



- (51) International Patent Classification:
A61K 39/02 (2006.01) *C12N 1/20* (2006.01)
- (21) International Application Number:
PCT/CN2014/083688
- (22) International Filing Date:
5 August 2014 (05.08.2014)
- (25) Filing Language: English
- (26) Publication Language: English
- (71) Applicants: **BGI SHENZHEN CO., LIMITED** [CN/CN]; Main Building 11F-3, Beishan Industrial Zone, Beishan Road 146, Yantian District, Shenzhen, Guangdong 518083 (CN). **BGI SHENZHEN** [CN/CN]; Main Building, Beishan Industrial Zone, Yantian District, Shenzhen, Guangdong 518083 (CN).
- (72) Inventors: **FENG, Qiang**; Main Building, Beishan Industrial Zone, Yantian District, Shenzhen, Guangdong 518083 (CN). **ZHANG, Dongya**; Main Building, Beishan Industrial Zone, Yantian District, Shenzhen, Guangdong 518083 (CN). **LIU, Chuan**; Main Building, Beishan Industrial Zone, Yantian District, Shenzhen, Guangdong 518083 (CN). **XIAO, Liang**; Main Building, Beishan Industrial Zone, Yantian District, Shenzhen, Guangdong 518083 (CN). **TANG, Longqing**; Main Building, Beishan Industrial Zone, Yantian District, Shenzhen, Guangdong 518083 (CN). **WANG, Jun**; Main Building, Beishan Industrial Zone, Yantian District, Shenzhen, Guangdong 518083 (CN).

- (74) Agent: **CCPIT PATENT AND TRADEMARK LAW OFFICE**; 8th Floor, Vantone New World Plaza, 2 Fuchengmenwai Street, Xicheng District, Beijing 100037 (CN).

- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: USE OF EUBACTERIUM IN THE PREVENTION AND TREATMENT FOR COLORECTAL CANCER RELATED DISEASES

(57) Abstract: The application of microorganisms, particular Eubacterium strains, in treatment and prevention of colorectal cancer-related diseases is disclosed. The composition comprising the Eubacterium strains is involved.



USE OF EUBACTERIUM IN THE PREVENTION AND TREATMENT FOR COLORECTAL CANCER RELATED DISEASES

CROSS-REFERENCE TO RELATED APPLICATION

None

FIELD

The invention relates to microbiology, specifically, this invention relates to *Eubacterium* bacterial strains in the treatment and prevention of colorectal cancer -related diseases in the application, and also involves the composition comprising *Eubacterium* bacteria and its application.

BACKGROUND

Colorectal cancer (CRC) is the third most common form of cancer and the second leading cause of cancer-related death in the Western world (Schetter AJ, Harris CRC (2011) Alterations of microRNAs contribute to colon carcinogenesis. *Semin Oncol* 38:734–742, incorporated herein by reference). A lot of people are diagnosed with CRC and many patients die of this disease each year worldwide. Although current strategies, including surgery, radiotherapy, and chemotherapy, have a significant clinical value for CRC, the relapses and metastases of cancers after surgery have hampered the success of those treatment modalities.

Chronic intestinal inflammation occurring in inflammatory bowel disease (IBD) induces persistent damage and enhanced mucosal permeability along the gastrointestinal tract, playing an important role in the development of colorectal cancer (CRC). Together with the hereditary syndromes of familial adenomatous polyposis and hereditary nonpolyposis, IBD is among the top three high-risk conditions for CRC (Xie J, Itzkowitz SH (2008) Cancer in inflammatory bowel disease. *World J Gastroenterol* 14: 378–389, incorporated herein by reference). Among ulcerative colitis (UC) patients, one of the two main manifestations of IBD, the relative risk of developing CRC correlates with the extent and duration of disease (Eaden JA, Abrams KR, Mayberry JF (2001) The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 48: 526–535, incorporated herein by reference). In patients with IBD, this risk increases by 0.5–1.0% yearly after 8–10 years (Munkholm P (2003) Review article: the incidence and

prevalence of colorectal cancer in inflammatory bowel disease. *Aliment Pharmacol Ther* 18 Suppl 2: 1–5, incorporated herein by reference).

The human gut microflora, which contains about 100 trillion microbial organisms, plays a critical role in maintaining host health, both in the gastrointestinal tract and systemically through the absorption of metabolites (e.g. vitamins and short chain fatty acids) (Moore WE, Holdeman LV (1974) Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl Microbiol* 27: 961–979, incorporated herein by reference). Recent studies have demonstrated that specific strains of bacteria are implicated in the regulation of the intestinal homeostasis, delivering regulatory signals to the epithelium, the mucosal immune system and to the neuromuscular activity of the gut (Shanahan F (2004) Probiotics in inflammatory bowel disease—therapeutic rationale and role. *Adv Drug Deliv Rev* 56: 809–818, incorporated herein by reference). Moreover, some commensal and pathogenic organisms of the human enteric microbiome are essential in the pathogenesis of IBD and CRC. Therefore, manipulating the gut bacterial composition and local metabolite production by using probiotic bacteria has been explored as a promising avenue for therapeutic intervention against CRC. Probiotics are live microbial feed supplements which beneficially impact on host health. They rely on introducing particular exogenous strains into the gut microflora. For the study of CRC, the inventors performed analysis in the metagenomics field, presenting data from deep metagenomic profiling of CRC gut microbiome,.

SUMMARY

Embodiments of the present disclosure seek to solve at least one of the problems existing in the prior art to at least some extent.

The present invention is based on the following findings by the inventors:

Assessment and characterization of gut microbiota has become a major research area in human disease, including colorectal cancer (CRC), one of the commonest causes of death among all types of cancers. To carry out analysis on gut microbial content in CRC patients, the inventors carried out a protocol for a Metagenome-Wide Association Study (MGWAS) (Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490, 55–60 (2012), incorporated herein by reference) based on deep shotgun sequencing of the gut microbial DNA from 128 Chinese individuals. The inventors identified 2 probiotic bacteria. Then the

inventors validated the probiotic bacteria in animal experiment related to colitis and CRC respectively. Results from the animal experiment demonstrated the ability of *Eubacterium ventriosum* and *Eubacterium eligens* to prevent and treat both colitis and colorectal cancer effectively.

BRIEF DISCRIPTION OF DRAWINGS

These and other aspects and advantages of the present disclosure will become apparent and more readily appreciated from the following descriptions taken in conjunction with the drawings, in which:

Fig.1 shows distribution of P-value association statistics of all microbial genes in this study. The association analysis of CRC p-value distribution identified a disproportionate over-representation of strongly associated markers at lower P-values, with the majority of genes following the expected P-value distribution under the null hypothesis. This suggests that the significant markers likely represent true rather than spurious associations.

Fig.2 Protective effects of intragastric administration of *Eubacterium ventriosum* ATCC 27560, *Eubacterium ventriosum* L2-12, *Eubacterium ventriosum* STAFF 1042, *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020, *Eubacterium eligens* TSDC10.2-1.1 respectively on DSS-induced colitis in C57Bl/ 6J considering (A) weight loss, (B) disease activity index and (C) Colon length. Data are represented as mean $\pm$ standard error. Different asterisks (*) indicate significant differences (*P<0.05, **P<0.01, ***p<0.001).

Fig.3 Quantification using ELISA of (A)TNF- α and (B) IL-10 in colons obtained at day 15 after induction of colitis. Data are represented as mean $\pm$ standard error. Different asterisks (*) indicate significant differences (*P<0.05, **P<0.01, ***p<0.001)

Fig.4 Effect of *Eubacterium ventriosum* ATCC 27560, *Eubacterium ventriosum* L2-12, *Eubacterium ventriosum* STAFF 1042, *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020, *Eubacterium eligens* TSDC10.2-1.1 respectively treatment on (A)_Disease activity indices and (B) colon histopathology. Different asterisks (*) indicate significant differences (*P<0.05, **P<0.01, ***p<0.001)

Fig.5 *Eubacterium ventriosum* ATCC 27560, *Eubacterium ventriosum* L2-12, *Eubacterium ventriosum* STAFF 1042, *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020, *Eubacterium eligens* TSDC10.2-1.1 respectively modulate colonic gene expression

in BABL/C mice (related with β -actin). Data are represented as mean $\pm$ standard error. Different asterisks (*) indicate significant differences (*P<0.05, **P<0.01, ***p<0.001).

DETAILED DESCRIPTION

Terms used herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as “a”, “an” and “the” are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

In one aspect, the present application provides *Eubacterium eligens* or *Eubacterium ventriosum* for use in the prevention or treatment of colitis and/or colorectal cancer. In another aspect, the present application provides use of *Eubacterium eligens* or *Eubacterium ventriosum* for manufacture of a medicament for prevention or treatment of colitis and/or colorectal cancer. In another aspect, the present application provides a method for the prevention or treatment of colitis and/or colorectal cancer comprising administering an effective amount of *Eubacterium eligens* or *Eubacterium ventriosum* to a patient in need thereof. In yet another aspect, the present application provides a kit comprising *Eubacterium eligens* or *Eubacterium ventriosum* and instructions for its use as a medicament. The present application further provides a pharmaceutical composition comprising *Eubacterium eligens* or *Eubacterium ventriosum* and a pharmaceutically acceptable vehicle.

Preferably, the *Eubacterium eligens* is a strain selected from the group consisting of *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020 and *Eubacterium eligens* TSDC10.2-1.1. Preferably, the *Eubacterium ventriosum* is a strain selected from the group consisting of *Eubacterium ventriosum* ATCC27560, *Eubacterium ventriosum* L2-12, and *Eubacterium ventriosum* STAFF 1042. In one embodiment, the *Eubacterium eligens* or *Eubacterium ventriosum* is administered in the form of living *Eubacterium eligens* or *Eubacterium ventriosum* or metabolites thereof.

The present invention is further exemplified in the following non-limiting Examples. Unless otherwise stated, parts and percentages are by weight and degrees are Celsius. As apparent to one of ordinary skill in the art, these Examples, while indicating preferred embodiments of the

invention, are given by way of illustration only, and the agents were all commercially available.

EMBODIMENT

Example 1. Identifying probiotic bacteria from 128 Chinese individuals

1.1 Sample collection and DNA extraction

Stool samples from 128 subjects, including 74 colorectal cancer patients and 54 healthy controls (Table 1) were collected in the Prince of Wales Hospital with informed consent. To be eligible for inclusion in this study, individuals have to fit the following criteria for stool sample collection: 1) no taking of antibiotics or other medications with no particular diets (diabetics, vegetarians, etc) and with normal lifestyle (without extra stress) for a minimum of 3 months; 2) a minimum of 3 months after any medical intervention; 3) no history of colorectal surgery, any kind of cancer, or inflammatory or infectious diseases of the intestine. Subjects were asked to collect stool samples before colonoscopy examination in standardized containers at home and store samples in their home freezer immediately. Frozen samples were then delivered to the Prince of Wales Hospital in insulating polystyrene foam containers and stored at -80°C immediately until use.

