AWARD NUMBER:	**W81XWH-17-1-0279**
TITLE:	**Invention of a Genetic Toolkit for Immunomodulatory Gut Bacteria to Expedite the Development of New Crohn's Disease Therapeutics**
PRINCIPAL INVESTIGATOR:	**Jakob Begun**
CONTRACTING ORGANIZATION:	**University of Queensland**
REPORT DATE:	**JULY 2020**
TYPE OF REPORT:	**Technical Report**
PREPARED FOR:	**U.S. Army Medical Research and Development Command** **Fort Detrick, Maryland 21702-5012**
DISTRIBUTION STATEMENT:	**Approved for Public Release;** **Distribution Unlimited**

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JULY 2020	Annual	15 June 2019 to 14 June 2020

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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Mater Research Institute-UQ
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Brisbane, Queensland 4101

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The incidence and prevalence of Crohn's disease has increased steadily over the past several decades in the developed world with a major impact on quality of life. Unfortunately, current therapies are often unsuccessful in controlling inflammation and there is an urgent need to develop more effective therapeutic strategies. Our team is addressing this challenge by pursuing the following innovative outcomes: (i) Identifying novel natural NF-kB suppressive bioactives to support the development of a new therapeutics that mimic immunoregulation in the healthy gut (ii) Establishing a rational genetic approach to effectively bioprospect the gut microbiota, and; (iii) Providing new insights into the NF-kB suppressive activities of the gut microbiota and the structural diversity of the molecules that underpin it. The significance of this research is that it will catalyse the transition of gut microbiota research from an observational and associative paradigm to a translational one.

15. SUBJECT TERMS
Crohn's disease, microbiome, bioactives, NF-kB

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	14	**19b. TELEPHONE NUMBER** *(include area code)*

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   Crohn's disease (CD) is a chronic relapsing and remitting inflammatory disease of the gut. The gut microbiota produces a plethora of chemical metabolites that have biological activity on the host, both locally and systemically. Metabolites produced by the gut microbiota promote intestinal homeostasis and balance inflammation in healthy patients. In CD, this balance is lost. While there have been huge advances in sequencing the microbiota, this has not yet translated to a bacteria-derived therapy for CD. The goal of this project is to develop tools and approaches to expedite the discovery of novel natural NF-κB suppressive bioactives from fastidious gut bacteria. We will use both *in vitro* and *ex vivo* samples to rationally choose the bacterial strains with the best suppressive characteristics.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   Crohn's disease, microbiome, bioactives, NF-κB

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   What were the major goals of the project?
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1*(specified in proposal)*	**Timeline**	**Site 1**
Major Task 1: Isolate genetically tractable human gut bacteria affiliated with the Firmicutes	Months	
Use our state-of-the-art facilities for anaerobic microbiology to isolate genetically tractable fastidious gut bacteria. ***Complete: 23 genetically tractable strains have been isolated for this project.***	21-27	Dr. Jakob Begun, Páraic Ó Cuív & Prof Mark Morrison
Major Task 2: Identify human gut bacteria which produce bioactive factors that suppress NF-κB in cell lines	Months	
Apply to the DoD Office of Research Protection HRPO for approval to use the human Caco-2 epithelial cell line for secondary research. ***Complete: Approval Memorandum dated Feb 2, 2018***	1-8	Dr. Jakob Begun & Dr. Páraic Ó Cuív
Apply to The University of Queensland Diamantina Institute to have the CaCo-2 epithelial cell lines declared as exempt ***Complete: Approval Memorandum dated Feb 2, 2018***	1-8	Dr. Jakob Begun & Dr. Páraic Ó Cuív
Use cell line based functional assays to identify bacteria secreting NF-κB suppressive bioactives ***Complete: All bacteria have been tested for suppressive function and results confirmed with independent assays.***	23-29	Dr. Jakob Begun, Dr. Páraic Ó Cuív,, Prof Michael McGuckin & Prof Mark Morrison

