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Diversity and antibiotic resistance in bacteria associated with symptoms of bacterial infection in Costa Rican crops

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ABSTRACT

Objetive/Background. The aim of this was to assess the diversity and antibiotic resistance of bacteria isolated from 19 crops with bacterial infection symptoms.

Material and Methods. This collection was identified using 16S rRNA gene sequencing and the Biolog system. Susceptibility and minimum inhibitory concentration (MIC) for streptomycin, tetracycline, and gentamicin were determined using disk diffusion and E-test methods, respectively.

Results. A total of 55 species belonging to 20 bacterial genera were identified, with *Pseudomonas*, *Serratia*, *Pantoea*, and *Stenotrophomonas* being the most abundant. Approximately 27% of the isolates were categorized as pathogenic through the hypersensitivity reaction test, including phytopathogenic species like *Pseudomonas syringae*, *P. cichorii, Pantoea anthophila, P. stewartii, Stenotrophomonas maltophilia, Dickeya oryzae, Erwinia billingiae, Pectobacterium aroidearum*, and *Enterobacter cloacae* subsp. dissolvens. Resistance to at least one antibiotic was detected in 60% of isolates from 17 crops, with tomatoes, heart of palm, and lettuce exhibited the highest proportion of resistant bacteria (>80%). Streptomycin resistance was most common (35%), followed by tetracycline (28%) and gentamicin (9%).

Conclusions. The findings indicate the presence of antibiotic resistance in saprophytic and pathogenic bacteria associated with 17 out of 19 assessed crops, posing risks to the environment, phytosanitary conditions, and public health.

Keywords. Bacteria, Phytopathogen, Antimicrobial, Phyllosphere, Control

Introduction

Bacteria associated with plants can thrive as epiphytes or endophytes (Agrios, 2005), and in most cases, they perform functions crucial for maintaining the system's equilibrium, such as nitrogen fixation and antagonism against phytopathogens (Arauz, 2011; Nion and Toyota, 2015; Compant *et al.,* 2019; Hashem *et al.,* 2019). These bacteria share their niche with phytopathogenic bacteria that utilize plants as a nutrient source and have specialized in evading their defenses, consequently invading the host plant's tissues (Vidaver and Lambrecht, 2004; Agrios, 2005; Kannan and Bastas, 2015).

In Costa Rica, several genera have been reported as pathogens of ornamental plants and foliage, including *Erwinia*, *Pectobacterium*, *Pseudomonas*, *Xanthomonas*, *Burkholderia*, and the *Ralstonia solanacearum* species complex (Arauz, 2011; Quesada-González and García-Santamaría, 2014; Cubero-Agüero *et al.,* 2021; López, 2021; Vidaurre-Barahona *et al.,* 2021). Infections caused by these bacteria can manifest as fruit spots (*Pseudomonas syringae* and *Xanthomonas campestris*), cankers (*Erwinia* and *Pseudomonas*), wilting (*Ralstonia solanacearum*), and soft rot (*Pectobacterium carotovorum* and *Dickeya dadanti*) (Vidaver and Lambrecht, 2004; Agrios, 2005; Arauz, 2011; Bellincampi *et al.,* 2014). The variety of symptoms and host plants make these pathogens responsible for severe damage to crops, thereby affecting the agricultural sector's economy. Additionally, these diseases are more severe and frequent in tropical and subtropical regions where the warm and humid conditions are ideal for their development, and there is no reduction in inoculum due to winter's low temperatures (Arauz, 2011; Kannan and Bastas, 2015; Miller *et al.,* 2022). Hence, combatting these pathogens is crucial for preserving agroecosystem productivity. However, only a few substances are effective or readily available to mitigate crop losses, among wich antibiotics are included. Presently, the most widely used antibiotics globally are streptomycin, oxytetracycline, penicillin, oxolinic acid, and gentamicin (McManus *et al.,* 2002; Mann *et al.,* 2021; Miller *et al.,* 2022). Among these, streptomycin and oxytetracycline are approved for agricultural use in the United States, in contrast to the European Union and other developed countries where their use is prohibited due to their medical significance (WHO, 2019).

The use of antibiotics is the prevailing approach for managing bacterial diseases in low and middle-income countries (LMICs) (Miller *et al.,* 2022). This is notably the case in Latin America and the Caribbean, where they are used without restrictions, despite the risk of resistance development (Rodríguez *et al.,* 2006; Rodríguez *et al.,*

2008; MSP, 2018; Taylor and Reeder, 2020), with varying outcomes in combating bacterial diseases in crops (McManus *et al.,* 2002; Stockwell and Duffy, 2012). Despite the capability of phytopathogenic bacteria to infiltrate plant tissues and multiply (Sundin *et al.,* 2016), most formulations are applied to the aerial parts, resulting in reduced absorption, translocation, and efficiency (McManus *et al.,* 2002; Agrios, 2005). For example, the foliar absorption of oxytetracycline in citrus is quite limited, necessitating trunk injection to combat *Candidatus* Liberibacter asiaticus in this crop (Killiny *et al.,* 2020).

Bactericides and antibiotics are the most commonly used compounds for controlling phytopathogenic bacteria in Costa Rica (Ramírez-Muñoz *et al.,* 2014; Durán-Quirós *et al.,* 2017; Blanco-Meneses *et al.,* 2023). These include oxytetracycline, gentamicin, as well as combinations of these with streptomycin (gentamicin and oxytetracycline in Agri-Gent Plus 4 and 8, streptomycin and oxytetracycline in Agry-mycin 16.5) (Rodríguez *et al.,* 2006; Galt, 2009; MSP, 2018). The absence of an integrated registration system in the country limits the control of efficient use of these products in humans, animals, and plants (MSP, 2018). De la Cruz *et al*. (2008) found that antibiotic usage in the cultivation of *Cucumis melo*, *Citrullus lanatus*, and *Oryza sativa* in the Arenal-Tempisque irrigation district ranged from 7.4-155.0 g ha^{-1} per year for both streptomycin and oxytetracycline; other studies indicate discrepancies among producers concerning dosage, frequency of application, and pre-harvest intervals (Durán-Quirós *et al.,* 2017; Blanco-Meneses *et al.,* 2023).

Antibiotics can remain active on plant surfaces for at least one week (Stockwell and Duffy, 2012), which, coupled with their frequent use, can lead to the development of resistant bacteria (Silbergeld *et al.,* 2008; Alós, 2014; FAO, 2021). In this regard, Rodríguez *et al.* (2006; 2008) identified epiphytic bacteria resistant to gentamicin and oxytetracycline in lettuce (*Lactuca sativa*) samples and agricultural soils, as well as genetic determinants of resistance, implying the dissemination of resistant bacteria across different environments and/or horizontal gene transfer. Nevertheless, the presence of antimicrobial-resistant phytopathogenic bacteria has not been studied. Therefore, this study aimed to analyze the diversity of a bacterial collection isolated from lesions in various crops exhibiting bacterial infection symptoms in Costa Rica and to evaluate their resistance to streptomycin, oxytetracycline, and gentamicin, antibiotics commonly used for managing plant bacterial diseases in this country.

Materials and Methods

Bacterial isolates. We analyzed 116 Gram-negative bacteria from the collection of the Environmental Microbiology Laboratory at the Center for Research in Cellular

and Molecular Biology. These bacteria were isolated between 2006 and 2009 from samples originating in various agricultural areas of Costa Rica. Tissue from the advancing lesion zone in plants displaying symptoms associated with bacterial pathogens was selected for analysis. Relevant information regarding this bacterial collection is presented in Table 1.

Table 1. Characteristics of bacterial isolates collection established from symptoms in 19 crops in different regions of Costa Rica between 2006 and 2009.

