PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

ESCUELA DE INGENIERIA

ENGINEERED BACTERIA FOR PROPIONATE-REPRESSED EXPRESSION OF sfGFP AND GM-CSF

MARIA JOSÉ BARRA

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Master of Science in Engineering

Advisor:

DANIEL GARRIDO

Santiago de Chile, March, 2021

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Dedicated to my parents and sister, for
their constant and limitless support.

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ABSTRACT

Inflammatory bowel diseases (IBD) are chronic diseases that currently have no cure. Their causes are multifactorial, but the composition of the gut microbiome is believed to play an important role in their development. Certain beneficial bacteria in the microbiome are able to ferment dietary fibers and produce short chain fatty acids (SCFA), such as propionate, that help protect intestinal integrity and prevent the colonization of pathogens. These are found in reduced concentrations in people who suffer from IBD. Taking advantage of the ability of bacteria to colonize the human body, they have been engineered to act as biosensors to detect specific biomarkers which can be used as complimentary diagnostic tools. They have also been genetically modified to act as drug delivery vehicles, helping to increase target specificity and avoid harmful side effects. In this work, we develop a two plasmid system in *E. coli* DH5α in which propionate induces the expression of the LacI repressor. In the second plasmid, the repressor controls the expression of the reporter protein, sfGFP. We observed that the genetic circuit functions between 0-110 mM of propionate with at least 0.1 mM generating a response. We then replaced *sfGFP* with the gene for the cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF), which was chosen due to its reported effects in strengthening the intestinal epithelium barrier. After 4 hours of growth and in the absence of propionate, 11.61 µg/mL of GM-CSF/OD_{600} were produced, while 8.959 µg/mL were produced in the presence of 100 mM of the inductor. Nevertheless, the differences in the concentration of GM-CSF produced were minimal between the two conditions after this time. Additionally, the concentration of GM-CSF produced rapidly declined after 4 hours, likely being degraded within the cell. Overall, the reporter strain may function as a complementary diagnostic tool for quantifying propionate and the therapeutic strain may work as a delivery vehicle for GM-CSF, although further studies are required to perfect the sense and respond mechanism.

1. INTRODUCTION

Inflammatory bowel diseases (IBD) are primarily composed of Crohn's disease and ulcerative colitis. These are chronic diseases characterized by severe inflammation in the gastrointestinal tract (Bernstein et al., 2009). Their causes are believed to be multifactorial including genetic, immunological and environmental aspects (Matsuoka and Kanai, 2015). Additionally, the composition of the gut microbiome is thought to play an important role in the homeostasis of the intestinal environment (Lakatos, 2009).

There are various inconveniences in diagnosing IBD. The procedures that are normally required, such as endoscopies and histological examinations, are expensive and highly invasive (Baumgart and Carding, 2007; Shergill et al., 2015). Alternatively, biomarkers indicative of these diseases have been studied for diagnostic means. For instance, fecal samples can be tested for the presence of these specific substances (Lopez et al., 2017). However, these samples may not accurately represent biomarker concentrations throughout the intestine due to their short half-lives or low concentrations *ex vivo*. In these cases, non-invasive methods for *in vivo* detection of IBD biomarkers should be developed.

IBD currently have no cure and treatments frequently have only a moderate impact considering their possible associated side effects and disease recurrence. Many commonly prescribed pharmaceuticals target inflammation, which is only a symptom and not a cause of the disease (Schölmerich, 2006). Immunosuppressive agents in high doses can have adverse side effects in the long term. Newer biologic treatments are generally considered to be more effective, although this is not the case in an important subset of patients who relapse when treatment is suspended (Coskun et al., 2017). Furthermore, these biologic treatments continue to be expensive (Holko et al., 2018). Fecal transplants have also been performed, but there is a lack of official protocols and guidelines for their execution

(Sunkara et al., 2018). Eventually, many patients undergo surgery during which portions of the gastrointestinal tract must be excised (Magro et al., 2017).

In experimental phases, bacteria are being engineered as diagnostic and therapeutic tools for IBD. Considering that microorganisms naturally colonize the human intestine, genetically modified bacteria can be used as *in situ* biosensors or delivery vehicles for therapeutic substances. As biosensors, they can detect IBD biomarkers and generate a quantifiable response which has the potential to act as a complementary diagnostic tool (Daeffler et al., 2017). Bacterial biosensors have the advantage of detecting substances that are present directly in the diseased environment. Additionally, certain substances may not be quantifiable by other means. Bacteria engineered for the delivery of therapeutic substances may be favorable in improving target specificity, which could increase the substances' efficacy in treatment. Furthermore, this would allow the use of lower doses and reduction of side effects. Biosensors and live biotherapeutics may be combined to create sense and respond systems. As a result, a therapeutic substance could be produced exclusively in the presence of a biomarker, adding specificity and regulation to engineered live biotherapeutic bacteria.

The microorganisms of the intestinal microbiota produce diverse metabolites that can directly or indirectly affect human health. The effects of the symbiotic relationship between these bacteria and the host may depend profoundly on the host diet (Thorburn et al., 2014). Particularly, dietary fibers consumed by humans can arrive intact to the intestine where they serve as a carbon source for many beneficial bacteria. When bacteria ferment these fibers they generate metabolites, among which short chain fatty acids (SCFA) are highly relevant (Thorburn et al., 2014). The most relevant and prominent SCFA are acetate, butyrate and propionate (Sun et al., 2017). These metabolites are the main energy source for colonocytes and have been shown to have anti-inflammatory and protective effects on the intestinal epithelium (Corrêa-Oliveira et al., 2016; Sun et al., 2017). In patients with IBD, reduced concentrations of SCFA have been observed (Parada

Venegas et al., 2019). Being able to detect a scarcity of SCFA in the intestine may be used, in addition to current IBD diagnostic methods, to help focus treatment options on a case-by-case basis.

Propionate is believed to have an important effect in controlling immune system responses. It was reported to decrease the release of TNF-α from neutrophils, inhibit NF-κB reporter expression in human colon cells and repress the expression of IL-6 in colon organ cultures *in vitro* (Tedelind et al., 2007). The main propionate producing bacteria in the gut microbiome belong to the Bacteroidetes phylum and Lachnospiraceae family from the Clostridia class (Reichardt et al., 2014). The former utilize the propanediol pathway to metabolize deoxy sugars into propionate while the latter produce the SCFA by consuming hexose sugars through the succinate pathway (Figure 8.1.1). In patients with IBD, both Bacteroidetes and Lachnospiraceae have been found in significantly lower amounts compared to healthy people (Frank et al., 2007, 2011). These findings suggest an important relationship between the members of these bacterial groups, the lack of production of propionate and the activity of IBD.

In this study, we create a two-plasmid system in *Escherichia coli* DH5α to detect reduced concentrations of propionate and produce either superfolder green fluorescent protein (sfGFP) or granulocyte macrophage-colony stimulating factor (GM-CSF) as a response. sfGFP is a quantifiable reporter protein and GM-CSF is a therapeutic protein. Specifically, GM-CSF is a cytokine that stimulates the innate immune system in intestinal cells, interacts with receptors on epithelial cells helping to improve mucosal barrier integrity and has shown promising results in clinical trials for treating IBD patients (Ramsay et al., 2004; Korzenik et al., 2005; Pizarro and Cominelli, 2007). In the first plasmid, a propionate-induced promoter controls the expression of the repressor protein, LacI. In the second plasmid, the P_{tac} promoter repressed by LacI controls the expression of sfGFP or GM-CSF. Therefore, higher concentrations of propionate produce more inhibitor and less sfGFP or GM-CSF and vice versa, supposedly responding to diseased states of intestinal

inflammation. The former acts as a biosensor for propionate with sfGFP as a quantifiable reporter. The latter aims to act as a biotherapeutic bacterium with a sense and respond mechanism.

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

We hypothesize that *E. coli* DH5α can be genetically engineered to produce increasing amounts of sfGFP and GM-CSF with decreasing concentrations of propionate.

2.2. Main Objective

The main objective of this work is to create a two plasmid system in *E. coli* DH5α in which expression of the LacI repressor is induced by propionate, which controls the expression of sfGFP or GM-CSF in inversely proportional amounts of the inducer.

2.3. Specific Objectives

1. Clone the *lacI* gene from *E. coli* K12 MG1655 into the pPro24 plasmid and transform *E. coli* DH5α with this and the reporter plasmid.
2. Evaluate and characterize the two plasmid system from the first objective.
3. Replace *sfGFP* with the *GM-CSF* gene in the reporter plasmid and transform *E. coli* DH5α with this and the pPro-lacI plasmid.

4. Quantify GM-CSF production from the two plasmid system from the third objective.

3. MATERIALS AND METHODS

3.1. Cloning of propionate-lacI system

The *lacI* gene was obtained by PCR with the *E. coli* K12 MG1655 genome as the template. The primers used contain overhangs from the backbone into which it was cloned for Gibson Assembly cloning (Gibson et al., 2009): 5'-AAGCTAGCAGGAGGAATTCAgtgaaaccagtaacgttatacgatg-3' and 5'-CCAAGCTTGCATGCCTGCAGtcactgcccgctttccagtc-3'. The backbone used was pPro24 purchased from Addgene (Addgene plasmid # 17805 ; http://n2t.net/addgene:17805 ; RRID:Addgene_17805, Lee and Keasling, 2005). The fragment of the backbone needed was obtained by PCR with the following primers: 5'-gactggaaagcgggcagtgaCTGCAGGCATGCAAGCTTGG-3' and 5'-tataacgttactggtttcacTGAATTCCTCCTGCTAGCTTGT-3'. The PCR products were gel purified and added to Gibson Assembly Master Mix in a 1:3 ratio of backbone to *lacI*. The mix was incubated at 50ºC for one hour.

Five microliters of the Gibson reaction were diluted in 15 μL of nuclease free water. Two microliters of the dilution were added to 50 μL of chemically competent *E. coli* DH5α and incubated on ice for 30 minutes. Heat shock was done at 42ºC for 50 seconds followed by two minutes on ice. One milliliter of SOC media was added and the bacteria were incubated at 37ºC with shaking at 200 rpm for one hour. The entire transformation volume was plated onto Luria-Bertani (LB) media agar plates with carbenicillin. Single colonies

were chosen to be grown in LB media for stock preparation and miniprep. Correct insertion of the *lacI* gene was verified through plasmid sequencing by Macrogen Inc.

Next, *E. coli* DH5α was transformed with the high copy propionate-lacI plasmid containing a carbenicillin resistance gene and with a low copy reporter plasmid. The latter includes *sfGFP* (Pédelacq et al., 2006) regulated by the P_{tac} promoter which is repressed by LacI (de Boer et al., 1983) and also contains a chloramphenicol resistance gene. This strain will be referred to as the prop-lacI-sfGFP strain.

The *sfGFP* gene was then replaced with the mouse *GM-CSF* gene obtained from a plasmid in which it was previously cloned, which was kindly donated by Dr. Tal Danino's laboratory at Columbia University. The following primers were used for Gibson Assembly cloning for the backbone and GM-CSF, respectively: 5'-AAAAGCCTGGACAGAAATAGaagcttaattagctgatctagacg-3' and 5'-GGAGAGCGTGTCGGCGCCATagaattctgtttcctgtgtga-3', 5'-cacacaggaaacagaattctATGGCGCCGACACGCTCTCC-3' and 5'-tagatcagctaattaagcttCTATTTCTGTCCAGGCTTTTTGCACTCG-3'. *E. coli* DH5α was transformed with the high copy propionate-lacI plasmid and the low copy pTac-GMCSF plasmid. Correct insertion of the *GM-CSF* gene was verified through plasmid sequencing by Macrogen Inc. This strain will be referred to as prop-lacI-GMCSF.

3.2. Prop-lacI-sfGFP strain functionality experiments

The prop-lacI-sfGFP strain was grown overnight in LB media with carbenicillin and chloramphenicol at 37ºC with shaking at 200 rpm. The next day, it was inoculated at a final concentration of 1% in 100 μL of fresh LB media in black 96 well plates with transparent bottoms. The culture media was supplemented with propionate ranging from 0-100 mM, as this range includes and supersedes concentrations that have been reported

to be physiologically relevant in the intestine (Parada Venegas et al., 2019). Each concentration was done in triplicate and absorbance and fluorescence measurements were taken every 30 minutes. The experiments were carried out in a Synergy H1 instrument at 37ºC with constant shaking. Sensitivity experiments were done in the same manner but increasing the concentration range of propionate. Specificity experiments followed the same procedure replacing propionate with 30, 60 and 90 mM of acetate or butyrate.

The growth kinetic parameters were calculated by fitting the absorbance data at 600 nm to the modified Gompertz equation (Zwietering et al., 1990):

$$OD_{600} = A \cdot \exp\left\{-exp\left[\frac{\mu_{max} \cdot e(1)}{A}(\lambda - t) + 1\right]\right\}$$

OD_{600} is the absorbance measured at 600 nm, A is the absorbance asymptote reached, μ_{max} is the maximum growth rate, λ is the duration of the lag phase and t is time.

The genetic circuit's sensitivity for propionate was quantitatively determined by fitting the fluorescence data to the Hill function for inhibition (as more propionate produces less sfGFP) as described by the following equation:

$$F = F_{min} + \frac{F_{max} \cdot K_{1/2}^{n}}{P^{n} + K_{1/2}^{n}}$$

F is fluorescence normalized by OD_{600} obtained at a certain concentration of propionate, F_{min} is the fitted minimum normalized fluorescence, F_{max} is the fitted maximum normalized fluorescence, P is propionate concentration, $K_{1/2}$ is the concentration at which half of the maximum fluorescence is produced and n is the Hill coefficient.

3.3. Prop-lacI-GMCSF strain protein production experiments

The prop-lacI-GMCSF strain was grown overnight in LB media with carbenicillin and chloramphenicol at 37ºC and shaking at 200 rpm. The following day fresh media either without propionate or with 100 mM of propionate were inoculated with the overnight culture at a final concentration of 1% in a 96 well plate. The plates were incubated at 37ºC with constant shaking in a Synergy H1 instrument for 4, 8, 12 and 25 hours. For each time point 1.2 mL were obtained and centrifuged. The supernatant was discarded and the pellet was resuspended in 60 μL of Bugbuster Protein Extraction Reagent to which 3 μL of lysozyme at 50 mg/mL was added. The lysed bacteria were then centrifuged for 15 minutes at 4ºC, after which the supernatant was stored. The supernatant was used to quantify GM-CSF production with the Invitrogen GM-CSF Mouse ELISA kit. Each time point was quantified in duplicates.

4. RESULTS AND DISCUSSION

4.1. Development of the propionate-lacI two plasmid system

The propionate-LacI system developed in this work was based on the previously constructed pPro24 plasmid (Lee and Keasling, 2005). This plasmid is based on the propionate catabolism genes found in *E. coli*'s prpBCDE operon, regulated by the transcriptional activator PrpR in the presence of propionate (Figure 8.1.2). The expression of the *PrpR* gene, which is a σ54 dependent regulator (Simonte et al., 2017), depends on the presence of the cAMP-CRP complex and the transcription factor becomes activated in the presence of 2-methylcitrate (2-MC) (Lee and Keasling, 2005; Lee et al., 2005). 2-MC is produced from propionate via propionyl-coenzyme A in the methylcitrate cycle (Figure

8.1.3). Therefore, the activation of the prpBCDE promoter (P_{prpB}) depends on the activated PrpR in addition to the cAMP-CRP complex itself, integration host factor (IHF) and σ54 (Lee and Keasling, 2005). The pPro24 plasmid, which was proved to be induced by propionate (Lee and Keasling, 2005), contains *PrpR* divergently transcribed from GFPuv, which is regulated by P_{prpB} (Figure 4.1A).

To create the first plasmid of the system (pPro-lacI), we replaced GFPuv with the *lacI* gene. The second plasmid (pTac-sfGFP) contains *sfGFP* controlled by the P_{tac} promoter which is repressed by LacI (Figure 4.1B). We chose to clone *lacI* in a high copy plasmid and *sfGFP* in a low copy plasmid in order to assure sufficient expression of the repressor and the desired functionality of the genetic system (higher expression of sfGFP under lower concentrations of propionate to reflect IBD conditions). *E. coli* DH5α was transformed with both plasmids to create the prop-lacI-sfGFP strain.

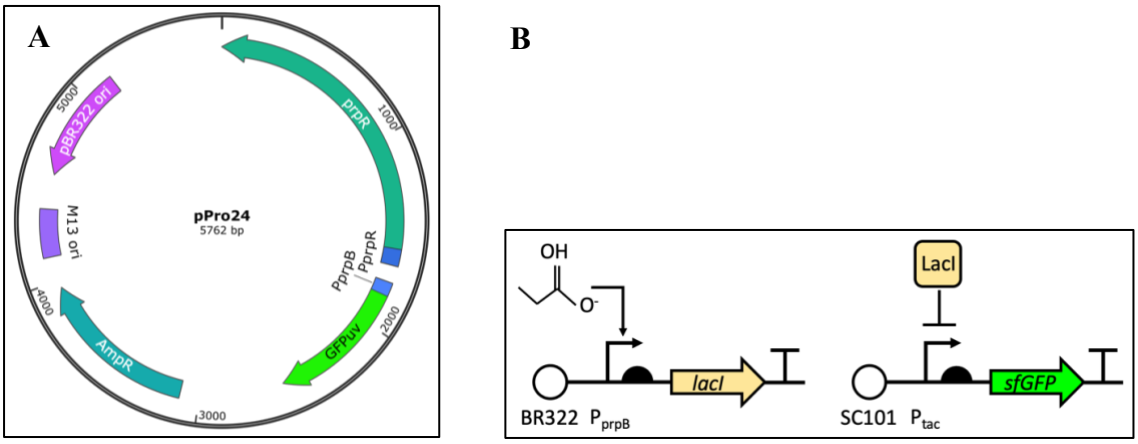


Figure 4.1. Plasmids used in this study. (A) pPro24 plasmid produces GFPuv when induced by propionate. (B) Two plasmid system developed in this study. Propionate

induces the expression of *lacI* in a high copy plasmid and LacI represses the expression of *sfGFP* in the low copy reporter plasmid.

4.2. Growth and functionality of the prop-lacI-sfGFP strain

Although it has been reported that *E. coli* can use propionate as its sole carbon source (Textor et al., 1997), its growth under these conditions is tediously slow. We observed low overnight growth in minimal media supplemented with a wide range of concentrations of propionate. We hypothesize that because the bacteria takes a prolonged amount of time to adapt to this media and because the SCFA was being used as a carbon source to generate biomass, it was not being used in the genetic system that we developed. Therefore, to assure adequate bacterial growth and the use of propionate to activate the genetic circuit, we decided to grow our strain in LB media supplemented with propionate.

The bacteria grew under every concentration of propionate, but the dynamics of the growth curves differed (Figure 4.2.1). The growth parameters obtained by fitting the data to the modified Gompertz equation are presented in Table 4.2 and the fitted curves are presented in Figure 8.1.4. The values of the OD_{600} asymptotes (A) reached generally increase with the concentration of propionate until 20 mM at which the value is 1.09. After this concentration the values are highly variable, with the lowest being 0.8403 at 30 mM (which is still higher than at 15 mM) and the highest being 1.119 at 45 mM. These results indicate that although LB media is rich in nutrients, it is likely that the bacteria are additionally using propionate as a carbon source to generate biomass. Specifically, concentrations of propionate greater than or equal to 20 mM appear to be necessary in order to maximize growth. On the other hand, the maximum growth rate (μ_{max}) only varied slightly comparing the different propionate concentrations with no clear pattern. For example, the minimum value of 0.1001 h^{-1} was obtained at 35 mM of propionate while the maximum value of 0.1473 h^{-1} was obtained at 40 mM. The duration of the lag phase

generally increased with the concentration of propionate, indicating that the bacteria took a longer time to adapt to the media when it was enriched with the propionate. It has previously been reported that very high concentrations of the SCFA can have an inhibitory effect on growth (Rocco and Escalante-Semerena, 2010). This is thought to be caused by an accumulation of 2-MC which may block fructose-1,6-biphosphatase, a key enzyme in the gluconeogenesis pathway (Rocco and Escalante-Semerena, 2010) and can explain the increasing duration of the lag phase with increasing concentrations of propionate.

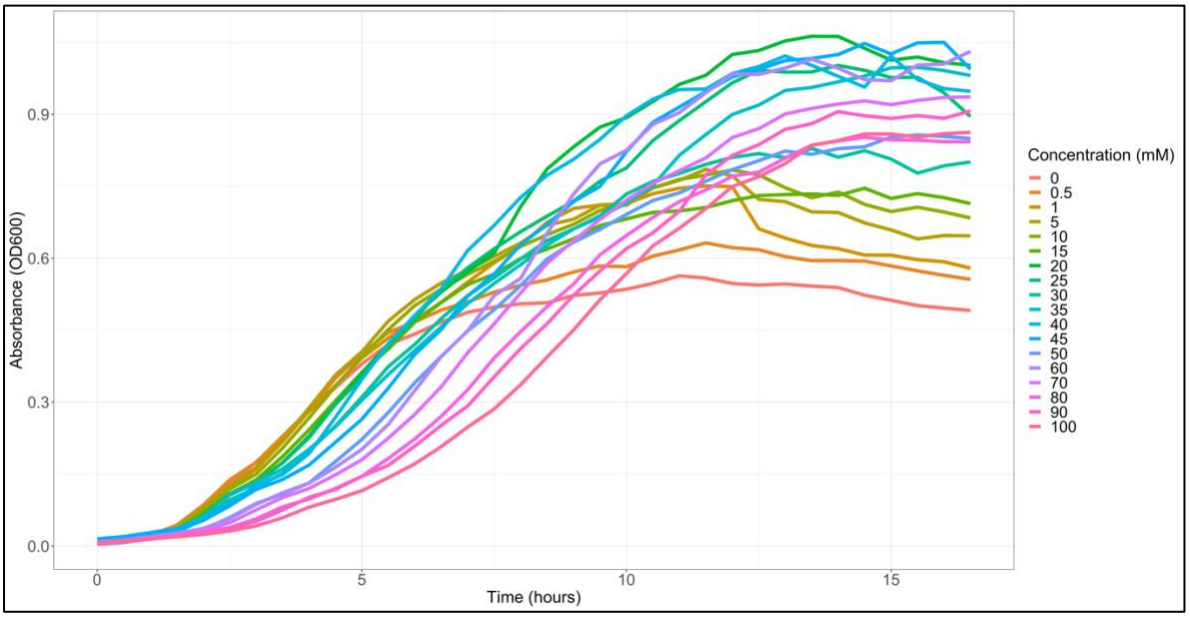


Figure 4.2.1. Growth curves of prop-lacI-sfGFP strain in LB media supplemented with various concentrations of propionate. Mean of triplicates is shown.

