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Lactobacillus rhamnosus Interactions With Foodborne Pathogens and Impact on Plant Productivity in a Model Hydroponic System

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LACTOBACILLUS RHAMNOSUS INTERACTIONS WITH FOODBORNE PATHOGENS AND IMPACT ON PLANT PRODUCTIVITY IN A MODEL HYDROPONIC SYSTEM

A Thesis

By

ESTHER OGINNI

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ABSTRACT

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Food production is a global problem, and this has been further compounded with changes in weather patterns necessitating the need for a sustainable approach to production. Chapter 1 introduces the broad concept of the Control environment agriculture (CEA) and then narrows it doe to the hydroponic system its food safety risks. The CEA has been employed alongside conventional practices methods to improve meet the demand for food without placing pressure on the land available. Foodborne outbreaks have been recorded in the production medium and are therefore susceptible to contamination. In Chapter 2, the study focused on using *Lactobacillus rhamnosus* and its cell-free supernatant (CFS) to inhibit the survival of pathogens in the hydroponic system while comparing its efficacy to other common sanitizers. The CFS was effective against Salmonella Typhimurium and Escherichia coli O157:H5 but not Listeria innocua. The study proceeded to apply these treatments on Lettuce to understand the implication of these treatments on plants and the physicochemical properties of the nutrient solution. The CFS treatments showed signs of stunted growth when compared to the control treatment and may not be suitable to be directly applied for pathogen control in a hydroponic growing system.

DEDICATION

To God, the giver of life, whose guidance and grace have been my constant source of strength.

To my family, whose unwavering support has empowered me to pursue my dreams. Your encouragement, love, and belief in me have been invaluable throughout this journey.

To my husband and his constant trust in my ability to do great things.

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CHAPTER 1

CONTROLLED ENVIRONMENT AGRICULTURE; AN OVERVIEW

Conventional Agriculture

Before the first evidence of farming 12,000 years ago in the Near East, Mesopotamia, and China (Barker, 2006), humans lived as foragers gathering, collecting, and hunting for their food. Farming also known as Agriculture, established the practice of growing crops on land which heavily relies on external environmental factors (Hussain et al., 2014). This method of agriculture is often referred to as conventional agriculture whereby the soil serves as the source of nutrients, supports the roots, and provides necessary plant growth promoters. Growing populations and the rising demand for food have led to adoption of practices in conventional agriculture that may not be sustainable to ensure food safety and food security. These challenges are further exacerbated due to factors such as climate change, resistant pests, and pathogens, depleting agricultural land and water resources. Thus, alternative production systems such as controlled environment gained prominence in recent times.

Controlled Environment Agriculture (CEA)

Unlike conventional agriculture, controlled environment agriculture (CEA) does not heavily rely on external environmental factors (Gómez et al., 2019).CEA adopts practices that address concerns associated with the use of harmful agrochemicals in conventional farming, and employs various techniques and technologies to ensure food safety and security (Barnhart et al., 2015; Hussain et al., 2014; Khan, 2018; Srivani & Manjula, 2019). Controlled environment agriculture (CEA) enables to achieve suitable growing conditions year-round by providing and monitoring required temperature, moisture, light, and nutrient availability for optimal plant growth. This system minimizes damages caused by unfavorable environmental factors such as flooding, drought, and extreme temperatures as well as biotic stresses such as pests, pathogens, and diseases(Gómez et al., 2019; Raviv & Lieth, 2007; Sardare & Admane, 2013). Studies have shown that CEA reduces the crop loss to pests and diseases (Gruda, 2008) and minimizes the risk of microbial contamination (Johannessen et al., 2005) In most indoor controlled environment systems, Light-emitting Diodes (LEDs) are utilized as lighting sources, and the environmental conditions are regulated using Heating Ventilation and Air Conditioning (HVAC) systems. The growth media provides root support while the nutrient solution avails plants with the necessary nutrients for the growth (Benke & Tomkins, 2017; Despommier, 2011). The use of artificial light helps to achieve year-round production, with increased duration of light exposure, both plant yield and quality can be improved (Marcelis et al., 2006). The microclimate removes the inconsistencies associated with changing weather conditions (Preite et al., 2023).CEA can also improve water and energy use efficiency ensuring sustainable production. The implications of adopting recirculatory systems in agriculture production to reduce the water input, fertilizer use, and effluent generation was widely discussed(Raviv 2007, Jensen, 1997; Khan, 2018; Martinez-Mate et al., 2018). The location of

CEA systems in urban settings is an additional benefit that reduces the energy consumption needed for mechanical farm practices and in the transportation of food products over long distances. This promotes local production and reduces the potential risk of food contamination or quality loss (Shamshiri et al., 2018; Stein, 2021).

Controlled Environment Agriculture Production Systems

Production systems are typically the structures put in place to support plant growth. This can range from building simple shed-like structures (Lenka, 2020; Tanny et al., 2009) to indoor and fully automated indoor agriculture (De Gelder et al., 2012; Pertry et al., 2018). Examples of CEA production systems include but are not limited to: (i) greenhouses, (ii) rooftop gardens, (iii) plant factories and/or vertical farming, (iv) hydroponics, (v) aquaponics, and (vi) aeroponics. Greenhouses evolved from covered rows of open fields to highly sophisticated tech plants to optimize the productivity of plants and human labor. A greenhouse may optimize natural and artificial light, temperature, moisture, and overall environment to achieve optimal plant growth conditions (Shamshiri et al., 2018). The Greenhouses are designed to prevent external contamination from insects, pests, and diseases. Furthermore, crop contamination by foodbome microorganisms will be considerably decreased since plants are shielded from the direct interactions with the environment, direct contact with animals and other elements. (Despommier, 2011). In rooftop gardening, crops are cultivated on existing buildings which can provide year round supply of fresh vegetables without putting pressure on the land (Quddus, 2022). This can be practiced in urban areas where land available for agricultural production is limited. A study by Astee & Kishnani (2010) projected that utilizing 661 hectares of roof-space would yield about 121,599 tons of vegetables which is estimated to meet about 30% of Singapore's vegetable need.

Grewal & Grewal (2012) compared vegetable yield from conventional urban farming, commercial farming, intensive urban gardening, and hydroponic rooftop gardening as 1.28, 2.42, 6.20, and 19.53 ($kg/m^2/year$), respectively.

Plant factories and vertical farming mostly urban, indoor, high-rise, climate-controlled, partially or fully automated form of production to cater for the increasing need for food production (Benke & Tomkins, 2017; Kozai & Niu, 2016). Vertical farming aims to increase the availability of agricultural land by planting upwards. This is done either by using tall buildings with multiple floors to where crops are planted or may entail horizontal grow beds where plants are stacked like shelves and often lined with artificial lights because only the upper tiers can access direct sunlight (Kotzen et al., 2019; Benke & Tomkins, 2017; Despommier, 2011). In hydroponic systems plants are cultivated in nutrient solutions instead of soil. This is achieved with or without the use of a growth media that provide mechanical support for plant root (Jensen, 1997; Maucieri et al., 2019; Rajan et al., 2019). The nutrient solution is supplied through a peristaltic pump and provides both the macro and micro nutrients and conducive environment required for optimal plant growth and development (Khan, 2018). Aeroponics does not use growth media and the nutrient solution is supplied as mists to the plant roots that are typically suspended in a dark enclosure(Maucieri et al., 2019; Rajan et al., 2019). Aquaponics integrates fish production often referred to as aquaculture within hydroponic systems. The waste products generated during fish production are recycled and used up as nutrients in plant cultivation (Lennard & Goddek, 2019).

Based on the above-mentioned reasons, controlled environment agricultural systems are gaining popularity to address the emerging needs of food safety and food security. For this thesis project, I focused on identifying food safety research needs pertaining to hydroponic systems. The

following sections provide an overview of hydroponic systems set-up, inputs, food safety concerns, and knowledge gaps to formulate research questions.

Hydroponic System Setup

As mentioned, one of the widely used systems in CEA is hydroponics in which seeds are first germinated using growth substrate(s). There are various substrate media such as vermiculite, pine bark, rockwool, peat moss, perlite, coir (cocopeat or coconut fiber), light expanded clay aggregate are used. Each substrate media has its own physical and chemical properties that are selected based on plant growth requirements. They are either used in the mixture at a different proportion to achieve high quality and quantity plant yield. Factors such as water retention capacity, air porosity, cation exchange, pH, electrical conductivity, and bulk density determine the type of growth media to be used for specific production (AlShrouf, 2017; Maucieri et al., 2019; Srivani & Manjula, 2019). Different substrate media might need a different environmental condition to perform the best. After the emergence of seedling, transferred to the system that will be used for growing plants using nutrient solution. These are broadly classified as open and closed loop systems.

The closed system uses circulation of nutrient solution (Fig. 1?) while the open system drains the solution and uses new nutrient solution in a defined interval of time. In an open system, nutrient solution is made available to the plant but does not require an active nutrient flow. The nutrient solution is monitored, and replenished when electrical conductivity increases to ensure plant growth. Aeration is provided to the plants through air pumps employing four techniques for plant growth namely root dipping, floating, wick, and capillary action (Khan, 2018; Sardare & Admane, 2013; Srivani & Manjula, 2019). A closed system alternatively uses a nutrient pump to circulate the nutrient solution in the plant roots, and the excess solution is drained and reused.

Figure. 2 shows different types of nutrient feeding techniques in hydroponic systems. Srivani & Manjula, (2019) provided a more detailed description of each of these types. Once these major inputs are provided, other factors such as temperature, pH, relative humidity, light conditions, etc. play a critical role in the plant growth.

Food Safety Concerns in Hydroponic Systems

Typically, hydroponic systems employ stringent measures to ensure the system remains free from external contaminants and foodborne pathogens by meticulous monitoring of nutrient flow and availability, system temperature, and pH levels. However, contaminated seeds, growth media, nutrient solutions, improper handling, harvesting, packing, cleaning and sanitation practices pose the risk of microbial contaminations. In 2021, packaged leafy greens produced in a CEA indoor hydroponic system are attributed to an outbreak of *Salmonella* (CDC, 2021). A traceback investigation by the Food and Drug Administration (FDA) found improper handling of seeds, growth media, irrigation water, washing and packing of harvested produce in the facility. *Salmonella enterica* serovar Liverpool was recovered from indoor water samples while a *Salmonella* strain related to the outbreak strain was recovered from stormwater drainage adjacent to the farm. However, testing of leafy greens, growth media, and seeds not identified presence of *Salmonella* that caused outbreak (McClure et al., 2023). In 2016, a multistate outbreak of *Salmonella* Muncheon and *Salmonella* Kentucky was linked to contaminated sprouts which was traced back to single contaminated seed lot (CDC, 2016). These examples highlight susceptibility of hydroponically grown produce to foodborne pathogen contamination.

Seeds and Growth media

Seed selection is a crucial step in setting up a hydroponic system. Seed quality determines the yield and can also serve as a conduit of pathogen spread within the system. Seed contamination can occur due to improper handling and storage. Pathogens have been shown to attach and survive for extended periods under normal seed storage conditions (NACMCF, 1999). The sprouting temperature, moisture levels, and nutrient availability are optimal for growth of pathogens and spoilage organisms (Riggio et al., 2019). Furthermore, these conditions pose a risk of cross-contamination. During germination of contaminated seeds; growth, multiplication, and spread of pathogens results in plant damage, product loss, or food poisoning (Stanghellini, 1994). Decontamination methods like photosensitization and heat treatment can help inactivate pathogens on sprouts (HU et al., 2004; Li et al., 2022; GU et al., 2014). Moist growth media impregnated with nutrients provide suitable environments for microorganisms to thrive (Dankwa et al., 2020). Table. 1 summarizes various studies focused on the effect of contaminated seeds and the survival of various human and plant pathogens in different crops.

Nutrient Solution

Plants require about 17 elements provided as nutrient solution to achieve high yield and optimum growth. These will include carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, copper, manganese, molybdenum, boron, chlorine, and nickel (Hussain et al., 2014). During plant growth, constant measurements of the physicochemical properties of the nutrient solution are taken to ensure that nutrient concentrations remain balanced throughout the growth phase (Son et al., 2020). Electrical conductivity (EC) is a primary indicator

of nutrient concentration and availability for plant uptake. Nutrients are available to plants in ionic form and the EC gives a measurement of the concentration of ions within the nutrient solution. EC is not an indicator of the distribution of ions buts gives us an assessment of nutrient depletion during plant growth. pH measurement is also necessary because some nutrients may become unavailable when the nutrient solution is within certain pH ranges (acidic or alkaline), a pH of 5.5-6.5 is considered optimum for nutrient uptake although this may vary depending on crop type (Srivani & Manjula, 2019).

Past studies have shown that pathogens can survive within the nutrient solution for over 14 days. During plant growth, pathogens move to regions around plant roots because of their dense nutrient concentration leading to pathogen growth and multiplication (Critzer & Doyle, 2010). As pathogens multiply, natural openings around plant roots can serve as ideal routes for pathogen internalization especially at the seedling stage. Additionally, injured plant roots leak nutrients from plant tissues acting as chemo-attractants to pathogens which attach to the tips of plant roots and gain access through the wound (Savatin et al., 2014). The primary method to cleaning fresh vegetables is surface decontamination, this is not effective when pathogens are internalized in plants. In open systems, recirculating nutrient solution may encourage biofilm formation which encourages pathogen proliferation (Guo et al., 2002; Wei et al., 2011; Xylia et al., 2022). Pathogen internalization occurs higher in seedlings when compared to older plants because their immunity strengthens over time (Hora et al., 2005). Practices like filtration, sonication, ozonation, ultraviolet irradiation, and thermal inactivation have been employed to eliminate pathogens in the nutrient solution (Maucieri et al., 2019; Stanghellini, 1994) each with their inherent benefits and limitations Table. 2, summarizes various studies focusing on effect of contaminated water or nutrient solution on the survival, growth, or death of foodborne pathogens.

