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FORUM FOR UNDERGRADUATE RESEARCH

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Gregory J. Falabella, Ph.D.
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Section I: The Natural Sciences & Quantitative Analysis

Characterization of Novel Seed Endophytes in *Medicago sativa*

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The microbiome of plants is essential in their development and health. Seed endophytes are microorganisms that live within the seeds of plants, the majority of which are believed to be non-pathogenic, although more research is needed to better understand the function of seed endophytes. They are present from the beginning growth stages and play many important roles. A variety of bacterial species have been found as seed endophytes. They have also been found to have common functions for colonization, benefiting plant growth, and antifungal properties. In this project, seed endophytes were isolated from surface sterilized *Medicago sativa* seeds on selective media for potassium solubilization, actinomycetes, and nitrogen free media. Sterilized seeds were germinated and planted in Yosida agar. The sterilized plants were then inoculated with the seed endophyte isolates and uptake of the isolates into the plant roots was measured. The methods were improved, germinating the seeds in sterile water often led to self-toxicity and the seeds germinated at a much higher rate using the Murashige and Skoog Medium. Several sources of contamination within the protocol were found and eliminated. The isolates also underwent DNA extraction and colony PCR for 16S rRNA gene sequencing. Using this method, a *Pseudomonas* species was identified. Seed endophytes from *Medicago sativa* were characterized.

I. Introduction

Members of Seed Endophyte Communities

Seed endophytes are microorganisms that can colonize the seeds of plants without causing damage to the plant (Truyens et al., 2014). These microorganisms can be beneficial to the seeds in many different ways. A great variety of organisms colonize seeds. One hundred thirty-one bacterial genera have been found as seed endophytes, according to a meta-analysis by Truyens and colleagues in 2014 (Truyens et al., 2014). This included bacteria, identified using many different methods such as 16S rRNA sequencing, which most commonly identified the phyla *Frimicutes*, *Actinobacteria*, and the genera *Pseudomonas*, *Enterobacter*, *Methylobacteria*, *Pantoea*, and *Sphingomonas*

(Ferreira et al. 2008, Mastretta et al., 2009, Johnston-Monje and Raizada 2011, Liu et al., 2012). 16S rRNA sequencing is the most common genetic tool of phylogeny and taxonomy, it looks at the 16S rRNA gene is found in all bacteria and archaea, is large enough for bioinformatics and the function hasn't changed (Janda and Abbott, 2007). PCR DGGE was used to find *Stenotrophomonas, Ochrobactrum, Pseudomonas, and Enterobacter* species (Hardoim et al., 2012). *Bacillus, Pseudomonas, Burkholderia, and Curtobacterium* were isolated from seeds using fatty acid methyl ester profiles (Vega et al. 2005, Graner et al., 2003). Overall this meta-analysis found that the most common phyla present in bacterial seed endophytes are *Proteobacteria, Actinobacteria, and Frimcutes* across 25 plant species (Truyens et al., 2014). A core microbiota of seed endophytes is conserved in maize and its ancestors from Mexico to Canada (Johnston-Monje and Raizada, 2011).

A study of fungal seed endophytes identified *Hormonema, Beauveria, Cladosporium, Geopyxis, Geomyces, and Sarcinomyces* from *Pinus monticola* seeds (Ganley and Newcombe, 2006). Pini and colleagues looked at the bacterial communities in *Medicago sativa* and found that *Alphaproteobacteria* dominated the plant tissues, but no bands of 16S rRNA were recovered from surface-sterilized seeds (Pini et al., 2012). MALDI-TOF has been used to find the phyla *Proteobacteria, Actinobacteria, Frimicutes,* and *Bacteroidetes* within surface sterilized *Medicago sativa* seeds (Lopez et al., 2017).

<div align="center">Vertical Transmission of Endophytes</div>

Johnston-Monje and Raizada found that the conserved core microbiota is vertically transmitted between generations (Johnston-Monje and Raizada, 2011). Endophytes can enter seeds in several ways. Vascular connections to the parent plant can act as a path for endophytes to get into seed endosperm. Shoot meristems which can develop into ovules can be colonized by endophytes causing the seeds to be colonized (Truyens et al., 2014). This allows plants to pass beneficial bacteria onto their offspring which might help maintain the stability of endophyte communities. The endophytes passed down have been shown to be shaped by environmental stressors experienced by the parent plant (Truyens et al., 2014). GFP gene tagging has been used to show the transfer of *Pantoea agglomerans* from inoculated seeds to seedlings in *Eucalyptus* (Ferreria et al., 2008). Endophytes from seeds were also found in the same switchblade plants a year later (Gagne-Bourgue et al., 2012). Mitter and colleagues found that by adding *Paraburkholderia phytofirmans* to the plants' flowers it would then be included in the microbiome of that plant's progeny seeds (Mitter et al., 2017).

Location of Endophytes within the Seed

Endophytes have been found in the seed coat, crease tissue, and endosperm (Robinson et al., 2016). FISH has also been used to detect bacteria along the cell walls inside of seeds (Compant et al., 2011). They have also been found in the intercellular spaces of the root cortex and vascular system (Puente et al., 2009). Several different endophyte isolates were found using GFP throughout the vascular system (Johnston-Monje and Raizada, 2011).

Seed Endophyte Functions

Metagenomic and bioinformatics studies have looked at bacterial endophyte genomes for common genes and functions. Current evidence does not suggest that endophytes are not specific to a plant host, so it makes sense that there is a general strategy for host colonization. Prominent features found across many different species were flagella, plant-polymer degrading enzymes, protein secretion systems, iron storage, quorum sensing, detoxification of reactive oxygen species, nitrogen fixation and denitrification, transporters, transcriptional regulators, redox potential maintenance (Sessisch et al., 2012, Ali et al., 2014). Johnston-Monje and Raizada tested isolated seed endophytes for similar traits and found that the ones most commonly observed were phosphate solubilization and the production of acetoin/butanediol (Johnston-Monje and Raizada, 2011). As discussed below, many of these can benefit plant growth, such as iron storage, nitrogen fixation, and phosphate solubilization. Others may be useful for colonizing the plants.

Interestingly, all known protein secretion systems other than type III were found in endophytes. The type III section system is often used by pathogenic bacteria to control host response (Sessisch et al., 2012, Ali et al., 2014). Several different plant-ployer dragging enzymes have been found to help endophytes enter and move through the plant. Similarly, flagella have been consistently found in seed endophytes; motility may likely be helpful for colonization. The presence of enzymes used for the detoxification of reactive oxygen species (ROS) have been found. Plants produce ROS when stressed, or as a response to colonization by microorganisms, so it seems these enzymes might help endophytes to survive (Sessisch et al., 2012, Ali et al., 2014). Transporter proteins for the MFS and ABC transport systems have been found in endophytes, which may allow them to take up nutrients from the plants (Ali et al., 2014). Many different translational regulators were also found, mostly global regulators that affect cellular metabolism, which may help respond to nutrients and the environment in the host (Ali et al., 2014).

<u>Plant Growth Promotion Among Seed Endophytes</u>

Seed endophytes can provide many different benefits to plants. Several studies have shown that they can stimulate plant growth. Many different plant growth-promoting traits have been found in seed endophyte isolates, such as indole-3-acetic acid (IAA) production, phosphate solubilization, siderophores production, nitrogen fixation, and 1-aminocyclopropane -1-carboxylate (ACC) deaminase in *Bacillus* species isolated from tomato seeds (Xu et al., 2014). IAA is naturally produced by plants to help with cell growth but can also be made by bacterial symbionts. It promotes plant growth by altering cell orientation, cell elongation and helping with organ development (Labeeuw et al., 2016). Plant-associated bacteria release organic acids, which solubilize phosphates into ortho-phosphate. Phosphate in the soil is insoluble making it unavailable to plants but when microbes convert it to soluble phosphate then it can be used by the plants (Otenio et al., 2015). Siderophores are used to uptake iron from the environment, which the plants can then use (Lurthy et al., 2020). Plants produce ethylene under stress conditions. Bacteria with the enzyme ACC deaminase can lower ethylene levels by hydrolyzing ACC, the precursor of ethylene, into ammonia and α-ketobutyrate, which can make the plant more resistant to stress (Gupta and Pandey, 2019). Some bacteria can produce the enzyme dinitrogenase, which reduces N2 to fixed inorganic nitrogen, putting the nitrogen in a form that the plants can use (Li et al, 2017). One study found that when cactus seeds were inoculated with isolated source endophytic bacteria, they grew for a year with no fertilization getting all of their nutrients from endophytes, the same seeds sterilized with antibiotics they were unable to grow, but if sterilized seeds were inoculated then growth resumed (Puente et al., 2009). *Bacillus pseudomycoides* strain BM1 was isolated from the rhizosphere of alfalfa and has the ability to produce IAA, siderophores, lipase, cellulase, and pectinase. It was also found that inoculating the seeds with BM1 increased root and shoot length by up to 21.43% (Knezevic et al., 2021).

Seed endophytes have also been shown to have antifungal properties. Studies have found well-known toxins from seed endophyte isolates such as surfactins (C 13, 14, 15), iturins, mycobacillin (Gagne-Bourgue et al., 2012). Iturnin and surfactins are lipopeptides. They primarily work by binding to the cell membrane, forming ion channel-like complexes, and releasing ions from cells, causing cell death (Tran et al., 2022). Surfactins have been shown to have antifungal properties against several plant pathogens in particular (Vitullo et al., 2012). Mycobacillin binds to ATP transports on the plasma membrane, resulting in over-release and cell starvation (Tran et al., 2022). Rice seed endophyte isolates were found to inhibit the growth of three plant pathogens, *Curvularia, Fusarium oxysporum,* and *Pythium ultimum* (Ruiz et al, 2011). The isolated endophyte *Bacillus pseudomycoides* strain BM1 inhibited *F. graminearum, F. proliferatum,* and *F.*

oxysporum in vitro. When seeds were inoculated, the number of seedlings infected *with F. oxysporum* were reduced by 25.41% (Knezevic et al., 2021).

Alfalfa as a Model Organism

Alfalfa (*Medicago sativa*) is a legume grown in temperate climates around the world and is often used in crop rotation. It is useful in crop rotation due to its symbiotic nitrogen fixing bacteria which can help add organic nitrogen to the soil (Pini et al., 2012). Alfalfa has also been investigated as a possible crop for biomass feedstock to be turned into bioenergy (Sanderson and Adler, 2008). Alfalfa has also been used in land restoration due to the deep taproot and nutrient cycling abilities. It is often used as a model species for studies of bacterial symbionts of plants, especially *Sinorhizobium meliloti* (Pini et al., 2012).

Summary

The microbiome of plants is essential in their development and health. Seed endophytes are microorganisms that live within the seeds of plants, the majority of which are believed to be non-pathogenic although more research is needed to better understand the function of seed endophytes. They are present from the beginning growth stages and play many important roles. They have been found to be vertically transmitted between generations. A variety of bacterial species have been found as seed endophytes. They have also been found to have common functions for colonization, benefiting plant growth, and antifungal properties.

II. Materials and Methods

Biological Materials

Alfalfa Seeds (*Medicago sativa*), Isolates 1-6

Other Materials

Media Ingredients

Magnesium sulfate ($MgSO_4$), Calcium Carbonate ($CaCO_3$), Potassium Feldspar ($AlKO_8Si_3$), Dextrose ($C_6H_{12}O_6$), Ferric Chloride ($FeCl_3$), Agarose ($C_{24}H_{38}O_{19}$), Sucrose ($C_{12}H_{22}O_{11}$), Magnesium Sulfate ($MgSO_4$), Sodium Chloride (NaCl), Ferrous Sulfate ($FeSO_4$), Sodium Molybdate (Na_2MoO_4), Difco Bacto Actinomycete Isolation Agar dehydrated, Ammonium nitrite (NH_4NO_2), Potassium sulfate (K_2SO_4), Calcium chloride ($CaCl_2$), Manganese(II) chloride ($MnCl_2$), $(NH_4)Mo_7O_2$, Boric acid (H_3BO_3), Copper sulfate (C_4SO_4), Potassium phosphate (KH_2PO_4), Ethylenediaminetetraacetic acid (EDTA), LB broth mix, TSA mix, Murashige and Skoog Medium.

Lab Equipment

Distilled water, Bleach, 1.5ml Eppendorf Tubes, Sterile Petri dishes, 70% ethanol, sterile 1X Phosphate Buffered Saline, p20/p200/p1000 pipettes and sterile tips, PCR tubes, agarose, 1X TAE, gel box, 15ml tubes, sterile mortar, mason jars, Autoclave, heat block, 28^0C incubator, Thermocycler, Spectrophotometer, grow lights, nanodrop, and qubit.

Molecular reagents

Invitrogen 27F, invitrogen 1492R, Master Mix, DNA Ladder, DNA loading dye, Promega Wizard SV Gel and PCR cleanup kit, Qiagen DNeasy Ultra Clean Microbial kit, ITS 4, ITS 9, and Qubit reagents.

Table 1: Primers used in these experiments

Primer Name	Sequence
27F	5'-AGAGTTTGATCCTGGCTCAG-3'
1492R	5'-AGAGTTTGATCCTGGCTCAG-3'
ITS4	5'-TCC TCC GCT TAT TGA TAT GC-3'
ITS9	5'-GAA CGC AGC RAA IIG YGA-3'

Techniques

Culture-based Methods

Media Preparation- Alexsandrow's Media/ Jensen's Media/ Actinomycetes Media

1000ml of Alexsandrow's Media (K solubilization) was made using 0.5g of Magnesium Sulfate, 0.1g of Calcium Carbonate, 2g of Potassium Feldspar, 5g of Dextrose, 0.005g Ferric Chloride, 20g of Agar. The 2g of Calcium Phosphate was left out because there was none in the lab. 1000 ml of Jesens Media (Nitrogen Free) was made using 20g of Sucrose, 1g of Potassium Phosphate, 0.5g of Magnesium Sulfate, 0.5g of Sodium Chloride, 0.1g of Ferrous Sulfate, 0.005g of Sodium Molybdate, 2g of Calcium Carbonate, and 15g of Agar (Jesen, 1941). Potassium Phosphate was substituted for dipotassium Sulfate. 1000ml of Actinomycetes Media was made using 22g of Difco Bacto Actinomycete Isolation Agar dehydrated, which contains 2g of Sodium Caseinate, 0.1g of Asparagine, 4g of Sodium Propionate, 0.5g of Dipotassium Phosphate, 0.1g of Magnesium

Sulfate, 0.001g of Ferrous Sulfate, and 15g Agar. All three media were made by dissolving the ingredients listed above in 1000ml of distilled water on a hotplate, once dissolved they were placed within the autoclave (Buxton 8200) and the autoclave was set to the liquid cycle. Once the autoclave cycle finished, they were all allowed to cool. Once cooled enough to touch the media was poured into sterile labeled petri dishes in the sterile media hood. Once the plates were cooled and solidified, they were placed into labeled sleeves and stored in the refrigerator. All other media for this experiment was prepared the same way.

Seed Surface Sterilization and isolation of organisms

Fresh 10% bleach solution was made. About 30 Alfalfa seeds were added to a sterile 1.5 ml eppendorf tube. 70% ethanol was added to the tube to cover the seeds and left at room temperature for five minutes. The ethanol was poured off and 10% bleach solution was added to cover the seeds and left at room temperature for five minutes. The bleach solution was poured off and the seeds were rinsed five times with sterile water. 1X PBS was added to the tube to cover the seeds and they were left overnight at room temperature. The next day a sterile mortar was used to homogenize the seeds. A p200 was used to pipet 100μl of homogenized material onto each plate. Three plates of each media (Alexandrow's, Jensen's, and Acintomyeyete) were used. Each of these plates plus one control per media were placed in a 28^0C incubator.

Six days later there was growth on all of the plates inoculated with homogenized seeds and no growth on the control plates. One organism was isolated from the Actinomycete media (Isolate 1). Two organisms were isolated from the Jensen's media plates (Isolate 2 and 3). Two organisms were isolated from the Alexdanrow's Media plates (Isolates 4 and 5). The organisms were isolated by touching a sterile loop to the colony and then performing a quadrant streak on the corresponding media. Each isolate was quadrant streaked twice. The new plates were incubated at 28^0C. The original plates were also incubated in order to look for slow growing organisms.

Media preparation- Yoshida Agar

Stock solutions were prepared for the Yoshida Agar in 50ml of distilled water. 2.84g of NH_4NO_2 for a 50X solution, 2.18g of K_2SO_4 for a 50X solution, 3.675g of $CaCl_2$ for a 50X solution, 6.16g of $MgSO_4$ for a 50X solution, 0.204g of $MnCl_2$ for a 250X solution, 0.01g of $(NH_4)Mo_7O_2$ for a 250X solution, 0.143g of H_3BO_3 for a 250X solution, 0.003g C_4SO_4 for a 250X solution, 1.07g KH_2PO_4 for a 250X solution, and 1.55g of EDTA for a 250X solution. Regular EDTA was substituted for Ferric EDTA and KH_2PO_4 was substituted for H_2PO_4, $ZnSO_4$ was left out.

Seed Sterilization and Germination

A 3% bleach solution was made. Alfalfa Seeds were added to a sterile 1.5ml eppendorf tube. Sterile water was added to the level of the seeds. The tube was then heated on a 40^0C heat block (VWR scientific products Select heatblock) for thirty minutes. The water was removed and 70% ethanol was added to cover the seeds and left at room temperature for one minute. The ethanol was removed and the freshly prepared 3% bleach was added to cover the seeds and left at room temperature for five minutes. The bleach was removed and the seeds were rinsed with sterile water. The seeds were left in a small approximately 8 ml petri dish with sterile water and covered with foil for two days then left to sit under the grow lights for one day.

The original sterilized seeds were not germinating, possibly due to self toxicity. The seed sterilization was performed again as above but this time they were put in a normal approximately 20 ml sized petri dish at the end with sterile water.

Colonization Experiment 1

LB broth was made using the mix. 5ml of LB broth was added to sterilized 15ml tubes and then inoculated with each of the isolates. They were incubated in the 28^0C incubator for two days. The optical density of each isolate was measured using the spectrophotometer. They were then standardized to 0.01 OD. The Yoshida agar was made by using 1ml of 50X stock solutions and 200μl of 250X stock solutions and the media preparation as described above. Three plates per isolate were prepared. Each plate was prepared by using sterilized tweezers to place one germinated seed on each plate. 100μl of each standardized isolate was pipetted onto the corresponding plant focusing on the root area. The plants were then placed under the grow lights for one week.

Tryptic Soy Agar was made using a mix and the media preparation as described above as a general media for the serial dilutions. Using sterilized tweezers the plants were placed into individual 1.5 ml eppendorf tubes and weighed, the weight was recorded. 500μl of 1X PBS was added to each tube; the tube was then vortexed for thirty seconds and poured off; this was repeated twice. 500μl of 1X PBS was added to each tube and the plant was homogenized with a sterile mortar. Once homogenized a serial dilution with 1X PBS was performed on each plant up to 10^{-6}. 100μl of each dilution was plated onto a TSA plate which was then spread with a sterile loop. The plates were then placed in the incubator at 28^0C. Two days later all of the plates were equally contaminated. A TSA plate and TSA plate with the 1X PBS were placed in the 28^0C incubator.

Seed Sterilization and Germination

Germination Agar was made using 2.22g Murashige and Skoog Medium and 5g of agar in 500ml distilled water and using media preparation as described above. Seed

sterilization was performed as described above. About ten sterilized seeds were placed on each of the four plates using sterile tweezers. The plates were wrapped in foil and left on the bench for two days. Then the plates were placed under the grow lights for one day.

Colonization Experiment 2

        Made LB broth and added 5ml to 15ml falcon tubes which were inoculated with the isolates and put in the 28^0C incubator for two days. The Yoshida agar was made again as described above but put into mason jars that had been sterilized in the autoclave. The isolates in LB broth had the optical density measured and were standardized as described above. The germinated seeds were placed in the mason jars and the rest of the inoculation was performed as described previously. Had two control plants which were placed in the mason jars but not inoculated. The Mason jars were placed under the grow lights for one week and the serial dilutions were performed as described above.

Seed Sterilization

        The seed sterilization used before germinating seeds was performed but at the end the seeds were homogenized and plated on LB agar. The plates were put in the 28^0C incubator for two days. The same procedure was done with new bleach at 3% and 10%.

        The seed sterilization was performed as described above using 3% of the new bleach. Three plates of Murashige and Skoog Medium with 10 sterilized seeds each were placed under the grow lights for three days.

Germination Experiment

        The seed sterilization was performed as described above using 3% of the new bleach. The isolates were grown in LB broth and standardized to 0.01 optical density using the spectrophotometer as described above. 15ml of each 0.01 OD isolate (other than isolate 4 which did not grow enough in the LB broth) were added to a sterile petri dish and twenty sterilized seeds were added to each dish along with a control petri dish with 15ml sterile water. The petri dishes were then left under the grow lights for one week.

Colonization Experiment 3

        One 1L of Yoshida was made as described above. Six of the germinated sterilized seeds were placed into their own mason jars with Yoshida agar. Three were left as controls and three were inoculated with 50 ul of 0.1 OD of isolate 2. The mason jars were left under the grow lights for one week. The plants were weighed and the homogenization and serial dilution were performed again as described above. The plants were homogenized in

200ul 1X PBS and 50ul of each dilution were plated on the LB plates and placed in the 28^{0}C incubator for two days.

In order to calculate the colony forming units (CFU) per gram the amount of colonies on each serial dilution plate was counted, anything over 300 colonies was considered too numerous to count (TNTC). The amount of CFU in 200ul was calculated by multiplying the amount on plates (amount in 50ul) by four. The CFU per gram was found by dividing the CFU in 200ul by the weight of the plant in grams.

Molecular Methods

Colony PCR and PCR Cleanup

After five days when the isolates had grown a colony PCR was performed. 1μl of invitrogen 27F, 1 μl of invitrogen 1492R, 12.5μl of Master Mix (Taq 2X Master Mix NEB #MO270S), 9.5μl dH$_2$O, were added to six PCR tubes. Each colony was touched using a sterile loop and then the loop was submerged in the corresponding PCR tube. The PCR tubes were put into the thermocycler (Applied biosystems GeneAmp PCR system 9700) and ran with the program: 94^{0}C- 3 minutes, (94^{0}C- 1 minute, 48^{0}C - 30 seconds, 72^{0}C - 1 minute) x35, 72^{0}C - 7 minutes, 4^{0}C. An agarose gel was made using 0.3g of agarose, 60ml of 1x TAE, combined in a 150ml flask and microwaved in thirty second increments until dissolved. The flask was left at room temperature until cooled and then poured into the gel box thing. The ladder was prepared by combining 8μl of dH$_2$O, 2μl DNA Ladder, 2μl loading dye in a PCR tube. After the cycle had run 5μl of DNA loading dye was added to each tube, and 5μl of each sample were loaded into the gel along with the ladder. The gel box was filled with 1x TAE and at 50 volts for thirty minutes. Once done the gel was imaged using the UV gel box (Fisher scientific Transilluminator FBTIV-88).

PCR Cleanup was performed using the Promega Wizard SV Gel and PCR cleanup kit. 10μl of PCR product from isolate 2 was used. The kit protocol was followed. The product was sent to Genewiz for sequencing and the results were visualized using Ugene and identified using NCBI BLAST.

DNA Extraction

Used Qiagen DNeasy Ultra Clean Microbial kit according to protocol to extract DNA from each of the isolates. Working stocks of ITS 4 and ITS were made. 10μl of Master Mix, 0.5μl of ITS 4, 0.5μl of ITS 9, and 2μl of DNA extraction product were added to each PCR tube. The PCR tubes were added to the thermocycler and run using the program: 95^{0}C - 3 minutes, (95^{0}C - 30 seconds, 55^{0}C - 30 seconds, 72^{0}C - 30 seconds) x25, 72^{0}C - 5 minutes, 4^{0}C. Made and loaded gel as described above. The DNA extraction

products were nanodroped (Thermo scientific nanodrop 2000c). The ITS PCR was performed again with a positive and negative control.

ITS PCR

A PCR was performed using the DNA extraction product. 0.5 µl of ITS 4, 0.5 µl of ITS 9, and 10µl of Master Mix, were added to 7 PCR tubes. 2µl of DNA extraction product from each isolate was added to the corresponding tube. The PCR tubes were put into the thermocycler (Applied biosystems GeneAmp PCR system 9700) and ran with the program: 95^0C- 3 minutes, (95^0C- 30 seconds, 55^0C - 30 seconds, 72^0C - 30 seconds) x25, 72^0C - 5 minutes, 4^0C. An agarose gel was made using 0.3g of agarose, 60ml of 1x TAE, combined in a 150ml flask and microwaved in thirty second increments until dissolved. The flask was left at room temperature until cooled and then poured into the gel box. The ladder was prepared by combining 8µl of dH_2O, 2µl DNA Ladder, 2µl loading dye in a PCR tube. After the cycle had run 5µl of DNA loading dye was added to each tube, and 5µl of each sample were loaded into the gel along with the ladder. The gel box was filled with 1x TAE and at 100 volts for twenty minutes. Once done the gel was imaged using the UV gel box (Fisher scientific Transilluminator FBTIV-88).

The exact same protocol was repeated using only a positive and negative control.

16S PCR and PCR Cleanup

A PCR was performed using the DNA extraction product. 1.5 µl of invitrogen 27F, 1.5 µl of invitrogen 1492R, 12.5µl of Master Mix, 8.5µl dH_2O, were added to 8 PCR tubes. 1µl of DNA extraction product from each isolate was added to the corresponding tube. The PCR tubes were put into the thermocycler (Applied biosystems GeneAmp PCR system 9700) and ran with the program: 94^0C- 5 minutes, (94^0C- 30 seconds, 58^0C - 30 seconds, 72^0C - 1 minute) x25, 72^0C - 10 minutes, 4^0C. An agarose gel was made using 0.3g of agarose, 60ml of 1x TAE, combined in a 150ml flask and microwaved in thirty second increments until dissolved. The flask was left at room temperature until cooled and then poured into the gel box. The ladder was prepared by combining 8µl of dH_2O, 2µl DNA Ladder, 2µl loading dye in a PCR tube. After the cycle had run 2µl of DNA loading dye was added to each tube, and 5µl of each sample were loaded into the gel along with the ladder. The gel box was filled with 1x TAE and at 75 volts for twenty minutes. Once done the gel was imaged using the UV gel box (Fisher scientific Transilluminator FBTIV-88).

