Investigation of the Mechanism by Which Bacteria Induce Dedifferentiation in Eukaryotic Cells

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ABSTRACT

This proposal describes experiments which observe the mechanism of the dedifferentiation of eukaryotic cells by bacteria**.** *H. pylori, L. acidophilus* and *M. leprae* all trigger dedifferentiation in their host cells to generate an environment most beneficial for their survival. Dedifferentiation of the host cells will be observed histologically and by TEM to see how the bacteria are physically interacting and signaling with the host cells. PCR, western blotting and RNAseq will be used to assess gene expression. Understanding the mechanism by which these bacteria dedifferentiate their host cells will give insight into how certain prokaryotes manipulate their hosts to fuel their growth.

INTRODUCTION

Eukaryotic cells have the ability to make much more complicated and intricate organisms than domain bacteria and archaea because of their cooperative capability to form multicellular organisms. These organisms consist of a variety of specialized cells that are all involved in different processes, and all of which contribute to maintaining a homeostatic condition. The process by which cells gain different components to increase functionality for their specific task is known as differentiation. Differentiation occurs through the expression of different genes. It is known that all cells in a eukaryotic organism contain the same genes, but each unique type of cell expresses these genes in its own specific way. Some mechanisms that encourage the downregulation and upregulation of certain genes include epigenetic regulation, RNA processing, and chromatin remodeling. While these mechanisms of differentiation occur throughout the lifespan of an organism, certain mechanisms that determine the fate of a cell occur early in development. Some vital genes that guide the growth of an organism are homeotic genes. Homeotic genes, or HOX genes, determine the positional identity and define the central axis of bilateral organisms (Seifert et. al 2015). Transcription factors, coded for by HOX genes, trigger the expression of morphogens that control the specialization of undifferentiated cells (Payne et al. 2013). These molecules can individually alter the fate of not only a single cell but also surrounding cells to induce specific forms of patterning (Sado and Tumbar 2013)

While all of this occurs in multicellular eukaryotes, prokaryotes are single-celled organisms that do not need differentiation or the determination of axes. However, they possess an unusual capability to produce and mimic the factors of differentiation in eukaryotes. Some bacteria, instead of differentiating themselves, induce the differentiation of eukaryotic cells through induction. This occurs through mechanisms that produce embryonic and senescence markers while influencing the homeobox. This can cause the reprogramming of cells in vivo, putting them in a transitional/progenitor-like cell state (Ito and Ohta 2015). This phenomenon is known to occur in *Helicobacter pylori, Lactobacillus acidophilus* (LAB), and *Mycobacterium leprae*.

Helicobacter pylori are helical bacteria cells that colonize over 50% of the global human population's intestines (Brown 2000). They have multiple virulence factors, including cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) (Anasari and Yamaoka 2020). CagA and VacA are genes that lack homologues across the other 2 prokaryote types and cause a surge in an infection's cancerous potential. CagA and VacA could be a possible differentiating factor when it comes to the dedifferentiation mechanism of the prokaryote. *H. pylori* utilizes urease to convert urea to ammonia and carbon dioxide which act as a buffer within the low pH of the stomach (Sachs et al. 2001). The infection of *H. pylori* in the human body can have a

broad array of outcomes from mucosa-associated lymphoid tissue (MALT) to extremely common ulcers and gastric adenocarcinoma (Keikha et al. 2022).

Lactobacillus acidophilus are facultatively anaerobic bacteria that play a large role in the human gastrointestinal tract, producing lactic acid from fermented carbohydrates (Dempsey and Corr 2022). They maintain an acidic environment which prevents the growth of other infectious pathogens like *E. coli* (Theriot and Young 2016). The genome of *L. acidophilus* is rather short, around 2.0 Mb, with the main focus of the genes being on immune adaptation, epithelial attachment, and lactic acid production through carbohydrate metabolism (Johnson and Klaenhammer 2014). Studies have shown the development of clusters (further discussed in 4.3.b) that specifically incorporate *L. acidophilus* into groupings of eukaryotic cells.

Mycobacterium leprae (*M. leprae*) is an obligate pathogen that is highly attracted to Schwann cells and macrophages, the key components of the neural and immune systems (Hess and Rambukkana 2019). It is an extremely slow-growing bacteria and its incubation period can last for years. It also has a selective zoonotic potential, meaning that using model organisms to study the infectious capabilities of *M. leprae* is extremely hard due to its lack of infectious ability.