Table 4.2. Growth kinetic parameters fitted to OD_{600} data.

Propionate	A (OD600)	μ_{max} (h^{-1})	λ (h)	R^2
0 mM	0.5351	0.1157	1.473	0.9925
0.5 mM	0.5998	0.1162	1.450	0.9940
1 mM	0.6705	0.1372	1.926	0.9600
5 mM	0.7131	0.1359	1.827	0.9816
10 mM	0.7443	0.1272	1.851	0.9906
15 mM	0.7428	0.1130	1.800	0.9992
20 mM	**1.090**	0.1356	2.393	0.9947
25 mM	1.0270	0.1199	2.111	0.9927
30 mM	**0.8403**	0.1143	2.236	0.9968
35 mM	1.101	**0.1001**	2.054	0.9977
40 mM	1.021	**0.1473**	2.638	0.9956
45 mM	**1.119**	0.1234	2.761	0.9967
50 mM	0.8897	0.1082	2.833	0.9993
60 mM	1.070	0.1397	3.566	0.9946
70 mM	1.003	0.1168	3.440	0.9979
80 mM	0.9178	0.1085	3.781	0.9970
90 mM	1.022	0.1074	4.019	0.9954
100 mM	0.9854	0.1035	4.438	0.9955

Throughout the 16 hour experiment, we also measured fluorescence in order to approximate sfGFP production. Because larger amounts of biomass should produce more sfGFP, fluorescence was divided by OD_{600}. The fluorescence values not divided by OD_{600} are presented in Figure 8.1.5. Additionally, background fluorescence from the growth media was subtracted from these measurements. Because of this subtraction, certain initial fluorescence/OD_{600} measurements were negative, in which cases the values were changed to zero. Furthermore, when OD_{600} was less than 0.09, certain fluorescence/OD_{600} measurements were unrealistically large due to the small denominator. Therefore, to

reduce noise in the graphic representations, these values were also changed to zero. The raw graphics are presented in Figures 8.1.6 and 8.1.7.

An inversely proportional relationship between propionate concentration and fluorescence is clearly observed, in accordance with our objective (Figure 4.2.2). The amount of time required for fluorescence to be observed increases considerably with propionate concentration, which appears to be related to the final hours of the bacterial exponential growth phase. In the absence of propionate, fluorescence is not observed until four hours of growth. Because sfGFP maturation takes only minutes after being expressed (Pédelacq et al., 2006), the P_{tac} promoter must be fully repressed until this time. Therefore, the LacI which represses it must come from the genome and/or from a basal leakiness in the P_{prpB} promoter from the first plasmid. Additionally, it is possible that the bacteria are only expressing essential genes during this time, which would not include *sfGFP*. On the other hand, in the presence of propionate the P_{prpB} promoter is likely activated from lag phase. It has been reported that promoters from operons for the catabolism of specific carbon sources present in media are active from this time forward, preparing bacteria for exponential growth (Madar et al., 2013). By the time the bacteria begin entering stationary phase, fluorescence begins to be expressed indicating decreasing effects of LacI. It is likely that the P_{prpB} promoter remains activated because higher concentrations of propionate produce considerably lower amounts of fluorescence, therefore, higher amounts of LacI. Nevertheless, its activation is clearly lower at this point considering the null fluorescence observed in earlier growth stages. With an excess of propionate (i.e. 100 mM) fluorescence is still observed which may be due to one of the following reasons: under this concentration not enough LacI is produced to fully block *sfGFP* expression or the system is effectively saturated by propionate but the P_{tac} promoter has a basal level of leakiness which outweighs the reduced levels of activation of the P_{prpB} promoter in the final stages of growth.

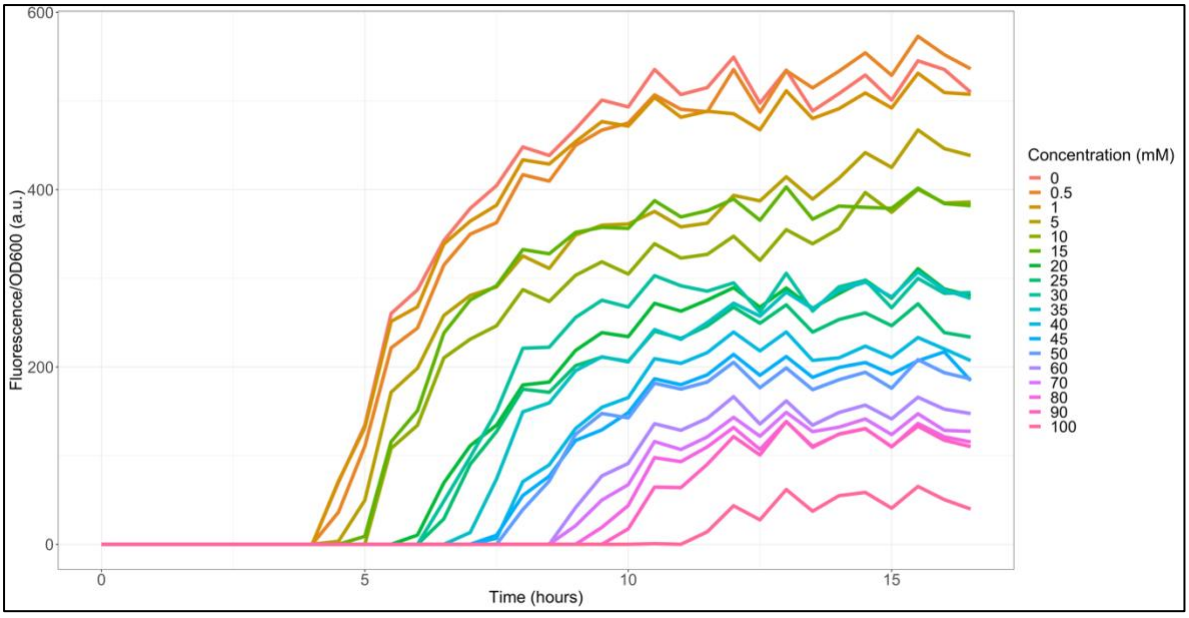


Figure 4.2.2. Prop-lacI-sfGFP strain functionality. Normalized fluorescence produced over time. Average of triplicates is shown.

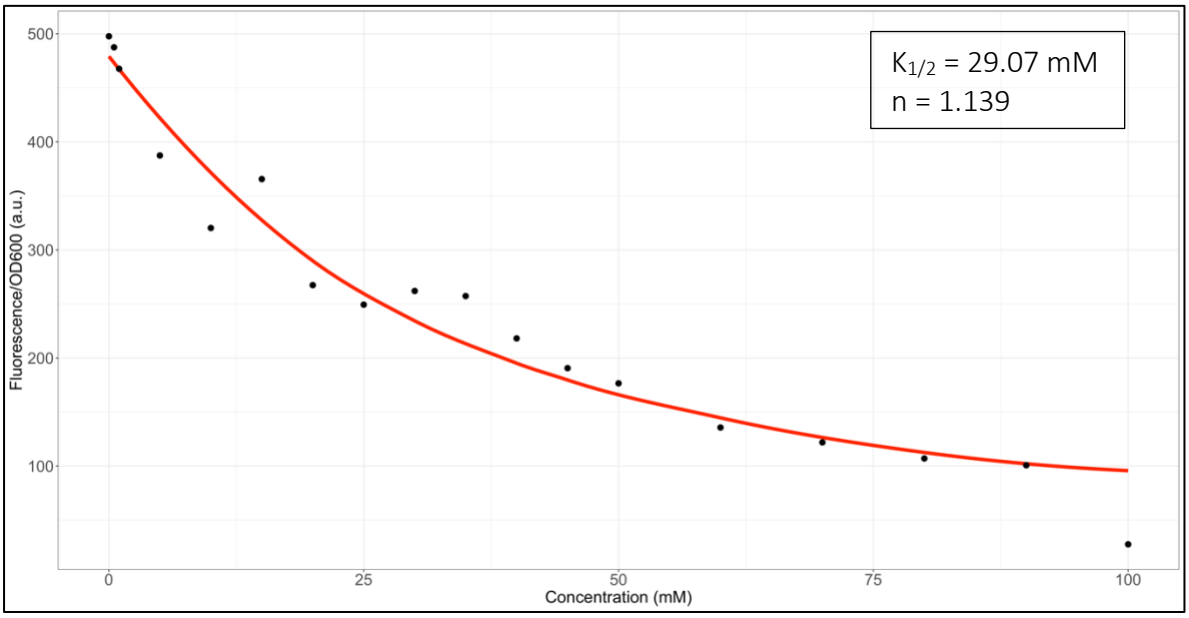
4.3. Sensitivity of the propionate-lacI system

In order to determine the genetic circuit's sensitivity to propionate, we fitted the Hill equation for inhibition to the data obtained at hour 12.5, having reached steady state (Figure 4.3A). The predicted half-maximal response ($K_{1/2}$) was 29.07 mM of propionate while the Hill coefficient had a value of 1.139. The value for $K_{1/2}$ is interesting considering that propionate is found in concentrations of approximately 20 mM in fecal samples of healthy humans (Parada Venegas et al., 2019), which is likely higher in the colon. This indicates that sfGFP production is considerably lower under concentrations of propionate that may be a signal of healthy gut conditions, which coincides with the objective of the system. On the other hand, the Hill coefficient (n) value of slightly higher than one indicates modest cooperativity or ultrasensitivity of the genetic circuit. This may be due

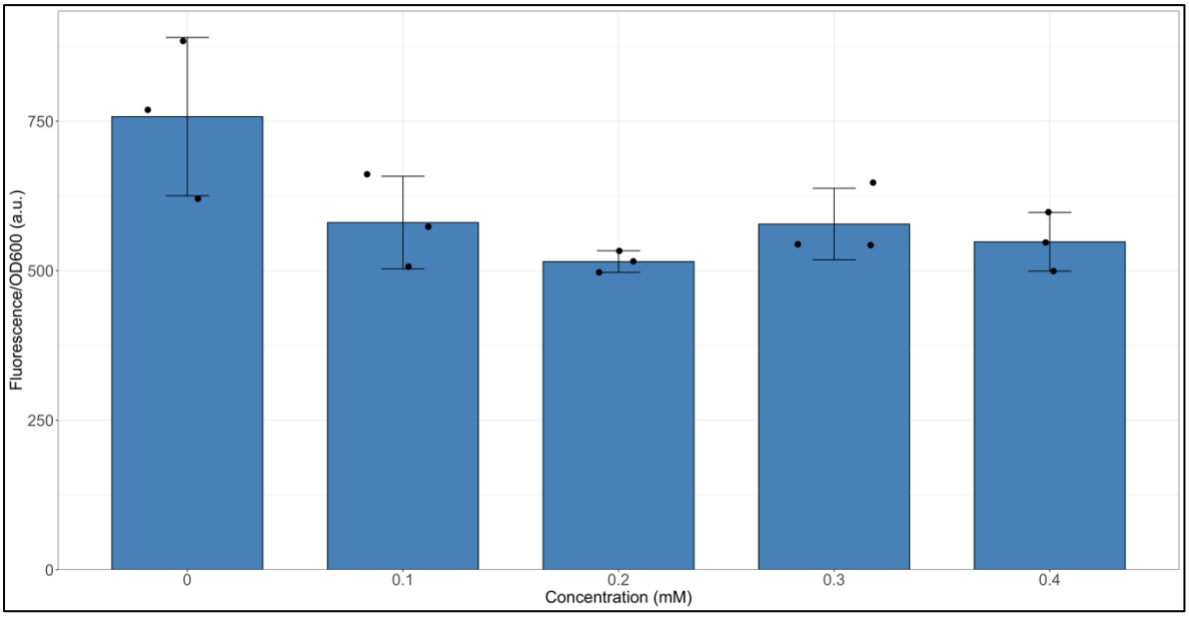
to the indirect response of sfGFP production to propionate concentration, because LacI as an intermediary is not considered in the model. This is because propionate concentration determines the degree of expression of *lacI*, and we are not measuring the amount of LacI produced, but rather, the amount sfGFP produced.

In a separate experiment, we tested the lower (Figure 4.3B) and upper limits (Figure 4.3C) of propionate concentration that are able to affect the genetic circuit after 12.5 hours of growth. An observable difference of the amount of fluorescence produced was registered between 0 and 0.1 mM of propionate, which suggests that the P_{prpB} promoter is sensitive to very low concentrations of the inducer. However, no noticeable differences in sfGFP expression were observed between 0.1 and 0.4 mM. Therefore, within this concentration range not enough LacI is expressed to inhibit sfGFP production an observable amount, but larger increments of propionate do appropriately affect the system as shown in Figure 4.2.2. Regarding the upper limits, a moderate difference was observed between the fluorescence produced under 105 and 110 mM of propionate. It is worth noting that this difference was reasonably smaller than the one observed in the lower concentration limits, considering the fluorescence scale. Between 110 and 130 mM there is a slight decrease in the measured fluorescence with the exception of 120 mM, although more variability (standard deviation) is generally observed in this range. Therefore, the concentration defining the circuit's saturation point appears to be approximately 110 mM of propionate, which far exceeds physiological conditions.

A


$K_{1/2}$ = 29.07 mM
n = 1.139
Fluorescence/OD600 (a.u.)
500
400
300
200
100
0
25
50
75
100
Concentration (mM)

B


Fluorescence/OD600 (a.u.)
750
500
250
0
0
0.1
0.2
0.3
0.4
Concentration (mM)

C

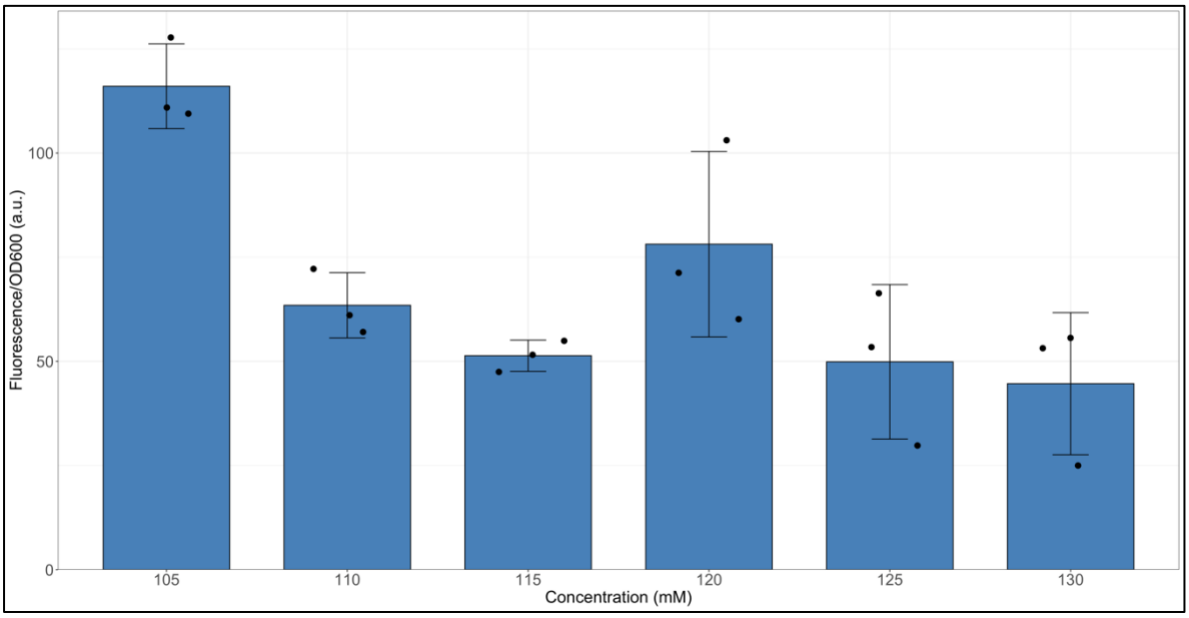


Figure 4.3. Sensitivity of the prop-lacI-sfGFP genetic system with data obtained at 12.5 hours of growth. (A) Hill function for inhibition fitted to the normalized fluorescence data. (B) Lower propionate concentration limits affecting the genetic circuit. (C) Upper propionate concentration limits affecting the genetic circuit.

4.4. Specificity of the propionate-lacI system

In order to test the genetic circuit's specificity, we grew the prop-lacI-sfGFP strain in LB supplemented with either acetate, propionate or butyrate. These are all SCFA with similar molecular structure but varying in number of carbons: two, three and four, respectively. They are all found abundantly in the healthy human intestine but have been reported to exist in considerably lower amounts in people with inflammatory bowel disease (Parada Venegas et al., 2019). First, the absorbance measurements at 600 nm were analyzed in order to determine the substrates' effects on the bacterial strain's growth (Figure 4.4.1).

The data was fitted to the modified Gompertz equation (Figure 8.1.8) in order to calculate the growth kinetic parameters (Table 4.4).

In the specificity experiment, the least amount of bacterial growth was observed in the absence of any SCFA, again suggesting the use of these as a carbon source. Similar maximum OD_{600} measurements were achieved for all concentrations of acetate which implies that 30 mM is enough for maximum growth and that 90 mM is an abundance but does not have an overall inhibitory effect under the specified growth conditions. The maximum growth rate decreased slightly with increasing concentrations of acetate, but the differences are minimal. On the other hand, for butyrate and propionate, 60 mM was the preferred concentration for maximal growth, with 30 mM appearing low and 90 mM too high, possibly having a slightly inhibitory effect. In these cases the maximum growth rate also decreased with increasing concentrations of SCFA, but more so comparted to acetate. In the presence of each SCFA, the duration of the lag phase increased as the concentration of the carbon source increased. This may be due to more significant pH changes in the media in the presence of larger amounts of SCFA, requiring a longer time to adapt.

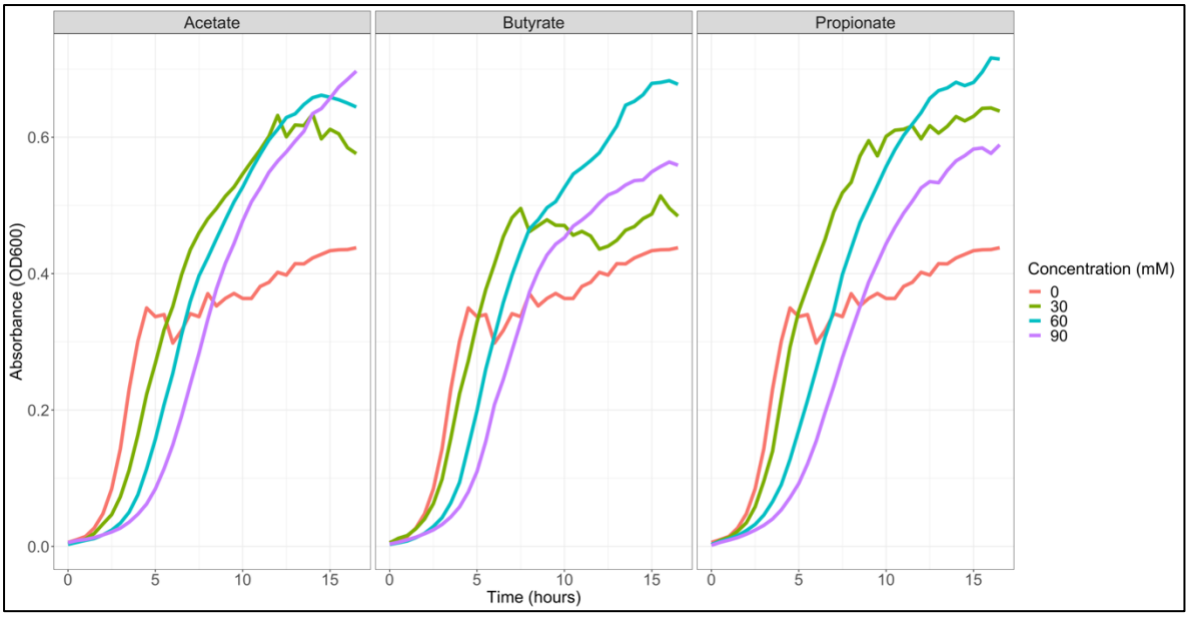


Figure 4.4.1. Growth curves of prop-lacI-sfGFP strain in LB media supplemented with various concentrations of SCFA. Average of triplicates is shown.

Table 4.4. Growth kinetic parameters fitted to OD_{600} specificity data

	A (OD600)	**μ_{max} (h^{-1})**	**λ (h)**	**R^2**
0 mM SCFA	0.3957	0.1019	1.404	0.9542
30 mM acetate	0.6162	0.09742	2.306	0.9960
60 mM acetate	0.6769	0.09229	3.264	0.9987
90 mM acetate	0.7188	0.08319	4.103	0.9986
30 mM butyrate	0.4758	0.1372	2.307	0.9878
60 mM butyrate	0.6770	0.08757	2.706	0.9940
90 mM butyrate	0.5598	0.08319	3.528	0.9981
30 mM propionate	0.6305	0.1159	2.155	0.9979
60 mM propionate	0.7291	0.09063	3.122	0.9995
90 mM propionate	0.6142	0.07713	3.875	0.9989

Analyzing the genetic circuit's behavior over time, maximum fluorescence was normally achieved by 10 hours of growth (Figure 4.4.2A). Additionally, both acetate and butyrate seem to activate the P_{prpB} promoter, because decreasing fluorescence was observed under higher concentrations of both SCFA, although considerably less compared to propionate. This may be due to an unknown subproduct of acetate and butyrate catabolism entering the methylcitrate cycle eventually leading to an increase in 2-MC and activation of the PrpR transcription factor. After 12.5 hours of growth, it can be observed that under 60 and 90 mM of acetate and butyrate almost the same amount of fluorescence is produced, and their effects are similar to that of 30 mM of propionate (Figure 4.4.2B). This indicates that the promoter's saturation point is likely less than or equal to 60 mM of acetate and butyrate but is more accurately regulated by propionate. If acetate and butyrate catabolism did lead to 2-MC production, it would be through a more indirect pathway than propionate, which would explain the considerably lower concentration of saturation in the presence of both SCFA. We do not consider the system's moderate specificity regarding these SCFA to be a problem considering that the three of these molecules are found in reduced concentrations in IBD patients. Additionally, this may help narrow down the system's concentration range of functionality and later avoid the over-production of GM-CSF.