Milestone Achieved: Completed Aim 1. **DONE**	29	
Specific Aim 2		
Major Task 1: Assess the ability of the bioactive factors to suppress the Crohn's disease (CD) inflammatory response		
Apply to the DoD Office of Research Protection HRPO for approval to use the ileal biopsies collected to support this study for secondary research. ***Complete: Approval Memorandum dated Feb 2, 2018***	1-8	Dr. Jakob Begun & Dr. Páraic Ó Cuív
Recruit and collect ileal biopsy samples from healthy human males and male CD subjects. ***Complete***	1-10	Dr. Jakob Begun
Determine the extent of NF-κB suppressive activity of the bioactives in healthy and CD derived peripheral blood mononuclear cells and gut epithelial enteroids ***Complete: Tested supernatants in both healthy and CD patient organoids and matched PBMCs***	28-32	Dr. Jakob Begun & Prof Michael McGuckin
Milestone Achieved: Completed Aim 2. **DONE**	32	
Specific Aim 3		
Major Task 1: Develop and apply genetic approaches to expedite the discovery of NF-κB suppressive bioactives		
Use a combination of genomic and reverse/forward genetic approaches to identify bacterial pathways underpinning the production of NF-κB suppressive secondary metabolites in select strains ***50% complete: WGS complete on strains. Identified candidate BGCs. Attempted forward genetic approach to identify genes underpinning bioactive production without successful identification.***	29-38	Dr. Jakob Begun, Dr. Páraic Ó Cuív, Prof Michael McGuckin & Prof Mark Morrison
Use functional genomics to identify NF-κB suppressive peptide bioactive factors ***50% complete. Functional genomics did not identify the genes underpinning bioactive function. We therefore transitioned to comparative metabolomics to identify the chemical structure of the bioactives. We have preliminarily identified the chemical structure of a suppressive bioactive.***	32-38	Dr. Jakob Begun, Dr. Páraic Ó Cuív, Prof Michael McGuckin & Prof Mark Morrison
Milestone Achieved: Prepare and submit paper ***Complete: Manuscript available on BioRxiv. Currently under review at Gastroenterology***	34-38	Dr. Jakob Begun
Milestone Achieved: Complete Aim 3 ***Ongoing: anticipate completion Dec 2020***	42	

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Reporting period: 15 June 2019 to 15 June 2020.

Major activities

Aim 1.
<u>Major Task 1: Isolate genetically tractable human gut bacteria affiliated with Firmicutes.</u>

Using our state-of-the-art anaerobic facility, we isolated bacterial strains from human stool samples. These were grown in conditions that we have previously optimized to support the specific isolation of anerobic bacteria from the Firmicutes family.
We performed meta-parental mating on these strains and isolated 23 genetically tractable strains of bacteria that grew under these conditions. Colonies were picked from agar plates into single wells of 96 deep well plates and grown for 72 hours. As we were interested in identifying bacterial strains that produced a secreted soluble bioactive, we collected the bacterial culture supernatant by high-speed centrifugation to produce a cell-free conditioned culture media to test in our cell culture assay. CaCo-2 cells bearing an NF-κB reporter were used to read-out changes in NF-κB activity. Cells were plated and then treated with 10% v/v of bacterial culture media (or sterile broth) for 30 minutes. Then the cells were stimulated with IL-1β. The activity of the NF-κB reporter (luciferase) as measured after 4 hours (Figure 1A). As illustrated in Figure 1A, the synthetic antagonist iC3 completely abolished NK-κB reporter activity. Sterile broth (MCM or BHI) did not alter NF-κB activity. However, we identified 5 strains of bacteria that were capable of decreasing NF-κB activity (AHG00XXX, is the in-house designation of the bacterial strains). F. prausnitzii A2-165 was used as a positive control for a bacterial strain capable of reducing NF-κB activity, as this has been previously published. We confirmed the reduction of NF-κB activity in the CaCo-2 cells by assessing gene expression by RT-qPCR. We examined the gene expression of *IL-6, IL-8 and CXCL10* which are all well-known NF-κB target genes. Similar to the reporter assay, the bacterial strain supernatants decreased the expression of these genes. Thus, we have identified at least 5 strains of genetically tractable bacteria that demonstrate NF-κB suppressive activity. Through biochemical studies including size fractionation and temperature and enzymatic sensitivity, we have been able to characterise the bioactives produced by these strains as either protein/peptide or small molecules (data not shown).

Thus, we have successfully completed all the tasks in Aim 1.