Experimental Design

H. pylori, L acidophilus (LAB), and *M. leprae* all target a different type of eukaryotic cell. The specific dedifferentiation mechanism that puts the target cells into a pluripotent stage upon infection by these species of bacteria will be observed. Because of its capability of dedifferentiating its target cell type (gastric epithelium) into three different layers of germ tissue, the main focus will be on *L. acidophilus* (Ito and Ohta 2015). The other bacteria will be identified and labeled by the germ layer they infect.

A culture with clusters (amalgamations of bacteria and eukaryotes) will be developed ensuring that all the appropriate conditions are met to trigger the specific dedifferentiation mechanisms for each species. Then to identify the gene expression patterns that are correlated with dedifferentiation, RNAseq/PCR and protein western blot will be utilized. To ascertain and confirm that dedifferentiation has occurred, cells will be examined histologically both before and after differentiation.

Western blot of cell surface markers will be used to determine the extent of differentiation. It will also be used to identify the mechanism by which dedifferentiation occurs by tracking proteins that are released by the bacteria and their concentrations. Examination of physical contact between the bacteria and host cells to determine whether the signaling is paracrine or juxtacrine will be examined by immunofluorescence and electron microscopy (determine the distance between cells to differentiate between cell to cell or small distance; juxtacrine will involve cell to cell whereas paracrine has signaling molecules that will move a distance). This will involve the usage of modeling tools to determine the approximate distance between the two cells, giving a clear identifier of the type of signaling that is occurring. To identify the receptors through which the bacterial substances interact, immunoprecipitation will be carried out, followed by mass spectroscopy to identify probable receptors on the cell membrane. Once the receptors are identified, phosphorylation of specific proteins in response to cell signaling will be detected by proteomic analysis. Gene expression will be examined by RNAseq.

METHODOLOGY

Bacterial Culture

Lactobacillus acidophilus

L. acidophilus are facultative anaerobes, meaning that they can thrive in both oxygen-rich and oxygen-poor environments. They also thrive in low PH, high carbohydrate density environments, in the presence of compounds involved in anabolism (Axelsson & Ahrné, 2000). This makes the infectious capability of lactic acid bacteria greatest in the stomach which fulfills all of these conditions (Troche et al. 2022).

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Since facultative anaerobes can thrive in oxygen-rich and oxygen-poor environments, the conditions of the culture are flexible. If the culture is conducted in an oxygen-lacking environment, an anaerobic glove box with an attached incubator will be used. The "SHEL LAB BACTRON300 Anaerobic Chamber" maintains high relative humidity and creates a no-moisture environment without the usage of artificial desiccants (Sheldon Manufacturing: LabRepCo). To maintain the simulated anaerobic environment, a vacuum pump will remove air and replace it with an inert gas of choice, such as nitrogen.

L. acidophilus will be grown in MRS broth medium (FisherSci) (Pal et al. 2010; Mészáros 2022). MRS is composed of proteose peptone, yeast extract, dextrose, polysorbate, ammonium citrate, magnesium sulfate, and dipotassium phosphate.

If the culture is being grown in an aerobic environment, a shaker incubator is required to allow for an equal supply of oxygen through the medium (Mészáros 2022). The incubation process for anaerobic bacteria varies primarily because the glove box/chamber will need an attached incubation to make sure oxygen or traces of oxygen don't make their way to the culture. Some methods to ensure that the only bacteria grown in the MRS broth are *L. acidophilus* is to utilize antibiotics to which *L. acidophilus* is resistant like norfloxacin and nalidixic acid (Gupta et al. 1995). Phages that do not target *L. acidophilus* can also be added into the media post autoclaving to prevent denaturation during this sterilization process (Mészáros 2022).

L. acidophilus will be procured from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, which provides a fully sequenced genome, relevant literature, and data sheets for further reference.

Helicobacter pylori

H. pylori is also a facultative anaerobe making it possible to manipulate the culture between aerobic and anaerobic (Percival and Williams 2014). The processes to ensure a non-oxygenated or an oxygenated environment for *H. pylori* are the same as those described in 4.1.b for *L. acidophilus*. It is grown on solid media, usually Brucella or Colombia agar, with cyclodextrins that have self-assembly functions in solution, allowing them to create aggregates as a result of hydrogen bonding (Cui et al. 2024; Olivieri et al. 1993; Andersen and Wadström 2001).

