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(54) Title: METHODS OF PREVENTING, TREATING AND DETECTING COLORECTAL CANCER USING BUTYRATE PRODUCING BACTERIA

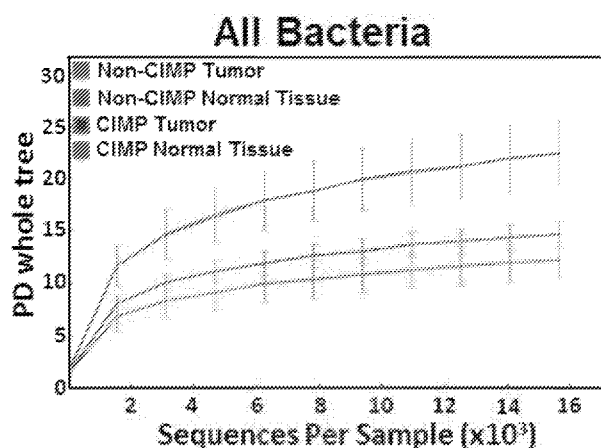


Figure 1A

(57) Abstract: The invention provides for methods for treating and preventing colorectal cancer, non-dysplastic serrated polyps, or serrated crypt foci in a subject by administering butyrate producing bacteria, dietary fiber, sodium butyrate, or a combination thereof. The invention also provides methods for detecting, determining and/or diagnosing colorectal cancer, non-dysplastic serrated polyps, or serrated crypt foci in a subject by detection of the level of butyrate producing bacteria in a sample, as well as the alpha diversity, the operational taxonomic units, the level of bacteria *Lactobacillales*, and the level of CoA and BcoA nucleic acid.



Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/24718

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.:
because they relate to subject matter not required to be searched by this Authority, namely:

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).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

----- SEE EXTRA SHEET -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1, 3-7, 9, 15, 18-21, 37, 38, 40, (42-43)(in part), 44, (48-49)(in part), 50, 52, 53, 67, 68, 70, 72, (73-76)(in part), 77, 79(in part), 80-83, 87, 88, (89-90)(in part) limited to administering *Clostridium* sp. str. M62/1 and detecting the alpha diversity of butyrate-producing bacteria.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/24718

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68, C12Q 1/04, C12Q 1/02 (2017.01)

CPC - C12Q 1/06, A61K 31/00, G01N 33/57419, G01N 2800/06, G01N 33/574

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2007/0258953 A1 (DUNCAN et al.) 8 November 2007 (08.11.2007) para [0023], [0025], [0031], [0038]	1, 5-6, 9, 50 ----- 3-4, 7, 15, 18-21, 37, 38, 40, (42-43)/(18-21), 44, (48-49)/(18-21), 89/(18-21), 90/(52-53, 80)
X --- Y	WO 2015/132273 A1 (INSTITUT D'INVESTIGACIO BIOMDICA DE GIRONA DR. JOSEP TRUETA) 11 September 2015 (11.09.2015) pg 1, para 1, pg 3, last para, pg 4, para 1, 2, 4, pg 6, para 1, pg 14, para 2, pg 15, para 5, pg 21, para 5, pg 28, para 2, pg 29 Table 9 legend, pg 30 para 1, Tables 1, 9	52, 67, 70, 72, (73-76)/52, 77, 80-82, 87-88 ----- 18, 19, 37, 40, (42-43)/(18-19), 44, (48-49)/(18-19), 79/52, 83, 89/(18-19), 90/(52,80)
X --- Y	US 2016/0032363 A1 (UNIVERSITY OF OTTAWA) 4 February 2016 (04.02.2016) para [0012], [0056], [0069], [0111], [0124], [0150], [0158], [0159], [0160], [0163]	53, 68, (73-76)/53 ----- 7, 15, 20, 21, 38, (42-43)/(20-21), (48-49)/(20-21), 79/53, 83, 89/(20-21), 90/53



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

2 October 2017

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18 OCT 2017

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/24718

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOJARAD et al. The CpG Island Methylator Phenotype (CIMP) in Colorectal Cancer. Gastroenterol Hepatol Bed Bench (Summer 2013) vol 6, no 3 pp 120-128, abstract, pg 124, col 2, para 2	3
Y	YAMANE et al. Serrated pathway in colorectal carcinogenesis. World J Gastroenterol. 14 March 2014 (14.03.2014) vol 20, no 10, pp 2634-2640, abstract	4
Y	WO 2008/091170 A1 (BIOLEK SP. Z O.O) 31 July 2008 (31.07.2008) abstract	89/(18-21)
Y	WEIR et al. Stool Microbiome and Metabolome Differences between Colorectal Cancer Patients and Healthy Adults. PLOS ONE 6 August 2013 (6.08.2013) vol 8, no 8, article e70803, pp 1-10, Abstract	79/(52-53)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/24718

Continuation of Box No. III, Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-91, directed to a method of treating and detecting colorectal cancer comprising:

--a method of treating colorectal cancer comprising administering to the subject, a therapeutically effective amount of butyrate producing bacteria, dietary fiber, or a combination thereof;

--a method of detecting butyrate-producing bacteria comprising:

- (i) detecting the alpha diversity of bacteria (claim 21);
- (ii) detecting the operational taxonomic units (OTUs) (claim 22);
- (iii) detecting the level of butyrate producing bacteria (claim 24);
- (iv) detecting the level of gene expression in butyryl pathways (claim 30);
- (v) detecting the cecal levels of short chain fatty acids (claim 46).

The method of treating colorectal cancer will be searched to the extent that the treatment method encompasses administering butyrate producing bacteria comprising *Clostridium* sp. str. M62/1.

The method of detecting butyrate-producing bacteria will be searched to the extent that the detection method encompasses detecting the alpha diversity of bacteria.