Stool samples were thawed on ice and DNA extraction was performed using the QiagenQIAamp DNA Stool Mini Kit according to manufacturer's instructions. Extracts were treated with DNase-free RNase to eliminate RNA contamination. DNA quantity was determined using NanoDrop spectrophotometer, Qubit Fluorometer (with the Quant-iTTMdsDNA BR Assay Kit) and gel electrophoresis.

Table 1 | Baseline characteristics of colorectal cancer cases and controls in the cohort I. BMI: body mass index; eGFR: epidermal growth factor receptor; DM: diabetes mellitus type 2.

Parameter	Controls (n=54)	Cases (n=74)
Age	61.76	66.04
Sex (M:F)	33:21	48:26
BMI	23.47	23.9
eGFR	72.24	74.15
DM (%)	16 (29.6%)	29 (39.2%)
Enterotype (1:2:3)	26:22:6	37:31:6
Stage of disease (1:2:3:4)	n.a.	16:21:30:7
Location (proximal: distal)	n.a.	13:61

1.2 DNA library construction and sequencing

DNA library construction was performed following the manufacturer's instruction (Illumina

HiSeq 2000 platform). The inventors used the same workflow as described previously to perform cluster generation, template hybridization, isothermal amplification, linearization, blocking and denaturation, and hybridization of the sequencing primers (Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490, 55–60 (2012), incorporated herein by reference).

The inventors constructed one paired-end (PE) library with insert size of 350bp for each sample, followed by a high-throughput sequencing to obtain around 30 million PE reads of length 2x100bp. High quality reads were extracted by filtering low quality reads with 'N' base, adapter contamination and human DNA contamination from the raw data, and by trimming low quality terminal bases of reads at the same time. 751 million metagenomic reads (high quality reads) were generated (5.86 million reads per individual on average, Table 2).

1.3 Reads mapping

The inventors mapped the high quality reads to the gene catalogue (Table 2) to a published reference gut gene catalogue established from European and Chinese adults (Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490, 55–60 (2012), incorporated herein by reference) (identity $\geq 90\%$), based on which the inventors derived the gene profiles using the same method of the published T2D paper in Qin et al. 2012, *supra*. From the reference gene catalogue as Qin et al. 2012, *supra*, the inventors derived a subset of 2,110,489 (2.1M) genes that appeared in at least 6 samples in all 128 Hong Kong samples.

Table 2. Summary of metagenomic data and mapping to reference gene catalogue. Fourth column reports results from Wilcoxon rank-sum tests.

Parameter	Controls	Cases	P-value
Average raw reads	60162577	60496561	0.8082
After removing low quality reads	59423292 (98.77%)	59715967 (98.71%)	0.831
After removing human reads	59380535 $\pm$ 7378751	58112890 $\pm$ 10324458	0.419
Mapping rate	66.82%	66.27%	0.252

1.4 Analysis of factors influencing gut microbiota gene profiles

To ensure robust comparison of the gene content of 128 metagenomes, the inventors created a set of 2,110,489 (2.1M) genes that were present in at least 6 subjects, and generated 128 gene abundance profiles using these 2.1 million genes. The inventors used the permutational

multivariate analysis of variance (PERMANOVA) test to assess the effect of different characteristics, including age, BMI, eGFR, TCHO, LDL, HDL, TG, gender, DM, CRC status, smoking status and location, on gene profiles of 2.1M genes. The inventors performed the analysis using the method implemented in package “vegan” in R, and the permuted p-value was obtained by 10,000 times permutations. The inventors also corrected for multiple testing using “p.adjust” in R with Benjamini-Hochberg method to get the q-value for each gene.

When the inventors performed permutational multivariate analysis of variance (PERMANOVA) on 13 different covariates, only CRC status was significantly associated with these gene profiles ($q = 0.0028$, **Table 3**), showing a stronger association than the second best determinant body mass index ($q = 0.15$). Thus the data suggest an altered gene composition in CRC patient microbiomes.

Table 3. PERMANOVA analysis using microbial gene profile. The analysis was conducted to test whether clinical parameters and colorectal cancer (CRC) status have significant impact on the gut microbiota with $q < 0.05$. BMI: body mass index; DM: diabetes mellitus type 2; HDL: high density lipoprotein; TG: triglyceride; eGFR: epidermal growth factor receptor; TCHO: total cholesterol; LDL; low density lipoprotein.

Phenotype	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	q-value
CRC Status	1	0.679293	0.679293	1.95963	0.015314	0.0004	0.0028
BMI	1	0.484289	0.484289	1.39269	0.011019	0.033	0.154
DM Status	1	0.438359	0.438359	1.257642	0.009883	0.084	0.27272
Location	1	0.436417	0.436417	1.228172	0.016772	0.0974	0.27272
Age	1	0.397282	0.397282	1.138728	0.008957	0.1923	0.4487
HDL	1	0.38049	0.38049	1.083265	0.010509	0.271	0.542
TG	1	0.365191	0.365191	1.039593	0.010089	0.3517	0.564964
eGFR	1	0.358527	0.358527	1.023138	0.009471	0.38	0.564964
CRC Stage	1	0.357298	0.357298	1.002413	0.013731	0.441	0.564964
Smoker	1	0.347969	0.347969	0.999825	0.013511	0.4439	0.564964
TCHO	1	0.321989	0.321989	0.915216	0.008893	0.6539	0.762883
LDL	1	0.306483	0.306483	0.871306	0.00847	0.7564	0.814585
Gender	1	0.267738	0.267738	0.765162	0.006036	0.9528	0.9528

1.5 CRC-associated genes identified by MGWAS

1.5.1 Identification of colorectal cancer associated genes. The inventors performed a metagenome wide association study (MGWAS) to identify the genes contributing to the altered gene composition in CRC. To identify the association between the metagenomic profile and

colorectal cancer, a two-tailed Wilcoxon rank-sum test was used in the 2.1M (2,110,489) gene profiles. The inventors got 140,455 gene markers, which were enriched in either case or control with $P < 0.01$ (**Fig. 1**).

1.5.2 Estimating the false discovery rate (FDR). Instead of a sequential P-value rejection method, the inventors applied the “qvalue” method proposed in a previous study (J. D. Storey, R. Tibshirani, Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences of the United States of America **100**, 9440 (Aug 5, 2003), incorporated herein by reference) to estimate the FDR. In the MGWAS, the statistical hypothesis tests were performed on a large number of features of the 140,455 genes. The false discovery rate (FDR) was 11.03%.

1.6 Data profile construction

Basing on the sequencing reads of the 128 microbiomes obtained above, the inventors examined the taxonomic differences between control and CRC-associated microbiomes to identify microbial taxa contributing to the dysbiosis. For this, the inventors used taxonomic profiles derived from three different methods, as supporting evidence from multiple methods would strengthen an association. First, the inventors mapped metagenomic reads to 4650 microbial genomes in the IMG database (version 400) and estimated the abundance of microbial species included in that database (denoted IMG species). Second, the inventors estimated the abundance of species-level molecular operational taxonomic units (mOTUs) using universal phylogenetic marker genes. Third, the inventors organized the 140,455 genes identified by MGWAS into metagenomic linkage groups (MLGs) that represent clusters of genes originating from the same genome, annotated the MLGs at species level using IMG database whenever possible, grouped MLGs based on these species annotations, and then estimated the abundance of these species (denoted MLG species).

1.6.1 Species annotation of IMG genomes

For each IMG genome, using the NCBI taxonomy identifier provided by IMG, the inventors identified the corresponding NCBI taxonomic classification at species and genus levels using NCBI taxonomy dump files. The genomes without corresponding NCBI species names were left with its original IMG names, most of which were unclassified.

1.6.2 Gene profiles

The inventors mapped our high-quality reads to the gene catalogue to a published reference

gut gene catalogue established from European and Chinese adults (Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490, 55–60 (2012), incorporated herein by reference) (identity $\geq 90\%$), based on which the inventors derived the gene profiles using the same method of the published T2D paper as Qin et al. 2012, *supra*.

1.6.3 mOTU profile

Clean reads (high quality reads in Example 1) were aligned to mOTU reference (total 79268 sequences) with default parameters (S. Sunagawa *et al.*, Metagenomic species profiling using universal phylogenetic marker genes. *Nature methods* **10**, 1196 (Dec, 2013) , incorporated herein by reference). 549 species level mOTUs were identified, including 307 annotated species and 242 mOTU linkage groups without representative genomes, which were putatively Firmicutes or Bacteroidetes.

1.6.4 IMG-species and IMG-genus profiles

Bacterial, archaeal and fungal sequences were extracted from IMG v400 reference database (V. M. Markowitz *et al.*, IMG: the Integrated Microbial Genomes database and comparative analysis system. *Nucleic acids research* **40**, D115 (Jan, 2012) , incorporated herein by reference) downloaded from <http://ftp.jgi-psf.org>. 522,093 sequences were obtained in total, and SOAP reference index was constructed based on 7 equal size chunks of the original file. Clean reads were aligned to reference using SOAP aligner (R. Li *et al.*, SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* **25**, 1966 (Aug 1, 2009), incorporated herein by reference) version 2.22, with parameters “-m 4 -s 32 -r 2 -n 100 -x 600 -v 8 -c 0.9 -p 3”. Then, SOAP coverage software was used to calculate read coverage of each genome, normalized with genome length, and further normalized to relative abundance for each individual sample. The profile was generated based on uniquely mapped reads only.

1.7 Identification of colorectal cancer associated species

Based on the identified 140,455 colorectal cancer associated maker genes profile, the inventors constructed the colorectal cancer associated MLGs using the method described in the previous type 2 diabetes study as Qin et al. 2012, *supra*. All genes were aligned to the reference genomes of IMG database v400 to get genome level annotation. An MLG was assigned to a genome if $> 50\%$ constitutive genes were annotated to that genome, otherwise it was termed as unclassified. Total 87 MLGs with gene number over than 100 were selected as colorectal cancer associated MLGs. These MLGs were grouped based on the species annotation of these genomes to

construct MLG species.

To estimate the relative abundance of an MLG species, the inventors estimated the average abundance of the genes of the MLG species, after removing the 5% lowest and 5% highest abundant genes. Relative abundance of IMG species was estimated by summing the abundance of IMG genomes belonging to that species.

These analysis identified 30 IMG species, 21 mOTUs and 86 MLG species that were significantly associated with CRC status (Wilcoxon rank-sum test, $q < 0.05$; see Tables 4, 5). *Eubacterium ventriosum* was consistently enriched in the control microbiomes across all three methods (Wilcoxon rank-sum tests – IMG: $q = 0.0414$; mOTU: $q = 0.012757$; MLG: $q = 5.446 \times 10^{-4}$), and *Eubacterium eligens* was enriched according to two methods (Wilcoxon rank-sum tests – IMG: $q = 0.069$; MLG: $q = 0.00031$). These results indicated that both of these two species may have great ability as probiotic bacteria in preventing and treating CRC related diseases.