H. pylori from the gastric mucosa of *M. nemestrina* will be obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH. *H. pylori* itself is classified under Risk Group 2 according to German TRBA guidelines because it causes an infectious disease, meaning it will need to be handled with proper protocols (DSMZ, 2024) (Biostoff-Verordnung, Bundesministerium der Justiz). While Risk Group 2 means that diseases caused by *H. pylori* are not of a serious threat level and usually have therapeutic interventions, the proper precautions include PPE, BSCs, and heat-based sterilization (PHE, 2015).

Mycobacterium leprae

M. leprae, the bacteria known to cause leprosy, was unculturable in vitro up to the late 70's but modern technology has opened up previously hidden avenues (Pattyn 1977). There is a reliable cultivation procedure involving M. leprae and Dubos-Lowenstein-Jenson medium (Biswas 1989). If the Dubos-Lowenstein-Jensen culture is utilized, then the culture would need to be grown at 27-30°C and plaques will be observed in the culture (this tries to recreate lesions or damaged tissues, which are infected by multibacillary leprosy) (Smith 2023).

Bacterial Growth

As mentioned above, with selective media a pure culture is attainable. However, other than a selective medium there are two methods by which a pure culture can be obtained and for the sake of culturing these bacteria, streaking will be used.

With the respective media for the bacteria (MRS broth, Brucella agar, Colombia agar, Dubos-Lowenstein-Jensons), the pellet of bacteria should be rehydrated and transferred to the broth. For facultative anaerobes,

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retrieve and transfer the bacteria in a totally anaerobic environment (proper hood/box) (ATCC 2013). Inoculate the petri dishes that have been prepared beforehand and incubate at 37°C overnight (Karcher 1995).

Cell Culture

The specific type of cells that will be cultured are gastric cells, skin fibroblasts, and Schwann cells for *H. pylori*, *L. acidophilus,* and *M. leprae* respectively (Ito and Ohta 2015).

H. Pylori and Gastric Epithelia

The gastric/gastrointestinal porcine tissue from both the large and small intestine needed for the *H. pylori* infection will be obtained through purchase from BioIVT. The gastric epithelium cells will be detached in Hank's Balanced Salt Solution (HBSS) (ThermoFisherSci) containing collagenase, dispase, and soybean trypsin inhibitor supplements along with 1.25 mg/mL bovine serum (Bautista-Amorocho et al. 2021). The cleaned gastric epithelium will be resuspended and agitated before a second centrifugation under the same conditions. The cells are seeded into plates coated with a bovine collagen type 1 solution and cultured at 37° C at 5% CO2 (Bautista-Amorocho et al. 2021).

Skin Fibroblasts

L. acidophilus will be cultured with human dermal fibroblasts (HDFs) (ATCC) and grown in Human Fibroblast Expansion Basal Medium (Medium 106) (ThermoFisherSci) at 37ºC and 5% CO2.

Schwann Cells

M.leprae is known to infect mainly macrophages and Schwann cells (Moura et al. 2013). They are gram-positive bacteria and are obligate parasites that can only grow in specific cell types (Ito and Ohta 2015). Currently there are two known methods of obtaining Schwann cells; (1) isolation from a tissue with a large concentration of Schwann cells or their progenitor cells or (2) artificially create Schwann cells from the directed differentiation of induced pluripotent stem cells (IPSCs) derived from differentiated somatic cells (Monje 2020).

Schwann cells will be isolated by collecting major pelvic ganglia from male Sprague-Dawley rats (Randolph, 2021). They are digested in collagenase, dispase, and grown in proper SC medium. Plating occurs onto poly-L lysine-coated coverslips before incubation (Randolph 2021). These cells are to be plated or for microscopic observation onto coverslips and with cells that are stained for beta-tubulin, myelination Schwann cell markers. This will be done with the 66240-1-Ig monoclonal antibody that targets the Tubulin-beta Fusion Protein (obtained from PTGlab) and TUNEL (to identify fragmented DNA present in apoptotic neuronal fibers) (Hara 1996; Randolph 2021).

The best method here is indirect immunofluorescence because it makes it easier to get a high sensitivity signal with minimal noise and change the secondary antibody to alter the color output. The TUNEL (Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) will be used to identify the DNA fragments *in situ*. This experiment will use the Click-iT™ Plus TUNEL Assay Kit (ThermofisherSci) and will follow the manufacturer's protocol.

Infecting Bacterial Cells with Target Eukaryotic Cells

Infecting Eukaryotic Cultures

The bacteria that don’t require a cluster will simply be added to the eukaryotic cultures.

