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Effects of Bacterial Metabolites in Conditioned Media on Colon Cancer Proliferation

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EFFECTS OF BACTERIAL METABOLITES IN CONDITIONED MEDIA ON COLON CANCER PROLIFERATION

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EFFECTS OF BACTERIAL METABOLITES

IN CONDITIONED MEDIA ON COLON CANCER PROLIFERATION

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ABSTRACT

Cancer is the second leading cause of death in the United States with colorectal cancer (CRC) being the third most common type. Available treatments include a combination of surgery and chemotherapy but have debilitating side effects. These also have limited effectiveness in some cases, creating the need for additional treatment options, or supplementary treatments to increase their effectiveness. This is leading scientists to consider the microbiome to fix this shortcoming. Current research is focusing on the microbiome and its interactions with certain diseases, which could lead to pro- or prebiotic therapies. This work aims to establish specific bacterial species can inhibit tumor cell growth and function. Conditioned media with a bacterial supernatant was added to cancerous and non-cancerous cells and their effect on the cell growth, proliferation, and invasiveness was assessed. It was determined that *Lactobacillus* intestinalis, *Bacteroides uniformis*, *Bacteroides thetaiotaomicron*, and *Prevotella copri* all show potential in reducing cancer cell growth. This work demonstrates the potential to significantly enhance current immunotherapies and/or replace chemotherapies as well as aid in the understanding of the role of the microbiome in cancer.

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INTRODUCTION

Cancer remains one of the more complex human diseases, affecting a large percentage of the world population. It is also one of the most deadly, being the 2nd leading cause of death in the United States [1]. Current treatment include a combination of surgery and chemotherapy. Although chemotherapy has shown some success in treating colorectal cancer (CRC) it comes with serious side effects that can reduce the quality of life of the patient. One study has reported side effect from cancer patients, showing a range of symptoms including chest pains, constipation, diarrhea, fatigue, general pain, rash, and vomiting among the most common [2]. The problem with these therapies is not only the debilitating side effects they induce, but also that they are not always effective. A new rise of immunotherapies are attempting to solve both of these problems including various program cell death protein 1 (PD-1) blocking strategies to increase the host immune response to CRC tumors[3–5]. This screening study attempts to supplement these immunotherapies through the utilization of the microbiome by identifying potential microbes that inhibit cancer cell division and viability.

As more research is being focused on the microbiome and its interactions with various diseases, it is a clear next step to look for its interactions with cancer cells and tumors that much microbiome research is now focusing on. Studies have found differences in bacteria when comparing CRC tumors and non-cancerous surrounding tissue[6–8]. Some of the primary bacteria found on healthy tissue include *Prevotella*, *Bacteroides*, *Feacalibacterium prausnitzii*, and *Bifidobacterium*[7,8]. These bacteria differ from those found on the CRC tumors through their production of butyrate.[6]. Butyrate is an essential energy source for colon cells[9], induce p21 dependent cell cycle arrest and apoptosis in cancer cells[6], and have anti-inflammatory effects[10]. Additionally, one study has used *Lactobacillus* (another butyrate producer) to create conditioned

media and showed inhibition of cancer cell progression in mice[11]. This screening study will assess the ability of various common human gut microbes to inhibit cancer growth in culture.

Colorectal Cancer

Being one of the most prevalent cancer types in the United States, being estimated to afflict ~150,000 people[12] and posing a large problem globally, there is much known about CRC. Both genetic and environmental links have been uncovered. Colorectal cancer results from the accumulation of both genetic and epigenetic changes within colonic epithelial cells that leads to a transformation into a cancerous cell[13]. Many factors can influence the formation of these cancerous cells, including dysregulation of several key signaling pathways including Wnt/β-catenin involved in intestinal epithelial regeneration[14], TGF-β/SMAD involved in cell proliferation, differentiation, migration and apoptosis[13,15], Notch signaling involved in intestinal epithelial proliferation[16], and Hedgehog signaling involved in cell proliferation[17]. These and others have been identified as improperly functioning due to one or several parts of the pathway containing a detrimental mutation creating a hyper- or hypoactive pathway leading to cancerous formation. Dysregulation in these pathways and others related to CRC can also create loss of function of pathways involved in cellular apoptosis and general tumor suppression.

Acquiring the types of mutations that result in cancer formation has been related to many factors identified as high-risk environmental factors. Risk factors can include a range of habits from dietary to lifestyle. Some of the most notable include smoking[18,19], alcohol consumption[19], excess body weight[20], and poor diet (high consumption of processed meat, low fruit/vegetable consumption, and low dietary fiber consumption)[21]. All these factors have been linked to increased risk of developing various types of cancer, including colorectal cancer though the mechanism through which these can increase risk is unknown to some extent.

To study the mechanisms of cancer progression and treatment, various model systems are used with associated benefits and pitfalls for each. One of the least invasive methods to study CRC are statistical analyses to identify strong correlations between cancer risks and genetic, environmental, and lifestyle habits[12]. These type of bioinformatic assessments give important insight and direction into future studies to further investigate these correlations with *in vitro* and *in vivo* methods. *In vivo* methods can be done in mice, one of the most common model organisms. Mouse models are continually used due to their many homologous genes and diseases that can model those present in humans. In the case of CRC, mouse models have been used to study the role of oncogenes like K-ras through the creation of knockout and knockdown models[22]. *In vitro* models can also be used for many studies using cell culture methods to look in-depth at the cellular responses. CRC cells in culture have been used to research many colorectal cancer therapies and mechanisms.

The SW480 cell line is a Human Duke's type B (stage II) colorectal adenocarcinoma which has been used to study the interaction with *Ferulago* species of bacteria originating from Turkey[23,24]. This line has also been the model for research into various potential anticancer compounds such as quercetin[25] and resveratrol[26]. SW480 cells have also been used to study interactions of receptors such as the estrogen receptor β and its role in tumor repression[27]. The LoVo cell line is a Human Duke's type C (stage III) colorectal adenocarcinoma cell line that has also been utilized as an *in vitro* model to develop a 3-D spheroid model for colorectal cancer[28] to more closely mimic the tumor environment *in vitro*. LoVo cells have also been used to study mutations associated with CRC like in the endoprotease furin[29], as well as responses to potential anticancer compounds like baicalein and luteolin[30]. In this study, cell culture was also used as the model of choice to understand at a fundamental level, the effects of the microbiome

macromolecular microbial components and metabolites on CRC. This fundamental look into interactions at the cellular level will expand on the findings of *in vivo* and computational studies to pave the way to potential anticancer strategies through specific microbial interactions.