PCR Cleanup was performed using the Promega Wizard SV Gel and PCR cleanup kit. 20µl of PCR product from isolates, 1, 4, 5, and 6 was used. The kit protocol

was followed. The PCR cleanup product was nanodropped and then a Qubit was also performed.

The 16S PCR was performed again exactly as described above. A PCR Cleanup was performed using the QIAGEN PCR cleanup kit, eluting in 30µl elution buffer and letting the elution buffer sit for one minute before centrifugation. The products were sent to Genewiz for sequencing and the results were visualized using Ugene and identified using NCBI BLAST.

III. Results

Isolation of organisms

Six different colonies were isolated from the surface sterilized alfalfa seeds. Isolate 1 grew on the Actinomycetes media, it was a small yellow round shiny colony. Two colonies were isolated from the Jensens Media. Isolate 2 was very small round white colonies, isolate 3 was a larger white fuzzy colony. Two colonies were isolated from the K Solubilization agar. Isolate 4 was a small round white colony, isolate 5 was a large fuzzy white colony with a slightly green center. There was no growth on any of the control plates. 5 days after the initial isolation another slow growing colony was isolated. Isolate 6 grew on the K solubilization agar and had small, tall, hard colonies that were gray with a white coating. These plates can all be seen in Figure 1.
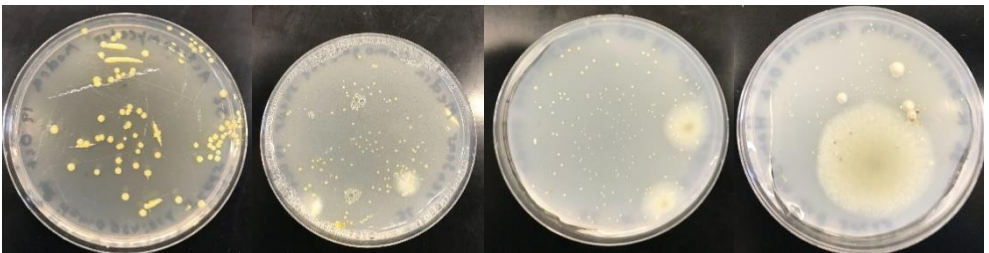

Figure 1: Left to right, Isolate 1 on Actinomycetes agar, Isolates 2 and 3 on Jensen's Media, Isolates 4 and 5 on K Solubilization Agar, Isolate 6 on K solubilization agar.

Seed Sterilization

After the alfalfa seeds were sterilized, they were placed in an 8ml petri dish with sterile water and 4 of the 88 seeds germinated. More alfalfa seeds were sterilized and then placed in a 20ml petri dish and 28 of the 66 seeds germinated (Figure 2).
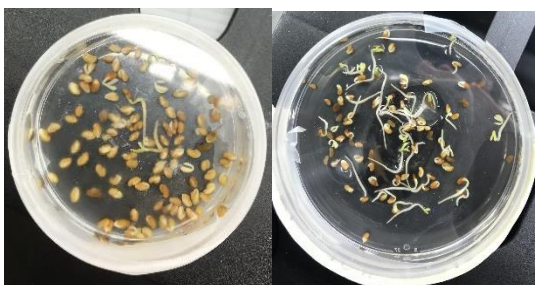
Figure 2: Left, Sterilized alfalfa seeds in an 8ml petri dish with sterilized water after 2 days in tin foil and 4 days under the grow lights. Right, sterilized alfalfa seeds in a 20ml petri dish with sterilized water after 2 days in foil and 1 day under the grow lights.

Colonization Experiment 1

After one week under the grow lights the inoculated alfalfa sprouts' roots and leaves had grown and the inoculated bacteria could be seen in a halo in the Yoshida agar surrounding the plant roots (Figure 3).

Figure 3: Alfalfa plants grown on yoshida agar and inoculated with isolates 4 and 5 after one week under grow lights.

In the first colonization experiment there was an even amount of contamination growth across all of the plates after two days in the 28^0C incubator. All plates of an isolate 6 replicate are shown as a representation but all of the plates looked very similar across all isolates and replicates (Figure 4). To find the contamination a plain TSA plate was incubated and no growth occurred, but there was growth on the plate with the 1X PBS used.

Seed Germination

Seeds were germinated using Murashige and Skoog Medium and 88% of the seeds germinated (Figure 5).
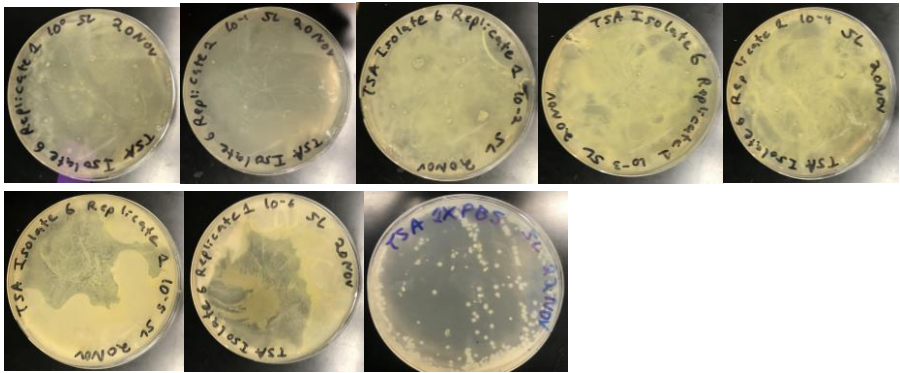
Figure 4: Serial Dilutions of homogenized alfalfa sprouts inoculated with isolate 6 on TSA after two days in the 28ºC incubator and 1X PBS on TSA after one day in the 28ºC incubator.
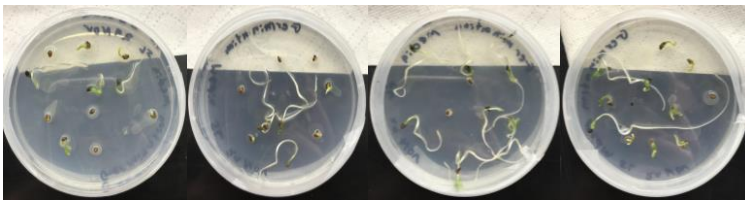
Figure 5: Sterilized alfalfa seeds germinated on Murashige and Skoog Medium after 2 days in tin foil and 3 days under the grow lights.

Table 2: Effectiveness of methods for germinating Alfalfa seeds

Method of Germination	Number of seeds germinated	Total number of seeds	Percent of seeds that germinated
Sterile water in 8ml petri dish	4	88	0.45%
Sterile water in 20ml petri dish	28	66	42%
Murashige and Skoog Medium	39	44	88%

Figure 6: Percent of alfalfa seeds germinated using different methods

Colonization Experiment 2

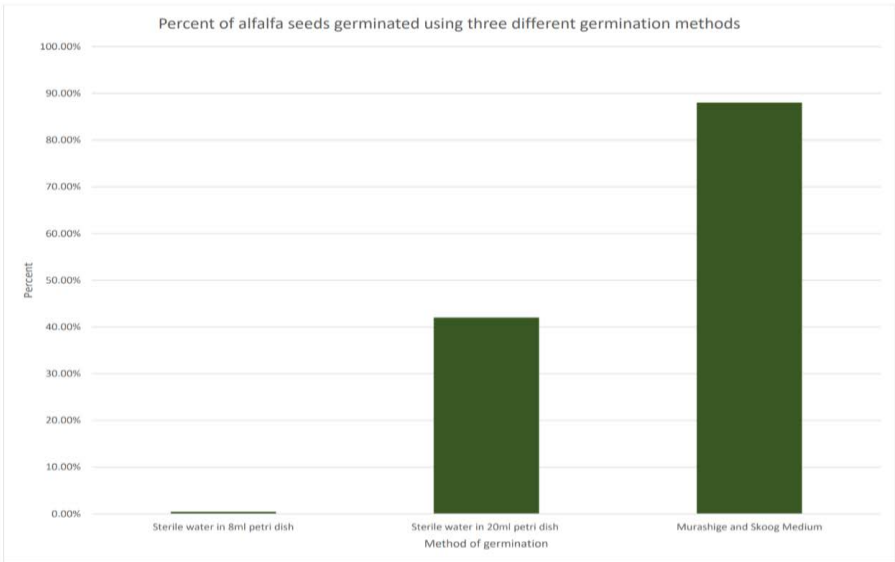In the second colonization experiment there was growth on all of the serial dilution plates including the control. There were multiple colonies with different colony morphology across all plates. The contamination was consistent with the dilutions, there was much more growth on the 10^0 plates than on the 10^6 plates.

Seed Sterilization

The alfalfa seed sterilization was performed exactly as before but the seeds were immediately homogenized and plated onto LB agar, there was growth on the plates. It was found that the bleach in the lab was one year expired. The serialization was performed again with new bleach at both 3% and 10% and there was no growth for either (Figure 7).
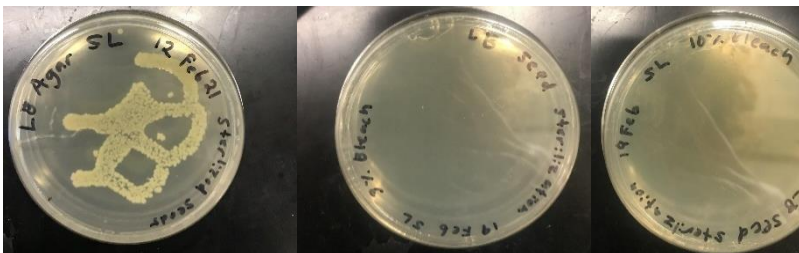

Figure 7: Left to right, homogenized seeds after seed sterilization using original bleach from the lab on LB agar after 2 days in the 28°C incubator, homogenized seeds after seed

sterilization using new bleach at 3% then 10% on LB agar after 2 days in the 28°C incubator.

Germination Experiment

Sterilized seeds were germinated in 0.01 OD of each of the isolates under grow lights for one week. There was only germination in the control with sterile water. The sterilized seeds in the inoculants did not germinate (Figure 8).
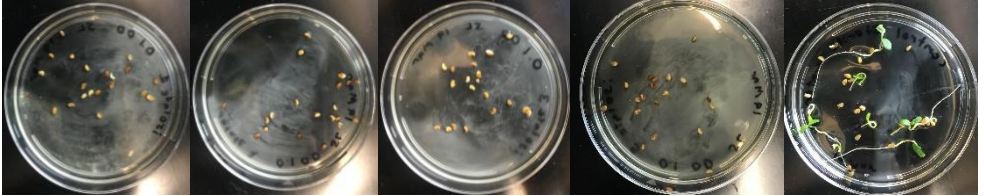

Figure 8: Left to right, 15 ml of isolates 1, 2, 3, 5, at 0.01 OD and control of sterile water. Each plate with 20 sterile alfalfa seeds after 1 week under grow lights

Colonization Experiment 3

In the third colonization experiment one of the controls got contaminated, the other two had no growth. For isolate 2, replicate 1 had 12 colonies on the 10^{-6} plate and 125 colonies on the 10^{-5} plate, replicate 2 had 18 colonies on the 10^{-5} plate and 178 colonies on the 10^{-4} plate, lastly replicate 3 had 2 colonies on the 10^{-6} plate and 46 colonies on the 10^{-5} plate. This can be seen in Figure 9.
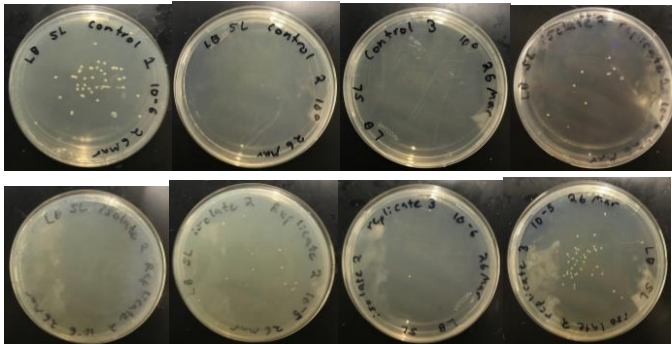

Figure 9: Results of third colonization experiment, serial dilutions on LB agar after 24 hours in 28°C incubator. From top to bottom, Control replicates 1-3 and Isolate 2 Replicates 1-3.

There was found to be 16×10^8 CFU/g for replicate 1, 18×10^7 CFU/g for replicate 2, and 2×10^8 CFU/g for replicate 3 for isolate 2 (Tables 3a and 3b).

Table 3a: Isolate 2 Colony Counts

Isolated Microbe	Replicate Number	Eppendorf Tube Weight (g)	Eppendorf Tube with Plant (g)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
2	1	1.09	1.12	TNC	TNC	TNC	TNC	125	12
	2	1.09	1.13	TNC	TNC	TNC	178	18	0
	3	1.09	1.13	TNC	TNC	TNC	TNC	46	2
Control	1	1.09	1.11						
	2	1.09	1.13	0	0	0	0	0	0
	3	1.09	1.11	0	0	0	0	0	0

Table 3b: Isolate 2 CFU Calculations

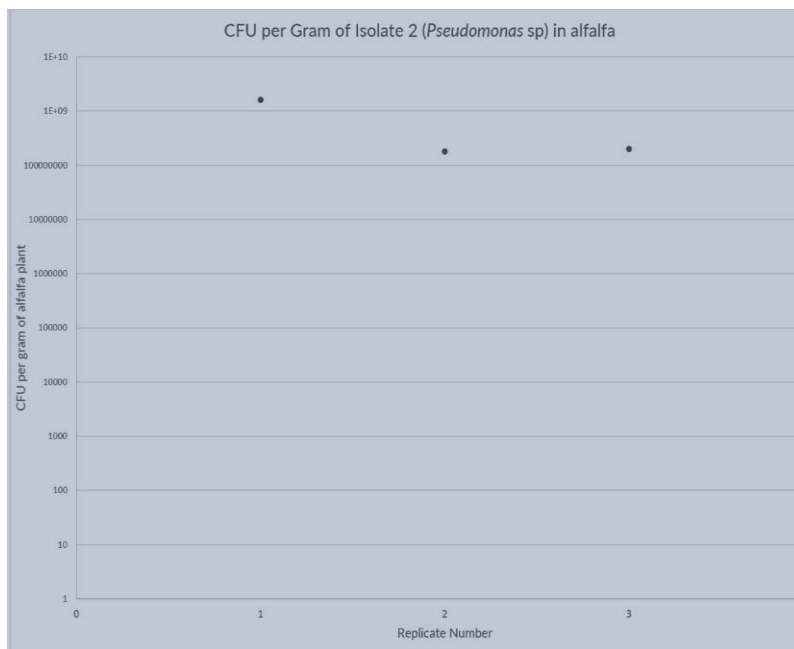| Isolated Microbe	Replicate Number	Eppendorf Tube Weight (g)	Eppendorf Tube with Plant (g)	CFU on Plate	CFL per 50ul	CFL per 200ul	CFL per gram
2	1	1.09	1.12	12×10^{-6}	12,000,000	4,800,000	1.6×10^{9}
	2	1.09	1.13	18×10^{-5}	1,800,000	720,000	1.8×10^{8}
	3	1.09	1.13	2×10^{-6}	2,000,000	800,000	2.0×10^{8}
Control	1	1.09	1.11				
	2	1.09	1.13	0			
	3	1.09	1.11	0			

Figure 10: CFU per gram of isolate 2 in alfalfa

Colony PCR

A colony PCR was performed and a band was there for the positive control and for isolate 2 (Figure 11).
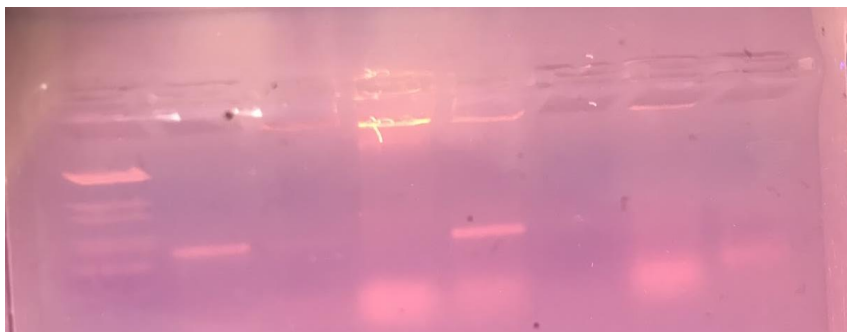
Figure 11: Gel from Colony PCR, from left to right Ladder, positive control, negative control, isolates 1, 2, 3, 4, 5, 6

DNA Extraction

A DNA extraction was performed and the DNA extraction product was nanodroped. The DNA concentration varied by isolates with some having very high concentrations of DNA and others with very little (Table 4).

ITS PCR

An ITS PCR was performed and there were bands for isolates 1 and 4. The bands appeared to be between 600-400bp (Figure 12).

Table 4: DNA concentration in extracted DNA product from each isolate

Isolate	Nanodrop DNA Concentration (ng/µl)	Graph
1	185	

2	132.7	
3	2.4	
4	242.9	
5	25	
6	56.3	
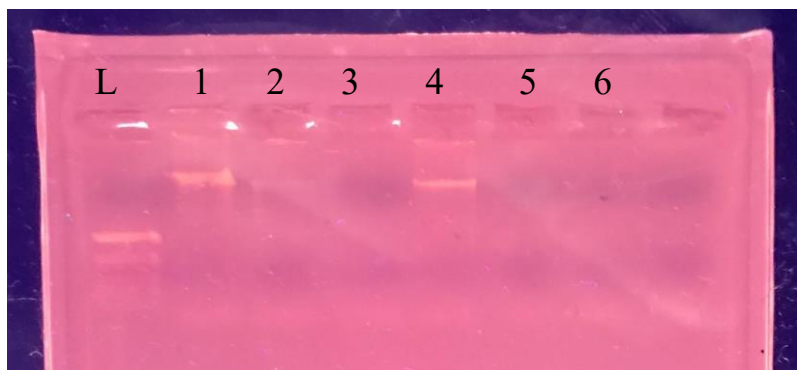
Figure 12: Gel from ITS PCR

The ITS PCR was performed again using just a positive and negative control; no bands for either control appeared (Figure 13).

Figure 13: Gel from ITS PCR with only controls

16S PCR and PCR Cleanup

A 16S PCR was performed using the DNA extraction product, there was a between 2640bp and 1400bp band for the positive control as well as all isolates other than isolate 3 (Figure 14).

The results from the 16S PCR Cleanup were nandroped and all isolates had very low concentrations of DNA. A Qubit was performed and very similar concentrations of DNA were detected for all samples (Table 5).

The 16S PCR was performed again with a modified PCR Cleanup and the nanodrop DNA concentrations were higher, between 31 and 16ng/µl (figure 15).
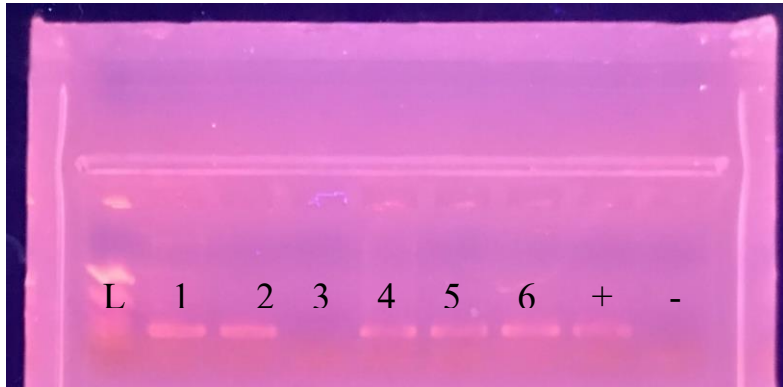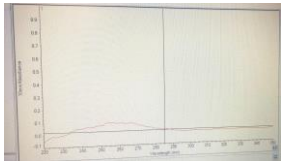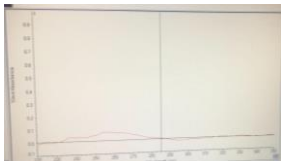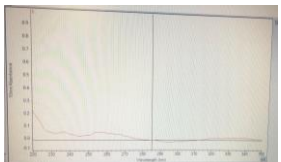
Figure 14: Gel from 16S PCR using DNA extraction product

Table 5: DNA concentration from 16S PCR Cleanup

Isolate	Nanodrop DNA Concentration (ng/µl)	Nanodrop Graph	Qubit DNA Concentration (ng/µl)
1	3.9		3.09
4	3.3		2.96
5	2.0		1.93
6	7.2		3.30
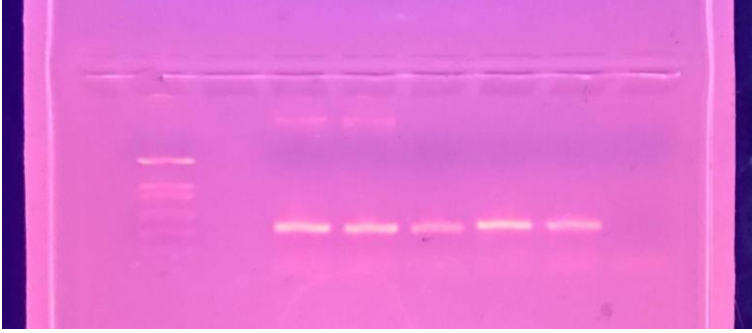
Figure 15: Gel from second 16S PCR using DNA extraction product

Table 6: DNA concentration from modified 16S PCR Cleanup

Isolate	Nanodrop DNA Concentration (ng/µl)	Graph
1	31.0	
4	23.9	
5	16.1	
6	22.0	

The PCR product from isolate 2 from the colony PCR was sent to Genewiz for sequencing and using NCBI BLAST was found to be a 99.35% match for several Pseudomonas partial sequences.

The PCR products for Isolates 1,4,5, and 6 from the 16S PCR were sent to Genewiz for sequencing. The samples were visualized using Ugene and appeared to be a little bit contaminated. The samples were identified using NCBI BLAST anyway. Isolate 1 had no BLAST matches. Isolate 4 was a 93% match for *Pseudomonas monteilii*. Isolate 5 was a 77% match for *Pseudarthrobacter sp.*L1SW. Isolate 6 was an 83% match for *Pantoea agglomerans*.
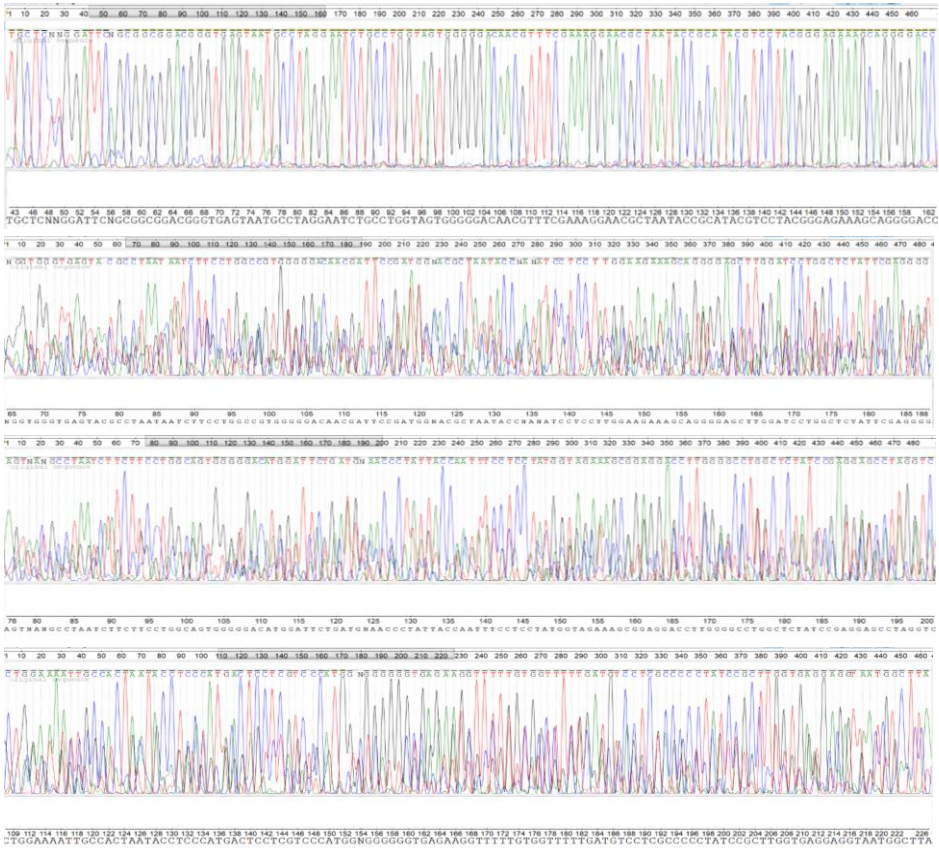
Figure 16: 16S sequences for (from top to bottom) isolate 2, 1, 4, 5, 6, visualized in UGene

IV. Discussion

Summary

Seed endophytes from alfalfa were isolated and characterized. The germination of sterilized seeds was optimized and the best germination was found using Murashige and Skoog Medium. Two different sources of contamination were identified and eliminated in the colonization experiment, 1X PBS and expired bleach. It was found that isolate 2 (*Pseudomonas sp*) colonized alfalfa at an average of $6x10^8$ CFU/g after 7 days. Using colony PCR and 16S PCR the isolates were identified as a probable Acintomcyeytes species, 99.35% match for *Pseudomonas* species, a 93% match for *Pseudomonas monteilii*, a 77% match for *Pseudarthrobacter sp*, and an 83% match for *Pantoea agglomerans*. An optimal PCR cleanup protocol was also developed. The protocols for these experiments were performed for the first time at this institution and optimized. This created a functioning framework of experiments for this lab.