Clustering Eukaryotic Cultures

L. acidophilus, as with any other lactic acid bacteria, needs to be incorporated into its specific target cell, which in this case is human dermal fibroblasts (HDFs) (Ito and Ohta 2015).

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To cultivate/culture a 3d tissue culture with planktonic bacteria in a collagenic based system, the fibroblasts will need to be treated with collagen gel and incubated at 37°C for 1 hour. This is to build a scaffold supported by collagen which in turn will brace the 3d culture, simulating an extracellular matrix (Donglai et al. 2016).

Allow the eukaryotic cells to face an air-liquid interface, where the upper surface of the cells is exposed to air, during the culture and the bacteria can then be prepared in an overnight culture. Then simply add the bacteria to the eukaryotic culture (Mountcastle et al. 2020).

Observation and Identification

To track the dedifferentiation of the eukaryotic cells, RNA-seq, polymerase chain reaction (PCR), western blotting, immunofluorescence, and histology will be utilized.

Histology

I Tissue Preparation

The cells will be fixed in neutral buffered formalin (NBF) (ThermoFisherSci) (Snyder 2022). The sample will be dehydrated and the ethanol replaced by xylene (Alturkistani 2015). Then the sample will be embedded in paraffin and sectioned into sections of thickness 4-5 micrometers (Gurina 2011).

II Histochemistry

To identify the distinct intracellular inclusions of these bacteria which allow for a conjectural analysis and prediction, commercially available antibodies exist for these bacteria making immunohistochemical analysis easier.

H. pylori is often detected with a simple hematoxylin and eosin tissue stain. Yet, with *H. pylori* specifically, there are a lot of false negatives and false positives, resulting in a rate of detection of roughly 66% (el-Zimaity 1996; Toulaymat 1996). Immunohistochemistry is far more reliable than traditional histochemical techniques. The commercially available antibody that will be used for this immunohistochemical analysis is a rabbit polyclonal at a 1:20 dilution with protease 1 pretreatment obtained from polyclonal rabbit anti-*H. pylori* (Dako, Carpinteria, CA, USA) (Eyzaguirre 2006) (Akeel et al. 2021). The sections of *H. pylori* and the gastrointestinal tissue are to be treated with DAKO Target retrieval solution, which improves staining results and the retrieval of epitopes, once rehydrated. Then, with a treatment of hydrogen peroxide (to prevent endogenous peroxidase activity), PBSm(works with the peroxides), and goat serum incubation(prevents non-specific binding of antibodies), the rabbit polyclonal dilution is applied to the sections. After 24 hours, the antibodies can be detected with EnVision Detection Systems Peroxidase/DAB, Rabbit/Mouse kit (Dako) (Akeel et al. 2021).

The basophilic structures of *Lactobacillus acidophilus* can be detected with Hematoxylin and Eosin stains, however, the biofilms that follow cannot (Gurina et al. 2023). The most viable form of immunohistochemistry in this case is the Cold Ziehl-Neelsen stain, which is traditionally used to stain tissues infected with leprosy. The stain involves using a Ziehl-Neelsen carbofuschsine that is filtered and is used to directly stain the sample. The c-myc epitope in the *L. acidophilus* will be detected with an anti-c-myc mouse monoclonal antibody (Thermo Fisher Scientific) or horseradish peroxidase (HRP)-conjugated anti-mouse IgG antibody (Thermo Fisher Scientific) and chemiluminescent detection kit (ECL Prime kit, Bio-Rad Laboratories) (Wakai et al. 2020). These bacteria will be placed onto polyvinylidene fluoride membrane (Bio-Rad Laboratories) and will be detected according to the ECL Prime kits manufacturers manual. With alcohol and water exposure as well as methylene blue, the bacteria can be observed in a 100x oil immersion (Trombone 2014).

III RNA-seq

The extraction of RNA involves using organic solvents or kits that are commercial (Croucher and Thomson 2010). The kit that will be used here is the Invitrogen PureLink RNA Mini Kit and the protocol will be that of the manufacturer (Thermo). The Nanopore Direct RNA Sequencing (DRS) method can be used post RNA

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extraction, for the sequencing. It requires the pre-processing of total RNA from the bacteria and sequences in a high throughput manner. This process also includes generating in vitro-transcribed RNA libraries, processing Nanopore DRS data, and finally analyzing transcriptomes and epitranscriptomes with computational equipment (Tan et al. 2024).