Equipment, tools and other abiotic surfaces

Water/Nutrient tanks, hanging rafts/troughs are employed during production and they are not often changed or decontaminated between planting cycles (Rajan et al., 2019). Microorganisms attach and colonize the equipment used during production by forming biofilms and little is known about biofilm formation of foodborne pathogens within the hydroponic system. Pathogen biofilm formation may adversely impact plant health and their elimination become more challenging due to extracellular polymeric substances that shield the pathogens from getting into direct contact with the treatments (Rodrigues et al., 2022). Other inputs such as tools, working surfaces, and workers wear should also be disinfected regularly to prevent cross-contamination and spread of pathogens within the hydroponic system (Barnhart et al., 2015; Paulitz, 1997; Stanghellini, 1994).

Challenges and Opportunities

The hydroponic production technique offers a viable solution to the challenges of land and water management in agricultural production that has been widely adopted as a more sustainable method to meet the increasing demand for food. However, these systems are not immune from potential risk of contamination with pathogenic microorganisms. (Gillespie et al., 2020; Lee & Lee, 2015). Plants grown in the hydroponic system have no contact with livestock, insect, and wildlife, but all the inputs used in crop production can facilitate the contamination and spread of pathogen (Barnhart et al., 2015). Literature reported in this chapter demonstrates that improper handling of seeds, growth media, and nutrient solution can pose a risk of direct and/or indirect contamination of hydroponically grown produce. Increasing evidence shows that foodbome pathogens such as *Salmonella* spp., *E. coli* O157:H7, and *Listeria monocytogenes* can proliferate well in these controlled environmental conditions.

A systems-based approach involving good agricultural practices supported by science-based information helps to minimize these risks. The CEA industry had adopted multiple strategies to mitigate these potential food safety risks. However, there exists several knowledge gaps to implement sustainable food safety practices in hydroponic systems. Among many, the ability of foodborne pathogens to survive in hydroponic nutrient solution is not well understood. Existing antimicrobial interventions such as chemical and UV light treatments are proven to be not sustainable and detrimental to plant and environmental health. Alternatively, biological interventions involving probiotic organisms have potential to minimize the risk of pathogens while maintaining plant health and promoting growth of beneficial organisms at the plant root microcosm. Thus, the main goal of this research is to investigate the effect of probiotic organisms in minimizing the risk of foodborne bacterial pathogens in hydroponic nutrient solutions. Among many probiotic organisms.

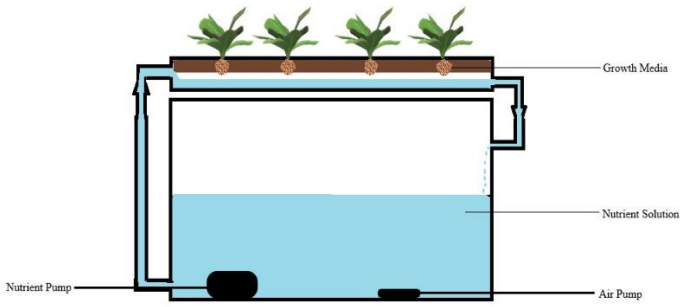

Figure 1.1 A model diagram of a recirculatory hydroponic system

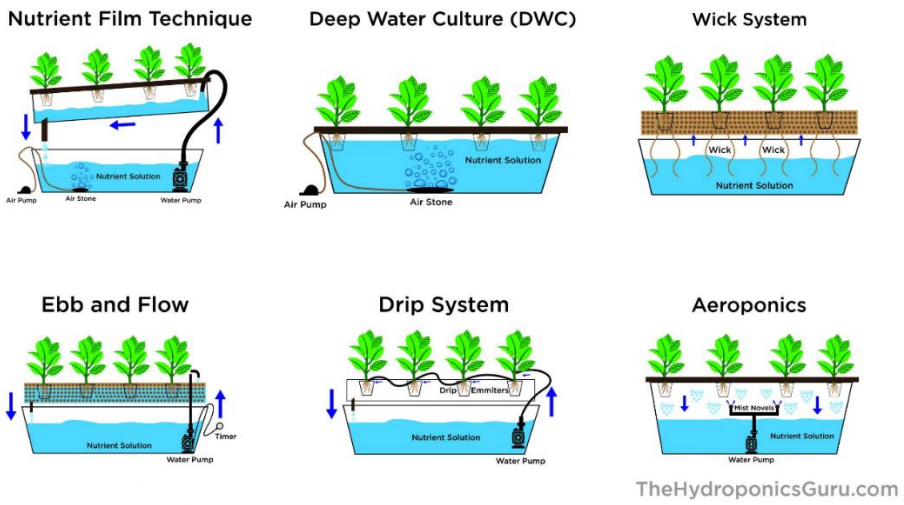

Figure 1.2 Schematic demonstration of different hydroponic cultivation system (Source: The hydroponicsguru.com)

Table 1.1 Survival of foodborne pathogens in hydroponic systems

Pathogen	**Produce type**	**Test conditions**	**Major Findings**	**Reference**
Escherichia coli O157:H7	Lettuce	50-day-old ice lettuce was irrigated with water containing 7.5×10^7 CFU of E. *coli*. Plants were harvested on days 1, 3, and 5 post-inoculations.	4/5 plants on day 1, 2/5 plants on day 3, and 2/5 plants on day 5 were contaminated	(Solomon et al., 2002)
Listeria monocytogenes and *Escherichia coli* O157:H7	Lettuce, basil, corn salad, cultivated rocket, and wild rocket	Seeds were germinated in sterile conditions; after the development of seed leaves, seedlings were transplanted and inoculated with 10^7 cells per mL. Plant samples were harvested to observe pathogen internalization.	Bacteria translocation and internalization within edible part of plants except for basil leaves that had pathogen undetected	(Wachtel et al., 2002)
Salmonella typhimurium	Mung bean	Sterile seeds were germinated in the dark and were inoculated via contaminated irrigation water until the concentration of 10^9 CFU/ml was achieved. Contaminated water was sprayed for 6 days before harvesting to check for pathogen internalization	Exposure to UV light reduced the amount of internalized *Salmonella*	(Ge et al., 2014)

Table 1.1 continued

Escherichia coli O157:H7 and *Salmonella enterica*	Lettuce	Potted lettuce plants were inoculated at their 10^{th} and 12^{th} leaf stages by inverting the pots and immersing the aerial part of the plant in the bacterial suspension for 3 s	E. *coli* and S. enterica increased in population sizes when inoculated on young potted plants; their population size on the old leaves also varied between replicates but did not exceed those on the young leaves	(Brandl & Amundson, 2008)
Escherichia coli O157:H7	Spinach	6 weeks old spinach with both damaged and undamaged roots was irrigated with 20ml of E. *coli* 10^6 cells per ml.	Pathogen was not internalized in the plant leaves, and the disrupted roots did not reveal the presence of E. *coli*; instead, E. *coli* was evenly distributed around the roots	(Hora et al., 2005)
Salmonella montevideo	Tomato	Sterilized seeds were grown for 2 weeks before they were irrigated with 350 ml of 7 log CFU of *Salmonella Montevideo* every 14 days for 70 days. Roots were sanitized prior to sampling	None of the treatment groups was positive for the presence of *Salmonella Montevideo* in their stem, leaf, and fruits of the plants, treatments with higher concentrations were positive for Salmonella	(Miles et al., 2009)
Escherichia coli O157:H7	Spinach	4 weeks old plants were inoculated with E. *coli* by bacterial suspension on spinach leaf to replicate contaminated Irrigation	Leaves were surface sterilized before pathogen detection. Day 0 had 0, day 7 had 4, and day 14 had 1 out of 20 positives of E. *coli* contamination.	(Mitra et al., 2009)
Escherichia coli O157:H7	Lettuce	Seeds were grown for 30 days before irrigating and spraying with 100ml of contaminated water at 10^7 CFU/ml on days 1, 7, and 14	Pathogens were persistent up to 20 days following the first exposure	(Solomon et al., 2003)
Canine calicivirus (CaCV)	Lettuce	Lettuce seeds were germinated in a microplate, after 5 days, CaCV was added at the rate of 10^6 or 10^9 PCR-U to cut off roots and intact roots. Sampling was carried out 1-, 2-, 3-, and 9-days post inoculation for seedlings with intact roots and 1- and 2-days post inoculation for seedlings with damaged roots	Low amounts of viruses were occasionally found in the upper edible parts of the plants for plants with damaged and intact roots	(Urbanucci et al., 2009)
Escherichia coli O157:H7	Lettuce	Seedlings grown in a growth chamber were inoculated with about 10^6 CFU/ml of irrigated water and incubated overnight; seedlings were surface sterilized before homogenizing and plating	Attachment levels were most significant at the roots and seed coats	(Wachtel et al., 2002)

Table 1.1 continued

Escherichia coli	Maize	E. *coli* cells were inoculated into a 4-liter solution to obtain a concentration of 10^7 CFU/ml and used for plant growth for 15 days	E. *coli* was internalized into the leaves of the maize plant, which was apparent in all inoculation treatments	(Bernstein, Sela, Pinto, et al., 2007)
Escherichia coli 0157:H7	Corn and beans	Crops were grown hydroponically for three weeks before inoculating with 100ml of phage per bucket; the roots of plants in specific buckets were severed.	A maximum amount of microbe was detected on day 4; corn plants with cuts had more pathogen uptake than the other	(Ward & Mahler, 1982)

CHAPTER II

LACTOBACILLUS RHAMNOSUS SUPERNATANT INHIBIT THE SURVIVAL OF FOODBORNE PATHOGENS IN HYDROPONIC NUTRIENT SOLUTION

Abstract

Nutrient solutions (NS) in hydroponic systems are ideal conduits for pathogen contamination, proliferation, and spread. This study sought to investigate the ability of *Lactobacillus rhamnosus* and its supernatant to exhibit antagonistic activity against foodbome pathogens in the NS as compared to conventional chemical treatments. *L. rhamnosus* live cells, cell-free supernatant (CFS) of *L. rhamnosus*, and various concentrations of peracetic acid were administered to *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria innocua* in a hydroponic NS over a period of 96 h at 21±2 ºC. *L. rhamnosus* and *L. innocua* cell counts significantly declined when administered to the NS as control treatments, while *Salmonella* and *E. coli* O157: H7 cell counts remained stable at 10^5 CFU/mL in the NS. *L. rhamnosus* live cells administered to *Salmonella* and *E. coli* did not decrease in cell counts compared to controls. However, *L. rhamnosus* CFS decreased *Salmonella* and *E. coli* O157: H7 cell by 2.69 and 0.60 logCFU/ml in the first 24 hr, while *L. innocua* remained stable. Peroxyacetic acid treatments at 12 mg/L reduced *Salmonella* and *L. innocua*, but not *E. coli* O157: H7 cell counts. These findings

suggest that *Lactobacillus* CFS should be further investigated as an antimicrobial intervention to reduce the survival of foodborne pathogens.

Keywords: Hydroponic Nutrient Solution, *Lactobacillus*, Metabolites, *Salmonella*, *Listeria*, *E. coli* O157:H7.

Introduction

Increased demands for food production in agriculture are exacerbated by rapid urbanization, climate change, food safety, and food insecurity i.e. population growth (Carstens et al., 2019; Srivani & Manjula, 2019; Shamshiri et al., 2018; Benke & Tomkins, 2017; Kroupitski et al., 2009). Controlled environmental agriculture (CEA) allows practitioners to manipulate variables such as temperature, light exposure and intensity, nutrients, and relative humidity to improve crop production and complement conventional farming. Hydroponics have gained much acceptance owing to the benefits of land and water conservation, efficient nutrient regulation, optimal environment for plant growth, increased and consistent food production, reduced agricultural footprints, and environmental contamination (Srivani & Manjula, 2019; Lee & Lee, 2015; Sardare & Admane, 2013).

Hydroponic systems utilize nutrient solutions rather than soil for nutrient uptake by plants. (McClure et al., 2023). Some challenges associated with the hydroponic system are high start-up costs, high energy inputs, and the ease of pathogen proliferation once contamination occurs (Benke & Tomkins, 2017; Sardare & Admane, 2013). Microbial contamination in hydroponic systems is rare when compared to soil-based crop production due to limited or no exposure to environmental factors that facilitate contamination. However, opportunities for pathogen contamination via different routes, such as seeds (Itoh et al., 1998; Jablasone et al., 2005; Miles et al., 2009; Warriner,

Ibrahim, et al., 2003), growth media (Deng et al., 2021), and nutrient solutions due to high nutrient concentrations still exist (Ilic et al., 2022; Xylia et al., 2022; Wang et al., 2020; Xiao et al., 2015).

Suitable environmental factors increase the likelihood of pathogen survival and proliferation, which may eventually lead to pathogen internalization and spread (Ilic et al., 2022; Son et al., 2020; Wang et al., 2020; Lee & Lee, 2015). Laboratory studies have demonstrated instances of pathogen contamination in lettuce, spinach, basil, and tomatoes grown in hydroponic systems (Humphrey, 2004; Kroupitski et al., 2009; Miles et al., 2009; Scattolini et al., 2020; Wang et al., 2020). In 2011, the *Escherichia coli* O104:H4 outbreak was linked to fenugreek sprouts produced in hydroponic cultures (Ilic et al., 2022). Recently, hydroponically grown packaged leafy greens were implicated in the outbreak of *Salmonella* Typhimurium (FDA, 2021).

To decontaminate water and nutrient solutions used in hydroponic production systems, antimicrobial interventions such as ultraviolet (UV) radiation (Kim et al., 2020; Son et al., 2020; Moriarty et al., 2018; Tsunedomi et al., 2018), heat (Son et al., 2020) and chemicals including ozone, hydrogen peroxide, and sodium hypochlorite (Allende & Monaghan, 2015; Ehret et al., 2001; Mensah et al., 2022; G. Riggio et al., 2019) have been commonly employed. However, these methods have inherent benefits and limitations. For example, UV LED lights are environmentally friendly and do not produce any known hazardous by-products (Kim et al., 2020; Tsunedomi et al., 2018). However, high operating costs, radiation-induced mutagenesis, the production of free radicals, and its interactions with chelating agents in the nutrient solution which can impact plant growth may limit its usage (Tsunedomi et al., 2018; Lee & Lee, 2015; Ehret et al., 2001; Buyanovsky et al., 1981). Similarly, heat and chemical treatments of nutrient solutions can result in residue buildup or halogenated by-products. Alternative sustainable interventions are in high demand to ensure the safety of hydroponically grown agricultural commodities. This suggests that

it is essential to adopt more effective methods to ensure microbial safety as well as to maintain plant health (Ehret et al., 2001).