Host plant	Common name	Nº samples	Plant tissue	Symptoms	Collecting site	Nº of isolates
Apium graveolens	Cellery	3	Stem	Soft rot (Figure 1D)	Cartago	6
Bactris gasipaes	Palm heart	3	Leaf	Necrosis	Limón	6
Brassica oleracea var. botrytis	Cauliflower	2	Leaf	Spot	Heredia	5
Brassica oleracea var. capitata	Cabbage	6	Leaf	Angular necrosis (Figure 1H), Spot, Soft rot	Heredia, Cartago	25
Capsicum annuum	Bell pepper	2	Fruit	Soft rot (Figure 1E)	Cartago	9
Cucumis melo	Cantaloupe	1	Fruit	Soft rot	Guanacaste	5
Cucurbita pepo	Summer squash	1	Fruit	Soft rot (Figure 1G)	Cartago	3
Curcuma longa	Turmeric	2	Root	Soft rot	Guanacaste	4
Daucus carota	Carrot	1	Root	Soft rot	Alajuela	1
Dracaena massangeana	Cornplant	2	Stem	Soft rot (Figure 1F)	Alajuela	7
Ficus carica	Fig	1	Leaf	Spot	Alajuela	1
Lactuca sativa	Boston lettuce	2	Leaf	Spot (Figure 1A)	Cartago	5
Lactuca sativa var. capitata	Iceberg lettuce	6	Stem, Leaf	Soft rot (Figure 1B), Spot	Cartago	11
Mangifera indica	Mango	2	Fruit	Spot (Figure 1I)	Alajuela	4
Musa paradisiaca	Banana	5	Stem, crown	Soft rot, Stem necrosis	Limón	12
Ornithogalum arabicum	Arabian startflower	1	Leaf	Soft rot	Alajuela	2
Phaseolus vulgaris	Bean	2	Leaf	Spot	Heredia	3
Solanum lycopersicum	Tomato	3	Fruit, Leaf	Fruit and leaf spots, Fruit soft rot	Alajuela, Cartago	6
Solanum tuberosum	Potato	1	Root	Soft rot	Cartago	1
Total		**46**				**116**

Identification of the Bacteria Collection by Analysis of the Ribosomal 16S RNA Gene. Each bacterium was inoculated into 3 mL of nutrient broth and incubated for 24-48 hours at 30 °C. Subsequently, the resulting growth was centrifuged for 5 minutes at 10,000 rpm to obtain biomass. DNA from this biomass was extracted following the protocol reported by Fontecha (2003). Amplification of the 16S rRNA gene was

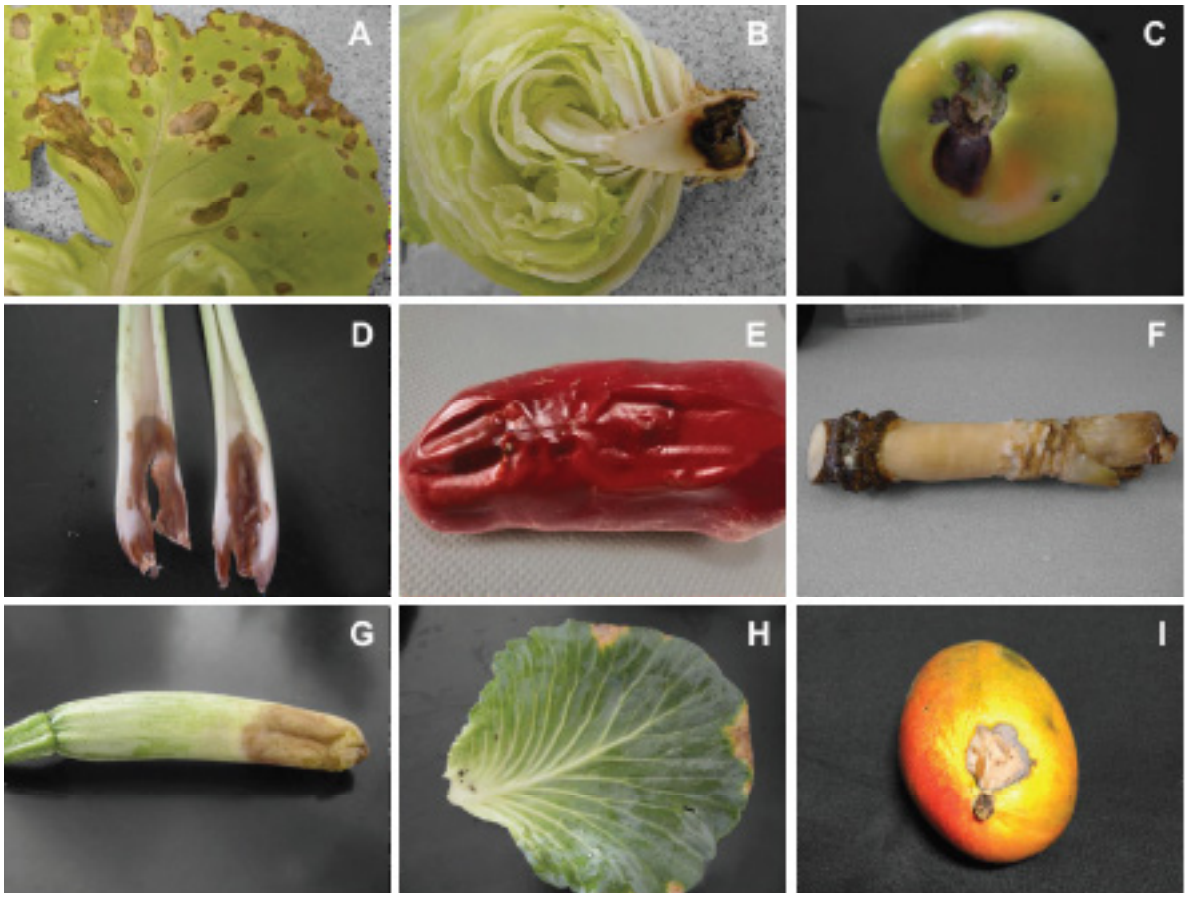

Figure 1. Symptoms of bacterial infection from which the bacterial collection were isolated. A. Leaf spot in Boston Lettuce, B. Soft rot in Iceberg Lettuce, C. Necrotic spot on Tomato. D. Soft rot in Celery. E. Soft rot in Pepper. F. Soft rot in Dracaena. G. Soft rot in Pumpkin. H. Angular necrosis in Cabbage. I. Fruit spot in Mango.

performed using universal primers: 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) (Weisburg *et al.,* 1991). Each PCR reaction was conducted in a 50 µL mixture containing 0.8 µL (200 µM) of each dNTPs (Thermo Scientific), 2.5 µL (1.25 mM) of MgCl2 (Thermo Scientific), 2.0 µL (0.4 µM) of each primer, 0.5 U of Taq DNA polymerase (Thermo Scientific), 5.0 µL of 1X KCl buffer without $MgCl_2$ (Thermo Scientific), and approximately 10 ng of genomic DNA. The reactions were amplified following the program and conditions reported by Fontecha (2003): an initial denaturation step at 94 °C for 4 minutes, followed by 34 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 90 seconds, with a final extension step at 72 °C for 10 minutes. The PCR products were visualized on a 1% agarose gel. Amplification products were purified using the Wizard PCR

Preps DNA Purification System kit (Promega, Madison, Wisconsin, United States) following the manufacturer's instructions. Sequencing was carried outusing an ABI PRISM 3130 sequencer with the BigDye® Terminator v3.1 Cycle Sequencing RR-100 kit (Applied BioSystems) or, alternatively, by Macrogen (Korea). The resulting sequences were assembled using BioEdit Sequence Alignment Editor Version 7.2 (Hall, 1999). Homology searches were performed using the NCBI BLASTN database (National Center for Biotechnology Information) to identify similarities with sequences deposited in GenBank (Altschul *et al.,* 1997) and the EzbioCloud extension (Yoon *et al.,* 2017), which stores 16S rRNA gene sequences from reference strains with valid taxonomic assignments.

Phylogenetic analysis. The sequences were aligned using ClustalX (Larkin *et al.,* 2007) within the MEGA program (Tamura *et al.,* 2013), and a phylogenetic analysis was conducted with sequences from reference-type strains and *Bacillus subtilis* (NR112116) as an outgroup. Distances were calculated using Neighbour Joining inference, and tree topology was assessed through 1,000 resamplings. The tree was visualized using the ITOL tool (https://itol.embl.de.com). The obtained sequences were deposited in the GenBank database of the NCBI under the bioproject PRJNA898399.

Identification of isolates by Biolog semi-automated system™. The Biolog™ system was employed for the identification of isolates that did not yield conclusive results in molecular identification. This system compares the redox reactions of 95 carbon sources and other substances with a database that includes environmental bacteria, including phytopathogens. To accomplish this, 24-hour bacterial cultures of each isolate were inoculated onto identification plates following the manufacturer's instructions. Readings were taken after 24 hours, and the closest identification according to the Biolog software was recorded.

