



First report of *Pseudomonas putida* affecting *Eryngium* spp., case study in Ixtapan de la Sal, State of Mexico

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ABSTRACT

Background/Objective. Plant species of the genus *Eryngium* are native to Mexico; their cultivation as ornamental cut flowers is a recent development, so the pathogens that affect them are largely unknown. In this context, a grower set out to innovate by using this plant as a cut flower but encountered a rot disease that decimated his field; therefore, the objective of this study was to isolate, identify, and characterize the causal agent of soft rot in the stems of *Eryngium* spp.

Experimental development. Plants exhibiting rot symptoms were collected from a cultivated plot in the municipality of Ixtapan de la Sal, State of Mexico. From ten symptomatic plants kept in a humid chamber, a bacterium was isolated and identified using biochemical analysis. For molecular identification, DNA was extracted, and the 16S ribosomal gene was amplified with primers 27F and 1492R. PCR products were sent to the Advanced Genomics Laboratory at LANGE BIO, CINVESTAV Irapuato, Guanajuato. Koch's postulates were also performed on twenty asymptomatic *Eryngium* spp. seedlings, which were inoculated by introducing a bacterial mass at the leaf base, the distal petiole, and the basal vein region.

Results. Based on biochemical analysis, the bacterium was identified as belonging to the genus *Pseudomonas*; molecular analysis revealed that the bacterium was *Pseudomonas putida*. According to Koch's postulates, 100% of the symptoms associated with soft rot were observed.

Conclusion. *Pseudomonas putida* was confirmed as the causal agent of soft rot in *Eryngium* spp. Its pathogenicity was confirmed in *Eryngium* spp. seedlings according to Koch's postulates. This is the first report of *P. putida* causing this disease in these plants.

Keywords: Soft rot, Toad herb, Koch's postulates, Sequencing



INTRODUCTION

The species of the *Eryngium* genus (1753) are grouped in the Apiaceae family (WFO, 2025); its distribution is broad and it is found in temperate and tropical areas (García-Ruiz, 2013). In Mexico, approximately 60 *Eryngium* species have been found (García-Ruiz, 2013), which are commonly known as the toad's herb. Some species are planted for their ornamental value, such as *Eryngium giganteum* and cultivars (Heywood *et al.*, 2007).

Among pests and diseases affecting *Eryngium* spp., the *Meloidogyne enterolobii* root-knot nematode has been reported in Pará, Brazil (Muniz *et al.*, 2024). González-Sandoval and Rivera-Vargas (2015) pointed out that *Colletotrichum* sp., *Sclerotium rolfsii*, *Xanthomonas campestris* and the Cucumber mosaic virus (CMV) are pathogens that have been found to affect *E. foetidum* in Costa Rica. In turn, Ratankumar *et al.* (2015) reported effects of powdery mildew caused by *Oidiopsis macrospora*.

Due to the lack of information on the diseases that affect *Eryngium* spp. in Mexico and to the intention of using registered varieties of this genus to produce cut flowers, it was necessary to review and analyze the origin of the soft rot of this species, which was reported by a producer in the municipal area of Ixtapan de la Sal, State of Mexico, Mexico.

One of the problems faced by producers of this plant is that its reproduction is asexual and is performed with rhizomes, which broadly complicates its management, since special attention must be paid to the health of the propagational material. This is because the seedlings in apparently good phytosanitary condition display initial symptoms of discoloration in the leaf venations as they develop in the field, followed by total plant rotting. Therefore, the aim of this study was to determine the etiology of soft rot in *Eryngium* spp. in Ixtapan de la Sal, State of Mexico.