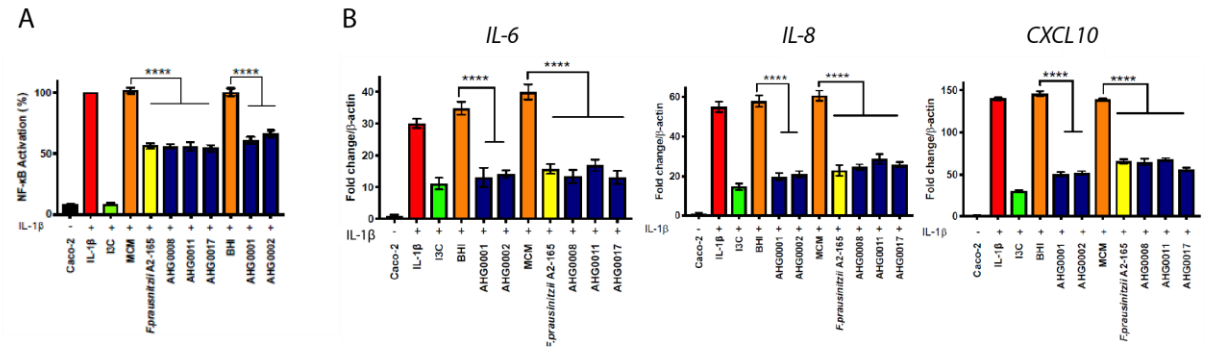

Figure 1. <u>NF-κB suppression by isolated bacterial strains</u>. CaCo-2 cells bearing an NF-κB luciferase reporter were pre-treated with cell-free bacteria culture supernatant or broth for 30 minutes prior to stimulation with IL-1β. **A)** Reporter expression 4 hours after IL-1β stimulation. **B)** Gene expression in cells after 4h stimulation. iC3 is a potent synthetic NK-κB inhibitor. BHI – brain heart infusion broth, MCM – modified clostridial media, AHG00XX – individual bacterial strains isolated from gut stool samples, F. prausnitzii A2-165 is a known suppressor of NF-κB activity. Significance determined by one-way ANOVA, Dunnet's post-hoc test.

Aim 2.

Major Task 1: Assess the ability of the bioactive factors to suppress the Crohn's disease (CD) inflammatory response

Biopsy specimens and blood from patients undergoing colonoscopy in CI Jakob Begun's clinic were collected (according to the approved ethical protocol 38409). Organoids were generated from the biopsy material, and PBMCs were isolated from the blood. Once established, organoids were treated as in Figure 1. IL-8 cytokine secretion was measured in the culture supernatant. To varying degrees, the bacterial culture supernatants could reduce IL-8 secretion in the primary cells. Interestingly, the organoids from the healthy patients exhibited stronger inhibition by the supernatants than did the organoids from CD patients. This was an interesting result and supported our proposed validation of the suppressive activity of the supernatants in patient samples. When we examined the response of matched PBMCs, we found that they also were inhibited from secreting IL-8 in the presence of the bacterial culture supernatants. At this point we identified several bacterial strains that have demonstrated activity in both epithelial cells (cell lines and organoids) and immune cells (PBMCs). We have also validated our results in both healthy and patient samples.

Thus, we have successfully completed all the tasks of Aim 2.