Antibiotic Susceptibility Profile and Minimum Inhibitory Concentration (MIC) Determination. Antibiotic susceptibility to streptomycin, gentamicin, and tetracycline was assessed using the Kirby-Bauer agar diffusion method. We employed antibiotic impregnated mono-discs with standard quantities (Oxoid): 10 mg for streptomycin and gentamicin, and 30 ng for tetracycline. These discs were placed on cultures of each isolate on Muller-Hinton agar, and subsequently, the growth inhibition zone was measured (Sánchez and Guerrero, 2006). The measurements were compared with arbitrary unique cutoff points based on recommendations for clinically relevant bacteria within the taxonomic families described below: Tetracycline-resistant (≤ 14 mm), intermediate (15-18 mm), susceptible (≥19 mm). Gentamicin-resistant (≤ 12 mm), intermediate (13-14 mm),

susceptible (≥15 mm). Streptomycin-resistant (≤ 11 mm), intermediate (between 12-14 mm), susceptible (≥15 mm), which correspond to the breakpoints for *Pseudomonas aeruginosa, Acinetobacter* sp., and enterobacteria (NCCLS, 2000). *E. coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-15442) strains were used as controls. Isolates categorized as resistant or intermediate were subjected to minimum inhibitory concentration (MIC) determination for streptomycin, gentamicin, and oxytetracycline using the E-test epsilometric method (AB Biodisk, Solna, Sweden). This method involves a solid strip featuring a reading scale and an exponential gradient of antimicrobial, facilitating MIC determination across suitable concentrations (Alippi *et al.,* 2013). We followed the protocol reported by Lang and García (2004), using LB agar as the culture medium and incubating at 30 °C. After the incubation period, the minimum inhibitory concentrations were documented.

Phytopathogenicity-Antibiotic Resistance Relationship. To investigate the potential relationship between resistance to the three antibiotics and the phytopathogenicity of the analyzed bacteria, we utilized hypersensitivity reaction (HR) data (Table 2) previously obtained (Herrera, 2009). For this purpose, 24-hour suspensions of each isolate were inoculated onto *Nicotiana tabacum* leaves using the imprinting technique described by Trigiano *et al.* (2004). HR was graded from levels 0 to 5, with negative reactions ranging from levels 0 to 2 indicating either no reaction to minimal chlorosis without necrosis. Positive reactions were categorized from level 3, displaying intense chlorosis with necrosis, to level 4, featuring typical HR necrosis confined within vascular bundles or leaf veins, or necrosis extending beyond that area (level 5).

Results

Identification of the Bacterial Collection through 16S rRNA Gene Analysis and Phylogenetic Analysis. We successfully obtained 90 sequences with the necessary quality for molecular identification, and the remaining 26 isolates were identified using the Biolog™ System (Table 2). The 116 isolates were grouped into 20 bacterial genera, with the majority classified as *Pseudomonas* (48%), followed by *Serratia* (12%), *Pantoea* (9%), *Stenotrophomonas* (6%), and *Psychrobacter* (5%). The other 15 genera represented less than 5% of the total collection, with 10 of them having only one representative (Table 2). A total of 55 bacterial species were identified, with the majority falling within the Proteobacteria phylum (53 species), specifically in the Gammaproteobacteria class, with one isolate each from Alfaproteobacteria (*Ochrobactrum*) and Betaproteobacteria (*Achromobacter*). Additionally, one

Table 2. Molecular identification and resistance levels to streptomycin, tetracycline, and gentamicin of bacteria associated with infection symptoms in crops collected from 2006-2009 in Costa Rica.

Code	Host	Sint.[a]	Plant tissue	HR[b]	Identification (RNA 16S and Biolog™)	MIC[c] (µg mL^{-1})		
						EST	TET	GEN
MA-6	*Apium graveolens*	SR	Stem	0	*Pseudomonas protegens* CHA0^T	>1024*		
MA-7	*Apium graveolens*	SR	Stem	2	*Pseudomonas capeferrum* WCS358^T	512*	-	
MA-15	*Apium graveolens*	SR	Stem	1	*Pseudomonas arauntiaca* (Biolog)*	-		
MA-16	*Apium graveolens*	SR	Stem	2	*Serratia marcescens* ATCC 13880^T	512*		
MA-79	*Apium graveolens*	SR	Stem	2	*Pseudomonas capeferrum* WCS358^T	-		
MA-86	*Apium graveolens*	SR	Stem	2	*Pseudomonas koreensis* Ps 9-14^T	128*	24	
MA-50	*Bactris gasipaes*	N	Leaf	4	*P. stewartii* sp. *indologenes* LMG 2632^T			
MA-52	*Bactris gasipaes*	N	Leaf	5	*Stenotrophomonas maltophilia* ATCC 19861^T	12	12	
MA-53	*Bactris gasipaes*	N	Leaf	2	*Stenotrophomonas maltophilia* ATCC 19861^T		12	
MA-54	*Bactris gasipaes*	N	Leaf	5	*Stenotrophomonas maltophilia* ATCC 19861^T	12	24	256
MA-55	*Bactris gasipaes*	N	Leaf	2	*Providencia rettgeri* DSM 4542^T	12	-	
MA-65	*Bactris gasipaes*	N	Leaf	5	*P. stewartii* sp. *indologenes* LMG 2632^T	12	12	
MA-30	*Brassica oleracea var. botrytis*	S	Leaf	0	*Serratia marcescens* ATCC 13880^T			
MA-95	*Brassica oleracea var. botrytis*	S	Leaf	5	*Pseudomonas protegens* CHA0^T	-		
MA-110	*Brassica oleracea var. botrytis*	S	Leaf	2	*Stenotrophomonas maltophilia* (Biolog)	-		
MA-142	*Brassica oleracea var. botrytis*	S	Leaf	0	*Stenotrophomonas rhizophila* DSM14405	>1024		
MA-143	*Brassica oleracea var. botrytis*	S	Leaf	0	*Acinetobacter lactucae* NRRLB-41902^T	12*	-	32
MA-9	*Capsicum annuum*	SR	Fruit	2	*Pseudomonas maumuensis* COW77^T			
MA-10	*Capsicum annuum*	SR	Fruit	2	*Serratia marcescens* ATCC 13880^T	16*	>256	-
MA-11	*Capsicum annuum*	SR	Fruit	2	*Pantoea vagans* LMG 24199^T	-		
MA-14	*Capsicum annuum*	SR	Fruit	1	*Acinetobacter lactucae* NRRL B-41902^T			
MA-19	*Capsicum annuum*	SR	Fruit	5	*Pantoea anthophila* LMG 2558^T	>1024	-	
MA-33	*Capsicum annuum*	SR	Fruit	4	*Pseudomonas punonensis* CECT 8089	-	>256	
MA-90	*Capsicum annuum*	SR	Fruit	4	*Pseudomonas fragi* ATCC 4973^T			
MA-120	*Capsicum annuum*	SR	Fruit	4	*Raoultella terrigena* ATCC 33257^T		>256	
MA-140	*Capsicum annuum*	SR	Fruit	5	*Pseudomonas protegens* CHA0^T	-	-	12
MA-128	*Cucumis melo*	SR	Fruit	2	*Klebsiella pneumoniae* DSM 30104^T			
MA-129	*Cucumis melo*	SR	Fruit	1	*Pseudomonas* sp. (Biolog)		-	
MA-130	*Cucumis melo*	SR	Fruit	5	*Pseudomonas protegens* CHA0^T	12	12	
MA-131	*Cucumis melo*	SR	Fruit	1	*Pseudomonas protegens* CHA0^T		12	
MA-132	*Cucumis melo*	SR	Fruit	3	*Achromobacter marplatensis* LMG 26685^T	>1024	12	
MA-2	*Curcuma longa*	SR	Root	4	*E. cloacae* subsp. *dissolvens* LMG 2683^T	-	12	
MA-8	*Curcuma longa*	SR	Root	1	*Serratia marcescens* (Biolog)	-		
MA-38	*Curcuma longa*	SR	Root	0	*Enterobacter huaxiensis* 090008^T	-		
MA-61	*Curcuma longa*	SR	Root	2	*Pseudomonas fragi* ATCC 4973^T	>1024*		
MA-4	*Dracaena massangeana*	SR	Stem	2	*Psychrobacter alimentarius* JG-100^T	12		
MA-27	*Dracaena massangeana*	SR	Stem	1	*Pantoea vagans* LMG 24199^T			
MA-42	*Dracaena massangeana*	SR	Stem	2	*Pseudomonas putida* NBRC 3738^T	48*		
MA-43	*Dracaena massangeana*	SR	Stem	2	*Comamonas koreensis* KCTC 12005^T	12	-	
MA-88	*Dracaena massangeana*	SR	Stem	0	*Pseudomonas fluorescens* (Biolog)	-		
MA-89	*Dracaena massangeana*	SR	Stem	2	*Pantoea vagans* LMG 24199^T	-		
MA-136	*Dracaena massangeana*	SR	Stem	0	*Pantoea agglomerans* (Biolog)			
MA-59	*Ficus carica*	S	Leaf	0	*O. pseudogrignonensis* CCUG 30717^T	-	96	
MA-5	*Lactuca sativa* var. capitata	SR	Leaf	0	*Pseudomonas putida* (Biolog)			
MA-13A	*Lactuca sativa* var. capitata	SR	Stem	4	*Pectobacterium aroidearum* SCRI 109^T	12		
MA-13B	*Lactuca sativa* var. capitata	S	Leaf	4	*Pseudomonas capeferrum* WCS358^T	96		
MA-14C	*Lactuca sativa* var. capitata	SR	Stem	5	*Stenotrophomonas maltophilia* ATCC 19861^T	-		48