On the other hand, *Parvimonas micra* ($q < 1.80 \times 10^{-5}$), *Peptostreptococcus stomatis* ($q < 1.80 \times 10^{-5}$), *Solobacterium moorei* ($q < 0.004331$) and *Fusobacterium nucleatum* ($q < 0.004565$) were consistently enriched in CRC patient microbiomes across all three methods. *P. stomatis* has been associated with oral cancer (S. Pushalkar *et al.*, Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma. *BMC microbiology* **12**, 144 (2012), incorporated herein by reference), and *S. moorei* has been associated with bacteremia (R. M. Pedersen, H. M. Holt, U. S. Justesen, Solobacterium moorei bacteremia: identification, antimicrobial susceptibility, and clinical characteristics. *Journal of clinical microbiology* **49**, 2766 (Jul, 2011), incorporated herein by reference). Recent work with 16S rRNA sequencing has observed significant enrichment of *F. nucleatum* in CRC tumor samples (M. Castellarin *et al.*, Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome research* **22**, 299 (Feb, 2012), incorporated herein by reference), and this bacteria has been shown to possess adhesive (G. Bachrach, C. Ianculovici, R. Naor, E. I. Weiss, Fluorescence based measurements of Fusobacterium nucleatum coaggregation and of fusobacterial attachment to mammalian cells. *FEMS microbiology letters* **248**, 235 (Jul 15, 2005), incorporated herein by reference), invasive (Y. W. Han *et al.*, Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. *Infection and immunity* **68**, 3140 (Jun, 2000), incorporated herein by reference) and pro-inflammatory (A. D. Kostic *et al.*,

Fusobacterium nucleatum Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment. *Cell Host Microbe* 14, 207 (2013), incorporated herein by reference) properties. These results confirmed this association in a new cohort with different genetic and cultural origins. However, a highly significant enrichment of *P. micra* – an obligate anaerobic bacterium that can cause oral infections like *F. nucleatum* (G. Sundqvist, Taxonomy, ecology, and pathogenicity of the root canal flora. *Oral surgery, oral medicine, and oral pathology* 78, 522 (Oct, 1994), incorporated herein by reference) – in CRC-associated microbiomes is a novel finding. *P. micra* is involved in the etiology of periodontitis (B. H. Kremer et al., *Peptostreptococcus micros* smooth and rough genotypes in periodontitis and gingivitis. *Journal of periodontology* 71, 209 (Feb, 2000) , incorporated herein by reference), and it produces a wide range of proteolytic enzymes and uses peptones and amino acids as energy source (D. A. Murdoch, H. N. Shah, Reclassification of *Peptostreptococcus magnus* (Prevot 1933) Holdeman and Moore 1972 as *Finegoldia magna* comb. nov. and *Peptostreptococcus micros* (Prevot 1933) Smith 1957 as *Micromonas micros* comb. nov. *Anaerobe* 5, 555 (10//, 1999), incorporated herein by reference). It is known to produce hydrogen sulphide (J. Carlsson, J. T. Larsen, M. B. Edlund, *Peptostreptococcus micros* has a uniquely high capacity to form hydrogen sulfide from glutathione. *Oral microbiology and immunology* 8, 42 (Feb, 1993), incorporated herein by reference), which promotes tumor growth and proliferation of colon cancer cells (C. Szabo et al., Tumor-derived hydrogen sulfide, produced by cystathionine-beta-synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer. *Proceedings of the National Academy of Sciences of the United States of America* 110, 12474 (Jul 23, 2013), incorporated herein by reference). Further research is required to verify whether *P. micra* is involved in the pathogenesis of CRC, or its enrichment is a result of CRC associated changes in the colon and/or rectum. Nevertheless, it may represent opportunities for non-invasive diagnostic biomarkers for CRC.

Example 2. Validation in animal experiment

In order to validate the ability of the probiotic bacteria, *Eubacterium ventriosum* and *Eubacterium eligens*, in preventing and treating CRC related diseases, the inventors performed animal experiments.

2.1 Validation in animal experiment related to colitis

2.1.1 Method

Ten to twelve weeks old C57BL/6J mice used for DSS-induced colitis study were prepared in SPF condition. After acclimatization, part of mice have ad libitum access to drinking water containing 3.5 - 5% DSS (dextran sodium sulfate) for five days for colitis induction. Bacterial suspensions of *Eubacterium ventriosum* ATCC 27560 , *Eubacterium ventriosum* L2-12 , *Eubacterium ventriosum* STAFF 1042, *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020, *Eubacterium eligens* TSDC10.2-1.1 (10^9 - 10^{10} cfu/ml , freshly prepared daily) ,or medium was administered respectively to mice daily by intragastric gavage from day 1 to day 14 after induction of colitis. No colitis and colitis control groups received medium intragastrically (500 μ l/50g.BW every day , n=10 in each group).

C57BL/6J mice were purchased from Laboratory Animal Center of Southern Medical University, China. *Eubacterium ventriosum* ATCC 27560 and *Eubacterium eligens* ATCC 27750 were purchased from American Type Culture Collection (ATCC).

Eubacterium ventriosum L2-12: Estelle Devillard, Freda M. McIntosh, Sylvia H. Duncan, and R. John Wallace (March 2007). Metabolism of Linoleic Acid by Human Gut Bacteria: Different Routes for Biosynthesis of Conjugated Linoleic Acid. Journal of Bacteriology 189 (6): 2566–2570.

Eubacterium ventriosum STAFF 1042, *Eubacterium eligens* STAFF 1020: Kageyama, A. et al. Rapid detection of human fecal *Eubacterium* species and related genera by nested PCR method, Microbiology and Immunology (2001) 45(4): 315-318.

Eubacterium eligens TSDC10.2-1.1 was purchased from Washington University in St. Louis (USA) <http://www.genomesonline.org/projects?id=47012>.

Mice were observed daily and recorded clinical measurements. Body weights and the disease activity index (Table 6) (Alex P, Zachos NC, Nguyen T, Gonzales L, Chen TE, Conklin ,LS, et al. 2009 Distinct cytokine patterns identified from multiplexprofiles of murine DSS and TNBS-induced colitis. Inflamm Bowel Dis; 15(3): 341-52. incorporated herein by reference.), a composite score reflecting clinical signs of the disease (i.e. perianal soiling, rectal bleeding and diarrhea) , were assessed for mice undergoing the DSS challenge , before DSS administration and weekly after intragastric gavage Inflammation was monitored at day 15 after induction of colitis when the mice were sacrificed by cervical dislocation.

Table 6 Clinical scoring standard

score	Body weight loss	Stool consistency	Fecal blood
0	None	Normal	No bleeding
1	0~5% weight loss	Wet stools	Occult bleeding

2	5%~10% weight loss	Soft stools with sticking surface	Slight bleeding
3	10%~15% weight loss	Moderate bleeding	Loose stools
4	More than 15% weight loss	Severe bleeding	Watery diarrhea

The colon was removed, dissected free of fat and mesentery, carefully opened, and cleaned with PBS (phosphate buffered saline). Colon length was measured. Colonic damage and inflammation were assessed blindly according to the Wallace criteria. Colon was also collected into liquid nitrogen and stored at -80°C for ELISA (Enzyme Linked Immunosorbent Assay) examinations.

2.1.2 Results

2.1.2.1 Severity of DSS-Induced Colitis in Mice Was Reduced by *Eubacterium ventriosum* ATCC 27560, *Eubacterium ventriosum* L2-12, *Eubacterium ventriosum* STAFF 1042, *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020, *Eubacterium eligens* TSDC10.2-1.1 respectively.

The inventors explored the anti-inflammatory effects in vivo by testing the ability of *E. eligens* ATCC 27750, STAFF 1020, TSDC10.2-1.1 and *E. ventriosum* L2-12, STAFF 1042, ATCC 27560 respectively to recover acute colitis induced by DSS. A severe acute inflammatory was observed in the colitis control group after DSS treated, and no significant protective effect was observed in mice receiving sterile culture medium. In contrast, daily intragastric administration of 6 living *Eubacterium* strains respectively all resulted in a marked attenuation of colitis with reduced weight loss (Table 7, Fig.2A), and decreasing disease activity index associated with colitis at the 7th and 14th day after intragastric gavage (the profiles of the data at 7th day were similar with those at the 14th day which was not shown here.). And partial colon length were normalized in comparison to the medium treated group (Table 8, Fig.2B、2C). There was no difference in body weight and disease activity index at the beginning of intragastric gavage. Data were represented as mean $\pm$ standard error.

Table 7 Data of Body weight (W0: body weight before DSS-treated ; W1: body weight at the 7th day after intragastric gavage)

Groups *	W0	W1	W1-W0	(W1-W0)/W0*100%
medium	28.06 $\pm$ 0.63	22.98 $\pm$ 0.95	-(5.08 $\pm$ 1.01)	-(18.12 $\pm$ 3.635)
ATCC 27750	27.83 $\pm$ 0.52	23.58 $\pm$ 1.73	-(4.25 $\pm$ 0.88)	-(15.29 $\pm$ 3.167)
L2-12	28.64 $\pm$ 0.65	24.00 $\pm$ 1.25	-(4.63 $\pm$ 0.62)	-(16.184 $\pm$ 2.178)

STAFF 1042	27.53±0.98	23.48±1.17	-(4.04±1.29)	-(14.693±4.701)
ATCC 27560	29.04±0.79	24.77±1.02	-(4.27±1.24)	-(14.69±4.267)
STAFF 1020	27.81±1.03	24.20±1.46	-(3.61±1.11)	-(12.967±4.009)
TSDC10.2-1.1	27.36±0.088	23.18±1.10	-(4.18±0.72)	-(15.278±2.667)
No colitis control	26.91±0.44	29.45±0.56	2.54±0.30	8.62±1.124
Colitis control	28.7±0.60	23.23±1.39	-(5.48±1.23)	-(19.08±4.27)

* ATCC 27560 represents *Eubacterium ventriosum* ATCC 27560; L2-12 represents *Eubacterium ventriosum* L2-12, STAFF 1042 represents *Eubacterium ventriosum* STAFF 1042, ATCC 27750 represents *Eubacterium eligens* ATCC 27750, STAFF 1020 represents *Eubacterium eligens* STAFF 1020, TSDC10.2-1.1 represents *Eubacterium eligens* TSDC10.2-1.1.

Table 8 Data of clinical measurements related to colitis

Groups	Colon length (cm)	Disease activity index	TNF α (pg/ μ g)	IL-10 (pg/ μ g)
medium	5.96±0.35	9.2±1.2	202.18±18.77	15032.2±254.9
ATCC 27750	6.55±0.38	5.8±1.5	175.44±28.14	15660.9±158.8
L2-12	6.61±0.57	7.5±2.6	161.06±25.89	15478.9±168.4
STAFF 1042	6.78±0.49	7.9±1.5	179.36±17.70	15347.3±190.6
ATCC 27560	6.38±0.39	6.4±2.1	145.44±24.14	15549.8±139.6
STAFF 1020	6.29±0.28	7.9±1.5	189.41±14.52	15373.3±186.1
TSDC10.2-1.1	6.36±0.32	7.4±2.7	186.13±20.10	15228.9±201.8
No colitis control	6.75±0.51	0	122.67±32.62	15658.3±230.2
Colitis control	6.11±0.27	9.8±2.0	216.15±24.86	14500.1±241.1

2.1.2.2 *Eubacterium ventriosum* ATCC 27560 , *Eubacterium ventriosum* L2-12 , *Eubacterium ventriosum* STAFF 1042, *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020, *Eubacterium eligens* TSDC10.2-1.1 modulate expression of colonic inflammatory respectively

Colonic proinflammatory TNF- α (tumor necrosis factor alpha) cytokines and anti-inflammatory IL-10 (interleukin-10) were quantified by ELISA. Compared with the no-colitis control group, TNF- α levels was increased, whereas IL-10 levels were barely modified in the colitis mice. After treated with suspensions of *E. eligens* ATCC 27750 , the secretion of TNF- α (Fig.3A , Table 8) was significantly lower than in the colitis control and medium groups. Meanwhile, IL-10 secretion was induced in the colon of mice treated with ATCC 27750 (Fig.3B , Table 8). Similar phenomenon ? were observed in C57Bl/ 6J mice administration with *E.*

ventriosum ATCC 27560 , *E. ventriosum* L2-12, *E. ventriosum* STAFF 1042 , *E. eligens* STAFF 1020 and *E. eligens* TSDC10.2-1.1 (Fig.3 , Table 8).