EXPERIMENTAL DEVELOPMENT

Ten *Eryngium* spp. plants with visible rotting of the root and stem were obtained on August 12, 2023, from a field in Ixtapan de la Sal, State of Mexico (18° 50' 40.73'' N and 99° 45' 13.75'' W). The soil in the production plot was clay-like and retained a high moisture content during the rainy season. During the inspection of the plants in the field, plants were observed to have symptoms of soft rot in rhizomes and roots, necrosis in the nervations and rotting of the rachis of the leaves. In the propagation site, the seedling produced from rhizomes appeared healthy, although the producer reported high mortality, reaching up to 70% and the diseased specimens were removed. At this production site, three registered varieties were managed, and all showed rot at varying levels.

The sampling was aimed at diseased plants; eight plants with rot in the stems and rhizomes, color changes in the leaves and plants with initial symptoms of color changes in the leaf nervations were collected. The specimens were kept in paper bags and placed in plastic bags, and transported in ice chests to be analyzed in the Plant Pathology Laboratory of the Department of Natural Sciences at the Autonomous University of Queretaro. Initially, fragments of rhizomes and roots with symptoms were washed with running water, then disinfected with 2% sodium hypochlorite for 2 minutes and rinsed with sterile distilled water. The pieces of tissue were placed in a humid chamber using filter paper, sterile distilled water and sterile Petri dishes to wait for the formation of the bacterial flow, which was achieved after incubating for three days.

The bacterial fluids were processed as proposed by Schaad *et al.* (2001) and Trigiano *et al.* (2003), therefore, streaks were made on the nutrient agar to obtain an axenic culture. From pure cultures, biochemical characterization was performed, including growth in King's B medium. From this axenic culture, a colony with registration number 1347 was selected for identification.

The DNA extraction from bacteria 1347 was carried out using the phenol chlorophorm method (Wilson, 1987), and the quality and amount of DNA was verified using Nanodrop™ 2000. The DNA was used to amplify a fragment of approximately 1.6 Kbp of the ribosomal RNA 16S by chain reaction of the polymerase (PCR, Biorad®, T100 Thermal Cycler). For this purpose, primers 27F AGA GTT TGA TCM TGG CTC AG and 1492R TAC GGY TAC CTT GTT ACG ACT T were used, along with the Taq DNA polymerase by ThermoScientific® following the company instructions. The conditions used in the thermocycler were 94 °C for 3 min, followed by 35 cycles: 94 °C 30 s, 62 °C 30 s, 72 °C 90 s, and a 7-minute cycle at 72 °C. The amplification of a single product was verified by electrophoresis in 1% agarose gel stained with ethidium bromide; the product of the PCR was purified using the Zymo Research kit (D4003S) and sequenced using Sanger's method in the advanced genomics laboratory of the LANGE BIO of the CINVESTAV Irapuato, Guanajuato. After the sequence was obtained, editing was performed using the Chromas Lite 2.1.1© program, followed by a Blast analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine the identity of the microorganism. Sequences from published species belonging to the identified genus were then unloaded onto the GenBank. The sequences were aligned using the Mesquite program (<https://mesquiteproject.org/>), and a phylogenetic analysis was carried out using the MrBayes program (MrBayes | index-nbisweden.github.io) using the maximum likelihood method with a bootstrap of 1,000 replications.

Due to limited information on the diseases that affect *Eryngium* spp. in Mexico, Koch's postulates were carried out to confirm the symptomatology and the causal agent of soft rot. Koch's postulates were implemented using 20 apparently healthy seedlings (without any signs of color changes in nervations), which were inoculated by injecting bacterial mass into the distal region of the petiole and the basal region of the nervations. For this purpose, a flamed dissecting needle was used, with its tip dipped in a bacterial culture. The inoculated seedlings were stored individually in plastic bags throughout the test, and two specimens were kept as controls, which were poked with a sterile needle, not dipped into bacterial growth, and under the same experimental conditions as the inoculated plants.