There are also other methods to maintain directional information and specifically ones used in *H. pylori* and *S. typhimurium*. With a dRNA-seq approach that chooses only the 5' end of transcripts and shows different levels and complexity of gene expression (Sharma et al. 2010) (Kurasz et al. 2018) or the Liu protocol which works through cDNA intermediates starting with one strand and ending with a double stranded cDNA, both methods can provide accurate results (Liu et al. 2021).

IV PCR

The 16S prokaryotic ribosomal RNA gene, which is obtained through the PCR, will be sequenced and searched for in a DNA prokaryotic database. There are many specific primers to find the 16S which makes this method universal. For the sake of this study, the primers that will be used are the Hp1-Hp2 primers which target a 109 bp segment of the 16S gene. These primers, once optimized, will then be synthesized by DNA Integrated Technologies. After the primers are obtained, PCR can be started and the 16S gene can be tracked throughout rRNAs. The significance of tracking 16S is to show the activity of specific proteins being produced by these rRNAs (activity of the ribosomes themselves) and observe the secretion of certain proteins to find patterns throughout the dedifferentiation process. Further sequencing can be done on the produced primer complexes to identify specifics (notable patterns about production of proteins, ribosomal activity, and increase in certain genetic upregulation) about this bacteria through BLAST (Ranjart et al. 2000) (Church et al. 2020).

V Western Blots

To western blot *H. pylori* in gastrointestinal tissue, *L. acidophilus* in fibroblasts, and *M. leprae* in Schwann cells, the eukaryotic cells will first be lysed with a RIPA buffer that has protease inhibitors (Zuo et al. 2022). Immediately after the lysis, the solution will be centrifuged to clarify the lysates. With a BCA or Bradford assay, protein concentration can be determined and then the appropriate amounts of protein can be transferred onto an SDS-PAGE gel to begin the actual blot (Hueso et al. 2022; Gavini and Parameshwaran 2023). Next, the proteins will be transferred onto a PVDF membrane with a transfer apparatus and blocked with 5% non-fat milk in TBST. Before incubation at 4°C, the following antibodies will be added: anti-*H. pylori* (targeting CagA or Urease B; IgG or IgA), anti-*L. acidophilus* (targeting surface proteins), and anti-*M. leprae* (targeting PGL-1 or LAM) antibodies (Yu et al. 2022) (Pierneef et al. 2021). The blot can then be developed using enhanced chemiluminescence (ECL) substrates to detect protein bands in an imaging system (Pillai-Kastoori et al. 2020). The luminescence will be achieved by using a secondary antibody, Goat anti-Human IgA Secondary Antibody, which will be labeled with CF dyes.

VI Electron Microscopy

The bacteria, present in a culture, will be fixed with 4% glutaraldehyde, and then stained with osmium tetroxide and uranyl acetate (Gothenburg 2022). Fixed samples will be dehydrated and embedded and then sectioned into thin sections. The sections will be further stained with lead citrate, placed on TEM grids, and viewed under a transmission electron microscope (Pandithage 2013).

Post-dedifferentiation Observation and Analysis

After following the protocol for 4.1-4.3, the bacteria should have induced differentiation in their target eukaryotic cells. Now they are ready for the post-differentiation analysis which will reutilize 4.4.a.1-4.4.a.VI. The methods of histology, blotting, and immunofluorescence will give an output that is comparable with the results obtained while using them for the pre-dedifferentiation observation. It will result in outputs which can be analyzed for significant differences which can be further targeted and investigated. The results of the tests can be used to compare the stage of dedifferentiation in the cell and the proteins being expressed (western Blot), the interactions it has made with the bacteria (electron microscopy), how the bacteria act after the

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differentiation (electron microscopy), and secretion of proteins that may or may not influence this differentiation by the bacteria (immunofluorescence).