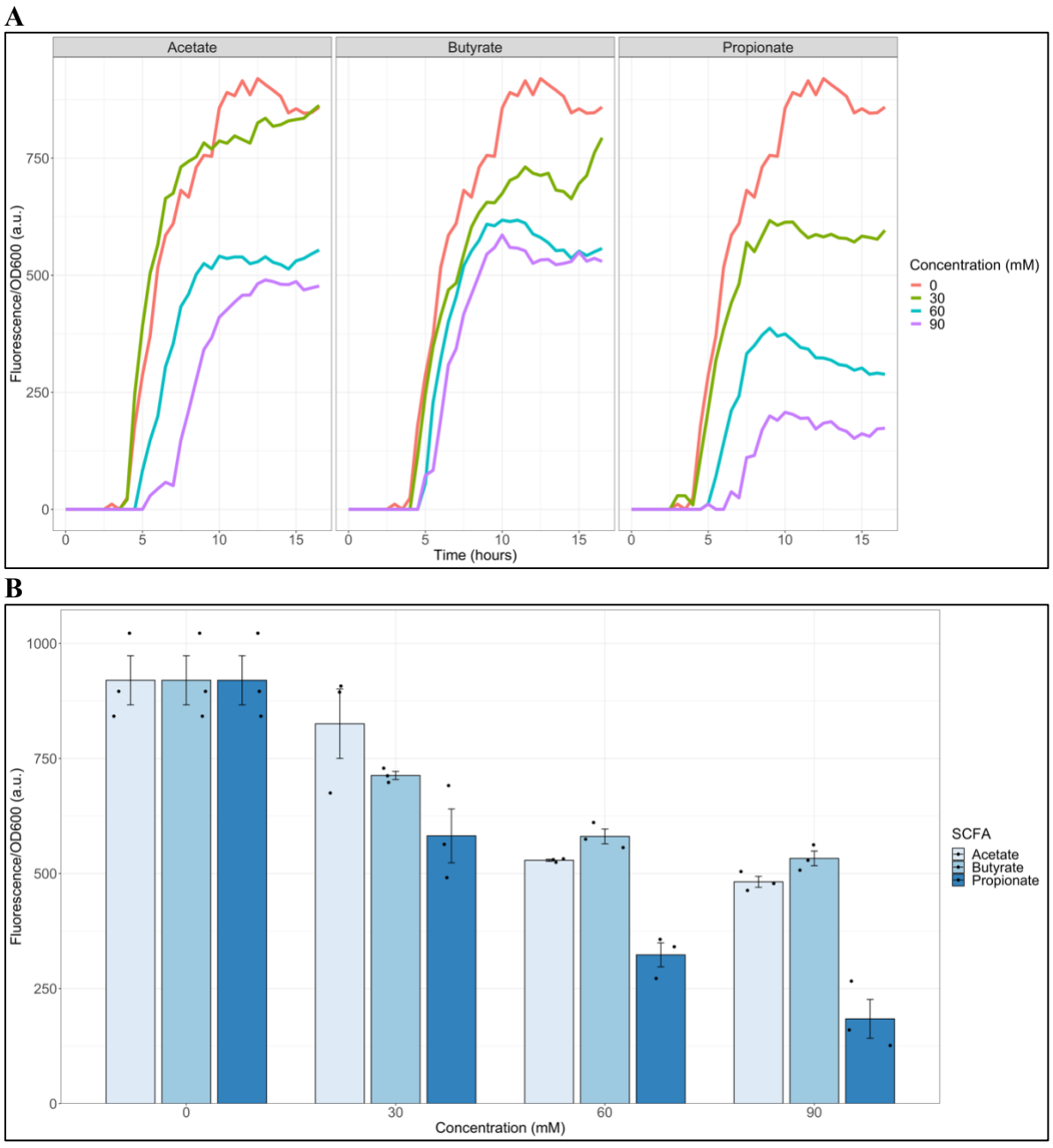


Figure 4.4.2. Specificity of the prop-lacI-sfGFP genetic system. (A) Fluorescence normalized by OD_{600} produced over time. Average of triplicates is shown. (B) Fluorescence normalized by OD_{600} produced after 12.5 hours.

4.5. Protein production by the prop-lacI-GMCSF strain

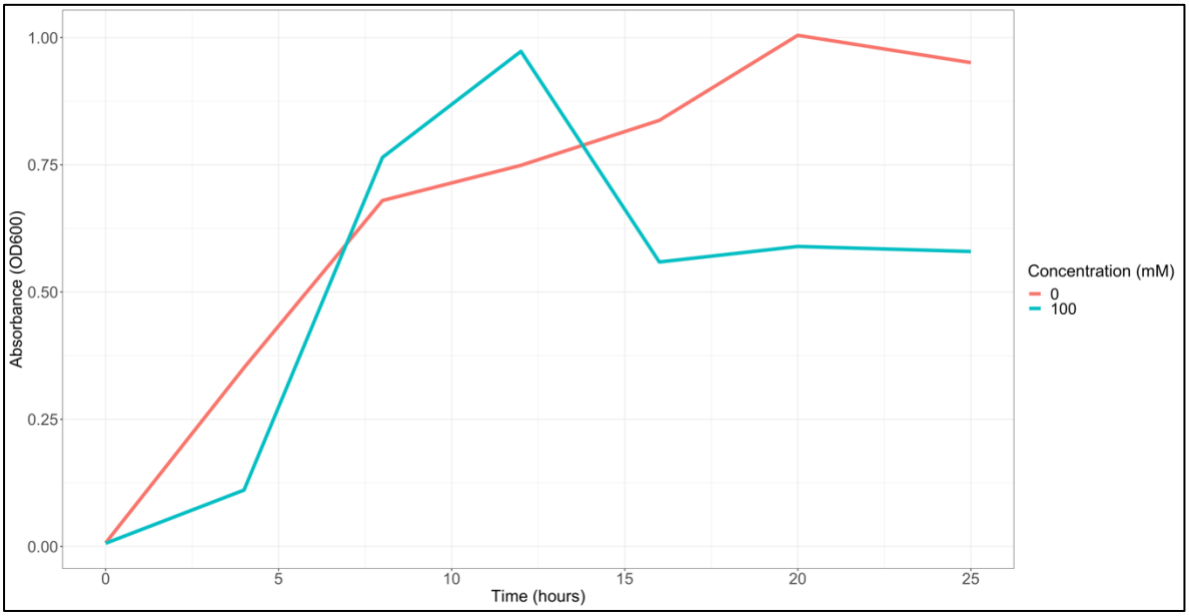
The prop-lacI-GMCSF strain experienced relatively regular growth in the absence of propionate reaching stationary phase at approximately 20 hours (Figure 4.5A). On the other hand, growth in LB media supplemented with 100 mM of propionate began slower but reached its maximum absorbance sooner than in the absence of propionate. Nevertheless, a sharp drop in OD_{600} was observed after 12 hours, reaching a lower stationary phase at approximately 16 hours. Because this same kinetic was not observed under 100 mM of propionate during the production of sfGFP, the combination of GM-CSF expression and a high concentration of inducer seems to have a detrimental effect on bacterial growth after some time. The simultaneous catabolism of propionate and production of GM-CSF may be a significant metabolic burden for the bacteria.

The production of GM-CSF was quantified after 4, 8, 12 and 25 hours of growth and divided by OD_{600} (Figure 4.5B). At each time point, more GM-CSF was produced in the absence of propionate than with 100 mM. However, the difference between the two was smaller than expected considering the large difference observed with sfGFP. Another difference with the prop-lacI-sfGFP strain is that sfGFP was just beginning to be expressed at approximately four hours while the prop-lacI-GMCSF strain's maximum production of the therapeutic protein was at that same time point. This may be due to the difference in the bacteria's growth kinetics that appear to be altered when containing the *GM-CSF* gene. In the latter strain, at the beginning of growth, considerable energy appears to be invested in producing GM-CSF rather than in propionate catabolism or LacI expression, either from the plasmid or the genome. Another possibility is that the small size of GM-CSF may lead to an expression time shorter than what it takes LacI to attach to its operator sequence, leading to a quick accumulation during the early stages of growth.

After four hours of growth, there is a quick decline in the concentration of GM-CSF/OD_{600} produced, and the values remain relatively constant in every time point observed. After

25 hours, the production of GM-CSF in the absence of propionate is almost 14 times less than after 4 hours, and the difference between 0 and 100 mM is negligible. It is likely that the protein is quickly degraded within the bacteria due to its proteases, which also makes it difficult to observe significant differences in its production when comparing it in the presence and absence of propionate. Therefore, the conditions regarding growth time, temperature and bacterial strain used should be optimized for the production of GM-CSF using this genetic system.

A



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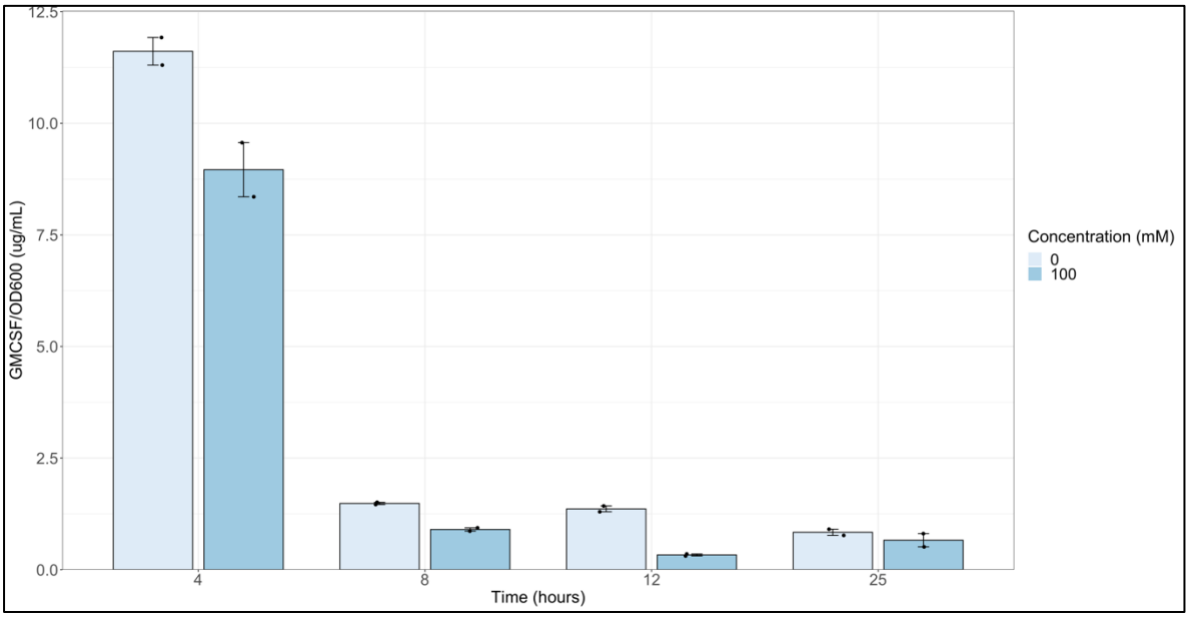

Figure 4.5. Prop-lacI-GMCSF strain performance. (A) Growth over time. (B) Quantification of normalized GM-CSF production.

5. CONCLUSIONS

With this work, we were able to prove our hypothesis by genetically engineering *E. coli* DH5α, first to produce increasing amounts of sfGFP with decreasing concentrations of propionate, and then, albeit less so, to produce GM-CSF. Our objectives were accomplished by cloning the *lacI* gene into the pPro24 plasmid and evaluating its performance with a reporter plasmid in the aforementioned bacteria. We observed that the first genetic circuit functions within a range of approximately 0-110 mM of propionate generating a response with as little as 0.1 mM of the inducer. Acetate and butyrate, the two other most common short chain fatty acids found in the human intestine were also shown to activate the P_{prpB} promoter, although considerably less so than propionate. With

the prop-lacI-GMCSF strain the therapeutic protein was successfully expressed, but the concentration of propionate present had a smaller effect than on the prop-lacI-sfGFP strain with the growth conditions used and at the times analyzed. Overall, we were able to successfully characterize our genetic circuit *in vitro*, which gives us an approximation towards its functionality in other environments. Currently, the prop-lacI-sfGFP strain may function as a diagnostic tool to quantify propionate levels. For the prop-lacI-GMCSF strain further studies should be performed to optimize the conditions for the differentiated expression of the protein under various concentrations of propionate. In order to evaluate the engineered bacteria's potential as a tool to sense and respond to SCFA levels in the intestine and improve mucosal barrier integrity, *in vivo* studies should also be carried out.

6. FUTURE WORK

The sense and respond genetic circuit developed in this work is an important step towards creating a diagnostic and therapeutic tool for IBD, though it can still be improved in various ways. For example, in order to fine tune the system's concentration range different genetic parts such as ribosome binding sites, terminators and origins of replication of varying strengths may be interchanged. Also, for *in vivo* studies avoiding antibiotic resistance genes would be preferable. This could be done by deleting an essential gene from the genome and including it in the plasmids or by integrating the genetic circuit into the bacterial genome.

Next, a means for the secretion of GM-CSF must be determined. The simplest solution may be to use the synchronized lysis circuit integrated into the genome of *E. coli* Nissle 1917 developed by Din et al. (2016) and Gurbatri et al. (2019), in which the bacteria lyse when reaching a certain density. This system has previously been used to liberate therapeutic substances for treating cancer.

Lastly, the system should be tested in a mouse model for colitis in order to test the sense and respond system *in vivo*. This would give a clearer idea on how a more realistic environment may interfere or possibly improve the dynamics of the genetic circuit. Additionally, the effects of GM-CSF could be observed on the intestinal epithelium to analyze its potential as a biotherapeutic produced *in situ*.

7. REFERENCES

Baumgart, D. C., and Carding, S. R. (2007). Inflammatory bowel disease: cause and immunobiology. *Lancet* 369, 1627–1640. doi:10.1016/S0140-6736(07)60750-8.

Bernstein, C. N., Fried, M., Krabshuis, J. H., Cohen, H., Eliakim, R., Fedail, S., et al. (2009). Inflammatory bowel disease : a global perspective. in *World Gastroenterology Organisation Global Guidelines*, 1–24. Available at: http://www.24hmb.com/voimages/web_image/upload/file/20140606/76761402057831265.pdf.

Bouchet, V., Hood, D. W., Li, J., Brisson, J. R., Randle, G. A., Martin, A., et al. (2003). Host-derived sialic acid is incorporated into Haemophilus influenzae lipopolysaccharide and is a major virulence factor in experimental otitis media. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8898–8903. doi:10.1073/pnas.1432026100.

Brock, M., Maerker, C., Schütz, A., Völker, U., and Buckel, W. (2002). Oxidation of propionate to pyruvate in Escherichia coli: Involvement of methylcitrate dehydratase and aconitase. *Eur. J. Biochem.* 269, 6184–6194. doi:10.1046/j.1432-1033.2002.03336.x.

Corrêa-Oliveira, R., Fachi, J. L., Vieira, A., Sato, F. T., and Vinolo, M. A. R. (2016). Regulation of immune cell function by short-chain fatty acids. *Clin. Transl. Immunol.* 5, 1–8. doi:10.1038/cti.2016.17.

Coskun, M., Vermeire, S., and Nielsen, O. H. (2017). Novel Targeted Therapies for Inflammatory Bowel Disease. *Trends Pharmacol. Sci.* 38, 127–142. doi:10.1016/j.tips.2016.10.014.

Daeffler, K. N., Galley, J. D., Sheth, R. U., Ortiz-Velez, L. C., Bibb, C. O., Shroyer, N. F., et al. (2017). Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. *Mol. Syst. Biol.* 13, 923. doi:10.15252/msb.20167416.

de Boer, H. A., Comstock, L. J., and Vasser, M. (1983). The tac promoter: a functional hybrid derived from the trp and lac promoters. *Proc. Natl. Acad. Sci. U. S. A.* 80, 21–25. doi:10.1073/pnas.80.1.21.

Din, M. O., Danino, T., Prindle, A., Skalak, M., Selimkhanov, J., Allen, K., et al. (2016). Synchronized cycles of bacterial lysis for in vivo delivery. *Nature* 536, 81–85. doi:10.1038/nature18930.

Frank, D. N., Robertson, C. E., Hamm, C. M., Kpadeh, Z., Zhang, T., Chen, H., et al. (2011). Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. *Inflamm. Bowel Dis.* 17, 179–184. doi:10.1002/ibd.21339.

Frank, D. N., St. Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., and Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl. Acad. Sci. U. S. A.* 104, 13780–13785. doi:10.1073/pnas.0706625104.

Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., and Smith, H. O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat. Methods* 6, 343–345. doi:10.1038/nmeth.1318.

Giron, L. B., Tanes, C. E., Schleimann, M. H., Engen, P. A., Mattei, L. M., Anzurez, A., et al. (2020). Sialylation and fucosylation modulate inflammasome-activating eIF2 Signaling and microbial translocation during HIV infection. *Mucosal Immunol.* doi:10.1038/s41385-020-0279-5.

Gophna, U., Sommerfeld, K., Gophna, S., Doolittle, W. F., and Veldhuyzen Van Zanten, S. J. O. (2006). Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J. Clin. Microbiol.* 44, 4136–4141. doi:10.1128/JCM.01004-06.

Goto, Y., Uematsu, S., and Kiyono, H. (2016). Epithelial glycosylation in gut homeostasis and inflammation. *Nat. Immunol.* 17, 1244–1251. doi:10.1038/ni.3587.

Gurbatri, C., Coker, C., Hinchliffe, T. E., Lia, I., Castro, S., Treuting, P. M., et al. (2019). Engineered probiotics for local tumor delivery of checkpoint blockade nanobodies. *bioRxiv*. doi:10.1101/562785.

Harel, J., Fairbrother, J., Forget, C., Desautels, C., and Moore, J. (1993). Virulence factors associated with F165-positive Escherichia coli strains isolated from piglets and

calves. *Vet. Microbiol.* 38, 139–155. doi:10.1016/0378-1135(93)90081-H.

Harvey, H. A., Swords, W. E., and Apicella, M. A. (2001). The mimicry of human glycolipids and glycosphingolipids by the lipooligosaccharides of pathogenic Neisseria and Haemophilus. *J. Autoimmun.* 16, 257–262. doi:10.1006/jaut.2000.0477.

Holko, P., Kawalec, P., and Mossakowska, M. (2018). Quality of life related to oral, subcutaneous, and intravenous biologic treatment of inflammatory bowel disease: A time trade-off study. *Eur. J. Gastroenterol. Hepatol.* 30, 174–180. doi:10.1097/MEG.0000000000001031.

Huang, Y.-L., Chassard, C., Hausmann, M., von Itzstein, M., and Hennet, T. (2015). Sialic acid catabolism drives intestinal inflammation and microbial dysbiosis in mice. *Nat. Commun.* 6, 8141. doi:10.1038/ncomms9141.

Korzenik, J. R., Dieckgraefe, B. K., Valentine, J. F., Hausman, D. F., and Gilbert, M. J. (2005). Sargramostim for Active Crohn's Disease. *N. Engl. J. Med.* 352, 2193–2201. doi:10.1056/NEJMoa041109.

Lakatos, P. L. (2009). Environmental Factors Affecting Inflammatory Bowel Disease: Have We Made Progress? *Dig. Dis.* 27, 215–225. doi:10.1159/000228553.

Lee, S. K., and Keasling, J. D. (2005). A Propionate-Inducible Expression System for Enteric Bacteria. *Appl. Environ. Microbiol.* 71, 6856–6862. doi:10.1128/AEM.71.11.6856-6862.2005.

Lee, S. K., Newman, J. D., and Keasling, J. D. (2005). Catabolite repression of the propionate catabolic genes in Escherichia coli and Salmonella enterica: Evidence for involvement of the cyclic AMP receptor protein. *J. Bacteriol.* 187, 2793–2800. doi:10.1128/JB.187.8.2793-2800.2005.

Lopez, R. N., Leach, S. T., Lemberg, D. A., Duvoisin, G., Gearry, R. B., and Day, A. S. (2017). Fecal biomarkers in inflammatory bowel disease. *J. Gastroenterol. Hepatol.* 32, 577–582. doi:10.1111/jgh.13611.

Madar, D., Dekel, E., Bren, A., Zimmer, A., Porat, Z., and Alon, U. (2013). Promoter activity dynamics in the lag phase of Escherichia coli. *BMC Syst. Biol.* 7.

doi:10.1186/1752-0509-7-136.

Magro, F., Dias, C. C., Coelho, R., Santos, P. M., Fernandes, S., Caetano, C., et al. (2017). Impact of Early Surgery and Immunosuppression on Crohn's Disease Disabling Outcomes. *Inflamm. Bowel Dis.* 23, 289–297. doi:10.1097/MIB.0000000000001007.

Matsuoka, K., and Kanai, T. (2015). The gut microbiota and inflammatory bowel disease. *Semin. Immunopathol.* 37, 47–55. doi:10.1007/s00281-014-0454-4.

McGovern, D. P. B., Jones, M. R., Taylor, K. D., Marciante, K., Yan, X., Dubinsky, M., et al. (2010). Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease. *Hum. Mol. Genet.* 19, 3468–3476. doi:10.1093/hmg/ddq248.

Ouwerkerk, J. P., De Vos, W. M., and Belzer, C. (2013). Glycobiome: Bacteria and mucus at the epithelial interface. *Best Pract. Res. Clin. Gastroenterol.* 27, 25–38. doi:10.1016/j.bpg.2013.03.001.

Pacheco, A. R., Munera, D., Waldor, M. K., Sperandio, V., and Ritchie, J. M. (2012). Fucose sensing regulates bacterial intestinal colonization. *Nature* 492, 113–117. doi:10.1038/nature11623.

Parada Venegas, D., De la Fuente, M. K., Landskron, G., González, M. J., Quera, R., Dijkstra, G., et al. (2019). Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front. Immunol.* 10. doi:10.3389/fimmu.2019.00277.

Pédelacq, J. D., Cabantous, S., Tran, T., Terwilliger, T. C., and Waldo, G. S. (2006). Engineering and characterization of a superfolder green fluorescent protein. *Nat. Biotechnol.* 24, 79–88. doi:10.1038/nbt1172.

Pickard, J. M., and Chervonsky, A. V (2015). Intestinal Fucose as a Mediator of Host–Microbe Symbiosis. *J. Immunol.* 194, 5588–5593. doi:10.4049/jimmunol.1500395.

Pickard, J. M., Maurice, C. F., Kinnebrew, M. A., Abt, M. C., Schenten, D., Golovkina, T. V., et al. (2014). Rapid fucosylation of intestinal epithelium sustains host–commensal symbiosis in sickness. *Nature* 514, 638–641. doi:10.1038/nature13823.