The bacterial fluids produced evenly colored yellowish white bacterial cultures, which in the Gram staining displayed Gram-negative bacilli. On the other hand, they displayed a facultative aerobic metabolism in the Hugh-Leifson medium and were fluorescent in King's B medium. Using different culture media, it was determined to belong to the genus *Pseudomonas*. The sequencing analysis determined that the bacteria isolated from *Eryngium* spp. is *Pseudomonas putida* (Figure 1). This bacterium is found in the Pseudomonadaceae family, which includes an important number of species that cause problems in plant production (e.g. *Pseudomonas syringae*) and others are of interest for human health (e.g. *Pseudomonas aeruginosa*). The taxonomic classification of the isolated strain named 1347 was determined with the maximum likelihood phylogenetic analysis of the 16S rRNA gene fragment sequence, the topology of which revealed strong evolutionary congruence with *Pseudomonas putida* group (Figure 1). The analysis of the genetic distances (represented by the scale bar of 0.9 substitutions per site) displays a minimal

divergence between isolate 1347 and reference strains *Pseudomonas putida* MF462927 and *Pseudomonas putida* PP095708, consolidating a terminal monophyletic clade with the maximum statistical support possible (bootstrap = 1.00) (Mallick *et al.*, 2025).

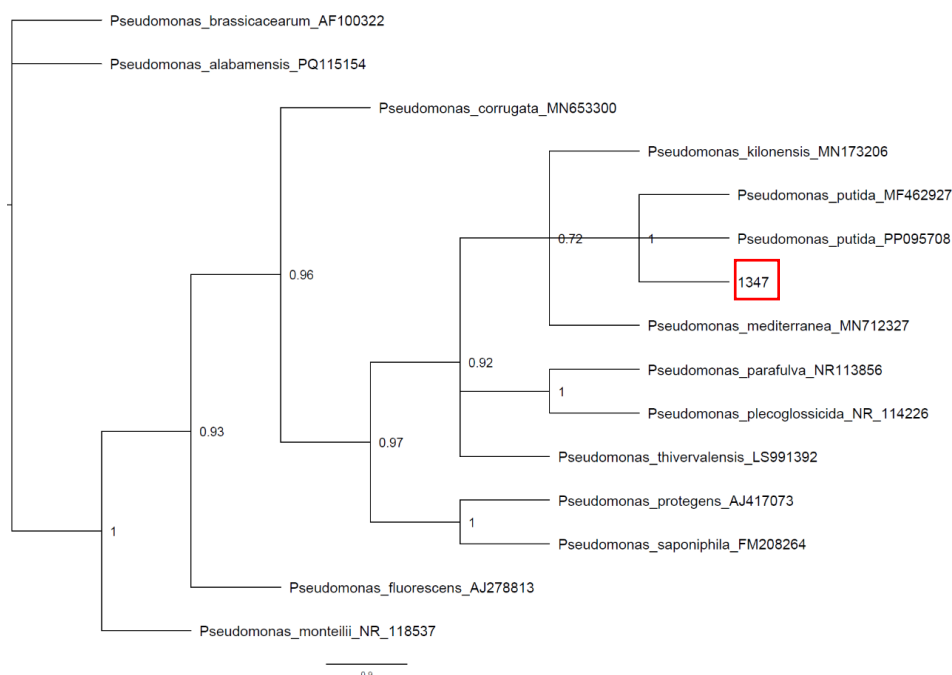


Figure 1. Phylogenetic relationship of bacterial strain 1347 and reference species from the genus *Pseudomonas*. The strain shows a correlation with *Pseudomonas putida* (MF462927, PP095708). The phylogenetic tree was constructed by the Maximum Likelihood method using 16S ribosomal DNA gene sequences. Bootstrap values at internal nodes represent support from 100,000 replicates. *P. brassicacearum* AF100322 and *P. alabamensis* PQ115154 served as the outgroup.

In *Eryngium* spp., the field symptoms observed are shown in Figure 2, beginning with color changes in the leaf veins (A). In the underside of the same leaves, the necrosis is visible in the same central nervation (B). The petioles (C) also displayed brown longitudinal grooves, and as the bacteria advances, leaves presented a loss of vigor and the leaves gradually wilted and died (D). Finally, the entire plant itself displayed wilting (E). In this final stage, when the plant was pulled out, the rhizomes displayed soft rot (Figure 3).

