the Dunn-Bonferroni post hoc method.

**Results:** The relative proportions of beneficial butyrate-producers *Kineothrix alysoides, Eubacterium rectale,* and *Roseburia inulinsivorans* were significantly higher in healthy control and colonic polyp groups compared with the CRC group at the top three lowest p-values. The recommended CRC biomarker *Clostridium symbiosum* was shown in a significantly higher proportion in the CRC group than in the normal control group. The prevalences and relative proportion of the novel CRC-associated species *Acutalibacter muris* and the familiar CRC-associated species *Christensenella massiliensis* and *Intestinimonas butyriciproducens* were significantly higher in the CRC group than in the normal control and colonic polyp groups at the top three lowest p-values.

**Conclusions:** A correlation between specific bacteria and clinical outcomes was found in this pilot study. The microbiome data revealed possible microbial biomarkers associated with CRC. Studies with larger numbers of stool samples are required to substantiate our findings.

Introduction

Colorectal cancer (CRC) is the second most deadly and the third most commonly diagnosed cancer in the world [1]. Approximately 1.97 million people worldwide or 10% of all cancer patients have been diagnosed with CRC in 2020 [1]. CRC is commonly found in people over 50 years of age. However, if a patient acknowledged any symptom and received treatment at the early stage, the patient would have a 93.2% of five years life extended whereas if a patient acknowledged cancer during the spreading stage, the curable rate was reduced to less than 10% [2]. The death rate is greater when CRC is detected in elderly patients. A recent study suggested that the gut microbiome could play a significant role in controlling the digestive system and thus in individual health [3]. In the human intestine, there are numerous microorganisms and up to 100 trillion cells, altogether weighing 1.5 kilograms, approximately three times the amount of cells in the individual human body [4]. The gut microbiome plays an important role in gut maturation, host nutrition, and pathogen resistance. It was found that the intestinal epithelial proliferation, fat collection in the metabolism system, and inflammatory immune response were controlled by the gut microbiome [5]. Scientific evidence found the relationship between the gut microbiome and CRC [6].
In recent years, following improvements in genetic engineering technology, a correlation between the gut microbiota and the CRC has been shown by different genetic analysis methods such as real-time PCR and next-generation sequencing. The microorganisms correlated with CRC were reported in the previous study such as *Fusobacterium*, *Providencia*, *Leptotrichia*, and *Campylobacter* genera. Most of the studies found that the amount of *Fusobacterium* spp. was significantly higher in CRC patients than in non-cancerous patients [7-9]. Nowadays, the studies of the correlation between microbiome from the feces and CRC patients had significantly shown an increased amount of the microbiome as well as the decline in gut microbiome diversity [7, 10]. Previous work found that the accuracy of fecal microbiota analysis for CRC screening in early and late stages was similar to the standard fecal occult blood test and when combining both assays the sensitivity could be increased by approximately 45% [11].

This present research aimed to identify appropriate biomarkers which could be used for potential CRC assessment in patients with gastrointestinal (GI) disorders and lead to further studies to investigate the scientific relationship of the surrounding factors in patients at risk for CRC.

**Methods**

**Sample collections and preparation**

The Human Research Ethics Committee, Faculty of Medicine, Prince of Songkhla University, reviewed and approved the study and all protocols and data collection forms (REC.62-327-10-4). This pilot study was conducted in Songklanagarind Hospital between July and December 2020. The clinical characteristics were collected in patients older than 50 years who were scheduled for elective colonoscopies at the surgery clinic. Forty-four stool samples were only collected from participants who agreed and signed an informed consent form. The specimens were divided into 3 groups based on colonoscopy and anatomic pathology reports. For stool preparation, at least 50 grams of stool specimen was collected in a plastic collection container then frozen immediately at -20°C at the outpatient clinic, and later transferred for storage at -20°C at the Office of Scientific Instrument and Testing, Prince of Songkhla University. For analysis, DNA was extracted from approximately 25 grams of stool sample by the GF-1 Bacterial DNA Extraction kit (Vivantis Technologies, Malaysia). A NanoDrop spectrometer (Thermo Fisher Scientific, USA) was used to evaluate the DNA concentration, which had to be not less than 5 ng/µL per 2.5 µL.