Pizarro, T. T., and Cominelli, F. (2007). Cytokine therapy for Crohn's disease: Advances in translational research. *Annu. Rev. Med.* 58, 433–444.

doi:10.1146/annurev.med.58.121205.100607.

Plumbridge, J., and Vimr, E. (1999). Convergent Pathways for Utilization of the Amino Sugars. *Microbiology* 181, 47–54.

Ramsay, R. G., Micallef, S. J., Williams, B., Lightowler, S., Vincan, E., Heath, J. K., et al. (2004). Colony-stimulating factor-1 promotes clonogenic growth of normal murine colonic crypt epithelial cells in vitro. *J. Interf. Cytokine Res.* 24, 416–427. doi:10.1089/1079990041535638.

Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K. P., et al. (2014). Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *ISME J.* 8, 1323–1335. doi:10.1038/ismej.2014.14.

Rocco, C. J., and Escalante-Semerena, J. C. (2010). In Salmonella enterica, 2-methylcitrate blocks gluconeogenesis∇. *J. Bacteriol.* 192, 771–778. doi:10.1128/JB.01301-09.

Schölmerich, J. (2006). Review article: Should we treat symptoms or lesions in Crohn's disease? The case for treating symptoms. *Aliment. Pharmacol. Ther.* 24, 33–36. doi:10.1111/j.1365-2036.2006.03057.x.

Scott, K. P., Martin, J. C., Campbell, G., Mayer, C. D., and Flint, H. J. (2006). Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium "Roseburia inulinivorans." *J. Bacteriol.* 188, 4340–4349. doi:10.1128/JB.00137-06.

Shergill, A. K., Lightdale, J. R., Bruining, D. H., Acosta, R. D., Chandrasekhara, V., Chathadi, K. V., et al. (2015). The role of endoscopy in inflammatory bowel disease. *Gastrointest. Endosc.* 81, 1101-1121.e13. doi:10.1016/j.gie.2014.10.030.

Simonte, F. M., Dötsch, A., Galego, L., Arraiano, C., and Gescher, J. (2017). Investigation on the anaerobic propionate degradation by Escherichia coli K12. *Mol. Microbiol.* 103, 55–66. doi:10.1111/mmi.13541.

Smith, N. W., Shorten, P. R., Altermann, E., Roy, N. C., and McNabb, W. C. (2019). The Classification and Evolution of Bacterial Cross-Feeding. *Front. Ecol. Evol.* 7, 1–15.

doi:10.3389/fevo.2019.00153.

Sun, M., Wu, W., Liu, Z., and Cong, Y. (2017). Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. *J. Gastroenterol.* 52, 1–8. doi:10.1007/s00535-016-1242-9.

Sunkara, T., Rawla, P., Ofosu, A., and Gaduputi, V. (2018). Fecal microbiota transplant – a new frontier in inflammatory bowel disease. *J. Inflamm. Res.* Volume 11, 321–328. doi:10.2147/JIR.S176190.

Tedelind, S., Westberg, F., Kjerrulf, M., and Vidal, A. (2007). Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: A study with relevance to inflammatory bowel disease. *World J. Gastroenterol.* 13, 2826–2832. doi:10.3748/wjg.v13.i20.2826.

Textor, S., Wendisch, V. F., De Graaf, A. A., Müller, U., Linder, M. I., Linder, D., et al. (1997). Propionate oxidation in Escherichia coli: Evidence for operation of a methylcitrate cycle in bacteria. *Arch. Microbiol.* 168, 428–436. doi:10.1007/s002030050518.

Thibault, R., Blachier, F., Darcy-Vrillon, B., De Coppet, P., Bourreille, A., and Segain, J. P. (2010). Butyrate utilization by the colonic mucosa in inflammatory bowel diseases: A transport deficiency. *Inflamm. Bowel Dis.* 16, 684–695. doi:10.1002/ibd.21108.

Thorburn, A. N., Macia, L., and Mackay, C. R. (2014). Diet, Metabolites, and "Western-Lifestyle" Inflammatory Diseases. *Immunity* 40, 833–842. doi:10.1016/j.immuni.2014.05.014.

Zhou, X., Yang, G., and Guan, F. (2020). Biological Functions and Analytical Strategies of Sialic Acids in Tumor. *Cells* 9, 273. doi:10.3390/cells9020273.

Zhu, Y., and Lin, E. C. C. (1988). A mutant crp allele that differentially activates the operons of the fuc regulon in Escherichia coli. *J. Bacteriol.* 170, 2352–2358. doi:10.1128/jb.170.5.2352-2358.1988.

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., and Van't Riet, K. (1990). Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* 56, 1875–1881.

doi:10.1128/aem.56.6.1875-1881.1990.

8. APPENDICES

8.1. Supplementary Images

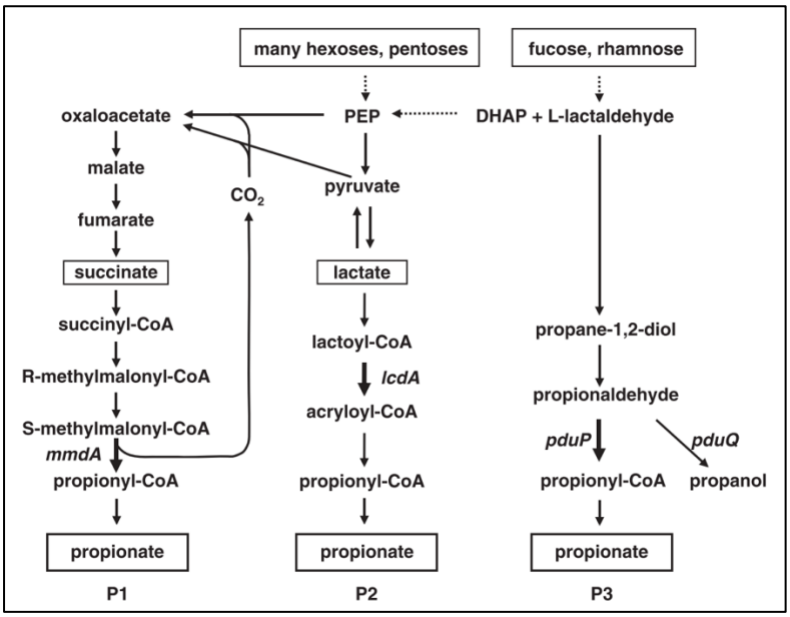

Figure 8.1.1. Metabolic pathways used by gut microbes for propionate production (Reichardt et al., 2014).

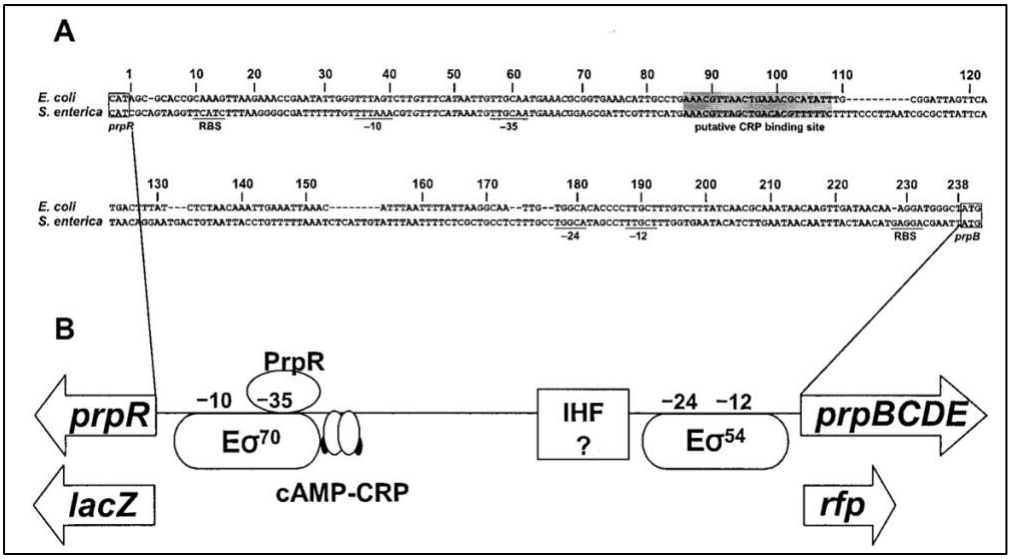

Figure 8.1.2. Propionate operon in *E. coli* (Lee et al., 2005).

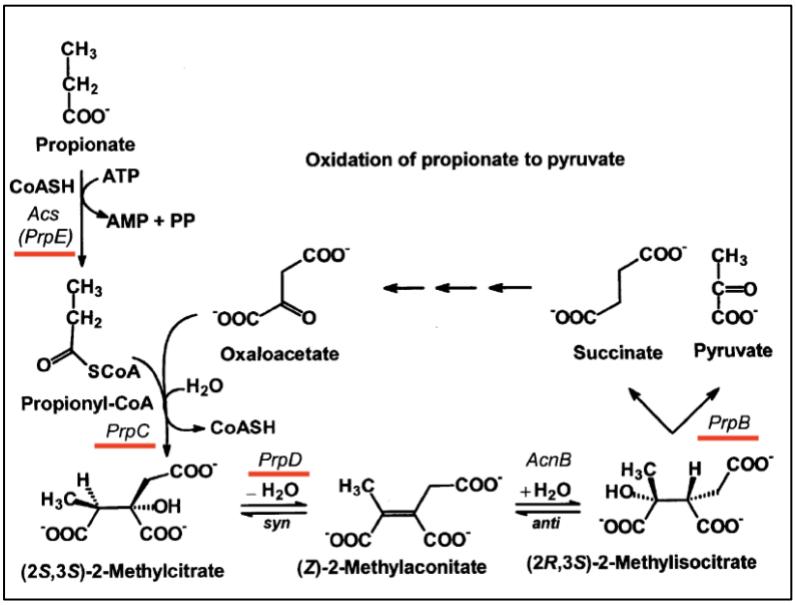

Figure 8.1.3. Propionate oxidation through the methylcitrate cycle (adapted from Brock et al., 2002).

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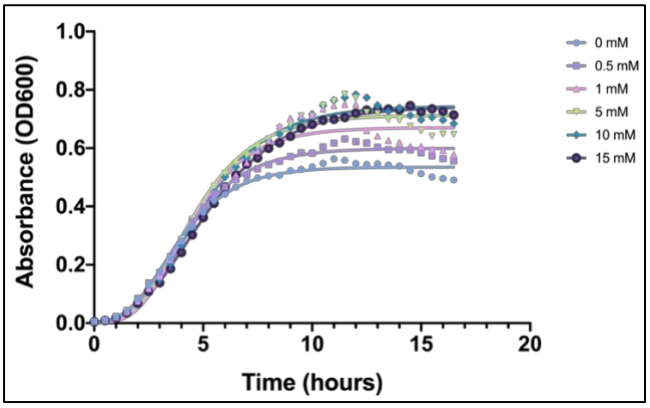

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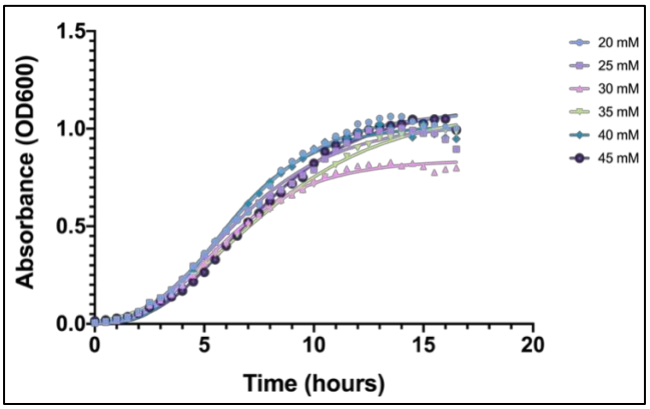

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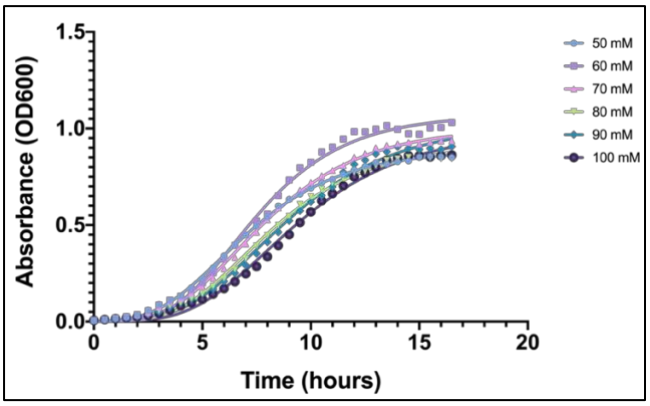


Figure 8.1.4. Modified Gompertz equation fitted to prop-lacI-sfGFP strain OD_{600} data under (A) 0-15 mM (B) 20-45 mM (C) 50-100 mM of propionate.

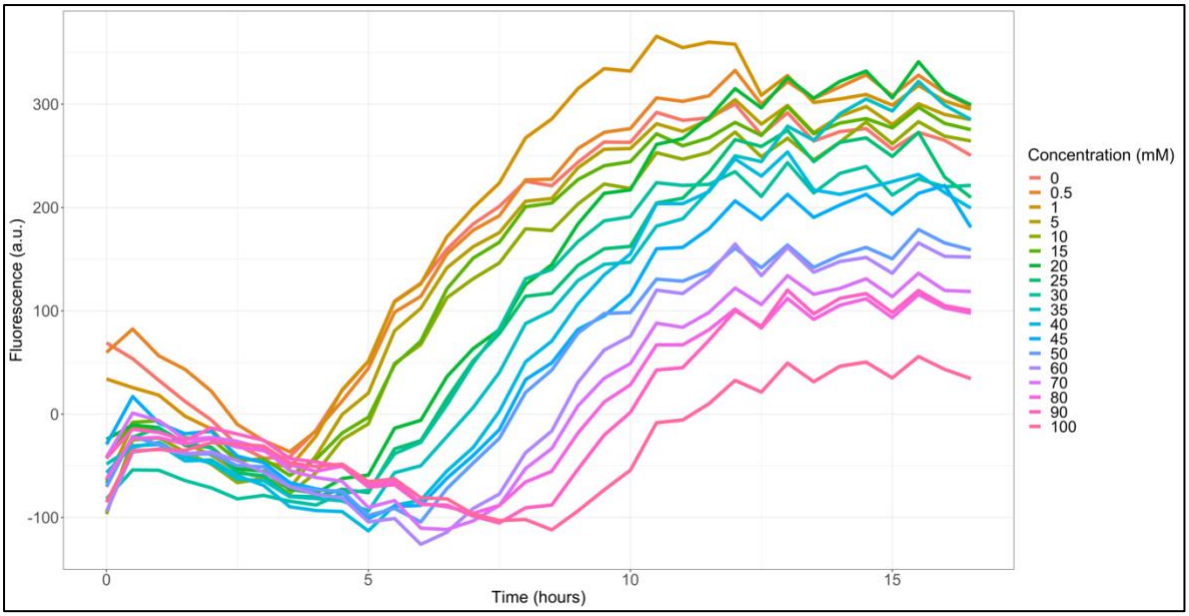


Figure 8.1.5. Prop-lacI-sfGFP strain fluorescence over time.

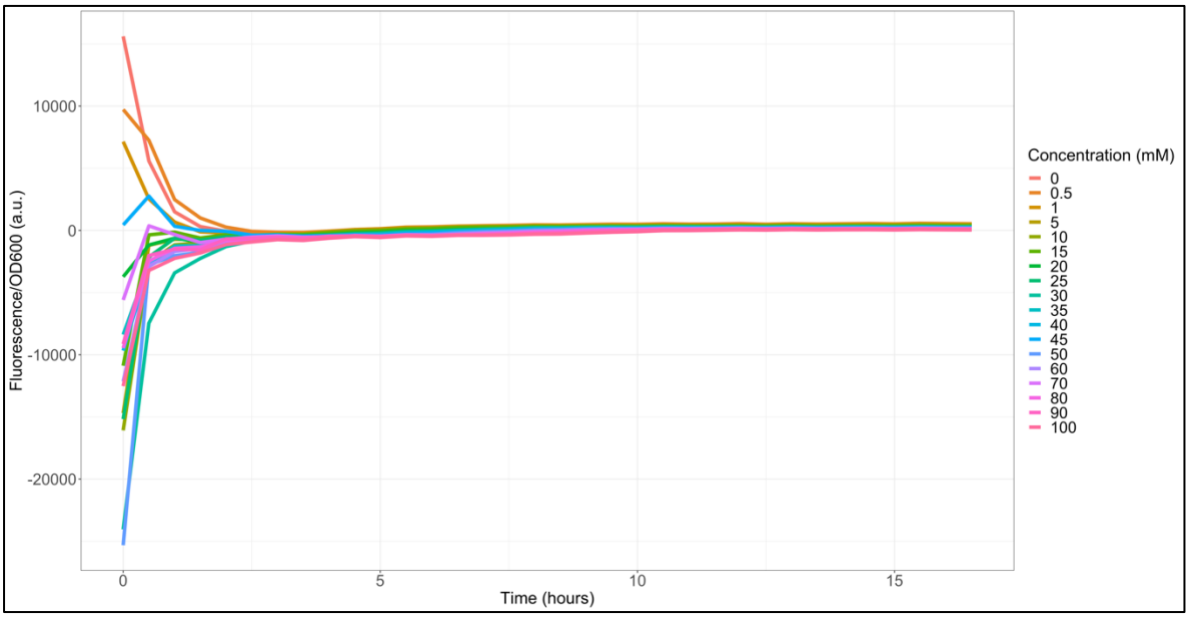


Figure 8.1.6. Prop-lacI-sfGFP strain raw normalized fluorescence graphics.

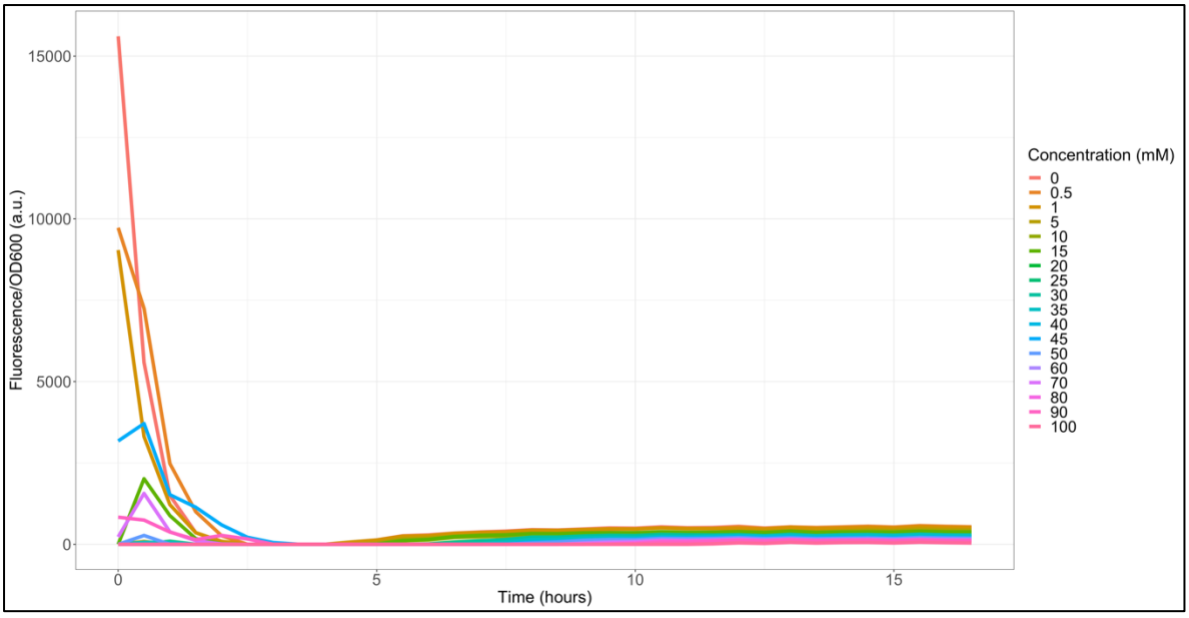


Figure 8.1.7. Prop-lacI-sfGFP strain positive normalized fluorescence values.

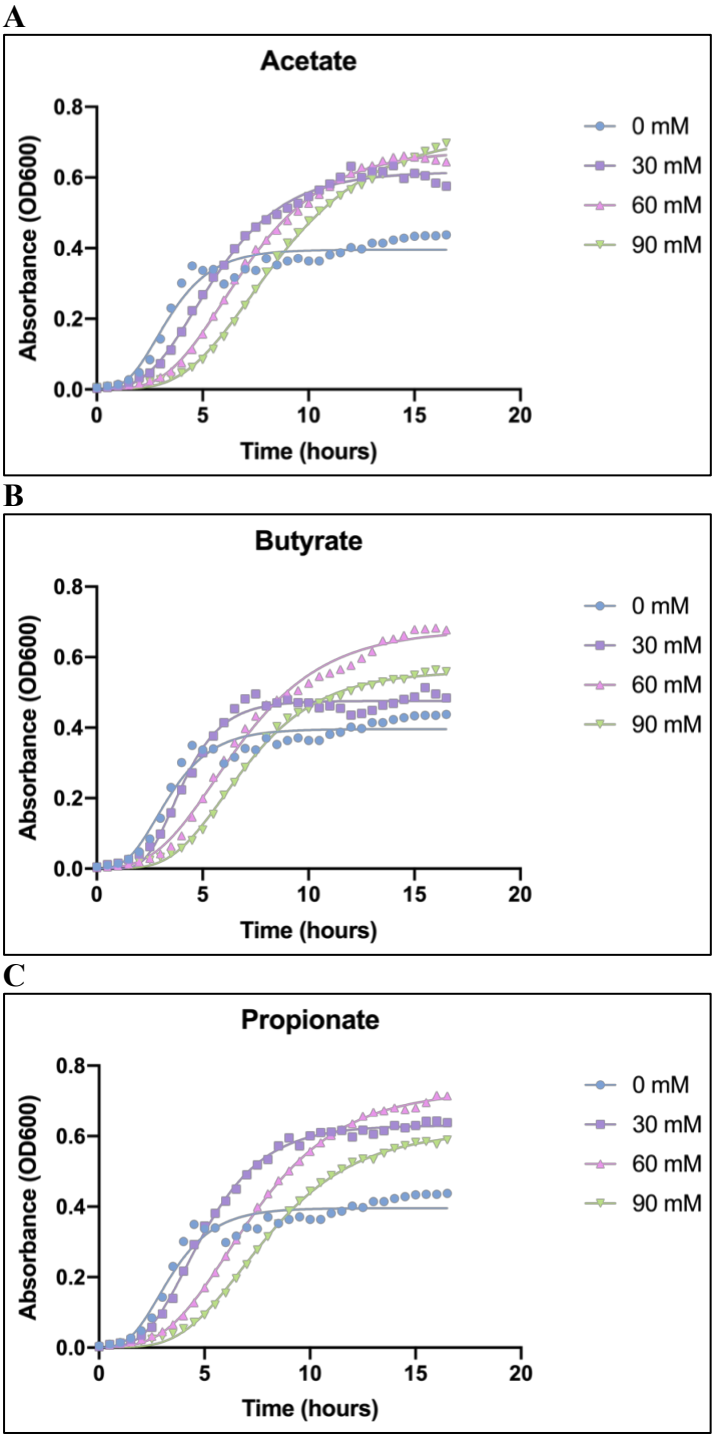


Figure 8.1.8. Modified Gompertz equation fitted to prop-lacI-sfGFP strain OD_{600} specificity data. Supplemented with (A) Acetate (B) Butyrate (C) Propionate.

