Intestinal bacteria detected in cancer and adjacent tissue from patients with colorectal cancer

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Abstract. Intestinal bacteria are symbiotic microbiota within the human gut and are implicated in the occurrence and development of colorectal cancer (CRC). The current study investigated the changes in bacterial composition prior to and following surgery, as well as the differences in the bacterial community structure between cancer tissue and adjacent normal tissue. The diversity of the bacterial community and the composition of the bacteria were assessed. In addition, phylogenetic analysis and principle component analysis (PCA) were performed. The results revealed that cancer tissue and adjacent normal tissue exhibited similar bacterial compositions. However, a significant difference was identified in the composition of intestinal bacteria in stool samples collected from patients following surgery compared with stool samples collected prior to surgery. Each patient had their own unique intestinal bacterial community, likely due to a number of factors, including diet, genetic factors and health status. In addition, phylogenetic trees revealed that the most abundant operational taxonomic unit, 0001, was associated with *Escherichia coli* in all samples. Finally, PCA suggested that the bacterial community structure in all patient stools was similar following surgery. The current study provides information regarding the diversity of the intestinal bacterial community of patients with CRC and provides a basis for postoperative intestinal assessments.

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Abbreviations: CRC, colorectal cancer; PCA, principle component analysis; OTU, operational taxonomic unit

Key words: colorectal cancer, surgery, bacterial composition, stool, tissue

Introduction

Approximately 10^{14} colony-forming units of bacteria colonize the human intestinal tract, particularly within the distal intestine, which contains 1,000-1,150 prevalent bacterial species (1,2). These bacteria degrade various indigestible polysaccharides, including plant-derived pectin, cellulose, hemicellulose and resistant starches, which is mutualistic with host health (1). Intestinal bacteria serve an important role in human health and dysbiosis of these bacteria is associated with obesity, type II diabetes, inflammatory bowel disease and colorectal cancer (CRC) (3-7).

CRC is one of the most common cancer types, with the third highest incidence rate and the fourth highest mortality rate of all cancer types worldwide (8). CRC initiation and development are associated with diet, environment and inheritance; however, only 20-25% of cases are associated with genetic factors (9). Previous studies have suggested that the western lifestyle is the most important risk factor for CRC, particularly improper diet, including the consumption of fat, alcohol and red meat (10,11). Improper diet leads to changes in intestinal microbiota, which is associated with CRC. CRC then causes further changes in intestinal microbiota (12). It has been reported that specific bacterial species serve important roles in CRC initiation and development. Enterotoxigenic *Bacteroides fragilis* can secrete *B. fragilis* toxin that induces colonic signal transducer and activator of transcription 3 activation, which activates the T helper 17 cell mucosal immune response and enhances colonic tumor formation (13). *Fusobacterium nucleatum* and *Escherichia coli* are implicated in CRC development and metastasis, respectively (14,15). Certain studies have confirmed that the occurrence of CRC affects the gut microbiota community structure. The diversity of microbiota is lower in CRC tissues compared with corresponding colorectal mucosal tissues from healthy individuals (3). Furthermore, Firmicutes and Fusobacteria are more highly abundant in patients with CRC compared with healthy individuals, whereas Proteobacteria are less abundant in patients with CRC (3). Lower levels of core species of bacteria have been identified in stool samples from patients with CRC compared with healthy individuals, including *Bifidobacterium longum*, *Clostridium clostridioforme* and *Ruminococcus* species (16). However, to the best of our

knowledge, the similarity of bacterial composition between cancer tissue and adjacent normal tissue remains unknown.

Colorectal tumors are typically surgically excised (17), however, it is not understood whether resection may induce changes in intestinal bacteria. In addition, the use of antibiotics following surgery may lead to changes in the intestinal bacteria (18), however, the bacterial community structure following surgery is unknown.

The aim of the current study was to determine the differences in the bacterial community structure between CRC tissue and adjacent normal tissue, as well as changes in intestinal bacteria in patients with CRC following surgery. Stool samples were collected prior to and following surgery, cancer tissue and adjacent normal tissue were obtained from patients with CRC, and the bacterial community structures were analyzed.

Materials and methods

Sample collection. The current study was reviewed and approved by the Ethics Committee of The First People's Hospital of Yunnan Province (Yunnan, China) and informed written consent was obtained from all participants. A total of 16 stool samples and 16 tissue samples were obtained from 8 patients with CRC at The First People's Hospital of Yunnan Province between January 2015 and December 2016. The patients' information is presented in Table I. Stool samples were collected ~7 days prior to surgery when a normal diet was received and ~10 days following surgery when a normal post-operative diet was received. All patients received cephamycin treatment for 3 days following surgery, consisting of 2 g twice a day. Tissue samples were collected during surgery, including cancerous tissue and adjacent normal tissue. The cancerous tissue consisted of the tumor and the corresponding intestinal wall, including the mucosal layer, the submucosal layer, the muscular layer and the serous layer. The adjacent normal tissue consisted of the normal intestinal wall, including the mucosal layer, the submucosal layer, the muscular layer and the serous layer, with a distance from the tumor of 2 cm. All samples were collected in a sterile sample box and stored at -80˚C until further experiments were performed.

Total DNA extraction. The layer of mucous membranes that surrounds tissues may affect the efficiency of DNA extraction. To minimize this, tissue samples were ground in liquid nitrogen. Then, DNA was extracted using a cetyltrimethylammonium bromide-based method (19), with slight modifications. Briefly, 0.2 g of sample was placed in a 2 ml sterile centrifugal tube and resuspended with 10 μl (50 mg/ml) lysozyme. Subsequently, 25 μl 20% SDS and 5 μl (20 mg/ml) protease K were added to the solution, which was then incubated in a water bath at 55˚C for 2 h. Finally, DNA was extracted and purified, as previously described (19). DNA samples were stored at -20˚C until subsequent experiments.

The genomic DNA of the stool samples was extracted using the QIAamp Fast DNA Stool Mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. DNA samples were stored at -20˚C until subsequent experiments.

Bacterial 16s ribosomal RNA (rRNA) gene amplification and analysis. The bacterial 16s rRNA gene was amplified using polymerase (Tiangen Biotech, Co., Ltd., Beijing, China) and the following barcoded primers: 343F, 5'-TACGGRAGGCAGCAG TCG-3' and 798R 5'-AGGGTATCTAATCCTGTCT-3' (20). The thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min followed by 30 cycles of 94˚C for 30 sec, 52˚C for 1 min and 72˚C for 1 min, and a final elongation step of 10 min at 72˚C. To eliminate heteroduplexes, the amplified reaction was diluted 10-fold into a fresh reaction mixture of the same composition, which was cycled five times using the aforementioned conditions (21). Polymerase chain reaction (PCR) products were purified using a Gel Extraction kit (BioTeke Corporation, Beijing, China) according to the manufacturer's protocol. Each purified PCR product was sequenced using the Illumina MiSeq™ system (Illumina, Inc., San Diego, CA, USA).