**Analysis of the microbiome**

An approximate 460 bp section of the V3–V4 region of the 16s rRNA gene was amplified by PCR analysis with the forward primer (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and reverse primer (5'-GTCTCGTGGCGACTACHVGGGTATCTAATCC-3') containing an Illumina adapter and Illumina index to mark each specimen. A Miseq Instrument (Illumina, California) with a Miseq v3 reagent kit (Illumina, California) was used for sequencing the 2 × 300 bp 16s rRNA amplicon. The sequencing data was analyzed using MiSeq Reporter software based on the Greengenes database to perform taxonomic classification showing phylum, genus, or species level classification. After quality
filtering, a total of 44 samples showing sequence 404,490 reads (average 9,192 reads) were included for downstream analysis.

**Statistical analysis**

The demographic and clinical characteristics of the study groups were analyzed using the ANOVA F-test for continuous variables and Fisher's exact test or Chi-Square test; \( \chi^2 \) for categorical variables. The microbiome data were analyzed to the Kruskal-Wallis test. The significant differences between groups were assessed by the Dunn-Bonferroni post hoc method. Statistical significance was assumed for adjusted p values <0.05 using IBM SPSS software ver. 22.

**Results**

Differences in bacterial community profiles between the normal control subject and colonic polyp and CRC patients were determined by 16s metagenomic sequencing. The demographic and clinical characteristics of the subject groups are shown in Table 1. A total of 17 normal control cases, 17 colonic polyp cases, and 10 CRC cases were analyzed in this study. The median body mass index (BMI) of the CRC group (20.9 ± 2.4) was significantly lower than in the normal control and colonic polyp groups (24.5 ± 3.5, 26.1 ± 3.4, respectively). The prevalence rates of patients with hypertension and who were taking aspirin in the CRC group were significantly higher than in the normal control and colonic polyp groups. The reasons for having a colonoscopy were significantly different, with clinical symptoms of gut obstruction and lower gastrointestinal bleeding which were higher in the CRC group than both the normal control and colonic polyp groups, while a regular colonoscopy screening was the least reported reason in the CRC group.

The comparison of microbiomes at the phylum level is shown in Fig. 1 and Table 2. The most abundant phyla in all groups were Bacteriodetes and Firmicutes. Phylum-level analyses identified one bacterial phylum, Synergistetes (p<0.05), that was significantly different between the normal control group and the CRC group. The relative abundance of phylum Euryarchaeota (p<0.01) in the CRC group was significantly higher than both the normal control and colonic polyp groups. The prevalence rates of both phyla in the CRC group were also higher in the normal control and colonic polyp groups. Other bacterial phyla were not significantly different between the groups.

Genus-level analyses as shown in Table 3 decreases in the levels of the genera *Kineothrix* (p<0.01), *Lactobacillus* (p<0.01), and *Lacrimsipora* (p<0.05) that were significantly different between the CRC group and both the normal control and colonic polyp groups, in contrast to the increased levels of the genera *Acutalibacter, Intestinimonas, Christensenella, Methanobrevibacter, Petrocella, Thermotalea, Comamonas,* and *Emergencia,* which were significantly different between the CRC group and both the normal control and colonic polyp groups at p-value<0.01. Likewise, the levels of the genera *Cloacibacillus, Culturomica, Anaerotruncus,* and *Neglecta* were significantly different (p<0.05) between the CRC group and the normal control group.
Table 4 demonstrates the comparison of microbiomes at the species level. The relative abundances of three bacterial species (Kineothrix alysoides, Eubacterium rectale, and Roseburia inulinivorans) were significantly lower in the CRC subjects than both the normal control and colonic polyp subjects at P-value<0.01. The relative abundances of four bacterial species (Lactobacillus rogosae, Lacrimispora xylanolytica, Roseburia intestinalis, and Lachnoclostridium pacaense) were significantly lower in the CRC subjects than both the normal control and colonic polyp subjects at P-value<0.05 while the relative abundances of ten bacterial species (Acatalibacter muris, Christensenella massiliensis, Intestinimonas butyriciproducens, Odoribacter laneus, Methanobrevibacter smithii, Thermodonta metallivorans, Comamonas kerstersii, Emergencia timonensis, Petrocella atlantisensis, and Butyricimonas virosa) were significantly higher in the CRC group than in the normal control and colonic polyp groups at P-value<0.01. The relative abundances of ten bacterial species (Cloacibacillus porcorum, Clostridium symbiosum, Parabacteroides chongii, Culturomica massiliensis, Oscillibacter valericigenes, Anaerotruncus colihominis, Christensenella minuta, Eubacterium limosum, Coprococcus catus, and Neglecta timonensis) were significantly higher between the CRC cases and normal control cases but not significantly different from the colonic polyp cases. Additionally, the prevalence rate data of the genus and species level analyses in Table 3 and Table 4 corresponded with the mean abundance values (MAV) as well.