8.2. Biosensors Project

8.2.1. Introduction

Gastrointestinal glycosylations serve diverse purposes as they are used in many ways by the intestinal microbiota. The abundance of different types of glycosylations affects the presence and diversity of bacteria in the gut (Giron et al., 2020). Polysaccharides can serve as a carbon source for the bacteria present, but in many cases terminal glycosylations must be cleaved first. The liberated monosaccharides can then be consumed by other bacteria, which creates a cross-feeding web between different species (Smith et al., 2019). This implies that the presence of specific types of glycosylations can affect the expression of certain metabolic pathways of the bacteria present, which also determines the byproducts generated. Additionally, glycosylated substances can act as a platform onto which certain bacteria can attach (Harel et al., 1993). The nature of the attached bacteria may influence the integrity of the intestinal epithelium barrier considering the proximity of the glycosylations to the barrier (Ouwerkerk et al., 2013). For example, beneficial bacteria that produce short chain fatty acids may be of great assistance in close proximity to the mucosal layer, since these metabolites are known to have protective and anti-inflammatory effects (Thibault et al., 2010). Meanwhile, polysaccharide-attached pathogens may quickly worsen inflammatory conditions and weaken the barrier.

Fucose is an abundant sugar found in glycans on proteins and lipids throughout the mammalian gastrointestinal tract. It is mainly found in the form of α-1,2-fucosylations on the intestinal epithelium oriented towards the lumen (Pickard and Chervonsky, 2015). The main enzyme responsible for these fucosylations is α-1,2-fucosyltransferase (Fut2), which is expressed in epithelial cells and links fucose to the terminal β-D-galactose of mucosal glycans (Goto et al., 2016). It is believed that this type of glycosylation in the small intestine is protective against pathogenic infection (Pickard et al., 2014). Terminal fucose

can be cleaved by certain gut microbes and is then free to be consumed by other bacteria. In addition to acting as an energy source, fucose is involved in diverse metabolic pathways including the regulation of quorum sensing and suppression of virulence genes in pathogens (Scott et al., 2006; Pacheco et al., 2012; Pickard and Chervonsky, 2015). Interestingly, loss of function mutations of Fut2 have been associated with Crohn's disease (McGovern et al., 2010). Therefore, being able to quantify the amount of fucose present in the gut could give insight to the conditions in the intestine, especially regarding the possible presence of pathogens and associated inflammation.

Another sugar abundantly found in glycans on the intestinal mucosa is sialic acid. Sialidases are enzymes that cleave terminal sialic acid monomers from the rest of the glycan. It has been shown that excessive sialidase activity in the intestine can lead to disproportionate sialic acid catabolism and cause detrimental conditions. Because larger amounts of the sugar are released, a niche for sialic acid consuming pathogens, especially *Enterobacteriaceae*, is generated and allows them to proliferate (Huang et al., 2015). This causes an exaggerated pro-inflammatory response from the host immune system. Additionally, pathogens can incorporate free sialic acid in their capsules and lipooligosaccharides which act as protection and help avoid detection from the host's immune system (Harvey et al., 2001; Bouchet et al., 2003). Many *Enterobacteriaceae*, including *Escherichia coli*, lack sialidase and depend on other bacteria, such as *Bacteroides*, to liberate the sugar for them to use. This may be related to the increase in both *Enterobacteriaceae* and *Bacteroides* in patients with colitis (Gophna et al., 2006). As with fucose, being able to quantify sialic acid in the intestine could be useful for studying the gut state.

Currently, a range of methods to quantify specific molecules, such as monosaccharides, in serum or plasma exist. Some of the most used are high performance liquid chromatography (HPLC) and mass spectrometry (MS) (Zhou et al., 2020). However, these methods do not permit *in vivo* quantification and can be costly. Methods used to detect

sugars *in vivo* include lectins, antibodies, recombinant binding proteins and chemical modification (Zhou et al., 2020). Nevertheless, these resources do not detect free monosaccharides, but rather those found as terminal glycosylations (Zhou et al., 2020). Therefore, there is a need for a tool that can quickly, accurately and at a low cost detect free fucose and sialic acid *in vivo*. Bacteria that can be consumed as probiotics have commonly been used as diagnostic tools or biosensors to detect molecules of interest. We created preliminary fucose and sialic acid biosensors in *E. coli* DH5α to detect these monosaccharides *in vitro*.

8.2.2. Materials and Methods

8.2.2.1. Biosensor construction

The fucose biosensor was created in a high copy plasmid backbone. The fucose-induced promoter was purchased as a gBlock from Integrated DNA Technologies, Inc. (IDT) including the PstI and EcoRI restriction sites at the 5' and 3' ends, respectively. The sequence was obtained from the *E. coli* K12 MG1655 genome, specifically from the fucose fukPIK operon (Zhu and Lin, 1988). The plasmid contains an ampicillin resistance gene as a selection marker and superfolder GFP (sfGFP) as a reporter molecule (Pédelacq et al., 2006). Both DNA fragments were digested with the PstI-HF and EcoRI-HF restriction enzymes for one hour at 37ºC, gel purified and ligated with T4 DNA ligase at room temperature for one hour (New England Biolabs, Inc.).

The sialic acid biosensor was initially created in the same high copy plasmid backbone as the fucose sensor. The sialic acid-induced promoter from the nanAT operon (Plumbridge and Vimr, 1999) was obtained through PCR with the *E. coli* K12 MG1655 genome as the template. The primers used included overhangs from the backbone into which the

promoter was inserted for Gibson Assembly cloning (Gibson et al., 2009). The primer sequences were 5'-AGTTCTTCTCCTTTGCTcataaatacctctgaagtgatgct-3', 5'-CACTATAGGGCGAATTGGAGtgccactttagtgaagcaga-3' for the promoter and 5'-GCATCACTTCAGAGGTATTtatgagcaaaggagaagaact-3', 5'-TCTGCTTCACTAAAgtggcactccaattcgccctatagtg-3' for the backbone. The promoter was cloned into the backbone with Gibson Assembly Master Mix 2X by incubating it at 50ºC for one hour (New England Biolabs, Inc.).

Because the high copy sialic acid sensor presented high levels of basal sfGFP expression, its promoter followed by the *sfGFP* gene were then cloned into a low copy plasmid with a chloramphenicol resistance gene. The primers used for Gibson Assembly cloning for the promoter/sfGFP sequence and the backbone were 5'-TTACACATGGCATGGATTAAttatctgttgtttgtcggtgaacg-3', 5'-tctgcttcactaaagtggcaACGTCGGAATTGCCAGCTGG-3' and 5'-CCAGCTGGCAATTCCGACGTtgccactttagtgaagcaga-3', and 5'-CACCGACAAACAACAGATAAttaatccatgccatgtgtaatc-3', respectively.

All plasmids were transformed into competent *E. coli* DH5α. Two microliters of ligation mixture or Gibson Assembly Master Mix were added to 50 μL of cells and incubated on ice for 30 minutes. Heat shock was done at 42ºC for 50 seconds followed by two minutes on ice. One milliliter of SOC media was added and the bacteria were incubated at 37ºC with shaking at 200 rpm for one hour. The entire transformation volume was plated onto LB agar plates with the corresponding antibiotic. Single colonies were chosen to be grown in LB media for stock preparation and miniprep. Correct insertion of genes of interest were verified through plasmid sequencing by Eton Bioscience Inc.

8.2.2.2. Biosensor functionality experiments

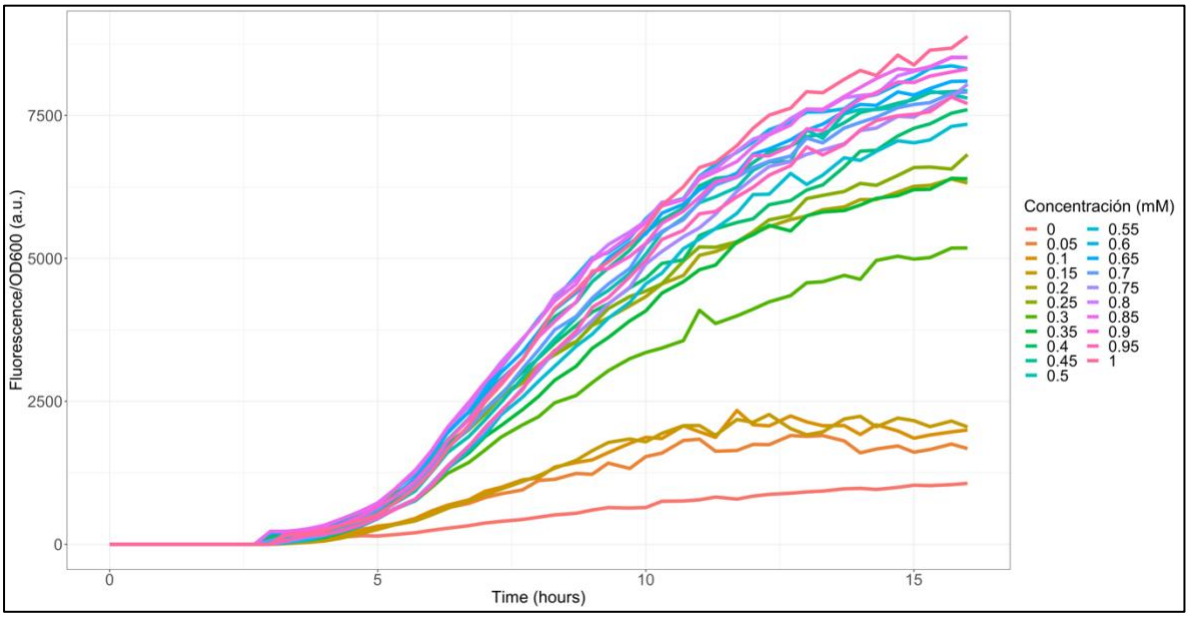
The biosensors were grown overnight in LB media at 37ºC with shaking. The next day, they were inoculated at a final concentration of 1% in 100 μL of fresh LB media in black 96 well plates with transparent bottoms. The culture media was supplemented with either fucose ranging from 0-1 mM with increments of 0.05 mM or with sialic acid with a range of 0-200 μM with increments of 10 μM. These concentrations were used as they have been reported to be physiologically relevant in the intestine (Pacheco et al., 2012; Huang et al., 2015). Each concentration was done in triplicate at 37ºC with constant shaking and absorbance and fluorescence measurements were taken every 20 minutes for 16 hours.

8.2.3. Preliminary Results

The fucose sensor presented obvious differences in the production of fluorescence between 0 and 0.3 mM of fucose (Figure 8.2.3.1A). Moderate differences in fluorescence are observed when induced with higher concentrations, although the concentration of saturation is unclear in this figure. A linear tendency can be observed in Figure 8.2.3.1B which shows the values obtained at each concentration after 15 hours of growth. However, it can be observed that for most of the concentrations tested two of the three triplicates presented fluorescence values considerably higher than the third. This may be due to complications in the instrument used to measure or external contamination of the 96 well plate used. Either way, the experiment should be repeated in order to obtain triplicates with a lower standard deviation. Nevertheless, a relatively linear and positive relationship between the concentration of fucose present and the amount of fluorescence produced can be observed mainly between 0 and 0.3 mM of fucose. Afterwards, a similar relationship can be observed but with a smaller slope meaning that the same increments of fucose concentration have a smaller effect nearing the concentration of saturation. However, with

these preliminary results said concentration remains unclear.

A



B

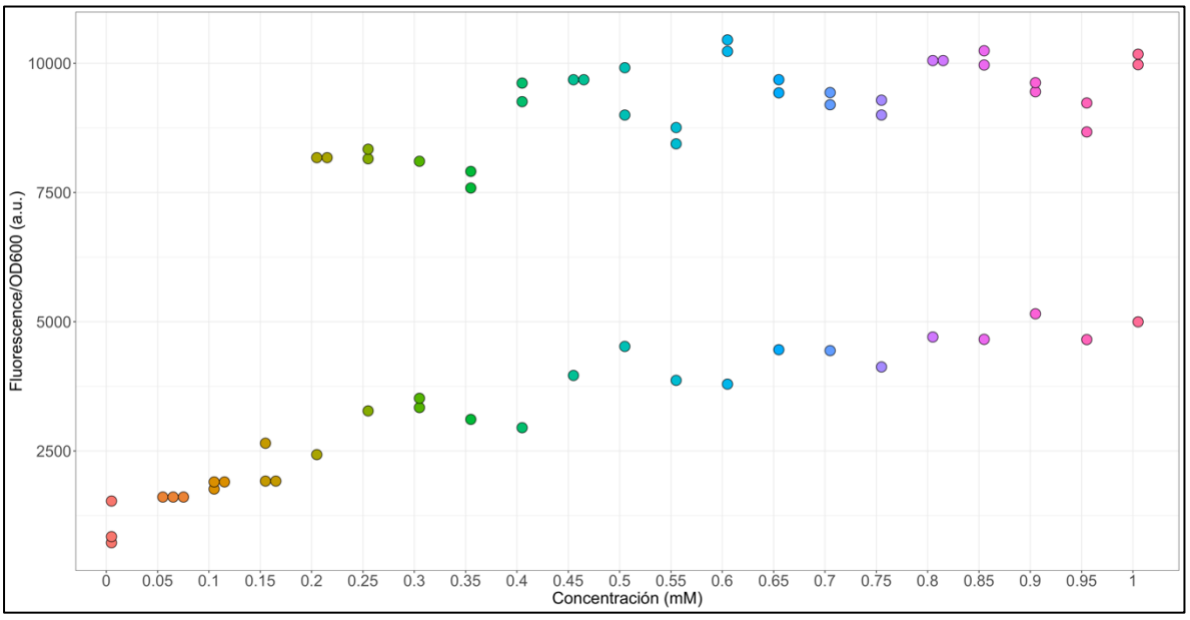


Figure 8.2.3.1. Fucose biosensor. (A) Fluorescence produced over time. (B) Fluorescence produced after 15 hours of growth.

With the sialic acid sensor, differences in the amount of fluorescence produced can be observed between approximately zero and 90 μM (Figure 8.2.3.2A). The initial peak observed is likely due to noise in the measurements. Because in initial growth stages the OD_{600} value will be very small, the little fluorescence produced will seem considerably larger when divided by these small values. To obtain neater results this experiment should also be repeated. However, after 15 hours of growth, a linear and positive relationship can be observed between the concentration of sialic acid and the amount of fluorescence produced until approximately 90 μM (Figure 8.2.3.2B). After this concentration there is more variability between triplicates, but the mean amount of fluorescence produced appears to be relatively constant. This suggests that the biosensor's concentration of saturation is approximately 90 μM.

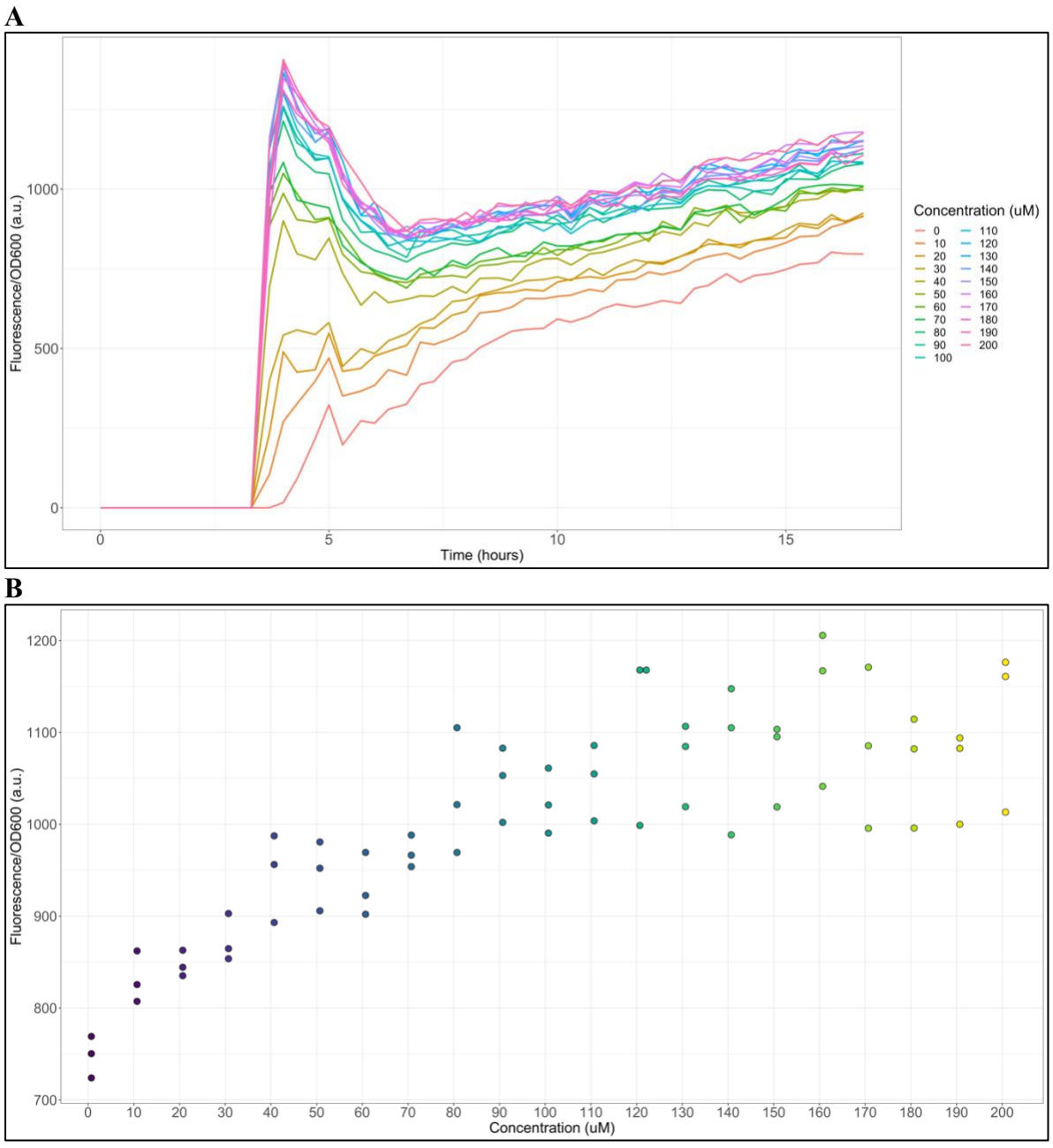


Figure 8.2.3.2. Sialic acid biosensor. (A) Fluorescence produced over time. (B) Fluorescence produced after 15 hours of growth.

8.2.4. Future Work

Although both biosensors are able to detect their respective monosaccharides, they can still be improved. The experiments should be repeated in order to obtain clearer measurements. They can also be cloned into different copy number plasmids which may lead to larger differences in the amount of fluorescence produced under different concentrations of inducer. Additionally, their respective transcription factors, which are found in the *E. coli* K12 genome next to the operons mentioned above, can be cloned into the plasmids. This should improve the regulation of the expression of sfGFP. Specificity experiments should also be carried growing the biosensors in media supplemented with different sugars to assure that the biosensor is not induced by other substances. Furthermore, samples containing glycosylated macromolecules should be inoculated with the biosensors and a fucosidase or sialidase. Eventually, these enzymes could be cloned into the biosensors so that they are able to liberate and quantify terminal fucose or sialic acid from macromolecules of interest.

8.2.5. References

Bernstein, C. N., Fried, M., Krabshuis, J. H., Cohen, H., Eliakim, R., Fedail, S., et al. (2009). Inflammatory bowel disease : a global perspective. in *World Gastroenterology Organisation Global Guidelines*, 1–24. Available at: http://www.24hmb.com/voimages/web_image/upload/file/20140606/76761402057831265.pdf.

Bouchet, V., Hood, D. W., Li, J., Brisson, J. R., Randle, G. A., Martin, A., et al. (2003). Host-derived sialic acid is incorporated into Haemophilus influenzae lipopolysaccharide and is a major virulence factor in experimental otitis media. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8898–8903. doi:10.1073/pnas.1432026100.

Chang, C., Tesar, C., Li, X., Kim, Y., Rodionov, D. A., and Joachimiak, A. (2015). A novel transcriptional regulator of L-arabinose utilization in human gut bacteria. *Nucleic Acids Res.* 43, 10546–10559. doi:10.1093/nar/gkv1005.

Chen, Y. M., Tobin, J. F., Zhu, Y., Schleif, R. F., and Lin, E. C. (1987). Cross-induction of the L-fucose system by L-rhamnose in Escherichia coli. *J. Bacteriol.* 169, 3712–3719. doi:10.1128/jb.169.8.3712-3719.1987.

Daeffler, K. N., Galley, J. D., Sheth, R. U., Ortiz-Velez, L. C., Bibb, C. O., Shroyer, N. F., et al. (2017). Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. *Mol. Syst. Biol.* 13, 923. doi:10.15252/msb.20167416.

Garschagen, L. S., Franke, T., and Deppenmeier, U. (2020). An alternative pentose phosphate pathway in human gut bacteria for the degradation of C5 sugars in dietary fibers. *FEBS J.*, 1–20. doi:10.1111/febs.15511.

Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., and Smith, H. O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat. Methods* 6, 343–345. doi:10.1038/nmeth.1318.

Giron, L. B., Tanes, C. E., Schleimann, M. H., Engen, P. A., Mattei, L. M., Anzurez, A., et al. (2020). Sialylation and fucosylation modulate inflammasome-activating eIF2 Signaling and microbial translocation during HIV infection. *Mucosal Immunol.*

doi:10.1038/s41385-020-0279-5.