Sequence analysis. All original sequence reads containing 'N' were discarded and sequences with <400 base pairs were omitted. All sequences were extracted using each sample's barcode and the barcode and primers were removed from the original sequences using BioEdit software (version 7.0.9.0; http://www.mbio.ncsu.edu/bioedit/bioedit.html). All sequences were aligned using the 'chimera.pueseus' command of mothur software (version 1.35.1; https://www.mothur.org/wiki/Download_mothur) (22). Sequences were assigned to an operational taxonomic unit (OTU) with 97% sequence homology. Small subunit ribosomal RNA sequence data and taxonomic information from the SILVA SSURef database (version 123) (23) were downloaded directly from the mothur website (https://www.mothur.org/).

An α-diversity analysis was performed using mothur (version 1.35.1) and included the following indexes: OTU number, Chao (24), ACE (25), Shannon (26) and Simpson (27). OTU numbers were divided on the basis of a similarity distance cut-off value of 0.03. The representative sequences of each OTU were aligned with the sequences of established taxonomic information obtained from the National Center for Biotechnology Information (NCBI) GenBank database using Clustal X software (version 1.83) (28). Phylogenetic trees were created using the neighbor-joining method (29) with MEGA software (version 5.0) (30) and the bootstrap number was 1,000. Finally, principle component analysis (PCA) was performed to evaluate variation between the bacterial composition from different patients and different samples.

Nucleotide sequences. The PCR product sequencing data obtained in the current study were deposited in the Sequence Read Archive of NCBI under the accession number SRP120060.

Results

Sequence information and diversity indices. Following the removal of replicates, duplicates and sequences with low quality or shortened read lengths (<400 bp), a total of 112,117 high-quality sequences with an average length of 424 bp were obtained. Diversity indices of OTU, Chao, ACE, Shannon and Simpson were determined and no significant differences were identified in the mean index for all samples (Table II). The coverage of all samples was >98%, indicating

Table I. Information regarding the 8 patients with colorectal cancer.

No.	Age, years	Sex	BMI	Location of tumor	Type of antibiotics following surgery	Duration of antibiotics following surgery	History of diabetes, hypertension or angiocardiopathy
Patient 1	74	F	17.78	Right colon	Cephamycin	3 days, 2 g two times a day	No
Patient 2	78	M	18.03	Sigmoid colon	Cephamycin	3 days, 2 g two times a day	No
Patient 3	51	F	22.31	Right colon	Cephamycin	3 days, 2 g two times a day	No
Patient 4	63	M	22.31	Rectum	Cephamycin	3 days, 2 g two times a day	Hypertension
Patient 5	59	M	21.95	Right colon	Cephamycin	3 days, 2 g two times a day	No
Patient 6	56	M	21.95	Rectum	Cephamycin	3 days, 2 g two times a day	No
Patient 7	50	M	22.71	Transverse colon	Cephamycin	3 days, 2 g two times a day	No
Patient 8	59	F	30.84	Right colon	Cephamycin	3 days, 2 g two times a day	Hypertension

F, female; M, male; BMI, body mass index.

Table II. Sequence numbers, lengths and α-diversity indexes for all samples (cut-off value, 0.03).

Sample	Sequence, n	Mean length, bp	OTU number	ACE index	Chao index	Shannon index	Simpson index	Coverage
Patient 1_B	2428	429	14	22	29	0.31	0.89	1
Patient 1_C	4308	429	37	165	75	1.07	0.51	1
Patient 1_N	1602	429	9	12	11	0.61	0.7	1
Patient 1_A	3641	427	48	144	84	1.52	0.3	0.99
Patient 2_B	2581	429	14	25	17	0.19	0.94	1
Patient 2_C	2607	430	14	14	14	0.93	0.56	1
Patient 2_N	2302	429	49	113	77	1.46	0.35	0.99
Patient 2_A	3322	427	67	146	96	1.88	0.27	0.99
Patient 3_B	3679	416	99	208	147	2.87	0.11	0.99
Patient 3_C	2393	418	97	162	132	2.67	0.13	0.99
Patient 3_N	3285	423	107	244	154	1.24	0.64	0.99
Patient 3_A	2261	419	35	133	69	1.65	0.27	0.99
Patient 4_B	3324	429	34	141	61	1.39	0.32	0.99
Patient 4_C	3269	417	49	163	112	2.34	0.15	0.99
Patient 4_N	3131	428	67	109	122	0.63	0.82	0.99
Patient 4_A	2809	428	33	82	46	1.45	0.34	0.99
Patient 5_B	3863	422	78	140	105	2.42	0.18	0.99
Patient 5_C	6224	424	98	192	162	2.61	0.12	0.99
Patient 5_N	6990	428	112	204	172	1.96	0.29	0.99
Patient 5_A	4434	418	58	137	87	2.16	0.23	1
Patient 6_B	4390	424	120	164	168	2.4	0.24	0.99
Patient 6_C	6399	427	92	220	170	1.72	0.41	0.99
Patient 6_N	6384	424	85	180	135	2.77	0.09	0.99
Patient 6_A	2996	422	95	206	161	2.3	0.21	0.99
Patient 7_B	3471	415	134	256	187	2.54	0.17	0.99
Patient 7_C	3004	418	156	221	243	3.4	0.09	0.98
Patient 7_N	3607	413	137	311	252	2.56	0.16	0.98
Patient 7_A	3076	426	114	149	136	1.58	0.53	0.99
Patient 8_B	2785	429	50	233	113	1.77	0.28	0.99
Patient 8_C	2756	429	23	27	26	0.73	0.68	1
Patient 8_N	2820	429	33	71	56	1.06	0.55	1
Patient 8_A	1706	421	34	120	69	1.57	0.37	0.99

N, normal tissue; C, cancerous tissue; B, before surgery; A, after surgery; bp, base pair; OTU, operational taxonomic unit.