Discussion

The data showed a statistically significant decrease of BMI in the CRC group as a result of poor oral intake and intestinal dysfunction in cancer patients. A previous study showed body weight loss in patients with CRC is correlated with tumor location, size, depth, and the prognostic factor for poor outcomes including overall survival and tumor relapse [12]. The clinical symptoms include abdominal pain, changes in bowel habits, constipation, and diarrhea, although these symptoms are not specific for the CRC. The symptoms of lower gastrointestinal bleeding and gut obstruction were found less in the normal control and colonic polyp groups than in the CRC group. Previous evidence suggests that the common practice of performing a colonoscopy to check for cancer in people with bowel symptoms is warranted only for gastrointestinal bleeding and the general symptom of weight loss [13].

This study found different bacterial flora between the normal control and CRC participants by next-generation sequencing analysis. Notwithstanding, this research didn't show statistically significant differences in gut microbiota between the normal control and colonic polyp groups. Only prominent gut microorganisms significantly associated with the normal control and CRC subjects would be indicated in Table 3 and Table 4.

Phylum-level investigations demonstrated that the relative proportions of the phyla Euryarchaeota and Synergistetes were significantly higher in the CRC group than in the normal control group. Previous studies show that the Methanobrevibacter genus in the Euryarchaeota phylum has been associated with CRC and linked to periodontitis and various GI disorders [14]. M. smithii is a colonic methane producer and it is a predominant methanogen in irritable bowel syndrome with constipation patients [15]. The level
of methane in CRC subjects was significantly higher than in healthy subjects [16]. A previous study indicated that individuals who high methane production showed high risks of colonic polyposis, ulcerative colitis, and colon cancer [17]. The genus *Methanobrevibacter* was found co-occurring with the genus *Christensenella*. Those genera were notably higher in the normal BMI (<25) subjects compared to the obese BMI (>30) subjects [18]. Previous studies found that the *Christensenella* genus was over-represented in CRC patients compared to the normal controls [19, 20]. The affluence of the genus *Christensenella* is higher in cancer patients with ZNF717 mutations. Burns et al. found that mutations in ZNF717, a transcription factor, regularly changed in numerous GI cancers such as CRC [21]. Alonso et al. reported *C. minuta* was isolated from a blood sample of an acute appendicitis patient [22]. In another study, the *Cloacibacillus* genus member of the Synergistetes phylum was higher in CRC patients than in healthy controls. This genus was at high levels in patients with CRC stage IV [23]. A previous study indicated that both *C. porcorum* and *C. evryensis* were potential human pathogens associated with bacteremia [24]. Yu et al. indicated that *C. evryensis* in the feces of CRC cases was significantly higher than in the control cases [25]. In another study, *C. porcorum*, a mucosa-associated degrading bacterium, was isolated from the swine intestinal tract [26].