Koch's postulates displayed the beginning of the infection process (Figure 4A) on the day after inoculation (dai), shown in Figure 4, whereas 2 dai, the place of injection displayed aqueous rot (Figure 4B), and later, 10 dai, the plants displayed all symptoms of the aqueous rot of *Eryngium* spp. (Figure 4C). Sections of petioles from seedlings with rot were placed in a moisture chamber as per the method described earlier and bacterial colonies with the same morphology were isolated, confirming the pathogenicity of *P. putida* against *Eryngium* spp. The control seedlings showed no symptoms during the experimental period. The plants were observed until day 19, when 100% of the inoculated plants died, whereas the control plants remained healthy.



Figure 2. Stages of soft rot development in *Eryngium* sp. under field cultivation; **A)** Leaf blade showing necrosis along the main veins; **B)** Underside of the same leaf with severe necrosis; **C)** Leaf petioles with necrotic streaks; **D)** Wilting and death of leaves; **E)** Wilting of the entire plant.



Figure 3. Soft rot in the stem and main root of *Eryngium* spp. associated with bacteria.

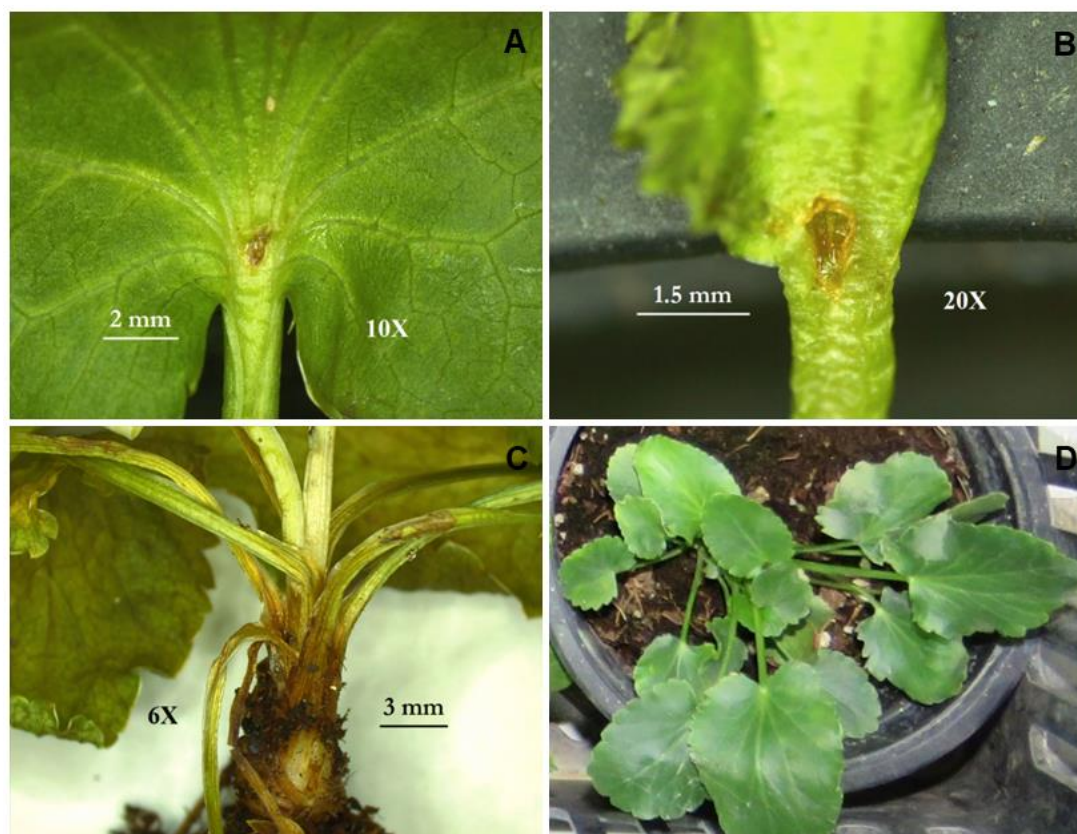


Figure 4. Koch's postulates in *Eryngium* spp. seedlings inoculated with bacterial strain 1347; **A)** Water-soaked lesions at the site of puncture inoculation on the leaf with infectious process the following day; **B)** Progression of severity on the second day; **C)** Dead plant on the tenth day; **D)** Control plant.