DISCUSSION

This study examines the ability of a *H. pylori*, *L. acidophilus*, and *M. leprae* to induce a pluripotent like state and mimic the actions of the Yamanka factors. The target eukaryotic cells should mimic IPSCSs, with differences such as being slightly further down the differentiation pathway. For example, once the gastrointestinal tissue has dedifferentiated due to interaction with the *H. pylori*, the cells would return to undifferentiated intestinal stem cells found in the intestinal crypt (Umar 2011). We predict this because, even though some Yamanaka factors might be present in the bacteria, there is no sign that all of the major factors are present. They still might possess the ability to induce the Yamanka factors within the eukaryotic cells through some other signaling molecules, making it so that the prokaryotes don't directly carry them. Even though the differentiation might not occur fully, the new multipotent cells have a vast capability. The lack of complete differentiation could aid the bacteria by allowing for damaged cells to slightly repair (in a multipotent phase) and then to begin taking advantage of the metabolic or replicative benefits a non-damaged cell would have originally provided. This would also apply to *L. acidophilus* which would return the human dermal fibroblasts to Dermal Fibroblast Progenitors (DFPs) and the Schwann cells which the *M. leprae* interact with would return to NG2+ progenitor cells (Phan et al. 2023).

Since the bacteria possess the main mechanism to initiate differentiation, not acting as carriers, their genome must have some overexpressed transcription factors. These transcription factors must cause the production of some signaling molecule that is made in higher than usual quantities to be used in this dedifferentiation. This relates to the analysis of results which would include looking at PCR and possible northern blots to observe any upregulated genes that are transcribed in excess. This could then be backed by mRNA-sequencing and secretion patterns of the bacteria which would also give information of the type of signaling, under which the mechanism operates (juxtacrine or paracrine). This signaling can also be observed based on the distance in which signaling molecules travel or if molecules are even present (juxtacrine signaling would induce a secondary messenger within the cell because the receptors on cell surfaces meet). If signaling occurs in a juxtacrine manner, focusing on proteins and carbohydrates in the bacteria would be a better path because juxtacrine signaling involves the interaction of membranes which are heavy in glycoproteins and glycolipids. On the other hand, with paracrine signaling different amino acids, peptides or steroids, nucleotides, retinoids, fatty acids, and even gasses can be used which makes it more challenging to track this process. The purpose of the electron microscopy in the pre-differentiation stage is to determine which signaling mechanism is being used and how the detection of secretory molecules (or membrane attachments) can be determined.

Continuing with alternate results, the respiration type, especially amongst the facultative aerobes or anaerobes, could lead to variations in the data collected. As mentioned earlier, there are no exact or specified conditions under which the cultures need to be run, and this includes the oxygen in the environment. With no previous experiments determining the possible variation of results, the only way to know whether or not alternate results will appear while investigating this mechanism is by performing both types of cultures.

Another possible alternative result could occur as a result of how the Schwann cells are obtained. This is true because artificial Schwann cells, made by differentiating stem cells, will have significant variations from isolated Schwann cells, and *M. leprae* may only induce its dedifferentiation in either one of the cases due to these observable variations.

As mentioned in section 4.3.b, the usage of a 2d culture to cluster eukaryotic cells is possible. While utilizing a 3d cell culture can give benefits to representing the complexity of these tissues, there is no set standard system to perform them universally (Duval et al. 2017). This means that 2d cell cultures are a good option to consider or have as a backup because the only difference is that no liquid air-liquid interface is necessary. While this process can work easily it does lack the complexity of a 3d culture and is not truly representative of an in vivo structure due to the fact that it does not factor in immune cells, mechanical signaling cues, nor the development of any bacteria specific structures such as biofilms (Mountcastle et al. 2020) (Sancilio 2014).

This study can be expanded by looking for specific factors in the genome that contribute to the mechanism individually. This could be done through metagenomics, where sequences are detected and cross referenced with those of the Yamanka factors to observe if the same or similar molecules are being produced. The main goal of this metagenomic sequencing would be to analyze which genomic components in the bacteria are enabling the multipotent transformation to occur in the eukaryotes which can be done easily by analyzing the known proteins that are involved. Another secondary study could be the genetic perspective of the eukaryotes. The incorporation of signaling molecules produced by the bacteria, or perhaps transcription factors and RNA molecules, could be observed in the nucleus of the eukaryotes to understand the mechanism that allows for the regression of the cell into multipotency.

The importance of this study is to attain an understanding on the evolution of a dedifferentiation mechanism. Further studies could help inquire why these prokaryotes create a microenvironment of stem cells and how it could benefit them. These could also be potentially powerful tools for inducing stem cell dedifferentiation in places where other vectors carrying dedifferentiation factors cannot reach. Since *H. pylori*, *L. acidophilus*, and *M. leprae* are all non-virulent prokaryotes that are known to interact in humans, this mechanism could help show how stem cells evolved in eukaryotes and their link to simpler, yet intriguingly powerful, organisms of another domain.

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