Genus-level and species-level analyses found that the genus *Sutterella* and the species *L. rogosae* and *C. spiroforme* occurred in greater abundances in the normal control group compared to the CRC group. Deng et al. reported that the *Sutterella* genus was found in greater abundance in the normal control group compared to in the esophageal cancer group [27]. Another study found that the *Sutterella* genus has a high association with the survival of CRC patients, as a higher level of this bacteria was related to better CRC patient survival [28]. The previous study indicated that the relative abundance of *Lactobacillus* genus was significantly higher in the normal control group than in the CRC group [29]. A dysbiosis study found that the relative proportion of *L. rogosae* was significantly higher in normal control cases compared with the CRC and obese cases [30]. Ningning et al. reported in the research square preprint services that the lower abundance of *C. spiroforme* was detected in the stool samples of gastrointestinal cancer patients with decreased FOXP3+CD4+ T regulated T cells (Treg) and natural killer (NK) cells. The Treg and NK cells were strongly associated with better CRC patient survival. There is an assumption that higher numbers of Treg and NK cells were associated with longer survival times [31]. Furthermore, the relative proportions of butyrate-producer species, including *K. alysoides*, *E. rectale*, *L. xylanolytica*, *L. pacaense*, *R. inulinivorans*, and *R. intestinalis*, were significantly higher in the normal control group than in the CRC group. Butyrate is an essential substrate for enterocytes that appears to have anti-oxidative, anti-inflammatory, and anti-carcinogenic activities and plays a beneficial role in regulating gut homeostasis and controlling immune development [32]. The butyrate-producing bacteria *K. alysoides* facilitates regulation of the gut barrier by serving as an energy source for colonocytes. Another study found that the relative abundance of *K. alysoides* gradually decreased as the liver dysfunction progressed to liver cirrhosis [33]. Stadler et al. reported strong evidence linking obesity with increasing the risk of CRC [34]. Park et al. found that participants who had reduced TC and LDL-cholesterol levels had increased *K. alysoides* [35]. Previous studies indicated the levels of *E. rectale* in inflammatory bowel disease (IBD) and CRC participants were significantly lower compared to healthy controls [36, 37].
Youssef et al. demonstrated that lower abundances of the genus *Lachnolclostridium* have been observed in stools of patients with stomach, small intestine, or colon neoplasm as compared to stools of healthy controls [38]. Japanese research reported that *L. pacaense* was at high levels in healthy centenarians compared with IBD patients [39]. Another previous study indicated the genus *Roseburia* was found in higher abundance in healthy controls compared with CRC and hepatocellular carcinoma patients [40]. *R. inulinivorans* acts as a probiotic associated with mucus production and is significantly more abundant in healthy controls than in rectal cancer patients [41]. Another species, *R. intestinaalis* could reduce levels of endotoxin, inflammatory cytokines, and the macrophages chemoattractant interleukin resulting in enhancement of gut barrier functions. In *vivo* analysis, higher levels of β-hydroxybutyrate and lower levels of lipopolysaccharide (LPS) were found in a mice model with a higher abundance of *R. intestinaalis*. The β-hydroxybutyrate inhibits the chronic inflammatory pathway in contrast to the LPS increase inflammatory response by activating arachidonic acid signaling in macrophages through the IL-12 expression [42]. A previous study found that the supernatant of *R. intestinaalis* could suppress signal transducer and activator of transcription 3 and decrease the IL-6 production by macrophage [43]. Another work indicated that this bacteria could be able to enhance the production of anti-inflammatory factors including TSLP, TGF-β, and IL-10 and increase the level of colonic Treg cells [44]. In another murine model, *R. intestinaalis* also down-regulated the expression of the oncostatin M and increased tight junction integrity for enhancing gut barrier function [45].

Genus-level and species-level analyses in this study indicated that several harmful bacteria were harbored in the CRC group. Decousser et al. reported that *C. symbiosum* was isolated from the bloodstream of a highly immunocompromised patient with metastatic CRC [46]. Another work indicated *C. symbiosum* was over-represented in CRC patients with early- and late-stage CRC compared to the normal controls in 335 participants from several countries [11]. Remarkably, *C. symbiosum* was recommended as a high-accuracy biomarker for both early- and late-stage CRC diagnosis in many previous reports [47, 48]. Other studies found that the relative abundance of *Comamonas* genus was higher in patients with CRC compared with healthy controls [14, 23, 49]. The level of this genus was significantly higher in patients with CRC stage IV [23]. Other studies found that *C. kerstersii* could be isolated from patients with intra-abdominal and urinary tract infections including peritonitis, diverticulosis, and appendix rupture [50-52]. Three previous reports demonstrated that the relative abundances of the genera *Odoribacter* and *Oscillibacter* were significantly higher in patients with CRC compared with normal controls [19, 23, 53]. In an *in vivo* investigation, the levels of *Odoribacter laneus* were significantly higher in colitis rats compared to wild-type rats [54]. The genus *Oscillibacter* was more abundant in patients with CRC stage III [23]. The negative relationship between the abundance of this genus and the gut barrier function in the proximal colon is interesting. The increased level of the genus *Oscillibacter* in obese and diabetic animal models had a relationship with increased gut permeability and decreased gut barrier integrity which was linked with metabolic dysfunction and mesenteric fat inflammation development [55]. Some species in this genus such as *Oscillibacter valericigenes* were in higher abundance in unilateral ureteral obstruction mice, which were associated with higher levels of the genus *Intestinimonas* [56]. Osman et al. revealed the over-representation of *I. butyriciproducens* was
detected in 88.9% of the CRC subjects [20]. In vivo study indicated the average abundance of Culturomica genus in mice with chronic kidney disease was higher than in normal control mice [57]. The previous studies reported the Parabacteroides genus was tightly associated with CRC patients. It was isolated from a biofilm-positive human CRC tumor when transporting it to a new cohort of germ-free mice, the CRC will have occurred. The relative abundance of this genus was strongly positively associated with increased tumors in a mice model [58, 59]. This genus was previously reported as a potential biomarker for cervical cancer diagnosis [60]. Another study indicated the genus Anaerotruncus was more prevalent in the intestinal lumen of CRC subjects compared to healthy subjects [61]. Both P. chongii and A. colihominis were previously isolated from the bacteremia patients [62, 63]. The USA microbiome study showed that N. timonensis was found in greater abundance in CRC groups compared to normal groups [64]. Previous works found that E. timonensis could produce significant amounts of trimethylamine N-oxide, a metabolite associated with an increased risk of CRC and cardiovascular disease [65, 66]. Infrequent human pathogens were reported in previous works including E. limosum were detected in bacteremia patients with CRC, gynecologic cancer, gastrointestinal disease, and diabetes mellitus [67]. B. virosa was isolated from bacteremia patients with CRC, peritonitis, and intestinal perforation [68, 69]. C. catus was reported as a dominant bacteria in the stools of breast cancer stage I patients [70]. T. metallivorans was isolated from the sputums of patients with respiratory tract infections [71]. Interestingly, we also found the levels of A. muris and P. atlantisensis were overrepresented in CRC patients that both bacteria have not been previously reported to be CRC-related bacteria. Studies with larger numbers of fecal samples are required to substantiate our findings. Pathological non-equilibrium of the gut microbiome or dysbiosis of the gut microbiome was found in CRC subjects. Intestinal bacteria might influence CRC directly or indirectly through secreting metabolites, invading intestinal tissues, and regulating host immune response. The underlying tumorigenesis of numerous CRC-correlated bacteria remains undescribed.