Lactic acid bacteria (LAB) are gram positive non-spore-forming bacteria known for their ability to ferment sugar into lactic acid and include common genera *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus,* and *Streptococcus.* LABs are known for their antagonistic activities against pathogenic bacteria due to the production of organic acids, mainly lactic acid. The decrease of pH below 4.0 is toxic to many pathogenic bacteria, whereas the LAB species are adapted to these environments (Zapaśnik et al., 2022). Lactic acid diffuses across cytoplasmic membrane of pathogenic bacteria, causing disruption of essential functions and structures of the cell. Hydrogen peroxide, ethanol, diacetyl, and bacteriocins are other compounds that have been associated with the antimicrobial effects of LAB and their metabolites (Laury-Shaw et al., 2019; Patel et al., 2012; Lanciotti et al., 2003; Alakomi et al., 2000; Brashears & Durre, 1999).

Bacteriocins, secreted proteins expressed by LAB have bactericidal activity can be applied either directly as a purified compound, as a crude bacterial metabolite, or by inoculation of the bacteria that produce them (Hartmann et al., 2011). LABs are generally regarded as safe and have been widely used in the food industry. For example, to inhibit the growth of pathogenic bacteria on animal (Aprea et al., 2023; Dejene et al., 2021; Dickson & Anderson, 1992; Kazemipoor et al., 2012; Lanciotti et al., 2003; Majamaa et al., 1995; Martín et al., 2022) and plant (Arellano-Ayala et al., 2020; Trias et al., 2008) products. Iglesias et al. (2017), evaluated the antagonistic activities of *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* against *Salmonella* and *Listeria monocytogenes* in minimally processed pears at different storage temperatures. Co-inoculation of the pathogens with *L. rhamnosus* significantly reduced *Salmonella* and *L. monocytogenes* counts,

while co-inoculation of the pathogens with *L. acidophilus* increased pathogen populations (Iglesias et al., 2017).

The application of lactic acid bacteria and/or its metabolites as a potential antimicrobial intervention in the hydroponic nutrient solution to mitigate the risk of foodborne pathogens has not been well explored. Thus, the main objective of this study was to determine the efficacy of *Lactobacillus rhamnosus* and its metabolites to inhibit foodborne bacterial pathogens in a hydroponic nutrient solution. *Listeria innocua* was used as a surrogate for *Listeria monocytogenes* in our study because *L. innocua* has exhibits a close genetic relationship with *L. monocytogenes* (Glaser et al., 2001). *S.* Typhimurium, *E. coli* O157:H7, and *L. innocua* were used as test organisms in the study.

Methodology

Bacterial cultures. Three bacterial species, namely *Salmonella* Typhimurium (ATCC 14028), *Escherichia coli* O157:H7 (ATCC 35150), *and Listeria innocu*a (ATCC 15742), were tested against *Lactobacillus rhamnosus* (ATCC 53103). Axenic *S.* Typhimurium, *E. coli* O157:H7, and *L. innocua* were originally stored at -70 ºC in Tryptic Soy Broth (TSB; BactoTM, Becton Dickinson, Sparks, MD, USA) containing 25% glycerol. Glycerol stocks were revived by first streaking a loopful (~10 µL) onto Xylose Lysine Deoxycholate (XLD; Hardy Diagnostics, Santa Maria, CA, USA), Sorbitol MacConkey (SMAC; Oxoid Ltd., Hants, UK), and Oxford (Neogen™, USA) agar plates, respectively, and at 37ºC for 24±2 h. While the pure strain of *L.* rhamnosus was streaked onto DeMan Rogosa and Sharpe (MRS; Oxoid™, Hampshire, England) agar and incubated at 37ºC for 48±2 h. Single colonies of each species of test organisms were inoculated

into either 10mL TSA and incubated at 37 ºC in an incubator shaker (New Brunswick Scientific: Excella E24 Incubator Shaker Series New Brunswick ScientificTM) for 24 h Similar procedure was followed for *L. rhamnosus* with MRS for 48h. After incubation, the cells were harvested at 4000 × g for 20 min at 4ºC in a centrifuge (Model 5920R, Eppendorf™, Germany), supernatants decanted, and the cell pellets resuspended in 10 mL of sterile Milli-Q™ (Model IX 7003, Millipore Sigma, MA, USA) water. Sterile water was employed as a diluent to prevent the introduction of additional buffer to the test organisms in the hydroponic NS. (Avery et al., 2008). Serial dilutions were performed to achieve a cell concentration of about 10^5 CFU/mL which was confirmed by serial dilution plating on selective media and incubated in a Steri-Cycle CO_2 incubator (Model 370 Series, Thermo Scientific, OH, USA) at 37ºC for 24±2 h (test pathogens) and 48±2 h (*L. rhamnosus*).

Treatment of pathogens with lactic acid bacteria in hydroponic nutrient solution. 1 L of sterile Hoagland's No. 2 Basal salts (Caisson Labs Inc., Smithfield, UT, USA) an inorganic hydroponic nutrient solution with nutrient composition shown in Table 2.1 was prepared as per the manufacturer's instructions. Reaction mixture of 30 mL were prepared for: (i) Control (27 mL NS + 3 mL of 10^5 CFU/mL test organism or *L. rhamnosus*), (ii) Treatment-1 (24 mL NS + 3 mL of 10^5 CFU/mL *S.* Typhimurium or *E. coli* O157:H7 or *L. innocua* + 3 mL *L. rhamnosus*), and (iii) Treatment-2 (26 mL NS + 3 mL of 10^5 CFU/mL *S.* Typhimurium or *E.* coli O157:H7 or *L. innocua* + 1 mL *L. rhamnosus*). Following the inoculation of the NS with test organisms, lactic acid bacteria was inoculated into the 50-mL centrifuge tube containing the treatments, the reaction mixtures were vortexed for 1 min and stored at 23 ± 2 ºC to determine the survival of the test organisms

over 96 h., 3 independent replicates were conducted for each treatment and test organism (Iglesias et al., 2017; Laury-Shaw et al., 2019).

Treatment of pathogens with CFS of lactic acid bacteria in hydroponic nutrient solution. During the preliminary studies, we found that the cell-free supernatants (CFSs) of *L. rhamnosus* inhibited *S.* Typhimurium. CFSs were prepared by inoculating 10 µL of *L. rhamnosus* into 10 mL of MRS broth and incubating at 37 ºC for 48±2 h. Cells were harvested by centrifugation at 4000 × g for 20 min, the supernatant decanted into a sterile 50 mL centrifuge and filtered twice (to ensure the absence of cells in the filtrate) through a 0.2 µm pore size acrodisc (Fisherbrand 13mm syringe filter, Ireland), and 100µl spread on MRS agar. CFS volumes identical to those of *L. rhamnosus* culture were used as described above. Following inoculation of the NS with the selected bacterial pathogens, CFS was aseptically introduced into a 50 mL sterile centrifuge tube, the reaction mixtures were vortexed for 1 min and stored at 23 ± 2 ºC to determine the survival of the test organisms over 96 h., 3 independent replicates were conducted for each treatment and test organism (Kohestani et al., 2018).

Treatment of pathogens with conventional sanitizers in hydroponic nutrient solution. To compare the efficacy of conventional sanitizers with the above-mentioned biological interventions, NS containing test pathogens were subjected to treatment with 4 and 12mg/L of sodium hypochlorite (RICCA, Arlington, TX, USA) and peracetic acid (PAA; SaniDate® 15, Biosafe Systems, East Hartford, CT, USA). Hence, the NS was prepared to a concentration of treatments at 4 and 12 mg/L of PAA as adjusted and determined using MQuant™ test strips (Millipore Sigma, Burlington, MA, USA). The treatments were then inoculated with the test organisms to a total volume of 30 mL each, comprising: (i) Treatment-5 (27 mL NS with 4 ppm

PAA + 3 mL *S.* Typhimurium or *E. coli* O157:H7, or *L. innocua*), and (ii) Treatment-6 (27 mL NS with 12 ppm PAA + 3 mL *S.* Typhimurium or *E. coli* O157:H7, or *L. innocua*). After inoculating the PAA-treated NS with the selected bacterial pathogens separately in a 50 mL sterile centrifuge tube, the reaction mixtures were vortexed for 1 min and stored at 23 ± 2 ºC to determine the survival of the test organisms over 96 h., 3 independent replicates were conducted for each treatment and test organism.

Determination of survival of test organisms. Following the respective treatments, the viability of *S.* Typhimurium, *E. coli* O157:H7, *L. innocua* and *L. rhamnosus* were determined by sampling 1 mL of treatment at different time intervals (0, 6, 12, 24, 48, 72, and 96 h). Treatments were serially diluted and plated on XLD, SMAC, Oxford, and MRS agar for *S.* Typhimurium, *E. coli* O157:H7, *L. innocua*, and *L. rhamnosus*, respectively, samples were incubated at 37 ºC for 24±2 h for test organisms and 48±2 h for LAB. Cell counts were reported as CFU/mL.

Measurement of physicochemical properties. To determine the consistency of the hydroponic NS over the experimental period, changes in temperature, pH, electrical conductivity, total dissolved solids, and % lactic acid (for CFS controls) were recorded and analyzed using a pH meter (Model A211, Orion™, MA, USA) and a conductivity meter (Thermo Scientific™, Eutech, Singapore) respectively. Acidity, expressed as % lactic acid, was determined by titration of a 1ml volume of CFS with 0.1N NaOH using the indicator phenolphthalein.

Statistics and data analysis. All data sets were analyzed using JMP®PRO 16 (SAS Institute, Inc., Cary, NC, USA) software. Three replicate (n = 3) experiments were performed the test organisms (*S.* Typhimurium, *E. coli* O157:H7, and *L. innocua*), and duplicate samples were analyzed at each sampling point. The survival data of each bacterium in the treatments was log transformed prior to analysis. The study was completely randomized, and the data was analyzed using ANOVA to compare the mean log survival CFU/mL obtained at each sampling treatment time point for each pathogen. Least mean squares were calculated to compare the means of log survival CFU/mL. Tukey-Kramer honest significant difference test was used to compare mean values. All the tests were performed with a 0.05 level of significance.

Results

Preliminary studies were conducted using 10, 20, and 30 mL of hydroponic NS consisting of varying ratios (1:1, 1:3, and 3:1 (v/v)) of *S.* Typhimurium to *L. rhamnosus*. A 30 mL reaction mixture volume was ideal for extended sampling times, and no significant difference in survival was observed with an increasing proportion of *L. rhamnosus* from 1 to 3 parts in the mixture (data not shown).

Bacterial viability in hydroponic NS. Fig. 2.1 shows the survival kinetics of tested organisms in the hydroponic NS with or without lactic acid bacteria (i.e., *L. rhamnosus*). *L. rhamnosus* treatment of the NS did not show a significant effect ($p > 0.05$) on *S.* Typhimurium when compared to the control (Fig. 2.1a). *S.* Typhimurium counts when maintained in NS by itself (control) decreased from 4.91 to 4.34 log CFU/mL in 48 h. Cell counts steadily increased to 4.93 log CFU/mL by 96 h. Similarly, no significant reduction in *S.* Typhimurium CFUs were observed

in the NS treated with *L. rhamnosus* (Fig. 2.1a) regardless of ratio (i.e. 1:1 or 3:1(v/v)) of *L. rhamnosus* in relation to *S.* Thyphimurium. Similarly, no significant difference ($p > 0.05$) in the CFUs of E. *coli* O157: H7 were observed comparing the treatments with the control (Fig. 2.1b). Overall, E. *coli* O157: H7 CFUs showed <0.5 log reductions over a 96-h treatment time. When compared to other test organisms, *L. Innocua* showed significant CFU reductions over a 96-h treatment period. *L. Innocua* cell counts decreased by 4.4 log CFU/mL in the control and about 2 log CFU/mL in the LAB-treated NS regardless of *L. rhamnosus* ratio (Fig. 2.1c).

***L. rhamnosus* persistence in hydroponic solutions in the presence/absence of pathogens.** *L. rhamnosus* CFUs dropped after 24 hours regardless of the presence or ratios of test organisms (Figs. 2.2a-c). However, after 48 h, *L. rhamnosus* became undetectable in the NS of the treatment with the higher ratio (3:1) of *S.* Typhimurium and after 72 h with the lower ratio (1:1) (Fig. 2.2a). Co-inoculation of *L. rhamnosus* with *E. coli* O157:H7, had no significant effect on *L. rhamnosus* CFUs for up to 48 h before strong reductions were observed. At 96 h, *L. rhamnosus* cell counts dropped to about 1.69 log CFU/mL when E. *coli O157:H7* and *L. rhamnosus* were co-inoculated at a ratio of 1:1 (v/v) while the cells of *L. rhamnosus* had become undetectable when *E. coli O157:H7* and *L. rhamnosus* were co-inoculated at a ratio of 3:1 (v/v) in the NS (Fig. 2.2b).

L. rhamnosus survival was comparatively improved in the presence of *L. innocua* (Fig. 2.2c). *L. rhamnosus* cell count decreased by 2.3 (for control and/or 1:1 treatment) and 1.7 (for 3:1 treatment) log CFU/mL.