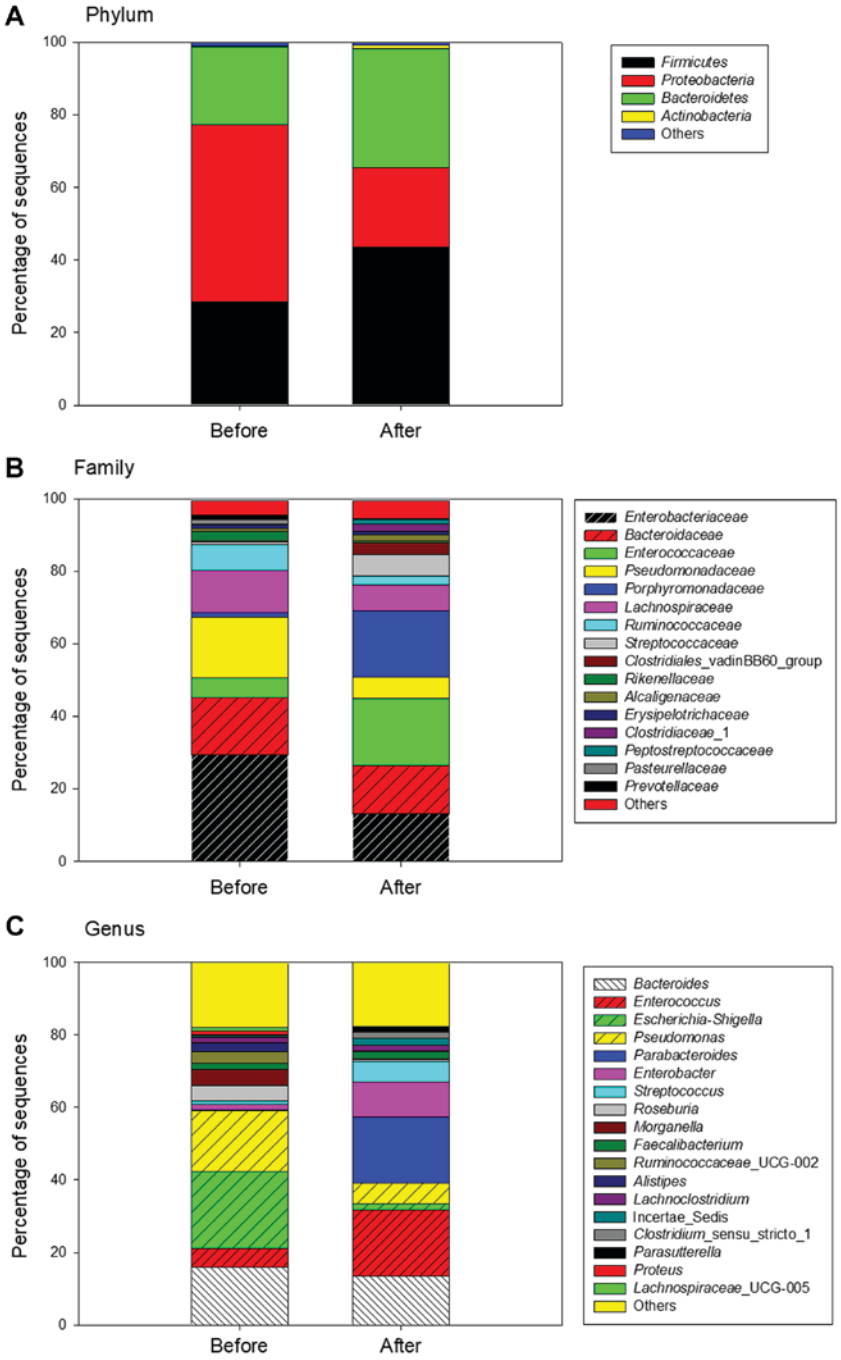

Figure 1. Bacterial composition of all stool samples obtained from patients prior to and following surgery. The bacterial community structures of stool samples were analyzed at the (A) phylum level, (B) family level and (C) genus level.

that the numbers of sequences were sufficient to demonstrate the diversity of the samples.

Bacterial composition of stool samples prior to and following surgery. Differences were identified between the bacterial composition of the stool samples obtained prior to and following surgery. At the phylum level, a total of 10 different bacterial phyla were identified in preoperative stool and 15 different bacterial phyla were revealed in postoperative stool. In all stool samples >98% of sequences belonged to the Proteobacteria, Firmicutes and Bacteroidetes, however, differences were identified in the proportions of the species present. Following surgery, the proportions of the phyla Firmicutes and Bacteroidetes increased, from 28 to 44% and from 21 to 33%, respectively. However, the proportion of Proteobacteria markedly decreased, from 49 to 22% (Fig. 1A).

At the family level, the most predominant sequences (>10%) belonged to Enterobacteriaceae (29%) and Bacteroidaceae (16%) prior to surgery. By contrast, following surgery the most predominant sequences belonged to Enterococcaceae (18%), Porphyromonadaceae (18%), Bacteroidaceae (13%) and Enterobacteriaceae (13%) (Fig. 1B). The percentage of the family Enterobacteriaceae decreased, but the family Enterococcaceae increased and became the most abundant family following surgery.