2.2 Validation in animal experiment related to colorectal cancer

2.2.1 Method

The BABL/c mice were challenged with azoxymethane (AOM, 10 mg/kg) at the age of 10 weeks followed by a 2.0% dextran sodium sulfate (DSS) treatment in the drinking water for 7 days beginning on week 2, week 5 and week 8 to induce colitis-associated colorectal cancer (CRC). Then mice were treated with *Eubacterium ventriosum* ATCC 27560 , L2-12, STAFF 1042, and *Eubacterium eligens* ATCC 27750, STAFF 1020, TSDC10.2-1.1 (10^9 - 10^{10} cfu/ml , freshly prepared daily) , or control medium respectively from week 9 of the study until killing by cervical dislocation on week 11(0.1ml /50 g.BW every day, n = 15 in each group). BABL/c mice were purchased from Laboratory Animal Center of Southern Medical University, China.

Disease activity index was calculated as previous described, which was assessed for mice undergoing the AOM+DSS challenge , before and weekly after intragastric gavage.

For histopathology examination, after the necropsy, colonic sections were fixed in 10% buffered neutral formalin, later embedded in paraffin, and then sectioned (6 μ m) and stained with H&E (Hematoxylin and eosin). Tissue slides were examined in an Olympus microscope (lympus America Inc., Dulles, VA). Specimens underwent blinded histological examination and were scored 1–4 on adenomas and adenocarcinomas.

For RNA isolation and real-time polymerase chain reaction of cytokines, tTotal RNA from colon was isolated using the Qiagen RNA isolation kit (Qiagen) according to the manufacturer's instructions, and then was used to generate the cDNA template using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) and real-time RT-PCR was performed as previously described (Bassaganya-Riera J, Reynolds K, Martino-Catt S, Cui Y, Hennighausen L, et al. (2004) Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. *Gastroenterology* 127: 777–791 , incorporated herein by reference). mRNA expression of CD36, and PPAR γ (peroxisome proliferator-activated receptor γ) were assessed by real-time quantitative PCR.

2.2.2 Results

2.2.2.1 After daily administrated with *E. ventriosum* L2-12 or *E. ventriosum* STAFF 1042 or

E. ventriosum ATCC 27560 or *E. eligens* STAFF 1020 or *E. eligens* TSDC10.2-1.1 or *E. eligens* ATCC 27750 for 2 weeks, the disease activity index were significantly lower than CRC control group (Table 9), while no difference was shown in the models of inflammation-driven colorectal cancer before intragastric gavage (data was not shown here). And the effect of six *Eubacterium* strains treatments respectively on colon histopathology of experimental azoxymethane-induced colorectal cancer were detected, and all these treatments diminished adenoma and adenocarcinoma formation when compared to the control (Fig.4, Table 9).

2.2.2.2 *E. ventriosum* L2-12 or *E. ventriosum* STAFF 1042 or *E. ventriosum* ATCC 27560 or *E. eligens* STAFF 1020 or *E. eligens* TSDC10.2-1.1 or *E. eligens* ATCC 27750 modulate expression of colonic inflammatory and carcinogenesis markers respectively.

Mice challenged with azoxymethane and DSS were euthanized in the tumor-bearing phase of the disease. Those mice treated with *E. eligens* ATCC 27750 or *E. eligens* STAFF 1020 or *E. eligens* TSDC10.2-1.1 or *E. ventriosum* L2-12 or *E. ventriosum* STAFF 1042 or *E. ventriosum* ATCC 27560 showed increased mRNA expression of CD36 and PPAR γ in the colon compared with medium-treated control group (Fig.5, Table 9).

Table 9 Data of clinical measurements related to CRC

Groups	Disease activity index	CD36	PPAR- γ	adenoma and adenocarcinoma
Healthy	0	0.92 $\pm$ 0.25	38.22 $\pm$ 7.06	0
CRC control	10.7 $\pm$ 3.0	0.40 $\pm$ 0.34	29.43 $\pm$ 8.14	2.3 $\pm$ 0.8
ATCC 27750	8.2 $\pm$ 2.5	0.67 $\pm$ 0.30	32.17 $\pm$ 8.03	1.2 $\pm$ 0.3
STAFF 1020	8.9 $\pm$ 2.2	0.72 $\pm$ 0.37	34.82 $\pm$ 5.79	1.7 $\pm$ 0.5
TSDC10.2-1.1	7.9 $\pm$ 3.0	0.65 $\pm$ 0.29	35.67 $\pm$ 3.96	1.5 $\pm$ 0.7
ATCC 27560	7.4 $\pm$ 2.1	0.75 $\pm$ 0.26	34.25 $\pm$ 5.23	1.6 $\pm$ 0.5
L2-12	9.1 $\pm$ 1.8	0.71 $\pm$ 0.39	36.11 $\pm$ 7.40	1.4 $\pm$ 1.1
STAFF 1042	8.8 $\pm$ 2.4	0.69 $\pm$ 0.40	35.93 $\pm$ 6.67	1.7 $\pm$ 0.6

Above all, results from this study demonstrate the ability of *E. ventriosum* L2-12 or *E. ventriosum* STAFF 1042 or *E. ventriosum* ATCC 27560 or *E. eligens* STAFF 1020 or *E. eligens* TSDC10.2-1.1 or *E. eligens* ATCC 27750 to ameliorate both colitis and colorectal cancer.

Table 4. IMG and mOTU species associated with CRC with q-value < 0.05

30 IMG species					
	Control rank mean	Case rank mean	Enrichment (1:Control;0:Case)	P-value	q-value
<i>Peptostreptococcus stomatis</i>	37.25926	84.37838	0	1.29E-12	3.34E-09
<i>Parvimonas micra</i>	38.43519	83.52027	0	1.13E-11	1.46E-08
<i>Parvimonas</i> sp. oral taxon 393	39.81481	82.51351	0	1.28E-10	1.10E-07
<i>Parvimonas</i> sp. oral taxon 110	43.52778	79.80405	0	4.71E-08	3.04E-05
<i>Gemella morbillorum</i>	43.87037	79.55405	0	7.77E-08	4.01E-05
<i>Burkholderia mallei</i>	45.19444	78.58784	0	4.84E-07	0.000156
<i>Fusobacterium</i> sp. oral taxon 370	45.02778	78.70946	0	3.93E-07	0.000156
<i>Fusobacterium nucleatum</i>	45.09259	78.66216	0	4.33E-07	0.000156
<i>Leptotrichia buccalis</i>	45.60185	78.29054	0	7.30E-07	0.000209
<i>Beggiatoa</i> sp. PS	46.53704	77.60811	0	2.79E-06	0.000601
<i>Prevotella intermedia</i>	46.47222	77.65541	0	2.67E-06	0.000601
<i>Streptococcus dysgalactiae</i>	47.06481	77.22297	0	3.09E-06	0.000613
<i>Streptococcus pseudoporcinus</i>	47.5	76.90541	0	8.58E-06	0.001581
<i>Paracoccus denitrificans</i>	47.48148	76.91892	0	9.35E-06	0.001608
<i>Solobacterium moorei</i>	47.66667	76.78378	0	1.17E-05	0.001884
<i>Streptococcus constellatus</i>	48.2037	76.39189	0	2.20E-05	0.003153
<i>Crenothrix polyspora</i>	48.76852	75.97973	0	4.20E-05	0.005697
<i>Filifactor alocis</i>	49.06481	75.76351	0	5.84E-05	0.007533
<i>Sulfurovum</i> sp. SCGC AAA036-O23	52.12037	73.53378	0	6.60E-05	0.008105
<i>Clostridium hathewayi</i>	49.68519	75.31081	0	0.000115	0.013431
<i>Lachnospiraceae bacterium 5_1_57FAA</i>	50.10185	75.00676	0	0.000178	0.019084
<i>Peptostreptococcus anaerobius</i>	50.14815	74.97297	0	0.000186	0.019221
<i>Streptococcus equi</i>	50.58333	74.65541	0	0.00029	0.027747

<i>Streptococcus anginosus</i>	50.66667	74.59459	0	0.000316	0.029114
<i>Leptotrichia hofstadii</i>	50.99074	74.35811	0	0.000342	0.030424
<i>Peptoniphilus indolicus</i>	51.2963	74.13514	0	0.000581	0.048307
<i>Eubacterium ventriosum</i>	80.98148	52.47297	1	1.77E-05	0.00269
<i>Adhaeribacter aquaticus</i>	77.06481	55.33108	1	0.000271	0.026839
<i>Eubacterium eligens</i>	77.90741	54.71622	1	0.000482	0.041404
<i>Haemophilus sputorum</i>	77.66667	54.89189	1	0.000608	0.048977
21 mOTU species					
	Control rank mean	Case rank mean	Enrichment (1:Control;0:Case)	P-value	q-value
<i>Parvimonas micra</i>	46.2963	77.78378	0	4.11E-08	1.80E-05
<i>Peptostreptococcus stomatis</i>	46.25	77.81757	0	6.56E-08	1.80E-05
motu_linkage_group_731	50.42593	74.77027	0	1.08E-06	0.000198
<i>Gemella morbillorum</i>	47.93519	76.58784	0	1.57E-06	0.000215
<i>Clostridium symbiosum</i>	48.66667	76.05405	0	1.89E-05	0.00173
<i>Solobacterium moorei</i>	51.22222	74.18919	0	6.31E-05	0.004331
<i>Fusobacterium nucleatum</i>	54.62037	71.70946	0	9.15E-05	0.004565
unclassified <i>Fusobacterium</i>	54.22222	72	0	0.000176	0.00806
<i>Clostridium ramosum</i>	50.92593	74.40541	0	0.000289	0.012202
<i>Clostridiales bacterium 1_7_47FAA</i>	51.27778	74.14865	0	0.000365	0.013366
<i>Bacteroides fragilis</i>	51.09259	74.28378	0	0.00045	0.01371
motu_linkage_group_624	51.01852	74.33784	0	0.000448	0.01371
<i>Clostridium bolteae</i>	51.81481	73.75676	0	0.000952	0.026134
motu_linkage_group_407	81.13889	52.35811	1	6.00E-06	0.000659
motu_linkage_group_490	80.46296	52.85135	1	3.06E-05	0.002403
motu_linkage_group_316	79.61111	53.47297	1	8.17E-05	0.004487
motu_linkage_group_443	79.66667	53.43243	1	7.63E-05	0.004487
<i>Eubacterium ventriosum</i>	78.09259	54.58108	1	0.000325	0.012757

motu_linkage_group_510	77.84259	54.76351	1	0.000443	0.01371
motu_linkage_group_611	77.2963	55.16216	1	0.000606	0.017499
motu_linkage_group_190	75.16667	56.71622	1	0.001694	0.044273

Table 5. List of 86 MLG species formed after grouping MLGs with more than 100 genes using species annotation when available.