Colonoscopy is the gold standard for sporadic CRC screening at age above 50 years. It is a minor invasive procedure, needing thorough bowel cleansing, sometimes performed under anesthesia or sedative, with a risk of post-procedural bleeding, and a small risk of gut perforation with the air or CO2 [72]. The fecal occult blood test is a non-invasive currently routine diagnostic method for screening for CRC which has a medium sensitivity (24% to 86%) [73]. Bleeding in the stool from a cancer cause is normally intermittent which can lead to false-negative results and lower sensitivity. Additionally, this test could be positive in other diseases with bleeding leading to false-positive results and lower specificity [74]. The current assay for CRC screening requires improvement. The previous study reported that a change of gut microbiota might be an indicator of the initiation and progression of CRC [10]. We found statistically significant differences in the microbiome between the normal control group and the CRC group, and suggest the four bacteria shown in Fig. 2 as a putative biomarker panel that might be useful in CRC screening including one recommended CRC biomarker species C. symbiosum (PR of normal controls: CRC = 18%: 70%, MAV of Normal Control: CRC = 0.022%: 0.157%) [47, 48] and the top three lowest p-values by Kruskal-Wallis test including a novel CRC-associated species A. muris (PR of Normal Control: CRC = 0%: 60%, MAV of Normal Control: CRC = 0%: 0.203%), and two familiar CRC-
associated species *C. massiliensis* (PR of Normal Control: CRC = 6%: 60%, MAV of Normal Control: CRC = 0.001%: 1.741%), and *l. butyriciproducens* (PR of Normal Control: CRC = 47%: 90%, MAV of Normal Control: CRC = 0.411%: 0.577%). Based on the microbiome analysis in our research, we propose the candidate bacterial biomarkers associated with CRC. The development of the CRC screening tool based on bacterial biomarkers is underway in the next study. The bacterial biomarkers combined with conventional screening tools and clinical risk factors could increase the accuracy of CRC diagnoses and might facilitate the physician's decision for early management and treatment of CRC patients.

**Conclusion**

The relative proportions of the beneficial butyrate-producer species such as *K. alysoides*, *E. rectale*, and *R. inulinsivorans* were significantly higher in the fecal samples of the healthy control and colonic polyp groups compared to the CRC group as indicated by 16s metagenomic sequencing. In addition, the relative abundances of several CRC-associated bacteria, including *C. symbiosum* as a recommended CRC biomarker species, *A. muris* as a novel CRC-associated species, and *C. massiliensis*, and *l. butyriciproducens* as familiar CRC-associated species, were statistically significantly higher in the feces of the patients with CRC compared to healthy controls and colonic polyp patients. The microbiome data in this work could be useful for potential CRC assessment in patients with GI disorders and lead to further studies to investigate the scientific relationship between gut biomarkers and CRC risk.