Colorectal Cancer Diagnosis and Treatments

Colorectal cancer, having a high incidence rate, requires early diagnosis and effective treatments for optimal patient outcomes. Diagnosis can occur with a variety of methods including the most common and widely accepted, colonoscopies. Colonoscopies can also be useful in the collection of biopsy samples and in reducing the incidence of CRC mortality through colonoscopic polypectomies[31,32]. This procedure allows for both rapid diagnosis as well as the potential for CRC prevention. Additional diagnostic tools include capsule endoscopy and CT colonography in addition to less invasive methods such as the detection of CRC biomarkers present in the blood, stool, and urine of patients[33].

Treatment options for CRC once identified are limited and are often the focus of research in this field to increase patient outcomes after diagnosis. The current standards of treatment include surgical intervention to remove all traces of the tumor and metastases as well as chemotherapy as either a supplemental treatment post-surgery to increase effectiveness, or as first-line treatments prior to surgical intervention[34–36]. In many cases these current treatments are not enough or in the case of chemotherapy, cause severe side-effects for the patient. For these reasons, continued research efforts are devoted to finding alternative treatments that are more effective and cause less severe side-effects. Immunotherapies are becoming one such treatment option that have been showing great promise in their long-term effectiveness against certain cancers. In CRC specifically, there has been some promise shown with anti-PD-1 therapies that had limited success with re-induction therapy to invoke the host immune response[4]. Problems

occur in tumors with high levels of mutations that lead to overcoming even anti-PD-1 therapies[5]. In many cases, the metastatic migration of cancer cells causes the main issue for all current therapy types. These treatment options are promising even with the known drawbacks, but this highlights the important need for more treatment options for individuals diagnosed with CRC.

Colorectal Cancer and the Microbiome

In the direction of combatting colorectal cancer, the microbiome has received attention, especially in recent years. Intense research into this area in recent years has uncovered numerous causal links between microbial composition and function, unveiling the etiology of many diseases, disorders and conditions and have further shown the molecular mechanisms behind these links. The microbiome has been demonstrated to work through various mechanisms which include modulation of the immune system through signaling to directly induce T-cell differentiation in the gut[37], as well as through their normal metabolic pathways that result in metabolite release into the body that can also play a role in T-cell development[38]. Manipulating this interplay between the microbiome and the immune system is the primary avenue to both prevent and combat human diseases. Fasting is one method that is showing promise through the increase in bacterial diversity in the gut as well as the specific outgrowth of bacteria that show therapeutic effects in diseases like multiple sclerosis (MS)[39]. Other methods of altering the microbiome are through pro- and pre- biotic treatment to directly introduce bacteria of interest. This can be an effective approach to supplement therapeutic bacteria into the human gut.

As it relates to CRC, the microbiome has been studied with the mindset of determining correlations with the various microbes and outcomes of the disease with the intention of finding bacteria that may have anticancer effects. Summaries of findings show an overall decrease in diversity in the microbiome of CRC patients alongside an increase in specific bacterial

abundance such as some species of *Bacteroides* and *Prevotella* that have been linked to CRC[40]. These can outcompete known therapeutic bacteria for the growth, which typically includes short-chain fatty acid (SCFA) producers. There are also studies that suggest dysbiosis of the gut microbiome is the main cause of CRC through inflammation that induces epithelial cell transformation into cancerous cells[41]. In the same light, diet has a large impact on both the microbiome and CRC as poor diet (high consumption of processed meat, low fruit/vegetable consumption, and low dietary fiber consumption) is a known risk factor for CRC[21,42] that may alter the gut microbiome. Diet has been shown to impact the microbiome and predict bacterial enterotypes. One study showed the link between two highly common enterotypes and diet where those with diets high in protein and animal fats predominantly had *Bacteroides* present in their microbiome, while those consuming more carbohydrates resulted in higher *Prevotella* abundance[43]. Therefore, it is reasonable to link poor diet and CRC via a microbiome mediation. Regardless of the role that the microbiome plays, though it is suggested to be a pivotal role in cancer progression, microbiome scientists tend to agree that the impact of the microbiome is substantial and requires investigation. This study strives to add to this pool of knowledge and aid in the discovery of the role of the microbiome in CRC as a therapeutic agent.

MATERIALS AND METHODS

Cell Culture

SW480 cells and LoVo cells, two human CRC cell lines, were utilized in this study donated from Dr. Daniel W. Rosenberg's lab (UConn Health Center). Each of these cell lines is a type of colorectal cancer with previous research behind them and are aggressive forms of CRC. Cells were cultured in DMEM/F12 media (Corning™ DMEM/Ham's F-12 50/50 Mix, Powder MT90091PB) supplemented with 10% Fetal Bovine Serum (Gemini, Cat# 100-106) and 1% penicillin streptomycin glutamine (ThermoFisher Scientific, Waltham, MA, USA, Cat# 10378016). Incubation was done at 37°C in 5% carbon dioxide (CO_2) in a humidified incubator.

Bacterial Species

Bacterial species used included six different bacteria already isolated and growing in Dr. Yanjiao Zhou's lab (UConn Health Center). Three *Lactobacillus* species including *L.* reuteri (ATCC 23272), *L.* johnsanii (ATCC 33200), and *L.* intestinalis (ATCC 49335) were all used and grown in both MRS media (MRS Broth modified, Vegitone, Millipore Sigma, 38944-F) supplemented with tween 80 (Millipore Sigma P4780) and TSB (Tryptic Soy Broth, Millipore Sigma, T8907). TSB was used prior to conditioned media creation as MRS broth is an acidic selection media for *Lactobacillus* species. *Prevotella copri* (DSM 18205) was isolated and grown in BHI media (Thermo Scientific™ Brain Heart Infusion Broth (Dehydrated), CM1135B) supplemented with 10mL/L Hemin Solution (Millipore Sigma H9039) and 0.2mL/L Vitamin K1 Solution (Millipore Sigma, 95271). Two *Bacteroides* species, *thetaiotaomicron* (ATCC 29148) and *uniformis* (ATCC 8492), were also grown in BHIS media. BHIS media was created using the ATCC formulation 1293 Brain heart infusion-supplemented (BHIS), which uses the BHI base supplemented with Hemin and Vitamin K1. All bacteria are anaerobic and were therefore

grown in a Bactronez anaerobic chamber with an anaerobic gas mixture of 5% carbon dioxide, 5% hydrogen, and 90% nitrogen from AirGas.