	Control rank mean	Case rank mean	Enrichment (1:Control;0:Case)	P-value	q-value
<i>Parvimonas micra</i>	38.40741	83.54054	0	3.16E-12	2.75E-10
<i>Fusobacterium nucleatum</i>	40.32407	82.14189	0	2.97E-11	1.29E-09
<i>Solobacterium moorei</i>	42.2037	80.77027	0	3.85E-09	1.12E-07
<i>Clostridium symbiosum</i>	46.31481	77.77027	0	1.64E-06	3.56E-05
CRC 2881	51.25926	74.16216	0	2.57E-06	4.46E-05
<i>Clostridium hathewayi</i>	46.77778	77.43243	0	3.92E-06	5.69E-05
CRC 6481	52.09259	73.55405	0	1.36E-05	0.000107
<i>Clostridium clostridioforme</i>	50.2037	74.93243	0	1.27E-05	0.000107
<i>Clostridiales bacterium 1_7_47FAA</i>	48.16667	76.41892	0	2.02E-05	0.000135
<i>Clostridium</i> sp. HGF2	48.27778	76.33784	0	2.36E-05	0.000147
CRC 2794	51.03704	74.32432	0	3.50E-05	0.000179
CRC 4136	50.99074	74.35811	0	5.22E-05	0.000233
<i>Bacteroides fragilis</i>	49.09259	75.74324	0	5.97E-05	0.000236
<i>Lachnospiraceae bacterium 5_1_57FAA</i>	49.96296	75.10811	0	7.37E-05	0.000273
<i>Desulfovibrio</i> sp. 6_1_46FAA	53.33333	72.64865	0	0.000214	0.000546
<i>Coprobacillus</i> sp. 3_3_56FAA	50.53704	74.68919	0	0.000265	0.000623
<i>Cloacibacillus evryensis</i>	52.73148	73.08784	0	0.000359	0.000801
CRC 2867	52.31481	73.39189	0	0.000552	0.001162
<i>Fusobacterium varium</i>	54.57407	71.74324	0	0.000586	0.001186
<i>Clostridium bolteae</i>	51.39815	74.06081	0	0.000647	0.001223
<i>Subdoligranulum</i> sp. 4_3_54A2FAA	51.56481	73.93919	0	0.000758	0.001373
<i>Clostridium citroniae</i>	51.71296	73.83108	0	0.000861	0.001529
<i>Lachnospiraceae bacterium 8_1_57FAA</i>	51.88889	73.7027	0	0.001024	0.001782
<i>Streptococcus equinus</i>	54.52778	71.77703	0	0.001581	0.002457
CRC 4069	53.7963	72.31081	0	0.001632	0.00249

<i>Lachnospiraceae bacterium 3_1_46FAA</i>	52.53704	73.22973	0	0.00178	0.002612
<i>Dorea formicigenerans</i>	52.98148	72.90541	0	0.002703	0.003409
<i>Synergistes</i> sp. 3_1 syn1	54.37963	71.88514	0	0.003358	0.004002
<i>Lachnospiraceae bacterium 3_1_57FAA_CT1</i>	54.07407	72.10811	0	0.004478	0.005109
CRC 3579	54.05556	72.12162	0	0.005638	0.006289
<i>Alistipes indistinctus</i>	54.50926	71.79054	0	0.008262	0.008766
Con 10180	82.03704	51.7027	1	4.87E-06	6.05E-05
<i>Coprococcus</i> sp. ART55/1	80.85185	52.56757	1	8.22E-06	8.94E-05
Con 7958	75.27778	56.63514	1	1.36E-05	0.000107
butyrate-producing bacterium SS3/4	80.57407	52.77027	1	1.98E-05	0.000135
<i>Haemophilus parainfluenzae</i>	80.49074	52.83108	1	2.54E-05	0.000148
Con 154	80.35185	52.93243	1	3.30E-05	0.000179
Con 4595	77.21296	55.22297	1	4.17E-05	0.000202
Con 1617	76.12963	56.01351	1	5.61E-05	0.000233
Con 1979	79.94444	53.22973	1	5.62E-05	0.000233
Con 1371	78.46296	54.31081	1	7.54E-05	0.000273
Con 1529	75.05556	56.7973	1	9.25E-05	0.00031
<i>Eubacterium eligens</i>	79.53704	53.52703	1	9.03E-05	0.00031
Con 1987	79.42593	53.60811	1	0.000101	0.000324
Con 5770	79.39815	53.62838	1	0.000104	0.000324
Con 1197	75.42593	56.52703	1	0.000128	0.000383
Con 4699	78.78704	54.07432	1	0.000152	0.000441
<i>Clostridium</i> sp. L2-50	76.37963	55.83108	1	0.000167	0.000469
Con 2606	77.5	55.01351	1	0.000189	0.000514
<i>Eubacterium ventriosum</i>	78.62963	54.18919	1	0.000207	0.000545
<i>Bacteroides clarus</i>	75.55556	56.43243	1	0.000247	0.000597
<i>Eubacterium bifforme</i>	74.68519	57.06757	1	0.000247	0.000597
<i>Faecalibacterium prausnitzii</i>	78.25926	54.45946	1	0.00034	0.000779
Con 563	72.7037	58.51351	1	0.000556	0.001162
Con 6037	77.5463	54.97973	1	0.000561	0.001162
Con 8757	77.17593	55.25	1	0.000634	0.001223
<i>Ruminococcus obeum</i>	77.53704	54.98649	1	0.000629	0.001223
Con 1513	76.59259	55.67568	1	0.000701	0.001298

<i>Roseburia intestinalis</i>	76.99074	55.38514	1	0.001079	0.001841
<i>Ruminococcus torques</i>	76.92593	55.43243	1	0.001186	0.001984
Con 4829	76.7963	55.52703	1	0.001335	0.002151
Con 569	73.41667	57.99324	1	0.001334	0.002151
Con 10559	76.59259	55.67568	1	0.001561	0.002457
Con 1604	71.92593	59.08108	1	0.001781	0.002612
Con 2494	74.35185	57.31081	1	0.001802	0.002612
Con 1867	76.38889	55.82432	1	0.001908	0.002722
Con 1241	76.27778	55.90541	1	0.002132	0.00294
Con 5752	73.65741	57.81757	1	0.002163	0.00294
Con 7367	76.23148	55.93919	1	0.002112	0.00294
Con 6128	76.22222	55.94595	1	0.002274	0.003043
Con 5615	76.07407	56.05405	1	0.002372	0.003104
<i>Klebsiella pneumoniae</i>	74.7037	57.05405	1	0.00239	0.003104
Con 4909	75.72222	56.31081	1	0.002685	0.003409
Con 356	75.94444	56.14865	1	0.002808	0.00349
<i>Eubacterium rectale</i>	75.90741	56.17568	1	0.002953	0.003619
Con 6068	75.74074	56.2973	1	0.003338	0.004002
Con 4295	74.98148	56.85135	1	0.004171	0.004904
Con 2703	74.55556	57.16216	1	0.00437	0.005069
Con 2503	74.14815	57.45946	1	0.004522	0.005109
Con 631	70.01852	60.47297	1	0.006178	0.006804
Con 561	70.5	60.12162	1	0.008137	0.00874
Con 8420	72.64815	58.55405	1	0.008068	0.00874
Con 425	73.19444	58.15541	1	0.008397	0.008802
Con 7993	73.74074	57.75676	1	0.009358	0.009692
<i>Burkholderiales bacterium 1_1_47</i>	72.37963	58.75	1	0.009707	0.009935
Con 600	69.53704	60.82432	1	0.026354	0.02666

Although explanatory embodiments have been shown and described, it would be appreciated by those skilled in the art that the above embodiments can not be construed to limit the present disclosure, and changes, alternatives, and modifications can be made in the embodiments without departing from spirit, principles and scope of the present disclosure.

WHAT IS CLAIMED IS:

1. *Eubacterium eligens* for use in the prevention or treatment of colitis and/or colorectal cancer.
2. The *Eubacterium eligens* for use according to claim 1, wherein the *Eubacterium eligens* is a strain selected from the group consisting of *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020 and *Eubacterium eligens* TSDC10.2-1.1.
3. The *Eubacterium eligens* for use according to claim 1 or claim 2, wherein the *Eubacterium eligens* is administered in the form of living *Eubacterium eligens* or metabolites thereof.
4. Use of *Eubacterium eligens* for manufacture of a medicament for prevention or treatment of colitis and/or colorectal cancer.
5. The use according to claim 4, wherein the *Eubacterium eligens* is a strain selected from the group consisting of *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020 and *Eubacterium eligens* TSDC10.2-1.1.
6. The use according to claim 4 or claim 5, wherein the *Eubacterium eligens* is administered in the form of living *Eubacterium eligens* or metabolites thereof.
7. A method for the prevention or treatment of colitis and/or colorectal cancer comprising administering an effective amount of *Eubacterium eligens* to a patient in need thereof.
8. The method of claim 7, wherein the *Eubacterium eligens* is a strain selected from the group consisting of *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020 and *Eubacterium eligens* TSDC10.2-1.1.
9. The method of claim 7 or claim 8, wherein the *Eubacterium eligens* is administered in the form of living *Eubacterium eligens* or metabolites thereof.
10. A kit comprising *Eubacterium eligens* and instructions for its use as a medicament.
11. The kit of claim 10, wherein the *Eubacterium eligens* is a strain selected from the group consisting of *Eubacterium eligens* ATCC 27750, *Eubacterium eligens* STAFF 1020 and *Eubacterium eligens* TSDC10.2-1.1.
12. A pharmaceutical composition comprising *Eubacterium eligens* and a pharmaceutically acceptable vehicle.
13. The pharmaceutical composition of claim 12, wherein the *Eubacterium eligens* is a strain selected from the group consisting of *Eubacterium eligens* ATCC 27750, *Eubacterium eligens*

STAFF 1020 and *Eubacterium eligens* TSDC10.2-1.1.