Culture Based Experiments

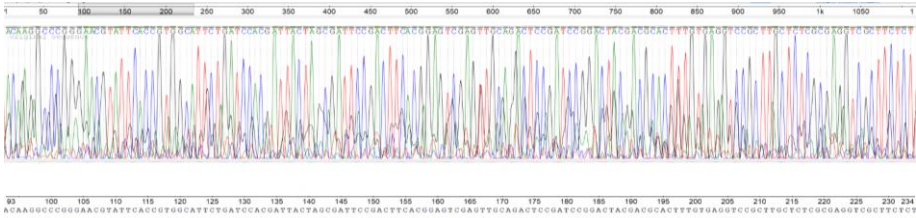The germination of the alfalfa seeds was optimized. In the first attempt to germinate the surface sterilized seeds only 4 of the 88 seeds germinated. This may have been due to alfalfa autotoxicity. Alfalfa are known to have autotoxicity where water soluble compounds are secreted which inhibit germination. These allelochemicals are likely produced in the shikimic acid or acetate pathway. They work by inducing oxidative stress and causing reactive oxygen species to build up which can result in membrane lipid peroxidation and even cell death (Zhang et al, 2021). When the alfalfa were germinated in a larger petri dish with less seeds 41% more of the seeds germinated. The highest percentage of seeds germinated when using Murashige and Skoog Medium, this is a medium for plant growth (Murashige and Skoog, 1962).

There were several sources of contamination that were found and eliminated. During the first colonization experiment the serial dilution plates were all equally overgrown with contamination. Since the contamination was the same throughout all of

the serial dilution plates the contamination most likely occurred after the dilution had taken place. The most likely sources were either the TSA plates themselves or the 1X PBS which was used to dilute the samples. A TSA plate and a TSA plate with 50ul of 1X PBS were left in the incubator. There was no growth on the plain TSA plate but there was growth on the 1X PBS plate (Figure 4). This indicates that the 1X PBS was the source of this contamination. The lab is a shared space among both research students and students using the space for lab assignments for classes. 1X PBS is very commonly used in many different experiments. There were many different opportunities for the 1X PBS to become contaminated.

In the second colonization experiment there was still contamination but the contamination was serially diluted. There were several different types of colonies on each plate including the control but the distribution through the dilutions was what was expected. The contamination being diluted means that the contamination had to occur before the serial dilution step. It also means that the original source of contamination had been eliminated. In order to find this new source of contamination the earlier steps within the protocol were evaluated. The first step is sterilizing the seeds which are then germinated. The seeds were sterilized according to the original procedure and immediately homogenized rather than germinated. The homogenization was plated on LB agar and there was growth so the original seed sterilization procedure was not working (Figure 7). The bleach used during this procedure was the bleach used to clean the labs. After examining all the reagents used to the procedure it was found that the bleach in the lab was over a year expired. The seed sterilization was performed again with fresh bleach at both 3% and 10% bleach concentrations and there was no growth on the plates (Figure 7). The issue with the procedure was the expired bleach and when fresh bleach was used the seeds were sterile so that source of contamination was eliminated. Since there was no growth on plates at both concentrations the issue was the bleach itself and not the concentration of the bleach so going forward 3% of the fresh bleach continued to be used for this procedure.

The colonization experiment was repeated but less seeds germinated. This could be due to the fresh bleach in the sterilization protocol. Due to the amount of germinated seeds, the colonization experiment was performed with only controls and isolate 2 since isolate 2 was able to be identified at that point. There was no growth on any of the plates for two of the controls but one of the controls had growth on the plates (Figure 9). The growth followed the serial dilutions and the colony morphology was the same as isolate 2. The serial dilutions and plating for the isolate 2 replicates was done before the controls. The control that was contaminated was the first control replicate so it is possible that the contamination occurred during the procedure. There was found to be 16×10^8 CFU/g for

isolate 2 replicate, 1. 18x10^7 CFU/g for isolate replicate 2, and 2x10^8 CFU/g for isolate 2 replicate 3 (Table 3). These values are similar to what has been found for other *Pseudomonas* species colonizing alfalfa. *P. fluorescens* F113 has been found to colonize alfalfa roots at a level of 2.9x10^7 CFU/g at day 7 (Villacieros et al., 2003).

None of the seeds germinated in the presence of 0.01 OD of the isolates (Figure 8). This could mean that the isolates are plant pathogens. Since all of them did not grow there also could have been an issue with the protocol or calculations. There was also an unexpected amount of turbidity in the water for 0.1 OD. This could indicate that the calculations were off or the spectrophotometer values were off. The spectrophotometer is quite old and infrequently used so it is very possible that the calibration is off. The control group of sterile water had an expected amount of germination which indicates that there was not an issue with the protocol. This experiment could be run again using a different spectrophotometer and having another person double check all calculations.

<div align="center">Molecular Experiments</div>

A colony PCR is performed by touching a sterile loop to an isolated colony on an agar plate and using that as the DNA template for the PCR. When this was done there was only a band for one of the isolates, isolate 2 (Figure 11). Colony PCR is a lab technique usually used for rapid screening of yeasts or bacteria. Since it relies on using an isolated colony as the DNA template rather than DNA that has been extracted, often they do not work. This is because the membrane of the cells is unable to be broken open during the PCR. Since gram positive bacteria have a thick peptidoglycan cell wall they are harder to break open using colony PCR. The one organism that was amplified during the colony PCR was later found to be a *Pseudomonas sp.* which are gram negative. Other than isolate 4, the other isolates that underwent the colony PCR were later identified as gram positive organisms which might explain why they were not amplified by the colony PCR.

The first time that the ITS PCR was performed there were bands for some of the isolates but they appeared to be the wrong length (Figure 12). The protocol was done again with a positive and negative control and no bands appeared for either (Figure 13). It was confirmed that the primers used were correct and the DNA extraction products were nanodropped and other than isolate 3 all had fairly high concentrations. Later bands were found for all isolates other than isolate 3 during the 16S PCR, meaning that the isolates were all either bacteria or archaea. This would explain why there was no amplification during the ITS PCR as the ITS region is found in fungi (Martin and Rygiewicz, 2005). If none of the isolates were fungi then there is no ITS region to amplify.

A 16S PCR was performed using the DNA extraction products and there were bands for all isolates other than isolate 3 which is to be expected since the initial DNA

concentration for that sample was very low (Figure 14). A PCR cleanup was performed using the Promega Wizard SV Gel and PCR cleanup kit and the DNA was eluted in 50ul sterile water. The DNA concentrations were very low using both the nanodrop and Qubit DNA concentrations between 1-3 ng/ul were found (Table 5). The PCR was performed again with the same results for the gel. The PCR cleanup was performed using the QIAGEN PCR cleanup kit and the DNA was eluted in 30ul of elution buffer and let to sit for one minute before the final centrifugation. After this change to the protocol DNA concentrations between 16-31 ng/ul were found (Table 6). For this kit DNA elution is most efficient under slightly basic conditions (pH between 7 and 8.5) with a low salt concentration (Kit handbook). DNA is more stable at this slightly basic pH because hydroxide ions can interrupt the hydrogen bonds in the DNA. It being more stable allows it to dissolve faster in the buffer which is important as the elution step is very quick. The pH of the sterile water used for the initial elution was not tested and if the pH was too acidic that could have interfered with the elution and caused the low DNA concentrations initially found.

Isolate 1 was unable to be identified using molecular techniques. It was amplified using 16S primers meaning that it is either a bacteria or archaea. It was also isolated on actinomycetes selective media. Therefore it is very likely an actinomycetes species. Actinomycetes are a common endophyte with 123 actinomycete strains having been found in over 113 species of plants (Matsumoto and Takahashi, 2017). They have been found to have some plant growth promoting properties along with antibacterial and antifungal activity (Shan et al., 2018). Of 46 actinomycetes isolates from tea plants 93.5% produced indole acetic acid (IAA) and 21.7% produced 1-aminocyclopropane- 1-carboxylic acid (ACC) deaminase.

Isolate 2 was identified using DNA sequencing as a 99.35% match for *Pseudomonas* species. Isolate 4 was also identified as a 93% match for *Pseudomonas monteilii*. These isolates both have very similar colony morphology so it makes sense that they are both Pseudomonas species. *Pseudomonas spp.* have been found as a seed endophytes in several different plants using many different technologies such as 16S rRNA Sequencing, PCR DGGE, and fatty acid methyl ester profiles (Ferreira et al. 2008, Mastretta et al., 2009, Johnston-Monje and Raizada 2011, Liu et al., 2012, Hardoim et al., 2012, Vega et al. 2005, Graner et al., 2003). Pseudomonas have been found using both MALDI-TOF and 16S rRNA gene sequencing in alfalfa seeds (Lopez et al, 2017). *P. monteilii* has been isolated from surface sterilized *Salvadora persica* (the toothbrush tree) leaves, shoots, and roots (Korejo et al., 2019). It has been shown to have a zone of inhibition and the lysis of fungal hyphae against *M. phaseolina, F. solani, F. oxysporum and R. solani*, which are all root infecting fungi. It was also found to have a positive effect

on sunflower growth. Similar results were found in Okra where *P. monteilii* was shown to reduce infection of *M. phaseolina*, *F. solani*, and *P. decumbens* in okra roots alongside *Penicillin* (Urooj et al., 2020). In a mixture with *Penicillin* it was also found to improve okra height, weight and polyphenol production. *Pseudomonas fluorescens* F113 has been found to have several plant growth promoting features and siderophore production, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, and inorganic phosphate solubilization (Lally et al., 2017). *P. fluorescens* F113 has been found to increase crop height, and stem, leaf and pod biomass in field experiments using *Brassica napus*.

Isolate 5 was identified as a 77% match for *Pseudarthrobacter sp.* L1SW complete genome. This species is unpublished and was uploaded directly to NCBI by Li, J at the School of Life Sciences and Biotechnology, Shanghai Jiaotong University (NCBI website). Another *Pseudarthrobacter species, Pseudarthrobacter phenanthrenivorans* strain MHSD1, has been isolated from surface sterilized leaves of *Pellaea calomelanos*, which is a species of fern (Tshishonga and Serepa-Dlamini, 2020). A different *Pseudarthrobacter* species has been identified in the rhizosphere of *D. antarctica* from King George Island in the Antarctic peninsula (Marian et al, 2022). *Pseudarthrobacter* have also been found in sterlized roots of tobacco plants (Shimasaki et al., 2021). While not much is known about the role of *Pseudarthrobacter* species as endophytes they have been found in a broad range of plants and environments. Isolate 3 was never able to be identified since there was a very low DNA concentration after DNA extraction. Isolate 3 had a very similar colony morphology to isolate 5 so it is possible that isolate 3 was also a *Pseudarthrobacter sp*.

Isolate 6 was identified as an 83% match for *Pantoea agglomerans*. *Pantoea agglomerans* YS19 isolated from rice plants has been shown to be a plant growth promoter (Shen and Song, 2006). It has been found to fix nitrogen and produce indole-3-acetic acid, abscisic acid, gibberellic acid and cytokinin which are all phytohormones. It was also found to increase plant biomass as well as enhance the transportation of the photosynthetic assimilation product. The sugarcane endophyte *Pantoea agglomerans* strain 33.1 has been found to colonize all areas of the sugarcane plant including the rhizosphere, inside the roots and aerial plant tissues (Quecine et al., 2012). It has been found to produce indole acetic acid (IAA) and solubilize phosphate and it has been suggested to stimulate chitinase and cellulase production in plant roots.

The 16S identifications come with an important caveat. The 16S samples had relatively low similarity to the sequences they matched to in BLAST and when visualized in UGene they appeared to be contaminated. There were multiple overlapping peaks for all of these samples (Figure 16). Overlapping peaks most commonly mean that there were two template DNA sequences present in the reaction. The most obvious cause of this is

contamination. The 16S reaction was run twice and both times the negative control did not have any amplification suggesting that contamination did not come from any of the reagents. One possible source of contamination would be intracellular bacteria, if two organisms with 16S regions are present then both would be amplified. Overlapping peaks can also mean that there are two priming sites present in the DNA template or too low of a primer annealing temperature was used (Nucleics DNA sequencing support, website). These isolates all came back as organisms that are known to be plant endophytes which could indicate that the identifications are correct. But all analysis on the identity of these organisms should be considered with the knowledge that these identifications could be incorrect.

<div align="center">Future Research</div>

There are many interesting directions for future research. Now that a working protocol has been developed the colonization experiment could be repeated for the rest of the isolates. This is the first time these experiments have been run at this institution and now there is a framework for all of these experiments to be run again in the future. The colonization experiment could also be repeated for different lengths of time. Due to time constraints the colonization experiment was only conducted over 1 week but there can be differences in endophyte colonization over different phases of plant growth. It would be interesting to look at colonization over a month or two and look at the changes in colonization week by week. The colonization in different parts of the plant could also be measured. The roots, leaves, and stem could be homogenized separately and colonization levels in these different areas of the plant could be measured. FISH could be used to visually examine the location of these endophytes within both the seeds and the plant's roots as they colonize. Various biochemical assays could be used to look for plant growth promoting activities in the isolates such as indole-3-acetic acid (IAA) production, phosphate solubilization, siderophores production, nitrogen fixation, and 1-aminocyclopropane -1-carboxylate (ACC) deaminase production.

V. Acknowledgements

which is on the land of the Lenepe people. I acknowledge the Lenni-Lenape as the original people of this land and their continuing relationship with their territory.

VI. References

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The Effect of Blockchain on Company Efficiency and Profit

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This paper takes a deep dive into blockchain infrastructure and how it could potentially benefit industries and businesses if they successfully implement it into their infrastructure. The breakdown of blockchain is explained through its molecular components and how secure blockchain is. A point was also made to include the negatives of blockchain to let the

reader decide for themselves if they feel blockchain can aid society.

I. Introduction

As we continue the technological revolution influenced by a mass pandemic, the world is yearning for innovations that allow us to communicate on a less personal and more mediated level while keeping the same sense of personal relationships. Although the thesis intends to present this technology to whoever is reading, everyone must understand the dynamics built around this technological advancement so that there are no predisposed biases. With the direction the world is heading toward when it comes to advancing technologically as a society, blockchain fits right into that progressive direction and can serve as a helpmate for increasing a company's efficiency. Many companies in various industries have adopted blockchain into their infrastructure to combat common problems their markets may face. A prime example of blockchain being adopted into an infrastructure would be the automotive industry, where they have been fighting against environmental issues and high manufacturing costs for the past decade. As a result, many of those automotive companies have collaborated to solve these problems using blockchain. With all the upside blockchain possesses, that does not exclude blockchain from its shortcomings. Blockchain is still relatively new and has some chinks in its armor. With any new product on the market, issues will happen, but with time and continuous research, the use of blockchain systems will improve the efficiency of companies.

The concept of blockchain increasing company efficiency is related to United Nations Sustainable Development Goal (SDG) number 9, Industry, Innovation, and

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Infrastructure. The consistent growth in technology has allowed companies to progress in their respective industries. The SDG proved an important point stating that higher-technology companies were more resilient towards crises than their counterparts of lower technology. They also stated that 1 in 3 manufacturing businesses are affected negatively by a crisis. With advancements blockchain has in itself, it can potentially serve as another technological alternative to potentially help companies for not just the present, but the future.

<p style="text-align:center">Components of Blockchain</p>

Components

A basic description of blockchain would be that it is a disseminated network of computer systems linked with the internet. These components rely on two fundamental concepts, consensus and trust to efficiently work together to keep track of electronic transactions for whoever implements it (YouTube, 2017). On a more complex level, a blockchain comprises a series of blocks linked together in a chain. Each block consists of three critical elements that allow the system to operate smoothly. The first element of the block makeup is its data, which is responsible for providing transaction details (YouTube, 2017). That data is stored and registered in the system-assigned blocks through the confirmation of nodes. Nodes serve as an intersection point for a blockchain network to help maintain security. A simple example of a block's data would be the detailed information of the sender, the receiver, and the total amount of money dealt with in the transaction.

The block's Hash is the second and most crucial element, also known as a code. A hash could be considered the fingerprint of a block, as each hash within the chain has its unique makeup (YouTube, 2017). Once the information about the block is confirmed by all of the nodes in this system, the block is solidified by being given a specific hash. The job of a hash is to identify the contents of each block registered in the network for the user. Depending on what type of blockchain system is being used, the user will ingrain specific information that they want to be put into the first block, Called the "genesis" block (YouTube, 2017). It is to be made known that blockchain is a very particular network that does not veer off its program. Changing information inside the block will cause the hash to change, making it easy to detect changes by the user or users of that chain (YouTube, 2017). Strict computer codes enforce the rules of the blockchain, and their codes do not possess any ambiguity in their meaning. Because of this process, they cannot be not subject to human discretion or interference (Xu & Zou, 2020). Bringing us back to the main point, whatever data the user puts inside that block must be

specific and align with their end goal and overall agenda. Otherwise, it could potentially lead to the failure of the individual's business infrastructure within the blockchain.

The final element in creating a blockchain Is the "hash of the previous block" because there would be no chain if only a single block of recorded transactions existed. The hash of the previous block is one of the keys to making blockchain as secure as it is, although there was a recent case where Binance, the world's largest cryptocurrency exchange, was recently hacked (Forbes.com, 2022). Once the next block in the chain is confirmed and put into the network, it receives its hash, but it also includes the hash of the block created before it. This is a vital security process because the more blocks added to the system, the higher the protection of the user's information from potential tampering (YouTube, 2017). Let us say there were five blocks of data within a chain; if someone were to tamper with the third block and change its hash information, it would then result in the change of all the blocks coming after because the fourth block contains the hash information of the third block. The fifth block has the knowledge of the fourth block. Once their hash information is altered, all the other blocks' code configurations change.

Security

An increase in crypto innovation over the past several years has also led to a significant increase in crypto crimes reported. In 2020 it was reported a 24,057% increase in crypto crimes from 2016 (Rotundu, 2022). What is also shocking about this statistic is that the increase is directly proportional to increases in the value of Bitcoin), although the cryptocurrency market took a huge hit recently (Rotundi, V., 2022). FTX, one of the largest known crypto exchanges, collapsed due to a lack of liquidity and mismanagement of funds (nerdwallet.com, 2022). Blockchain should not be held responsible for the collapse of the crypto market for two reasons. First, blockchain is not limited to just one market. Multiple industries can use blockchain depending on the business's needs within that industry. Second, blockchain follows a strict algorithmic protocol that does not allow it to act maliciously. Part of blockchain's security relies on a 3-part operating consensus. The names of these operating systems are machine consensus, governance consensus, and market consensus (Xu & Zou, 2022). A consensus is a decision or an opinion that is accepted by a group of people, or in blockchain's terms, by the system itself.

Machine Consensus

Blockchain's technological structure allows it to be useful in many different industries and is a key part of making blockchain what it is. Strictly referring to blockchain's molecular makeup, it aims to ensure the nodes in its peer-to-peer network share the same copy of a distributed ledger in case of harmful cyber-attacks and irregular

communication within the chain (Xu & Zou, 2022). To get a better understanding of this process, you must understand what nodes are. A node's job is to serve as a gateway within the blockchain network to confirm a transaction. Nodes can also be physical equipment connected to a network, in which they can perform specific duties like creating, receiving, or ascending data across a blockchain channel (Abrol; blockchain-council.org, 2022).

An example could be a person who is running the blockchain network from their home, and they are using another physical system like a laptop with its own MAC address so that the laptop's identification can be verified. The user would now have access to use that laptop as a physical node for producing, receiving, or moving data. The action of a user controlling the physical node is called a "miner". On a deeper note, a node's function occurs when a miner seeks to add a new block of transactions to the blockchain. The legitimacy of a block determines whether the miner accepts or declines the transaction (Abrol; blockchain-council.org, 2022).

An internal form of machine consensus is proof of work (PoW). PoW is a mechanism within a blockchain network that slows down the creation of new blocks by requiring miners to solve a mathematical puzzle to confirm the transaction's legitimacy (YouTube, 2018). Making the user complete a puzzle seems childish but doing so allows for others participating on the network to be notified quickly enough of the change so that the rest of the users can make a consensual decision to confirm or deny any change (YouTube, 2018). The first miner within the PoW network to solve the hash equation receives an award from the network. Because the PoW network gives larger rewards to miners with better quality equipment, it has led to a situation where miners are creating mass mining farms that use an excess of electricity to solve the equations (YouTube, 2018). This is an extremely important topic because technology is not the only important application of knowledge. The knowledge of our world and how to preserve its health is an integral part of our existence and improving one form of the world at the cost of another is potentially catastrophic to our people's well-being and safety. A recent study identified a significant increase in energy consumption from December 1st, 2020 – December 1st, 2022. PoW miners have also been known for creating what is known as mining pools to raise their hash power and increase their chances of receiving mining rewards that could be distributed evenly amongst the group (YouTube, 2018). The act of a person strengthening their processing power irregularly through mining pools contradicts blockchain's intended duty. The actions conducted within mining pools create a more centralized operating system leaning away from the blockchain's intended decentralized infrastructure (YouTube, 2018). The environmental and structural

drawbacks of PoW systems have sparked other crypto exchanges to make the switch over to a new system of consensus.

PoW was heavily used by nodes in the earlier stages of Ethereum (crypto exchange platform) to find the hash of their transactions, but the environmental hazards motivated the company to switch its machine consensus to a more productive and safer version called Proof-of-Stake (PoS). Ethereum believed it would help prevent users from double spending on their network (etherum.org, 2022). Unlike the system of PoW, PoS uses validators to confirm transactions and requires them to mint or forge new blocks (YouTube, 2018). For a user (node) or users to become a validator, they are required to make a deposit to the network that way every other node within the network is held responsible if the system fails (YouTube, 2018). Smaller businesses are great examples to help gain a better understanding of what PoS is. For example, a well-known independent contractor of a small sports performance business wants to promote a product for a respected company. The two parties then come to an agreement to use a blockchain platform to finalize their agreement. Depending on what platform the two companies choose, whether it be Coinbase, Ethereum, or any other exchange platform using PoS, the agreement is installed in the blockchain system and stored as a hash in a block, making them nodes. If the two nodes would like to become validators over the block, whoever of the two invested more into the network as a stake is given a higher chance of being randomly selected to check over the validity of transactions within the block. Once the transactions are successfully processed, the chosen node becomes the validator and can sign off on the block and add it to the chain. You might now ask, how can the other node in the agreement tell if the validator is being truthful about the validity of the block? PoW regulates the nodes by penalizing the offender with a percentage of their stake significantly reduced if fraudulent transactions were confirmed. With a multitude of machine consensus options, blockchain has to offer, blockchain can serve as a useful tool in ensuring the protection of companies (Youtube, 2018).

Governance Consensus

Another valuable process in blockchain's consensus info structure is governance consensus. Governance consensus pertains to members of a blockchain community that all agree on a decision or decisions that benefit the group as a whole (Xu & Zou, 2022). The unified decisions of the group are a crucial element in the stability and security of blockchain. If individuals fail to come to an agreement or deviate from the consensus rules, the possibility of governance consensus is broken, leaving companies susceptible to collapsing (Xu & Zou, 2022).

Market Consensus

Market consensus deals with transactions of tokens that are traded with each other or with assets outside of the blockchain system. Depending on what the equilibrium of the market price is, it determines the state of the market consensus (Xu & Zou, 2022). Each form of consensus has some type of relationship with the other that affects blockchain as a whole. If there was malicious behavior taking place amongst the members of governance consensus and the system were to fail for a company using blockchain, it could lead to malfunctions within machine consensus. The failure of the machine consensus then would have an effect on the integrity of the market consensus.

Difference Between Blockchain Platforms

Today, new technology is often complex and is not created in a way to complete a particular task. New technology is structured and coded to take care of a multitude of needs for a consumer regularly; the same goes for blockchain and its platforms. Depending on what type of network a company conducts business on, whether it be a public, permissioned, or private network, or what the company needs to be protected from or track within the industry, determines which blockchain platform is selected (leewayhertz.com, 2022).

Small business owners and large companies need to know what type of blockchain platform they want before picking one. You can install a platform whose ledger type is permissionless, like Ethereum so that you can have more control over the decisions of your transactions made (leewayhertz.com, 2022). You can want the opposite in your ledger type and get a blockchain platform that requires permission to maintain equilibrium between a partnership. If a company wants to have the ability to configure official contractual electronic agreements on the blockchain network called a smart contract, the company selects a certain platform that provides that need.

Smart Contracts

This brings us to our next topic of discussion, the use of smart contracts within the system of blockchain. Smart contracts are decentralized agreements with built-in computer code stored on a blockchain (Sklaroff, 2017). The goal of smart contracts is to remove some of the inefficiencies that paper contracts may face. Examples of inefficiency for traditional contracts can be breaches of contracts, slow processing, or manipulation tactics. Smart contracts can mitigate the possibility of a breach by forcing the people agreeing to the contract to honor their original agreement (Sklaroff, 2017). The rigid quota of smart contracts reduces the negotiation flexibility of nodes once they have finalized the smart contract.

<div align="center">Advantages and Disadvantages of Blockchain</div>

Disadvantages

As much as blockchain may get recognized for its benefits, some factors about blockchain are not too appealing. As was mentioned before, the process of mining blockchain blocks has had considerable negative effects on energy consumption for the past several years (digiconomist.net). Large amounts of energy being consumed could potentially lead to power outages or even fire hazards if one of the mining farms were not kept under safe conditions. Blockchain's strict infrastructure might not be conducive for everyone, as some people might want the ability to opt out of a contract. Once the people within the network agree to a contract, they are bound to the contract by being confirmed as nodes in a blockchain system (YouTube, 2018). Being an integral part of the machine consensus, they are held to certain rules backed by the system. Blockchain being recognized as a useful system in the tech world is still relatively new due to it just recently bursting onto the scene around four years ago. The lack of expertise in the technology has left blockchain vulnerable to hackers and skilled criminal organizations (Rotundu, 2022). This is the most alarming problem because blockchain's reputation relies on its word of being a highly secure AI network.