Conditioned Media

Conditioned media was made for each of the bacteria less than one week prior to use. Bacteria were grown in their appropriate broth media at a final volume of 50mL. *Lactobacillus* species were grown for 48 hours prior to isolation of supernatant, while the *P. copri* and *Bacteroides* species required only 24 hours before they were ready to process. After the growth period, the tubes were sealed and spun for 5 minutes at 1,000xg before being filtered through a 0.2um polyether sulfone (PES) filter. The cell-free bacterial supernatant preparation was adapted from previous studies[44,45]. After preparation of cell-free bacterial supernatant was complete, three conditioned media formulations were made containing 50%, 25%, and 10% bacterial supernatant in complete F12 cell media which was stored at 4°C until used within a week of creation. Sterile media was used to create the same three concentrations of control conditioned media to exclude effects of the bacterial media on the CRC cells.

Cell Proliferation Assessment

Effects of the conditioned media on each CRC cell line was observed via confluence and morphological changes. Baselines of each cells' growth was noted to establish normal growth confluence changes and morphology. Additionally, the control conditioned media was used to exclude effects of the bacterial media on the CRC cells. Cell counts and viability determinations were preformed after confirmation of effects using the Countess™ II Automated Cell Counter to avoid bias during cell counting and maintain consistency both before and after the addition of conditioned media. Trypan blue was used for viability determination. Graphs were created using the GraphPad Prism 9.1 software.

RESULTS

Growth Changes with *Lactobacillus* Conditioned Media

As both a confirmatory test for the model system used, and an experimental test, several *Lactobacillus* species were used to create conditioned media. Both SW480 and LoVo cells were grown with the varying concentrations (50%, 25%, and 10% bacterial supernatant) alongside TSB controls to exclude bacterial media related effects on growth. Only one well of a six-well plate was used for each of the conditions. *L.* reuteri and *L.* johnsanii species showed no visible effect on the growth or morphology of either cell lines after five days.

L. intestinalis was also tested and showed slight growth reduction in SW480 cells. As shown in Figure 1, a comparison between the TSB controls (Figure 1A, C, and E) and the *L.* intestinalis (Figure 1B, D, and F) shows slight confluence reduction between 3-5% in most cases. Each concentration shows two images of the same well in different locations to ensure uniformity throughout the well and avoid misleading results due to varying cell confluence across the well. Comparing Figure 1E and 1F, 10% TSB and 10% *L.* intestinalis respectively, there is a more noticeable difference of 10-15% confluence change. In addition to the confluence changes, cell morphology is noticeably more spherical indicating potential attachment issues in the cells cultured with *L.* intestinalis conditioned media. This morphology is present in the TSB controls, but to a lesser extent indicating either growth delay and delay in attachment, or prevention of growth and/or attachment.

Similar results are indicated with the LoVo cell line, but current results are unclear. Grown in parallel with the SW480 cells, the LoVo cells were plated in varying concentrations of the *L.* intestinalis conditioned media and TSB control conditioned media to visualize changes in growth and/or morphology. In Figure 2A, C, and E, cells were grown in 50%, 25%, and 10%

TSB respectively as points of comparison for growth in the same concentrations of bacterial conditioned media. Figures 2B, D, and F show growth in 50%, 25%, and 10% of *L.* intestinalis conditioned media. When comparing the growth conditions, changes are subtle and may be related to the bacterial supernatant or may be a result of outside factors. The 50% wells show some confluence reduction of approximately 5%, however, no other concentrations have noticeable reduction in confluence. Additionally, no clear morphological changes can be seen in any concentration of bacterial supernatant.

SW480 Cell Response to Bacterial Conditioned Media

In addition to *Lactobacillus* species, the SW480 cell line was tested with three other bacteria including *Bacteroides uniformis*, *B. thetaiotaomicron*, and *Prevotella copri*. Noticeable differences were seen with all three bacterial conditioned medias. In terms of morphological changes, the 10% *P. copri* supernatant well showed a clear change from individually attached cells to clustered cell groups (Figure 3). The morphology change is clear starting at day three post-plating (Figure 3A and B) and continued to move towards tumor-like formations at day five (Figure 3C and D). This was the only morphological change seen specifically in the SW480 cells with 10% *P. copri* supernatant in the growth media.

Confluence changes were tracked for three timepoints at day two, three, and five post-plating (Figure 4). Increases in confluence can be seen with all control BHIS groups, but most notably with 10% BHIS. This may indicate slight inhibition of growth due to the bacterial media supplementation with the standard F12 growth media. *B. uniformis* conditioned media shows reduced confluence overtime in the 50% and 25%, while the 10% group shows an increase comparable to the 10% BHIS control group. Potential growth inhibition can be seen at the higher concentrations of *B. uniformis* conditioned media. Observing the *B. thetaiotaomicron* and *P.*

copri conditioned media confluence changes, similar results to each other are found. Confluence appears to increase slightly, but plateau at about 50% confluence. In all cases excluding the 10% *B. thetaiotaomicron* group, the confluence percentages are lower than all BHIS control groups indicating some effect for almost all groups. This is corroborated with live cell counts showing similar changes comparing the cell counts of the control groups with the *B. uniformis*, *B. thetaiotaomicron*, and *P. copri* groups (Figure 5). Viability did not appear to correlate with any bacterial conditioned media (Table 1).

LoVo Cell Response to Bacterial Conditioned Media

Similarly, to the SW480 cells, LoVo cells were tested under the same conditions in parallel. Unlike the SW480 cells however, the LoVo cells showed unclear and inconsistent results with conditioned media from *B. uniformis*, *B. thetaiotaomicron*, and *P. copri*. No morphological changes were visible, and cells had an overall low density in all conditions including control plates.