Gophna, U., Sommerfeld, K., Gophna, S., Doolittle, W. F., and Veldhuyzen Van Zanten, S. J. O. (2006). Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J. Clin. Microbiol.* 44, 4136–4141. doi:10.1128/JCM.01004-06.

Goto, Y., Uematsu, S., and Kiyono, H. (2016). Epithelial glycosylation in gut homeostasis and inflammation. *Nat. Immunol.* 17, 1244–1251. doi:10.1038/ni.3587.

Harel, J., Fairbrother, J., Forget, C., Desautels, C., and Moore, J. (1993). Virulence factors associated with F165-positive Escherichia coli strains isolated from piglets and calves. *Vet. Microbiol.* 38, 139–155. doi:10.1016/0378-1135(93)90081-H.

Harvey, H. A., Swords, W. E., and Apicella, M. A. (2001). The mimicry of human glycolipids and glycosphingolipids by the lipooligosaccharides of pathogenic Neisseria and Haemophilus. *J. Autoimmun.* 16, 257–262. doi:10.1006/jaut.2000.0477.

Huang, Y.-L., Chassard, C., Hausmann, M., von Itzstein, M., and Hennet, T. (2015). Sialic acid catabolism drives intestinal inflammation and microbial dysbiosis in mice. *Nat. Commun.* 6, 8141. doi:10.1038/ncomms9141.

Kalivoda, K. A., Steenbergen, S. M., and Vimr, E. R. (2013). Control of the Escherichia coli sialoregulon by transcriptional repressor NanR. *J. Bacteriol.* 195, 4689–4701. doi:10.1128/JB.00692-13.

Lakatos, P. L. (2009). Environmental Factors Affecting Inflammatory Bowel Disease: Have We Made Progress? *Dig. Dis.* 27, 215–225. doi:10.1159/000228553.

Matsuoka, K., and Kanai, T. (2015). The gut microbiota and inflammatory bowel disease. *Semin. Immunopathol.* 37, 47–55. doi:10.1007/s00281-014-0454-4.

McDonald, N. D., Lubin, J. B., Chowdhury, N., and Boyd, E. F. (2016). Host-derived sialic acids are an important nutrient source required for optimal bacterial fitness In Vivo. *MBio* 7, 1–10. doi:10.1128/mBio.02237-15.

McGovern, D. P. B., Jones, M. R., Taylor, K. D., Marciante, K., Yan, X., Dubinsky, M., et al. (2010). Fucosyltransferase 2 (FUT2) non-secretor status is associated with

Crohn's disease. *Hum. Mol. Genet.* 19, 3468–3476. doi:10.1093/hmg/ddq248.

Ouwerkerk, J. P., De Vos, W. M., and Belzer, C. (2013). Glycobiome: Bacteria and mucus at the epithelial interface. *Best Pract. Res. Clin. Gastroenterol.* 27, 25–38. doi:10.1016/j.bpg.2013.03.001.

Pacheco, A. R., Munera, D., Waldor, M. K., Sperandio, V., and Ritchie, J. M. (2012). Fucose sensing regulates bacterial intestinal colonization. *Nature* 492, 113–117. doi:10.1038/nature11623.

Pédelacq, J. D., Cabantous, S., Tran, T., Terwilliger, T. C., and Waldo, G. S. (2006). Engineering and characterization of a superfolder green fluorescent protein. *Nat. Biotechnol.* 24, 79–88. doi:10.1038/nbt1172.

Pickard, J. M., and Chervonsky, A. V (2015). Intestinal Fucose as a Mediator of Host–Microbe Symbiosis. *J. Immunol.* 194, 5588–5593. doi:10.4049/jimmunol.1500395.

Pickard, J. M., Maurice, C. F., Kinnebrew, M. A., Abt, M. C., Schenten, D., Golovkina, T. V., et al. (2014). Rapid fucosylation of intestinal epithelium sustains host–commensal symbiosis in sickness. *Nature* 514, 638–641. doi:10.1038/nature13823.

Plumbridge, J., and Vimr, E. (1999). Convergent Pathways for Utilization of the Amino Sugars. *Microbiology* 181, 47–54.

Schneider, M., Al-Shareffi, E., and Haltiwanger, R. S. (2017). Biological functions of fucose in mammals. *Glycobiology* 27, 601–618. doi:10.1093/glycob/cwx034.

Scott, K. P., Martin, J. C., Campbell, G., Mayer, C. D., and Flint, H. J. (2006). Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium "Roseburia inulinivorans." *J. Bacteriol.* 188, 4340–4349. doi:10.1128/JB.00137-06.

Smith, N. W., Shorten, P. R., Altermann, E., Roy, N. C., and McNabb, W. C. (2019). The Classification and Evolution of Bacterial Cross-Feeding. *Front. Ecol. Evol.* 7, 1–15. doi:10.3389/fevo.2019.00153.

Thibault, R., Blachier, F., Darcy-Vrillon, B., De Coppet, P., Bourreille, A., and Segain, J. P. (2010). Butyrate utilization by the colonic mucosa in inflammatory bowel diseases: A transport deficiency. *Inflamm. Bowel Dis.* 16, 684–695.

doi:10.1002/ibd.21108.

Zhou, X., Yang, G., and Guan, F. (2020). Biological Functions and Analytical Strategies of Sialic Acids in Tumor. *Cells* 9, 273. doi:10.3390/cells9020273.

Zhu, Y., and Lin, E. C. C. (1988). A mutant crp allele that differentially activates the operons of the fuc regulon in Escherichia coli. *J. Bacteriol.* 170, 2352–2358. doi:10.1128/jb.170.5.2352-2358.1988.

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MINI REVIEW
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8.3. Published Review

Engineered Probiotics for Detection and Treatment of Inflammatory Intestinal Diseases

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Inflammatory intestinal diseases such as Crohn's disease and ulcerative colitis have seen an increase in their prevalence in developing countries throughout the current decade. These are caused by a combination of genetic and environmental factors, altered immune response, intestinal epithelium disruption and dysbiosis in the gut microbiome. Current therapies are mainly focused on treating symptoms and are often expensive and ineffective in the long term. Recently, there has been an increase in our understanding of the relevance of the gut microbiome and its impact on human health. Advances in the use of probiotics and synthetic biology have led to the development of intestinal biosensors, bacteria engineered to detect inflammation biomarkers, that work as diagnostic tools. Additionally, live biotherapeutics have been engineered as delivery vehicles to produce treatment *in situ* avoiding common complications and side effects of current therapies. These genetic constructs often express a therapeutic substance constitutively, but others could be regulated externally by specific substrates, making the production of their treatment more efficient. Additionally, certain probiotics detecting specific biomarkers *in situ* and responding by generating a therapeutic substance are beginning to be developed. While most studies are still in the laboratory stage, a few modified probiotics have been tested in humans. These advances indicate that live biotherapeutics could have great potential as new treatments for inflammatory intestinal diseases.

Keywords: probiotics, live biotherapeutics, biosensors, inflammatory bowel disease, intestinal inflammation, gut microbiome

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INTRODUCTION

The composition and function of the gut microbiome have important effects on diverse aspects of human health. The extensive network of metabolites produced by intestinal microbes can affect the integrity of the gut epithelium, energy balance and host immune responses (Matsuoka and Kanai, 2015). While certain genera are known to be dominant in the microbiomes of most adults, the diversity of bacteria that colonize the human intestine, particularly at the species level, is highly variable. A dysbiosis of the gut microbiota, the rupture of homeostasis between harmful and protective intestinal bacteria, can correlate and may be causative of certain disease states (Lakatos, 2009). These alterations have been linked to diabetes, obesity, asthma, allergy, inflammatory bowel disease (IBD), among others (Bäckhed et al., 2012).

Attempts to restore unhealthy microbiomes have been made by using probiotics. Probiotics are live microorganisms that upon consumption in adequate amounts provide beneficial effects on health (Hill et al., 2014). They have been shown to improve diseased states in the intestine, such as pouchitis, infectious diarrhea, Irritable Bowel Syndrome, *Helicobacter pylori* infection, *Clostridium difficile* infection, and antibiotic-associated diarrhea (Ritchie and Romanuk, 2012). Nevertheless, they often only transiently colonize the host and are not retained in the long term (Derrien and van Hylckama Vlieg, 2015). Additionally, current probiotics are not designed to treat a specific condition; they instead provide general health benefits. This problem raises the opportunity to use genetic engineering to develop more pragmatic probiotics that can produce substances that are relevant to treating specific conditions.

With the increased knowledge of the gut microbiome and the role of specific keystone microbes in our health, combined with the development of new synthetic biology tools, probiotic microorganisms have been engineered to diagnose and treat intestinal inflammation. These microorganisms are being designed for the sensitive and precise detection of inflammation-related biomarkers *in situ*. Besides, live biotherapeutics have been engineered with diverse functions ranging from the constitutive expression of a therapeutic substance to more complex sense/respond/record mechanisms. The aim of this review is to provide a current view of advances regarding the applications of live biotherapeutics in the diagnosis and eventual treatment of inflammatory intestinal diseases.

INFLAMMATORY BOWEL DISEASES

The two most prevalent inflammatory bowel diseases are Crohn's disease (CD) and ulcerative colitis (UC). Both UC and CD are chronic disorders characterized by severe intestinal inflammation, but they also have significant differences. UC is characterized by the formation of superficial mucosal ulcerations and is limited to the proximity of the rectum (Xavier and Podolsky, 2007). Significant amounts of neutrophils form micro-abscesses in the lamina propria and the crypts. CD can be manifested elsewhere in the gastrointestinal tract, although the terminal ileum is most commonly affected. It is characterized by the accumulation of macrophages forming granulomas, and inflammation is usually transmural (Xavier and Podolsky, 2007). IBD symptoms could include bleeding, diarrhea, anemia, weight loss, and high levels of pain (Pithadia and Jain, 2011).

These diseases have a higher prevalence in North America and northern Europe and lower prevalence in developing countries (Baumgart and Carding, 2007). While the incidence of IBD has reached a plateau in the former, there has been a rise in the number of cases in South America, Eastern Europe, and Asia in the current decade (Burisch and Munkholm, 2013). It is estimated that approximately 6.8 million people worldwide are living with IBD (Jairath and Feagan, 2019).

The causes of IBD are believed to be multifactorial including genetic predisposition, environmental factors, alterations in the immune system, disruption in the integrity of the intestinal epithelium and dysbiosis in the gut microbiome (Matsuoka and Kanai, 2015; Martini et al., 2017). The susceptibility genes that have been identified include several pathways relevant to intestinal homeostasis. Nevertheless, these do not explain the increase in IBD cases that have been reported in developing countries suggesting the relevance of environmental factors (Khor et al., 2011). These include diet, smoking, geography and hygiene, among others (Baumgart and Carding, 2007; Lakatos, 2009). Additionally, IBD patients have been shown to have an overreactive immune system that leads to exacerbated intestinal inflammation (Baumgart and Carding, 2007). There is also a malfunctioning of the intestinal epithelium and barrier function. The epithelium acts typically as a semipermeable barrier keeping pathogens out while allowing the entrance of selective nutrients (Martini et al., 2017). It also acts as a receptor of signals from the intestinal microbiome and the immune system maintaining homeostasis. When its integrity is compromised, alterations in immune responses may occur, leading to IBD symptoms. Together, the immune system, as well as genetic and environmental factors, influence the composition of the gut microbiome, and in turn these microbes influence immune responses.

The proliferation of certain species and overproduction or lack of specific metabolites could also contribute to the development of IBD. For example, *Faecalibacterium prausnitzii* is an intestinal microbe known to have anti-inflammatory properties by secreting metabolites that block nuclear factor κB (NF-κB) and interleukin-8 (IL-8) production (Sokol et al., 2008). The numbers of this particular microbe are significantly reduced in patients with IBD (Sokol et al., 2008). Additionally, short chain fatty acids (SCFA), particularly butyrate, show protective and anti-inflammatory properties in the intestine, and they are present in lower concentrations in IBD patients (Parada Venegas et al., 2019). Therefore, studying the relevance of particular protective gut bacteria could be important for reverting dysbiosis.

Current therapies used for IBD alleviate inflammation and help to prevent flare-ups; these diseases presently have no cure (Caprilli et al., 2008). Symptoms are generally treated with corticosteroids, aminosalicylates and immunomodulators (Stein and Hanauer, 1999). Unfortunately, these drugs do not treat the cause of the disease and induce undesirable side effects, being sometimes ineffective. More recently, certain biologic treatments, usually antibodies that target specific inflammatory pathways, have been proven to be more effective. These alternatives, however, are costly and frequently delivered subcutaneously, which may increase the possibility of adverse side effects (Paramsothy et al., 2018). Another approach has been attempting to improve the composition of the gut microbiome of IBD patients through fecal microbiome transplants. This approach has been successfully used on various occasions for treating *Clostridium difficile* infection and associated diarrhea (Lopez and Grinspan, 2016). Nevertheless, this is an invasive procedure still in clinical trials with unestablished protocols and specifications, making this a riskier option (Sunkara et al., 2018). Despite receiving various treatments throughout their lives, many IBD patients eventually must undergo surgery to treat complications and alleviate symptoms (Caprilli et al., 2008). Therefore, new

treatment options focusing on improving intestinal epithelium integrity rather than merely treating symptoms are necessary. Live biotherapeutics with targeted delivery and action in the intestine could be an exciting option for fulfilling the current requirements of IBD treatments.

BIOSENSORS

Establishing an accurate diagnosis of gut-related diseases such as IBDs is usually difficult. First, invasive and costly procedures such as endoscopies and biopsies are normally required (Shergill et al., 2015). Second, substances indicative of disease sometimes have short half-lives or are too unstable to be easily detected. Bacterial biosensors that act *in situ* could be crucial for the future of non-invasive and precise diagnostics. Biosensors are live microorganisms engineered to detect specific biomarkers suggestive of certain disorders. Upon detection, they generate a marker that can be easily quantified, such as fluorescent proteins or colored substrates (**Figure 1A**).

High sensitivity and specificity toward the biomarker they recognize are important requirements for biosensors to be used as diagnostic tools. Sensitivity can be optimized by combining different genetic parts such as promoters, ribosome binding sites and terminators that confer varying strength to the output they produce. Sensitivity must also be adjusted to the biological concentrations of the molecules being sensed, which could range from pM to mM concentrations. Specificity requests the detection of the specific biomarker and no other substances with similar molecular structures that are not indicative of disease. Additionally, these must be detected in specific sections of the body and not where the biomarker is irrelevant. In order to increase specificity, biosensors can be genetically modified to sense physicochemical parameters in certain tissues, for example low oxygen tension in the intestine and tumors or certain pH. It is important for biosensors to produce a specific response exclusively when needed in order to optimize their energy resources and achieve a correct diagnosis.

The ability to optimize biosensors induced by small molecules (although not IBD biomarkers), was demonstrated in *E. coli* MG1655, DH10B, and BL21 (Meyer et al., 2019). The objective was to comply with the high standards required of biosensors including reduced promoter leakiness, high dynamic range, high sensitivity and high specificity. Different biosensors detecting relevant molecules such as 2,4-diacetylphophloroglucinol, cuminic acid, 3-oxohexanoyl-homoserine lactone, vanillic acid, isopropyl β-D-1-thiogalactopyranoside, anhydrotetracycline, L-arabinose, choline chloride, naringenin, 3,4-dihydroxybenzoic acid, sodium salicylate, and 3-hydroxytetradecanoyl-homoserine lactone were fine-tuned through directed evolution. Additionally, promoters and ribosome binding sites of varying strengths were tested in order to obtain optimal constructs. While *in vivo* studies are still required, this work shows the possibility of optimizing small molecule induction in bacteria.

Different molecules have been considered as biomarkers of gut inflammation and are used for the development of biosensors. Archer et al. (2012) used genetic parts naturally found in *Escherichia coli* to create a biosensor in the same species to detect nitric oxide (NO), a marker of intestinal inflammation (Kimura et al., 1997). NorR is a bacterial enhancer-binding protein that binds to transcription factor σ^{54} in the presence of NO, therefore activating transcription regulated by the promotor pNorV. In this biosensor pNorV regulated the expression of the DNA recombinase FimE, which activated a bidirectional circuit that in the absence of FimE (therefore, in the absence of NO) produced a yellow fluorescent protein and a cyan fluorescent protein in the presence of NO. This sensor could be an important diagnostic tool considering that NorR is highly specific toward NO and not toward other reactive oxygen species that might not necessarily be biomarkers of inflammation (Tucker et al., 2008; Bush et al., 2011).

Biosensors have also been constructed to detect thiosulfate and tetrathionate (Daeffler et al., 2017; Riglar et al., 2017). It is believed that during colitis, sulfate-reducing bacteria (mostly from the *Desulfovibrio* genus) produce hydrogen sulfide, which is converted to thiosulfate by host enzymes (Roediger et al., 1997; Levitt et al., 1999; Blachier et al., 2010; Jackson et al., 2012; Rey et al., 2013). Daeffler and colleagues computationally identified a thiosulfate sensor in *Shewanella halifaxensis* HAW-EB4 (a marine bacteria), based on a two-component system. The respective genes were cloned and optimized in *E. coli* Nissle 1917 by combining different strengths of promoters and ribosome binding sites that resulted in the best dynamic range of ligand activation. Its activation by thiosulfate was demonstrated in mice with dextran sodium sulfate (DSS)-induced inflammation (Daeffler et al., 2017). A different system has been developed to detect tetrathionate, another potential biomarker of intestinal inflammatory conditions (Riglar et al., 2017). During infection by *Salmonella typhimurium* in the mouse intestine, reactive oxygen species produced by the host convert thiosulfate to tetrathionate, which triggers inflammatory processes. Interestingly, tetrathionate is used as an alternative electron acceptor by *Salmonella*, thereby creating a niche for infection (Winter et al., 2010). Riglar and colleagues used the TtrSR two-component system from *S. Typhimurium* to create a tetrathionate biosensor in *E. coli* NGF-1, which also encoded a phage-lambda based memory circuit (Hensel et al., 1999; Riglar et al., 2017). The engineered strain was able to colonize and detect the biomarker in mice for six months under infection-induced and genetic models of inflammation. In summary, both genetic systems were highly sensitive and specific toward inflammation-triggered molecules in animal models initially making them exciting candidates for diagnosis. However, the actual relevance of thiosulfate and tetrathionate as inflammation biomarkers has not been fully studied. Particularly, tetrathionate has not been evaluated in non-mouse models due to the invasive means for its detection (Daeffler et al., 2017; Riglar et al., 2017).

There is clearly a limited knowledge regarding relevant biomarkers for gut inflammation. Recently a memory-based circuit was created to identify biosensor triggers in *E. coli* (Naydich et al., 2019). The bacteria was orally administered to healthy mice and to those with intestinal inflammation. A genetic library was created and computationally analyzed to detect these activators or repressors by comparing both conditions. Each

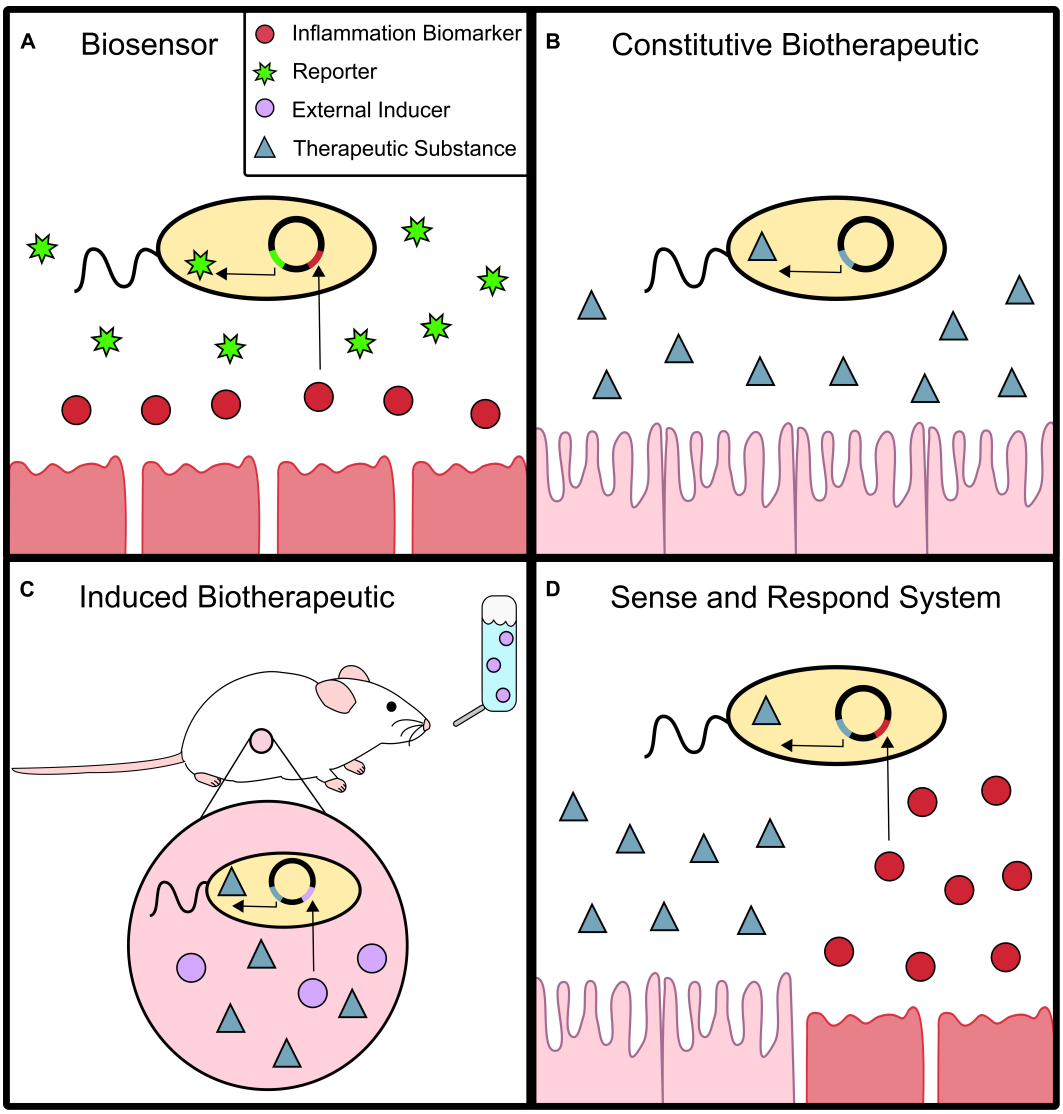

FIGURE 1 | Biosensor and Live Biotherapeutics. **(A)** Biosensors can detect an inflammation biomarker which activates the expression of a reporter molecule, such as green fluorescent protein. **(B)** Constitutive biotherapeutics are probiotics that constantly produce a therapeutic substance to treat inflammation. **(C)** Induced biotherapeutics produce a therapeutic substance when activated by an external signal, commonly added to food or water. **(D)** Sense and respond systems combine biosensors and live biotherapeutics. The therapeutic substance is produced only when the probiotic detects an inflammation biomarker *in situ*.

library included a promoter and ribosome binding site, and the latter was in some cases modified to increase sensitivity to the promoter's regulator. This is an important study considering the number of genes and operons found. However, their identity or function is not fully understood. This work provides insights to find novel biomarkers that may be indirectly related to intestinal inflammation.