Effect of *L. rhamnosus* CFS on bacterial pathogens. Table. 1 compares the physicochemical properties of control samples without any bacteria as well as control samples with just CFS. As shown in the table, no significant changes in temperature, pH, electrical

conductivity, or total dissolved solids were observed during the testing period. The lactic acid concentration in the hydroponic solution containing CFS was within the range of 0.16 to 0.12%. Fig. 2.3 shows the survival kinetics of the test organisms in the hydroponic NS containing the *L. rhamnosus* (CFS) of *L. rhamnosus*. CFS treatment of the hydroponic NS showed a significant ($p<0.05$) reduction of *S.* Typhimurium CFUs when compared to the control (without CFS) (Fig. 2.3a). Treatment time and ratios of CFS in the treatment solution had a significant effect ($p<0.05$) on *Salmonella* CFU reduction. *Salmonella* cell counts in 1:1 (v/v) CFS ratio decreased 2.69 log CFU/mL within the first 24 h of treatment. Beyond the 24-h treatment time, *Salmonella* was no longer detected in the NS. A post-hoc test showed that 0 to 12 h and 48 to 96 h of treatment times differed significantly. However, *Salmonella* and CFS at a ratio of 3:1 (v/v), led to a reduction of 1.84 log CFU/mL at 96 h treatment. A significant difference in the reduction was observed between the treatments (Fig. 2.3a). No significant difference in *E. coli* O157:H7 CFUs between the control and treatment samples was evident up to 24 h (Fig. 2.3b). However, at 96 h, a reduction of 1.16 log CFU/mL was observed in the NS with CFS and *E. coli* O157:H7 at a 3:1 (v/v) ratio. At a CFS to *E. coli* O157:H7 1:1 (v/v) ratio, bacterial counts decreased by 2.28 log CFU/mL at 72 h and then became undetectable at 96h (Fig. 2.3b). Compared to *S.* Typhimurium (Fig. 2.3a) and *E. coli* O157: H7 (Fig. 2.3b), *L. innocua* survival improved in the NS containing CFS (Fig. 2.3c). No significant difference in *L. innocua* cell counts could be discerned between the CFS treated NS at a ratio of 1:1 or 3:1. *L. innocua* displayed improved survival in CFS-treated nutrient solution compared to control treatment without CFS which led to undetectable CFUs by 96 h post-inoculation (Fig. 2.3c).

Effect of conventional sanitizer-treated hydroponic NS on test organisms. Upon treatment, none of the test organisms were able to survive 4 mg/L sodium hypochlorite from the

beginning of time (data not shown). A concentration of 4 ppm in the PAA in the NS did not affect *S.* Typhimurium CFU compared to the control (Fig. 2.4a). However, increasing the PAA concentration from 4 to 12 ppm yielded a significant reduction of 3.83 log CFU/mL within 24 h, and beyond that time, *Salmonella* became undetectable (Fig. 2.4a). The least squares mean of difference shows that *S.* Typhimurium CFUs at 12 ppm PAA did not differ significantly at 0 to 12 h and 24 to 96 h. In contrast, E. *coli* 0157:H7 was unaffected by the PAA-treated NS either at 4 or 12 ppm concentrations under the tested conditions. Further at 12 ppm PAA, an increase of 0.67 log CFU/mL was observed after 48 h exposure. Similarly, PAA treatment of NS showed no significant effect on the survival of *L. innocua* when compared with the control without PAA treatment (Fig. 2.4c). However, *L. innocua* levels gradually decreased by about 2.5 (for 12 ppm) to 4 (for 4 ppm and control) from 24 h onwards. However, these differences were not statistically different from each other by the end of the 96-h treatment time (Fig. 2.4c).

Discussion

NS used in hydroponic production systems are a possible source of microbial contamination. (Ilic et al., 2022; Coleman et al., 2017; Sharma et al., 2009; Warriner et al., 2003; Guo et al., 2002). Previous studies demonstrated the ability of foodborne pathogens to adhere to plant surfaces or become internalized via contaminated hydroponic NS (Ilic et al., 2022; Li et al., 2022; Xylia et al., 2022; Sharma et al., 2009; Bernstein et al., 2007; Guo et al., 2002). In this study, we investigated the survival kinetics of *S.* Typhimurium, *E. coli* O157:H7, and *L. innocua* in an inorganic hydroponic NS; to determine the efficacy of lactic acid bacteria-based interventions to mitigate the

risk and compare it with conventional chemical treatments. The findings indicate distinct survival patterns exhibited by the test organisms within this hydroponic NS. Among the test organisms, *S.* Typhimurium was displayed improved survival in the hydroponic NS under the tested conditions. In contrast, a study by Ilic et al. (2022) reported a 90% reduction in *S.* Typhimurium cell counts within 24 h post-inoculation in a hydroponic NS although it persisted over the 28-day test period. However, other studies suggest that *S.* Typhimurium can adapt to their environment quickly even in the presence of other bacteria, leading to a long persistence period as observed in our study (Xylia et al., 2022; Shaw et al., 2016). Similarly, *E. coli* O157:H7 cell counts remained stable throughout the experimental period which showed the ability of these two enteric pathogens to persist in an inorganic hydroponic NS. *E. coli* O157:H7 can resist various environmental stresses and can survive in nutrient-deficient environments (Puligundla & Lim, 2022). *E. coli* O157:H7 was reported to have survived within water samples for up to 91 days (Shaw et al., 2016; Avery et al., 2008; G. Wang & Doyle, 1998). Unlike *S.* Typhimurium and *E. coli* O157:H7; *L. innocua* cell counts displayed a significant reduction in this hydroponic NS. The hydroponic NS employed in this study did not support the persistence of *L. Innocua*, as seen by a 4.4 log CFU/mL reduction over 72 h period.

These observation differs from previous findings where *L. innocua* was able to survive for up to 28 days in stored irrigation water at 6.88 °C (Machado-Moreira et al., 2021). The Ilic et al. (2022) study also reported that *Listeria monocytogenes* cell counts persisted for 28-day within a NS reservoir at the end of the experiment. Though *L. innocua and L. monocytogenes* are phylogenetically closely related, genetic divergence easily explains observable phenotypic differences. For example, distinct phosphotransferase systems (responsible for sugar uptake in

bacteria) of *L. innocua* and *L. monocytogenes* likely impact their fitness in certain environments. (Milillo et al., 2012).

The availability of nutrients is crucial for an organism's survival in the environment. Soluble carbon sources support the survival of organisms. In anaerobic conditions, *S.* Typhimurium and *E. coli* O157:H7 can utilize nitrate as an alternate electron acceptor (Hoagland et al., 2018). However, *L. monocytogenes,* and *L. innocua* cell counts were reported to decline rapidly in untreated water but survival improved in autoclaved well water and seawater for up to 80 days (Hansen et al., 2006). Physicochemical properties such as temperature, pH, and salt also exert significant effects on bacteria. Xylia et al. (2022) observed that *Salmonella* enteritidis in hydroponic NS displayed higher survival rates at pH 7 and 8, than at pH 5. In addition, *E. coli* survival across multiple water sources was highest at 8°C for up to 91 days, notably in municipal water, which provided a survival advantage when compared to other water sources at a pH of 7.4. However, at 35°C using same water source, *E. coli* O157:H7 was undetectable between 49 and 84 days, suggesting a higher survival rate at lower temperatures (G. Wang & Doyle, 1998). Persistence of *L. monocytogenes* improved at 5°C when compared to 20°C. A rapid decline was observed in untreated water when compared to autoclaved water. In seawater, *L. monocytogenes* was able to persist for up to 30 days, while *L. innocua* was undetectable after 19 days (Budzińska et al., 2011; Hansen et al., 2006). Electrical conductivity indicates the presence of soluble salts and quantifies the total electrical current generated by both positively and negatively charged ions (Ali Al Meselmani, 2023). All these factors can influence the survival kinetics of microorganisms leading to either a longer persistence time or a shorter survival time.

L. rhamnosus-treated NS showed consistent cell reduction in all the treatments, suggesting no specific observable antagonistic activity of *L. rhamnosus* against the test organisms. Antagonistic

activities of *L. rhamnosus* against *S.* Typhimurium, *E. coli* O157:H7 and *L. innocua* have been reported by other yet could not be validated here (Alakomi et al., 2000; Brashears & Durre, 1999; Castellano et al., 2017; Iglesias et al., 2017; Serna-Cock et al., 2019; Zapaśnik et al., 2022). Both *Salmonella* and *E. coli* O157:H7 survival improved in the hydroponic NS for up to 96 h, while L. *rhamnosus* experienced a consistent decline. Enteric's ability to thrive in a nutrient-deficient environment is responsible for their resilience against numerous treatment inventions. *L. rhamnosus* requires sugar source for fermentation for metabolic activities therefore, the absence of carbon sources in the hydroponic NS may contribute to cell death (Martín et al., 2022; Russo et al., 2014) and the inability to display (Zapaśnik et al., 2022; Russo et al., 2014; Khalid, 2011).

L. rhamnosus displayed antagonistic activities against *Salmonella* survival co-inoculated with *L. rhamnosus* on fresh-cut pear at 10 and 20^{o} C. A 2- and 3-log reduction was reported in *Salmonella* or *L. monocytogenes* survival respectively when co-inoculated with *L. rhamnosus* (Iglesias et al., 2017). While these findings suggest that *S.* Typhimurium and *E. coli* O157: H7 can survive in the hydroponic nutrient solution, the conditions were found to be challenging for the survival of *L. innocua* and *L. rhamnosus.* Studies have reported that *L. rhamnosus* metabolites and reduced pH have antagonistic effects against bacteria (Martín et al., 2022; Shi et al., 2022; Castellano et al., 2017; Khalid, 2011). Thus, CFS of *L. rhamnosus* (i.e., metabolites produced because of *L. rhamnosus* growth in nutrient-rich growth media) were applied to the hydroponic nutrient solution to assess its efficacy against test organisms and compared with peracetic acid. A quantitative analysis of a strain of *L. rhamnosus*, using chromatographic methods, revealed the production of DL-p-Hydroxy-Phenyllactic acid and ferulic acid. Both substances displayed substantial inhibitory effects on the tested gram-positive and gram-negative pathogens (Vougiouklaki et al., 2022). *E. coli* O157:H7 may exhibit acid tolerance (Benjamin & Datta, 1995); however, lactic acid bacteria

metabolites from our study showed antagonistic properties against *E. coli* O157:H7 (Puligundla & Lim, 2022; Zapaśnik et al., 2022; Khalid, 2011; Alakomi et al., 2000). Analysis of the cell-free extracts from LAB by Dimitrijevic et. al. (2009), isolated a novel bacteriocin from *L. rhamnosus* 68 NCQ 1872, the resulting peptide displayed inhibitory activities against *Micrococcus lysodeikticus*. De Keersmaecker et. al. (2006), also tested the antimicrobial activities of *L. rhamnosus* 53103, a higher pH reduced the antimicrobial activities previously observed in the CFS with *Salmonella* displaying a faster regeneration time at a pH of 6.6 when compared with pH 5. Additional tests were performed to confirm the source of the detected antibacterial activity, it was determined that the antagonistic activity was caused by a heat stable non proteinaceous low molecular weight compound(s). Xu et al. (2021) employed ethyl acetate extraction to obtain the crude extract of bacteriocin from *L. rhamnosus*. The bacteriocin named 1.0320 was diluted into various gradients and added to an *E. coli* bacterial suspension to determine its antibacterial activity. Results indicated that bacteriocin 1.0320 exhibited strong antimicrobial activity against all the tested gram-negative bacteria except *Salmonella pullorum* but showed a weaker antimicrobial activity against the tested gram-positive bacteria. It was also noted that bacteriocin 1.0320 showed no antimicrobial activity against three of the tested strains of *Staphylococcus aureus* (Xu et al., 2021). In this study, the pH of the CFS-treated hydroponic nutrient solutions was less than 4 (data not shown) depending on the ratio, and lactic acid concentrations were in the range of 0.12 to 0.16%. Contrastingly, CFS treatment did not show any significant antagonistic effect on *L. innocua* in the hydroponic nutrient solution when compared to the control treatment, which showed a significant reduction. Studies have reported *L. innocua*'s acid tolerance and ability to synthesize proteolytic enzymes, Metabolites produced by *L. rhamnosus* may not impact the metabolic of *L. innocua* because strains of *L. innocua* have been observed to be resistant to multiple antimicrobials

which has been reported to be transferable among *Listeria* spp. (Martín et al., 2022; Webb et al., 2022; Kasra-Kermanshahi & Mobarak-Qamsari, 2015; Hartmann et al., 2011). Additionally, these observations seem to follow a similar pattern to what was observed in a study carried out by Xu et. al. (2021), purified strain of bacteriocin obtained from *L. rhamnosus* 1.0320 displayed weak antagonistic activity against gram-positive bacteria compared to strong antagonistic activity observed in gram-negative bacteria which was associated to LAB strain, peptide sequence, hydrophobicity and the electric charge of the bacteriocins (Xu et al., 2021). Another study observed that *L. innocua* isolates produce inhibitors such Listeriocin 743A, which showed similarities with other types IIa or pediocin, a bacteriocin widely produced by LAB. (Kalmokoff et al., 2001). The persistence of *L. innocua* in the CFS treatment of hydroponic nutrient solution could be attributed to the presence of residual nutrients from the nutrient broth present in the cell-free extract and the inherent characteristics of the organism.

The survival of the test organisms in the CFS-treated hydroponic nutrient solution was compared with that of the PAA-treated hydroponic nutrient solution. These results indicate that PAA was only effective at 12 ppm against *S.* Typhimurium while CFS treatments reduced the cell counts of *E. coli* O157:H7 and *S.* Typhimurium. This may show promise for the treatment of hydroponic NS using LAB CFS. However, it should be noted that this study was conducted using an inorganic NS and select organisms under laboratory conditions which may not replicate real-world hydronic systems. Therefore, additional research is warranted to gain a better understanding of metabolite profiles, their activities against human and plant pathogens and other beneficial organisms in the hydroponic environment. This will help determine the efficacy of these treatments in a system closer to what is obtainable in hydroponic systems.

