United States Department of Agriculture
Project Initiation

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Project Title

Impact of substrate microbiomes on biofilm formation and persistence of Listeria monocytogenes in hydroponic systems

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
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Project Director

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Recipient Organization

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Performing Department

(N/A)

Non Technical Summary

Foodborne pathogens such asListeria monocytogenescan contaminate fresh produce and cause serious illness. As hydroponic farming becomes more common, it is important to understand the potential food safety risks associated with these systems. Hydroponic farms grow plants without soil, often using materials such as rockwool or coconut fiber to support plant roots. These materials can contain natural communities of bacteria. Some of these bacteria can form persistent slimy layers called biofilms that help pathogens survive longer. However, very little research has examined how these microbial communities affect the survival of harmful bacteria in hydroponic farming environments. This project will examine the bacteria naturally present in hydroponic growing materials and test whether they help or hinder the survival ofListeria monocytogenes.The study will identify bacteria commonly found in hydroponic substrates. Next, laboratory experiments will test whether these bacteria form biofilms and how they interact

withListeria.Finally, the research will evaluate these interactions in a working hydroponic system with and without plants.These results will help scientists, farmers, and food safety professionals better understand how microbes behave in hydroponic farming systems. This knowledge can support the development of improved food safety practices that protect consumers while helping hydroponic agriculture continue to grow as a sustainable method of producing fresh food.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	20%
Applied	60%
Developmental	20%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
712	1430	1040	100%

Knowledge Area

[712] Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

Subject Of Investigation

[1430] Greens and leafy vegetables (includes endive, lettuce, spinach, turnip-greens, celery,rhubarb, parsley, asparagus)

Field Of Science

[1040] Molecular biology

Keywords

Food safety, Listeria monocytogenes, biofilms, hydroponic farming, microbiome

Goals / Objectives

To determine how microbial communities associated with hydroponic substrates influence the formation of multispecies biofilms and the growth and persistence of Listeria monocytogenes in hydroponic systems, in order to inform targeted strategies to mitigate pathogen contamination and improve food safety in controlled environment agriculture.1. Objective one of this project aims to analyze the microbiota associated with commonly used hydroponic substrates to enhance our understanding of the microbial communities present in these systems. Furthermore, it is essential to determine whether these bacteria form biofilms and how they affect the Listeria monocytogenes growth and survival.2. Objective two will evaluate the biofilm formation capacity of bacteria identified in Objective 1 and assess their impact onListeria monocytogenesgrowth and survival in multispecies biofilms.3. Objective three will explore the interactions between these biofilms and Listeria monocytogenesgrowth in a deep water culture system

with and without plants. This research will ultimately provide insight into the behavior ofListeria monocytogenesin complex hydroponic environments, which is needed for the development of targeted intervention strategies to mitigate contamination in hydroponic systems to enhance food safety.

Project Methods

Microbiome characterizationMicrobial communities in commonly used hydroponic substrates from multiple vendors will be characterized using 16S rRNA amplicon sequencing. Sequencing data will be processed using the DADA2 pipeline in R to generate amplicon sequence variants and assign taxonomy. Differences in microbiota composition among substrates will be assessed using PERMANOVA and differential abundance analysis. Dominant taxa will be identified for isolation and further study. Dominant bacterial taxa will be isolated using nutrient-poor media to improve the recovery of environmental bacteria. Colonies will be purified and identified using PCR and Sanger sequencing of the 16S rRNA gene, followed by BLAST analysis.Biofilm and pathogen interaction experimentsBiofilm formation will be evaluated using a crystal violet assay conducted in technical and biological replicates. To assess interactions withListeria monocytogenes,multispecies biofilms will be grown using MBEC plates. Samples will be collected over time and plated on ALOA to quantify pathogen survival and growth.Hydroponic system experimentsDeep water culture systems will be used to evaluate microbial interactions under realistic conditions. Four treatments will be tested: (1)L. monocytogenesalone, (2)L. monocytogeneswith biofilm-forming bacteria, (3)L. monocytogeneswith plants, and (4)L. monocytogeneswith biofilm-forming bacteria and plantsData analysisMicrobiome data will be analyzed in R using diversity metrics, ordination methods, and PERMANOVA to assess differences in community composition. Experimental data from biofilm and hydroponic trials will be analyzed using appropriate statistical tests (e.g., t-tests or ANOVA) to compare pathogen survival among treatments. Growth curves will be generated to evaluate changes in bacterial populations over time.Efforts to increase knowledgeThis project will provide experiential training for undergraduate students in microbiological techniques, experimental design, and data analysis. Research findings will be disseminated through conference presentations, seminars, and peer-reviewed publications, ensuring that results reach researchers, food safety professionals, and stakeholders in controlled environment agriculture.Evaluation of outcomesProject success will be evaluated through completion of experimental milestones, generation of microbiome datasets, and identification of bacterial taxa influencing L. monocytogenes survival. Additional indicators include peer-reviewed publications, conference presentations, and undergraduate research training outcomes. These measures will assess both scientific progress and knowledge dissemination related to microbial interactions in hydroponic food production systems.