Looking at confluence changes, effects of the conditioned media was unclear. In all control BHIS groups, confluence had a net loss indicating there may be an effect of the BHIS media itself on the LoVo cell growth, or outside factors contributed. The cell counts substantiate the role of outside factors on the cell growth showing an increase in live cells only in the 25% BHIS control group, yet a loss in live cells in all other groups. Viability was on average, lower than SW480 cells in the same conditions, but does not show any relationships with either the bacterial conditioned media types, or the overall lower cell counts.

DISCUSSION

The goal of this screening study was to determine if bacteria commonly found in the human gut had an anticancer effect on colorectal cancer cells in culture. Determination of bacterial species that may exhibit therapeutic effects in relation to CRC can direct future studies. Using isolated bacterial supernatants to create conditioned media, we found potential therapeutic candidates that likely secrete a metabolite able to reduce CRC growth in culture. Similar studies have found butyrate and other SCFAs to invoke therapeutic responses such as p21 mediated or ferrichrome induced apoptosis[6,11]. Similar mechanisms may be used by the identified anticancer bacteria in this study including *Lactobacillus* intestinalis, *Bacteroides uniformis*, *Bacteroides thetaiotaomicron*, and *Prevotella copri*.

Three *Lactobacillus* species were tested, being reuteri, johnsanii, and intestinalis. Despite *Lactobacillus* being a family of bacteria that is associated with therapeutic responses to cancer[11,44], only one strain, intestinalis was identified as showing a slight therapeutic effect. This was observed with 10-15% confluence reduction in the SW480 cell line, but only a 3-5% reduction with the LoVo cell line (Figure 1 and 2). These results were seen with the 10% and 50% *L.* intestinalis media with SW480 cells and LoVo cells, respectively. This indicates *L.* intestinalis may share the positive effect with previously studied *Lactobacillus* species. From current results it is not clear whether *L.* reuteri or *L.* johnsanii have similar effects, but more evidence may support this.

The remaining three bacteria tested, *B. uniformis*, *B. thetaiotaomicron*, and *P. copri*, all showed stronger results than the *Lactobacillus* species tested. In the SW480 cell line, several noteworthy observations were made when cultured with the *P. copri* conditioned media. A clear morphological change can be seen in Figure 3B and D, where the 10% *P. copri* conditioned

media likely caused the SW480 cells to grow into tumor-like clusters. This is atypical of this cell line as shown in Figures 3A and C where individual cells are easily observed with an epithelial-like morphology. This morphological change did not, however, occur in any other concentrations of *P. copri* conditioned media or any other growth condition indicating this may be an isolated event, but still warrants further investigation. Additionally, the LoVo cell line did not exhibit any clear morphological changes with any of the conditioned medias.

Alongside morphology, cell confluence changes were monitored during cell culturing with conditioned medias, and cell counts were determined after five days in culture. Conclusions can be drawn from the results of the SW480 cell line experiments, however, the results of the LoVo cell line are unclear. Cell counts from the control groups are unclear and therefore comparisons are unable to be made. This may be due to significant effects of the BHIS media on the LoVo cell growth, however, additional experiments must be run to confirm this. In the SW480 cell line, a potential reduction in growth may have been observed due to the bacterial control media group, as the 10% BHIS control group showed the largest confluence increase overall in these cells (Figure 4). Analyzing the cell counts, the initial plated cell count was $1x10^6$ cells, indicated by the grey bars in Figure 5. As shown by this figure, all conditioned medias appear to have reduced growth in the SW480 cells when compared to the BHIS controls. There also appears to be a dose-dependent effect moving from 10% conditioned media to 50% that is observed to a lesser extent in the BHIS control groups. This could be a result of higher concentrations of the key therapeutic metabolite that each bacterium is producing, leading to cellular death in the CRC cell line. Key therapeutics could range from SCFAs like butyrate that has been identified as therapeutic[6,9,10] or other potential unidentified compounds that may have anti-inflammatory affects, or otherwise affect the CRC cells more like butyrate through p21

mediated apoptosis. This could also be attributed to high concentrations of lipopolysaccharide (LPS) which is a known component of gram-negative bacterial outer membranes. Other studies investigating the microbiome have identified the *Bacteroides* and *Prevotella* families of bacteria to be therapeutic through both meta-analysis[40] and through correlations with lessened MS disease severity[46]. One unpublished work from Dr. Zhou's lab implicates increased red meat intake with reduced *B. thetaiotaomicron* and increased severity of MS, also showing *B. thetaiotaomicron* as a potential therapeutic bacteria[47]. Evidence of other *Bacteroides* species exhibiting pro-inflammatory responses, however, such as *B. fragilis* that have also been linked to CRC[41,48]. Additional research must be done in relation to CRC to show the interactions in this type of cancer as either therapeutic or antagonistic overall.

Moreover, the results obtained through this study pave the way for future studies to further the knowledgebase of the microbiome and CRC. An avenue for continuing includes exploring the interactions between the microbiome and T-cells specifically as there is already evidence of the microbiome influencing T-cell differentiation in the gut[37,38]. Utilizing *in vivo* mouse studies to colonize with each individual bacterium followed by subsequent isolation and characterization of the T-cells could discern alternative mechanisms through which the tested bacteria can play a therapeutic role in the context of CRC. In the same light, further *in vitro* testing can be done on both T-cells in culture and non-cancerous colorectal cells. Observing and characterizing T-cells in culture can give insight into the direct interactions between the bacterial metabolites and immune cell differentiation. Observations of non-cancerous cells would show any potential adverse effects on surrounding tissue if bacterial therapies were to move forward as a potential therapeutic for CRC patients.

In summary, the results obtained from this screening study are limited, but justify the further exploration of how the microbiome interacts with colorectal cancer. The bacteria that show noticeable differences in cell morphology and cell numbers, after conditioned media treatment, especially warrant further investigation to confirm the results of this study and determine potential mechanisms for their inhibition of tumor cell growth. Many potential treatments for CRC and other human cancers are being explored, and the microbiome is yet another promising avenue.