**Abbreviations**

CRC: Colorectal cancer; BMI: Body Mass Index; IBD: Inflammatory Bowel Disease; ANOVA: Analysis of Variance; SD: Standard Deviation; MRS: MiSeq Reporter Software; MAV: Mean Abundance Value; PR: Prevalence; LPS: Lipopolysaccharide; GI: Gastrointestinal; DLP: Dyslipidemia; ACS: Acute Coronary Syndrome; HT: Hypertension; ASA: On Aspirin; DM: Diabetes Mellitus.

**Declarations**

**Ethics approval and consent to participate**

This study was performed in line with the principles of the Declaration of Helsinki. The Human Research Ethics Committee (HREC), Faculty of Medicine, Prince of Songkhla University, Thailand, reviewed and approved the study and all protocols and data collection forms (REC.62-327-10-4). All subjects were asked for their permission to participate, and signed consent forms.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analyzed during this study are included in the published article.
Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WW designed and conducted the study, provided the cases, reviewed and edited the manuscript. KU analyzed and interpreted data, statistical analysis of the data, and drafted the manuscript. KB interpreted data, statistical analysis of the data, and helped draft the manuscript. TH helped plan the study, revised the article critically. All authors have read and approved the final manuscript.

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**Tables**

**Table 1** Demographic and clinical characteristics of the study groups
| Characteristic                          | NC (N=17) | CP (N=17) | CRC (N=10) | P-value |
|----------------------------------------|-----------|-----------|------------|---------|
| Age, mean (SD)                         | 62.1 (8.4)| 65.1 (8.8)| 69.9 (10.2)| 0.106   |
| BMI, median (SD)                       | 24.5 (3.5)| 26.1 (3.4)| 20.9 (2.4)| 0.001   |
| Gender                                 |           |           |            | 0.112   |
| Female                                 | 10 (58.8)| 4 (23.5)  | 4 (40)     |         |
| Male                                   | 7 (41.2) | 13 (76.5) | 6 (60)     |         |
| Preexisting diseases                   |           |           |            |         |
| Diabetes mellitus (DM)                 | 1 (5.9)   | 3 (17.6)  | 4 (40)     | 0.106   |
| Hypertension (HT)                      | 2 (11.8)  | 8 (47.1)  | 7 (70)     | 0.007   |
| Dyslipidemia (DLP)                     | 4 (23.5)  | 8 (47.1)  | 7 (70)     | 0.058   |
| Acute coronary syndrome (ACS)          | 0 (0)     | 1 (5.9)   | 1 (10)     | 0.695   |
| On Aspirin (ASA)                       | 0 (0)     | 1 (5.9)   | 3 (30)     | 0.032   |
| Reason for colonoscopy                 |           |           |            |         |
| Abdominal pain                         | 2 (11.8)  | 1 (5.9)   | 0 (0)      | 0.782   |
| Bowel habit change                     | 0 (0)     | 4 (23.5)  | 2 (20)     | 0.122   |
| Historical polyps                      | 3 (17.6)  | 1 (5.9)   | 0 (0)      | 0.427   |
| Gut obstruction                        | 0 (0)     | 0 (0)     | 3 (30)     | 0.009   |
| Lower gastrointestinal bleeding        | 1 (5.9)   | 5 (29.4)  | 5 (50)     | 0.029   |
| Screening colonoscopy                  | 11 (64.7) | 6 (35.3)  | 0 (0)      | 0.003   |

Data are shown as n (%) unless indicated otherwise. P-values are based on ANOVA F-test for continuous variables and Fisher's exact test or Chi-Square test for categorical variables. NC = normal control, PC = colonic polyps, CRC = colorectal cancer.

**Table 2** Prevalence of phylum and average abundance distribution of gut microbiomes of normal control, colonic polyp, and colorectal cancer groups