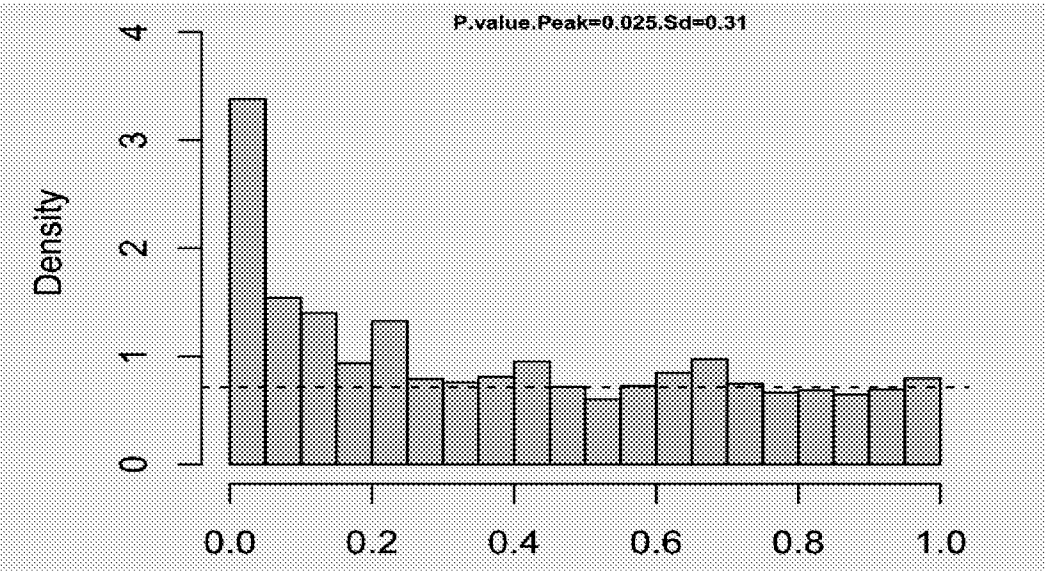


Fig.1

(A)

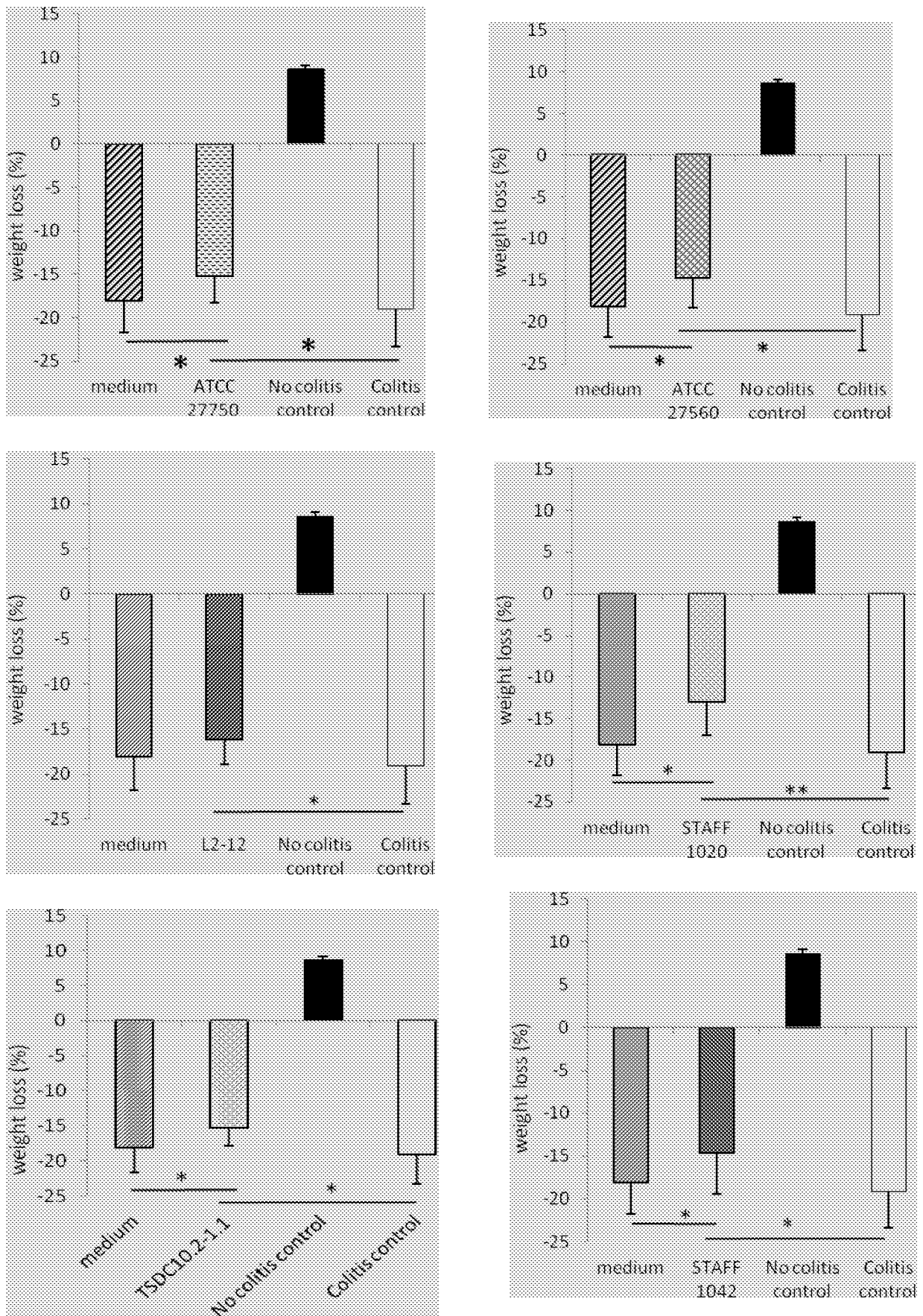


Fig.2

(B)

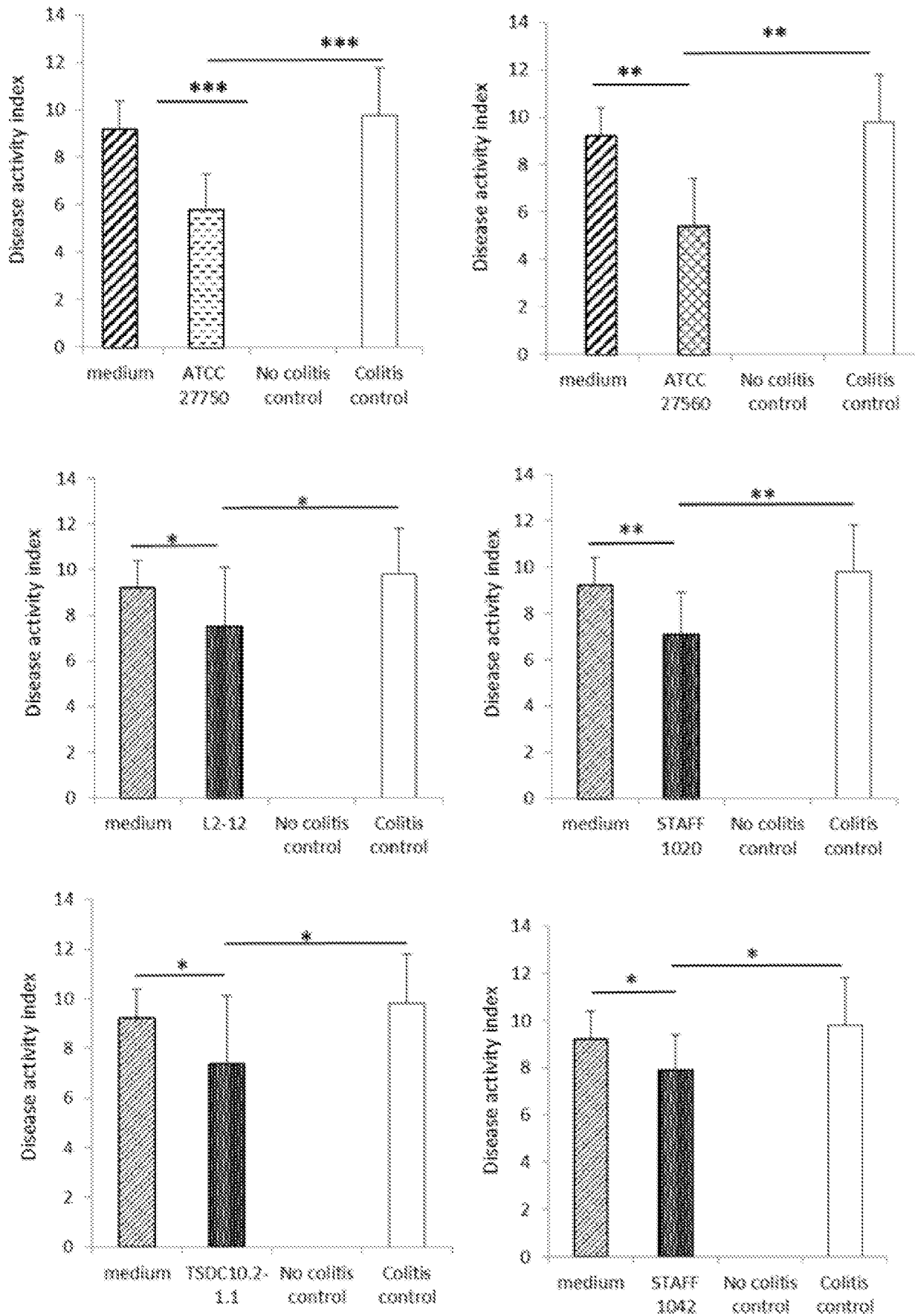


Fig.2 (cont'd)

(C)