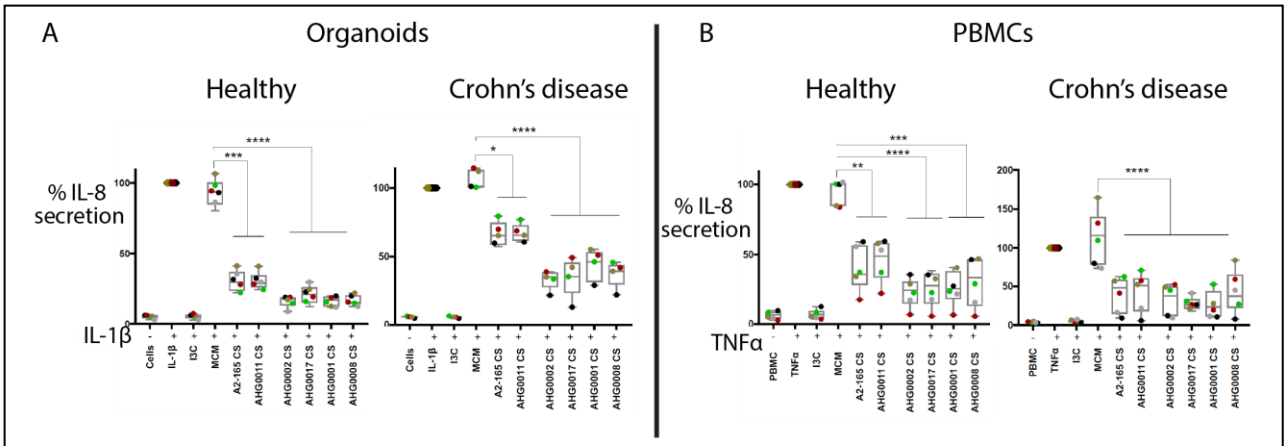

Figure 2. Bacterial stains decrease NF-κB activity in human organoids and PBMCs. **A)** Healthy and Crohn's disease patient biopsies were collected (according to our approved ethics) and intestinal organoids grown *ex vivo.* Following establishment, these organoids were then treated as in Figure 1. The supernatants were collected and assessed for secretion of IL-8 by ELISA. Each dot represents an individual patient sample. **B)** Matched PBMCs from the patients were isolated and stimulated as in Figure 1. Supernatants were collected and IL-8 secretion determined by ELISA. CS = culture supernatant, A2-165 is F. prausnitzii A2-165. Significance by one-way ANOVA, Dunnet's post-hoc test.

Aim 3.

Major Task 1: Develop and apply genetic approaches to expedite the discovery of NF-κB suppressive bioactives

To identify the suppressive bacterial strains, we performed 16S rRNA sequencing to generate the phylogenic tree (Figure 3A). This allowed us to confirm our preliminary analysis which indicated that each of the bacterial strains that we had identified was unique. For each suppressive strain, we identified a closely related species that we would use for comparative genomic analysis of the suppressive strains. We performed whole genome sequencing of the suppressive strains and *de novo* assembled the sequencing data. CheckM was used to evaluate the genome sequencing quality by estimating the completeness and contamination based on the phylogenetic assignment of a broad set of marker genes. The contigs were ordered using Mauve using the identified type-strains for comparison. Genome based phylogeny was determined using Genome Taxonomy Database (GTDB) (Figure 3B). The extent

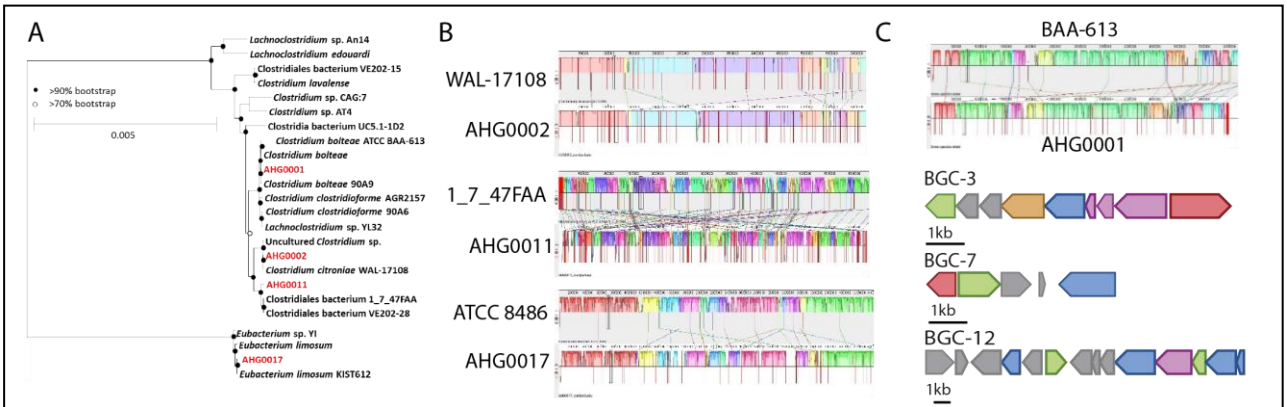

Figure 3. Genetic identification of NF-κB suppressive bacterial strains. **A)** 16S rRNA gene sequencing was conducted to identify each bacterial strain. Phylogenetic tree of the isolates in comparison with other annotated bacterial strains. **B)** Whole genome sequencing was conducted on the suppressive strain, their genomes assembled and compared against closely relatives. **C)** Whole genome sequencing of the small molecule bioactive producing strain AHG001 compared to a non-suppressive strain BAA—613. We identified 5 non-conserved BGCs. We identified 3 BGCs with potential as the genes controlling bioactive production.

of protein (Genes conserved) and syntenic gene pair (syntenous pairs) conservation was assessed. Comparative genomic analyses revealed that AHG0001 carries 19 predicted

biosynthetic gene clusters (BGCs) of which 14 are either highly or partially conserved in the closely related non-suppressive strain BAA-613. We identified three candidate BGCs which may drive bioactive production in the suppressive strain. This BGC was then cloned into a pEHR plasmid and transferred into a non-suppressive strain to test for acquisition of the suppressive function. The transferred BGC did not confer suppressive function in the new strain, suggesting that bioactive production was not driven by the candidate BGC (data not shown).