Table 2. Continue...

Code	Host	Sint.[a]	Plant tissue	HR[b]	Identification (RNA 16S and Biolog™)	MIC[c] (µg mL^{-1}) EST	TET	GEN
MA-17	*Lactuca sativa* var. capitata	SR	Leaf	1	*Pseudomonas azotoformans* LMG 21611^T			
MA-23A	*Lactuca sativa* var. capitata	S	Leaf	3	*Pantoea vagans* LMG 24199^T	12		
MA-224	*Lactuca sativa* var. capitata	S	Leaf	4	*Pseudomonas oryzihabitans* NBRC 102199^T	12		
MA-43A	*Lactuca sativa* var. capitata	SR	Leaf	4	*Raoultella terrigena* ATCC 33257^T	12		
MA-82	*Lactuca sativa* var. capitata	SR	Leaf	1	*Pseudomonas extremorientalis* KMM 3447^T	-	24	
MA-60	*Lactuca sativa* var. capitata	SR	Leaf	1	*Pseudomonas* sp. (Biolog)			
MA-84	*Lactuca sativa* var. capitata	SR	Leaf	2	*Pseudomonas putida* (Biolog)	-		
MA-40	*Lactuca sativa*	S	Leaf	1	*Pseudomonas fragi* ATCC 4973^T	32*		
MA-44	*Lactuca sativa*	S	Leaf	2	*Pseudomonas punonensis* CECT 8089^T	-	>256	-
MA-49	*Lactuca sativa*	S	Leaf	4	*Pseudomonas cichorii* ATCC10857^T			
MA-111	*Lactuca sativa*	S	Leaf	0	*Serratia marcescens* ATCC 13880^T	-	>256	
MA-112	*Lactuca sativa*	S	Leaf	2	*Pantoea vagans* LMG 24199^T	-	128	
MA-115	*Mangifera indica*	S	Fruit	2	*Erwinia billingiae* CIP 106121^T			
MA-124	*Mangifera indica*	S	Fruit	2	*Pseudomonas putida* NBRC 3738^T			
MA-125	*Mangifera indica*	S	Fruit	0	*Pseudomonas* sp. (Biolog)			
MA-127	*Mangifera indica*	S	Fruit	1	*Pseudomonas putida* (Biolog)			
MA-24	*Musa paradisiaca*	SR	Stem	1	*Pseudomonas* sp. (Biolog)			
MA-51	*Musa paradisiaca*	N	Stem	1	*Pseudomonas* sp. (Biolog)	-	12	
MA-68	*Musa paradisiaca*	SR	Stem	2	*Acinetobacter* sp. (Biolog)			
MA-72	*Musa paradisiaca*	SR	Stem	1	*Pseudomonas* sp. (Biolog)	-	-	
MA-77	*Musa paradisiaca*	N	Stem	2	*Aeromonas caviae* (Biolog)	-		12
MA-87	*Musa paradisiaca*	SR	Stem	0	*Serratia marcescens* ATCC 13880^T	>1024	>256	
MA-97	*Musa paradisiaca*	SR	Stem	2	*Pseudomonas* sp. (Biolog)	-	12	
MA-101	*Musa paradisiaca*	SR	Stem	2	*Pseudomonas protegens* CHA0^T		12	
MA-117	*Musa paradisiaca*	SR	Stem	1	*Achromobacter spanius* LMG 5911^T	-		
MA-118	*Musa paradisiaca*	SR	Stem	4	*Pseudomonas protegens* CHA0^T	-	>256	-
MA-119	*Musa paradisiaca*	SR	Stem	1	*Pseudomonas putida* NBRC 3738^T	12	-	
MA-139	*Musa paradisiaca*	N	Stem	1	*Pseudomonas* sp. (Biolog)	-	-	-
MA-1	*Ornithogalum arabicum*	SR	Leaf	0	*Psycrobacter faecalis* Iso-46^T	-		
MA-13	*Ornithogalum arabicum*	SR	Leaf	1	*Pseudomonas putida* NBRC 3738^T	24*	-	-
MA-37	*Phaseolus vulgaris*	S	Leaf	4	*Pseudomonas syringae* ATCC 19304^T	24*		
MA-70	*Phaseolus vulgaris*	S	Leaf	5	*Pseudomonas syringae* ATCC 19304^T			
MA-81	*Phaseolus vulgaris*	S	Leaf	1	*Pseudomonas azotoformans* DSM 18862^T	-		
MA-12	*Solanum tuberosum*	SR	Root	0	*Psychrobacter aquaticus* CMS 56^T	-		
MA-18	*Brassica oleracea* var. capitata	SR	Leaf	3	*Sphingobacterium kitahiroshimense* 10C^T	12	-	256
MA-22	*Brassica oleracea* var. capitata	N	Leaf	2	*Psychrobacter pulmonis* CECT 5989^T			
MA-23	*Brassica oleracea* var. capitata	S	Leaf	2	*Pseudomonas* sp. (Biolog)			
MA-26	*Brassica oleracea* var. capitata	N	Leaf	1	*Pseudomonas moraviensis* CCM 7280^T	-		
MA-28	*Brassica oleracea* var. capitata	N	Leaf	0	*Serratia marcescens* ATCC 13880^T	-	>256	
MA-29	*Brassica oleracea* var. capitata	N	Leaf	0	*Serratia marcescens* (Biolog)	-	>256	
MA-32	*Brassica oleracea* var. capitata	SR	Leaf	1	*Pantoea vagans* LMG 24199^T	-	-	256
MA-34	*Brassica oleracea* var. capitata	N	Leaf	1	*Pseudomonas protegens* CHA0^T	48*		24
MA-35	*Brassica oleracea* var. capitata	S	Leaf	1	*Serratia marcescens* ATCC 13880^T		>256	
MA-36	*Brassica oleracea* var. capitata	SR	Leaf	5	*Pantoea vagans* LMG 24199^T			
MA-39	*Brassica oleracea* var. capitata	N	Leaf	1	*Psycrobacter faecalis* Iso-46^T			
MA-41	*Brassica oleracea* var. capitata	N	Leaf	2	*Pseudomonas capeferrum* WCS358^T	>1024*	-	-
MA-46	*Brassica oleracea* var. capitata	S	Leaf	0	*Pseudomonas saponiphila* DSM 975^T			
MA-48	*Brassica oleracea* var. capitata	S	Leaf	0	*Pseudomonas laurylsulfativorans* AP3_22^T	-	-	

Table 2. Continue...