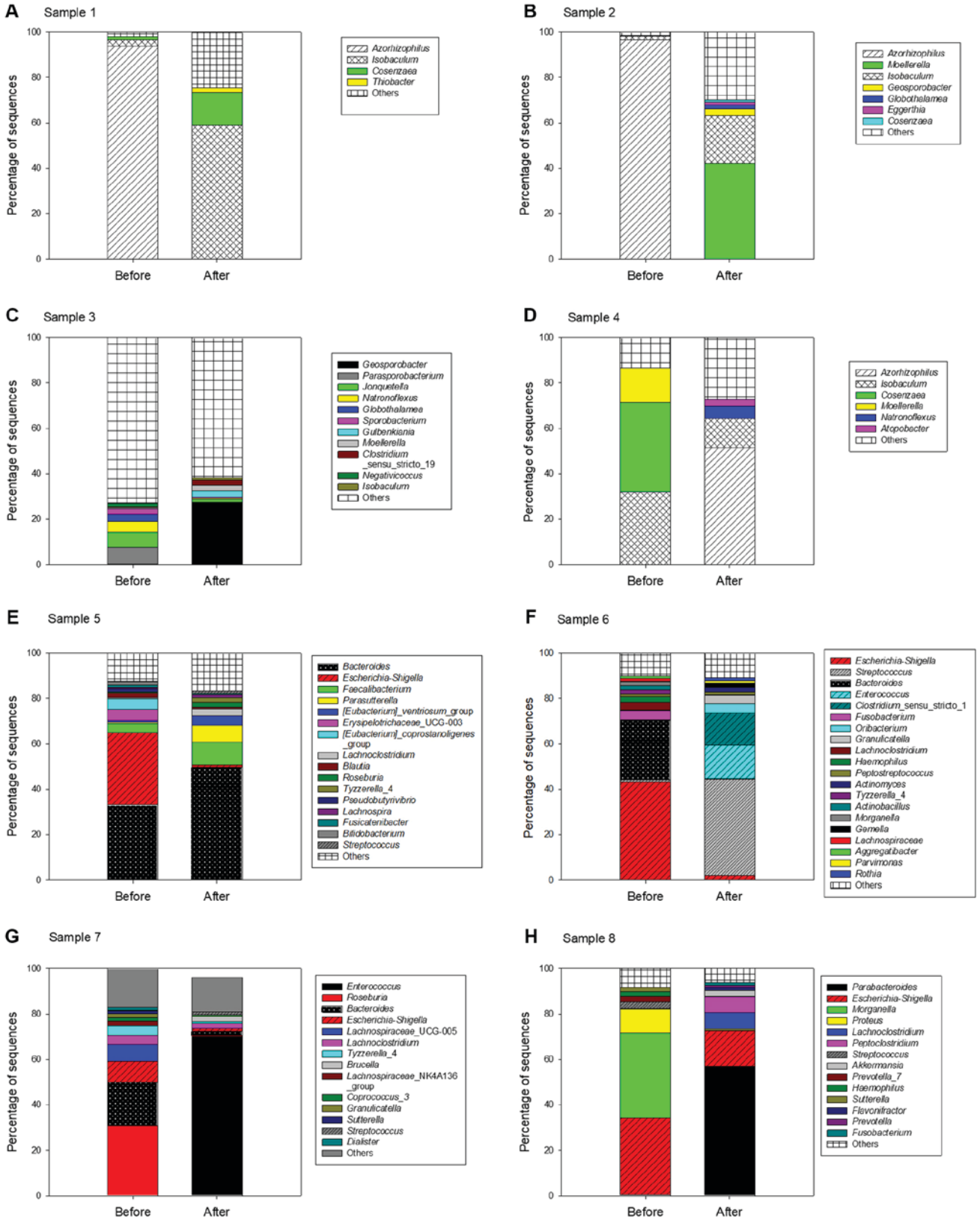

Figure 2. Bacterial compositions of stool samples obtained from each patient prior to and following surgery. (A-H) The bacterial composition of stool samples from every patient was analyzed at the genus level.

Finally, at the genus level, the most predominant bacterial genera were *Escherichia-Shigella* (21%), *Pseudomonas* (17%) and *Bacteroides* (16%) prior to surgery. However, following surgery, the most predominant bacterial genera were *Enterococcus* (18%), *Parabacteroides* (18%) and *Bacteroides* (13%) (Fig. 1C). Whereas, the abundance of *Escherichia-Shigella* significantly decreased from 21 to 1.7%, the abundance of *Enterococcus* and *Parabacteroides* markedly

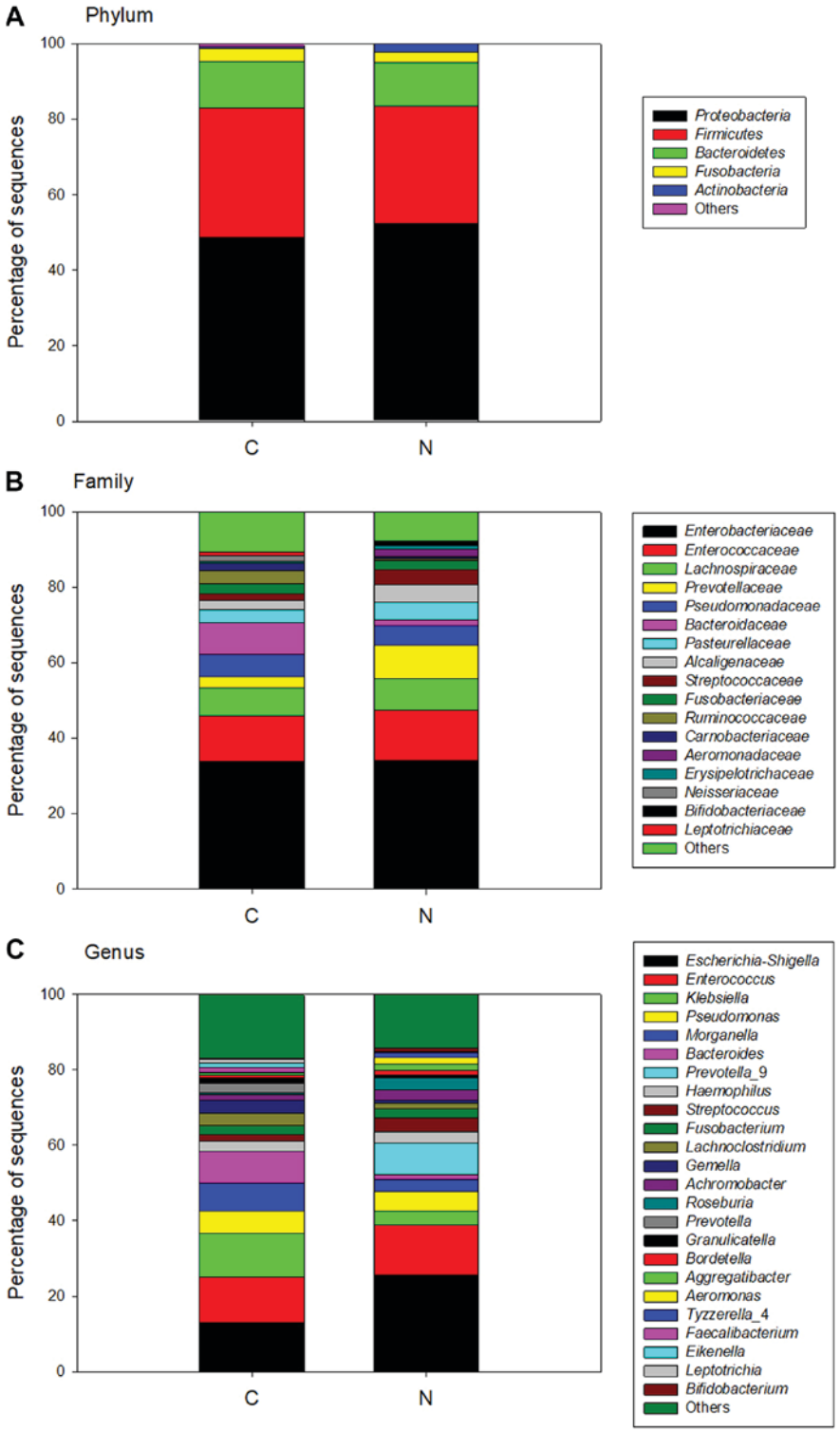

Figure 3. Bacterial composition of all tissue samples obtained from the patients. All sequences were analyzed at the (A) phylum level, (B) family level and (C) genus level. C, cancerous tissue; N, adjacent normal tissue.

increased following surgery. The bacterial composition of each patient was evidently different; therefore, the sequences of the stool samples from each patient were analyzed at the genus level (Fig. 2).