Table 2.1 Hoagland's No. 2 Basal Salt Mixture

Ingredients	**Milligrams/liter**
Potassium nitrate	505.50
Calcium nitrate	820.75
Magnesium sulphate	240.94
Potassium dihydrogen phosphate	136.10
Manganese chloride.4H2O	1.81
Boric acid	2.86
Molybdenum trioxide.2H2O	0.02
Zinc sulphate.7H2O	0.22
Copper sulphate.5H2O	0.08
Ferric tartarate	5.00
TOTAL gm/liter	**1.71**

Figures

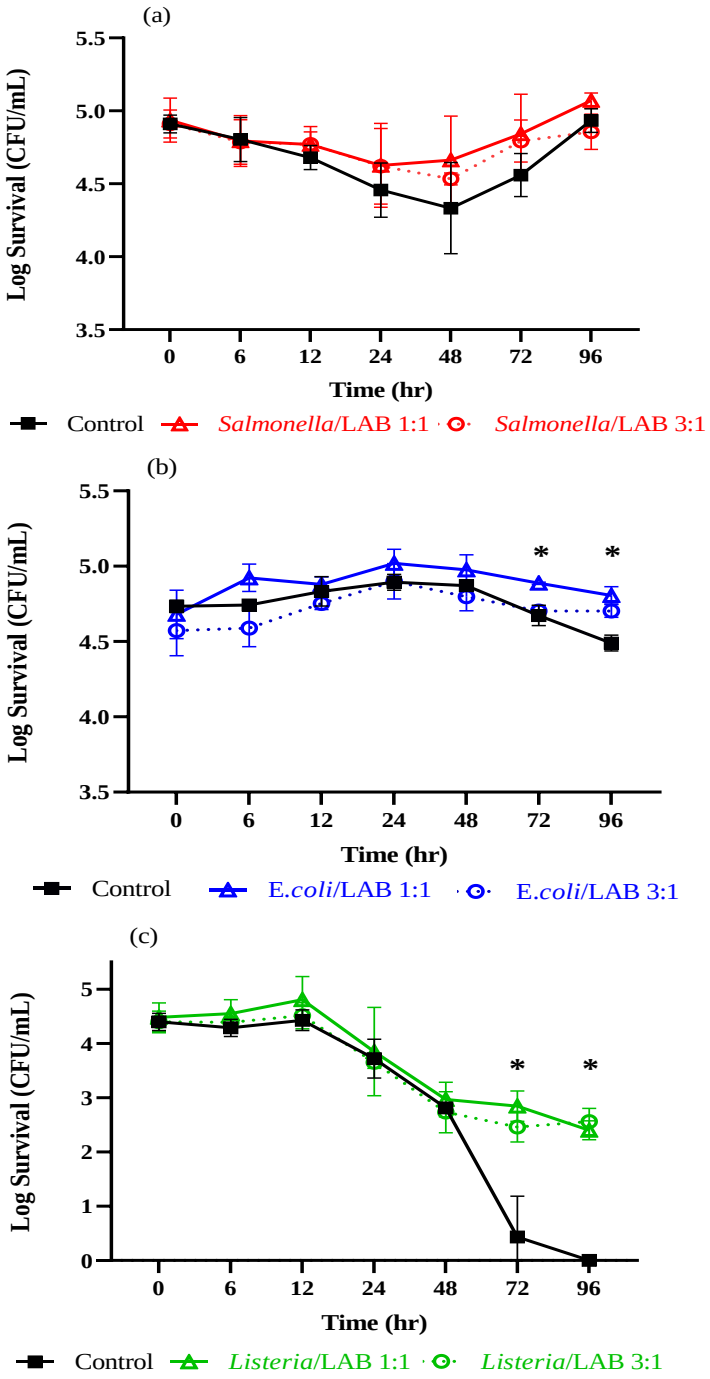

Fig 2.1. Growth, survival, and death kinetics of *Salmonella* Typhimurium (a), *E. coli* O157:H7 (b), and *Listeria innocua (*c) in *L. rhamnosus* treated hydroponic nutrient solution. Data was analyzed using ANOVA and means were separated using Tukey's HSD post hoc test at P value of 0.05. Means with an asterisk above indicate a statistical significance between treatments at individual time points at $p < 0.05$.

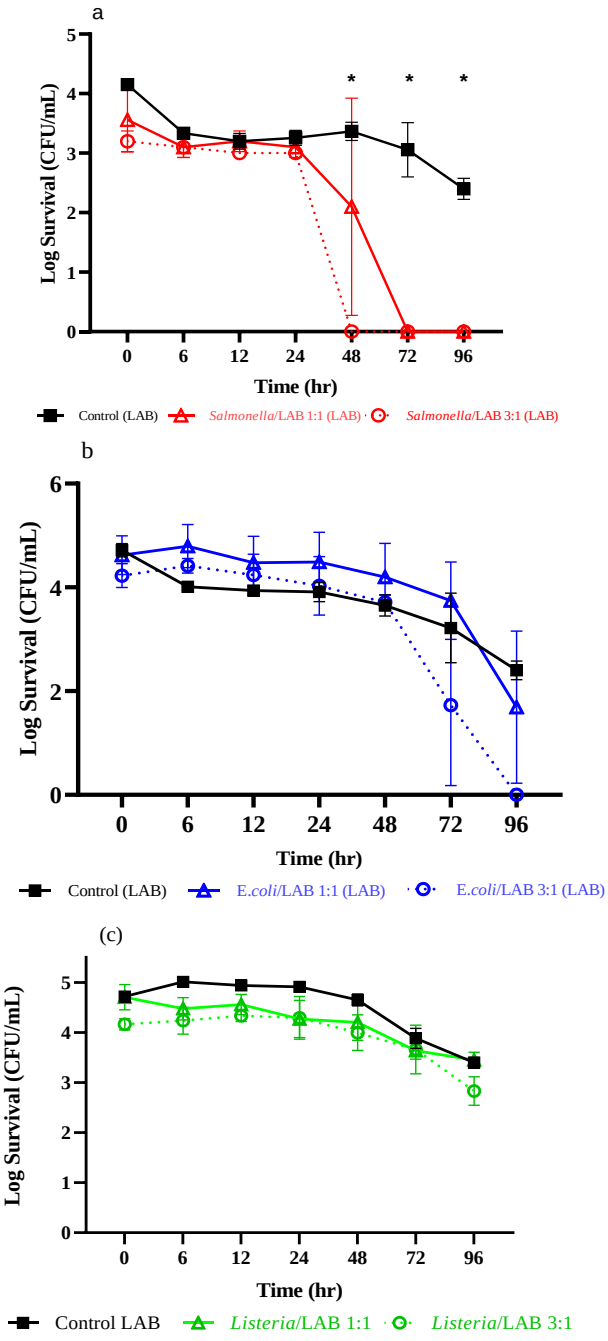

Fig 2.2. Survival kinetics of *L. rhamnosus* in hydroponic nutrient solution containing *Salmonella* Typhimurium (a), *E. coli* O157:H7 (b), and *Listeria innocua (*c). Data was analyzed using ANOVA and means were separated using Tukey's HSD post hoc test at P value of 0.05. Means with an asterisk above indicate a statistical significance between treatments at individual time points at $p < 0.05$.

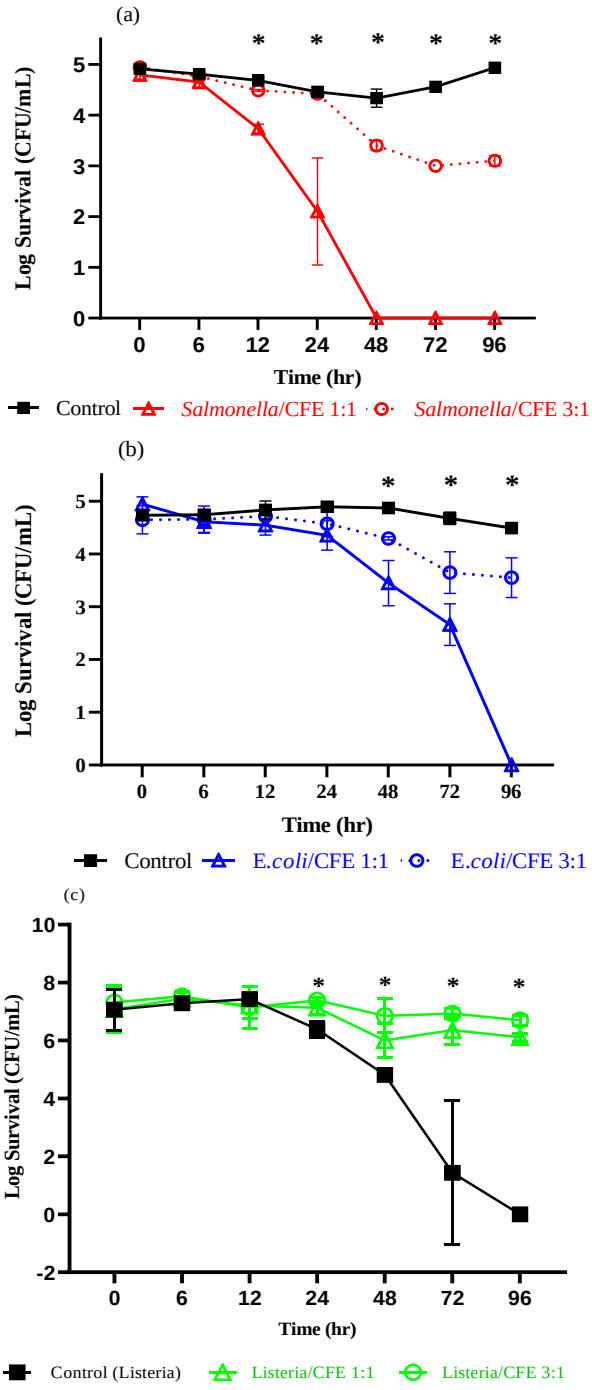

Fig 2.3. Growth, survival, and death kinetics of *Salmonella* Typhimurium (a), *E. coli* O157:H7 (b), and *Listeria innocua (*c) in *L. rhamnosus* cell free extract treated hydroponic nutrient solution. Data was analyzed using ANOVA and means were separated using Tukey's HSD post hoc test at P value of 0.05. Means with an asterisk above indicate a statistical significance between treatments at individual time points at $p < 0.05$.

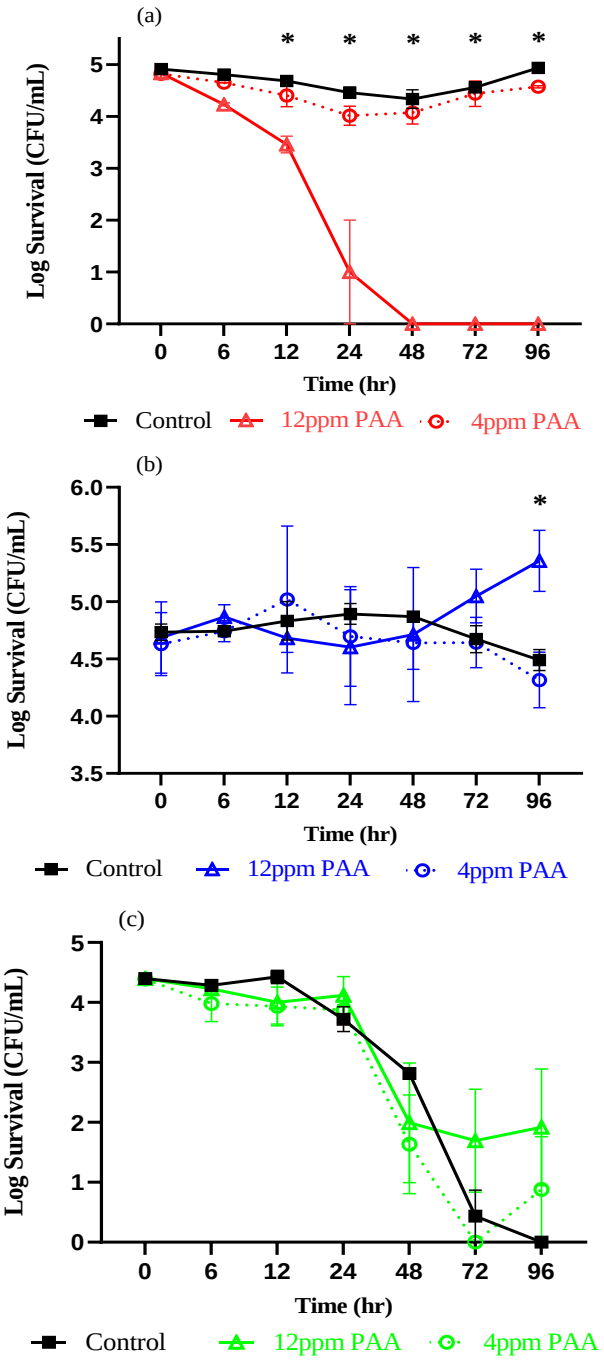

Fig 2.4. Growth, survival, and death kinetics of *Salmonella* Typhimurium (a), *E. coli* O157:H7 (b), and *Listeria innocua (*c) in Peracetic acid (PAA) treated hydroponic nutrient solution. Data was analyzed using ANOVA and means were separated using Tukey's HSD post hoc test at P value of 0.05. Means with an asterisk above indicate a statistical significance between treatments at individual time points at $p < 0.05$.

CHAPTER III

THE IMPACTS OF *LACTOBACILLUS RHAMNOSUS* ON PLANT GROWTH CHARACTERISTICS OF A HYDROPONIC PLANT

Abstract

The solution of a hydroponic system is the vehicle that supplies essential nutrients for plant growth and water for metabolic processes such as photosynthesis and respiration. Treating the NS is necessary to prevent contamination and the spread of pathogens within the production system. However, commercial chemical treatments like chlorination may alter the physicochemical properties of the solution, potentially impacting nutrient availability for plant uptake. While nutrients may be present, factors such as pH, electrical conductivity, and temperature can affect the plants' ability to utilize these nutrients effectively. This study compared the effects of Peroxyacetic acid (PAA), Sodium hypochlorite (NaOCl), Cell-free supernatant (CFS) obtained from *Lactobacillus rhamnosus*, and *Lactobacillus rhamnosus* on growth characteristics of lettuce plant. Lettuce seedlings were randomly assigned to PAA, NaOCl, CFS, and *L. rhamnosus* treatments, and growth measured every three days post-treatment for a duration of 21 days. The treatments affected the growth properties of this plant model, as measured by weight, height, dry-matter weight, leaf, and shoot length. Controls had the highest mean values among the treatments.