It is believed that claims 1, 3-7, 9, 15, 18-21, 37, 38, 40, (42-43)(in part), 44, (48-49)(in part), 50, 52, 53, 67, 68, 70, 72, (73-76)(in part), 77, 79(in part), 80-83, 87, 88, (89-90)(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that method encompasses administering *Clostridium* sp. str. M62/1 and detecting the alpha diversity of butyrate-producing bacteria. Applicants must specify the claims that encompass any additionally elected method(s) of treatment and/or detection. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched.

An exemplary election would be a method of treatment comprising administering dietary fiber and detecting the operational taxonomic units of butyrate-producing bacteria (claims 1-7, 9, 15, 18-23, 37-40, (42-43)(in part), 44, (48-49)(in part), 50-54, 67-70, 72, (73-76)(in part), 77, 79(in part), 80-83, 87, 88, (89-90)(in part)).

Another exemplary election would be a method of treatment comprising administering butyrate producing bacteria comprising *Roseburia hominis* and detecting the levels of butyrate producing bacteria (1, 3-7, 9, 10, 15, 18-21, 24-29, 37, 38, 40, (42-43)(in part), 44, (48-49)(in part), 50, 52, 53, 55-60, 67, 68, 70, 72, (73-76)(in part), 77, 79(in part), 80-83, 87, 88, (89-90)(in part)).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is the specific methods of treating colorectal cancer comprising administering butyrate producing bacteria or dietary fiber; and methods of detecting butyrate-producing bacteria. Each invention of Group I+ requires a specific therapeutic agent and a specific method of detecting colorectal cancer, a genus/species of bacteria being detected and/or administered, a specific method of detecting butyrate-producing bacteria, not required by any of the other inventions.

Common Technical Features

The inventions of Group I+ share the technical feature of detecting colorectal cancer (CRC) in a fecal or tissue sample from the subject and measuring the level of a gene/nucleic acid from a butyrate producing bacteria in the subject sample compared to the level in a control sample, and further treating said subject comprising administering a butyrate producing bacteria, dietary fiber, sodium butyrate or a combination thereof if said subject sample bacteria is lower than said control sample bacteria. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by WO 2015/132273 A1 to INSTITUT D'INVESTIGACIO BIOMEDICA DE GIRONA DR. JOSEP TRUETA et al. (hereinafter 'IDIBGI') in view of US 2007/0258953 A1 to Duncan et al. (hereinafter 'Duncan') and the article entitled "Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors" by Ishikawa et al. (hereinafter 'Ishikawa').

IDIBGI teaches detecting CRC is said sample by measuring 16S rRNA sequences (pg 1, para 1 "The present invention relates to the field of detection of colorectal cancer (CRC). Specifically it relates to methods for the early detection, risk screening and monitoring of CRC and/or adenomatous polyps in a human subject based on the quantification of one or more 16S rDNA bacterial sequences in feces") including sequences from butyrate-producing bacteria in healthy subjects (pg 4 para 2 "a study conducted by one of the inventors in mucosal biopsy samples ... shows the association of abundance of firmicutes, actinobacteria and *Escherichia coli* in patients with colorectal cancer and of *Faecalibacterium prausnitzii* in healthy subjects", pg 4 para 4 "quantification by real-time quantitative PCR [qPCR] of specific bacteria with metabolism products which are known to have a protective effect (i.e., *Eubacterium rectale* and *Faecalibacterium prausnitzii*, which are butyrate-producing bacteria) or to be toxic or carcinogenic (i.e., *Desulfovibrio* (sulphate-reducing bacteria) and *Enterococcus faecalis* (that produces extracellular superoxide) in the feces of patients with colorectal cancer"), and further teaches identifying [16S] sequences that are lower in CRC than in controls (pg 15, para 5 "In a preferred embodiment, wherein concentration levels of one or more of said sequences in said subject sample are lower than the median(cut-off) value minus the standard deviation of the levels of the respective sequence(s) measured in a control sample is indicative of CRC").

I

----- SEE NEXT EXTRA SHEET -----

Continuation of Box No. III, Observations where unity of invention is lacking:

IDIBGI does not expressly teach further treating said subject comprising administering a butyrate producing bacteria if said subject sample bacteria is lower than said control sample bacteria, however, Duncan teaches administering butyrate producing bacteria to mammals having low butyrate in the colon (para [0031] "a prophylactic method to reduce the incidence or severity of colorectal cancer or colitis in mammals caused in part by high lactic acid and low butyric acid concentrations, which method comprises the administration of a therapeutically effective dose of at least one above identified strains of live lactic acid utilizing bacteria and/or butyric acid producing bacteria mentioned above para [0007] "Butyrate also helps to protect against colorectal cancer"). IDIBGI and Duncan do not expressly teach administering fiber with bacteria, however, Ishikawa teaches treating CRC with butyrate producing bacteria and fiber (abstract "We examined whether dietary fiber and *L. casei* prevented the occurrence of colorectal tumors. Subjects were 398 men and women presently free from tumor who had had at least 2 colorectal tumors removed. Subjects were randomly assigned to 4 groups administered wheat bran, *L. casei*, both or neither. ... our results suggest that *L. casei* prevented atypical of colorectal tumors", pg 762, col 1, para 2 "since [it was] proposed that a diet high in dietary fiber prevented colorectal cancer, basic studies have suggested the possibility of prevention of colorectal cancer by dietary fiber"). IDIBGI, Duncan and Ishikawa do not expressly teach optionally treating subjects with sodium butyrate, however, would have been obvious to an artisan of ordinary skill to experiment with supplementing butyrate produced by bacteria with another source, because as taught by Duncan, butyrate protects against CRC.

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Group I+ therefore lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.