Code	Host	Sint.[a]	Plant tissue	HR[b]	Identification (RNA 16S and Biolog™)	MIC[c] (µg mL^{-1}) EST	TET	GEN
MA-57	*Brassica oleracea* var. capitata	S	Leaf	1	*Serratia marcescens* ATCC 13880^T	-	>256	
MA-76	*Brassica oleracea* var. capitata	S	Leaf	1	*Serratia marcescens* (Biolog)	-		
MA-85	*Brassica oleracea* var. capitata	S	Leaf	4	*Pseudomonas protegens* CHA0^T	-		
MA-91	*Brassica oleracea* var. capitata	N	Leaf	4	*Psychrobacter pulmonis* CECT 5989^T		24	
MA-96	*Brassica oleracea* var. capitata	S	Leaf	2	*Pseudomonas hunanensis* LV^T	-	18	
MA-98	*Brassica oleracea* var. capitata	N	Leaf	2	*Carnobacterium inhibens* DSM 13024^T	-	>256	
MA-103	*Brassica oleracea* var. capitata	S	Leaf	0	*Serratia marcescens* ATCC 13880^T	32*		16
MA-104	*Brassica oleracea* var. capitata	S	Leaf	4	*Escherichia hermannii* CIP 103176^T		-	>1024
MA-105	*Brassica oleracea* var. capitata	S	Leaf	1	*Pseudomonas fluorescens* (Biolog)	24*		
MA-106	*Brassica oleracea* var. capitata	N	Leaf	1	*Acinetobacter johnsonii* CIP 64.6^T	-	>256	
MA-107	*Brassica oleracea* var. capitata	S	Leaf	3	*Stenotrophomonas rhizophila* DSM14405^T		12	
MA-31	*Solanum lycopersicum*	S	Leaf	1	*Pseudomonas fulva* (Biolog)	>1024*		
MA-56	*Solanum lycopersicum*	N	Fruit	2	*Pseudomonas fulva* 12-X^T	>1024*		
MA-74	*Solanum lycopersicum*	SR	Fruit	3	*Dickeya oryzae* ZYY5^T	>1024		
MA-83	*Solanum lycopersicum*	N	Fruit	1	*Pseudomonas sp.* (Biolog)	>1024*		
MA-113	*Solanum lycopersicum*	S	Leaf	3	*Serratia marcescens* ATCC 13880^T	-	-	-
MA-141	*Solanum lycopersicum*	S	Leaf	2	*Serratia marcescens* ATCC 13880^T	-	>256	-
MA-3	*Daucus carota*	SR	Root	1	*Pseudomonas azotoformans* LMG 21611^T	32*		
MA-20	*Cucurbita pepo*	SR	Fruit	0	*Klebsiella michiganensis* W14^T	48		
MA-75	*Cucurbita pepo*	SR	Fruit	1	*Pseudomonas corrugata* (Biolog)	-*		
MA-80	*Cucurbita pepo*	SR	Fruit	2	*Pseudomonas protegens* CHA0^T	16*		

[a] Symptoms. Type of lesion in plant tissue from which the bacterium was isolated. S: Spot, N: Necrosis, SR: Soft Rot

[b] RH: Hypersensitive Reaction. Data taken from Herrera (2009)

[c] Determination of Minimum Inhibitory Concentration using E-Test (Solna). EST: Streptomycin, TET: Tetracycline, GEN: Gentamicin

* Data taken from Méndez (2010)

isolate was obtained from the Bacteroidetes phylum (*Sphingobacterium*) and one from Firmicutes (*Carnobacterium*).

With the exception of samples of *Ficus carica* (fig), *Bactris gasipaes* (palm heart), and *Solanum tuberosum* (potato), *Pseudomonas* isolates were recovered from all other analyzed crops (Table 2), and originated from all sites except one. Among this isolates, recognized plant pathogens such as *P. syringae*, *P. cichorii*, *P. corrugata*, and *P. orizihabitans* were identified, along with 18 other environmentally-derived species. Isolates of *Serratia marcescens* were recovered from samples of *Apium graveolens* (celery), *Musa paradisiaca* (banana), *Capsicum annuum* (bell pepper), *Brassica oleracea* var. botrytis (cauliflower), *Curcuma longa* (turmeric), *Lactuca sativa* (Boston lettuce), *Brassica oleracea* var. capitata (cabbage), and *Solanum lycopersicum* (tomato), from the provinces of Cartago, Limón, Heredia, and Guanacaste. Meanwhile, *Pantoea*, including pathogenic species like *P. anthophila*, *P. stewartii*, *P. agglomerans*, and *P. vagans*, were obtained from various crops such as sweet pepper, Iceberg and Boston lettuce, cabbage, palm heart, and *Dracaena massangeana* (dracaena), also in the same provinces. *Stenotrophomonas* isolates, the next most abundant genus in the collection, were found in palm heart, cauliflower, cabbage, and lettuce in the provinces of Cartago, Limón, and Heredia, with most of these strains classified as *S. malthophilia* (Table 2). Additionally, Gram-negative phytopathogens such as *Dickeya oryzae*, *Enterobacter cloacae* subsp. dissolvens, and *Pectobacterium aroidearum* were identified in tomato, turmeric, and Boston lettuce samples, respectively. Phylogenetic relationships between the sequences of the isolates and reference sequences obtained from curated databases such as EzbioCloud (Yoon *et al.,* 2017) are presented in Figure 2.

Antibiotic Susceptibility Profile and Minimum Inhibitory Concentration (MIC) Determination Profile. For the purposes of this study, isolates classified as resistant or intermediate by the Kirby-Bauer test, which also presented a MIC $\geq$ 12 ng mL^{-1} (Figure 3) (Miernik and Rzeczycka, 2007; Rodríguez *et al.,* 2008), were considered resistant. This concentration was chosen based on the CLSI (2017) recommendations for enteric, non-enteric, and anaerobic human pathogens, as it is higher than the intermediate MIC for these bacterial groups. According to this classification, bacteria resistant to the studied antibiotics were observed in samples from all crops except mango and potato (Table 3). The highest proportion of resistant isolates was found in tomato (83.3%), heart of palm (83.3%), and Boston lettuce (80%) samples (Figure 4). Isolates obtained from cabbage, banana, bell pepper, and Iceberg lettuce exhibited the same resistance phenotypes to streptomycin, tetracycline, and gentamicin, including multiple resistances such as streptomycin-tetracycline (Estr-Tet) (bell pepper and banana) and streptomycin-gentamicin (Str-Gent) in cabbage. In the case of heart of palm, the highest number of bacteria

Figure 2. Cladogram constructed using the nearest neighbor method from 70 partial sequences of the 16S rRNA gene of bacteria isolated from plant lesions and sequences of reference strains. The tree topology was assessed through 1,000 resamplings, with the sequence of *Bacillus subtilis* used as an outgroup. Symbols on the outer part of the tree indicate isolates classified as resistant to Streptomycin, Tetracycline, and Gentamicin, as well as their combinations (circles), and those with a positive Hypersensitive Reaction (triangles).

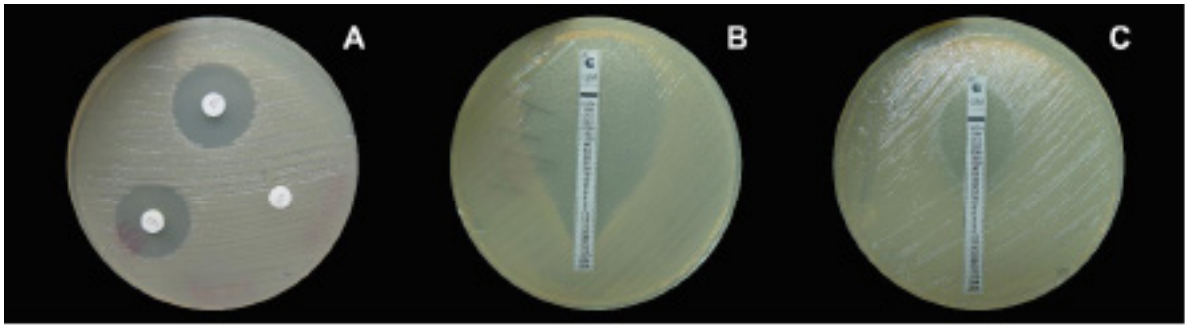

Figure 3. A. Halos indicating sensitivity to antibiotics on Oxoid discs for gentamicin and tetracycline, and resistance to streptomycin (absence of a halo) in the Kirby Bauer disk diffusion test. B. Bacteria with a minimum inhibitory concentration (MIC) of 0.25 µg mL^{-1} for gentamicin, determined by the E-test method. C. Bacteria with a MIC of 32 µg mL^{-1} for the same antibiotic.