Different letters indicate significant differences according to Kruskal–Wallis test with the Dunn-Bonferroni post hoc method. (* = p < 0.01, ** = p < 0.05). PR = prevalence, MAV = mean abundance value, NC = normal control, PC = colonic polyps, CRC = colorectal cancer.
| Phylum            | PR: MAV of NC (%) | PR: MAV of PC (%) | PR: MAV of CRC (%) | P-value |
|-------------------|-------------------|-------------------|--------------------|---------|
|                   | (N=17)            | (N=17)            | (N=10)             |         |
| Bacteroidetes     | 100: 47.53        | 100: 36.17        | 100: 42.40         | 0.171   |
| Firmicutes        | 100: 39.88        | 100: 50.98        | 100: 36.50         | 0.072   |
| Proteobacteria    | 100: 10.17        | 100: 7.543        | 100: 10.52         | 0.604   |
| Actinobacteria    | 88: 1.281         | 82: 3.007         | 100: 1.526         | 0.885   |
| Fusobacteria      | 53: 0.618         | 47: 0.702         | 80: 0.594          | 0.552   |
| Verrucomicrobia   | 47: 0.366         | 24: 1.229         | 50: 6.199          | 0.248   |
| Synergistetes**   | 12: 0.002 a       | 18: 0.011 ab      | 50: 1.952 b        | 0.024   |
| Euryarchaeota*    | 0: 0.000 a        | 6: 0.008 a        | 40: 0.068 b        | 0.004   |
| Tenericutes       | 6: 0.056          | 0: 0.000          | 0: 0.000           | 0.452   |
| Cyanobacteria     | 0: 0.000          | 12: 0.005         | 0: 0.000           | 0.197   |
| Unclassified      | 53: 0.091         | 53: 0.342         | 60: 0.243          | 0.817   |

**Table 3** Prevalence of genera and average abundance distribution representative of gut microbiomes from normal control, colonic polyp, and colorectal cancer groups
| Genus                  | PR: MAV of NC (%) (N=17) | PR: MAV of PC (%) (N=17) | PR: MAV of CRC (%) (N=10) | P-value |
|-----------------------|--------------------------|--------------------------|---------------------------|---------|
| **Kineothrix**        | 100: 1.066 b             | 94: 1.417 b              | 90: 0.187 a              | 0.002   |
| **Lactobacillus**     | 88: 0.481 b              | 76: 0.494 b              | 30: 0.057 a              | 0.012   |
| **Lacrimispora**      | 94: 0.610 b              | 76: 0.426 b              | 50: 0.051 a              | 0.014   |
| **Sutterella**        | 65: 0.556 b              | 47: 0.190 ab             | 20: 0.007 a              | 0.027   |
| **Acutalibacter**     | 0: 0.000 a               | 6: 0.001 a               | 60: 0.203 b              | 0.000   |
| **Intestinimonas**    | 47: 0.411 a              | 18: 0.014 a              | 90: 0.577 b              | 0.001   |
| **Christensenella**   | 6: 0.002 a               | 18: 0.009 a              | 60: 1.762 b              | 0.001   |
| **Methanobrevibacter**| 0: 0.000 a               | 6: 0.008 a               | 40: 0.068 b              | 0.004   |
| **Petrocella**        | 0: 0.000 a               | 0: 0.000 a               | 30: 4.724 b              | 0.005   |
| **Thermotalea**       | 0: 0.000 a               | 0: 0.000 a               | 30: 0.009 b              | 0.005   |
| **Comamonas**         | 0: 0.000 a               | 0: 0.000 a               | 30: 0.411 b              | 0.005   |
| **Emergencia**        | 0: 0.000 a               | 0: 0.000 a               | 30: 0.013 b              | 0.005   |
| **Cloacibacillus**    | 6: 0.002 a               | 18: 0.011 ab             | 50: 1.526 b              | 0.011   |
| **Culturomica**       | 0: 0.000 a               | 6: 0.001 ab              | 30: 3.320 b              | 0.025   |
| **Anaerotruncus**     | 0: 0.000 a               | 6: 0.003 ab              | 30: 0.017 b              | 0.028   |
| **Neglecta**          | 18: 0.005 a              | 35: 0.024 ab             | 70: 0.020 b              | 0.044   |

Different letters indicate significant differences according to Kruskal–Wallis test with the Dunn-Bonferroni post hoc method. (* = $p< 0.01$, ** = $p< 0.05$). PR = prevalence, MAV = mean abundance value, NC = normal control, PC = colonic polyps, CRC = colorectal cancer.