Advantages

On the other hand, the problems that blockchain may face do not entirely define the potentialities of what it can bring to companies. Blockchain still offers services that help many different companies across several different industries. A consensus of 37 major automotive and tech companies, Toyota and General Motors being of the group, have banded together to help tackle the challenge of making vehicles more widely accessible to the public with blockchain technology (Dobreva, 2019). The ability to create affordable cars would be able to significantly help automotive companies because that could potentially increase sales rates of companies, generating more money for each other. Blockchain has also played a role in the retail industry. Companies like Home Depot and Walmart have joined blockchain systems themselves to help ensure the safe travel of their products being transferred from destination to destination (Brown, 2021). Blockchain offers a flexible system that gives entrepreneurs or companies the option of tailoring the blockchain system to their business infrastructure depending on the individual's particular needs.

<u>Versatility of Blockchain</u>

Ways Blockchain is Used

As mentioned before, blockchain is a very flexible system that can be used in many different industries. Automotive and Retail industries have formulated business plans revolving around blockchain, but blockchain can offer so much more. The county of Jordan in the Middle East has proposed ideas to fully integrate the blockchain system into the government's financial department, amid the mass pandemic that took place back in 2020 (Khasawneh, Oquab, 2022). By Jordan linking blockchain to its governmental e-accounting systems, Khasawneh believed that they would be able to improve policies and decisions that help manage Jordan's health crisis from COVID-19 (Khasawneh, Qquab, 2022).

II. Hypothesis

After concluding my research and taking the pros and cons of the structure of blockchain, I believe that if companies were to implement blockchain into their platform then the company's efficiency would increase. The ability to measure efficiency for a company can be difficult, so my data research consisted of me analyzing the financial data of a company's annual profit, revenue, and income numbers. I planned on calculating the productivity of a company, but that too became difficult to analyze because productivity is another element that is difficult to judge solely by numbers.

III. Methods

<u>Subjects</u>

As part of my research, I looked at the financial data of five companies in four separate industries. I wanted to gather more companies to research but due to time constraints, it was very difficult for me to do so. Home Depot, AT&T, Ford Motors, T-Mobile, and Pfizer were all the companies that I studied and analyzed the data for.

Home Depot

Home Depot is the world's largest home improvement retailer in the U.S. founded in June 1978. Their specialties consist of building materials, home improvement supplies, hardware, and various other products that help customers with their daily needs.

AT&T

AT&T is an American multinational telecommunications company. The company was established in October 1983 and its main headquarters is located in the downtown Dallas area of Texas. They are currently the world's largest

telecommunications company in revenue and the third-largest provider of U.S. telephone services.

Ford Motors

Ford Motors is an American multinational automobile company established on June 16th, 1903. They are headquartered in Dearborn, Michigan, and sell automobiles as well as commercial vehicles under the brand.

T-Mobile

T-Mobile is a multinational telecommunication company established in December 1999 and headquartered in Bonn, Germany. T-Mobile offers mobile broadband internet access for smartphones, basic phones, and tablets.

Pfizer

Pfizer is one of the world's largest biopharmaceutical companies. Founded in 1849, they serve to deliver breakthroughs in the pharmaceutical industry using science and technology.

<div align="center">Measures</div>

Because efficiency is a difficult variable to calculate, the variables that I will use for my data research are income, revenue, and gross profit. Income is an important part of this data research because the use of blockchain directly correlates with the efficiency of a company. If a company is more efficient in its work, it will then gain more income from its products. Revenue is important to the research because it serves as an indicator for a company based on the other two variables. If a company's income and profit are up, likely, its revenue will also increase. This leads back to the hypothesis that the efficiency of that particular company will increase. Gross Profit gives a clear indication of how a company stands in the ranks within its respective industry, making this data extremely important to the research.

Components of Study				
Data	**Variables**			
		Efficiency		
	Blockchain	Income	Gross Profit	Revenue
Instrument	Horrmann Library, Peer reviewed topic articles	annual financial reports of Ford, Home Depot, AT&T, T-Mobile, and Pfizer companies	annual financial reports of Ford, Home Depot, AT&T, T-Mobile, and Pfizer companies	annual financial reports of Ford, Home Depot, AT&T, T-Mobile, and Pfizer companies
Data Source	annual reports of publicly traded companies	annual reports of publicly traded companies	annual reports of publicly traded companies	annual reports of publicly traded companies
Type of data gathered	Quantitative	Quantitative	Quantitative	Quantitative
Types of scores produced	Total GNP	Indexed to 1982		
Adapted from: Rudestam, K.E. & Newton, R.R. (1992). Surviving Your Dissertation. Newbury Park, California: Sage Publications, Inc. page 140.				

Hypothesis, Instruments, and Statistical Analysis						
Hypothesis	Instrument				Variable Relationships	Statistical Test
	Blockchain	Efficiency				
		Income	Revenue	Gross Profit		
H1: The use of blockchain technology will improve the efficiency for a company.	Horrmann Library, Peer reviewed topic articles	annual financial reports of Ford, Home Depot, AT&T, T-Mobile, and Pfizer companies	annual financial reports of Ford, Home Depot, AT&T, T-Mobile, and Pfizer companies	annual financial reports of Ford, Home Depot, AT&T, T-Mobile, and Pfizer companies	SC=PF+ PD+RV	correlation; regression; line charts
Adapted from: Rudestam, K.E. & Newton, R.R. (1992). Surviving Your Dissertation. Newbury Park, California: Sage Publications, Inc. page 138.						

<div align="center">Procedure</div>

The statistical procedure I will use is to look at the financial records of each company from before and after they applied blockchain into their business. Then I will look to see if there is any legitimate documentation from the companies stating whether the implementation of blockchain has positively or negatively affected the company. The approach that I used to locate the journal articles consisted of me using the Horrmann Library to direct myself to the business source premier. There, I looked up in the search bar blockchain technology "and" efficiency and got 541 articles. I found that the articles became too general and limited my search results by applying full text and peer reviewed to my search results. When I applied those two factors the search results went down to 105, where I found 10 solid articles to support my hypothesis. To conclude my hypothesis, I utilized the annual financial reports of the companies that were previously mentioned. Each report provides the company's net income, revenue, and gross profit. Based on the company's annual reports, it determines how much of a positive or negative change blockchain may have had. A two-year period was recorded before and after the year each company implemented blockchain into the business, giving a balanced outlook on the data.

IV. Results

Table 1 shows the financial data of Ford Motors from the years 2017–2021. Ford implemented blockchain into their company in 2019, so the numbers provided are two years before the implementation and two years after.

Table 1: Ford Financial Income Statement, 2017–2021, In Millions

Income Statement	Dec. 31, 2017	Dec. 31, 2018	Dec. 31, 2019	Dec. 31, 2020	Dec. 31, 2021
Net Income	$7,757	$3,695	$84	($1,276)	$17,910
Revenue	$145,653	$160,338	$143,604	$115,894	$126,150
Gross Profit	$25,455	$24,069	$21,207	$14,392	$21,690

(Sec, 2022)

Figure 1 shows that for the years 2017 and 2018, all of Ford's financial recordings were for the most part at its highest. From 2019 to 2020, their revenue and net income dropped significantly before picking back up slightly in 2021.
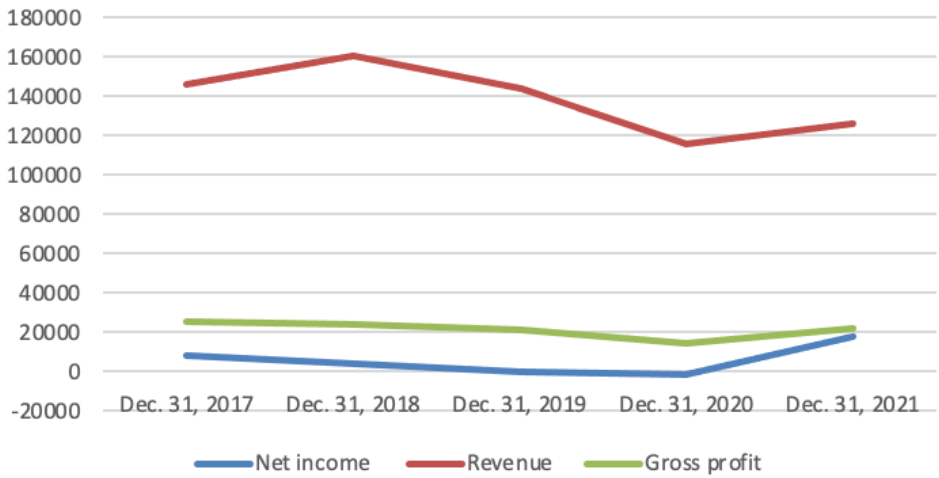
Figure 1. Depiction of Ford's Net Income, Revenue, and Gross profit from 2017–2021 in Millions.

Table 2 shows the financial data of Home Depot from the years 2016–2020. They implemented blockchain into their company in 2018, so the numbers provided are two years before the implementation and two years after. Figure 2 depicts that all of Home Depot's financial stats had increased steadily before taking a small, but significant jump in revenue in 2019. This was one year after the implementation of blockchain.

Table 2: Home Depot Financial Income Statement, 2016 – 2020, In Millions

Income Statement	Jan. 31, 2016	Jan. 29, 2017	Jan. 28, 2018	Feb. 03, 2019	Feb. 02, 2020
Net Earnings	$7,009	$7,957	$8,630	$11,121	$11,242
Revenue	$88,519	$94,595	$100,904	$108,203	$110,225
Gross Profit	$30,265	$32,313	$34,356	$37,160	$37,572

(Sec, 2022)

Figure 2: Home Depot Financials 2016–2020 in Millions.

Table 3 shows the financial data of AT&T from the years 2016–2020. They implemented blockchain into their company in 2018, so the numbers provided are two years before the implementation and two years after. Figure 3 shows that from 2016 to 2018 AT&T's financial stats fluctuated several times between each other. In 2019, one year after they implemented blockchain, their stats increased before plummeting the next year.

Table 3: AT&T Financial Income Statement, 2016–2020, In Millions

Income Statement	Dec. 31, 2016	Dec. 31, 2017	Dec. 31, 2018	Dec. 31, 2019	Dec. 31, 2020
Net Income	$13,333	$29,847	$19,953	$14,975	($3,821)
Revenue	$163,786	$160,546	$170,756	$181,193	$171,760
Gross Profit	$23,543	$19,970	$26,096	$27,955	$6,405

(Sec, 2022)
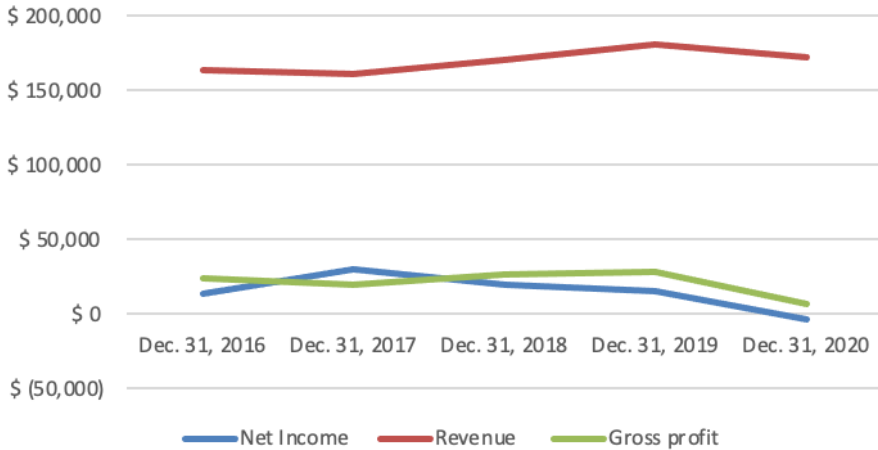
Figure 3: AT & T Financials 2016–2020 in Millions.

Table 4 shows the financial data of T-mobile from the years 2016–2020. They implemented blockchain into their company in 2018, so the numbers provided are two years before the implementation and two years after. Figure 4 shows that T-mobiles numbers of its revenues, gross profit, and net income all increased through the years 2016 and 2017 and fluctuated between gradual increases and decreases throughout the next two years. In 2020 All of AT&T's finances dramatically increased.

Table 4: T-Mobile Financial Income Statement, 2016-2020, In Millions

| Condensed Consolidated Statements of Comprehensive Income-In millions	Dec. 31, 2016	Dec. 31, 2017	Dec. 31, 2018	Dec. 31, 2019	Dec. 31, 2020
Net Income	$1,460	$4,536	$2,888	$3,468	$3,064
Revenues	$37,490	$40,604	$43,318	$44,998	$68,387
Gross Profit	$26,671	$28,996	$24,964	$26,477	$40,121

(Sec, 2022)
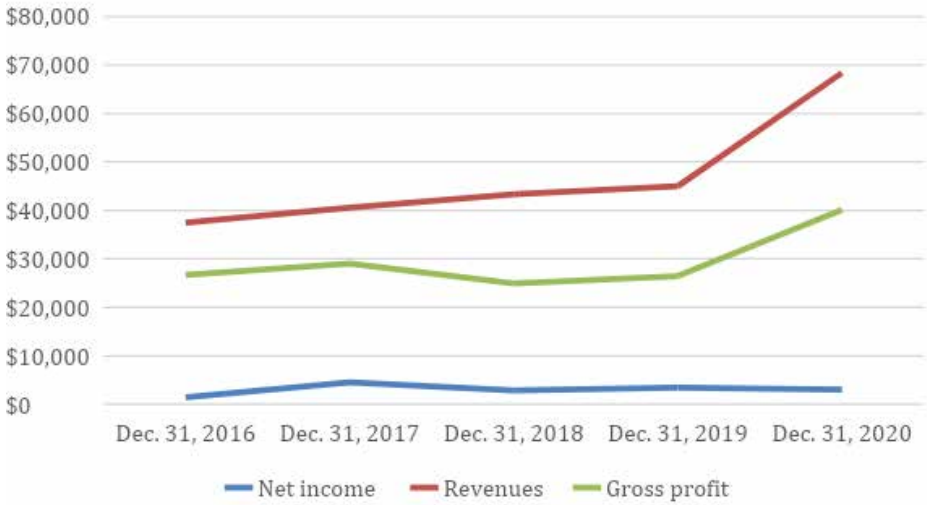
Figure 4: T-Mobile Financials 2016–2020 in Millions.

Table 5 shows the financial data of Pfizer from the years 2017–2021. They implemented blockchain into their company in 2019, so the numbers provided are two years before the implementation and two years after. Figure 5 shows that Pfizer was in fact going through some financial troubles, but in 2020 the company's finances increased significantly and shows that the numbers are likely to keep increasing.

Table 5: Pfizer Financial Income Statement, 2017-2021, In Millions

Income Statement	Dec. 31,2017	Dec. 31,2018	Dec. 31,2019	Dec. 31,2020	Dec. 31,2021
Net income	$11,188	$16,056	$9,195	$22,025	$21,979
Revenue	$52,546	$40,825	$41,172	$41,908	$81,228
Gross Profit	$41,318	$42,399	$32,851	$33,167	$50,467

(Sec, 2022)
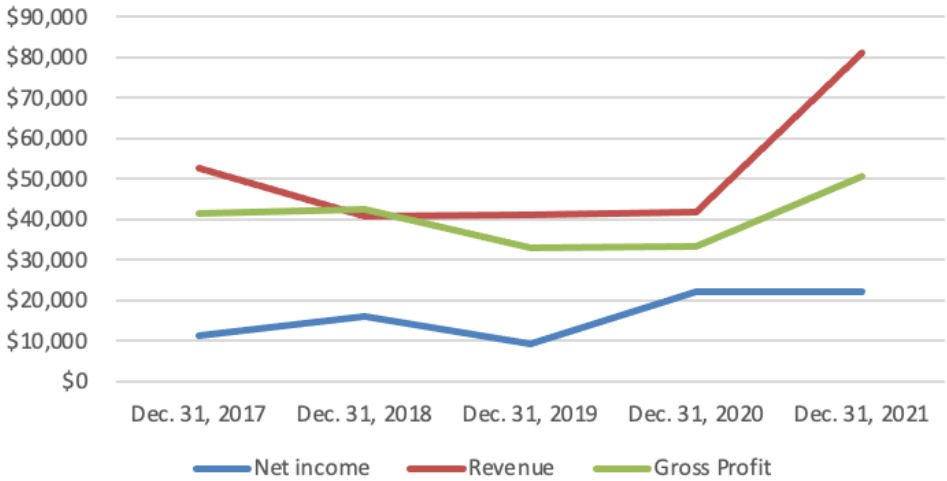
Figure 5: Pfizer Financials 2017–2021 in Millions.

V. Discussion

Through the past several weeks, gathering and deciphering the financial data of companies to support my hypothesis stating, "smart contracts within the system of blockchain improve efficiency for a company", has been interesting. I went into the research process assuming that every company implementing blockchain into their business would have immediate success because of the upside of blockchain's security system. What I learned provided insight into how a company may or may not need blockchain based on non-controllable factors for financial success. The five companies researched showed insightful information. An important statistic to bring up is all the companies reviewed all implemented blockchains before the start of the COVID virus. This is a significant point to consider because the entire dynamic of business interaction was disrupted during this time, resulting in mass disruption of corporate stability. To fully grasp the importance of each company's stat, it is necessary that "non-controllable factors" be broken down. A non-controllable factor is a phrase that I made up initially to help me to understand how circumstance (positive or negative) a company has no control over affects their profit.

An example of this can be natural disasters, where floods destroying a large sum of telephone poles in a specific area can directly affect the payment plan of members, disrupting the expected income for a significant telephone company in that area. Or a mass pandemic that altered how businesses (small or large) connect and interact with

each other. That said, the reader impacts how much of an effect blockchain had on their company. I came up with my opinions on the data given by finding out when each company officially implemented the software. I then created a line graph posting the company's net income, revenue, and profit two years before and after the system's implementation to find results to prove my hypothesis.

Automobile companies like BMW, Toyota, and Ford have served as essential forms of travel for years now. With the increase in technology came the need for higher-quality parts to help newer models of cars to run efficiently. Because most automobile companies sell worldwide, they often transport many parts to different headquarters. To help prevent expensive manufactured items from being misplaced, leading to unnecessary expenses. After researching financial data of automobile companies using blockchain, I realized and feel that automobile companies that incorporated blockchain into their company were successful during the pandemic because of how much of a safety net blockchain's system offered, mitigating parts being lost by tracking parts since most companies had to lay-off many employees. I believe this prevented significant decreases in the financial deficits of companies.

The pandemic contributed significantly to the success of the home retail market, and Home Depot was one of the retail stores that experienced immediate success after installing blockchain. The transportation of products became a problematic way of business due to person-to-person restrictions. The initial intentions of Home Depot using blockchain were to help ensure all inventory was well-maintained and fully traceable. People around the world were forced to stay indoors from the virus, which forced them to stay in the house unless they needed important materials like food, home appliances, and tools. Home Depot's specialty is supplying home appliances and tools, so the need for retail stores seemingly increased overnight. I feel as though blockchain fits the need of where Home Depot wanted to progress, and COVID-19 contributed to their goal by naturally forcing consumers to rely on their products and services.

VI. Conclusion

Based on the evidence presented to me over the course of this semester, I was able to prove that my hypothesis was correct, but to a certain extent. I realized that the success of many businesses was predicated by factors that could not be controlled, like environmental disasters or market crashes within an industry. A particular incident in the FTX collapse opened my eyes to see that people or companies that use blockchain to take advantage of the system often backfired and left businesses empty-handed. I learned a lot of new information that I plan to carry over into my next career.

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Section II: The Social Sciences

The Effects of Influencer Marketing on Instagram and TikTok on Brand Awareness in the Fashion Industry

Yasmin Rieger (Business Administration)[1]

Increasing brand awareness is the top goal that marketers seek to achieve when working with influencers on social media. To illustrate the effects of influencer marketing on brand awareness on TikTok and Instagram, this thesis analyzes ten fashion influencers that worked with the fashion retailer, Shein, in the past. One post per influencer on Instagram and TikTok was used to calculate variables like reach and engagement. The results show that both Instagram and TikTok are great platforms for fashion brands and retailers to promote their products through influencers. While TikTok showed a greater reach and engagement per post, Instagram is more predictable regarding the success of each post. Instagram shows a positive correlation between followers and reach, which implies that companies will reach a greater audience when working with macro-influencers. TikTok works differently due to a complex algorithm. This is why, in some cases, micro-influencers might reach more people than macro-influencers.

I. Introduction

Influencer marketing has become a popular marketing tool in the past years. Influencers are individuals on social media that are seen as trustworthy and are considered to be experts in their field by their followers (Scholz, 2020). Companies cooperate with these influencers to promote their products. Brands seek to achieve different goals with their influencer marketing strategy. The main objective in 2022 for companies was to raise brand awareness, followed by sales, and user-generated content (Influencer Marketing Hub, 2022). With brand awareness, companies try to link their brand in the consumer's mind so that they remember the brand when they make a purchase decision (Azzari & Pelissari, 2020). Through influencers, companies hope to expose their brand to many potential customers and, thus, increase brand awareness. This paper intends to measure the effectiveness of influencer marketing when it comes to increasing brand awareness on Instagram and TikTok in the fashion industry. Influencers have changed the fashion industry in the past years. They serve as trendsetters and people

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[1] Written under the direction of Dr. Shani Carter in partial fulfillment of the Senior Program requirements.

take fashion advice from the influencers (Fashion Gone Rouge, 2020). Because customers primarily seek fashion inspiration from social media influencers, most fashion brands and retailers work together with these individuals.

II. Literature Review

<div align="center">Brand Awareness</div>

What is Brand Awareness

Brand awareness is the conscious recollection of a brand (Renchen, 2020). It shows how strong a brand is linked in a customer's mind (Azzari & Pelissari, 2020). When a consumer then wants to make a purchase decision, all brands one is aware of will be available for selection (Langaro et al., 2015).

Nowadays, new brands emerge constantly which means that simply being aware of a brand does not yet lead to a positive or negative perception. Brand awareness does, however, serve as the first step toward building positive attitudes and behaviors towards a brand (Azzari & Pelissari, 2020).

Creating Brand Awareness

Brand awareness is created by constantly repeating the exposure of a brand to consumers. The exposure should be memorable so that the consumers will remember the brand (Langaro et al., 2015).

Brand awareness can be divided into two dimensions: brand recall and brand recognition. One speaks of brand recall when a customer remembers a brand when they encounter the product category or usage situation the brand covers. Brand recognition, on the other hand, describes the ability to recognize a brand's elements and products. Communication of the brand is the key to building brand recall and brand recognition (Langaro et al., 2015).

<div align="center">Influencer Marketing</div>

Definition of Influencers

Influencers are individuals on social media who produce original content displaying their lifestyle and expertise (Scholz, 2020). Their content and portrayal of themselves allow them to accumulate a large network of followers. Their followers see them as trusted tastemakers and experts. Other resources call influencers opinion leaders that exercise a significant social influence on their followers (Leung et al., 2022).

Actively interacting with their followers allows influencers to build an active relationship. Just like companies, influencers build their brand by carefully managing

their social media content and image. Followers then voluntarily decide to follow influencers with whom they feel a personal connection (Leung et al., 2022).

Influencer Marketing

Influencer marketing is a strategy where companies carefully select individuals to promote their products on social media with the hope of increasing the company's performance (Leung et al., 2022). Companies work with these opinion leaders, that are followed by many people on social media, to post about the brand's interest and generate positive behavioral actions in their followers towards the brand. This leads to a co-creation of the brand by the influencers and followers (Martínez-Lopez, 2020). Influencer marketing can be seen as a hybrid of the "old" marketing tool of celebrity endorsement and new modern content-driven marketing campaigns (Influencer Marketing Hub, 2022).

In 2022, the Influencer Marketing Industry is expected to grow to around $16.4 billion. In comparison, the industry was valued at $1.7 billion in 2016 (Influencer Marketing Hub, 2022). The industry has been growing rapidly over the last years and that is why 75% of brand marketers locate some of their budgets to influencer marketing in 2022 (Influencer Marketing Hub, 2022). Some marketers even spend 75% of their total marketing budget on influencer marketing (Scholz, 2021).

Influencer Categories

Brands have been using celebrities or other well-known opinion leaders as influencers for a while. These people, however, usually come with very high prices and are mostly too expensive for smaller brands. That is why smaller brands have started to leverage micro-influencers. Micro-influencers are social media personalities that are less known than celebrities but still have a big following (Appel et al., 2020).

Influencers are often categorized based on their number of followers (Santora, 2021). Nano influencers have one thousand to ten thousand followers, micro-influencers have ten thousand to 100 thousand followers, macro-influencers have 100 thousand to one million followers, and celebrity influencers have over one million followers. Influencers can also be differentiated based on the content they post, such as fashion, fitness, or food (Santora, 2021).

When it comes to choosing what influencer to work with, companies must look at the goals of the influencer marketing strategy. If the goal is brand awareness, it is recommended to leverage influencers with a large following as the reach of these influencers is higher. However, if a company would like to reach a specific target audience in a specific area, a smaller influencer is the better option (Haenlein et al. 2020).

Word-of-Mouth Marketing

Traditional word-of-mouth (WOM) occurs when a current customer shares their experience with a product without any influence from the company (Leung et al., 2022). WOM is appealing to marketers because it helps convince customers of a company's product fast and at a low cost (Trusov et al., 2009).