Pseudomonas putida is a usual inhabitant of soil and water. It has even been documented as having benefits when added to the soil as part of the microbiota which will colonize the rhizosphere in vegetables such as the bell pepper (*Capsicum annuum*) (Hernández-Montiel *et al.*, 2018) and in grains such as maize (*Zea mays*) (Molina-Romero *et al.*, 2017). It has been suggested that some strains can serve as biological control agents against other bacteria that affect plants (Purtschert-Montenegro *et al.*, 2022). On the other hand, the potential of *P. putida* has been studied for some strains to be used in the production of bioplastics (Obeso, 2017) and potentially, in the bioremediation of soils contaminated with aromatic compounds such as toluene and xylene (Volke *et al.*, 2020).

Despite the benefits documented in investigations, it is now known that *P. putida* can be a pathogen to plants and even humans (Goenaga- Sánchez *et al.*, 2005). In the case of problems associated to humans, it has been reported as a problem for the respiratory tract, endocarditis, meningitis, contaminant in endoscopies and in patients with catheterization, which, in time, causes infections at different levels (Goenaga-Sánchez *et al.*, 2005).

In cases involving effects on plant production, recent publications have reported that *P. putida* is now registered as a pathogenic agent. Thus, despite the broad distribution and presence of the bacteria in the soil, water and the rhizosphere, it has been reported to have adverse effects on plants, on which several authors point out that it may produce phytotoxins that interfere with plant regulation mechanisms, presenting wilting, abnormal development and soft rot, among other symptoms (Environmental Canada, 2017). Color

changes have been reported in vascular bundles in tomato plants (*Solanum lycopersicum*), which result in disease, a situation which was evaluated in seedlings of the same species and chlorosis and necrosis were recorded, along with the death of the seedlings (Dimartino *et al.*, 2011). Likewise, Mota *et al.* (2021) reported necrosis in tomato plants, being the first report of pathogenicity of *P. putida* in Brazil.

Due to the asexual reproduction of *Eryngium* spp. by rhizomes, the study reported a mortality of 70% in seedlings in the sampling site. This situation can be a determining factor for the dissemination in the field, according to reports by the producer. Additionally, due to the favorable conditions that accelerate bacterial growth and reproduction. Knowledge of *P. putida* as a plant pathogen is scarce, indicating that further investigation is required to determine its effects on *Eryngium* spp. plants.

CONCLUSIONS

According to the biochemical and molecular analysis, *Pseudomonas putida* was identified as the causal agent of soft rot in *Eryngium* spp. In Koch's postulate, 100% of inoculated plants developed soft-rot symptoms, similar to those observed in the field. This is the first report of *P. putida* causing rot in *Eryngium* spp.

Limitations. The access to a greater amount of apparently healthy seedlings to carry out Koch's postulates is one of the problems found. It was not possible to indicate the varieties due to rights.

Conflict of interest. The parties involved do not present any conflict of interest. The manuscript does not indicate the variety of the ornamental plant; on the other hand, the people dealt with reviewed the document before it was sent to be published.

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Contribution of the authors. Santiago Vergara-Pineda participated by taking simples and in the conventional laboratory procedures. Paola S. Puga-Guzmán participated by taking simples and in the follow-up of Koch's postulates. Daniel Mendoza Jiménez participated in the molecular diagnostic procedures. José Antonio Cervantes Chávez participated in the computer analysis of the results.

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