**Table 4** Prevalence of species and average abundance distribution representative of gut microbiomes from normal control, colonic polyp, and colorectal cancer groups
| Species                                      | PR: MAV of NC (%) | PR: MAV of PC (%) | PR: MAV of CRC (%) | P-value |
|----------------------------------------------|-------------------|-------------------|--------------------|---------|
| **Kineothrix alysoides**                     | 100: 1.066 b      | 94: 1.417 b       | 90: 0.187 a        | 0.002   |
| *Eubacterium rectale*                       | 94: 2.106 b       | 88: 3.610 b       | 60: 0.227 a        | 0.005   |
| *Roseburia inulinivorans*                   | 76: 0.648 b       | 94: 1.184 b       | 50: 0.075 a        | 0.005   |
| **Lactobacillus rogosae**                   | 88: 0.481 b       | 76: 0.494 b       | 30: 0.057 a        | 0.012   |
| **Lacrimispora xylanolytica**               | 94: 0.610 b       | 76: 0.426 b       | 50: 0.048 a        | 0.012   |
| *Roseburia intestinalis**                   | 71: 0.126 b       | 65: 0.418 b       | 20: 0.004 a        | 0.015   |
| **Lachnoclostridium pacaense**              | 76: 0.851 b       | 59: 0.871 b       | 20: 0.044 a        | 0.024   |
| **Clostridium spiroforme**                  | 82: 1.559 b       | 41: 0.359 ab      | 20: 0.392 a        | 0.036   |
| *Acutalibacter muris*                       | 0: 0.000 a        | 6: 0.001 a        | 60: 0.203 b        | 0.000   |
| *Christensenella massiliensis*              | 6: 0.001 a        | 6: 0.006 a        | 60: 1.741 b        | 0.000   |
| *Intestinimonas butyriciproducens*          | 47: 0.411 a       | 18: 0.014 a       | 90: 0.577 b        | 0.001   |
| *Odoribacter laneus*                        | 0: 0.000 a        | 6: 0.001 a        | 40: 0.334 b        | 0.004   |
| *Methanobrevibacter smithii*                | 0: 0.000 a        | 6: 0.008 a        | 40: 0.068 b        | 0.004   |
| *Thermotalea metallivorans*                 | 0: 0.000 a        | 0: 0.000 a        | 30: 0.009 b        | 0.005   |
| *Comamonas kerstersii*                      | 0: 0.000 a        | 0: 0.000 a        | 30: 0.411 b        | 0.005   |
| *Emergencia timonensis*                     | 0: 0.000 a        | 0: 0.000 a        | 30: 0.013 b        | 0.005   |
| *Petrocella atlantisensis*                  | 0: 0.000 a        | 0: 0.000 a        | 30: 4.724 b        | 0.005   |
| *Butyricimonas virosa*                      | 18: 0.055 a       | 12: 0.002 a       | 60: 0.213 b        | 0.006   |
| **Cloacibacillus evryensis**                | 6: 0.002 a        | 6: 0.001 a        | 40: 0.128 b        | 0.014   |
| **Cloacibacillus porcorum**                 | 0: 0.000 a        | 12: 0.010 ab      | 40: 1.398 b        | 0.010   |
| **Clostridium symbiosum**                   | 18: 0.022 a       | 59: 0.134 ab      | 70: 0.157 b        | 0.015   |
| **Parabacteroides chongii**                 | 0: 0.000 a        | 12: 0.012 ab      | 40: 0.056 b        | 0.019   |
Culturomica massiliensis**  
0: 0.000 a  
6: 0.001 ab  
30: 3.320 b  
0.025

Oscillibacter valericigenes**  
65: 0.348 a  
65: 0.632 ab  
90: 3.745 b  
0.028

Anaerotruncus colihominis**  
0: 0.000 a  
6: 0.003 ab  
30: 0.012 b  
0.032

Christensenella minuta**  
6: 0.001 a  
12: 0.004 ab  
40: 0.021 b  
0.038

Eubacterium limosum**  
12: 0.011 a  
29: 0.126 ab  
60: 0.096 b  
0.038

Coprococcus catus**  
24: 0.026 a  
29: 0.049 ab  
70: 0.191 b  
0.043

Neglecta timonensis**  
18: 0.005 a  
35: 0.024 ab  
70: 0.020 b  
0.044

Different letters indicate significant differences according to Kruskal–Wallis test with the Dunn-Bonferroni post hoc method. (* = p< 0.01, ** = p< 0.05). PR = prevalence, MAV = mean abundance value, NC = normal control, PC = colonic polyps, CRC = colorectal cancer.

Figures

Figure 1

The gut microbiome profiling at the phylum level. Mean abundance value (%) of the microbiota at the phylum level in fecal samples of the normal control (n = 17), colonic polyp (n = 17), and colorectal cancer
(n = 10) groups.

**Figure 2**

The candidate CRC-associated bacteria biomarkers. The bacterial abundance of a recommended CRC biomarker species *Clostridium symbiosum*, a novel CRC-associated species *Acetalibacter muris*, and the familiar CRC-associated species *Christensenella massiliensis*, and *Intestinimonas butyriciproducens* that were analyzed from the fecal samples of the normal control (n = 17), colonic polyp (n = 17), and colorectal cancer (n = 10) groups.