The internet has always been a popular tool for WOM marketing. Consumers can easily share their views and experiences with others (Trusov et al., 2009). Electronic word-of-mouth (eWOM) reaches more people and spreads more quickly than traditional WOM. Also, it allows customers to share their thoughts anonymously (Ishida et al., 2016).

While influencer marketing is also considered word-of-mouth marketing, it differs from traditional WOM since the influencer is purposely chosen and paid by the company to share their thoughts on the products (Leung et al., 2022). However, it is recognized as personal WOM since the influencer posts their thoughts on a personal channel, not through a commercial channel which makes it more trustworthy to customers (Ishida et al., 2016).

Impact on the Consumer Decision-Making Process

The consumer decision-making process consists of five stages: problem recognition, information search, alternative evaluation, purchase transaction, and post-purchase experience evaluation (Avery et al., 2016).

Influencers play an important role when it comes to problem recognition. They can be a source of external problem recognition (Avery et al., 2016). The need for a product can be activated by an influencer in a consumer sees them trying out a new product (Appel et al., 2019).

Influencers also help as a source of information. Even though they are controlled by the marketer and would, thus, be considered marketer sources, consumers see them more as personal sources even though they do not know the influencer personally (Avery et al., 2016). The relationship between influencers and consumers is often described as a parasocial relationship, which is a one-sided relationship where the consumer develops a friendship with an influencer (Scholz, 2020). This leads to the consumer seeing the influencer as a trusted source of information. That is why influencers are considered a personal source since the consumer feels as if they would know the influencer (Scholz, 2020). Parasocial relationships are long-term relationships that are equivalent to friendship (Liebers et al., 2019).

Additionally, if a consumer can trust the influencer, they are more likely to form a positive attitude toward the brand the influencer is presenting and consider the brand in their alternative evaluation (Chetiousi et al., 2019).

<p align="center">Source credibility</p>

Consumers follow influencers because of their independence from brands and expertise (Scholz, 2020). Since the consumers are aware of the fact that most of the influencers get paid for their posts, they choose carefully which influencers to follow. Consumers will neglect influencers that never say anything negative, only wear one single brand, and post about this one brand all the time (Scholz, 2020).

Trust is an important factor when it comes to credibility. Consumers have to be able to trust what the influencer is saying is true (Chetiousi et al., 2019). Thus, Influencers seem more credible and trustworthy when they give balanced arguments about products and try products from different brands. By doing so, influencers present themselves as consumers rather than agents of brands (Scholz, 2020).

Credibility increases if the brand product and the influencer show similarities, meaning they target the same audience (Liebers et al., 2019). An influencer will not seem credible if they endorse incongruent brands. With the industry rising, it gets more difficult for businesses to choose the right influencer that will best promote their products to the right audience (Liebers et al., 2019). That is why companies have to look at their brand personality, which is the human characteristics associated with a brand. Defining the brand personality makes it easier to find an influencer that is a good fit for the brand (Von Mettenheim et al., 2021)

A study has shown that consumers perceive the trustworthiness and the level of expertise as higher if the influencer matches the brand they are promoting (Liebers et al., 2019). If an influencer promotes a product with a low congruence to the influencer itself, followers perceive the message shared more as commercial intent (Von Mettenheim et al., 2021). Another study conducted by Liebers et al. (2019) showed that the familiarity of the influencer is also very important when it comes to credibility, meaning that consumers trust influencers more if they have been following them for a while (Von Mettenheim et al., 2021).

Fashion Influencers

Fashion is one of many niches for influencers. Fashion influencers post content that revolves around fashion and are often considered trendsetters. This allows them to influence their follower's opinions and purchase behavior (Chetioui et al., 2019). While trends were introduced on catwalk shows in the past, they are now presented on social

media by influencers. Around 50% of all Instagram users follow some kind of fashion account for outfit inspiration (Fashion Gone Rouge, 2020).

<div align="center">Social Media Platforms</div>

Instagram

Instagram is a social media platform that is mostly focused on picture sharing, even though short videos are slowly gaining importance (Haenlein et al., 2020). Instagram's goal is to bring people closer to things they love (Instagram, n.d.). The app was launched in October 2010. The platform shows its users the content of users they follow and so have a "connection" with (Haenlein et al., 2020).

When posting a picture, users usually use hashtags. Hashtags are words with a "#" in front of them. By putting the pound sign in front of text on Instagram, it turns the text into a link. When a user clicks on a hashtag, they will see all the photos that were posted using the same hashtag (Instagram, n.d.). This allows users to reach people that are not following them. An artificial intelligence algorithm decides the order and content of the posts a user gets to see on their feed which leads to lower exposure of posts (Haenlein et al., 2020).

There are different ways to advertise on Instagram. One way is to create a sponsored post. These posts will then reach people who are not following the account the advertisement is coming from (Haenlein et al., 2020). The platform offers an Ads Manager which allows for more advanced multi-platform campaigns. This means that you can launch the same campaign on Facebook and Instagram from one place. Businesses can decide when and where to launch the campaign and they offer insights into the success of the campaign. On the ad manager, one can also specify their demographics to reach the target audience with the ad. Instagram also offers an even easier and faster way to create ads. It introduced the "boost" feature which turns any post into an advertisement within seconds (Instagram, n.d.). When it comes to influencer marketing, Instagram drives $6.6 million in sales for every million dollars spent (O'Brien et al., 2021).

The main target audience for Instagram is 18 – 34 years. There is an even balance between female and male users on Instagram (Influencer Marketing Hub, 2022). There are around two billion monthly active users on the app which makes Instagram one of the most popular social media sites worldwide. In the U.S. there are 157 million Instagram users (Statista, 2022).

TikTok

TikTok emerged in 2018. It was the second most downloaded social platform in the first quarter of 2020 and in January 2021 it was ranked seventh among all social platforms when it comes to worldwide users (Peña-Fernandez et al., 2022). TikTok has been the most downloaded app and it reached more than one billion monthly active users globally in September of 2021 (O'Brien et al., 2021). The focus of TikTok is short videos. Users often create these videos based on trending songs, dialogues from movies or TV shows, or certain sound effects. These sounds are crucial for linking content on the platform and reaching a wide audience. The app also uses hashtags, however, the sounds are more important. All videos that use a certain sound are combined in a "folder". Users can search for the sounds and then find all the videos using them (Haenlein et al., 2020).

TikTok is very fast-paced. New trends emerge every day and will only last for a short period. TikTok is divided into different subcultures with their trends, which makes it very important for businesses to figure out which niche they want to work in. Examples of subcultures are fashion, fitness, healthy recipes, and so on (Haenlein et al., 2020).

The TikTok feed is divided into two parts. The "Following" feed shows the user videos of people they follow whereas the "For You" feed shows videos that the algorithm chose for the user based on certain actions on the app (Haenlein et al., 2020). The algorithm works very proficiently. The app's "For You" page is very explosive compared to Instagram and Facebook, which work more linear and incremental. Not much is known in detail about the algorithm, however, it is speculated that every video is sent to a minimum number of users. Depending on the performance of the video, it will be promoted more or less. Normally, engagement, such as likes and views, goes up as followers go up. However, the algorithm also allows new users to get millions of views without any followers. This is a big challenge for content creators since they must produce videos that are relevant to many users to receive views and likes (O'Brien et al., 2021).

The number of companies that use the platform for advertisement has increased by 500% in 2020. 70% of the top 500 brands have an account on TikTok (O'Brien, 2021). TikTok wants to point out the fact that brands will be more successful if they create videos that are entertaining to their target audience rather than a classic commercial (TikTok, n.d.).

TikTok supports brands to work with content creators by providing a Creator Marketplace that provides analytics tools. The app also pays users with more than 10,000 followers and more than 100,000 views in the last month. The CEO of ROI Influencer,

Seth Kean says that for every million dollars spent on influencer marketing on TikTok, the brand sees $ 7.2 million in sales over the first 90 days (O'Brien, 2021).

TikTok targets Generation Z. The main age group represented in the app is 13 – 24 years old (Influencer Marketing Hub, 2022). This age group represents 63.5% of the US. In 2020, the US had 45.6 million active users. The app surpassed 100 million monthly active users in February 2021 (Peña-Fernandez et al., 2022). TikTok is female-dominant when it comes to the age group of 24 and younger. The gender bias balances out for the age group of 24 – 34 years (Influencer Marketing Hub, 2022).

<center>UN Sustainable Development Goals</center>

Influencer marketing is all about a partnership between the brand and the influencer. That is why it is linked to the United Nations development goal number 17: Partnership for the goals. It says that the 17 sustainable development goals can only be achieved with strong global partnerships and cooperation. The same is true for a business. In order to achieve their goals, partnerships are very important. Influencers can help companies to reach their marketing goals especially when it comes to increasing awareness globally. The UN states that inclusive partnerships at the global, regional, national, and local levels that share the same principles and values and follow the same vision are crucial for successfully executing the agenda (United Nations, n.d.). When it comes to influencer marketing, it is also very important that the company and influencer share the same values to assure credibility. Without credibility, the partnership would not be successful.

III. Hypotheses

H1: Promoting products through influencers on Instagram will increase brand awareness in the fashion industry.

H2: Promoting products through Influencers on TikTok will increase brand awareness in the fashion industry.

IV. Method

<center>Subjects</center>

Fashion Brands

The subjects included in this study are fashion brands and fashion retailers in the United States. To show the effect of Influencers on brand awareness, the online fast-fashion retailer Shein was used as an example in this Study. Shein was founded in 2012. The company targets the Gen Z and millennial generation with its cheap prices and wide

range of products (Shein, n.d.). Their Instagram account has 23.7 million followers and their TikTok account has 3.8 million followers.

Influencers

Other subjects that are important to the study are fashion influencers. The influencers promote the brand's product on their social media and, thus, share it with their followers. Shein has different ways of collaborating with influencers. They work with celebrities, like Addison Rae or Katy Perry, as well as micro-influencers. On Instagram, they use the hashtag #SHEINgals for their micro-influencers. Shein was the third most mentioned brand on Instagram in 2021 ahead of Nike and H&M (Influencer Marketing Hub, 2022). The fashion retailer worked with over 50,000 Instagram influencers in 2021(Influencer Marketing Hub, 2022). On TikTok, the hashtag #sheinhaul is very popular. Influencers post videos of them showing what they have ordered on Shein. The hashtag has 4.8 billion views and #SHEINgals has 2.4 billion views on TikTok

Social Media

Social media platforms are the "workplace" for Influencers. They provide a place to connect with their followers and to "influence" them. While multiple social media platforms are used for influencer marketing, this study focuses on Instagram and TikTok. According to a study conducted by Influencer Marketing Hub (2022), Instagram was the most popular platform for companies for influencer collaborations. TikTok placed third after Facebook (Influencer Marketing Hub, 2022).

<u>Measures</u>

Table 1: Components of Study

Hypothesis	Variables							Hypotheses & Variable Relationships	Statistical Tests
	1	2	3	4	5	6	7		
H1	IF	I	IEI			IBA	MU	IF + I + IEI + MU = IBA	correlation; regression; line charts
H2	IF			IET	V	IBA	MU	IF + IET + V + MU = IBA	correlation; regression; line charts
Adapted from: Rudestam, K.E. & Newton, R.R. (1992). Surviving Your Dissertation. Newbury Park, California: Sage Publications, Inc. page 138.									

Table 2: Hypothesis, Instruments, and Statistical Analysis

Data	Variables						
	1	2	3	4	5	6	7
Instrument / Survey	average number followers of brand Influencers	Statistics	Statistics	Statistics	Analysis of views of videos from Influencers	Statistics	Analysis of variables
Author / Publisher	TikTok/Instagram	Tribegroup	Gothivarekar	Influencer Marketing Hub	TikTok	Statista	Statista
Type of data	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative

Adapted from: Rudestam, K.E. & Newton, R.R. (1992). Surviving Your Dissertation. Newbury Park, California: Sage Publications, Inc. page 140.

Variable 1: Influencer Followers

This variable gives the number of followers an influencer has on TikTok and Instagram. This variable affects other variables in the study such as reach and engagement rates. The average number of followers of 10 Influencers on Instagram and TikTok will be used to represent this variable.

Variable 2: Influencer Engagement Rate Instagram

The engagement rate on Instagram describes how many people engaged with an influencer's post. Engagement includes likes, comments, saves, and shares. Engagement mostly comes from but is not limited to followers.

Variable 3: Influencer Reach Instagram

The reach of a post measures the number of users that have seen the post. Due to the algorithm, a post is not shown to all of a user's followers, however, the post can also be shown to non-followers.

Variable 4: Influencer Engagement Rate TikTok

The engagement rate on TikTok measures, as for Instagram, the number of likes, comments, shares, and saves for a video.

Variable 5: Views on TikTok (Reach)

On TikTok, the reach is measured by views. Each video a user posts shows how many people have seen it and, thus, measures how many people the video reached.

Variable 6: Monthly users on Instagram and TikTok

This variable shows how many people are using each app monthly. This gives an idea of the potential for reaching new customers through influencers.

Variable 7: Increased Brand Awareness

The goal of the study is to show whether the variables above will have a positive effect on brand awareness.

V. Procedure

Procedure To Locate Journal Articles and Data

For the location of journal articles, the Business Premier Database of the Wagner College Library as well as Google Scholar were used. The search was filtered to "peer-reviewed" articles. I looked at more general articles about brand awareness, influencer marketing, and social media marketing when it comes to search terms. Next, I narrowed down the search by focusing on Marketing on Instagram and Marketing on TikTok. Other search terms used were "influencer marketing in the fashion industry" and "the effect of influencers on brand awareness". The articles I chose presented a good idea and different opinions on the subject matter. The journal articles were retrieved on March 20th, 2022. Other sources of data will be the social media platforms Instagram and TikTok.

Statistical Methods to Present and Analyze the Data

For the presentation of the data, tables, line charts, correlations, and regression charts will be used.

Analysis of Fashion Brands' Social Media Accounts

To find a correlation between Influencers and increased brand awareness, the average number of followers of 10 Shein influencers will be used to calculate the engagement rate on Instagram and TikTok as well as the reach on TikTok. To calculate the average reach on TikTok, the average number of views will be calculated by using 10 influencer accounts and their average views per video.

VI. Results

Table 3 shows the number of followers each of the selected influencers has on Instagram and TikTok. The reach was calculated using the Instagram reach rate depending on an influencer's number of followers. For the reach on TikTok, the number of views on a Shein video was used.

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Table 3: Followers and Reach on Social Media

Influencer	Followers TikTok	Reach TikTok	Followers Instagram	Reach Instagram
1	46,200	7,065	2,200,000	279,400
2	30,500	11,700	23,700	5,830
3	4,333	3,956	189,000	36,099
4	1,600,000	274,000	372,000	71,052
5	57,700	488,200	31,100	7,651
6	84,000	504,400	397,000	75,827
7	97,300	850,600	2,361	857
8	6,159	4,200,000	1,500,000	190,500
9	4,300,000	295,000	674,000	98,404
10	1,300,000	534,900	32,200	7,921
Total	7,526,192	7,169,821	5,421,361	773,541

Source: Instagram, TikTok, and Gothivarekar (2021)

Table 4 and figure 1 show the reach rate on Instagram based on the number of followers. The reach describes how many people a post on Instagram reaches.

Table 4: Reach Rate on Instagram

Followers Instagram	Reach Rate Instagram
<10,000	36.30%
10,000 - 50,000	24.60%
50,000 - 100,000	21.30%
100,000 - 500,000	19.10%
500,000 - 1,000,000	14.60%
>1,000,000	12.70%

Source: Gothivarekar (2021)

Figure 1: Reach rate on Instagram based on number of followers.
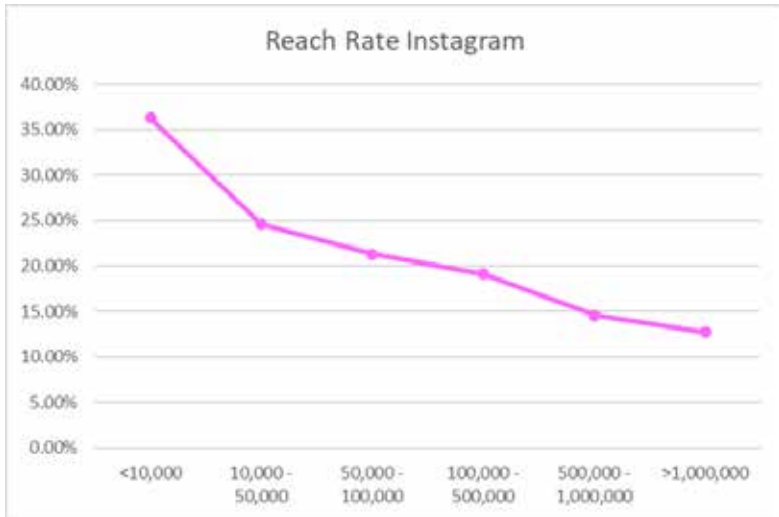
Source: Gothivarekar (2021)

Figure 2: The average number of followers of Shein Influencers on TikTok and Instagram.

Source: Instagram and TikTok

Table 5: Engagement in Social Media

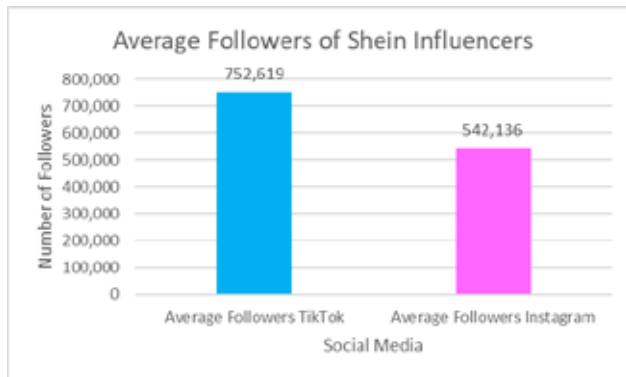|	Engagement on TikTok	Engagement on Instagram
Engagement rate	13.09%	1.06%
Average Followers	752,619	542,136
Average Engagement per post	98,518	5,747

Source: Influencer Marketing Hub (2022)

Figure 3: This chart shows the average engagement an Influencer's post generates on TikTok and Instagram. An engagement rate of 13.09% was used for TikTok and a rate of 1.06% for Instagram. The average number of followers on Instagram and TikTok of Shein influencers was used to calculate the engagement per post.
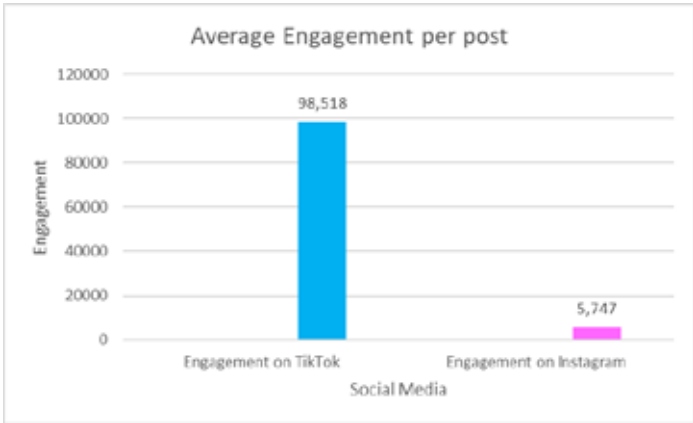
Source: Influencer Marketing Hub (2022)

Figure 4: The bar chart shows the comparison between TikTok and Instagram regarding reach per post. The reach on TikTok is measured by the average number of views per video that a Shein Influencer posted promoting the fashion retailer. The reach on Instagram is calculated using a reach rate between 12.70% and 36.30% depending on the number of followers of each Influencer used in the sample.
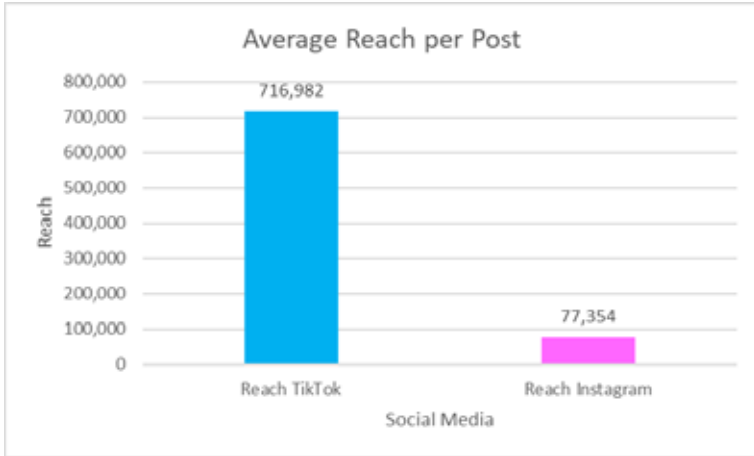
Source: TikTok and Gothivarekar (2021)

Figure 5: The Scatter Diagram shows that there is no correlation between the number of followers of an influencer and the reach per post on TikTok. The correlation coefficient for both variables is -0.18.

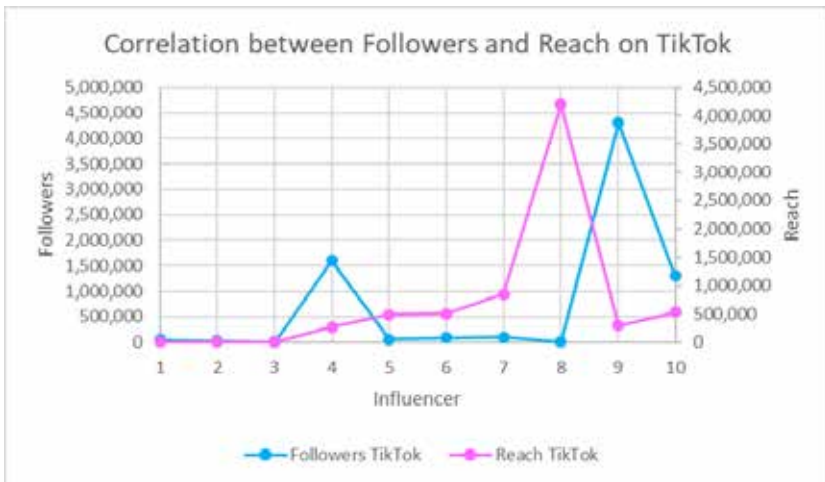Figure 6: The Scatter Diagram shows a positive correlation between the number of followers and the reach per post on Instagram. The correlation coefficient for the two variables is 0.99.
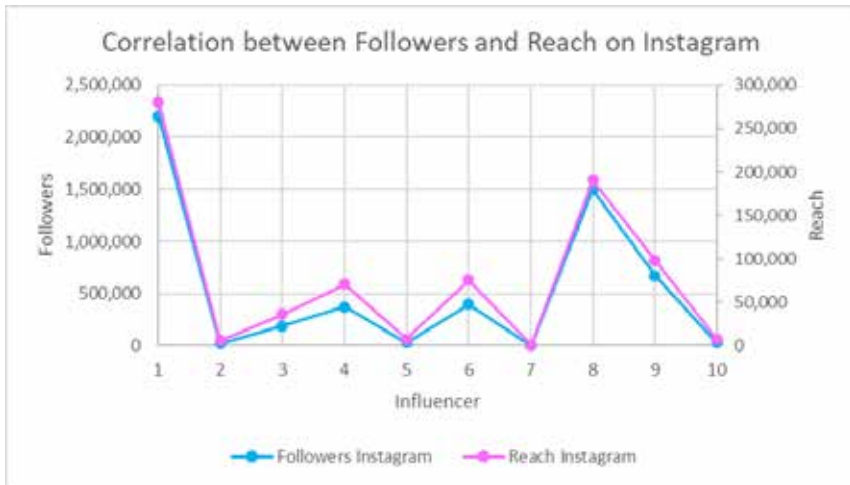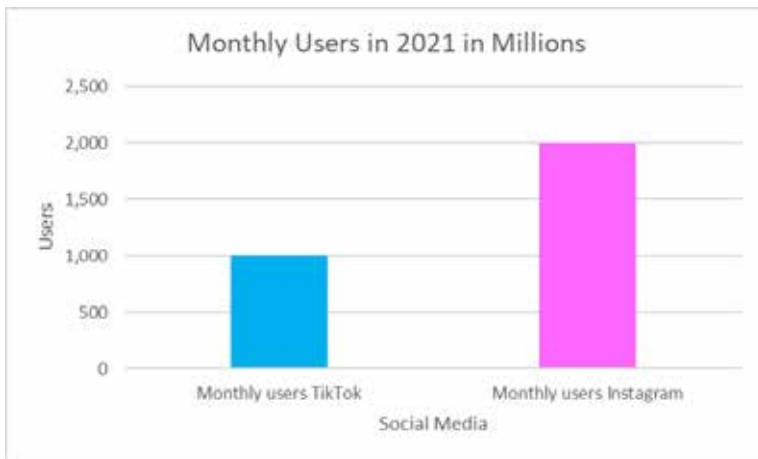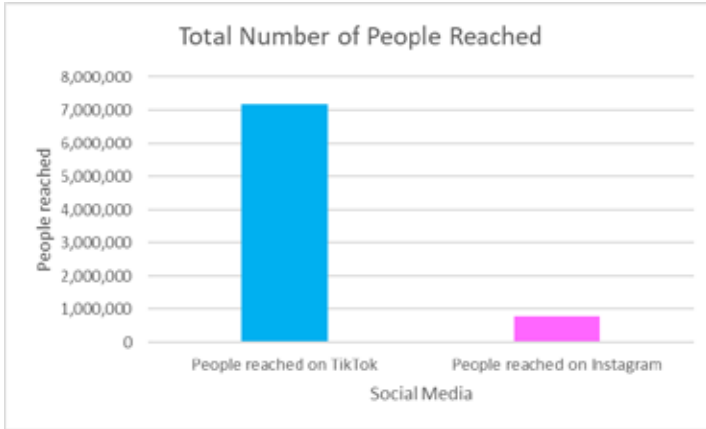

Figure 7: This chart compares the number of monthly users on both social media apps in 2021.