Bacterial differences between cancer tissue and adjacent normal tissue. Comparison of the sequences identified in cancer tissue and adjacent normal tissue revealed that both bacterial community structures were similar. At the phylum level, a total of 18 different bacterial phyla were identified in cancer tissues and 15 different bacterial phyla were identified in adjacent normal tissue. The majority of sequences were from Proteobacteria (49 and 52% in cancer and adjacent normal tissue, respectively), Firmicutes (34 and 31% in cancer and adjacent normal tissue, respectively) and Bacteroidetes (12% in cancer tissue and adjacent normal tissue; Fig. 3A). At the

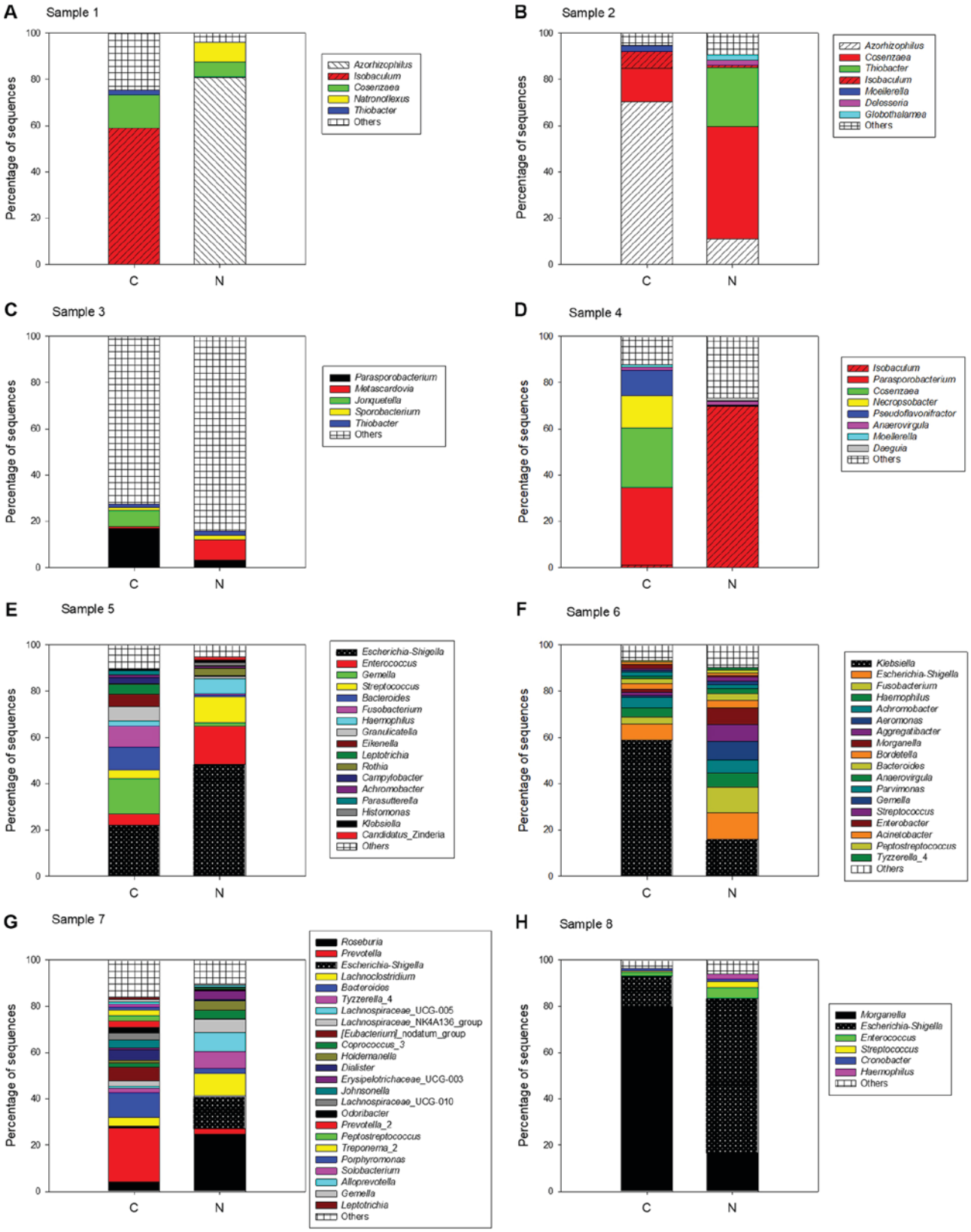

Figure 4. Bacterial compositions of all tissue samples obtained from each patient. (A-H) All sequences of every patient were analyzed at the genus level. C, cancerous tissue; N, adjacent normal tissue.

family level, the majority of the sequences were restricted to Enterobacteriaceae (34% in cancer tissue and in adjacent normal tissue) and Enterococcaceae (12% in cancer tissue and in adjacent normal tissue); the percentages were similar in both tissue types (Fig. 3B). At the genus level, the predominant genera in cancer tissues were *Escherichia-Shigella* (13%), *Enterococcus* (12%) and *Klebsiella* (12%), whereas in the adjacent normal tissues, the main genera were *Escherichia-Shigella* (26%) and

Enterococcus (13%), but the abundance of *Klebsiella* was low (Fig. 3C). The bacterial community structures of cancer tissue and adjacent normal tissue were analyzed and compared in every patient to further investigate the differences (Fig. 4). The results demonstrated that the bacterial composition of each patient was markedly different.