FIGURES

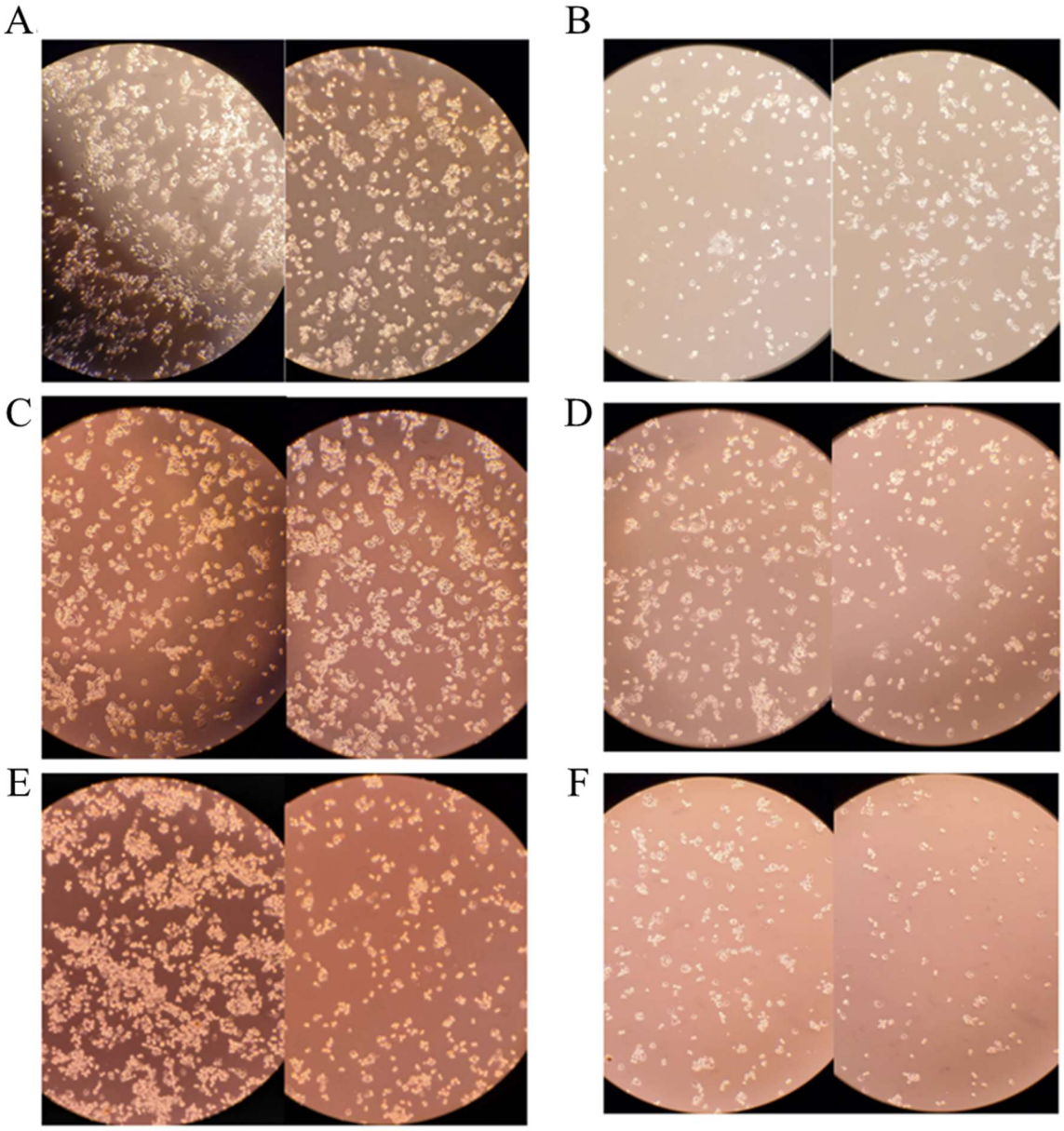

Figure 1: Images depicting the growth of SW480 cells in TSB control and *L.* intestinalis conditioned media three days after plating. A, C, and E show the TSB control conditioned media at 50%, 25%, and 10% TSB, respectively. B, D, and F show the *L.* intestinalis conditioned media at 50%, 25%, and 10% *L.* intestinalis supernatant, respectively.