Source: Statista (2021)

Figure 8: The bar chart shows the total number of potential customers who were reached with 10 posts by 10 different Shein Influencers on TikTok and Instagram.

VII. Discussion

H1: Promoting products through influencers on Instagram will increase brand awareness in the fashion industry.

       According to Figure 2, a Shein influencer has an average of 542,136 followers on Instagram. On average, one post on Instagram reached 77,354 people per influencer as shown in Figure 4. This makes a total of 773,541 (Figure 8) potential new customers who could have seen one of the influencers promoting Shein on their accounts. Taking these numbers into consideration, it is obvious that influencers help brands like Shein to increase brand awareness on Instagram. The average engagement of 5,747 per post on Instagram (Figure 3) is low compared to the average number of people reached. However, a person does not have to like or comment on a picture to become aware of a new brand. The reach shows how many people actually saw the post which further represents the increase in awareness. Instagram had 2 billion monthly users in 2021, as seen in Figure 7, which makes it a great platform for advertisement as there is great potential for reaching a large number of people.

       The benefit of using an influencer on Instagram is that a company can target a specific market. Followers of fashion influencers are very likely interested in fashion, otherwise, they would not follow the influencer. The followers of these fashion influencers want to see what the influencers wear and how they style certain clothes. They want to stay up to date about what is considered in trend at the moment. This means that if the influencer posts a picture and mentions where they bought the clothes from, the followers can purchase the same articles and discover new brands.

There is also a positive correlation between the number of followers and the reach per post, as shown in figure 6, meaning as the number of followers increases, the number of people reached increases as well. This implies that if a company decides to cooperate with an influencer with a large following, it is guaranteed that the post will reach more people than a post from an influencer with fewer followers would reach. Thus, considering the goal of increasing brand awareness, it is better to work with macro-influencers.

<u>H2: Promoting products through Influencers on TikTok will increase brand awareness in the fashion industry.</u>

Figure 2 and Figure 4 show that on average, a Shein influencer has a greater following and a higher reach per post on TikTok than on Instagram. The same is true for the average engagement per post (Figure 3). With one post per influencer, ten Shein influencers have reached a total of 7,169,821 people on TikTok. This number clearly shows that TikTok is also a great platform to leverage influencers if a company wants to increase brand awareness. However, there are some differences compared to Instagram. There is no linear relationship between the number of followers and the reach on TikTok as represented in Figure 5. An almost 0 correlation coefficient means that the number of followers per influencer does not dictate the number of views an influencer gets on their video. This is due to the complex algorithm of the app which makes the work with influencers a lot riskier on TikTok than on Instagram. The video of the fashion influencer on TikTok must be relevant enough to be shown to the right audience. This makes it difficult for fashion brands and retailers to predict how many people they will reach when they work with influencers. The brands could save money by working with micro-influencers because their video has the potential to reach more people than a macro-influencer. On the other hand, the company could also lose money if the video of the influencer does not perform well and, thus, does not reach any people. Working with macro-influencers is more expensive, however, would guarantee the brand to reach the current followers of the influencer, which would be a greater number than they would reach with a micro-influencer.

VIII. Conclusions

<div align="center">General Conclusions</div>

The research shows that working with influencers can have a great impact on brand awareness for fashion brands. However, it has to be mentioned that not every brand might see success when working with influencers. Brands must think about what target

audience their brand attracts. For example, if the company seeks to reach an older population, it might be difficult to increase brand awareness due to the lack of social media use in populations over 40-years-old. On the other hand, for fashion retailers like Shein, which mostly targets Generation Z, influencers are a great marketing tool since this generation spends a lot of time on social media and relies on the opinion of influencers. Since Shein works with a large number of influencers, these customers are constantly exposed to the brand which ultimately builds brand awareness.

Implications for Practice

The results of this thesis show companies that are still hesitant about working with influencers that influencer marketing is an effective way to reach the younger generation and increase the brand's awareness among this audience.

Additionally, the research helps companies choose the right influencers and platforms for their marketing strategy. It also gives insight into how the social media platforms work and how to use them as a company. This is crucial for the success of an influencer marketing strategy.

Implications for Research

Further research can be done to go into more depth when it comes to the increase of brand awareness through influence marketing. While the reach shows how many people have seen a post from an influencer, it does not indicate how many of these people will recall the brand promoted by the influencer after seeing their post. Thus, research can be done on how well people remember brands after seeing a post from an influencer.

Furthermore, more companies with different target audiences can be analyzed to draw a comparison to Shein. This would give insights into how the success of influencer marketing is dependent on the target audience that a company wants to reach.

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Providing Mobile Healthcare to the
Homeless Population of East Harlem

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On a daily basis, the average individual does not realize the impact having a house to come home to each night has on their health and wellness. Most people might not think about the immense privilege it is to turn one's keys into the lock and walk into an insulated, open space with running water and electricity. Similarly, one does not usually ponder how easy it could be to tip the scale from having a home to not having one. There are many factors that may lead to homelessness other than simply not having enough money to pay for, or continue to pay for, a living space. Some heads of household may have to choose between buying nutritious foods for the week or paying the rent. Others may be considering going a week or two without picking up their medication so that they do not have to face the looming copay. Others may simply be trying to hold onto a job, for losing the job not only means losing a source of income, but losing their shelter as well. Every individual deserves a house to come home to each night. However, not everyone can always afford that luxury. In the Upper Manhattan neighborhood of East Harlem, many members of the adult population are at risk for, or are already experiencing, homelessness. In a city and a country filled with numerous resources and an abundance of wealth, it is difficult to imagine how some may experience such ordeals regarding stable housing. Aspects such as gentrification, high unemployment rates, rent burden, and food insecurity can easily lead to homelessness.

Each of these factors impacting homelessness has a profound impact on the community of East Harlem, especially the adult population. When faced with homelessness, adults often have two options: move into homeless shelters (which might not be in the best conditions), or simply live on the streets. Not much data is even available on the homeless population, as they are most likely not counted in census data and not much research has been done on them. There is also a pervasive negative stigma surrounding homeless adults in the U.S., especially in areas like NYC. The health of adults in East Harlem is negatively impacted by homelessness as well. One's health is

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[1] Written under the direction of Professor Josephine Marcantonio and Professor Tinamarie Petrizzo-Hughes for NR400: Nursing Research.

greatly influenced by the environment one surrounds oneself with, and being exposed to an environment where safety, support, and shelter is not guaranteed can have detrimental effects on one's health and wellness. It would be unethical for health professionals such as nurses to ignore the inhumane issue of human beings living on the streets rather than in affordable dwellings they rightfully deserve access to.

This problem within this community begs the question: amongst adults in East Harlem, how does providing access to mobile healthcare improve the health outcomes of the homeless population of East Harlem? After taking into account information on this issue and the numerous health problems that may arise from homelessness, nurses may be able to come together to develop a solution. A proposed solution, entitled SMILE, may be able to help. SMILE (Screening, Medication, Immunization, Learning, Eats) is a mobile healthcare system that provides comprehensive complementary healthcare for homeless adults in East Harlem. This program provides an array of services ranging from primary to tertiary interventions such as vaccinations, commonly needed medications, and education.

<div align="center">The Community</div>

Ethnic and Cultural Patterns of the Community

East Harlem is a place known for its rich culture and ethnic diversity. With a total population of 123, 582; 43% of residents are Hispanic, 29% are Black, 14% are White, and 10% are Asian, with 4% identifying with two or more races (*Census Reporter*, 2021). This neighborhood is made up of a majority of ethnic minorities, giving the area a richness in cultural expression that cannot be replicated elsewhere. In addition, 49.7% of residents speak a language other than English at home, which is more than 1.5 times the rate in New York state itself and more than double the rate in the whole country (*Census Reporter*, 2021). Overall, East Harlem is known for having a dominant Spanish and Latino culture, having the nickname El Barrio. Even with the Puerto Rican and a few other ethnic groups' population declining since the early 2000s, the, "immigrants' imprint is still fully alive and apparent throughout the neighborhood streets, storefronts, arts, and politics" (Guerrero, 2017, para. 18). The neighborhood is rooted in such cultural enrichment that all the cultural influences still persist despite the changing demographics, showing how powerful the community values are. Walking through East Harlem streets, one may encounter Dominican restaurants, Mexican vendors, Puerto Rican marketplaces, and even more, all sharing a single block (Guerrero, 2017). The combination of a multitude of cultures and ethnic groups brought together in a small area has created a unique identity for East Harlem and East Harlemites themselves. The livelihood and

diversity is palpable in this community as many different people are brought together to form one unified neighborhood.

East Harlem has also gone through cultural and ethnic changes over the last few years, largely due to gentrification. Written in 2021, "Across East, Central and West Harlem, the neighborhood's Black population declined by 10,805 people between 2010 and 2020, according to newly released data from last year's census. The number of Hispanic people dropped by 2,015. Meanwhile, the number of white residents increased by 18,754" (Garber, 2021, para. 2-3). In order to make room for brand new wealthier, often white, residents moving into the neighborhood, many members of minority groups have left. Whether this change is attributed to an increase in rent, a lack of affordable housing, or displacement of residents to make room for said housing, this marks a change in the community culture. A large influx of white residents accompanies the migration of a new culture into the community, which could grow to dominate the Latin American influence.

Economic Environment of the Community

In terms of economy, East Harlem is well-known as a poverty-stricken neighborhood. Living in poverty, facing high unemployment rates, and facing rent burden can easily lead an individual to homelessness. *Community Health Profiles* for East Harlem states, "Living in high-poverty neighborhoods limits healthy options and makes it difficult to access quality health care and resources that promote health. In East Harlem, 23% of residents live in poverty, compared with 20% of NYC residents" (New York City Department of Health and Mental Hygiene, 2018, p. 7). Even if certain residents of East Harlem want to make healthy lifestyle choices and participate in their own health promotion, they may be unable to simply due to economic circumstances. The rising cost of healthcare services may push residents in poverty to make sacrifices to keep up with their health, or force them to not do so at all. Living in poverty could also indicate risk for homelessness as residents are most likely unable to afford housing. East Harlem's unemployment rate is 11%, higher than both Manhattan and NYC overall (New York City Department of Health and Mental Hygiene, 2018). This highlights how wealth and opportunity are distributed disproportionately across areas of NYC, which could contribute to the economic status of East Harlem. Without a stable source of income, residents may be unable to afford their housing and may be forced to relocate.

Although gentrification may be seen as beneficial in that it transforms previously low-income neighborhoods into higher income ones, the changes that occur are inequitable and often displace residents and businesses who have occupied the area

for decades, East Harlem being one of the 17 affected neighborhoods in NYC (New York City Department of Health and Mental Hygiene, 2018). East Harlemites might find themselves becoming the collateral damage of gentrification as they are inevitably forced to make room for wealthier new residents. 48% of East Harlemites are also rent burdened, meaning residents pay more than 30% of their total income for housing (New York City Department of Health and Mental Hygiene, 2018). Putting such a large percentage of their income towards housing leaves little room for purchasing necessities such as food and medication. Rent burden may also be pushing certain East Harlemites into homelessness as more individuals are faced with rising rent prices while their incomes remain the same (Calmes, 2016). This highlights the inequality present within this community's economy, showing how easy it can be for an individual to slip into homelessness.

Health of the Community

The overall health of a community is influenced by the environment surrounding the residents and the economic status of the area, and East Harlem is no exception. "A quarter of East Harlem children in grades K through 8 has obesity. This is higher than the citywide rate of one in five" (New York City Department of Health and Mental Hygiene, 2018, p. 12). This could be due to food insecurity in that it is easier to access fast food than nutritious food, which would be more expensive as well. Despite the disparities, 76% of East Harlem residents reported their own health as "excellent," "very good," or "good," comparing reasonably to the citywide rate of 78% (New York City Department of Health and Mental Hygiene, 2018, p. 13). East Harlemites may have a different perception of health or may not realize health problems they face, which could lead to this statistic. 68% of East Harlem adults report engaging in physical activity in the last 30 days and 84% report having at least one serving of vegetables or fruit each day (New York City Department of Health and Mental Hygiene, 2018, p. 13). These percentages are fairly consistent with good health habits. "The rate of avoidable hospitalizations among adults in East Harlem is more than double the citywide rate" (New York City Department of Health and Mental Hygiene, 2018, p. 14). This shows that health promotion or screening tactics within East Harlem could use improvement, possibly with more health education readily available to residents. When it comes to statistics on vaccinations, East Harlem ranks well when compared to Manhattan and NYC as a whole. 71% of teens receiving all doses of the HPV vaccine and 46% of adults having received their flu vaccine (New York City Department of Health and Mental Hygiene, 2018, p. 15). The rate of diabetes in East Harlem is higher than the NYC average with 17% of

adults having been diagnosed, and the rate of hypertension is 34% amongst adults, which is similar to the NYC average (New York City Department of Health and Mental Hygiene, 2018, p. 16). When it comes to psychiatric hospitalizations, East Harlem's rate is significantly higher when compared to that of Manhattan and NYC overall. This reflects hardships residents in poorer neighborhoods face, such as, "difficulty accessing preventive services and early care, greater exposure to stressors and interruptions in health insurance coverage" (New York City Department of Health and Mental Hygiene, 2018, p. 17). East Harlem residents die prematurely at a much higher rate, with lung, breast and colorectal cancer being the "three leading causes of cancer-related premature death" (New York City Department of Health and Mental Hygiene, 2018, p.18). This could be rectified with more accessible screening services in combination with education about healthier diets, exercise, and the importance of regular screenings. Health outcomes are largely due to racial inequalities as well, research showing that poorer health outcomes congregate in places where there are more residents living in poverty and where more people of color reside (Johnson, 2017). Therefore, in an area like East Harlem with a large population of minorities, many residents of color are likely to experience poor health outcomes which may be evident in higher incidences of chronic and preventable diseases.

Resources Available in the Community to Combat Homelessness

There are a few programs attempting to help community members who are already homeless, and also help them combat factors that may lead to homelessness. Like other NYC neighborhoods, East Harlemites have access to an East Harlem Neighborhood Action Center that provides services such as car seat distribution, nicotine replacement therapy, and a family wellness suite that helps families avoid eviction and find jobs (New York City Department of Health and Mental Hygiene, 2022). This program also offers many other services that may help decrease individuals' risk for homelessness. East Harlemites are also provided with the Project Renewal Support and Connection Center that houses citizens experiencing homelessness, mental illness exacerbations, substance abuse disorder, and those with a criminal history for five night periods (Moses, 2022). This service is an inclusive, welcoming, and judgment-free zone staffed with nurses and other medical professionals (Moses, 2022). This program truly works to help the people of East Harlem and aims to provide the personal touch and human connection that homeless shelters often lack. East Harlem also participates in a homelessness prevention program called Homebase that houses families in crisis and helps them overcome problems that cause them to lose their home (Goodman et. al, 2016). This program is

especially unique as it aims to help families who are specifically eligible for their criteria, meaning they are most likely helping those at high risk for homelessness. East Harlem also has a few food stamp distribution centers, most notably the presence of SNAP Centers. However, many of these centers have recently been facing closures which directly impact those who experience homelessness (*Harlem World Magazine*, 2019). Without access to these vital food stamps, many individuals are robbed of their main source of food. It has even been known that people who participate in SNAP are less likely to return to homeless shelters than those who do not (*Harlem World Magazine*, 2019). Even programs created by government officials aimed to help the homeless are not as effective as they appear to be when put into practice. In 2016, Mayor de Blasio's plan to build 200,000 units of affordable housing in neighborhoods like East Harlem has actually caused residents to fear change as they would consequently be displaced from their homes in order to make room for new residents and the infrastructure necessary for this plan (Calmes, 2016). Therefore, improved solutions are needed to combat this issue, specifically solutions that would be rooted in solving the issues not only homeless citizens face, but also those who are at risk for homelessness. In order for a solution to this problem to be successful, program starters and government officials need to exercise empathy and understanding through direct communication and interaction with populations at risk for, or already experiencing, homelessness.

<div align="center">Problems in the Community</div>

Among adults in East Harlem, homelessness has been a pervasive problem that negatively affects the community's health and wellness. People experiencing homelessness do not have access to long-term, stable housing, including those residing in shelters, subway stations, or on the streets. Homelessness is a complex issue that can have many different causes, including high unemployment rates, food insecurity, rent burden, and gentrification.

Causes of Homelessness

Gentrification, or when wealthier people move into a poorer neighborhood in an attempt to make "improved" changes in the community, is a huge leading cause of homelessness (Figueroa, 2022). Residents at risk for homelessness related to gentrification may start seeing signs such as new tenants moving into the neighborhood paying a higher rent, more and more neighbors moving out or being displaced, and the development of luxury housing and more established buildings or businesses (Figueroa, 2022). In addition to the physical neighborhood itself changing, the neighborhood's

culture can also change through gentrification as wealthier, often white, residents and their businesses come and overtake both the housing and the local businesses of the previous residents. Rent burden can also lead to homelessness, as NYC residents might end up becoming unable to afford the increasing rent payments as the cost of living increases, but their income does not. In terms of income, a stable source of income can easily become unstable if employees lose their job or are forced to stop working due to their own illness or the need to become a caretaker. Therefore, high unemployment rates may also be linked to homelessness. Food insecurity and the closing of several food stamp centers impacts homelessness as well, making the problem worse (*Harlem World Magazine*, 2019). Saving money on food through the assistance of food stamps may keep families from homelessness since that extra cost can perhaps go towards paying the rent. The closure of food stamp centers in East Harlem takes away a main source of food for many families who are homeless or who are at risk for homelessness. Many families may rely on the help of food stamps and similar programs in order to put food on the table. If these programs are defunded, it may push certain families to either cut back on their meals or begin paying more money for food instead of rent, both of which are options that affect their health and wellness.

Homelessness Data

East Harlem has a high rate of homelessness and poverty. The median household income in East Harlem in 2019 was $34,060, about 53% less than citywide median household income ($72,930). The poverty rate in East Harlem was 34.0% in 2019 compared to 16.0% citywide. In 2019, 24.7% of renter households in East Harlem were severely rent burdened (spent more than 50% of household income on rent). In 2019, the homeownership rate in East Harlem was 5.5%, lower than the citywide share of 31.9%. The homeownership rate in the neighborhood has decreased by 1.1 percentage points since 2010 (*East Harlem Neighborhood Profile,* 2022). According to the US census, the national poverty rate was 12.8% in 2021 (Benson, 2022). This is a 21.2% difference compared to East Harlem alone. In 2021 an estimated 698 million people, or 9% of the global population, are living in extreme poverty – that is, living on less than $1.90 a day. Over one-fifth of the global population live below the higher $3.20 poverty line (1,803 million people), and over two-fifths (3,293 million people) live below $5.50 a day (Suckling et al., 2021). Poverty and homelessness is a global problem, but East Harlem is one of the most affected areas by these issues.

Homelessness is a problem that can happen to anyone, however, New Yorkers of color and people living with disabilities are disproportionately affected among those experiencing homelessness. 86% of homeless single adults and 93% of heads-of-household in family shelters identify as Black or Hispanic – significantly higher than the 53% of New York City's population overall who identify as Black or Hispanic (Routhier, 2020). East Harlem's population is primarily comprised of Black or Hispanic individuals. In 2019, the Black population of East Harlem made up 35.9% of the whole population, and the Hispanic population made up 43%. The disabled population makes up 12.5% of the area as well. Because of this, East Harlem has many risk factors for having homelessness in the area (*East Harlem Neighborhood Profile*, 2022).

Homelessness is a lot more than not having somewhere to call home. It affects people's psychological state and can lead to poor health outcomes. A study about HIV diagnoses in the East Harlem area accentuates that homelessness is very multifaceted. The study discusses the relationship between stable housing and HIV diagnoses. It looked at people who were eligible for and applied to a permanent supportive housing intervention in NYC between 2007 and 2013. People included in the evaluation had to be 18 or older and had not received an HIV diagnosis or HIV supportive housing services. The research focused on if there was a decrease in risks for new HIV diagnoses after giving participants stable housing. After looking at the results, the new HIV diagnosis rate for those who were provided housing was lower than the comparison group. The study found a significant reduction in risk for a new HIV diagnosis among those who received housing placement compared to those who did not (Lee et al., 2018). This supports the hypothesis that long-term and stable housing decreases the HIV risk for those experiencing homelessness. This can be applied to the concept that homelessness leads to unfavorable health outcomes. Unfortunately, HIV is just one of the diseases that homeless individuals have an increased risk of developing.

Homeless individuals also suffer disproportionately high rates of diet-related chronic illness such as hypertension, hypercholesterolemia, and diabetes mellitus but are often poorly diagnosed and treated (Dewi, 2022). Screenings and vaccinations are imperative to providing top quality care to patients. Preventable deaths include heart diseases, cancer, injuries, strokes, and lower respiratory diseases. These issues have many modifiable risk factors, including diet, weight, and lack of physical activity. For people experiencing homelessness, these risk factors are difficult to control. These individuals are more worried about their living situation or if they can eat anything at all than the

quality of the food they can obtain. When there is a limited amount of money available, it is difficult for people to choose healthier options since they are usually more expensive. Fast food restaurants have large amounts of food for a small cost, while organic and more nutritious foods or meals are more expensive. This contributes to obesity and health issues in homeless people, since, many times, they will have to settle with the cheaper, less nutritious meals rather than not eating anything at all. Most homeless people can not afford a monthly gym membership, and they might not have the time, energy, or money for exercise programs. This contributes to a lack of physical activity in their population. Overall, while these risk factors are something that are "modifiable" to the general population, for a lot of the homeless population, they are uncontrollable. This leads to poor health outcomes and a higher rate of disease.

The U.S. Department of Health and Human Services performed a study evaluating the health records of homeless people between 2015 and 2019 (Sutherland et al., 2021). A list of the 15 most prevalent chronic conditions among people with a history of homelessness, and the rates of those conditions in the comparison group was compiled. The study found that most of the conditions, 10 out of 15, had higher rates in the homeless population than the comparison group. These conditions included asthma, diabetes, heart disease, and hepatitis. The research shows that chronic health conditions are more likely to be present among people with a history of homelessness compared to people without a history of homelessness. Further investigation also found that homeless people have a higher rate of traumatic injuries, such as fracture of bone, injury of head, and broad traumatic injury. People experiencing homelessness also had a higher substance abuse rate compared to the control group (Sutherland et al., 2021). This study highlights the fact that homeless people are more at risk for injury and illness than those who are not homeless, which can further show that homelessness negatively affects health outcomes.

While it is clear that homelessness individuals struggle with higher rates of physical illnesses, they also have high rates of mental health illnesses. Two-thirds of homeless New Yorkers have some measure of "mental health needs" and about 17% have a "severe mental illness" (*Improving Care Coordination for Homeless Individuals with Severe Mental Illness in NYC,* 2022). This is an issue because they do not have the same access to care as people who are not homeless. They are most likely not receiving the mental health care that they need, which can lead to safety issues for themselves or others. This also contributes to the stigma that homeless people are "crazy," meanwhile,

they just don't have access to the resources that people who are not experiencing homelessness can access.

Nursing Care for Homeless Individuals

Nursing is vital in the care of those experiencing homelessness. We, as nurses, can be homeless patients' biggest advocates by continuing to defend their rights, especially in terms of access to high-quality shelters and programs that give them food and other necessities. Many homeless people do not have the means to look up places that can give them resources, so we can also provide them with those resources, or come up with a list ourselves to provide them with. Advocating for healthy eating to become more widespread and more available to people is essential too, since poor diet is a key risk factor for acquiring diseases. Nurses can volunteer their time by participating in available clinics or places that provide affordable healthcare by actively helping or by finding these places and letting patients know about them. Patient education is so important, especially for the homeless population, since they are at a disadvantage of not always having information available to them at their fingertips. Nurses can help close that gap for homeless patients in order to give them the best care that they can. Collaborating with social workers is so important as well to ensure patients go to safe and high-quality places after discharge. If a homeless patient is residing in a shelter that has unpleasant conditions, a social worker may be able to help them find another place to live that can better serve them.

Within this particular community, the rate of pregnant community members who receive late prenatal care, or just a complete lack thereof, is about 2% higher than the citywide rate (New York City Department of Health and Mental Hygiene, 2018, p. 11). This could be due to a lack of financial resources amongst pregnant residents or a lack of prenatal health education readily available within the community. As nurses, we can advocate for increased prenatal care and education for residents that these issues can affect. It is essential to try to help patients as much as possible, and nurses play such a large role in advocating, providing resources, and instilling education for all patients, but especially for the patients that may need the help more than others.

<center>Proposed Solution/Program</center>

The adult homeless population of East Harlem too often face barriers that deprive them of their fundamental right to healthcare, food, water, education, and nondiscrimination. The proposed solution is a program with the initiative to eliminate these barriers and advocate for the basic human rights of unsheltered individuals. SMILE

<center>90</center>

(Screening, Medication, Immunization, Learning, Eats) is a mobile medical clinic that provides comprehensive free healthcare and resources for the adult homeless population in East Harlem. The mobile clinic will visit homeless encampment sites daily throughout East Harlem and is staffed with a physician, mental health professional, substance abuse counselor, social worker, and nurse. Private examination rooms will be available and equipped for the clinical team to perform screenings, prescribe common medications, administer vaccines, provide education and referrals, and offer food, water, and other basic necessities. The clinic will work closely with our community partners including emergency shelters, substance abuse treatment facilities, psychiatric hospitals, food pantries, dental providers, and mental health providers to provide the homeless with various resources to meet the immediate needs of the adult homeless population in East Harlem.

SMILE proposes the offering of many screenings on-site to assess for common health concerns identified in the homeless. The clinical team will evaluate each patient to determine which screenings best address their needs. Screenings will be available for cardiovascular disease risk factors including hypertension, hyperlipidemia, and diabetes, depression, infectious diseases including COVID-19, hepatitis C, and tuberculosis, sexually transmitted infections, intimate partner violence, and substance abuse. These screenings are reported by the HCH's Preventive Medicine Task Force as the highest priority screenings for the homeless population (Jean, 2017, p. 3). If a screening detects a health problem, unsheltered individuals can reduce their risk through immunizations, medications, education, and outside resources provided by the clinic.