Only plants treated CFS ratios differed significantly from controls, while PAA, NaOCl, and *L. rhamnosus* did not. The only physicochemical properties of NS impacted by any treatment was pH in the CFS which had the lowest mean.

Keywords: Peroxyacetic acid, Sodium hypochlorite, *Lactobacillus rhamnosus*, Nutrient solution, pH, Electrical conductivity.

Introduction

To sustainably feed the world's growing population, agricultural methods must evolve to accommodate the decreasing availability of arable land and the increasingly unfavorable climatic conditions. Soilless agriculture is gaining prominence as a viable alternative to produce leafy vegetables, strawberries, cucumbers, and other crops. This method offers a successful solution to the challenges faced by traditional farming practices (Sardare & Admane, 2013; N. Sharma et al., 2018). Plant nutrients used in hydroponics are dissolved in water and can be supplied in either organic or inorganic forms to provide a suitable ion ratio for plant development. Nutrient uptake is only possible when these nutrients are available in a form that can be absorbed by the plants (Ali Al Meselmani, 2023).

Plant nutrients have specific roles in the physiological growth of plants, and their absence can impact the plant's life cycle for example, phosphorus deficiency can lead to stunted growth, poor flowering and fruiting, other deficiencies like nitrogen and iron can cause leaf discoloration. Various standard nutrient solutions, such as Hoagland and Snyder, Hoagland and Arnon, Steiner, and Bollard, are commonly used in hydroponic systems because they supply the appropriate balance of nutrients for optimal development (Asao, 2012; N. Sharma et al., 2018). These nutrients are absorbed in ionic forms, i.e. either positively charged (cation) or negatively charged (anions).

Nitrogen for example is absorbed as (NH_4^+, NO_3^-), Phosphorus (HPO_4^{-2}, $H_2PO_4^-$), Potassium (K^+) (Ali Al Meselmani, 2023).

When nutrient concentrations become imbalanced either due to deficiencies or excesses, ion composition in the solution is disrupted. The way plants respond to changes in nutrient concentrations and balance dictates how these interactions affect their growth and development and may lead to either nutrient toxicity or deficiency. Thus, understanding nutrient interactions is crucial for optimizing plant nutrition and ensuring healthy growth in hydroponic systems (Ali Al Meselmani, 2023).

Nutrients availability for growth, developments and plant production in the NS is assessed through Electrical Conductivity (EC), which measures the total amount of ions of dissolved salts. The EC in the NS exerts osmotic pressure depending on the amount of dissolved nutrients (Iris & Carlos, 2012). pH, on the other hand, indicates the relationship between the concentration of free H^+ and OH^- present in NS measured as either the acidity or alkalinity of the solution. pH plays a crucial role in nutrient availability for plant uptake during production, by affecting nutrient composition, distribution of elements among the various forms (free ions, soluble complexes, ion pair, and different oxidation states), and bioavailability (Iris & Carlos, 2012). In the NS, NH_3 can form a complex with H^+ to make NH_4^+ only between pH 2 to 7. Beyond that, concentration of NH_3 increases in the NS. Phosphorus (P)is available in a form that can be taken up by plants in acidic around pH 5 but decreases in alkaline and highly acidic solutions. While nutrients like P and nitrogen are in available forms at limited pH ranges, potassium has a wider pH range (2 to 9). When NS solution has a pH above 7, some nutrients precipitate and become insoluble and this can alter the nutrient composition in a way that affects plant absorption capabilities, potentially leading to deficiency symptoms. Maintaining a pH between 5.5 and 6.5 is recommended for optimal

development across most plant species because in that range ions in the solution are available in forms that plants can absorb (Iris & Carlos, 2012; N. Sharma et al., 2018). Most nutrient solutions will fall in the of 5.0-6.0 pH changes in the nutrient solution are dependent on factors such as temperature, content of organic and inorganic ions, types of ions present, and CO_2 content. During plant growth, the physicochemical properties of the nutrient solution will change reflecting the depletion of the nutrient's availability in the solution. (Ali Al Meselmani, 2023; Jones, 2014).

In a hydroponic system, plants are shielded from external influences, yet the inputs into the system can serve as pathways for pathogen contamination. Irrigation water, for instance, is recognized as a source of produce contamination and serves as a significant input in hydroponic production. The application of irrigation water in hydroponic systems may differ slightly from conventional agriculture because of the introduction of plants nutrients to make the NS. However, the collection, replenishment, and storage processes are similar in both practices and can be monitored to reduce the risk of microbial contamination, using untreated wastewater can substantially increase the risk of contaminating produce during cultivation (Alegbeleye et al., 2018).

In our previous study, we used *Lactobacillus rhamnosus* and its metabolite to mitigate foodborne pathogens in the hydroponic NS and compared its efficacy with some conventional chemical sanitizers. Sodium hypochlorite (NaOCl) inhibited survival of *Salmonella*, *Listeria*, and *E. coli* at a concentration as low as 4ppm, *L. rhamnosus* cell free supernatant (CFS) inhibited *Salmonella* and *E. coli* survival but not that of listeria. Peroxyacetic acid (PAA) inhibited survival of *Salmonella* while *L. rhamnosus* had no significant effect on the survival of the three pathogens when compared to the controls i.e. pathogen in NS without treatment. This study aims to

understand the effect of these treatments on plant growth performance over a 21-day period. Additionally, the physicochemical properties of the NS will be monitored over time.

Methodology

Preparation of the treatments; control (NS without treatment), NaOCl, PAA, CFS (1, 0.5, and 0.25 mL), *and L. rhamnosus* followed the same procedure described earlier in chapter 2.

Seedling germination and measurements. Lettuce seeds of the Butterscotch variety (*Latuca sativa*) were aseptically germinated in sterile Petri dishes and sterile filter papers using sterile water for seven days in the dark at 25^{o}C. Seedlings were watered regularly to prevent wilting, at about 3 cm height at first leaf formation, seedlings were transferred to 50mL Falcon tubes wrapped with aluminum foil (to keep the root region dark) and filled with the appropriate treatments. Treatments were transferred to a growth chamber (Thermo Scientific PR505755L Precision Incubator 17.79) previously set to a photoperiod of 16h light at 21^{o}C and 8h dark at 19^{o}C for the whole experiment period (Xylia et al., 2022).

Three days after transferring the seedlings to their respective treatments, plant weight was measured using measuring scale (Mettler Toledo ME104TE/00 Analytical balance, Allendale MI) and plant height, shoot length, root length, leaf height, and leaf width were measured with caliper (VINCA DCLA-0605 Digital Caliper, Valencia CA) every 3 days for a total period of 21days. Plant dry weight was measured after desiccation in stove at 65^{o}C for 72h.

NS properties. To determine the consistency of the hydroponic NS over the experimental period, changes in temperature, pH, electrical conductivity, and total dissolved solids were recorded and analyzed using a pH meter (Model A211, Orion™, MA, USA) and a conductivity meter (Thermo Scientific™, Eutech, Singapore) respectively. This was done every 3 days for a total period of 21 days.

Results

Plant Height. As shown in Fig 3.1 LAB, PAA, and NaOCl showed significant increases in plant height at 6.74, 5.88, 5.96, and inches respectively at day 21 when compared to the CFS treatments. When compared to the control, LAB, PAA, and NaOCl showed no statistical significance, they exhibited an average increase of 0.6 inches on day 9 and day 12 post-transfer into the growth chamber. The highest plant height difference within this group was observed on day 15, with NaOCl showing the highest increase of 2.24 inches. After day 15, NaOCl showed an average decline of about 0.6 inches in plant height on day 18 and 21, PAA and LAB showed 0.7 and 0.18 inches decrease in plant height on day 21 only, while the control treatment showed no average decrease in plant height.

On the other hand, the CFS treatment showed an average increase in plant height up to day 6 post-transfer into the growth chamber. Treatment with 1ml CFS exhibited an average decrease of 0.1 inches on day 9 and 12, with an increase in plant height observed from day 15 onwards. The 0.5ml CFS treatment exhibited a decrease in average plant height on days 9, 18, and 21. The 0.25ml CFS treatment showed a decrease in average plant height on days 12 and 21.

Plant Weight. As shown in Fig 3.2, a significant weight increase was recorded in PAA, NaOCl, and control treatments, with average plant weights of 0.19, 0.13, and 0.16 g respectively at day 21 post-transfer into the growth chamber. When compared to the control, PAA and NaOCl did not show significant differences as opposed to the LAB and CFS treatments. PAA exhibited an overall increase in weight, except on day 18 where a 0.002g decrease was recorded. Similarly, the NaOCl treatment showed a slight decrease in weight (about 0.0014g) on day 9 but exhibited an increase on days 3 to 21. The control, weight increased on all days except on day 12, which showed a decrease of 0.15g compared to day 9.

The LAB treatment demonstrated a significant difference in plant height compared to other treatments, with an average of 0.09 g at day 21. Conversely, no differences were evident within the CFS groups but differed significantly when compared to the control and other treatments. The 1ml, 0.5ml, and 0.25ml CFS treatments showed average weights of 0.05, 0.03, and 0.06g respectively. The 1ml treatment experienced a weight decrease on days 6 and 12 post-transfer compared to previous weights, but an increase in weight was observed on other days. Similarly, the 0.5ml CFS treatment yielded a weight decrease on days 12 and 18 post-transfer, with an increase in weight on other days. The 0.25ml CFS treatment exhibited a decrease of about 0.0025 g compared to the average weight observed on day 3, with an increase in weight on other days.

Dry matter weight (DMW). Fig 3.3 shows the DMW of treatments across the time points, 0.5ml CFS and PAA did not differ significantly from each other in their DMW. The other treatments had a p value < 0.005 across the time points. A significant increase was observed in LAB, PAA, NaOCl, and Control treatments, with average DMW of 0.0039, 0.0068, 0.0058, and

0.0054 g respectively at day 21 post-transfer into the growth chamber. Within each treatment, there was no significant effect of time on DMW in 0.5ml CFS while other treatments showed significant differences at different time points.

Physicochemical Properties. This study aimed to assess how PAA, NaOCl, *L. rhamnosus* and CFS affected nutrient availability and uptake by plants.

pH. The pH of each treatment was first recorded to assess if a significant difference in pH over the experiment period would nutrient uptake by lettuce observed in the plant growth. Table 3.1 indicates that no statistical significance was found across the measured day points. On day 0, pH of control 5.3 did not differ significantly from LAB 4.97 and NaOCl 4.56 but was significantly different from PAA 4.4 and all the CFS (1ml 3.93, 0.5ml 3.95, and 0.25ml 4.27) treatments. LAB 5.18 & 5.31 and NaOCl 4.6 & 4.57 did not differ significantly from the control 5.34 & 5.36 respectively, while PAA 4.21 & 4.34 and the CFS groups (1ml CFS 3.98 & 4.12, 0.5ml CFS 3.98 & 4.12, and 0.25ml CFS 4.16 & 4.32) differed significantly from the control on days 3 and 6 respectively after introducing treatments. 9- and 12-days post treatment, LAB 5.29 & 5.44, NaOCl 4.57 & 4.76, PAA 4.32 & 4.41, 0.5ml CFS 4.31 & 4.49, and 0.25ml CFS 4.64 & 5.27did not differ significantly while 1ml CFS 3.45 & 3.97 differed significantly from the control 5.39 & 5.30. Beyond day 12, pH did not differ significantly among all treatments.

Electrical Conductivity. Table 3.2 indicates that no statistical significance was found in the EC of each treatment across the measured day points. Immediately after treatments were

prepared (day 0) and day 3, EC of nutrient solution was recorded, LAB 111.27 & 87.63 mV did not differ significantly from the control 90 & 88.17 mV but significantly differed from NaOCl 133.71 & 121.17 mV, PAA 126.93 & 143 mV, 1ml CFS 162.9 & 163.4 mV, 0.5ml CFS 160.23 & 157.43 mV, and 0.25ml CFS 155.47 & 148.1 mV. On the 6^{th} day, LAB 75.67 mV and NaOCl 122.73 mV did not differ significantly from the control 85.5 mV which significantly differed from PAA 132.8 mV, 1ml CFS 161.7 mV, 0.5ml CFS 149.13 mV and 0.25ml CFS 161mV. Beyond day 6 EC did not differ significantly among all treatments

TDS. Table 3.3 summarizes the TDS, on days 0 and 3 the LAB 1.118 & 1.105ppt, NaOCl 1.084 & 1.081ppt, PAA 1.126 & 1.091ppt, 0.5ml CFS 1.194 & 1.178 ppt, and 0.25ml CFS 1.155 & 1.167 ppt did not differ significantly from the control 1.084 & 1.082ppt which significantly differed from 1ml CFS 1.219 & 1.178ppt respectively. Within treatments statistical significance was observed in LAB and PAA treatments, in the LAB treatment, post-hoc test indicated that TDS did not significantly differ on days 12 (1.142ppt), 18 (1.167ppt) and 21 (1.133ppt), days 0 (1.118ppt), 3 (1.105ppt), and 15 (1.120ppt), and days 6 (1.064ppt) and 9 (1.077ppt). Day 18 exhibited the highest TDS measured with an average of 1,167ppt. In the PAA treatment, TDS differed statistically between day 21 (1.173ppt) having the highest mean value and days 6 (1.064ppt), 12(1.062ppt), and 18(1.084ppt).