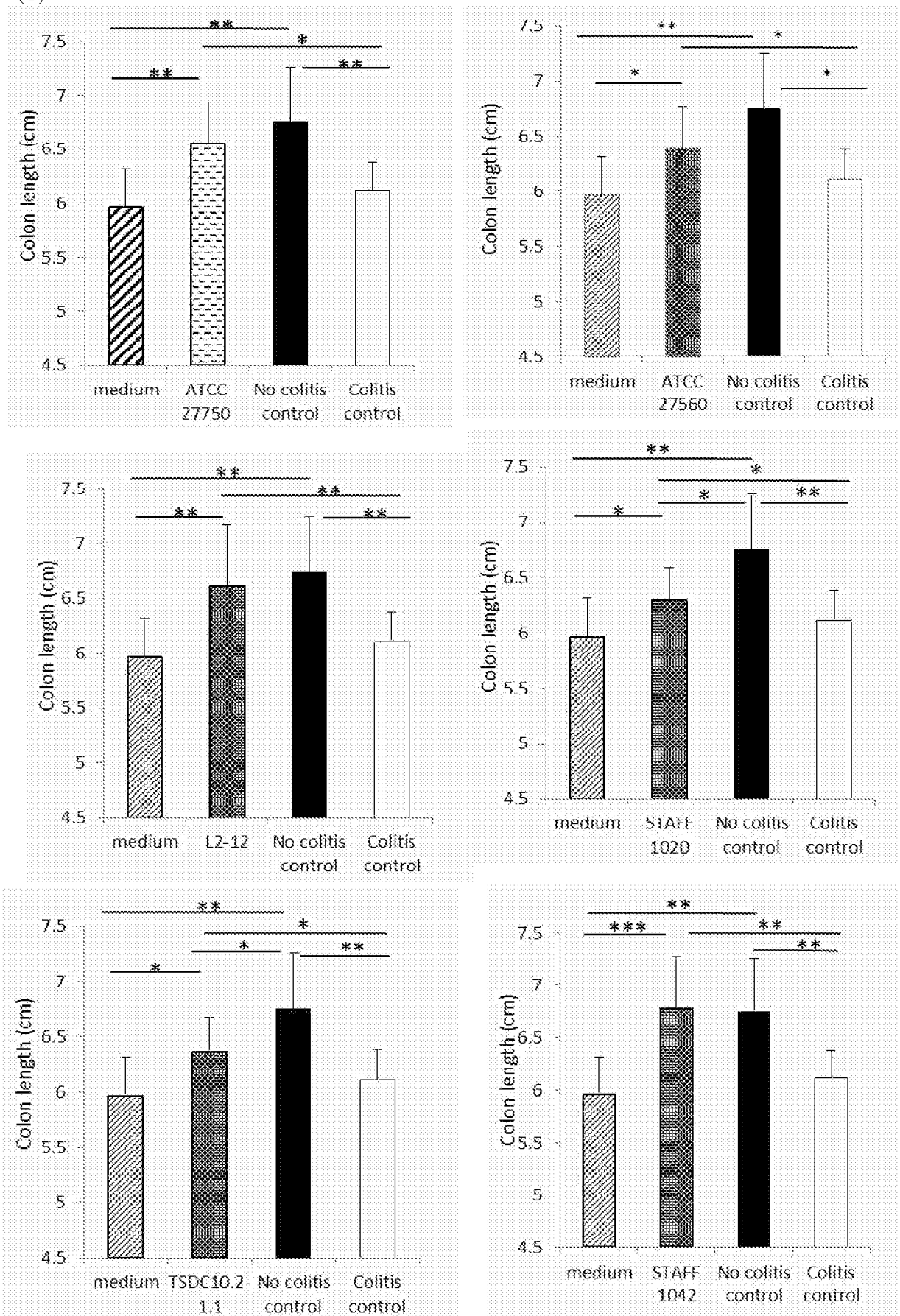


Fig.2 (cont'd)

(A)

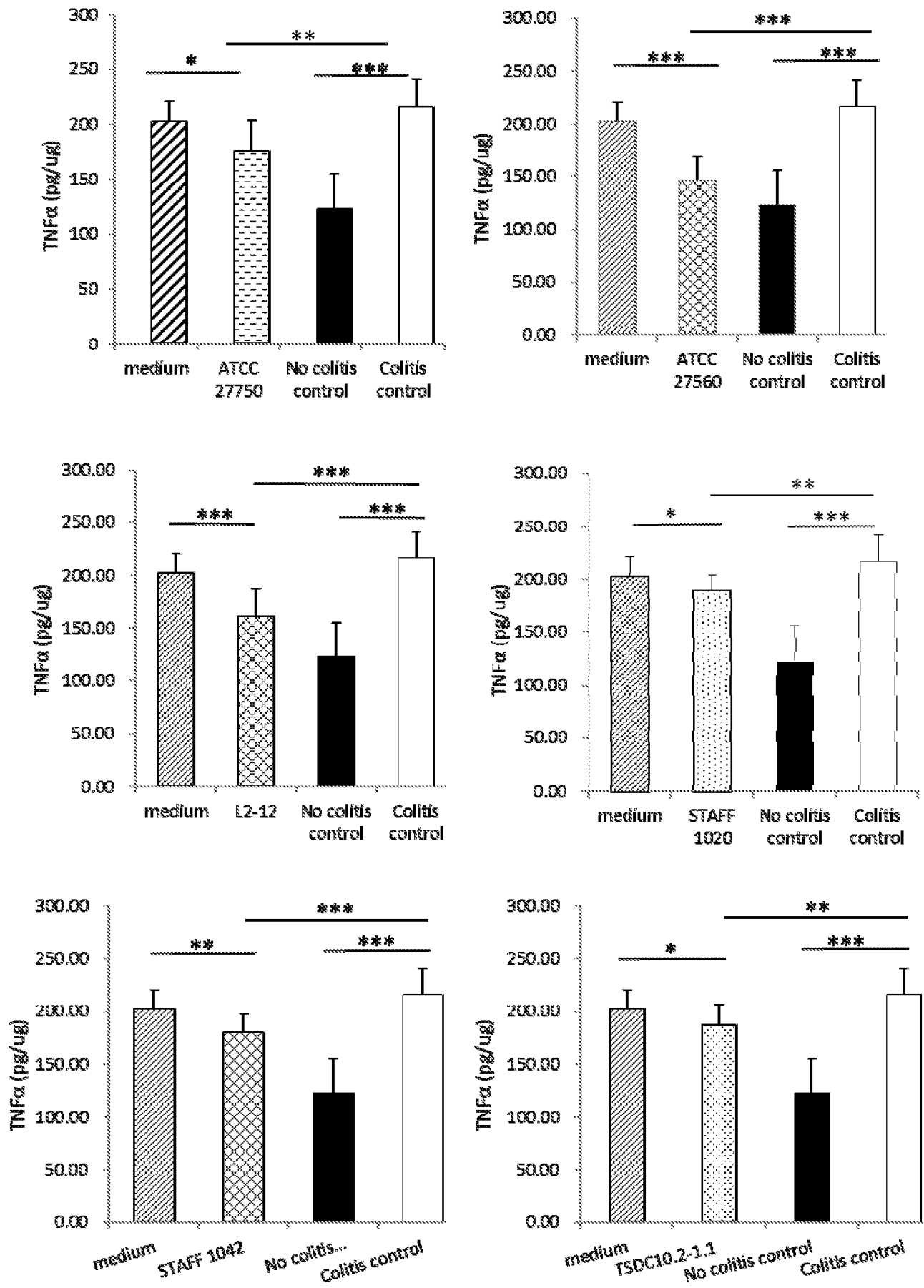


Fig.3

(B)

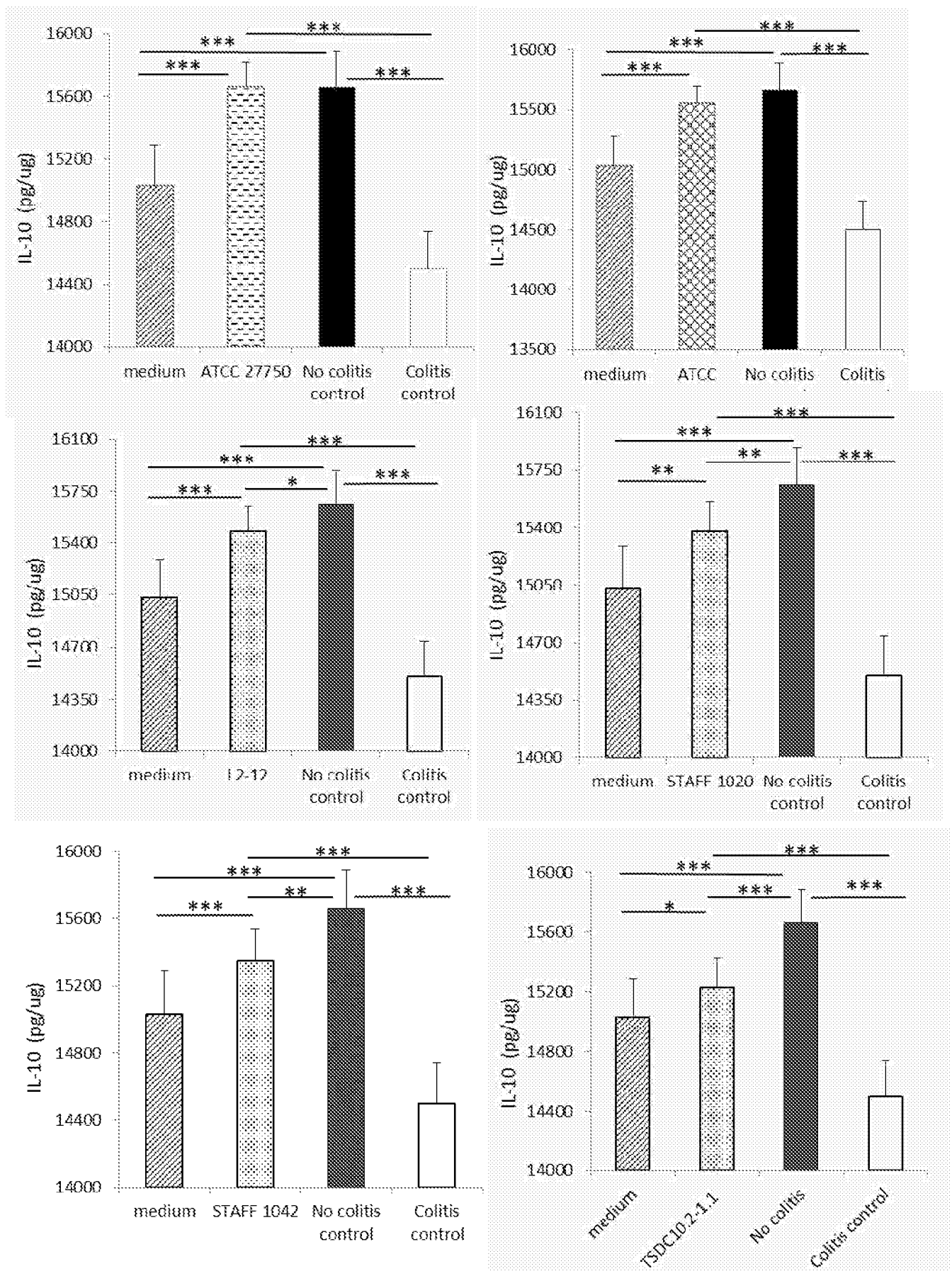


Fig.3(cont'd)