The homeless can be treated with medications at the clinic if recommended by the healthcare provider following their assessments and appropriate screenings. Available medications will include antibiotics, common over-the-counter medications including antihistamines, antipyretics, analgesics, and antiviral treatment for patients that test positive for COVID-19. A recent study interviewing New York City homeless on the barriers faced when entering shelters found that many homeless individuals choose not to live in shelters because their complex health needs could not be met, especially medication needs (Wusinch, 2019). SMILE aims to make medication more accessible to the homeless by providing common medications on-site and recommending local clinics and hospitals that address the complex health problems of the homeless. Immunization can also be administered on-site for vaccine-preventable diseases that the homeless have a higher rate of contracting including hepatitis A/B, influenza, invasive pneumococcal disease, tuberculosis, and COVID-19 (McCosker,

2022). Immunized patients will be asked to sit outside the clinic in a waiting area under a tent for 15 minutes to monitor for vaccine reactions. For patients reluctant to receive vaccines, education will be provided on the high risks of being unvaccinated in a vulnerable population.

Social workers, substance abuse counselors, and mental health professionals will be available on-site to work with patients experiencing intimate partner violence, mental health disorders, and substance abuse. Patients will work alongside healthcare professionals to develop an individualized plan and discuss the option of transitioning to a shelter, rehabilitation facility, or psychiatric facility. The clinic will have naloxone on hand to reverse overdoses from opioids, including heroin, fentanyl, and prescription opioid medications that occur while the mobile clinic is at the encampments. The opioid antidote will also be given out to the homeless to keep on hand in case an overdose occurs when the mobile clinic is closed. Before giving out the naloxone, a member of the clinical team will provide a quick demonstration on how to administer it as well as a pamphlet that reviews the steps of proper administration. Drug overdose remains the leading cause of death among the New York City homeless population, accounting for at least 237 deaths between July 1, 2020 and June 30, 2021, up from 131 the previous year (Brand, 2022). Food and water will be provided, as well as information to connect them to East Harlem food pantries. Patients will also be supplied with hygiene kits containing toothbrushes, toothpaste, soap, surgical masks, baby wipes, tissues, socks, feminine hygiene products, and first-aid kits.

Other cities like Los Angeles have already successfully launched similar solutions to address the health needs of its homeless population. In September of 2022, Los Angeles County launched Housing for Health, a program that brings mobile medical clinics to homeless encampments to provide primary, urgent, and specialty care (*LA County*, 2020). The clinic offers patients primary care, including vaccines and medications, and urgent care procedures such as wound care, ultrasounds, and blood work. Los Angeles' efforts have led to more than 70% of its homeless population being vaccinated for COVID-19 and models the success that can come with direct outreach and integrated medical care (*LA County*, 2020). Los Angeles will continue to serve as a leading example to SMILE and will hopefully encourage other cities to fight for its homeless populations' right to healthcare.

Community Health Goal

SMILE's community health goal is to screen, medicate, immunize, educate, and provide food to homeless encampments in East Harlem. In order to meet this goal,

SMILE is aiming to eradicate healthcare barriers among the homeless. Common barriers include a lack of information, limited transportation to clinics, and reluctance to trust healthcare professionals (McCosker, 2022). The homeless often misunderstand their infection risk and the importance of comprehensive healthcare due to limited education. A recent study found that two-fifths of the homeless population are unsure of where to receive vaccines, and more than half of homeless people do not have access to transportation to travel to a clinic (McCosker, 2022). SMILE removes the need for transportation to a clinic and brings care directly to East Harlem encampments. Another common barrier is hesitancy to trust healthcare. The homeless population often has limited interactions with healthcare providers, leading to mistrust and skepticism. SMILE hopes that over time with regular visits to encampment sites, the homeless will recognize the clinical team's efforts to bring them complementary care and will be willing to build a trusting relationship with the healthcare professionals.

Learning Objectives

SMILE developed several learning objectives that the homeless are expected to learn after receiving care at the clinic. The first learning objective is that the homeless will be able to verbalize an understanding of the risks that come with being unvaccinated in a vulnerable population. If an individual declines a vaccine at the clinic, they will be educated on the risk of acquiring a preventable disease, as well as the risk of spreading the disease to other homeless individuals. The second learning objective is that the homeless will be able to verbalize preventable diseases that often spread in encampments as well as symptoms that require prompt treatment. The third learning objective is that the homeless will be able to verbalize how to properly administer naloxone after watching a demonstration. Due to the overwhelming number of overdoses that occur in the homeless population, the clinic hopes to provide East Harlem encampments with the resources to save the life of someone overdosing on opioids. Education is a valuable tool that SMILE will utilize to optimize the health of the East Harlem homeless population.

Theory/Theorist

A theory that can be applied to East Harlem's community problem of homelessness among adults is Martha Rogers' theory of "Science of Unitary Human Beings." Rogers viewed the human being and their environment as a single unit, and therefore, must be studied together. The theory's main assumption is that the environment is an integral part of a patient and therefore must be considered to ensure a

successful recovery (Gonzalo, 2021). Rogers believes that a human being is an individual who is the sum of all of their complex parts, and these individuals fit into a society that makes up a larger group of people, and the health of all of these individuals is intrinsically linked to the environment they have surrounded themselves with (Vince, 2022).

Rogers' theory is an excellent framework for SMILE because homelessness is closely related to a patient's environment. Her theory underlines that the environment an individual resides in can have a significant impact on their health, therefore, having a lack of a home environment or being surrounded by an environment that has rampant issues of high unvaccinated rates, rise in vaccine-preventable diseases, limited access to health care, and lack of education on health could consequently create more health issues and decrease the life expectancy of unsheltered individuals. Inspired by Roger's theory, SMILE highlights the importance of improving a patient's overall environment in order to improve the health of more patients and members of the community. SMILE hopes to fulfill this initiative by bringing a clinical team directly to underserved encampments to provide free comprehensive healthcare, resources, and compassion.

Conclusion

The adult homeless population of East Harlem continues to face a homeless crisis as many residents are at risk for or currently experiencing homelessness. Unsheltered individuals too often meet barriers that deny them access to basic necessities such as food, water, safety, housing, and healthcare. The proposed program, SMILE, aims to challenge these barriers and the stigma that comes with being homeless by bringing complementary healthcare directly to encampments. The mobile clinic hopes to provide care on the frontline to bring hope and healing to East Harlem's most vulnerable patient population.

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Section III: Critical Essays

Goblin Market Dramaturgy Packet for Wagner College Theatre Production Stage One Theatre

Madeline Lee (Theatre and Childhood Education)[1]

Origins of *Goblin Market*

Goblin Market is based on an 1859 poem of the same title, written by British lyric poet Christina Rossetti (1830-94). Though she wrote over one thousand poems (Rosenblum 2), "Goblin Market" is regarded as her most famous poem, and was acclaimed by critics and the public, though it received some critique concerning the irregularities in the poem's meter. During the early 20th century, Rossetti's "Goblin Market" was typically regarded as a poem for children, though this innocence has been largely lost to modern scholars. (Rosenblum 63).

Christina Rossetti and *Goblin Market*

In reading "Goblin Market," it is also important to examine Rossetti's life and consider the parallels between the two. Like Laura and Lizzie, Rossetti also had siblings. She was the youngest of four children, having two brothers, Dante Gabriel and William, and a sister, Maria. According to Jan Marsh's book, *Christina Rossetti: a Literary Biography*, Rossetti had an individual relationship with all of her siblings, united with each of them by shared personality, age, or gender, respectively.

Rossetti was raised in a God-fearing household, and, from an early age, was taught that the omnipresent God would reward or punish her actions. Her mother also taught her that girls were "supposed to suppress desire and ambition, told that wishing and wanting were greedy and selfish" (Marsh). These principles are seen in "Goblin Market," when Laura indulges her desire for the goblin fruit, and is punished as a result.

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[1] Written under the direction of Prof. Theresa McCarthy as a research project for the Wagner College Theatre Production Goblin Market by Polly Pen and Peg Harmon. The department presented this musical theatre work in Stage One Theatre October 20-23, 2022. Madeline's dramaturgy provided the actors, director, musical director, designers and technicians crucial background understanding of the original source material, historical context, sociological interpretations, religious connotations along with other curiosity provoking discoveries.

"Goblin Market" was adapted into a musical by Peggy Harmon (book) and Polly Pen (book and music), first published in 1987 by Dramatists Play Service. Both Harmon

Fig. 1. The Rossetti family. From left to right: Christina, Dante Gabriel, Frances (their mother), William, and Maria.

and Pen were better known as actors (Gussow), though Pen later found success with her 1996 score for *Bed and Sofa* (Dramatists Play Service).

Goblin Market made its debut in the 1985 season of New York's Vineyard Theatre ("Goblin Market Author Bios"), and transferred to the Circle in the Square Theater in 1986 ("Goblin Market - 1986"). This production, directed by Andre Ernotte, featured Terri Klausner as Laura and Ann Morrison as Lizzie ("Goblin Market - 1986").

The show was generally well-received, and nominated for several awards, including Drama Desk Nominations for Best Musical, Best Score, and Best Actress in a Musical for both Terri Klausner and Ann Morrison; and was named in the year's *10 Best Plays Volume* ("Goblin Market - 1986"). In his 1985 review, Mel Gussow, for the *New York Times*, writes that the production was effective in not physicalizing the goblins, instead allowing the audience to "feel their presence through the wide eyes and incantatory memories of the sisters" (Gussow para. 4).

Themes

Temptation and Sin

With an overtone of religion, the themes of temptation and sin are seen throughout the poem. The goblin's fruit and Laura's longing for it mirrors the Biblical

fall of man, where humans transitioned from purity and innocence to disobedience. In the Book of Genesis, God creates "plants yielding seed of every kind, and trees of every kind bearing fruit with the seed in it" (*The New American Bible,* Genesis 1.11). This echoes the wide selection of wares the goblins bear, with "gifts both choice and many" (Rossetti line 149). Upon placing Adam and Eve in the Garden of Eden, God tells them "of the tree of the knowledge of good and evil you shall not eat, for in the day that you eat of it you shall die" (Gen. 2.17). Similarly, Lizzie warns Laura about the dangers of eating the goblin fruit, reminding her of Jeanie, who, after eating the fruit, "dwindled and grew grey; / Then fell with the first snow" (Rossetti lines 156-157). Despite God's commandment, the smooth-talking serpent convinces Eve to try the fruit, tempting her with the promise of enlightenment: "when you eat of it your eyes will be opened, and you will be like God, knowing good and evil" (Gen. 3.5). Laura is similarly convinced to try the fruit, despite her sister's warnings, through the goblins' calls: "Sweet to tongue and sound to eye; / Come buy, come buy" (Rossetti lines 30-31). Laura and Adam and Eve are all punished for their disobedience, though in different ways. While Laura falls into a physical illness, Adam and Eve are expelled from the Garden of Eden, condemned to live a life of pain and toil (Gen. 3.16-23).

According to the *Catechism of the Catholic Church*, which summarizes the primary beliefs of the Catholic faith, several wounds afflict the human race as a result of original sin. However, in *Goblin Market*, Laura and Lizzie overcome all of these effects. It states that the entire human race, due to Adam's sin of eating from the tree of knowledge, is "subject to ignorance, suffering and the dominion of death, and inclined to sin - an inclination to evil that is called concupiscence" (*Catechism* 404). In *Goblin Market*, Lizzie illustrates the first wound, ignorance. At the beginning of the story, she refuses to look at the goblins to avoid being tempted by their wares, and "cover'd up her eyes, / Cover'd close lest they should look" (Rossetti lines 50-51), and, in the play, sings that "Their evil gifts would harm us. / We must not look at goblin men, / We must not look. We must not buy" (Harmon and Pen 16). With her eyes shut, Lizzie is ignorant of the world and its offerings, even if they are harmful. However, when she goes to the goblins to save her sister, Lizzie "for the first time in her life / Began to listen and look" (Rossetti lines 327-328). Despite her ignorant beginnings, Lizzie, motivated by her desire to save Laura, sets aside her cautious nature, begins to examine the world and its perils, and finally becomes enlightened.

The second wound named in the *Catechism of the Catholic Church*, suffering and death, is illustrated by Laura's illness. After committing the sin of eating the goblin

fruit, Laura falls ill until she "Seem'd knocking at Death's door" (Rossetti line 321). In her sickness, Laura thinks of Jeanie, who also became ill and died after eating the fruit (Rossetti line 315). However, unlike Jeanie, Laura survives her encounter with the goblin fruit after Lizzie braves the goblins' market to bring her another piece of fruit, which serves as an antidote, bringing "Life out of death" (Rossetti lines 524). Laura, though she committed the sin of eating the goblin fruit and suffered because of it, defied death with her sister's help.

Concupiscence– the inclination to sin, defined as a strong desire ("Concupiscence")–, is seen in the difference between Laura and Lizzie's encounters with the goblin fruit. While Laura and Lizzie both go to the goblins' market for fruit, their individual desires for fruit alter their fates. Laura, tempted by the goblins' calls, "Long'd" (Rossetti line 106) for the fruit, and, once she tastes it, sings about its pleasures:

> O tell me now, o tell me
> Why thy sweetness lures me so, so
> That in thee all bliss is mine
> All bliss is mine
> Ah, ah, ah, fount of joy divine. (Harmon and Pen 17)

After eating the fruit, Laura, wanting more, but unable to obtain it, falls into a state of sorrow, caught in a "passionate yearning, / And gnash'd her teeth for baulk'd desire, and wept / As if her heart would break" (Rossetti lines 266-268). Laura's intense desire for the fruit causes her to suffer and sin again, illustrating the principle of concupiscence.

Lizzie, on the other hand, spurns the goblins' fruit, which saves her and Laura. In the play, the goblins try to convince Lizzie to eat their fruit by daring to "Hug her and kiss her, / squeeze and caress her" (Harmon and Pen 28). Lizzie is harassed by the goblins, who touch her inappropriately to get her to eat their fruit. The goblins' assault on Lizzie illustrates concupisence, which carries the connotation of sexual desire ("Concupiscence"). Through their molestation, the goblins tempt Lizzie to commit the same sin that her sister committed– to open her mouth and eat the fruit, preying on her concupiscence– the inclination to sin, specifically in a sexual manner.

Despite the goblins' harassment, Lizzie remains steadfast in her quest. As she endures the goblins' assault, she "utter'd not a word; / Would not open lip from lip / Lest they should cram a mouthful in" (Rossetti lines 430-432). Unlike Laura, who "suck'd and suck'd and suck'd the more / Fruits which that unknown orchard bore" (Rossetti lines 134-135), Lizzie refuses to taste the goblin fruit, instead enduring the goblins' attempts to entice her, so she can bring Laura another piece of fruit. By not giving into the goblins'

temptations, Lizzie overcomes concupiscence– the propensity to sin–, defying the last of the wounds resulting from original sin.

Sisterhood

In the author's note, Harmon and Pen write that *Goblin Market* is, at its core, "the story of a very real and intricate relationship between two sisters" (Harmon and Pen 5). The importance of sisterhood in the story can be seen in Dante Gabriel Rossetti's 1862 frontispiece for the poem (see fig. 4). Dante Gabriel Rossetti, Christina Rossett's brother, chose to illustrate Laura and Lizzie, locked in an embrace, captioned with the line from the poem, "Golden head by golden head."

Fig. 2. Illustration for *Goblin Market* by Arthur Rackham, 1933, depicting Lizzie refusing the goblin's fruit, therefore refusing their temptations.

Fig. 3. *Adam and Eve* by Titian, circa 1550, depicting Adam and Eve giving into temptation by eating from the tree of knowledge.
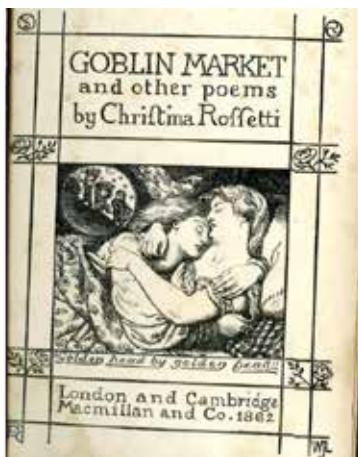
Fig. 4. Frontispiece for *Goblin Market and Other Poems*, illustrated by Dante Gabriel Rossetti.

Dante Gabriel Rossetti gives Laura and Lizzie almost identical facial features, and tangles their limbs under loose clothes and blankets, so it is difficult to tell where one sister ends and the other begins. The goblins are isolated into a small circle in the upper left corner, while Laura and Lizzie take up the majority of the image. According to this image, the

dominant theme in the poem does not concern the goblins, who are almost an afterthought in the illustration, but is about the bond that Laura and Lizzie share.

Laura and Lizzie, despite the challenges they face throughout the story, are very close, both in appearance and their relationship. Simultaneously, they sing that they are "like as like can be … you can't tell her from me" (Harmon and Pen 21), and both have "hair nut brown and fine" (Harmon and Pen 21). As Laura is affected by the goblin fruit, their appearances begin to differ, her hair growing "thin and grey" (Harmon and Pen 25). The goblin fruit, in addition to making her lethargic and delusional, separates her from Lizzie in appearance, physically showing that the fruit has the power to divide their sisterly bond. Later, after Lizzie saves Laura, they again sing that they have "hair nut brown and fine, / You twine it in a single braid, a single braid, / And hers is just like mine, / And hers is just like mine" (Harmon and Pen 35). After Laura recovers from her illness, the sisters return to their similar appearances, and their relationship is strengthened. This difference is shown in Laurence Housman's 1893 illustrations for the poem. He first shows their similarities by illustrating the lines "Cheek to cheek and breast to breast / Lock'd together in one nest" (Rossetti lines 197-198), depicting two identical faces pressed together (see fig. 5). Like Dante Gabriel Rossetti's illustration (see fig. 4), the two girls are intertwined, making it difficult to discern where one sister stops and the other begins.

Fig. 5.

However, after Laura eats the goblin fruit and falls into a depression, Housman depicts them very differently (see fig. 6). In this illustration, though Lizzie embraces Laura, their bodies are less intertwined. Additionally, while Lizzie's face is still youthful and full, Laura's is gaunt, giving her the appearance of an old woman. Housman's illustrations depict the separation between the sisters due to the goblin fruit.

Fig. 6.

At the end of the play, the sisters reflect on how their experience influenced their relationship. They pass the lessons that they learned onto their own children, telling them to "cling together / For there is no friend like a sister / In calm or stormy weather … to strengthen whilst one stands" (Harmon and Pen 36). As sisters, they stand together not only in good times, but also support one another in hard times, as proven by their ordeal with the goblin fruit.

The World of *Goblin Market*

The Market

Despite the poem's rural setting, Christina Rossetti may have drawn upon urban London to create the world of *Goblin Market*. Coming from an affluent family, Rossetti was known to visit Covent Garden Market, and was once noted as going there "twice within one week in December 1883 to buy a wreath, a cross, and flowers" (Tarr 298), though her shopping habits prior to writing *Goblin Market* in 1859 are difficult to trace. Though the bustling urbaneness of Covent Garden Market seems like a contrast to *Goblin Market*'s bucolic setting, many people comment upon the Market's rural aura. In his 1858 poem, "Convent-Garden Market," John Ashford writes "The country's here in town, its fresh sweet ware, fragrant of fields, perfumes the smoky air" (qtd. in Tarr 301).

Despite its location in the middle of London, Covent Garden Market conjures a bit of countryside, creating a location not unlike the goblin's market in the glen.

Like the goblins' market, Covent Garden Market was full of things to see, exotic goods, and danger. In 1873, *Punch* called it "the sweetest, nastiest, prettiest, dirtiest, brightest, dullest, beautifullest, beastliest place in all London" (qtd. in Tarr 299). Of all the markets in London, Covent Garden Market alone offered both exotic fruits and greenhouse-grown flowers. This is significant, as the goblins, too, sell imported fruits and hothouse flowers, including "Rare pears and greengages" (Rossetti line 23), "Citrons from the South" (Rossetti line 29), and "flowers / Pluck'd from bowers / Where summer ripens at all hours" (Rossetti lines 150-152).

Though many families shopped at Covent Garden Market, children had to learn to be wary of the dangers lurking there. Guidebooks, such as John Frost's 1844 *City Scenes*, showed children what they might encounter there, encouraging them to take caution at the Market (Tarr 299). Covent Garden Market attracted some unscrupulous vendors, "cut off from the rest of metropolitan society by their low habits, general improvidence, pugnacity, love of gambling, total want of education, disregard for lawful marriage ceremonies, and their use of a peculiar slang language" (Hotten qtd. in Tarr 306). Given the distrust of these vendors, Rossetti's *Goblin Market* may function as a warning for children to be wary in the Market, lest the end up like Lizzie, after the goblins had "cuff'd and caught her, / Coax'd and fought her, / Bullied and besought her, / Scratch'd her, pinch'd her black as ink, / Kick'd and knock'd her, / Maul'd and mock'd her" (Rossetti lines 424-429).

Fig. 6. Drawing of Covent Garden Market by William Havell (1782-1857)

Food Anxieties and Laura's Illness

Aside from the religious and moral symbolism of the goblin's fruit, *Goblin Market*'s warning about caution in the marketplace has its roots in the concerns with food safety and addiction in the Victorian era. During this time, food was often altered, producing harmful effects. In 1820, the German chemist Frederick Accum wrote that "Numerous facts are on record, of human food, contaminated with poisonous ingredients, having been vended to the public; and the annals of medicine record tragical events ensuing from the use of such food" (qtd in Stern 486). By 1855, the issues with food tampering had become so prevalent that Parliament formed a Select Committee to handle the situation (Stern 487). The average Victorian person could expect to consume a plethora of toxic pigments and other harmful substances, including lead, mercury, and copper, on a daily basis (Stern 487). While many of the toxins came from potted meats and fruits, fresh fruits were not entirely safe. Produce was often colored with harmful substances to make them more appealing. An 1855 pamphlet titled "How to Detect Adulteration" comments upon the "abominable practice of adulterating all green fruits with copper. Gooseberries, greengages, olives, limes and rhubarb are almost invariably coppered to give them a false colour. The purchaser of these fruits is advised to abstain from any that have a bright green look, for it is impossible to preserve greenness in preparations of this kind without the use of copper; but a bad colour is preferable to poison" (qtd. in Stern 488). Like the goblins, luring Laura and Lizzie with their fruits "so luscious" (Harmon and Pen 15), Victorian fruit vendors tempted their customers with visual appeal, though at the expense of the consumer's safety.

The focus on toxic foods and Laura's resulting illness also reinforces the allusions to Covent Garden Market. In 1857, the *Parliamentary Papers* reported on a poisoning from a purchase at Covent Garden Market (Tarr 308). Though accidental, the victim ingested belladonna, a plant with sweet-tasting– and toxic– dark purple berries. In 1859, several London news outlets again reported on a case of poisoning after a group of boys stole belladonna extract from a Covent Garden vendor, then, thinking it was licorice, sold it to other children, who ingested it (Tarr 308). According to an 1854 medical journal, victims of belladonna poisoning can experience "heat and dryness of the mouth, throat, and fauces,—giddiness, double vision, with delirium, convulsions, stupor, and lethargy; sometimes nausea and vomiting. The pupils are much dilated, and the eyes are insensible to light" (*Medical Jurisprudence* qtd. in Tarr 308). Laura, after eating the goblins' fruit, experiences many of the same symptoms. She is described as "longing for the night" (Rossetti line 214), which, if her pupils were dilated, would allow her to see

more comfortably in the low light. She becomes lethargic, unwilling to fulfil her typical household duties, and refuses to eat:

> She no more swept the house,
> Tended the fowls or cows,
> Fetch'd honey, kneaded cakes of wheat,
> Brought water from the brook:
> But sat down listless in the chimney-nook
> And would not eat. (Rossetti lines 293-298)

Instead of going about her normal routine, Laura becomes lost in visions, dreaming "of melons, as a traveller sees / False waves in desert drouth / With shade of leaf-crown'd trees, / And burns the thirstier in the sandful breeze" (Rossetti lines 289-292). Not only is Laura experiencing hallucinations, but these visions suggest thirst– yet another symptom Laura shares with the victims of the Covent Garden Market belladonna poisonings.

Poetic Language

Much of *Goblin Market*'s text is taken verbatim from Rossetti's poem, or, at the very least, imitates her style of writing. This poetic quality not only drives the piece's impulse and pace, but also helps to create the fictitious market. In Victorian England, rhyming and alliteration were commonly used by vendors to hawk their wares in the marketplace, and "costermongers [one who sells goods, especially fruits or vegetables, from a handcart], were among the earliest users of rhyming slang" (Lobdell 5). Like market vendors, the goblins use rhyme and alliteration to lure the girls to give into their temptations as they hawk their wares. The goblins call to Laura, crying "'Pretty goblin' still for 'Pretty Polly'" (Pen and Harmon 16), and later tempt Lizzie with the words "Come take a seat with us, / Honour and eat with us" (Pen and Harmon 30). In both instances, the goblins use poetic devices– alliteration and internal and end rhyme, respectively– to coerce Laura and Lizzie to take their fruits. By reproducing merchant calls to sell harmful fruits, Rossetti, Pen, and Harmon create a cautionary tale about the Victorian marketplace: keep your wits about you in the market, for not all calls come from trustworthy vendors.