Nutrient Availability. Table 3.4 indicated that treatments significantly differed on days 0, 3, and 9 post treatment. On day 0, 1ml CFS 2.23mScm^{-1} differed significantly from the control while LAB 2.09mScm^{-1}, NaOCl 2.03mScm^{-1}, PAA 2.10mScm^{-1}, 0.5ml CFS 2.23mScm^{-1}, and 0.25ml CFS 2.15mScm^{-1} did not differ. On days 6 and 9 1ml CFS (2.27 & 2.29mScm^{-1}), 0.5ml

CFS (2.20 & 2.21mScm^{-1}), and 0.25ml CFS (2.18 & 2.24mScm^{-1}) differed significantly from the control while LAB (2.06 & 2.01mScm^{-1}), NaOCl (2.02 & 2.03mScm^{-1}), and PAA (2.03 & 2.05mScm^{-1}) did not differ. Beyond day 9, treatments did not differ statistically. Within treatments statistical significance was observed in LAB and PAA treatments, in the LAB treatment, post-hoc test indicated that nutrient availability did not differ in the LAB treatment on days 12 (2.13mScm^{-1}), 18 (2.18mScm^{-1}) and 21 (2.15mScm^{-1}), days 0 (2.09mScm^{-1}), 3 (2.06mScm^{-1}), and 15 (2.09mScm^{-1}), and days 6 (1.99mScm^{-1}) and 9 (2.01mScm^{-1}). Day 18 exhibited the highest condition measured with an average of 2.18mScm^{-1}. In the PAA treatment, condition also differed statistically between day 21 (2.19mScm^{-1}) having the highest mean value and days 6 (1.99mScm^{-1}), 12(1.99mScm^{-1}), and 18(2.03mScm^{-1}).

Discussion

The effects of LAB, NaOCl. PAA, and CFS treatments on overall plant health, as indicated by measures such as plant fresh weight, height, and dry matter weight was assessed. For plant height, the control having no form of treatments had the highest value while the 0.5ml CFS treatment had the lowest mean value among all the treatments. However, in the plant fresh weight and DMW, PAA had the highest mean value while CFS has the lowest mean value.

Lykogianni et al., (2023) reported similar results where NaOCl application at 7.5mg/L did not affect plant growth. In general, 2-4 mg/L of chlorine is allowable and should not lead to phytotoxicity in plants. However, some plants are more sensitive to chlorine at low concentrations. Cayanan et al., (2009) observed visual injuries on some nursery plant at concentrations of ≤ 2.5ml/L, phytotoxic effects and growth reductions were observed in deciduous but not the

evergreen shrubs. This observation was attributed to plant structure enabling the retention of irrigation water on plant surfaces, length of exposure, and the age of plants.

Vines et al., (2003) observed phytotoxic effects on tomato seedlings when PAA was applied hydroponically at concentrations of 1, 2 and 5µg/mL, they noted a reduction in plant growth based after 4 weeks of culture yielding a decrease in the dry matter compared to controls. Despite hydrogen peroxide and acetic acid in PAA solutions showing phytotoxic properties (Vines et al., 2003), experiments presented here show that PAA treatment yields the highest plant weight and plant dry matter. Factors such as plant type, exposure length, and differences in protocol may be responsible for the differences observed in plant yield.

1ml CFS had an initial pH average of 3.93 and this gradually increased with time to 5.27 (Table 3.1). P is usually found in the root zone of plant are present as PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$ ions, P is available for plant uptake in HPO_4^{2-} and $H_2PO_4^-$ ion form. An acidic or alkaline solution will decrease the availability of P in the nutrient solution (Asao, 2012). Average EC was highest in 1ml CFS treatment 162.9 and lowest in the control treatment 90 (Table 3.2). High EC may provide an environment unsuitable for nutrient uptake and may also put osmotic pressure on plant roots(Iris & Carlos, 2012). Absorption of water and nutrients within the NS changes the ion balance, causing an increase in the EC of the NS. EC increase was observed in the Control, NaOCl and PAA treatments but decreased with treatments 0.25ml CFS and 1ml CFS over the 21 days experimental trial.

Figures

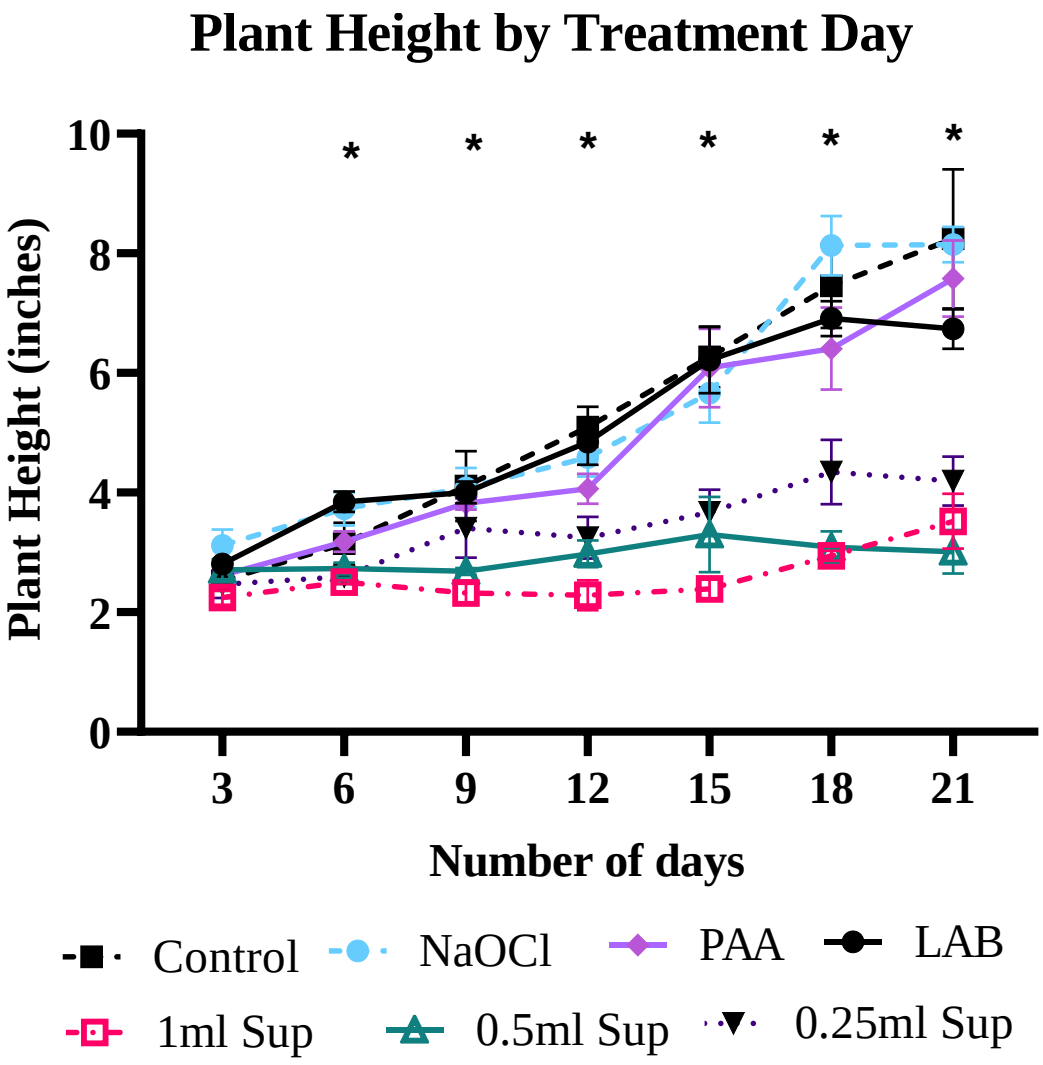

Fig 3.1. Plant Height of Treatments across day points. Data was analyzed using ANOVA and means were separated using Tukey's HSD post hoc test at P value of 0.05. Means with an asterisk above indicate a statistical significance between treatments at individual day points at $p < 0.05$.

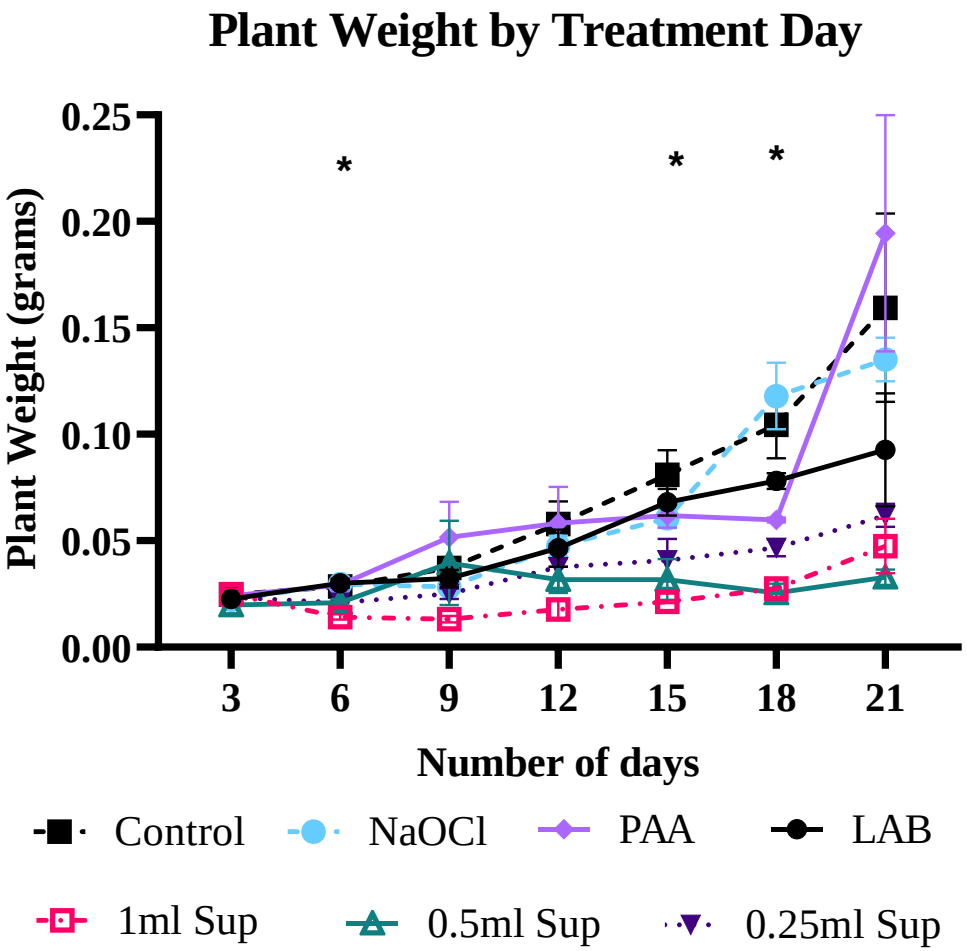

Fig 3.2. Plant Weight of Treatments across day points. Data was analyzed using ANOVA and means were separated using Tukey's HSD post hoc test at P value of 0.05. Means with an asterisk above indicate a statistical significance between treatments at individual day points at $p < 0.05$.

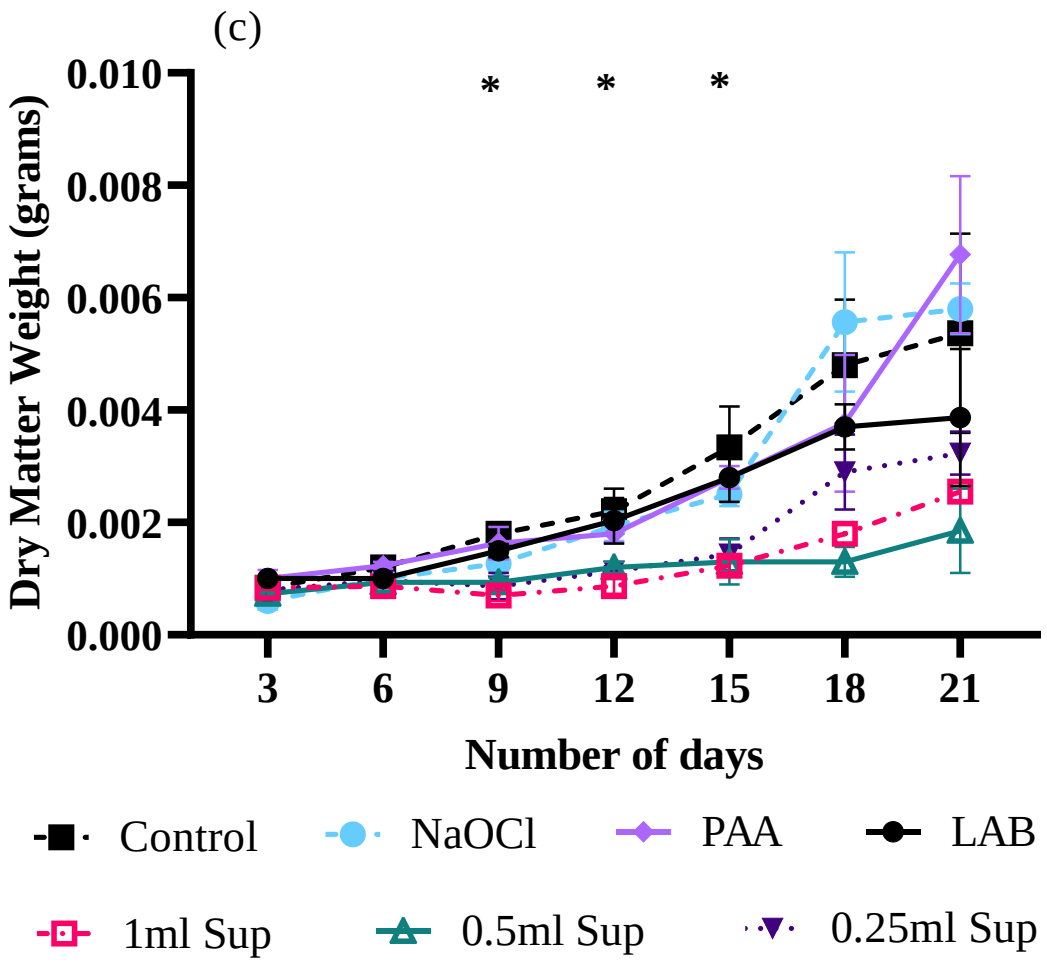

Fig 3.3. Plant Dry Matter Weight of Treatments across day points. Data was analyzed using ANOVA and means were separated using Tukey's HSD post hoc test at P value of 0.05. Means with an asterisk above indicate a statistical significance between treatments at individual day points at $p < 0$.