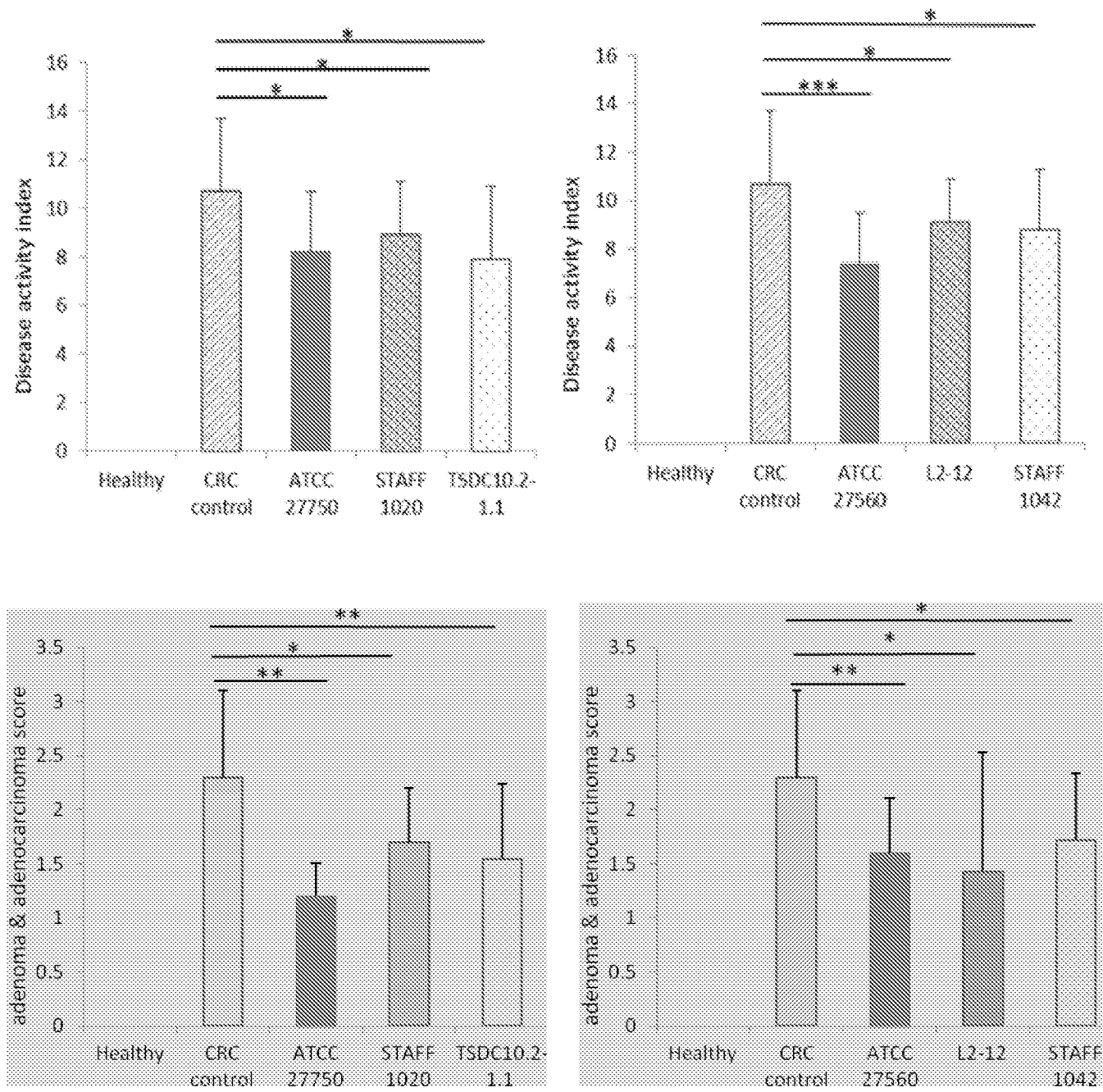


Fig.4

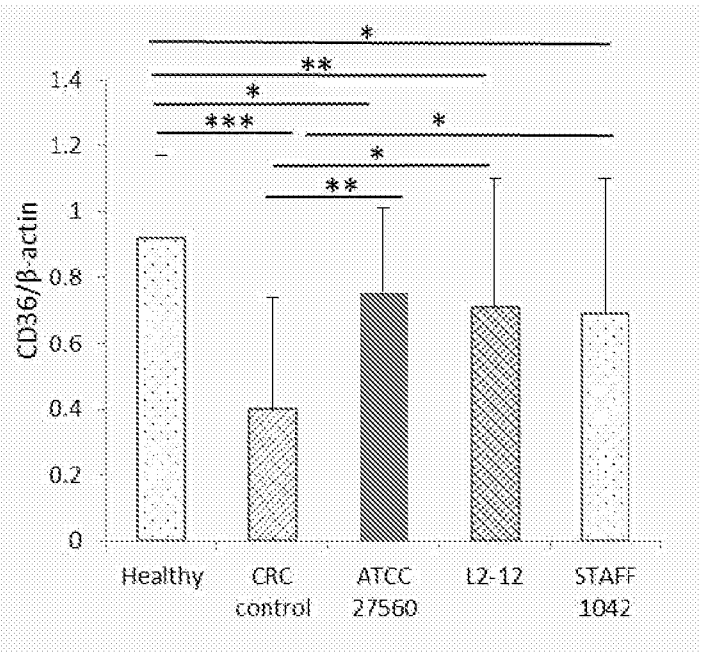
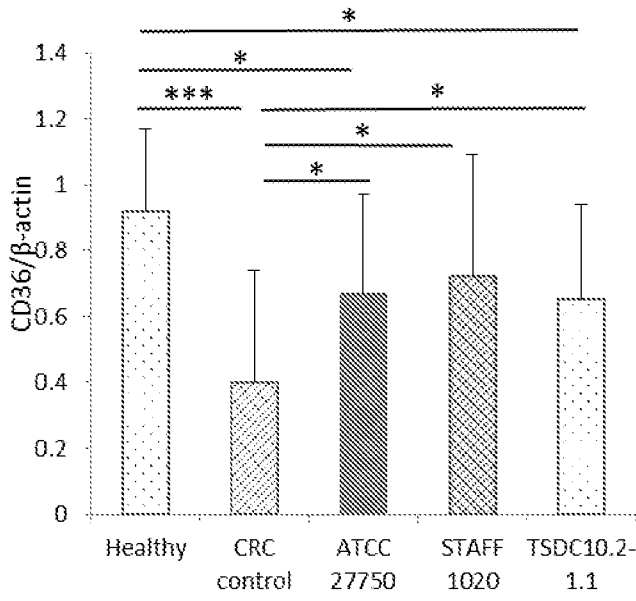
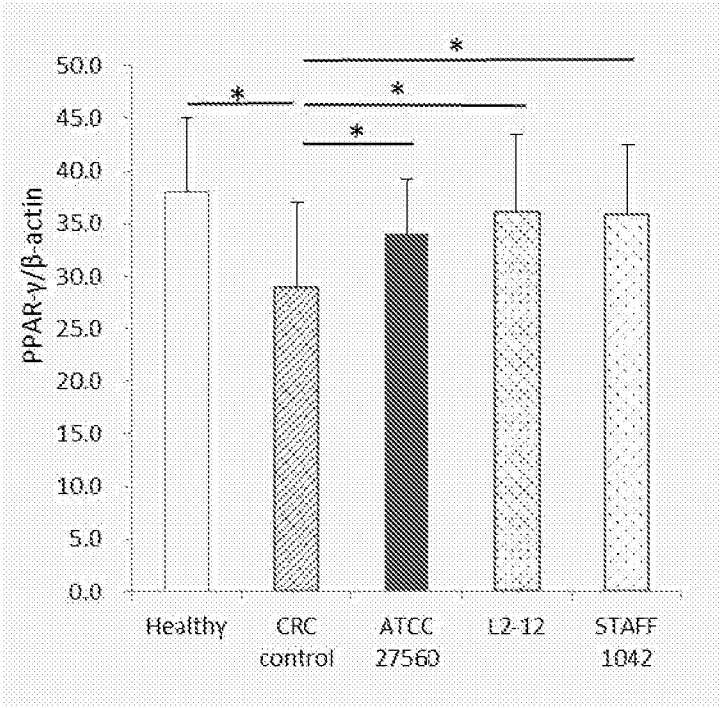
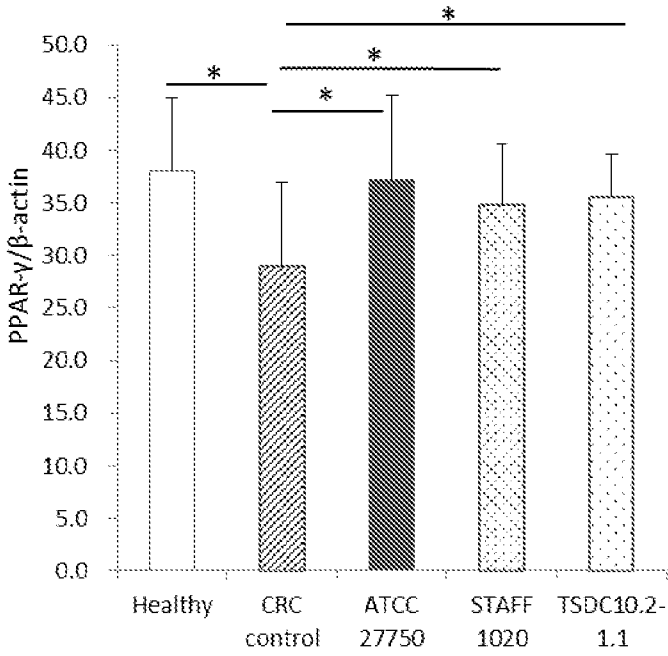


Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2014/083688

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/02(2006.01)i; C12N 1/20(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K; C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CPEA;DWPI;SIPOABS;EPODOC;CNPAT;GOOGLESCHOLAR;PUBMED:eubacterium, colitis, colorec+, cancer, CRC, eligens, colon.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012142605 A1 (SAMARITAN HEALTH SERVICES) 18 October 2012 (2012-10-18) abstract; claims 1-6; description, page 1 line 19-page 3 line 2	1-13
X	WO 2013037068 A1 (QUEEN'S UNIVERSITY AT KINGSTON ET AL.) 21 March 2013 (2013-03-21) abstract, description, page 4 paragraph 3, page 5 paragraph 2	1-13
X	WO 9001335 A1 (BORODY T.J.) 22 February 1990 (1990-02-22) abstract, claims 6, 9	1-13
A	CN 101590081 A (QINGDAO EASTSEA PHARMACEUTICAL ET AL.) 02 December 2009 (2009-12-02) the whole document	1-13
X	PETROF E.O. et al. "Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut." <i>MICROBIOME</i> , Vol. vol.1, No. no.3, 31 December 2013 (2013-12-31), abstract, Table 1	1-13



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 April 2015

Date of mailing of the international search report

08 May 2015

Name and mailing address of the ISA/CN

STATE INTELLECTUAL PROPERTY OFFICE OF THE
P.R.CHINA(ISA/CN)
6,Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088, China

Authorized officer

ZHONG,Hui

Facsimile No. (86-10)62019451

Telephone No. (86-10)62413728

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2014/083688

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **1-3,7-9**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 1-3 direct to the use of Eubacterium eligens for preventing or treating colitis and/or colorectal cancer. Claims 7-9 direct to a method for preventing or treating colitis and/or colorectal cancer.
The search has been carried out and based on the use of the microorganisms for manufacturing of a medicament for preventing and/or treating the disease.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2014/083688

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YANG L.Y. et al. "Bacteria, inflammation, and colon cancer." <i>WORLD JOURNAL OF GASTROENTEROLOGY</i>, Vol. vol 12, No. no.42, 14 November 2006 (2006-11-14), the whole document	1-13

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2014/083688

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2012142605	A1	18 October 2012	None			
WO	2013037068	A1	21 March 2013	US	2014363397	A1	11 December 2014
				US	2014342438	A1	20 November 2014
				CA	2848762	A1	21 March 2013
				EP	2744890	A1	25 June 2014
				EP	2756071	A1	23 July 2014
				WO	2013037067	A1	21 March 2013
				CA	2848757	A1	21 March 2013
WO	9001335	A1	22 February 1990	CA	1333564	C	20 December 1994
				AT	165738	T	15 May 1998
				EP	0433299	A4	08 April 1992
				DE	68928665	D1	10 June 1998
				EP	0433299	B1	06 May 1998
				DE	68928665	T2	12 November 1998
				EP	0433299	A1	26 June 1991
CN	101590081	A	02 December 2009	None			