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Alive with Color: How Color
Brings the Day of the Dead to Life

Diana Vidals[1]

The Day of the Dead, also known as el Día de Los Muertos, is one of Mexico's most well-known cultural celebrations, with growing popularity throughout Latin America. Celebrated from October 31st to November 2nd, the holiday is recognized for its colorful *ofrenda*s and recognizable symbols such as the colorfully decorated sugar skulls. The holiday has roots tracing back to pre-Columbian Mesoamerica and has even survived the Spanish's attempt to eradicate Mexico's indigenous roots through Catholicism. Despite Mexico being a predominantly Catholic country, the Day of the Dead is often seen as separate from the church. This distance can be viewed through the customs and practices of the holiday, rather than mourning the loss of a loved one, the Day of the Dead celebrates them with bright colors that can be found in flowers, food, decor, and art. Through the use of color, the Day of the Dead festively celebrates a morbid event, ultimately changing the narrative in which death is portrayed.

What is the Day of the Dead?

Starting at midnight on October 31st, the Day of the Dead is a time when it is believed that the deceased can cross from their supernatural world into ours, "there is a very fine line that separates the world of the living from the supernatural world where the dead live. On El día de Muertos (the Day of the Dead), that line dissolves and, for a time, there is only one world" (Hayley and Fukuda 1). During this brief period, the dead can return home and reunite with their loved ones. The Day of the Dead is deeply rooted in Mesoamerican traditions that date back to 3,000 years (Mejorada). The practice of honoring the dead through altars and offerings stemmed from the Aztec custom of Miccaihuitl, a ritual to honor the dead (Mittan) and honor the Aztec goddess Mictēcacihuātl, the goddess of the underworld. Traditionally, Miccaihuitl is a month-long celebration that was done in August instead of November, which also marked the end of the harvest (Richman-Abdou). However, the standard way in which the holiday is currently celebrated differs from the pre-Columbian Mesoamerican way of celebrating

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the deceased, due to colonization at the hands of the Spanish. Most notably, the days it is celebrated have been changed to fit in line with the Catholic holidays, *All Saints* and *All Souls day*. These changes were made in an attempt to colonize the holiday, however, instead, it created "a syncretic holiday, meaning it's a cultural product of two different religious traditions that hybridized during the European colonization of the Americas" (Sandoval).

Three millennia later there are still a few pre-Hispanic elements that can be found in the Day of the Dead, a prime example of this is the use of *ofrendas.* The practice of honoring the dead through offerings originated from Mexico's indigenous people, Mesoamericans such as the Aztecs and Toltecs who through offerings celebrated the lives of their deceased rather than grieve them (PBS). Tied to the "pan-Mexican Tree of Life" (Hayley and Fukuda 135), three-tier *ofrendas* were made to symbolize life and the world, "The floor represents the underworld. The tabletop is the world of men and the arch represents the sky stretching overtop of it all. The ofrenda is their universe in miniature" (Hayley and Fukuda 135). Today, the size of an *ofrenda* is simply based on the taste of whoever is constructing it. A tier on an *ofrenda* is typically dedicated to a single family member, thus, *ofrendas* with three tiers or more are common.

Another pre-Hispanic element that can be found in modern celebrations is the skeleton iconography that is representative of the Day of the Dead. The connection between skeletons and death is linked to pre-Hispanic Mesoamerican art with "pre-Columbian stone sculptures of deities and humans with skull-like faces" (Brandes 47) produced by ancient Aztecs and Mayans. This skeletal art also had ties with the cultural belief shared by indigenous groups in Mexico such as the Nahuatl people who saw skeletons as a symbol of life (Paz). Today, the Day of the Dead is known for its skeleton iconography in the form of paper-mache skulls, clay Calaveras and sugar skulls. Sugar skulls, like the skeleton art, also date back to pre-Hispanic times, however, sugar skulls have a history of being "an indigenous response to repressive overlords" (Hayley and Fukuda 133). Prior to the arrival of the Spanish, indigenous groups in Mexico would celebrate their dead by digging up "the remains of their ancestors, particularly the skull, and clean the bones, paint them bright colors and put a prayer and the name of the person to whom the skull belonged on the forehead" (Hayley and Fukuda 133). This was put to a stop, along with many other spiritual indigenous practices, by the Spanish government. However, the Mexican people found a way around the ban on their traditional practices with the creation of sugar skulls. Sugar skulls are made of granulated sugar mixed with water, and decorated with floral details with colorful icing, contrary to popular belief, the

skulls are meant to be decor, not food. Today, while the practice of cleaning bones is continued in Pomuch, a Maya town in Mexico ("In Pomuch.."), sugar skulls have become an important aspect of the celebration and a famous symbol of the Day of the Dead.

While many traditions have been lost or altered, a still-standing tradition is the use of the color yellow as a marker of death. Yellow can be found throughout the traditions of the Day of the Dead, from the sugar skulls to the cempasúchil flowers, this is due to the Mesoamerican belief that it is the "color of death and the color that coincides with the direction south (where the Village of the Dead is located)" (Hayley and Fukuda 74). Along with this, many people believe that the vivid color cheers up the deceased (Richman-Abdou). Despite the changes made throughout the centuries, the importance of color and art on the Day of the Dead is undeniable.

<div align="center">The Day of the Dead and Catholicism</div>

While Mexico does not have an official religion, the country and culture are predominantly Catholic, with over 80% of the population identifying as Catholic (Evanson). Catholicism was first introduced in Mexico in the 16th Century by the Spanish, this has led the religion to increase in popularity as well as become ingrained in the country's culture. However, there are many differences in the way that Mexicans practice Catholicism, one prime example is their treatment of death. In Catholicism, death is traditionally observed somberly with a series of masses, prayers, and the color black, "the color most often associated with death for Catholics" (Hayley and Fukuda 134). It is often treated as a serious and grim event as many of the rituals such as the Last Rites determine whether the soul of the deceased goes to heaven or hell. The disparity in the treatment of death between both cultures is seen distinctly in how both holidays are observed. Differing greatly from the lively celebrations of the Day of the Dead, *All Souls* and *All Saint's Day*, which fall on the same days as the Day of the Dead, " is bleak and dismal, requires one to go to the cemetery to pray and to mourn once again for lost loved ones" (Hayley and Fukuda 2). This is not the case when celebrating the Day of the Dead as it is not seen as "mourning of lost loved ones, but a celebration, a reunion with the dead" (Hayley and Fukuda 2). The different emotions that both celebrations evoke ultimately display how the perception of death in each culture is vastly different, "because it is a flamboyant, colorful holiday of considerable renown, the Day of the Dead is often cited as a manifestation of a uniquely Mexican view of death" (Brandes 273).

Despite the inclusion of religious icons, the Day of the Dead is often seen as separate from the church, "the church does not identify itself with nor does it support the celebration as a Catholic event. Occasionally, individuals within the church will attempt

to participate but they do so without the sanction of their superiors" (Hayley and Fukuda 123). This separation is particularly astonishing as "once the Spanish conquered the Aztec empire in the 16th century, the Catholic Church moved indigenous celebrations and rituals honoring the dead throughout the year to the Catholic dates commemorating *All Saint's Day and All Souls* Day on November 1 and 2" (Román). Despite the Spanish's attempt to eradicate the Mesoamerican celebration, the two holidays are seen as two separate entities as "nominally this is the Christian feast of the All Saints' and Souls', but it is celebrated in Mexico as nowhere else in the Catholic world" (qtd. In. Brandes 187). Despite having similar objectives, the Day of the Dead is detached from the typical Catholic holiday due to the striking and festive nature of its celebration and view of death.

<div align="center">Color Significance on the Day of the Dead</div>

As previously mentioned, color is vital to the festivities as they bring the celebration to life. Bright colors on the Day of the Dead are traditionally preferred over colors with washed-out appearances such as pastels. As a symbol of death, with cultural ties to Mexico's indigenous roots, yellow is always present in the form of cempasúchil flowers. The color is often tied to another important symbol of the Day of the Dead, the sun. Yellow is believed to represent unity "because under the sun, we're all the same" (Incorvaia). Other colors that are commonly used in the celebration are pink and white. Pink which can be found on Papel Picado and adorning sugar skulls is believed to symbolize the happiness that the souls and family feel celebrating the Day of the Dead. White is meant to depict spirit, purity, and hope ("Day of the Dead:.."), this can be tied back to Mexico's religious culture through Catholicism and even the nation's flag, whose white stripe also represents purity. Other prominent colors are red, which is meant to represent blood and life, and purple, which typically represents royalty, symbolizes mourning, suffering, and loss (Incorvaia). Many colors such as yellow are used throughout the holiday traditions due to the cultural symbolism behind them.

<div align="center">The Traditions</div>

Many traditions make up the Day of the Dead, *ofrendas* are the most important, serving as the epicenter of the celebration. *Ofrenda* in Spanish means offering, set up on the 30th of October and taken down on the 2nd of November, *ofrendas* are created by the family members of the deceased to place offerings for them to enjoy upon their arrival home. *Ofrendas* serve as a symbol of remembrance of the dead, "The *ofrendas* speak of affection toward the deceased and are an expression of love towards life" ("What is

dia…"). varying in size, some families create elaborate multilevel *ofrendas*, with one level for each deceased family member while others make simpler ones that consist of just one. The size of *ofrendas* can also vary depending on the age of the deceased, *ofrendas* for children are simpler, "Usually, the altar was either a single wooden chair with the back placed against the wall or a wooden crate…The children's altars were small, about 18 inches (45 cm) above the floor (to make it easier for the 'little ones' to reach)" (Hayley and Fukuda 67). Each *ofrenda* is made with the utmost care and attention to detail, hoping to satisfy the dead who are believed to be "capable of bringing prosperity (e.g. an abundant maize harvest) or misfortune (e.g. illness, accidents, financial difficulties) upon their families depending on how satisfactorily the rituals are executed" (*UNESCO - Indigenous Festivity Dedicated to the Dead*). There are a few items that are staples in *ofrendas* such as a cup of water, "The dead are always thirsty"…Hot chocolate is almost as common…The more modern families would place bottles of soft drinks on their *ofrendas*. The flavors or kinds depended on the ones the dead preferred while alive" (Hayley and Fukuda 77). Objects such as photos of the deceased, religious icons, "fanciful ceramic skulls or tiny skeletal figurines to, as they say, add a bit of color to the ofrenda…" (Hayley and Fukuda 76). Toys such as brightly painted skeletal figurines are included on the *ofrendas* for the deceased children to play with, while these toys seem morbid, the colorful skeletal figurines are often portrayed in a comical way reflecting "a peculiarly Mexican view of death- an "acceptance of death… the expression of a unique and creative philosophy of life and death" (Brandes 182).

Along with food, toys, pictures, and religious icons, *ofrendas* famously include colorful decorations. *ofrendas* are created to be colorful and eye-catching, typically using bright pinks, and oranges "along with purple and white, are commonly attributed symbolic meanings: the life-giving properties of the sun, celebration, mourning, and purity" (Sunnucks 3). The most popular decorations are Papel Picado, banners made of tissue paper that have designs cut out of them. Papel Picado is known for its bright colors, each symbolizing a different theme in the celebration, such as mourning, celebration, hope, and sun. Another popular decor that is used is fruits and vegetables. Some families simply lay the fruits and vegetables on the *ofrenda* while those with more elaborate setups hang them on arches, "The most common were bunches of bananas, limes, oranges, manzanitas, nísperos, and jicama. In some homes, apples, grapefruit, or lemons are also added" (Hayley and Fukuda 75).

While the majority of the Day of the Dead is celebrated at home alongside the *ofrendas*, a big portion of the celebration happens at the cemetery. Along with making

ofrendas, families also decorate the graves of the deceased and spend the night in the cemetery celebrating. As with *ofrendas*, the complexity of the setup is up to each family, however, a lot of time and care is still put into cleaning and colorfully decorating the graves with religious icons such as the Virgin of Guadalupe, photos of the deceased, candles, flowers, and even sand paintings. Families spend their time at the cemetery sharing meals and stories, further linking the living with the dead. Despite the morbid setting, the visits to the cemetery are treated as a celebration, complete with music, food, and in some cases art. In certain towns such as Xoxocotlán, the graves are also decorated with paintings "using multicolored or painted sand" (Hayley and Fukuda 107), known as *tapetes de arena*. In other places such as San Felipe del Agua, artists use flowers to create these portraits as well, while others even use broken glass. These paintings are sometimes religious, depicting the Virgin Mary or Jesus Christ, or they are portraits of the deceased, whatever the subject the paintings use saturated colors such as blue, pink, and red in order to stand out. Decorating the graves is seen as another way to welcome the dead back home, in many places, even those who don't have families in town, otherwise known as *Los olvidados*, have their graves decorated by those who had a few flowers and candles to spare. The presence of color at the cemeteries is just as important as with the *ofrendas*, the warm and vibrant colors from the orange flowers and yellow candlelight turn an otherwise bleak setting into a celebration of life and death.

The Cempasúchil Flower

While there are many iconic symbols that the Day of the Dead is recognized for, the cempasúchil flower, otherwise known as the marigold flower, serves as one of the most important. The strong smell and the vibrant orange color of the cempasúchil flower are inescapable during the Day of the Dead as they are used in many of the traditions. Due to their strong scent and bright appearance, cempasúchil flowers are placed on the ofrendas to attract the dead, "The strong scent of cempasúchil, the prepared foods, the bright colors, and the candlelight are popularly thought to attract the spirits of the dead and lead the way home" (Sunnucks 3). Their presence can also be found adorning the graves of the dead in flower pots and art. In some cases, "women sometimes wear the flower-heads in their hair" ("Discovering Cempaxochitl…") this is typically seen in those who dress up as Catrinas, another famous symbol of the Day of the Dead, to join in the celebration. Whether they are used as decor or an accessory, the cempasúchil flower remains a symbol of light and death. According to Aztec tradition, the flower and its bright hue "contains the connotation of a compass that leads the souls of the dead to the material world" ("Day of the Dead Colors…"). The flower serves as a tie between the

dead and the living, as well as a tie between the indigenous and modern celebrations of the Day of the Dead.

Known as the flower of the dead, the cempasúchil flower has had associations with death since pre-Hispanic times. The belief that the flower signifies death stems from the Aztec legend of the young Aztec couple, Xóchitl and Huitzilin. The couple would often climb up to the top of a mountain where it was believed that the sun god, Tonatiuh, lived and they would offer him flowers. On one of their hikes up the mountain, they asked the sun god for his blessing in their union. However, shortly after the couple received his blessing, they were torn apart by war and death. Devastated by the loss of Huitzilin, Xóchitl climbed the mountain, this time to ask to be reunited with her love, and was turned into an unbloomed flower. Ultimately, the two were reunited in death when Huitzilin, now reincarnated as a hummingbird, perched himself on the flower that then bloomed into a cempasúchil ("The Legend of..."). The flower's ability to reunite the couple has made cempasúchil a symbol of death and it became known as a device to reunite the dead with the living as "yellow is a color that attracts the spirits of the dead and brings them to the altars to enjoy the feast that has been prepared for them and placed on the altar for them" (Hayley and Fukuda 74).

Because of the belief in the cempasúchil's ability to guide the dead, it is customary to create a trail of its petals to lead the dead home. On November 1st, trails of marigold petals that begin in front of the *ofrendas* and end in the cemeteries at the graves of the deceased, adorn the floors and streets, "This magic path now shows the way from the grave to the ofrenda and the family's home… The grave acts as a homing beacon, attracting the dead to the right place. Once there, the soul follows the appropriate path to the home of his or her loved ones and he cannot get lost because of the path's magic" (Hayley and Fukuda 87). While they make great decorations for a festive *ofrenda*, cempasúchil flowers hold cultural and spiritual significance in bringing the dead home.

Death is traditionally depicted as sad, serious, and alongside the color black. During Mexico's Day of the Dead, death isn't treated with fear but instead with warmth as families get ready to welcome back their deceased loved ones. The festive decor, colors, traditions, and overall atmosphere change how death is viewed and portrayed. Many traditions on the Day of the Dead use color to do this, such as the *ofrendas* which utilize colorful fruits, flowers, and objects to welcome the dead home. Similarly, families also decorate their graves as well and use the bright orange petals of the marigold flowers to guide their ancestors back home. The lack of dark colors like black depicts this holiday to be more like a party rather than another cycle of mourning. Thus showing that the use

of bright colors can change and evoke different feelings as they help create a festive atmosphere, turning a morbid holiday into something festive celebrating both life and death.

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More than a Nurse, More than a Doctor:
The Leadership of Dr. Hadassah Bimko Rosensaft

Lejla Ivackovic[1]

When thinking of World War II and the Holocaust, our minds often go straight to the number of casualties - six million Jews and millions of other civilians - or to the Nazi perpetrators, without considering who showed bravery during these times and in their aftermath. During World War II, women of all faiths did invaluable service as both nurses and doctors. Thousands of nurses aided those targeted by the Nazis, including Allied soldiers, and significantly decreased their death rate. Dr. Hadassah Bimko Rosensaft (1912-1997) was one of these extraordinary people. She was extremely selfless and used her medical skills to help aid Holocaust survivors back to health. On arrival in Auschwitz, she endured news of her husband and child's gassing, yet found a new purpose in saving women and children there and in Bergen-Belsen. Dr. Rosensaft and many other women showed extreme courage during these times and made an impact on thousands of lives.

Across the world, female nurses were helping injured soldiers fighting in the war. Between 1942 and 1945, 59,000 women were recruited in the Army and Navy Nurses Corps as part of the Red Cross Team to help wounded soldiers.[2] Although nurses were not fighting directly in the war, they too were at risk because of the constant attacks and bombings, but leaders wanted to be sure to keep them safe. On June 12, 1944, six days after the D-Day landing, 1st Lt. Edna Haddick Dorsman landed on Omaha Beach, where her unit was immediately besieged with patients. As she recalled in her memoir,

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[2] Duquesne University School of Nursing, "The History of Wartime Nurses: World War II" and National Museum of the United States Air Force, "Winged Angels: USAAF Flight Nurses in WWII." For example, 2nd Lt. Geraldine Dishroon served on the first air evacuation team to land on Omaha Beach after the D-Day invasion in June 1944. African-American female nurses were segregated until 1947. Bullough, Bonnie. "Nurses in American History: The Lasting Impact of World War II on Nursing." *The American Journal of Nursing*, 1976, pp.120.

"All the nurses just jumped right in and did whatever needed to be done, all urgent stuff like give IVs, administer oxygen, pass stomach tubes and set up suctions, possibly change bandages, and give shots."[3] Not only were these nurses helpful on the battlefield, but they were also influential as role models because they proved just how useful women were and that this was a job they could manage.

According to Duquesne School of Nursing, due to the skills of nurses "the U.S. military had an astonishingly low rate of death following injury."[4] These women were able to use their skill and decrease death rates despite a shortage of resources. Not only was this an extremely risky job, but they also developed diseases and infections due to the substantial number of patients they were treating. Since so many men were recruited to the war, many jobs were left empty and women had to step up and take their spots, proving that they were just as capable to do that job as a man would be. After years of being underestimated, women finally began to earn recognition for all their challenging work on the battlefield.

From October 1943-November 1944, in Auschwitz-Birkenau, a woman named Dr. Hadassah Bimko (later, Rosensaft) was also responsible for saving lives. Born in Poland in 1912, Dr. Rosensaft completed her dental surgery training in France. She practiced her dentistry in Poland up until the outbreak of World War II, when she was deported to Auschwitz, the largest Nazi death camp. Tragically, her five-year old son, her parents, her husband and her neighbors were murdered at Auschwitz. Dr. Rosensaft's main goal after her deportation was to use her expertise and help aid inmates of the camps.[5] In addition to the Auschwitz camp, she also helped save hundreds of lives at Bergen-Belsen Concentration Camp, in Northern Germany. Even though she had been a dental surgeon, she collaborated with the British Army medical staff and was able to save the lives of thousands of sick and wounded survivors. Upon finding more than 13,000 lifeless bodies in Bergen-Belsen, and another 15,000 sick Jewish and non-Jewish prisoners, British troops asked Dr. Rosensaft to put together a medical team to assist in

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[3] As cited by Megan Harris, "D-Day Journeys: Nurses on the Ground and in the Sky," *Library of Congress*, April 15, 2019.

[4] Duquesne University School of Nursing, "The History of Wartime Nurses: World War II."

[5] Eric Pace, "Hadassah Rosensaft, 85, Dies, Saved Auschwitz Inmates." *The New York Times*, October 8, 1997l.

aiding the survivors back to health.[6] As mentioned previously, this medical team was filled with some people who had little knowledge when it came to the medical field, but this did not discourage them in any way. They were determined to help as many people as possible and that they did.

Not only did Dr. Rosensaft help save the lives of survivors, but she was also a key witness for the prosecution at the first trial of Nazi war criminals. Dr. Rosensaft along with other doctors and nurses of Auschwitz and Bergen-Belsen talked about the horrible acts of violence they witnessed during the Holocaust. She recalls, "women coming into the infirmary with abscesses, furuncles, and wounds inflicted by dogs as well as from the infirmary's guards who watched the women at work."[7] She mentions how the infirmaries were not well equipped and they struggled looking for supplies such as aspirin, scissors and bandages. She started off at Birkenau's Jewish infirmary but was then moved to the concentration camp in Bergen-Belsen on November 14, 1944. In December of that year, forty-nine Dutch Jewish children were placed in her care by Nazi S.S. guards and more children continued to come as well. One of the barracks were then used as a children's home for around 150 boys and girls. She was able to work with inmates to provide these children with additional necessities such as food, clothing, and medication. Inmates recall that she "walked from block to block, found the children, took them, lived with them, and took care of them. … The children were very small and sick, and we had to wash them, clothe them, calm them, and feed them. That they survived was due to Ada Bimko and her helpers."[8] This was yet another reason for Dr. Rosensaft to be seen as heroic. She not only helped save hundreds of lives by treating the ill, but she took in hundreds of sick children as well and treated them as her own.

In T*he Shalvi/Hyman Encyclopedia of Jewish Women*, a reference tool, her son Dr. Menachem Rosensaft writes proudly: "Because of her countless acts of bravery Dr.

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[6] Jane Brooks, "'The nurse stoops down … for me': Nursing the liberated persons at Bergen-Belsen," in *One Hundred Years of Wartime Nursing Practices, 1854-1953* edited by Jane Brooks and Christine E. Hallett (Manchester University Press, 2015), 213. At liberation, 90% of those in Bergen-Belsen were Jewish victims of the Holocaust.

[7] Menachem Z. Rosensaft, "Hadassah Rosensaft." *Shalvi/Hyman Encyclopedia of Jewish Women* (Jewish Women's Archive, 31 December 1999). See also his article "Hadassah Bimko Rosensaft: Saving Others" in Linda Stein. *Holocaust Heroes: Fierce Females*: Tapestries and Sculptures (Old City Publishing, 2016), 55-58.

[8] Rosensaft, "Dr. Hadassah Rosensaft." *Shalvi/Hyman Encyclopedia of Jewish Women* (Jewish Women's Archive, 31 December 1999); Brooks, "'The nurse stoops down … for me', pp.213, 218.

H. Rosensaft was appointed to the United States Holocaust Memorial Council by President Jimmy Carter in 1980 and then reappointed in 1989 by President Reagan."[9] It is refreshing to know that Dr. Rosensaft was alive when she received her recognition because so many heroines and survivors of the Holocaust died without knowing their story has been shared all across the world and that they have created an impact on so many people's lives.

The term "ordinary people doing extraordinary things" has so much meaning behind it not only as a general statement, but when it is applied to the Holocaust. Leo Ullman is a well-educated man who, at the age of three, went into hiding in the Netherlands. In his memoir, he shares his story of how he was split from his family and placed to live with Hendrik and Jannigje Schimmel. They raised him for 796 days while his family was in separate hiding places in Amsterdam. The Schimmels along with any other non-Jews who helped Jewish families hide are great examples of ordinary people doing extraordinary things.[10] These families could have ignored the struggles of the Jews, just like the rest of the world, but they decided to take a leap of faith and help them. Because of these ordinary people's amazing deeds, they were able to save a life which is a truly extraordinary thing. I am a firm believer of the fact that we are all ordinary people, but it is our actions that make us extraordinary.

In 2023, the Holocaust Memorial Day Trust, an organization in England devoted to lessons of genocide, chose to promote the theme of "Ordinary People" for the following reasons:

> Ordinary people were involved in all aspects of the Holocaust. Nazi persecution of other groups, and in the genocides that took place in Cambodia, Rwanda, Bosnia, and Darfur. Ordinary people were perpetrators, bystanders, rescuers, witnesses – and ordinary people were victims… ordinary people have choices.[11]

This is a notable example to show how we hold our destiny and fate in our own hands. It is up to us to decide whether we want to do good with our lives. So many heroes like Dr. Hadassah Rosensaft, the Schimmel family, the partisan Vitka Kempner, or the British special agent Noor Khan, among others, all saw themselves as regular people but to the

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[9] Rosensaft, "Dr. Hadassah Rosensaft."

[10] Leo Ullman, *796 Days: Hiding as a child in occupied Amsterdam during WWII and then coming to America* (Conte Q Publishing, 2015); Diane D'Amico. "Expansion of Holocaust Resource Center Tells Story of Those Who Helped Family Survive." *Jewish Community Voice,* October 23, 2019.

[11] Holocaust Memorial Day Trust, Theme of 2023 Holocaust essay contest.

rest of the world they are true inspirations.[12] The women healthcare workers all tie into this aspect. At the time their struggles went unnoticed, and they were underappreciated but if you were to do any research on them you would see just how resourceful they were and learn how they put themselves in dangerous positions and sacrificed their lives just to help others. Like the famous saying goes, "Not all heroes wear capes."

Overall, it is important to dig deep beyond the first impressions of history because so many heroic people go unnoticed when past events are being taught. Dr. Rosensaft along with so many other women on different battlefields, used their incredible skill and resilience to save thousands of lives. Although they were not fighting in the military sense, they dealt with the aftermath, which was difficult and heart-breaking. They certainly did not have to choose to sacrifice their lives, but they made the brave decision to step up and do what was right in their hearts, which was to become quiet heroes and save lives.

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[12] Kempner and Khan (a British SOE agent later killed in Dachau Concentration Camp) are among the eleven women depicted in Stein, *Holocaust Heroes: Fierce Females, Sculptures and Tapestries*.

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