Phylogenetic analysis. A phylogenetic tree was constructed using the OTU sequences based on a 3% cut-off value and the associated sequences were obtained from the NCBI GenBank database. A total of 44 OTUs with a sequence percentage >1% were selected for constructing the phylogenetic tree (Fig. 5). The OTUs were named based on the number of sequences; therefore, the lower the OTU number, the higher its abundance. The most abundant OTU0001 demonstrated 100% identity to *E. coli*. OTU0002, 0012 and 0024 were similar to *Enterococcus faecalis* and *Enterococcus faecium*. A high similarity was identified between OTU0003 and *Pseudomonas azotoformans*. OTU0004 and *Klebsiella pneumoniae* demonstrated 99% identity. OTU0005 was similar to *Morganella morganii*. OTU0010, OTU0013, OTU0014 and OTU0023 were similar to *Streptococcus tigurinus*, *B. fragilis*, *Bacteroides dorei* and *Parabacteroides distasonis*, respectively.

PCA. PCA on weighted UniFrac dissimilarities was also performed to evaluate variation between the bacterial composition from different patients and different samples (Fig. 6). The total percentage of principle components PC1, PC2 and PC3 was 60.24%, which could better explain the distribution of the bacterial composition. The dots represent different samples, and the dots from same plane showed the different samples had similar bacterial composition. The results demonstrated that the postoperative stool samples (green circles) were clustered together and are more similar compared with preoperative stool samples (yellow circles) and cancer tissue (red circles). However, the bacteria of adjacent normal tissue (blue circles) were distinct from other samples (Fig. 6A). For the majority of patients, including patients 3, 5, 6, 7 and 8, the four samples from the same patient were clustered together (Fig. 6B).

Discussion

The current study provided information regarding the intestinal bacteria community of both cancer tissue and adjacent normal tissue, as well as the bacterial intestinal status of patients with CRC prior to and following surgery. It was demonstrated that the bacterial composition of stool samples prior to and following surgery were markedly different and that the bacterial composition greatly improved following surgery. However, the bacterial community structures of cancer tissues and adjacent normal tissues prior to surgery were similar, which suggested that the bacterial composition of adjacent normal tissue may not be as healthy as previously considered.

The dominant bacteria of all samples at the phylum level were identified as Proteobacteria, Firmicutes and Bacteroidetes. Proteobacteria has previously been demonstrated to have a higher abundance in intestinal inflammation and in patients with tumors (4,31,32). In the current study, the abundance of bacteria from the phylum Proteobacteria decreased in stool samples following surgery, while those in the phylum

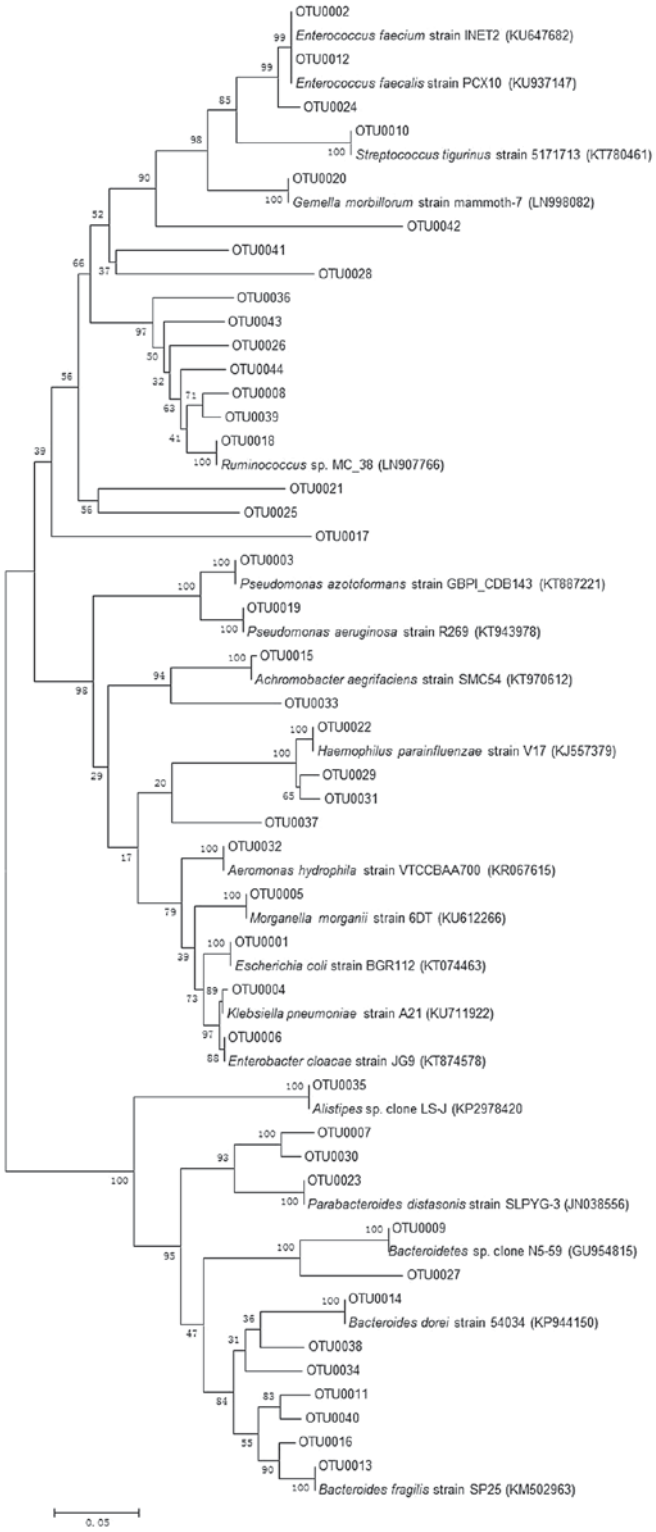

Figure 5. Phylogenetic tree of 16S ribosomal RNA gene sequences based on a cut-off value of 0.03. A total of 44 OTUs were used to construct a phylogenetic tree. The OTU names were based on the number of sequences, therefore, the lower the number, the higher its abundance. OTU, operational taxonomic unit.