While we have specifically designed our screen to isolate genetically tractable bacteria to facilitate identification of the genes driving bioactive production, testing a single BGC from AHG001 proved extremely labour intensive. To expedite bioactive discovery, we employed a different strategy. As we have identified a non-suppressive close relative of AHG001 we were able to use comparative metabolomics to begin to identify the chemical structure of the small molecule bioactive (Figure 4). Using HPLC fractionation we identified the fraction which contained the suppressive bioactive (Figure 4A). We next used UPLC-QTOF single ion extraction which found 6 small molecules i-vi that were present in the NF-κB suppressive HPLC

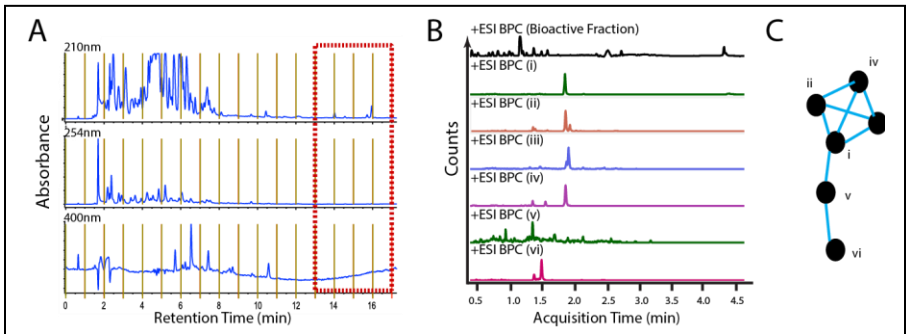

Figure 4. Metabolomic identification of small molecule bioactive. **A)** HPLC fractionation of supernatant from the suppressive stain was conducted. The fraction from 13-17 was determined to contain suppressive activity using our reporter assay (data not shown). **B)** UPLC-QTOF of the suppressive fraction identified 6 small molecules that may contain suppressive properties. **C)** Global natural products social molecular networking analysis of the UPLC-QTOF ms/ms demonstrated the relationships between the identified potential suppressive bioactives. BPC = selected base peak (m/z molecular ion) chromatogram.

fractions (Figure 4B). The acquired MS/MS data was converted from Agilent MassHunter data file (.d) to the mzXML file format using the software MS-Convert. Molecular networks were generated using the online Global Natural Products Social molecular networking web-platform (GNPS) (gnps.ucsd.edu) (Figure 4C). Metabolomics, rather than genetics, has allowed us to approach the identity of the small molecule produced by strain AHG001. As the overall goal of the project was to

identify the suppressive bioactives, metabolomics has proved more successful and more rapid than genetic analysis. We will continue this method to confirm the structure the bioactive and optimize its chemical properties using structure-activity relationship studies.

Thus, we have completed 50% of the tasks for this aim.

Key Outcomes and Achievements to Date: We have successfully completed a significant amount of the tasks set out in this grant proposal. We have isolated gut-derived bacterial strains that inhibit inflammatory signalling in both cell lines and primary patient samples (Aim 1 and Aim 2). We have identified the strains by 16S rRNA sequencing and have catalogued their genome by Whole genome sequencing. This allowed us to identify genetically closely related strains that did not demonstrate suppressive activity. This has been an important step, as having non-suppressive strains to compare against has allowed us to perform powerful metabolomic analysis. Upon completion of this grant we will have validated the chemical structure of one NF-κB suppressive small molecule. While we have not successfully identified the genetics underpinning bioactive production, this has not prevented us from accomplishing the over-arching goal of this project, which is identifying naturally occurring NF-κB suppressive bioactives from the gut bacteria.

Currently, a manuscript relating to this work is under review at *Gastroenterology*. Additionally, a pre-print version of the paper is available on BioRxiv (Giri R, Hoedt EC, Khushi S, McGuckin MA, Morrison M, Capon RJ, et al. Secreted microbial metabolites modulate gut immunity and inflammatory tone. bioRxiv. 2019).

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Training activities were undertaken through presentations of the results of this project. Ms. Rabina Giri, the Ph. D student on this project has presented her results both orally and in posters at a number of conferences including; AGW2019: Oral presentation, AGW 2018: Oral presentation, GESQ Noosa update: Oral Presentation, BIG2019: Oral presentation, APDW2019: Oral presentation, DDW 2017,2018,2019: Poster Presentations

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

As previously described these results have been presented at international conferences and will be published in a peer-reviewed journal.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next reporting period, we will continue with the comparative metabolomic strategy to identify the structure of the bioactive produced by AHG001. We have identified a preliminary structure that we will optimize with repeated rounds of structure-activity relationship testing to optimize the chemical characteristics of the bioactive (solubility, potency, stability, tagging). At the end of the project we will have identified a naturally produced anti-inflammatory with optimized chemical properties, ready for *in vivo* testing.