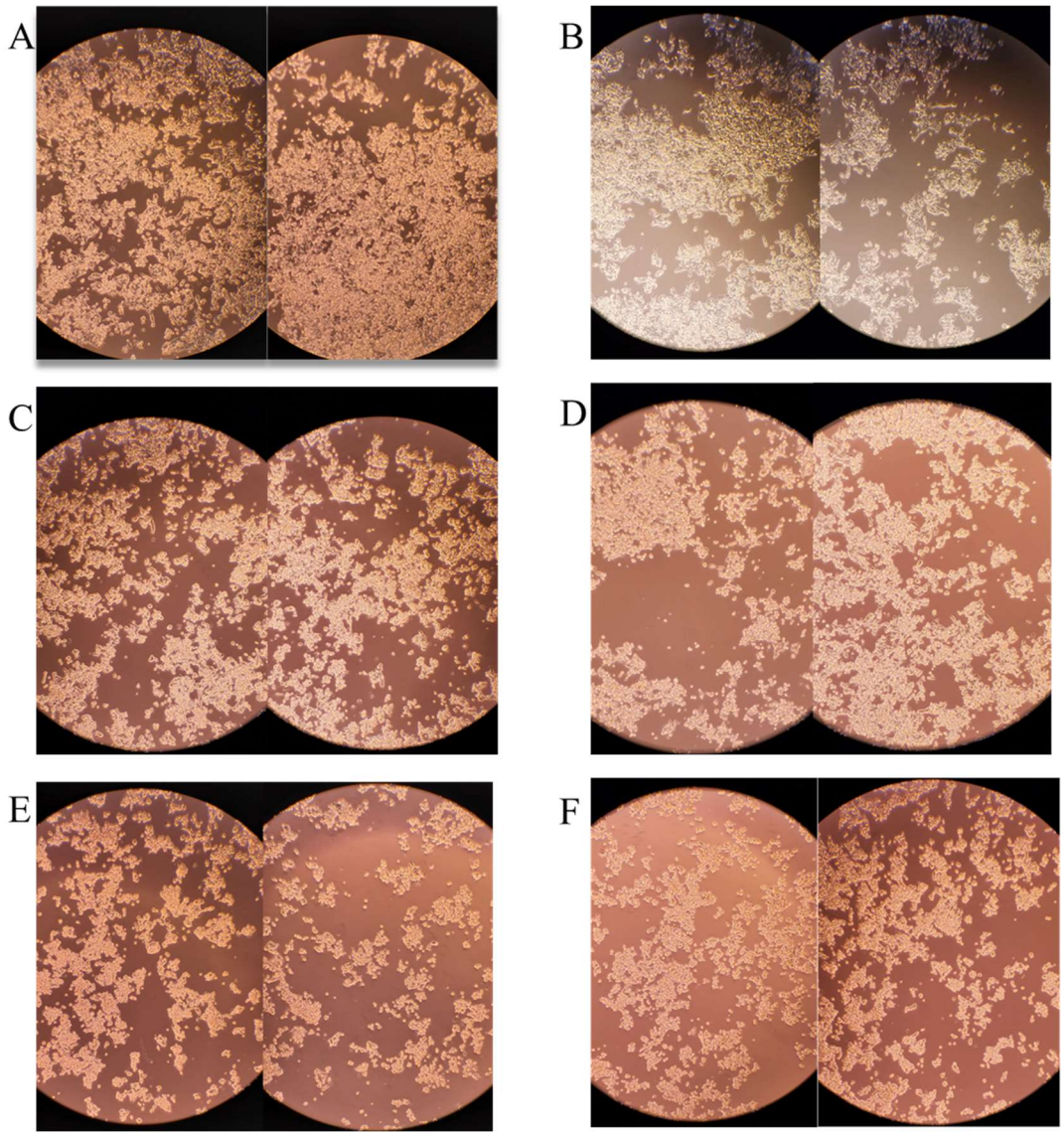

Figure 2: LoVo cell growth three days after passaging into conditioned media. A, C, and E show TSB control conditioned media at 50%, 25%, and 10% concentrations, respectively. B, D, and F depict growth in 50%, 25%, and 10% concentrations of *L.* intestinalis conditioned media, respectively.

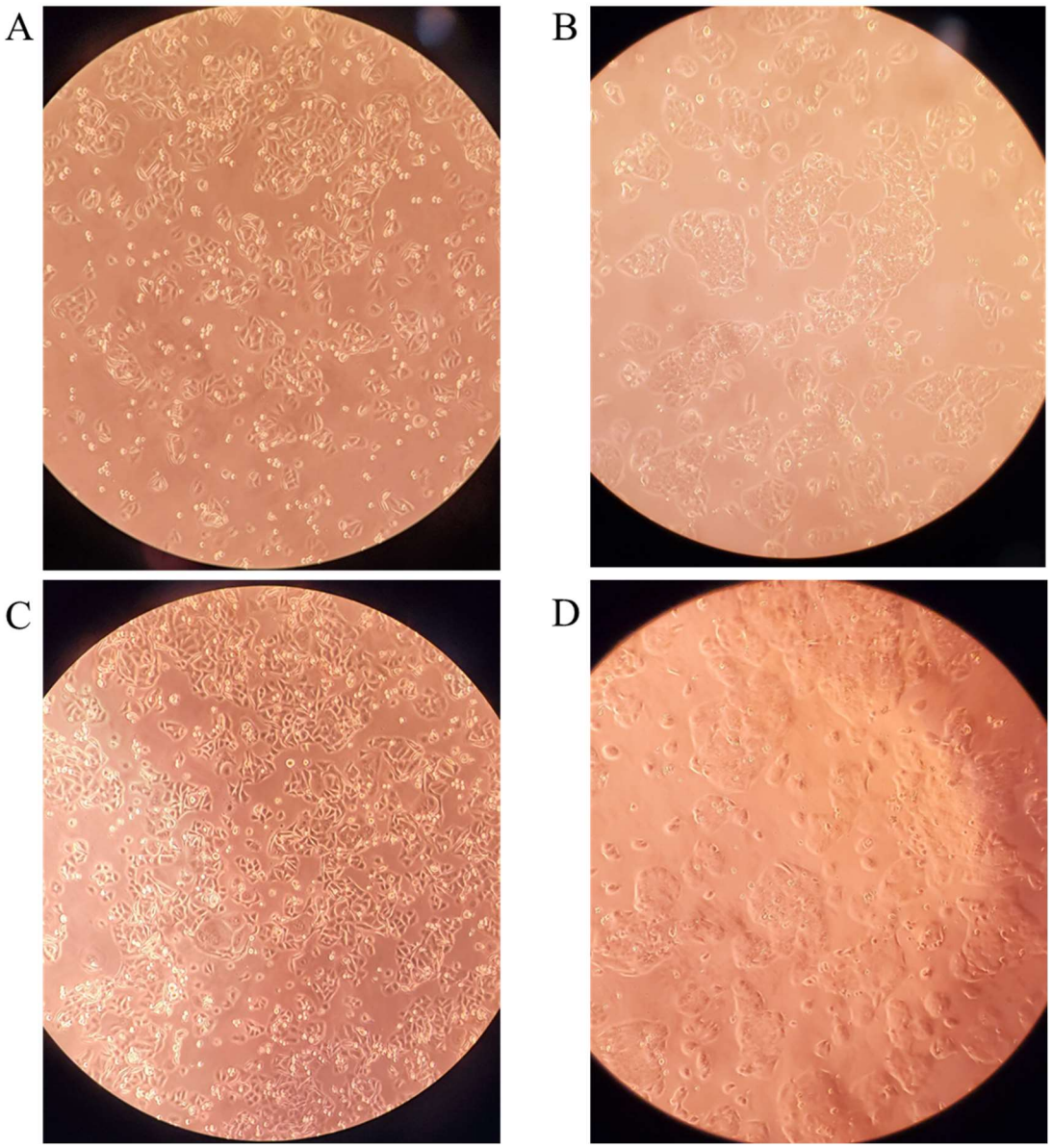

Figure 3: Morphology of SW480 cells in BHIS control and *P. copri* conditioned media. (A) BHIS control conditioned media at 10% BHIS three days after plating. (B) 10% *P. copri* conditioned media three days after plating. (C) 10% BHIS control conditioned media five days after plating. (D) 10% *P. copri* conditioned media five days after plating.

