

Research Note

SEED-BORNE ENDOPHYTIC AND PATHOGENIC BACTERIA ISOLATED FROM ONION IN PUERTO RICO^{1,2}

Myrna Alameda³ and Lydia I. Rivera-Vargas³

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Onion is an important crop grown in southern Puerto Rico. Previous studies have reported seventeen bacterial genera isolated from foliar lesions in commercial onion fields on the island (Calle-Bellido et al., 2006). Pathogenicity test showed that *Acidovorax avenae* subsp. *citrulli*, *Burkholderia glumae*, *Pantoea agglomerans*, *Pantoea dispersa*, *Pseudomonas* sp., *Xanthomonas* sp. and *Xanthomonas*-like bacteria caused foliar lesions (Calle-Bellido, 2005). Contaminated seeds and plant tissue could be the primary source of bacteria inoculum and dissemination. *Pantoea ananatis* responsible for leaf and seed stalk necrosis is seed-borne and seed-transmitted in onions. This pathogen was introduced into the United States in infected seed lots from South Africa (Goszczyńska et al., 2006). *Pantoea ananatis*, *P. agglomerans*, *Xanthomonas campestris*, and *Pseudomonas viridiflava* were also detected in onion seeds in Venezuela (Rodríguez and Contreras, 2006). *Xanthomonas axonopodis* pv. *allii* has been reported in onion seed worldwide (Roumagnac et al., 2004) and *Erwinia carotovora* var. *carotovora* was isolated in New Zealand (Sendhilvel and Marimuthu, 2007). *Bacillus* spp. were isolated from endophytic tissues and extensively studied as a biocontrol agent (Bacon and Hinton, 2002). The purpose of this study was to isolate and identify bacterial microflora from onion seeds and to relate these species to symptoms observed in onion foliage under field conditions.

In this study, two grams of seeds from three different onion cultivars (i.e., Candy, Excalibur, and Mercedes) were surface-disinfested (two minutes with 70% ethyl alcohol, two minutes with 5% sodium hypochlorite, and rinsed five times with sterile distilled water), and ground in a blender for 30 min in 50 ml of a 0.1 M phosphate buffer. The suspension was serial-diluted and plated on Tryptic Soy Agar (TSA). Seventy-four bacterial colonies were randomly selected and characterized by using the Biolog MicroStation® System⁴.

Fifteen different pathogenic endophytic plant growth promoters and biocontrol species were isolated and identified (Table 1). Estimated bacterial colony counts were 14×10^2 , 8.5×10^1 and 10 cfu/g for onion cultivars Excalibur, Candy and Mercedes, respectively. The greatest bacterial diversity was observed in Excalibur; 66.6% of these isolates were gram-negative. *Enterobacter cloacae* was the most frequently isolated species (51.8%) and the corn pathogen, *Pantoea stewartii* subsp. *stewartii*, was isolated only from Excalibur (7.4%). *Pantoea stewartii* transmission has been reported from corn seeds in Iowa, USA, (Block et al., 1998). *Pantoea agglomerans* (69.5%) and *P. dispersa* (17.3%) were isolated only from onion cv

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³Professors, Department of Crops and Agroenvironmental Sciences, College of Agricultural Sciences, University of Puerto Rico-Ma yagüez Campus, P.O. Box 9000, Mayagüez, Puerto Rico 00681. e-mail: myrna.alameda@upr.edu; lydia.i.rivera@upr.edu

⁴Biolog MicroStation® System was mentioned to provide specific information and do not constitute a warranty by the University of Puerto Rico, nor is this mention a statement of preference over other identification services.

TABLE 1.—*Bacterial identification from onion seeds of cultivars Candy, Excalibur and Mercedes.*

Onion Cultivar	Identification	No. of bacterial isolates
Candy	<i>Bacillus licheniformis</i>	2
	<i>Bacillus pumilus</i>	19
	<i>Bacillus subtilis</i>	3
Subtotal	three species; G+	24
Excalibur	<i>Actinobacillus hominis</i>	3
	<i>Curtobacterium pusillum</i>	1
	<i>Enterobacter cloacae</i>	14
	<i>Klebsiella pneumoniae</i> ss. <i>pneumoniae</i>	1
	<i>Micrococcus luteus</i>	1
	<i>Pantoea stewartii</i> ss. <i>stewartii</i>	2
	<i>Pasteurella multocida</i> ss. <i>multocida</i>	2
	<i>Pasteurella trehalosi</i>	1
Subtotal	10 species; 2 G+ and 8 G-	27
Mercedes	<i>Bacillus subtilis</i>	1
	<i>Enterobacter gergoviae</i>	1
	<i>Pantoea agglomerans</i>	16
	<i>Pantoea dispersa</i>	4
	<i>Rahnella aquatilis</i>	1
Subtotal	five species; 1 G+ and 4 G-	23
Total		74

Mercedes. Both species were shown pathogenic to onion foliage under field conditions in Puerto Rico (Calle-Bellido, 2005). In Venezuela, *Pantoea agglomerans* was reported as a pathogen of the ornamental plant *Gloxinia alba* (Jiménez et al., 2007) and also was isolated from onion seeds (21.6%) (Rodríguez and Contreras, 2006). In onion cultivar Candy, all bacterial strains isolated were gram-positive, *Bacillus pumilus* being the most prevalent (79%). *Bacillus pumilus* has been reported as seed endophytic bacteria in *Phaseolus vulgaris* (López-López et al., 2010) and as an antifungal antibiotic producer (Leifert et al., 1995).

Enterobacter cloacae, *Bacillus* spp. and *Micrococcus luteus* can be found in association with many crops, such as beans, cucumber, radish, peas, soybeans, sunflowers, sweet corn, hibiscus, pepper and wheat seeds (Mundt and Hinkle, 1976; Roberts et al., 1999). Some endophytic bacteria, such as *Klebsiella pneumoniae*, enhance plant growth and nutrition in *Medicago* sp. (Iniguez et al., 2005). *Pantoea agglomerans*, *P. dispersa*, *E. cloacae*, *Pasteurella* spp. and *Curtobacterium* sp. isolated from onion seeds in this study were also isolated from foliar lesions in commercial onion fields in Puerto Rico (Calle-Bellido, 2005). The importance of these findings is that pathogen-free seed will be critical to prevent bacterial disease outbreaks in the field.

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