Quorum sensing has also been studied as a way to detect bacterial signals and interactions in the gut. It was demonstrated that traditionally non-quorum sensing bacteria can be engineered to utilize signaling pathways to transfer information to each other in the gut (Kim et al., 2018). Native gut *E. coli* and attenuated *S. enterica* serovar Typhimurium were used as the signalers or responders. When externally induced by anhydrotetracycline, the signaler produced acyl-homoserine lactone, which was received and recorded by the responder. This system was implemented in mice and was functional throughout the gut. It could eventually be used to detect important disease biomarkers produced by pathogens and produce therapeutic substances by the responder. An example of the implementation of a quorum sensing system was developed in *L. lactis* genetically modified to detect quorum sensing signals specifically from the diarrhea-producing pathogen *Vibrio cholerae* (Mao et al., 2018). These signals activated the expression of an enzymatic reporter which was detectable in fecal samples.

Progressing toward medically applied biosensors, an ingestible probiotic and electrical based system was created to detect intestinal bleeding, wirelessly communicating the detected results to an external device (Mimee et al., 2018). *E. coli* Nissle 1917 was engineered to produce luciferase under the regulation of a synthetic promoter [$P_{L(HrtO)}$], which was modulated by heme-responsive repressor HrtR from *Lactococcus lactis* (Lechardeur et al., 2012). The extracellular transporter ChuA from *E. coli,* which allows diffusion of heme into the cell was also included in the circuit (Nobles et al., 2015). Therefore, heme was able to enter the cell and interact with the HrtR repressor, which liberated the $P_{L(HrtO)}$ promoter to express luciferase. The system was able to correctly diagnose gastrointestinal bleeding in swine, also proving to be adaptable for the detection of other biomarkers and possible diagnoses of other disorders. While the system's size, shelf-life and length of residency are factors to be improved, this innovative tool is an important example of the practicality and potential of long-studied biosensors. It represents a critical step toward fast, accurate and less invasive diagnoses.

LIVE BIOTHERAPEUTICS

Constitutive Systems

Bacteria have also been engineered as delivery vehicles to produce different therapeutic substances to treat intestinal inflammation *in situ* (**Figure 1B**). The traditional oral or systemic delivery of many of these substances can be problematic, considering they are often unstable with short half-lives and require high doses that may cause unwanted side effects. Considering that certain bacterial strains are well suited to colonize the intestinal epithelium, live biotherapeutics have the opportunity to proliferate and simultaneously produce a desired molecule *in situ*.

Earlier attempts to develop synthetic probiotic bacteria focused on the cytokine IL-10 to reduce gut inflammation. In certain studies, the protein was expressed in genetically modified *L. lactis* (Fedorak et al., 2000; Schotte et al., 2000; Schreiber et al., 2000; Steidler et al., 2003). This lactic acid bacterium could help avoid complications presented by traditional methods of delivery, such as sensitivity to low pH and dose-dependent side effects when delivered by injection. Lactic acid bacteria have historically been used in fermented foods and are generally regarded as safe (GRAS) (del Carmen et al., 2011; Benbouziane et al., 2013). Additionally, a wide variety of genetic engineering tools have been developed for this species, and several therapeutic proteins have been produced in *L. lactis* (Benbouziane et al., 2013). Nevertheless, after a phase II clinical trial it was concluded that an IL-10 producing *L. lactis* strain was safe but ineffective at improving mucosal healing compared to a placebo (Actogenix, 2009).

More recently, the immunosuppressive cytokines IL-27 and IL-35 were expressed in *L. lactis* and non-pathogenic *E. coli*, respectively (Hanson et al., 2014; Zhang et al., 2018). IL-27 producing *L. lactis* proved more effective than both its IL-10 producing counterpart and systemic administration of IL-27 in colitis mouse models. It was shown that this strain increased the production of IL-10 in the intestinal epithelium, contributing to the effectiveness against colitis. The IL-35-producing *E. coli* not only suppressed pro-inflammatory cytokine levels, but also increased anti-inflammatory cytokine activity. Nevertheless, these mechanisms of action are not yet fully understood requiring further studies. Additionally, it would be preferable to test the construct in food-grade bacteria or in a more prominent gut microbe.

Trefoil factors (TFF) and anti-tumor necrosis factor-α (TNF-α) nanobodies (single domain antibody fragments) are other therapeutic substances that have been constitutively expressed in *L. lactis* and tested in DSS-induced colitis in mice (Vandenbroucke et al., 2004, 2010). The former are peptides that are differentially produced in specific sections in the gastrointestinal tract and have protective and reparative properties on the intestinal epithelium (Playford et al., 1996; Vandenbroucke et al., 2004). Specifically, TFF-1 and TFF-2 are produced in the stomach and duodenum in mucus-producing cells, while TFF-3 is produced in the small and large intestines, predominantly in goblet cells (Vandenbroucke et al., 2004). The peptides produced *in situ* by *L. lactis* were considerably more effective at healing colitis than the oral or rectal administration of the purified peptides (Vandenbroucke et al., 2004).

A different construct was created to counteract TNF-α (Vandenbroucke et al., 2010). It is known that levels of TNF-α are augmented in IBD patients and that this cytokine is linked to the disease's symptoms (Vassalli, 1992; Papadakis and Targan, 2000; Adegbola et al., 2018). Antibodies for this cytokine are currently used as a treatment for IBD. Nevertheless, this treatment is expensive and can be associated with diverse systemic administration related side effects (Vandenbroucke et al., 2010). The *L. lactis* construct that produced the anti TNF-α nanobodies proved to have the beneficial effects of the aforementioned antibodies without adverse side effects.

Other possible inflammation treatments include the use of interference RNA (RNAi). Engineered *E. coli* expressing invasin and listeriolysin O are able to invade mammalian cells and therefore facilitate the transfer of genetic material (Grillot-Courvalin et al., 1998). Cyclooxygenase-2 (COX-2) is an enzyme induced by proinflammatory cytokines including TNF-α and is overexpressed in the colonic mucosa of IBD patients (Singer et al., 1998). Using the two genes previously mentioned, non-pathogenic invasive *E. coli* was engineered to transfer anti COX-2 RNAi to silence the expression of this enzyme resulting in positive effects on DSS-induced colitis in mice (Spisni et al., 2015).

While most studies utilize bacterial systems with established genetic modification systems, the cognate microorganisms are generally not dominant in the gut microbiome and their relative impact is small. This is why it is necessary to study prominent intestinal microbes as engineered probiotics for treatment of gut inflammation. For example, *Bifidobacterium longum* subsp. *longum* is a dominant microorganism found in most individuals' microbiomes, and therefore an interesting target for delivery of biotherapeutics (Arboleya et al., 2016). This bacterium was modified to produce α-melanocyte-stimulating hormone (α-MSH), a peptide with protective and anti-inflammatory properties. α-MSH acts by increasing IL-10 and down-regulating

the production of pro-inflammatory cytokines (such as TNF-α) and nitric oxide (Brzoska et al., 2010; Wei et al., 2016). The engineered strain showed significant anti-inflammatory effects in DSS-induced colitis in mice.

Other studies have focused on modifying bacteria to produce substances that counteract the action of reactive oxygen species (ROS) (Bruno-Bárcena et al., 2004; Han et al., 2006; Carroll et al., 2007; Watterlot et al., 2010; LeBlanc et al., 2011; del Carmen et al., 2014; Liu et al., 2018). Gastrointestinal tract inflammation has been associated with an overactive immune system and the accumulation of ROS. These species can damage proteins, lipids and DNA (Grisham et al., 1990; Seguí et al., 2005). ROS are usually neutralized by antioxidant enzymes, such as catalases and superoxide dismutases, which are produced *in situ* in healthy humans (Carroll et al., 2007). Therefore, increasing the production of these enzymes could improve inflammatory conditions. However, the traditional delivery of these proteins is complicated due to their short circulation half-life (Turrens et al., 1984). Ideally, they must be produced and secreted only where they are required to act, making programmable engineered probiotics an interesting option. Most studies involving ROS have genetically modified lactic acid bacteria to be used as delivery vehicles for antioxidants, achieving reduced inflammation in different *in vitro* and *in vivo* models. However, one study did utilize the more dominant bacterium, *B. longum,* to express the antioxidant enzyme manganese superoxide dismutase, reducing DSS-induced colitis in mice (Liu et al., 2018).

In a different approach, the use of a biosensor in a transgenic mouse model was combined with the production of a therapeutic peptide by intestinal epithelial cells (Breyner et al., 2017). Microbial Anti-inflammatory Molecule (MAM) is a peptide produced by *Faecalibacterium prausnitzii* that has been shown to have anti-inflammatory properties on the intestinal epithelium and was shown to block NF-κB activation *in vitro* (Quévrain et al., 2016). After failed attempts of heterologous and chemical synthesis of MAM, the authors modified *L. lactis* to carry a plasmid with MAM's cDNA under the control of a eukaryotic promoter. The plasmid was transfected into intestinal epithelial cells, which successfully produced the peptide in a mouse model. In this model, luciferase was produced under the control of an NF-κB promoter allowing the observation of MAM's interference with the production of the nuclear factor *in vivo*. MAM's anti-inflammatory properties were confirmed in dinitrobenzene sulfonic acid (DNBS) and DSS mouse models.

Inducible Systems

There are several advantages in the application of inducible probiotics instead of microorganisms producing therapeutic substances constitutively (**Figure 1C**). The probiotics mentioned previously have been developed using relatively simple genetic modifications, mostly expressed in a constitutive fashion and including their native transcription and translation signals. It is important to consider that constantly generating these therapeutic substances requires a large amount of energy for the probiotic. This can be detrimental for bacterial fitness where constitutive expression places a substantial cost with no major benefit. In contrast, inducible systems are easier to control and can help prevent the overproduction of the substance, which can have unknown consequences at elevated concentrations.

Lactococcus lactis has been engineered to produce IL-10 in a regulated manner under the control of inducible promoters. The *L. lactis* xylose inducible expression system (XIES) was used to genetically modify this same bacteria, regulating the expression of the cytokine by modifying the concentration of xylose present (del Carmen et al., 2011). This system was chosen because of its well-controlled regulation and efficiency in the production of long-term complex proteins (Miyoshi et al., 2004). The bacteria was used to ferment milk containing xylose and given to mice as a treatment for trinitrobenzenesulfonic acid (TNBS)-induced colitis. The authors hypothesized that the food matrix protected IL-10 through the gastrointestinal tract where it was able to act. Although this system is easily regulated, it is limited to the food matrix used and does not respond automatically to host signals, but rather to the concentration of xylose present.

Bacteroides species are dominant in the gut microbiome representing up to 25% of total microorganisms (Salyers, 1984). A xylan-inducible system has been developed in *B. ovatus* to produce human keratinocyte growth factor 2 (KGF-2; Hamady et al., 2010) and transforming growth factor β (TGF-β; Hamady et al., 2011). Both growth factors are important for maintaining intestinal integrity, being essential for epithelial cell proliferation (Barnard et al., 1989; Werner, 1998). They were both regulated by the xylanase promoter from the xylan operon in *B. ovatus*. Xylan is a fiber found in plants and is utilized by certain gut bacteria, including *B. ovatus* (Hespell and Whitehead, 1990; Thomson, 1993). The fiber was added to the experimental mice's food and water to induce the expression of the therapeutic substances. Using this bacterium as a delivery system has several advantages. It is an important anaerobic human gut commensal and certain *Bacteroides* have been found in the intestinal mucin layer (Croucher et al., 1983). Therefore, its survival and location of action are safer and more specific than other potential probiotics such as *L. lactis*. Both constructs were easily regulated and showed improved colitis conditions in mice.

Recently, *E. coli* Nissle 1917 was engineered to produce an extracellular matrix containing all three trefoil factors to treat inflammation and help re-build the intestinal epithelium (Praveschotinunt et al., 2019). CsgA is the monomer unit for the curli fibers that make up the fibrous matrix. The trefoil factors were fused to the CsgA C-terminus. Along with other genes required for the assembly and secretion of the modified polymer, they were placed under the control of an arabinose-induced promoter and incorporated into a plasmid with kanamycin resistance. The modified probiotic was tested in mice with DSS-induced colitis. In order to induce expression and assure plasmid stability the mice were given water infused with kanamycin and arabinose. The biotherapeutic improved inflammation, intestinal epithelium integrity and correlated with a decrease in the production of inflammatory cytokines and enzymes. While this is an interesting combination of delivery technique and therapeutic action, the authors note the necessity

of replacing antibiotic-based selection and using induction based on environmental signals rather than an external substance.

Sense and Respond Systems

With the objective of creating more specific, efficient and well-regulated live biotherapeutics, sense and respond genetic mechanisms are beginning to be developed. These systems are a more specific type of inducible probiotic, considering that their objective is to respond to the state of a certain organ and/or to a disease biomarker, rather than to an external activation source (**Figure 1D**). For example, a method that has been used to regulate IL-10 expression is through a stress-induced system (Benbouziane et al., 2013). Benbouziane and colleagues used the *L. lactis* groESL operon promoter, which has been shown to respond to low pH, heat shock and UV-radiation *in vitro* (Arnau et al., 1996; Hartke et al., 1997). Its activation has also been reported *in vivo* in a murine model (Roy et al., 2008). This system increases the chances that the therapeutic substance is produced *in situ* considering that the administered bacteria automatically goes through stressful conditions compared with laboratory growth conditions. Nevertheless, this circuit is not specific to gut inflammation conditions, and several stresses could be found along the gastrointestinal tract.

Recently, a genetic circuit based on NO detection, pseudotaxis and secretion was created in *E. coli* to produce and export granulocyte macrophage-colony stimulating factor (GM-CSF) *in situ* (McKay et al., 2018). NO activated the expression of CheZ, a motility regulator protein in an *E. coli* strain otherwise lacking this gene. Because NO acted as an attractor, the modified bacteria moved toward higher concentrations of this biomarker, where inflammation should be more prevalent. GM-CSF was chosen because it is reported to have therapeutic effects in patients with Crohn's disease by helping restore the mucosal barrier, stimulate neutrophils and sensitize pathogenic bacteria (Dieckgraefe and Korzenik, 2002; Korzenik et al., 2005; Choudhary et al., 2015). Its extracellular release was allowed through the inclusion of *tolAIII*, the gene of a pore-forming protein, in the genetic circuit. The release was confirmed with higher concentrations of GM-CSF in the supernatant of bacteria that included *tolAIII*. This engineered probiotic can respond directly to environmental inflammation conditions but must be tested *in vivo* in order to evaluate its therapeutic potential for IBD patients.

Another sense and respond system was engineered to treat *Salmonella* infection. As mentioned previously, *Salmonella typhimurium* can utilize tetrathionate as an electron acceptor creating a niche for infection and producing gut inflammation (Riglar et al., 2017). The tetrathionate biosensor system was incorporated into a plasmid controlling the expression of microcin H47 and transformed into *E. coli* Nissle 1917 (Palmer et al., 2018). Microcin H47 is a peptide originally obtained from *E. coli* H47 that was confirmed to inhibit growth of *Salmonella in vitro*. The modified strain of *E. coli* Nissle effectively produced the microcin in the presence of tetrathionate inhibiting *Salmonella* growth *in vitro*. While this is an interesting example of a sense and respond construct, it must also be tested *in vivo* before extrapolating further conclusions about its therapeutic effectiveness.

In theory, sense and respond systems have a higher impact and are more beneficial for inflammation treatment than simpler constructs. Nevertheless, this also implies more extensive genetic modifications that can weaken the circuit's durability and bacterial fitness. Still, because so few of these types of circuits have been developed, they should be expanded upon and tested in mice. It is important to quickly confirm whether they are more effective as treatment in order to validate or reject their further development.

CHALLENGES REGARDING LIVE BIOTHERAPEUTICS

While there is great potential in using engineered bacteria as diagnostic tools and live biotherapeutics, there is much left to improve and achieve. Although most biosensor and live biotherapeutic studies have focused on modifying *L. lactis* and *E. coli*, it is essential to develop new genetic engineering tools in more dominant microorganisms from the gut microbiome. These species may be more effective in treating gut inflammation since they have several adaptations for colonization of the human intestine, safe interaction with the immune system and reach high cell numbers, therefore, increasing the rate of success. Nevertheless, these species are usually anaerobic, more difficult to manipulate and their efficacy as genetically modified probiotics is strain-dependent. Additionally, in the gut microbiome introduced engineered bacteria could be more prone to genetic mutations, loss of therapeutic functions and decreased growth rates due to the energetic burden caused by synthetic circuits. Barriers including the lack of reliable genetic engineering tools for these species and methods for their encapsulation and delivery must be overcome.

Safety issues regarding containment, specificity and toxicity issues also arise. An engineered bacteria ingested by a person must be contained within that person and unable to transfer its modified DNA to the environment where it could have unpredicted consequences. Bacterial kill switches and quorum sensing-based autolysis systems have been incorporated into genetic circuits in order to avoid these issues (van de Poel and Robaey, 2017; Chowdhury et al., 2019). It is also fundamental for the biotherapeutic to be secreted directly to its target. In this sense, detecting biomarkers is not only important for diagnosing gut inflammation, but also for correctly generating treatment. This will allow the sidestepping of the substance acting in an incorrect organ and high doses that can have side effects. Additionally, the engineered probiotics must be toxin-free in order to avoid creating more damage than benefits, which is why food-grade bacteria initially seem appealing. A hesitant reception may be generated toward the use of new and generally unheard of gut-relevant probiotics.

It may be challenging to achieve colonization of individual bacteria through probiotics due to the difficulty of finding a niche to survive in the gut microbiome. The design of microbial consortia could be useful for this purpose. This would ideally depend on an individual IBD patient's needs regarding the cause of his or her symptoms. For example, genetically

modified SCFA-producing live biotherapeutics could eventually contribute to maintaining a balanced and healthy microbiome. Additionally, if multiple complementing species were to be ingested simultaneously, their chances of survival could improve. Further, different parts of a genetic circuit could be distributed among different species, increasing their co-dependence and relieving the metabolic strain on each one. The concept of personalized consortia is a future possibility that will depend on medical validity, safety and the future of personalized medicine.

CONCLUSION

Current advances in developing live biotherapeutics indicate they are a promising treatment for IBD. With synthetic biology tools, scientists have the ability to rapidly create various genetic circuits at a time and by high throughput screening, multitudes of options can be evaluated. The best candidates should then go on to clinical trials. Having a variety of options may be essential in the future of personalized medicine in which an individual's symptoms could be treated in a specific manner. This is crucial considering the diversity of the human gut microbiome and the plethora of possible causes that generate IBD. Additionally, the treatment would be non-invasive, direct and fast. Depending on the species and the therapeutic substance used, they could help restore normal microbiome conditions and heal the intestinal epithelium rather than simply treat recurring symptoms.

AUTHOR CONTRIBUTIONS

TD and DG conceived the manuscript and reviewed the last version. MB and DG wrote the manuscript.

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REFERENCES

Actogenix, O. (2009). *ActoGeniX Completes Phase 2A Clinical Trial of AG011*. Available at: http://www.aescap.com/pdf/actogenix_press_release_october_9.pdf (accessed October 9, 2009).