Firmicutes increased. Previous studies have indicated that the incidence and mortality rates of patients of African-American background with CRC are higher compared with other racial and ethnic groups in the USA and these patients demonstrate a lower five-year survival rate compared with Caucasian

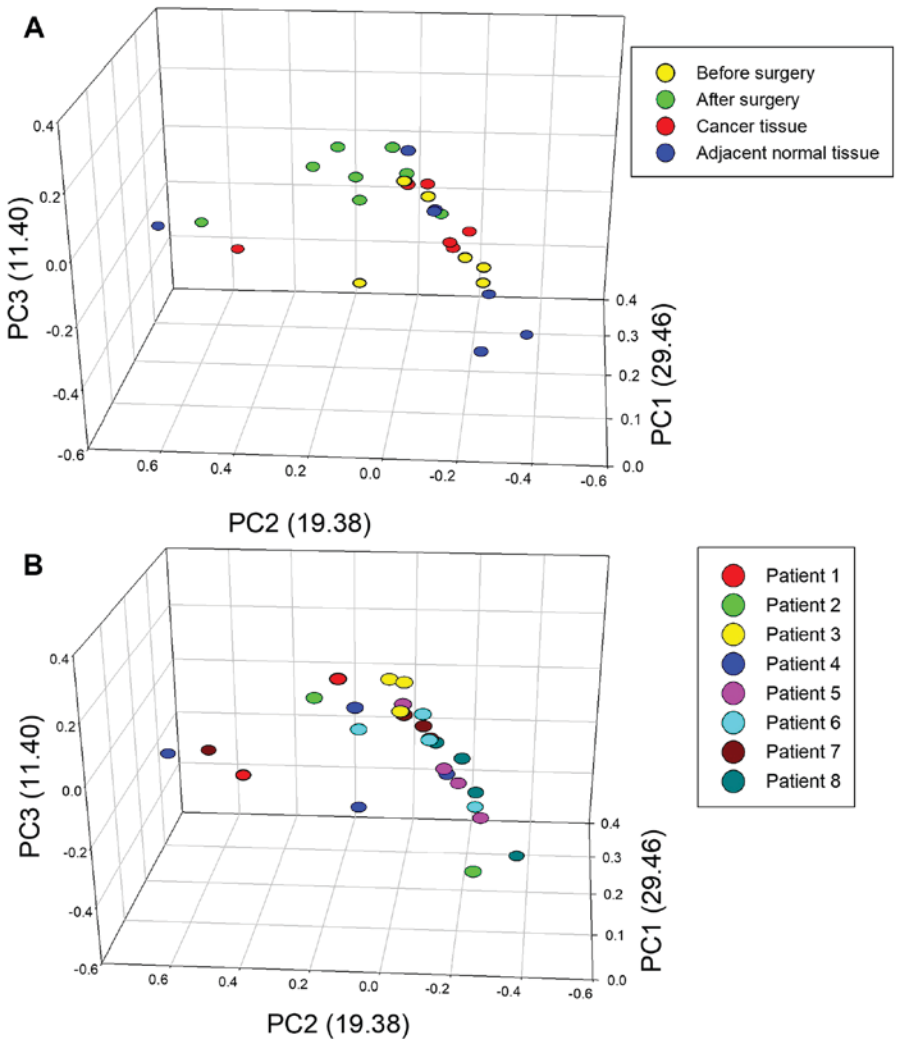

Figure 6. PCA based on different sample sources and different patients. PCA was performed based on (A) different sample sources, including cancer tissue, adjacent normal tissue and stool samples obtained prior to and following surgery, and (B) different patients. PCA, principle component analysis; PC, principle component.

patients (33-36). Compared with the intestinal microbes and metabolites of Caucasian patients, African-American patients exhibit significantly lower levels of short chain fatty acids and significantly higher levels of *Firmicutes* species (37). Another study that compared the intestinal microbes of patients with CRC and healthy individuals suggested that the phylum *Firmicutes* is more abundant in patients with CRC (3).

Enterobacteriaceae (phylum Proteobacteria) are normal commensal bacteria in the gut of humans, however, the family consists of numerous genera of bacteria that are potentially pathogenic, including *Salmonella*, *Shigella*, *Escherichia*, *Proteus* and *Klebsiella* (38). It has been reported that Enterobacteriaceae is more abundant in patients with inflammatory bowel disease (IBD) or CRC compared with healthy individuals (39-41). The Enterobacteriaceae bacteria, including *Proteus* and *Klebsiella* are associated with colitis in T-bet$^{-/-}$ Rag2$^{-/-}$ ulcerative colitis mice, due to intestinal inflammation that contributes to the high abundance of Enterobacteriaceae (42,43). In the current study, Enterobacteriaceae abundance decreased following surgery, in contrast to Enterococcaceae and Porphyromonadaceae, which increased. Mira-Pascual *et al* (41) have identified that the proportion of the family Enterococcaceae is higher in patients with polyps compared with healthy controls, suggesting that this could be used as a biomarker to detect polyps, which are a risk factor for CRC. Enterococcaceae includes four genera; *Enterococcus*, *Melissococcus*, *Tetragenococcus* and *Vagococcus*, and the genus *Enterococcus* has been most extensively studied (44). *E. faecalis* has been identified at a higher proportion in patients with CRC compared with healthy individuals (45,46). In addition, *E. faecalis* has been revealed to induce IBD in interleukin-10 knockout mice (47). Sun *et al* (48) revealed that the family Porphyromonadaceae is significantly enriched in an inflammatory group of 1,2-dimethylhydrazine (DMH)-induced mice compared with hyperproliferation and adenoma groups. The authors suggested that the family Porphyromonadaceae serves a protective role by mediating the microbiota balance in the murine gut. The current study demonstrated that following surgery, samples from patients contained a higher percentage of potential pathogens, including members of Enterobacteriaceae and Enterococcaceae. This may be due to the tumor resection time being too short to detect a change of bacteria or due to the use of antibiotics following surgery. Therefore, future studies should collect more samples at different time points following surgery.

The current study identified that the predominant genera were *Escherichia-Shigella* and *Enterococcus* in all samples analyzed. The 16S rRNA genes of *Escherichia* and *Shigella* have a high similarity, therefore more detailed classified information requires identification of other functional genes. The *Escherichia-Shigella* genera are considered to be potential pathogens of CRC; *Escherichia-Shigella* species are typically abundant in the colon and may promote tumor formation (3,40). The genera *Escherichia-Shigella* may produce bacteriocins in the intestine (49). Bacteriocins are antibacterial and are induced during apoptosis, however, to the best of our knowledge, their association with eukaryotic hosts remains unclear. An *in vitro* study identified several types of bacteriocins that are considered to promote tumor cell proliferation (50). *E. coli* was revealed to be more prevalent in stage III/IV CRC compared with stage I CRC and an association was identified between poor prognosis of CRC and colonization of mucosa by *E. coli* (51). The current study identified a large number of *Escherichia-Shigella* species in the stool samples of patients 5, 6, 7 and 8 prior to surgery, and the proportions decreased following surgery, which may suggest that the factors causing inflammation and tumor formation were reduced within the intestinal microflora following surgery.