4. IMPACT: D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Currently, the field of microbiota research is hampered by a lack of functional annotation for the genes that comprise the microbiome. Without this knowledge, sequencing efforts are unlikely to uncover how bacteria function in the intestine. We have developed a novel discovery approach to find bacteria that have a specific function in the gut. This is a departure from traditional microbiota studies. We hope that our successful approach will inspire other groups to functionally characterize the microbiota, in addition to genetic sequencing, to more fully understand the relationship between the microbiota and the host.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

This project has required expertise from three different scientific disciplines; chemistry, biology and bacterial genetics. Cross-discipline collaboration has enabled a significant body of work to be conducted on this project, and we anticipate it will lead to the successful development of a lead compound for the treatment of CD.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report at this stage. Future work may lead to commercialization if the results of this study result in improvement in a pre-clinical model.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

The over-arching goal of this proposal was to identify bioactives produced by gut-derived bacteria capable of suppressing NF-κB activity. We proposed to do this by identifying the genes underlying bioactive production. However, this turned out to be laborious, time-consuming and ultimately ineffective. Therefore, we have changed our approach to achieve the same goal. We have employed comparative metabolomics to identify the chemical structure of the bioactive. Using this method, we successfully isolated the active fraction by HPLC and by UPLC-QTOF have identified a putative structure for the bioactive. The preliminary genetic analysis supported this method as we needed to identify a closely related non-suppressive stain for the comparison.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report in addition to the above comments

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Giri R, Hoedt EC, Khushi S, McGuckin MA, Morrison M, Capon RJ, et al. Secreted microbial metabolites modulate gut immunity and inflammatory tone. bioRxiv. 2019.

This manuscript is currently under review at Gastroenterology; acknowledgement of federal support = yes.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

<u>Poster presentations:</u>

R., Cuiv, P., & Begun, J. (2018). Tu1852 - Role of Anti-Inflammatory Gut Bioactives in the Modulation of Immune Response in Crohn's Disease. *Gastroenterology*, 154, S-1038. doi:10.1016/S0016-5085(18)33478-4

Giri, R., Cuiv, P., & Begun, J. (2019). Mo1930 – Harnessing Anti-Inflammatory Gut Bioactives to Modulate the Immune Response in IBD. *Gastroenterology*, 156, S-890. doi:10.1016/S0016-5085(19)39197-8

Giri, R., Hoedt, E., Khushi, S., Salim, A., Capon, R., Morrison, M., . . . Begun, J. (2020). Tu1302 Investigating the role of bioactives produced by gut bacteria to modulate immune response in IBD. *Gastroenterology*, 158, S-1050. doi:10.1016/S0016-5085(20)33310-2

Oral abstract presentations:

Giri, R., Khushi, S., Salim, A., Capon, R., Morrison, M., Cuiv, P., & Begun, J. (2020). OP36 Investigating the role of bioactives produced by gut bacteria to modulate immune response in inflammatory bowel disease. *Journal of Crohn's and Colitis*, 14, S037-S038. doi:10.1093/ecco-jcc/jjz203.035

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

The whole genome sequencing that we have completed for this project has been deposited at NCBI. The sequences will be release upon manuscript acceptance. They are QYRW00000000, QYRX00000000, QYRY00000000 and QYRZ00000000, and are the first versions.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Rabina Giri
Project Role: PhD student
Nearest person month worked: 12
Contribution to the project:
Ms Giri has collected, archived, and stored patient derived samples. Ms Giri has isolated different bacterial strains from a human stool sample and prepared the bacterial supernatants and tested those on Caco-2 epithelial cells in vitro. She had performed the testing on the human patient samples.

Name: Jakob Begun
Project Role: Principle Investigator
Nearest person month worked: 1
Contribution to the project:
Dr Begun has been responsible for patient recruitment and obtaining of biopsy samples. His other involvement included but was not limited to project supervision, data interpretation and manuscript preparation.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name: University of Queensland – Institute for Molecular Biology
Location: Brisbane Queensland, Australia
Contribution to project: Collaboration and facilities. Use of metabolomic equipment and staff participation for bioactive identification were undertaken in collaboration with partners at IMB.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.