Tables

Table 3.1 NS's pH mean and SD. Observations not connected by the same letter are significantly different. Significant difference between treatment represented with uppercase letters and differences within treatment represented by lowercase letters.

pH measurement							
Days	**0.25ml CFS**	**0.5ml CFS**	**1ml CFS**	**Control**	**LAB**	**NaOCl**	**PAA**
0	4.27 ± 0.44^{BCa}	3.95 ± 0.13^{Ca}	3.93 ± 0.21^{Ca}	5.30 ± 0.26^{Aa}	4.79 ± 0.27^{ABa}	4.56 ± 0.30^{ABCa}	4.40 ± 0.24^{BCa}
3	4.16 ± 0.26^{Ba}	3.98 ± 0.21^{Ba}	3.88 ± 0.14^{Ba}	5.34 ± 0.36^{Aa}	5.18 ± 0.50^{Aa}	4.60 ± 0.21^{Aba}	4.21 ± 0.07^{Ba}
6	4.32 ± 0.40^{Ba}	4.12 ± 0.46^{Ba}	3.97 ± 0.27^{Ba}	5.36 ± 0.31^{Aa}	5.31 ± 0.37^{Aa}	4.57 ± 0.07^{Aba}	4.34 ± 0.18^{Ba}
9	4.64 ± 0.46^{ABa}	4.31 ± 0.72^{ABa}	3.45 ± 0.99^{Ba}	5.39 ± 0.25^{Aa}	5.29 ± 0.41^{ABa}	4.57 ± 0.08^{Aba}	4.32 ± 0.11^{Aba}
12	5.27 ± 1.07^{ABa}	4.49 ± 0.22^{ABa}	3.97 ± 0.13^{Ba}	5.30 ± 0.62^{Aa}	5.44 ± 0.47^{Aa}	4.76 ± 0.18^{Aba}	4.41 ± 0.25^{Aba}
15	5.05 ± 0.94^{Aa}	4.09 ± 0.34^{Aa}	4.52 ± 1.21^{Aa}	5.04 ± 0.34^{Aa}	5.07 ± 0.29^{Aa}	4.67 ± 0.25^{Aa}	4.39 ± 0.32^{Aa}
18	4.93 ± 0.74^{Aa}	4.75 ± 0.25^{Aa}	3.96 ± 0.23^{Aa}	4.89 ± 0.39^{Aa}	4.94 ± 0.29^{Aa}	4.44 ± 0.16^{Aa}	4.33 ± 0.17^{Aa}
21	5.26 ± 0.70^{Aa}	3.89 ± 0.04^{Aa}	5.27 ± 1.79^{Aa}	4.57 ± 0.99^{Aa}	4.91 ± 0.36^{Aa}	4.69 ± 0.36^{Aa}	4.53 ± 1.04^{Aa}

Table 3.2. NS's EC mean and SD. Observations not connected by the same letter are significantly different. Significant differences between treatment are represented with uppercase letters and differences within treatment are represented by lowercase letters.

Electrical conductivity (EC) measurement (mV)							
Days	**0.25ml CFS**	**0.5ml CFS**	**1ml CFS**	**Control**	**LAB**	**NaOCl**	**PAA**
0	155.47±3.86^{Aa}	160.23±7.22^{Aa}	162.90±8.92^{Aa}	90.00±8.94^{Ba}	111.27±15.88^{Ba}	133.71±3.67^{Aba}	126.93±25.48^{Aba}
3	148.10±14.81^{Aa}	157.43±12.02^{Aa}	163.40±8.08^{Aa}	88.17±23.96^{Ba}	87.63±29.38^{Ba}	121.17±12.91^{Aba}	143.00±5.65^{Aa}
6	142.97±24.75^{Aa}	149.13±26.44^{Aa}	161.70±11.53^{Aa}	85.40±24.28^{BCa}	75.67±17.57^{Ca}	122.73±7.26^{ABCa}	132.80±11.08^{Aba}
9	161.00±1.84^{Aa}	147.40±25.92^{Aa}	160.67±12.82^{Aa}	77.17±13.16^{Aa}	78.53±24.74^{Aa}	121.17±10.9^{Aa}	134.23±9.76^{Aa}
12	113.70±60.76^{Aa}	141.40±2.08^{Aa}	157.83±8.20^{Aa}	80.73±36.08^{Aa}	72.10±27.89^{Aa}	102.00±27.19^{Aa}	131.43±15.30^{Aa}
15	93.87±56.39^{Aa}	148.95±18.74^{Aa}	131.90±59.69^{Aa}	108.33±19.82^{Aa}	93.03±16.92^{Aa}	116.33±25.00^{Aa}	128.23±13.90^{Aa}
18	95.90±53.33^{Aa}	161.60±1.83^{Aa}	106.75±87.33^{Aa}	106.43±22.82^{Aa}	102.20±17.37^{Aaa}	131.53±14.89^{Aa}	138.23±10.50^{Aa}
21	78.73±40.07^{Aa}	163.30±2.26^{Aa}	157.20^{Aa}	123.53±54.50^{Aa}	95.27±16.57^{A}	136.03±16.90^{Aa}	141.80±34.17^{Aa}

Table 3.3. NS's TD mean and SD. Observations not connected by the same letter are significantly different. Significant differences between treatment are represented with uppercase letters and differences within treatment are represented by lowercase letters.

Total dissolved solids measurement (ppt)							
Days	**0.25ml CFS**	**0.5ml CFS**	**1ml CFS**	**Control**	**LAB**	**NaOCl**	**PAA**
0	1.155 ± 0.07^{ABa}	1.194 ± 0.01^{ABa}	1.219 ± 0.05^{Aa}	1.084 ± 0.05^{Ba}	1.118 ± 0.01^{ABbc}	1.084 ± 0.05^{Ba}	1.126 ± 0.02^{ABab}
3	1.167 ± 0.04^{ABa}	1.178 ± 0.02^{ABa}	1.213 ± 0.04^{Aa}	1.082 ± 0.06^{Ba}	1.105 ± 0.02^{ABcd}	1.081 ± 0.05^{Ba}	1.091 ± 0.03^{Bab}
6	1.145 ± 0.06^{Aa}	1.172 ± 0.03^{Aa}	1.174 ± 0.11^{Aa}	1.054 ± 0.01^{Aa}	1.064 ± 0.01^{Ae}	1.076 ± 0.03^{Aa}	1.064 ± 0.02^{Ab}
9	1.195 ± 0.02^{Aa}	1.184 ± 0.02^{Aa}	1.201 ± 0.04^{Aa}	1.076 ± 0.01^{Aa}	1.077 ± 0.01^{Ade}	1.086 ± 0.19^{Aa}	1.096 ± 0.03^{Aab}
12	1.212 ± 0.03^{Aa}	0.9841 ± 0.29^{Aa}	0.980 ± 0.25^{Aa}	1.119 ± 0.05^{Aa}	1.142 ± 0.01^{Aabc}	1.113 ± 0.03^{Aa}	1.062 ± 0.04^{Ab}
15	1.078 ± 0.09^{Aa}	1.068 ± 0.15^{Aa}	1.061 ± 0.09^{Aa}	1.113 ± 0.05^{Aa}	1.120 ± 0.02^{Abc}	1.103 ± 0.03^{Aa}	1.101 ± 0.03^{Aab}
18	0.824 ± 0.23^{Aa}	0.821 ± 0.33^{Aa}	0.949 ± 0.43^{Aa}	1.165 ± 0.03^{Aa}	1.167 ± 0.02^{Aa}	1.101 ± 0.06^{Aa}	1.084 ± 0.04^{Ab}
21	0.993 ± 0.34^{Aa}	0.933 ± 0.39^{Aa}	1.199 ± 0.01^{Aa}	1.133 ± 0.08^{Aa}	1.153 ± 0.01^{Aab}	1.139 ± 0.01^{Aa}	1.173 ± 0.03^{Aa}

Table 3.4. NS's Condition mean and SD. Observations not connected by the same letter are significantly different. Significant difference between treatment are represented with uppercase letters and differences within treatment are represented by lowercase letters.

Conditions($mScm^{-1}$)							
Days	**0.25ml CFS**	**0.5ml CFS**	**1ml CFS**	**Control**	**LAB**	**NaOCl**	**PAA**
0	2.15 ± 0.13^{Aba}	2.23 ± 0.02^{ABa}	2.28 ± 0.09^{Aa}	2.04 ± 0.10^{Ba}	2.09 ± 0.02^{ABbc}	2.03 ± 0.09^{Ba}	2.10 ± 0.03^{ABab}
3	2.18 ± 0.07^{Aba}	2.20 ± 0.04^{Aba}	2.27 ± 0.08^{Aa}	2.02 ± 0.10^{Ba}	2.06 ± 0.04^{Bcd}	2.02 ± 0.10^{Ba}	2.03 ± 0.05^{Bab}
6	2.14 ± 0.11^{Aa}	2.19 ± 0.06^{Aa}	2.19 ± 0.21^{Aa}	1.97 ± 0.02^{Aa}	$1.99\pm.01^{Ad}$	2.01 ± 0.05^{Aa}	1.99 ± 0.05^{Ab}
9	2.23 ± 0.04^{Aa}	2.21 ± 0.04^{Aa}	2.29 ± 0.07^{Aa}	2.01 ± 0.02^{Ba}	2.01 ± 0.02^{Bd}	2.03 ± 0.04^{Ba}	2.05 ± 0.04^{Bab}
12	2.26 ± 0.05^{Aa}	1.84 ± 0.54^{Aa}	1.16 ± 0.69^{Aa}	2.09 ± 0.10^{Aa}	2.13 ± 0.01^{Aabc}	2.08 ± 0.06^{Aa}	1.99 ± 0.08^{Ab}
15	2.02 ± 0.17^{Aa}	1.98 ± 0.25^{Aa}	1.98 ± 0.16^{Aa}	2.07 ± 0.10^{Aa}	2.09 ± 0.05^{Abc}	2.06 ± 0.05^{Aa}	2.06 ± 0.06^{Aab}
18	1.69 ± 0.47^{Aa}	1.50 ± 0.55^{Aa}	1.78 ± 0.81^{Aa}	2.18 ± 0.06^{Aa}	2.18 ± 0.03^{Aa}	2.06 ± 0.11^{Aa}	2.03 ± 0.08^{Ab}
21	2.06 ± 0.83^{Aa}	1.74 ± 0.72^{Aa}	2.24 ± 0.01^{Aa}	2.13 ± 0.17^{Aa}	2.15 ± 0.03^{Aab}	1.82 ± 0.55^{Aa}	2.19 ± 0.05^{Aa}

CHAPTER IV

CONCLUSIONS

Controlled environment agriculture (CEA) has emerged as a promising alternative, offering increased control factors, and reduced environmental dependencies. CEA offers diverse production techniques that vary in their input usage and nutrient delivery methods. While CEA systems prioritize enhancing produce quality and yield through precise control of environmental factors and nutrient management, challenges still exist in managing foodborne pathogens and plant diseases.

The persistence of pathogens in seed during storage, nutrient solutions, and growth media presents ongoing challenges for CEA systems because pathogens can survive for extended periods on these inputs, the potential for contamination and proliferation during plant growth and the difficulty in decontamination during production may contribute to food safety concerns. Various antimicrobial interventions like UV radiation, heat treatment, and chemical applications are employed to mitigate these risks, each method has its own set of advantages and limitations.

Our research explored the potential of lactic acid bacteria and its CFS to inhibit foodbome pathogens in hydroponic NS. The studies revealed varying pathogen survival patterns and the efficacy of different treatments. Notably, CFS of *L. rhamnosus* showed promise in inhibiting

certain pathogens, while the bacteria itself demonstrated consistent cell reduction across treatments. While cell counts of Salmonella and E. coli reduced when treated with CFS, Listeria showed a distinct interaction with the CFS. This distinction can be considered from various angles.

Listeria is a gram-positive (+ve) bacterium while both Salmonella and E. coli are gram-negative (-ve) implying different cell structures that may either enhance their survival or susceptibility in their environment. Listeria and L. rhamnosus gram (+ve) bacteria with similar cell structures, which might be responsible for both microorganisms to survive the CFS that caused cell death in the other pathogens. Studies that have been conducted to test the effects of CFS or pure bacteriocins on pathogenic bacteria have shown greater effectiveness when applied to gram-ve pathogens than the gram +ve pathogens.

Additionally, Listeria's ability to produce proteolytic enzymes that not only break down protein but are also important for immunity which may be responsible for its high resistance to multiple antimicrobials. Its ability to produce Listeriocin which is like bacteriocins produced by LAB can also improve its resistance to our CFS.

Further investigations into plant growth performance and NS properties under various treatments yielded mixed results. PAA has the highest plant weight and DMW while CFS treatments significantly differed from the control, suggesting potential impacts on plant growth. This raises some questions about what might be responsible for the impaired growth observed in the CFS groups. PAA had a pH of 4.4 while the least concentrated CFS has a pH of 4.27. However, a distinct growth difference was observed between the two treatments. E.C of the NS follows a similar pattern where that PAA treatment has similar EC values to the least concentrated CFS. The expected outcome would have been to observe more growth in the treatment with the lower EC values vice versa however this was not the case.

Based on these observations, the CFS might contain other metabolic constituents that can affect nutrient availability or absorption in the nutrient solution. The application of CFS in the NS may affect the nutrient balance showing symptoms such as stunted growth.

These findings emphasize the complexity of balancing pathogen control with optimal plant growth in CEA systems as seen in the impact of CFS on plant growth and NS. Additional research is required to isolate and identify the specific components of CFS responsible for pathogen inhibition which may be responsible for the growth patterns observed in the CFS treatments. With this understanding, interventions that effectively control pathogens while minimizing adverse effects on plant growth can be developed.

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