Enterococcus species are also commensal bacteria within the human intestinal tract, serving as facultative anaerobes (52). *Enterococcus* are considered to benefit from an inflammatory niche in the intestine (53,54). A number of studies have demonstrated that the abundance of *Enterococcus* is higher in stool samples from patients with CRC compared with healthy controls (45,46,55). In addition, *Enterococcus* often develop antibiotic resistance due to abuse of antibiotics and may cause fatal nosocomial infections in humans (40,56,57). In the current study, the genera *Escherichia-Shigella* was abundant in stool samples prior to surgery, cancer tissues and adjacent normal tissues, while the genus *Enterococcus* was abundant in stool samples following surgery, cancer tissue and adjacent normal tissue. These two genera contain potential pathogens of CRC, which may indicate a poor prognosis or longer recovery time for patients with a high abundance.

In the present study, it was found that the composition of the bacterial community in every patient was different, and certain novel bacteria have been revealed in the current study that have rarely been identified in previous intestinal bacterial studies, including *Azorhizophilus*, *Isobaculum*, *Moellerella*, *Streptococcus* and *Klebsiella*. Previously, *Azorhizophilus* has not been detected in the gut; however it was identified at a higher level in the current study in patients 1, 2 and 4. *Azorhizophilus*, a member of the Pseudomonadaceae family, is a nitrogen-fixing bacteria associated with the legume species (58). Recently, the intestinal microbiota was also detected by the presence of genes associated with nitrogen-fixing bacteria, in populations that eat a large number of legumes (59). *Isobaculum* was also detected at a high level in patients 1, 2 and 4. *Isobaculum* is phylogenetically similar to lactic acid bacteria and the type of glucose metabolism is also similar, where acetate and lactate are produced (60). This suggests that *Isobaculum* may be a potential probiotic bacterium. *Moellerella* is a commensal bacterium in the gut but is often isolated from clinical specimens of patients with diarrhea (61,62). This bacterial genus was increased in the stool sample from patient 2 following surgery, which may suggest an unhealthy intestinal state. Another potential pathogenic bacteria identified was *Streptococcus*, which has a close association with the occurrence of CRC and intestinal inflammation (63,64). Finally, *Klebsiella* was detected in cancer tissues at 12% but was not detected in stool samples. *Klebsiella* is a classical respiratory pathogen (65), therefore the reason for its high abundance in cancer tissue is unclear.

According to phylogenetic analysis, the sequences of OTU0001 were similar to *E. coli*. It has previously been indicated that colorectal carcinoma mucosa, but not normal colonic mucosa, is colonized by intracellular *E. coli* (66). The DNA repair gene MUTYH in intestinal epithelial cells is a homologue of the *E. coli* gene mutY and is associated with CRC (67,68). The sequences of OTU0002, OTU0012 and OTU0024 were similar to *E. faecalis* or *E. faecium*. These two bacteria are common bacteria in the gut and resistance of these two strains frequently occurs due to antibiotics abuse (56,57). In addition, *F. nucleatum* promotes chemoresistance to CRC by modulating autophagy (69). *F. nucleatum* was identified in OTU0017 in the current study but the sequence ratio was very low. Furthermore, the sequences of OTU0010 and *S. tigurinus* were identified to be similar. *S. tigurinus* is associated with numerous clinical infections, including endocarditis, meningitis and oral infection, and in the USA, the spread of infectious disease is commonly associated with this strain (70-72). The sequences of OTU0005 demonstrated 100% identity to *M. morganii*, which can infect insects (73). Murphy *et al* (74) performed a study on neonatal fecal microbiota and identified that *M. morganii* is often associated with sulfhemoglobinemia. *M. morganii* is an opportunistic pathogen that may cause serious infection, particularly in immunocompromised hosts, including neonates (75). The immune systems of patients with CRC are impaired following surgery and during chemotherapy, therefore, infection with *M. morganii* must be strictly avoided. The sequences of OTU0023 were similar to *P. distasonis*, which is a normal intestinal bacterium (76). A previous study demonstrated that *P. distasonis* may be a colitis-promoting species (77). There were nine major OTUs that were prevalent in the intestinal bacteria and similar to *Bacteroides* species, including *B. dorei* and *B. fragilis*. *B. dorei* is dominant in the intestinal tracts of children with type I diabetes, with the highest level observed in 7.6-month-old infants, which suggests that it may be associated with a solid diet (78). *B. fragilis* is considered to be associated with intestinal inflammation and intestinal tumors due to the production of enterotoxin (79).

PCA revealed that the bacterial community structures were similar within the stool samples of all patients following surgery. However, the bacterial community structures between the stool samples prior to surgery and tissue samples from each patient were even more similar. There are two possible explanations for this result. Firstly, the impact of CRC is very complex. An obvious association was not identified from intestinal microbiota analysis alone, but several factors, including diet, genetics and lifestyle are likely associated with CRC. Secondly, the stool samples from all patients following surgery may be more consistent in their bacterial community structures since patients consumed a lighter diet and used antibiotics following surgery.

In summary, certain bacterial species have previously been identified to serve a role in the occurrence and development of CRC, including *Streptococcus* species, *Helicobacter pylori*, *E. faecalis*, *B. fragilis*, *Clostridium septicum* and *E. coli* (63). Almost all these bacteria were detected in the current study, which emphasizes the importance of studying the association between these bacteria and CRC to prevent infection of patients with CRC in the future. A number of conclusions have been made by the current study. Firstly, in terms of the intestinal microbiota present, cancer-adjacent tissues are not as healthy as previously considered. Furthermore, following surgery, there is an increase in patients' bacteria diversity index and the species of the bacterial community may be more abundant. The current study suggested that the bacterial community of the intestinal tract of patients with CRC may not be healthy. Finally, a number of drug-resistant bacteria were identified in the intestinal tract following surgery, which may possibly be caused by routine treatment with antibiotics. In the future, it may be necessary to study safe and effective clinical anti-infection methods, including the use of probiotics.

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Availability of data and materials

The datasets generated during the current study are available in the Sequence Read Archive of NCBI under the accession number SRP120060 and all data analyzed during this study are included in the published article.

Authors' contributions

CJL and XRL contributed to the conception and design of the study. YS and BW assisted with the collection of samples. YLZ acquired the data. XRL, EY and YYL analyzed the data. YLZ, CJL, XRL, EY and YYL contributed to the writing, reviewing and/or revision of the submitted manuscript.

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of The First People's Hospital of Yunnan Province and informed consent was obtained from all participants prior to engaging in any study activities.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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