Table 3. Bacterial genera identified and the frequency of bacteria resistant to the antibiotics Streptomycin (Strept), Tetracycline (Tetra), and Gentamicin (Gent).

Phylum	Class	Genus	Nº isolates	Isolates with MIC (E-test) ≥ 12 µg mL^{-1}						Nº (%) of resistance
				Estr	Tet	Gent	Estr-Tet	Estr-Gent	Estr-Tet-Gent	
Proteobacteria	Alfaproteobacteria	*Ochrobactrum*	1	0	1	0	0	0	0	1 (100.0 %)
Proteobacteria	Betaproteobacteria	*Achromobacter*	2	0	0	0	1	0	0	1 (100.0 %)
Proteobacteria	Gamaproteobacteria	*Acinetobacter*	4	0	1	0	0	1	0	2 (50.0 %)
Proteobacteria	Gamaproteobacteria	*Aeromonas*	1	0	0	1	0	0	0	1 (100.0 %)
Proteobacteria	Betaproteobacteria	*Comamonas*	1	1	0	0	0	0	0	1 (100.0 %)
Proteobacteria	Gamaproteobacteria	*Dickeya*	1	1	0	0	0	0	0	1 (100.0 %)
Proteobacteria	Gamaproteobacteria	*Enterobacter*	2	0	1	0	0	0	0	1 (50.0 %)
Proteobacteria	Gamaproteobacteria	*Erwinia*	1	0	0	0	0	0	0	0
Proteobacteria	Gamaproteobacteria	*Escherichia*	1	0	0	1	0	0	0	1 (100 %)
Proteobacteria	Gamaproteobacteria	*Klebsiella*	2	1	0	0	0	0	0	1 (50.0 %)
Proteobacteria	Gamaproteobacteria	*Pantoea*	11	2	1	1	1	0	0	5 (45.0 %)
Proteobacteria	Gamaproteobacteria	*Pectobacterium*	1	1	0	0	0	0	0	1 (100 %)
Proteobacteria	Gamaproteobacteria	*Providencia*	1	1	0	0	0	0	0	1 (100 %)
Proteobacteria	Gamaproteobacteria	*Pseudomonas*	56	17	8	1	3	1	0	30 (53.6 %)
Proteobacteria	Gamaproteobacteria	*Psychrobacter*	6	1	1	0	0	0	0	2 (33.3 %)
Proteobacteria	Gamaproteobacteria	*Raoultella*	2	1	1	0	0	0	0	2 (100.0 %)
Proteobacteria	Gamaproteobacteria	*Serratia*	14	1	6	0	2	1	0	10 (71.4 %)
Proteobacteria	Gamaproteobacteria	*Stenotrophomonas*	7	1	2	1	1	0	1	6 (85.7 %)
Bacteroidetes	Sphingobacteriia	*Sphingobacterium*	1	0	0	0	0	1	0	1 (100 %)
Firmicutes	Bacilli	*Carnobacterium*	1	0	1	0	0	0	0	1 (100 %)
	TOTAL		**116**	**28**	**23**	5	8	4	**1**	**69**

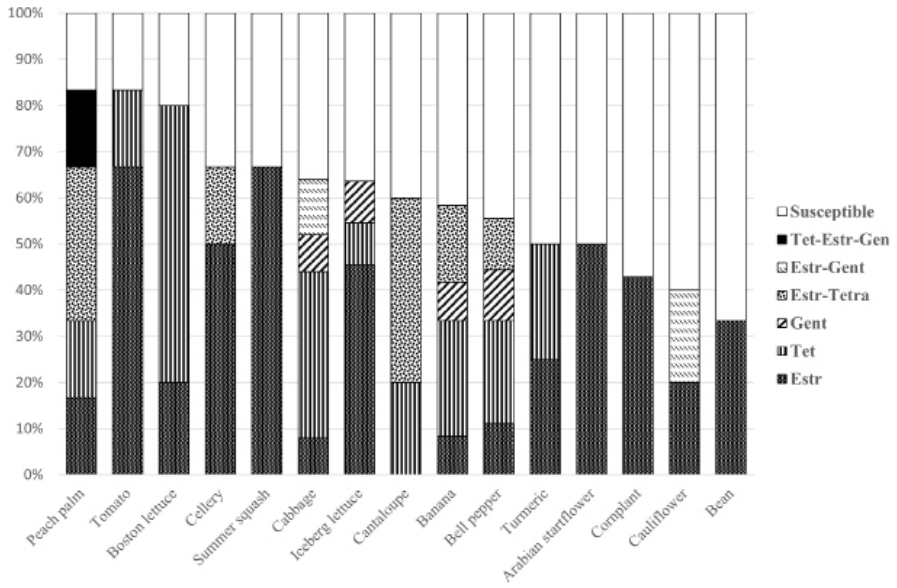

Figure 4. Proportion of bacteria resistant (MIC ≥ 12 µg mL^{-1}) to the antibiotics Streptomycin (Str), Tetracycline (Tet), and Gentamicin (Gent), as well as their combinations, in the analyzed hosts that had more than one isolation.

with multiple resistance to Estr-Tet and Estr-Tet-Gent was isolated (Table 3, Figure 4). Another crop that showed bacteria resistant to all three analyzed antibiotics was Iceberg lettuce, with samples coming from different farms in the province of Cartago. Multiple resistance was also observed in cauliflower (Estr-Gent), celery, and melon (Estr-Tet), while crops such as tomato, Boston lettuce, and turmeric had isolates resistant to streptomycin and tetracycline. Resistance to streptomycin was found in bacteria isolated from all crops except fig. For this antibiotic, all isolates from tomato and turmeric had a maximum MIC of >1024 µg mL^{-1}, those from celery had an MIC of 128 to >1024 µg mL^{-1}, and in the case of cabbage, banana, melon, and cauliflower, it ranged from 12 to >1024 µg mL^{-1}, sweet pepper from 16 to >1024 µg mL^{-1}, and Iceberg lettuce from 12 to 96 µg mL^{-1}. MICs between 12 and 48 µg mL^{-1} were observed in other crops isolates. Tetracycline resistance was detected in bacteria from 10 of the 19 crops, and isolates from cabbage, banana, bell pepper, Boston lettuce, and carrot had a maximum MIC of >256 µg mL^{-1}. Although less frequent, gentamicin-resistant isolates had MICs of 256 in heart of palm, 16 to 256 in cabbage, and 12 to 48 µg mL^{-1} in banana, bell pepper, and cauliflower (Table 2). Among the genera with a higher number of resistant isolates were *Pseudomonas*, *Serratia*, *Pantoea*, and *Stenotrophomonas*, all of which included phytopathogenic and saprophytic members characterized by molecular identification and hypersensitivity reaction (Figure 2 and 5).

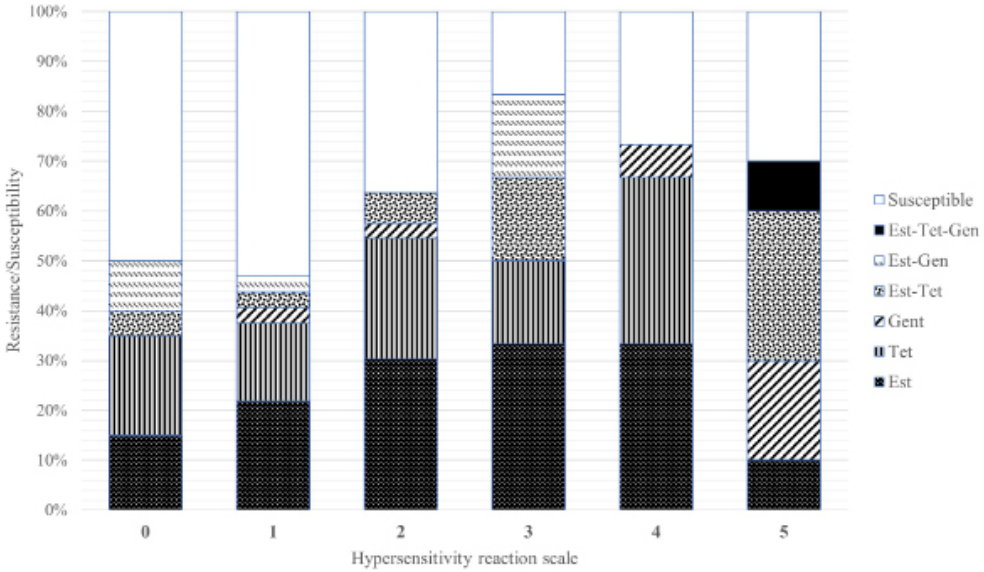

Figure 5. Types of Hypersensitivity Reactions (HR) in the most frequently observed bacterial genera in the analized crop samples. Isolates with an HR ranging from 3 to 5 are considered to be phytopathogenic bacteria (Herrera, 2009). HR levels from 0 to 2 are grouped together and indicate non-pathogenicity.