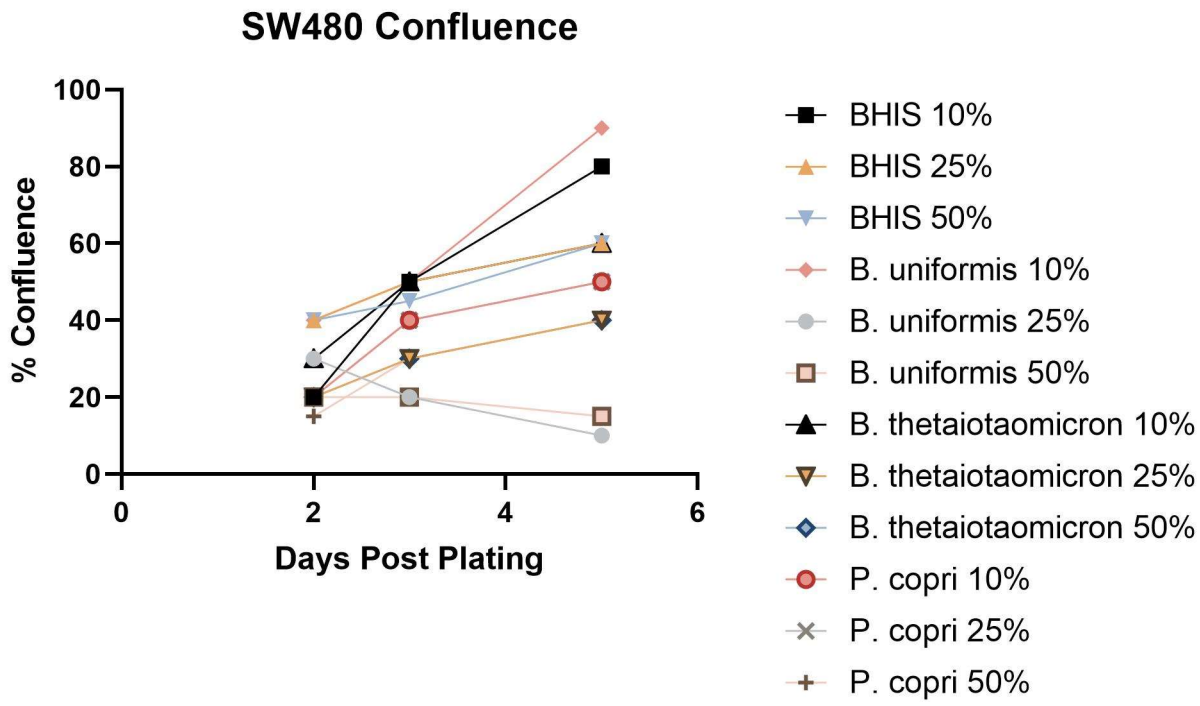

Figure 4: Descriptive graph of SW480 cell confluence changes over the course of five days being cultured in conditioned media. Cells were grown in a six-well plate and seeded at $1x10^6$ cells per well in their respective medias.

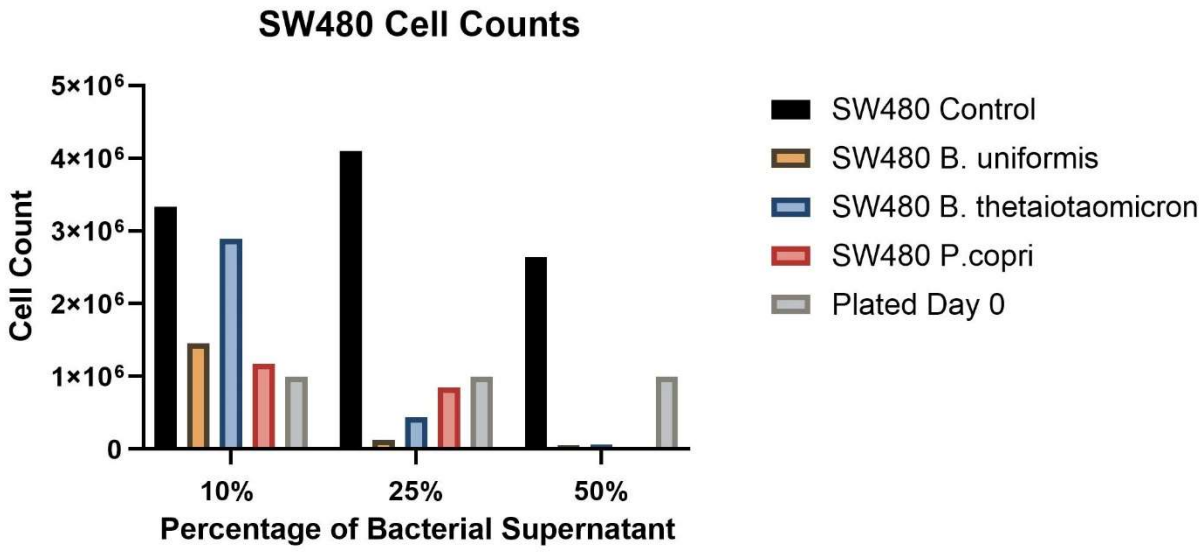

Figure 5: Descriptive graph of cell counts of live SW480 cells plated in varying conditioned media including BHIS (SW480 control), *B. uniformis* supernatant, *B. thetaiotaomicron* supernatant, and *P. copri* supernatant alongside the initial plated cell count of $1x10^6$ cells. Data represented is only one replica.