Adegbola, S. O., Sahnan, K., Warusavitarne, J., Hart, A., and Tozer, P. (2018). Molecular sciences anti-TNF therapy in Crohn's disease. *Int. J. Mol. Sci.* 19:2244. doi: 10.3390/ijms19082244

Arboleya, S., Watkins, C., Stanton, C., and Ross, R. P. (2016). Gut bifidobacteria populations in human health and aging. *Front. Microbiol.* 7:1204. doi: 10.3389/fmicb.2016.01204

Archer, E. J., Robinson, A. B., and Süel, G. M. (2012). Engineered *E. coli* that detect and respond to gut inflammation through nitric oxide sensing. *ACS Synth. Biol.* 1, 451–457. doi: 10.1021/sb3000595

Arnau, J., Sorensen, K. I., Appel, K. F., Vogensen, F. K., and Hammer, K. (1996). Analysis of heat shock gene expression in *Lactococcus lactis* MG1363. *Microbiology* 142, 1685–1691. doi: 10.1099/13500872-142-7-1685

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., et al. (2012). Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe* 12, 611–622. doi: 10.1016/j.chom.2012.10.012

Barnard, J. A., Beauchamp, R. D., Coffey, R. J., and Moses, H. L. (1989). Regulation of intestinal epithelial cell growth by transforming growth factor type beta. *Proc. Natl. Acad. Sci. U.S.A.* 86, 1578–1582. doi: 10.1073/pnas.86.5.1578

Baumgart, D. C., and Carding, S. R. (2007). Inflammatory bowel disease: cause and immunobiology. *Lancet* 369, 1627–1640. doi: 10.1016/s0140-6736(07)60750-8

Benbouziane, B., Ribelles, P., Aubry, C., Martin, R., Kharrat, P., Riazi, A., et al. (2013). Development of a stress-inducible controlled expression (SICE) system in *Lactococcus lactis* for the production and delivery of therapeutic molecules at mucosal surfaces. *J. Biotechnol.* 168, 120–129. doi: 10.1016/j.jbiotec.2013.04.019

Blachier, F., Davila, A.-M., Mimoun, S., Benetti, P.-H., Atanasiu, C., Andriamihaja, M., et al. (2010). Luminal sulfide and large intestine mucosa: friend or foe? *Amino Acids* 39, 335–347. doi: 10.1007/s00726-009-0445-2

Breyner, N. M., Michon, C., de Sousa, C. S., Vilas Boas, P. B., Chain, F., Azevedo, V. A., et al. (2017). Microbial anti-inflammatory molecule (MAM) from *Faecalibacterium prausnitzii* shows a protective effect on DNBS and DSS-induced colitis model in mice through inhibition of NF-κB pathway. *Front. Microbiol.* 8:114. doi: 10.3389/fmicb.2017.00114

Bruno-Bárcena, J. M., Andrus, J. M., Libby, S. L., Klaenhammer, T. R., and Hassan, H. M. (2004). Expression of a heterologous manganese superoxide dismutase gene in intestinal lactobacilli provides protection against hydrogen peroxide toxicity. *Appl. Environ. Microbiol.* 70, 4702–4710. doi: 10.1128/aem.70.8.4702-4710.2004

Brzoska, T., Böhm, M., Lügering, A., Loser, K., and Luger, T. A. (2010). "Terminal signal: anti-inflammatory effects of α-melanocyte-stimulating hormone related peptides beyond the pharmacophore," in *Melanocortins: Multiple Actions and Therapeutic Potential*, ed. A. Catania (New York, NY: Springer), 107–116. doi: 10.1007/978-1-4419-6354-3_8

Burisch, J., and Munkholm, P. (2013). Inflammatory bowel disease epidemiology. *Curr. Opin. Gastroenterol.* 29, 357–362. doi: 10.1097/MOG.0b013e32836229fb

Bush, M., Ghosh, T., Tucker, N., Zhang, X., and Dixon, R. (2011). Transcriptional regulation by the dedicated nitric oxide sensor, NorR: a route towards NO detoxification. *Biochem. Soc. Trans.* 39, 289–293. doi: 10.1042/BST0390289

Caprilli, R., Angelucci, E., and Clemente, V. (2008). Recent advances in the management of Crohn's disease. *Dig. Liver Dis.* 40, 709–716. doi: 10.1016/j.dld.2008.02.042

Carroll, I. M., Andrus, J. M., Bruno-Bárcena, J. M., Klaenhammer, T. R., Hassan, H. M., and Threadgill, D. S. (2007). Anti-inflammatory properties of *Lactobacillus gasseri* expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis. *Am. J. Physiol. Liver Physiol.* 293, G729–G738. doi: 10.1152/ajpgi.00132.2007

Choudhary, G. S., Yao, X., Wang, J., Peng, B., Bader, R. A., and Ren, D. (2015). Human granulocyte macrophage colony-stimulating factor enhances antibiotic susceptibility of *Pseudomonas aeruginosa* persister cells. *Sci. Rep.* 5:17315. doi: 10.1038/srep17315

Chowdhury, S., Castro, S., Coker, C., Hinchliffe, T. E., Arpaia, N., and Danino, T. (2019). Programmable bacteria induce durable tumor regression and systemic antitumor immunity. *Nat. Med.* 25, 1057–1063. doi: 10.1038/s41591-019-0498-z

Croucher, S. C., Houston, A. P., Bayliss, C. E., and Turner, R. J. (1983). Bacterial populations associated with different regions of the human colon wall. *Appl. Environ. Microbiol.* 45, 1025–1033. doi: 10.1128/aem.45.3.1025-1033.1983

Daeffler, K. N., Galley, J. D., Sheth, R. U., Ortiz-Velez, L. C., Bibb, C. O., Shroyer, N. F., et al. (2017). Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. *Mol. Syst. Biol.* 13:923. doi: 10.15252/msb.20167416

del Carmen, S., de Moreno de LeBlanc, A., Martin, R., Chain, F., Langella, P., Bermúdez-Humarán, L. G., et al. (2014). Genetically engineered immunomodulatory *Streptococcus thermophilus* strains producing antioxidant enzymes exhibit enhanced anti-inflammatory activities. *Appl. Environ. Microbiol.* 80, 869–877. doi: 10.1128/AEM.03296-13

del Carmen, S., de Moreno de LeBlanc, A., Perdigon, G., Bastos Pereira, V., Miyoshi, A., Azevedo, V., et al. (2011). Evaluation of the anti-inflammatory

effect of milk fermented by a strain of IL-10-producing *Lactococcus lactis* using a murine model of Crohn's disease. *J. Mol. Microbiol. Biotechnol.* 21, 138–146. doi: 10.1159/000333830

Derrien, M., and van Hylckama Vlieg, J. E. T. (2015). Fate, activity, and impact of ingested bacteria within the human gut microbiota. *Trends Microbiol.* 23, 354–366. doi: 10.1016/j.tim.2015.03.002

Dieckgraefe, B. K., and Korzenik, J. R. (2002). Treatment of active Crohn's disease with recombinant human granulocyte-macrophage colony-stimulating factor. *Lancet* 360, 1478–1480. doi: 10.1016/s0140-6736(02)11437-1

Fedorak, R. N., Gangl, A., Elson, C. O., Rutgeerts, P., Schreiber, S., Wild, G., et al. (2000). Recombinant human interleukin 10 in the treatment of patients with mild to moderately active Crohn's disease. *Gastroenterology* 119, 1473–1482. doi: 10.1053/gast.2000.20229

Grillot-Courvalin, C., Goussard, S., Huetz, F., Ojcius, D. M., and Courvalin, P. (1998). Functional gene transfer from intracellular bacteria to mammalian cells. *Nat. Biotechnol.* 16, 862–866. doi: 10.1038/nbt0998-862

Grisham, M. B., Gaginella, T. S., von Ritter, C., Tamai, H., Be, R. M., and Granger, D. N. (1990). Effects of neutrophil-derived oxidants on intestinal permeability, electrolyte transport, and epithelial cell viability. *Inflammation* 14, 531–542. doi: 10.1007/BF00914274

Hamady, Z. Z. R., Scott, N., Farrar, M. D., Lodge, J. P. A., Holland, K. T., Whitehead, T., et al. (2010). Xylan-regulated delivery of human keratinocyte growth factor-2 to the inflamed colon by the human anaerobic commensal bacterium *Bacteroides ovatus*. *Gut* 59, 461–469. doi: 10.1136/gut.2008.176131

Hamady, Z. Z. R., Scott, N., Farrar, M. D., Wadhwa, M., Dilger, P., Whitehead, T. R., et al. (2011). Treatment of colitis with a commensal gut bacterium engineered to secrete human TGF-β1 under the control of dietary xylan. *Inflamm. Bowel Dis.* 17, 1925–1935. doi: 10.1002/ibd.21565

Han, W., Mercenier, A., Ait-Belgnaoui, A., Pavan, S., Lamine, F., van Swam, I. I., et al. (2006). Improvement of an experimental colitis in rats by lactic acid bacteria producing superoxide dismutase. *Inflamm. Bowel Dis.* 12, 1044–1052. doi: 10.1097/01.mib.0000235101.09231.9e

Hanson, M. L., Hixon, J. A., Li, W., Felber, B. K., Anver, M. R., Stewart, C. A., et al. (2014). Oral delivery of IL-27 recombinant bacteria attenuates immune colitis in mice. *Gastroenterology* 146, 210–221.e13. doi: 10.1053/j.gastro.2013.09.060

Hartke, A., Frère, J., Boutibonnes, P., and Auffray, Y. (1997). Differential induction of the chaperonin GroEL and the co-chaperonin GroES by heat, acid, and UV-irradiation in *Lactococcus lactis* subsp. *lactis*. *Curr. Microbiol.* 34, 23–26. doi: 10.1007/s002849900138

Hensel, M., Hinsley, A. P., Nikolaus, T., Sawers, G., and Berks, B. C. (1999). The genetic basis of tetrathionate respiration in *Salmonella typhimurium*. *Mol. Microbiol.* 32, 275–287. doi: 10.1046/j.1365-2958.1999.01345.x

Hespell, R. B., and Whitehead, T. R. (1990). Physiology and genetics of xylan degradation by gastrointestinal tract bacteria. *J. Dairy Sci.* 73, 3013–3022. doi: 10.3168/jds.s0022-0302(90)78988-6

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11, 506–514. doi: 10.1038/nrgastro.2014.66

Jackson, M. R., Melideo, S. L., and Jorns, M. S. (2012). Human sulfide:quinone oxidoreductase catalyzes the first step in hydrogen sulfide metabolism and produces a sulfane sulfur metabolite. *Biochemistry* 51, 6804–6815. doi: 10.1021/bi300778t

Jairath, V., and Feagan, B. G. (2019). Global burden of inflammatory bowel disease. *Lancet Gastroenterol. Hepatol.* 1253, 9–10.

Khor, B., Gardet, A., and Xavier, R. J. (2011). Genetics and pathogenesis of inflammatory bowel disease. *Nature* 474, 307–317. doi: 10.1038/nature10209

Kim, S., Kerns, S. J., Ziesack, M., Bry, L., Gerber, G. K., Way, J. C., et al. (2018). Quorum sensing can be repurposed to promote information transfer between bacteria in the mammalian gut. *ACS Synth. Biol.* 7, 2270–2281. doi: 10.1021/acssynbio.8b00271

Kimura, H., Miura, S., Shigematsu, T., Ohkubo, N., Tsuzuki, Y., Kurose, I., et al. (1997). Increased nitric oxide production and inducible nitric oxide synthase activity in colonic mucosa of patients with active ulcerative colitis and Crohn's disease. *Dig. Dis. Sci.* 42, 1047–1054. doi: 10.1023/a:1018849405922

Korzenik, J. R., Dieckgraefe, B. K., Valentine, J. F., Hausman, D. F., and Gilbert, M. J. (2005). Sargramostim for active Crohn's disease. *N. Engl. J. Med.* 352, 2193–2201. doi: 10.1056/NEJMoa041109

Lakatos, P. L. (2009). Environmental factors affecting inflammatory bowel disease: have we made progress? *Dig. Dis.* 27, 215–225. doi: 10.1159/000228553

LeBlanc, J. G., del Carmen, S., Miyoshi, A., Azevedo, V., Sesma, F., Langella, P., et al. (2011). Use of superoxide dismutase and catalase producing lactic acid bacteria in TNBS induced Crohn's disease in mice. *J. Biotechnol.* 151, 287–293. doi: 10.1016/j.jbiotec.2010.11.008

Lechardeur, D., Cesselin, B., Liebl, U., Vos, M. H., Fernandez, A., Brun, C., et al. (2012). Discovery of intracellular heme-binding protein HrtR, which controls heme efflux by the conserved HrtB-HrtA transporter in *Lactococcus lactis*. *J. Biol. Chem.* 287, 4752–4758. doi: 10.1074/jbc.M111.297531

Levitt, M. D., Furne, J., Springfield, J., Suarez, F., and DeMaster, E. (1999). Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. *J. Clin. Invest.* 104, 1107–1114. doi: 10.1172/JCI7712

Liu, M., Li, S., Zhang, Q., Xu, Z., Wang, J., and Sun, H. (2018). Oral engineered *Bifidobacterium longum* expressing rhMnSOD to suppress experimental colitis. *Int. Immunopharmacol.* 57, 25–32. doi: 10.1016/j.intimp.2018.02.004

Lopez, J., and Grinspan, A. (2016). Fecal microbiota transplantation for inflammatory bowel disease. *Gastroenterol. Hepatol.* 12, 374–379.

Mao, N., Cubillos-Ruiz, A., Cameron, D. E., and Collins, J. J. (2018). Probiotic strains detect and suppress cholera in mice. *Sci. Transl. Med.* 10:eaao2586. doi: 10.1126/scitranslmed.aao2586

Martini, E., Krug, S. M., Siegmund, B., Neurath, M. F., and Becker, C. (2017). Mend your fences: the epithelial barrier and its relationship with mucosal immunity in inflammatory bowel disease. *Cell. Mol. Gastroenterol. Hepatol.* 4, 33–46. doi: 10.1016/j.jcmgh.2017.03.007

Matsuoka, K., and Kanai, T. (2015). The gut microbiota and inflammatory bowel disease. *Semin. Immunopathol.* 37, 47–55.

McKay, R., Ghodasra, M., Schardt, J., Quan, D., Pottash, A. E., Shang, W., et al. (2018). A platform of genetically engineered bacteria as vehicles for localized delivery of therapeutics: toward applications for Crohn's disease. *Bioeng. Transl. Med.* 3, 209–221. doi: 10.1002/btm2.10113

Meyer, A. J., Segall-Shapiro, T. H., Glassey, E., Zhang, J., and Voigt, C. A. (2019). *Escherichia coli* "Marionette" strains with 12 highly optimized small-molecule sensors. *Nat. Chem. Biol.* 15, 196–204. doi: 10.1038/s41589-018-0168-3

Mimee, M., Nadeau, P., Hayward, A., Carim, S., Flanagan, S., Jerger, L., et al. (2018). An ingestible bacterial-electronic system to monitor gastrointestinal health. *Science* 360, 915–918. doi: 10.1126/science.aas9315

Miyoshi, A., Jamet, E., Commissaire, J., Renault, P., Langella, P., and Azevedo, V. (2004). A xylose-inducible expression system for *Lactococcus lactis*. *FEMS Microbiol. Lett.* 239, 205–212. doi: 10.1016/j.femsle.2004.08.018

Naydich, A. D., Nangle, S. N., Bues, J. J., Trivedi, D., Nissar, N., Inniss, M. C., et al. (2019). Synthetic gene circuits enable systems-level biosensor trigger discovery at the host-microbe interface. *mSystems* 4:e00125-19. doi: 10.1128/mSystems.00125-19

Nobles, C. L., Clark, J. R., Green, S. I., and Maresso, A. W. (2015). A dual component heme biosensor that integrates heme transport and synthesis in bacteria. *J. Microbiol. Methods* 118, 7–17. doi: 10.1016/j.mimet.2015.07.011

Palmer, J. D., Piattelli, E., McCormick, B. A., Silby, M. W., Brigham, C. J., and Bucci, V. (2018). Engineered probiotic for the inhibition of *Salmonella* via tetrathionate-induced production of microcin H47. *ACS Infect. Dis.* 4, 39–45. doi: 10.1021/acsinfecdis.7b00114

Papadakis, K. A., and Targan, S. R. (2000). Tumor necrosis factor: biology and therapeutic inhibitors. *Gastroenterology* 119, 1148–1157. doi: 10.1053/gast.2000.18160

Parada Venegas, D., De la Fuente, M. K., Landskron, G., González, M. J., Quera, R., Dijkstra, G., et al. (2019). Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front. Immunol.* 10:277. doi: 10.3389/fimmu.2019.00277

Paramsothy, S., Rosenstein, A. K., Mehandru, S., and Colombel, J. F. (2018). The current state of the art for biological therapies and new small molecules in inflammatory bowel disease. *Mucosal Immunol.* 11, 1558–1570. doi: 10.1038/s41385-018-0050-3

Pithadia, A. B., and Jain, S. (2011). Treatment of inflammatory bowel disease (IBD). *Pharmacol. Rep.* 63, 629–642.

Playford, R. J., Marchbank, T., Goodlad, R. A., Chinery, R. A., Poulsom, R., and Hanby, A. M. (1996). Transgenic mice that overexpress the human trefoil peptide pS2 have an increased resistance to intestinal damage. *Proc. Natl. Acad. Sci. U.S.A.* 93, 2137–2142. doi: 10.1073/pnas.93.5.2137

Praveschotinunt, P., Duraj-Thatte, A. M., Gelfat, I., Bahl, F., Chou, D. B., and Joshi, N. S. (2019). Engineered *E. coli* Nissle 1917 for the delivery of matrix-tethered therapeutic domains to the gut. *Nat. Commun.* 10:5580. doi: 10.1038/s41467-019-13336-6

Quévrain, E., Maubert, M. A., Michon, C., Chain, F., Marquant, R., Tailhades, J., et al. (2016). Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. *Gut* 65, 415–425.

Rey, F. E., Gonzalez, M. D., Cheng, J., Wu, M., Ahern, P. P., and Gordon, J. I. (2013). Metabolic niche of a prominent sulfate-reducing human gut bacterium. *Proc. Natl. Acad. Sci. U.S.A.* 110, 13582–13587. doi: 10.1073/pnas.1312524110

Riglar, D. T., Giessen, T. W., Baym, M., Kerns, S. J., Niederhuber, M. J., Bronson, R. T., et al. (2017). Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. *Nat. Biotechnol.* 35, 653–658. doi: 10.1038/nbt.3879

Ritchie, M. L., and Romanuk, T. N. (2012). A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLoS One* 7:e34938. doi: 10.1371/journal.pone.0034938

Roediger, W. E. W., Moore, J., and Babidge, W. (1997). Colonic sulfide in pathogenesis and treatment of ulcerative colitis. *Dig. Dis. Sci.* 42, 1571–1579. doi: 10.1023/a:1018851723920

Roy, K., Meyrand, M., Corthier, G., Monnet, V., and Mistou, M.-Y. (2008). Proteomic investigation of the adaptation of *Lactococcus lactis* to the mouse digestive tract. *Proteomics* 8, 1661–1676. doi: 10.1002/pmic.200700698

Salyers, A. A. (1984). *Bacteroides* of the human lower intestinal tract. *Annu. Rev. Microbiol.* 38, 293–313. doi: 10.1146/annurev.mi.38.100184.001453

Schotte, L., Steidler, L., Vandekerckhove, J., and Remaut, E. (2000). Secretion of biologically active murine interleukin-10 by *Lactococcus lactis*. *Enzyme Microb. Technol.* 27, 761–765. doi: 10.1016/s0141-0229(00)00297-0

Schreiber, S., Fedorak, R. N., Nielsen, O. H., Wild, G., Williams, C. N., Nikolaus, S., et al. (2000). Safety and efficacy of recombinant human interleukin 10 in chronic active Crohn's disease. *Gastroenterology* 119, 1461–1472. doi: 10.1053/gast.2000.20196

Seguí, J., Gil, F., Gironella, M., Alvarez, M., Gimeno, M., Coronel, P., et al. (2005). Down-regulation of endothelial adhesion molecules and leukocyte adhesion by treatment with superoxide dismutase is beneficial in chronic immune experimental colitis. *Inflamm. Bowel Dis.* 11, 872–882. doi: 10.1097/01.MIB.0000183420.25186.7a

Shergill, A. K., Lightdale, J. R., Bruining, D. H., Acosta, R. D., Chandrasekhara, V., Chathadi, K. V., et al. (2015). The role of endoscopy in inflammatory bowel disease. *Gastrointest. Endosc.* 81, 1101–1121.e13. doi: 10.1016/j.gie.2014.10.030

Singer, I. I., Kawka, D. W., Schloemann, S., Tessner, T., Riehl, T., and Stenson, W. F. (1998). Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease. *Gastroenterology* 115, 297–306. doi: 10.1016/s0016-5085(98)70196-9

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L. G., Gratadoux, J.-J., et al. (2008). *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc. Natl. Acad. Sci. U.S.A.* 105, 16731–16736. doi: 10.1073/pnas.0804812105

Spisni, E., Valerii, M. C., De Fazio, L., Cavazza, E., Borsetti, F., Sgromo, A., et al. (2015). Cyclooxygenase-2 silencing for the treatment of colitis: a combined in vivo strategy based on RNA interference and engineered *Escherichia Coli*. *Mol. Ther.* 23, 278–289. doi: 10.1038/mt.2014.222

Steidler, L., Neirynck, S., Huyghebaert, N., Snoeck, V., Vermeire, A., Goddeeris, B., et al. (2003). Biological containment of genetically modified *Lactococcus lactis* for intestinal delivery of human interleukin 10. *Nat. Biotechnol.* 21, 785–789. doi: 10.1038/nbt840

Stein, R. B., and Hanauer, S. B. (1999). Medical therapy for inflammatory bowel disease. *Gastroenterol. Clin. North Am.* 28, 297–321.

Sunkara, T., Rawla, P., Ofosu, A., and Gaduputi, V. (2018). Fecal microbiota transplant – a new frontier in inflammatory bowel disease. *J. Inflamm. Res.* 11, 321–328. doi: 10.2147/JIR.S176190

Thomson, J. (1993). Molecular biology of xylan degradation. *FEMS Microbiol. Lett.* 104, 65–82. doi: 10.1016/0378-1097(93)90504-U

Tucker, N. P., D'Autréaux, B., Yousafzai, F. K., Fairhurst, S. A., Spiro, S., and Dixon, R. (2008). Analysis of the nitric oxide-sensing non-heme iron center in the NorR regulatory protein. *J. Biol. Chem.* 283, 908–918. doi: 10.1074/jbc.M705850200

Turrens, J. F., Crapo, J. D., and Freeman, B. A. (1984). Protection against oxygen toxicity by intravenous injection of liposome-entrapped catalase and superoxide dismutase. *J. Clin. Invest.* 73, 87–95. doi: 10.1172/JCI111210

van de Poel, I., and Robaey, Z. (2017). Safe-by-design: from safety to responsibility. *Nanoethics* 11, 297–306. doi: 10.1007/s11569-017-0301-x

Vandenbroucke, K., de Haard, H., Beirnaert, E., Dreier, T., Lauwereys, M., Huyck, L., et al. (2010). Orally administered *L. lactis* secreting an anti-TNF Nanobody demonstrate efficacy in chronic colitis. *Mucosal Immunol.* 3, 49–56. doi: 10.1038/mi.2009.116

Vandenbroucke, K., Hans, W., Van Huysse, J., Neirynck, S., Demetter, P., Remaut, E., et al. (2004). Active delivery of trefoil factors by genetically modified *Lactococcus lactis* prevents and heals acute colitis in mice. *Gastroenterology* 127, 502–513. doi: 10.1053/j.gastro.2004.05.020

Vassalli, P. (1992). The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.* 10, 411–452. doi: 10.1146/annurev.iy.10.040192.002211

Watterlot, L., Rochat, T., Sokol, H., Cherbuy, C., Bouloufa, I., Lefèvre, F., et al. (2010). Intragastric administration of a superoxide dismutase-producing recombinant *Lactobacillus casei* BL23 strain attenuates DSS colitis in mice. *Int. J. Food Microbiol.* 144, 35–41. doi: 10.1016/j.ijfoodmicro.2010.03.037

Wei, P., Yang, Y., Ding, Q., Li, X., Sun, H., Liu, Z., et al. (2016). Oral delivery of *Bifidobacterium longum* expressing α-melanocyte-stimulating hormone to combat ulcerative colitis. *J. Med. Microbiol.* 65, 160–168. doi: 10.1099/jmm.0.000197

Werner, S. (1998). Keratinocyte growth factor: a unique player in epithelial repair processes. *Cytokine Growth Factor Rev.* 9, 153–165. doi: 10.1016/s1359-6101(98)00010-0

Winter, S. E., Thiennimitr, P., Winter, M. G., Butler, B. P., Huseby, D. L., Crawford, R. W., et al. (2010). Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature* 467, 426–429. doi: 10.1038/nature09415

Xavier, R. J., and Podolsky, D. K. (2007). Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 448, 427–434. doi: 10.1038/nature06005

Zhang, B., Liu, Y., Lan, X., Xu, X., Zhang, X., Li, X., et al. (2018). Oral *Escherichia coli* expressing IL-35 meliorates experimental colitis in mice. *J. Transl. Med.* 16:71. doi: 10.1186/s12967-018-1441-7

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