Out of the 19 crops sampled, *Pseudomonas* genus comprised 56 isolates identified in 16 of them. Among these, 30 had a MIC greater than 12 µg mL^{-1} (Table 3), and were considered resistant. Five of the six studied resistance phenotypes were observed, with streptomycin resistance being the most prevalent, ranging from 12 to >1024 µg mL^{-1}, with 65% of the isolates having a MIC greater than 32 µg mL^{-1}, including four bacteria with maximum MIC levels (>1024 µg mL^{-1}). Regarding tetracycline resistance, three out of the 11 resistant isolates showed a maximum MIC of >256 µg mL^{-1}. Only two bacteria exhibited gentamicin resistance with MICs ranging from 12 to 24 µg mL^{-1} (Table 2).

Ten out of 14 isolates identified as *Serratia* displayed resistance (Table 3), originating from six out of eight crops from which isolates were obtained (cabbage, tomato, bell pepper, Boston lettuce, celery, and banana). Commonly observed resistance was to tetracycline, with eight resistant isolates having the maximum MIC for this antibiotic. Additionally, three strains showed resistance to streptomycin (MIC ranging from 12 to 512 µg mL^{-1}), and one to gentamicin (16 µg mL^{-1}). Bacteria classified as *Stenotrophomonas* exhibited MICs ranging from 12 to >1024 µg mL^{-1} for streptomycin, between 12 and 24 µg mL-1 for tetracycline, and from 48 to >256 µg mL^{-1} for gentamicin, with only one isolate (MA-110) being susceptible to all three antibiotics. Among the 11 bacteria identified in the *Pantoea* genus, five presented resistance, with maximum values observed only for streptomycin in the case of MA-19 (bell pepper) (Table 2, 3).

Phytopathogenicity-Antibiotic Resistance Relationship. According to Herrera's reported hypersensitivity reaction data (2009), 73% of the bacteria in this collection were classified as non-plant pathogens or RH negative, displaying no chlorosis or necrosis around the inoculation point (levels 0 to 2). When comparing the taxonomy of the isolates with this classification, a higher proportion of isolates with a positive RH (levels 3 to 5) was observed in the genera *Stenotrophomonas* (57.2%), *Pantoea* (45.5%), *Pseudomonas* (21.4%), and *Serratia* (7.1%). However, some of the species that exhibited this reaction were not recognized as plant pathogenic but rather environmental species, such as *P. protegens*, *P. fragi*, and *P. punonensis* (Table 2).

When comparing the degree of pathogenicity rating with the number of identified resistant strains in each RH category (Figure 6), it was observed that 45% of the population with RH negative exhibited resistance to some of the studied antibiotics. However, this proportion was lower than that found in pathogenic isolates (RH+), where resistant bacteria represented 70 to 83%, corresponding to levels 3 and 5, respectively. Resistance to all three antibiotics was detected at all levels of phytopathogenicity, and a bacterium with RH 5 (*Stenotrophomonas malthopilia* MA-54) was the only one found to be resistant to all three. The highest level of pathogenicity exhibited a higher proportion of bacteria with multiple resistance.

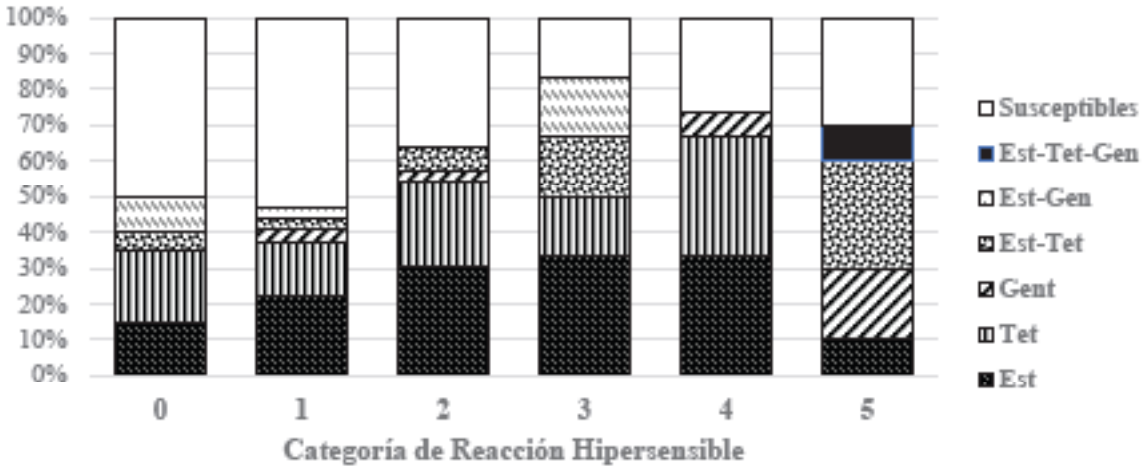

Figure 6. Proportion of strains resistant to the studied antibiotics according to the HR scale (levels 0 to 5) as determined by Herrera (2009). The Y-axis shows the percentage of susceptible isolates within each category.

Discussion

The diversity of symptoms related to plant diseases of bacterial origin increases the difficulty of management in agricultural systems (Aráuz, 2011). Furthermore,

some symptoms such as fruit and leaf spots and soft rot are shared by different pathogens, making laboratory identification necessary using various methodologies, as early and accurate identification allows for better disease control (Kannan *et al.,* 2022).

On the other hand, the aboveground part of plants is predominantly colonized by a diverse bacterial community, both in the form of epiphytes on the plant surface and endophytes in plant tissues. While some plant-associated bacteria promote plant growth, others can be plant or even human pathogens (Lindow and Brandl, 2003; Jackson *et al.,* 2013). In this regard, Jackson et al. (2013), when analyzing the bacterial community composition of leafy vegetables through pyrosequencing, identified eleven different phyla, where Gamaproteobacteria, Betaproteobacteria, and Bacteroidetes were the dominant lineages. These phyla align with what was observed in this study, where the same groups of Proteobacteria were found, in addition to Alphaproteobacteria.

Among the identified genera, *Pseudomonas*, *Serratia*, *Pantoea*, *Stenotrophomonas*, *Klebsiella*, and *Enterobacter* are Gram-negative bacteria that have plant pathogenic species. It is important to remember that these isolates come from the edge of the lesion area, and due to the isolation process in which the sample is disinfested with hypochlorite before dissection, endophytic and opportunistic bacteria can also be isolated. To discriminate plant pathogens in this collection, Herrera (2009) used the hypersensitive reaction (HR) technique on the indicator plant *Nicotiana tabacum*. This technique, entails inoculating bacteria on the undersides of plant leaves to assess their phytopathogenic capacity (Zurbriggen *et al.,* 2010). This method is characterized by cell death near the pathogen recognition site and the development of delimited chlorosis and necrosis in the leaves (Bellincampi *et al.,* 2014). In this study, the most abundant genera comprised isolates classified as both phytopathogens and saprophytic representatives.