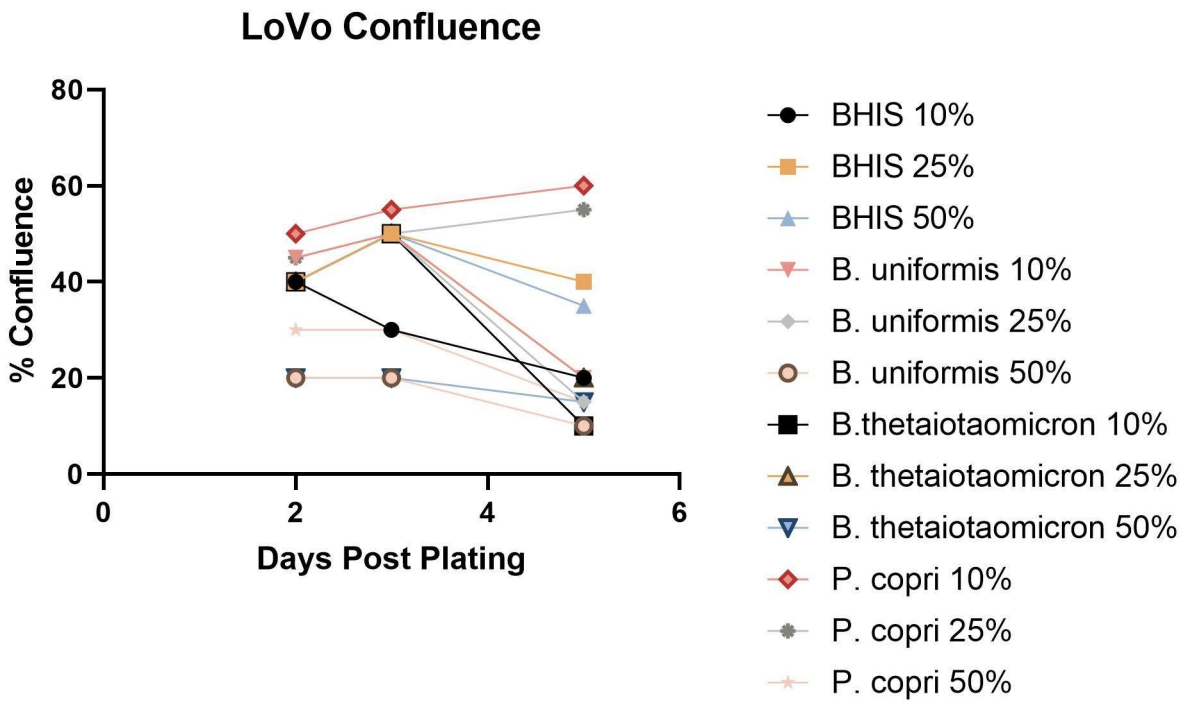

Figure 6: Descriptive graph of LoVo cell confluence changes over the course of five days being cultured in conditioned media. Cells were grown in a six-well plate and seeded at $1x10^6$ cells per well in their respective medias.

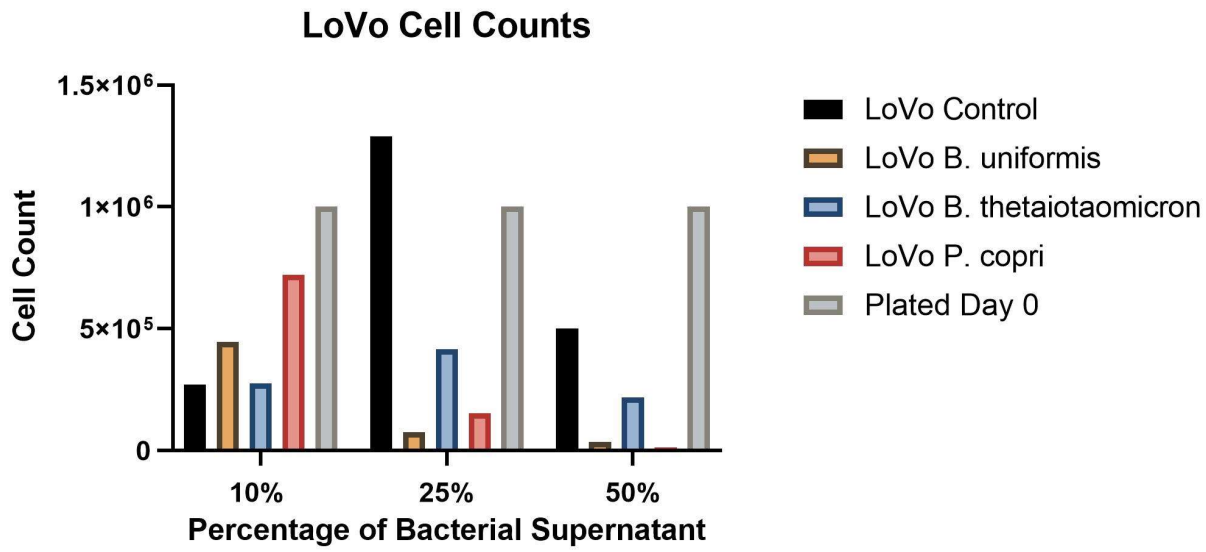

Figure 7: Descriptive graph of Live cell counts of LoVo cells plated in varying conditioned media including BHIS (LoVo control), *B. uniformis* supernatant, *B. thetaiotaomicron* supernatant, and *P. copri* supernatant alongside the initial plated cell count of $1x10^6$ cells. Data represented is only one replica.

	SW480 Cells		LoVo Cells	
Condition	**Live Count**	**Viability**	**Live Count**	**Viability**
BHIS 10%	3.33E+06	98.00%	2.70E+05	98.00%
BHIS 25%	4.10E+06	100.00%	1.29E+06	80.00%
BHIS 50%	2.64E+06	94.00%	5.00E+05	91.00%
B. uniformis 10%	1.45E+06	100.00%	4.46E+05	92.00%
B. uniformis 25%	1.29E+05	55.00%	7.62E+04	92.00%
B. uniformis 50%	5.28E+04	100.00%	3.52E+04	50.00%
B. thetaiotaomicron 10%	2.89E+06	100.00%	2.76E+05	96.00%
B. thetaiotaomicron 25%	4.34E+05	86.00%	4.16E+05	93.00%
B. thetaiotaomicron 50%	6.45E+04	82.00%	2.17E+05	92.00%
P. copri 10%	1.17E+06	100.00%	7.21E+05	100.00%
P. copri 25%	8.45E+05	89.00%	1.52E+05	88.00%
P. copri 50%	5.86E+03	100.00%	1.17E+04	50.00%

Table 1: Live cell counts, and viability percentages determined via trypan blue staining for each cell line under each condition. Cell count values used in Figure 5 and Figure 7.

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