The diversity of *Pseudomonas* species in the soil ecosystem influences both plant growth and their pathogenicity (Kumar *et al.,* 2017). This is due to their metabolic versatility and genetic adaptability, and this characteristics may explain why we isolated *Pseudomonas* from 16 out of the 19 crops analyzed. *P. syringae*, the species that tops Mansfield et al.'s (2012) list of the top 10 bacterial plant pathogens, was identified in this study, along with *P. cichorii*, *P. corrugata*, and *P. orizihabitants* (Höfte and De Vos, 2007; Pauwelyn *et al.,* 2013; Li *et al.,* 2021). While other *Pseudomonas* species not identified in this collection have been reported to exhibit natural resistance to aminoglycosides (Krahn *et al.,* 2012), a wide range of MIC values was found for streptomycin, with over half of the isolates having MIC values greater than 32 µg mL^{-1} for streptomycin and most being susceptible to gentamicin. Given the ubiquity of this genus in different environments and its use as a biofertilizer and biocontroller, it is important to investigate whether the

resistance-conferring genes are present in the chromosome or in horizontal gene transfer determinants in *Pseudomonas* isolates.

Serratia marcescens is a genetically variable bacterial species found in different environments, including water, soil, plants, and as an opportunistic pathogen in humans and animals (Besler and Little, 2017). This species has been associated with nosocomial infections in the medical field as well as diseases in plants. For example, strains of *S. marcescens* have been identified as the causative agents of cucurbit yellow vine disease and soft rot in bell peppers (Zhang *et al.,* 2005; Gillis *et al.,* 2017). In this study, 14 *S. marcescens* isolates from different hosts showed high similarity to the type strain *S. marcescens* ATCC 13880(T) sequence, which was isolated from a wastewater treatment tank. Therefore, its role in the host plant needs to be studied in more detail. This species had a CMI $\geq$ 12 µg mL^{-1} in 10 out of 13 isolates, and its resistance level was maximum for tetracycline and within a wide range for streptomycin, with only one isolate being resistant to gentamicin. These findings align with a study conducted in Costa Rica where *Serratia* isolates obtained from soil treated with gentamicin and tetracycline were tetracycline-resistant and gentamicin-sensitive (Rodríguez *et al.,* 2007). Despite *S. marcescens* exhibiting natural resistance to aminoglycosides (Sandner-Miranda *et al.,* 2018), most of the isolates analyzed in this study were susceptible to streptomycin and gentamicin, and some showed maximum tetracycline MIC values, necessitating further investigation of their resistance determinants.

The genus *Pantoea* encompasses bacteria with various roles in plants, including their roles as phytopathogens, endophytes, and epiphytes (Doni *et al.,* 2021). Among plant pathogens, *P. citrea*, *P. ananas*, *P. eucalypti*, *P. stewartii*, *P. agglomerans*, *P. vagans*, and *P. antophila* have been documented (Schaad *et al.,* 2001; Brady *et al.,* 2009), with the latter four being identified in this study. In particular, *Pantoea stewartii* sp. *indologenes* MA-65, isolated from lesions on palm leaflets, is the causal agent of palm bacterial wilt, which affected plantations in Costa Rica in the 2000s (Mora-Urpí *et al.,* 2008). In this study, isolates classified as pathogens through phylogenetic and phytopathogenicity analyses exhibited resistance to streptomycin and tetracycline.

Species within the genus *Stenotrophomonas* include endophytic representatives and are used as biocontrol agents in sustainable agriculture (Berg and Martinez, 2015). *S. maltophilia* is considered an emerging opportunistic pathogen in clinical settings, with natural resistance to aminoglycosides, albeit to a lesser extent for gentamicin (Antón *et al.,* 2005; Berg and Martinez, 2015). A study conducted in Costa Rica found that *Stenotrophomonas* sp. isolates from lettuce plants exhibited resistance to various antibiotics, including tetracycline and gentamicin (Rodríguez *et al.,* 2007). In this work, the majority of the isolates showed resistance to the antibiotics tested, with MIC values ranging between 12-24 µg mL^{-1} for tetracycline

and streptomycin, except for MA-142, which had a maximum MIC (>1024 µg mL^{-1}) for streptomycin and gentamicin. Among the resistant isolates are bacteria with a high level of pathogenicity, recovered from palm leaflets (Table 2). Additionally, isolate MA-54 was the only one in the collection resistant to all three antibiotics studied (Table 2). Given their intrinsic resistance, a detailed analysis of resistance determinants in *S. maltophilia* isolates, particularly regarding streptomycin and tetracycline, is important.

Most of the phylogenetically identified and RH-tested phytopathogenic bacteria have not been described in Costa Rica, except for the isolates of *Erwinia billingiae* MA-115 and *Raoultella terrigena*. These isolates were subjected to pathogenicity tests in mango and bell pepper plants, respectively (the crops from which they were isolated), and upon proving their virulence using Koch's Postulates, they were described as phytopathogens (Vidaurre-Barahona *et al.,* 2021; Cubero *et al.,* 2021). In this diverse collection, bacteria with positive RH, considered plant pathogens, exhibited a higher proportion of resistance (Figure 4). This could be due to their adaptation to survive on the plant surfaces and in plant tissues, where they are more exposed to antimicrobials applied to the leaves (Stockwell and Duffy, 2012).

The resistance observed in 60% of the isolates to the antibiotics studied may result from the use of products in the crops from which the bacteria were obtained. It is important to consider that the 12 µg mL^{-1} threshold used to define resistance in this study surpass the MIC for susceptible bacteria but falls below that for resistant bacteria in human setting. However, this criterion has been used in previous studies in different environments, as environmental microorganisms are considered to be exposed to much lower concentrations than clinical bacteria, but they can also develop resistance in natural settings (Popowska *et al.,* 2012; Sandegren, 2014; Nogrado *et al.,* 2021). The lower number of bacteria resistant to gentamicin observed (10 out of 116) may result from less frequent use of this antibiotic or intrinsic factors related to the molecule and its persistence in environmental conditions.

The presence of resistant bacteria in 90% of the analyzed hosts, which constitute a diverse set of plants, raises concerns regarding the current status of antibiotic resistance, as these isolates were collected between 2006 and 2009. Crops with a higher prevalence of resistant bacteria and phenotypes resistant to the three studied antibiotics included cabbage, banana, Iceberg lettuce, palm heart, and bell pepper. This could be associated with more aggressive management practices to combat bacterial diseases affecting these crops. For instance, cabbage may face angular leaf spot caused by *Xanthomonas campestris*, banana may be susceptible to wilt (*Ralstonia solanacearum*), lettuce could suffer from leaf spots caused by *Pseudomonas* species, palm heart may be affected by bacterial wilt (*P. stewartii*), and various vegetables in tropical climates may experience soft rot (Bhat *et al.,* 2010). The high proportion of resistant bacteria identified in 16 out of 19 crops

from diverse regions of the country, may result from the widespread agricultural use of antibiotics such as tetracycline, streptomycin, and gentamicin without adequate regulation, dosing, and record-keeping, a common phenomenon in other Latin American countries as well. These findings provide a foundation for investigating the evolution of antimicrobial resistance in both phytopathogenic and non-pathogenic bacteria present in vegetables destined for human consumption in Costa Rica. It underscores the necessity for more comprehensive studies due to their impact on disease control in crops and associated microbial communities.

Conclusions

The analysis of 116 isolates from a collection derived from symptoms of soft rot, necrosis, and leaf, stem, and fruit spots across 19 crops revealed the presence of 20 genera, with *Pseudomonas* (48%), *Serratia* (12%), *Pantoea* (9%), and *Stenotrophomonas* (6%) being the most abundant. *Pseudomonas* had the highest number of species and isolates among them. Among the phytopathogenic bacteria in the collection, *Pseudomonas syringae*, *P. cichorii*, *P. corrugata*, *Pantoea stewartii* subsp. *indologenens*, *P. anthophila*, *Dickeya oryzae*, *Enterobacter cloacae* subsp. *dissolvens*, and *Pectobacterium aroidearum* were identified, accounting for 23% of isolates positive for the hypersensitive reaction (HR). In this study, resistance to at least one of the three antibiotics was detected in 60% of the evaluated isolates, with streptomycin resistance being the most common. Resistance was observed in bacteria isolated from 17 plant species, with tomatoes, palm hearts, lettuce, celery, squash, and cabbage showing the highest proportion of resistant isolates. This suggests a greater risk of selecting antibiotic-resistant bacteria in the production of these foods. The information obtained highlights the necessity for stricter regulation in the sale and usege of antimicrobial products to mitigate their impact on the environment, animals, and humans.

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