









## Pathological and genetic variability of bacterial strains causing vegetable soft rot in west Pará region, Brazil

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**ABSTRACT:** Seventy-five pectinolytic strains collected from vegetables grown in the counties of Santarém, Belterra, and Mojuí dos Campos, located in the western region of the Pará State, Brazil, were studied according to their pathological and genetic variability. The strains were grouped in 5 clusters according to pathogenicity in potato, pepper, carrot, and onion, and 38 strains were selected for genetic analysis using rep-PCR. These strains were divided into 35 genetic groups according to rep-PCR at 70% similarity. These results indicated high pathological and genetic variability of the strains causing soft rot in vegetables in the western region of the Pará State, which will be used in etiological research and for the development and assessment of management techniques for the soft rot in vegetables in this region.

**Key words:** pectinolytic bacteria, pathogenicity, rep-PCR, host range.

### Variabilidade patológica e genética de isolados bacterianos causadores de podridão mole em hortaliças na região oeste do Pará, Brasil

**RESUMO:** Setenta e cinco isolados de bactérias pectinolíticas coletados de hortaliças cultivadas nos municípios de Santarém, Belterra e Mojuí dos Campos, região oeste do Estado do Pará, Brasil, foram estudadas de acordo com sua variabilidade patológica e genética. Os isolados foram agrupados em cinco grupos de acordo com a patogenicidade em batata, pimenta, cenoura e cebola, e 38 isolados foram selecionados para análise genética por rep-PCR. Esses isolados foram divididos em 35 grupos genéticos de acordo com rep-PCR a 70% de similaridade. Esses resultados indicaram alta variabilidade patológica e genética dos isolados causadores da podridão mole em hortaliças da região oeste do Pará, os quais serão utilizados em pesquisas etiológicas e para o desenvolvimento e avaliação de técnicas de manejo da podridão mole em hortaliças nesta região.

**Palavras-chave:** bactérias pectinolíticas, patogenicidade, rep-PCR, gama de hospedeiros.

Soft rot caused by pectinolytic bacteria is a globally important disease that occurs in several vegetables, and the principal genera related to this disease are *Pectobacterium* and *Dickeya* (CHARKOWSKI, 2018). However, more genera in the *Enterobacteriaceae* family have been found associated with vegetables soft rot, such as *Kluyvera*, *Klebsiella*, *Leliottia*, *Morganella*, *Rahnella* (MORAES, 2018). In Pará, there is a report of *Dickeya dadantii* causing soft rot in cariru in the city of Ananindeua (MORAES et al. 2023). However, there is no information about the pathogenic and genetic variability of strains causing soft rot in vegetables in counties of the western region of Pará State.

Currently, the use of molecular markers has stood out in studies of genetic characterization of microorganisms (ALVARADO, 2011). Among them, the REP, ERIC, and BOX-PCR markers (collectively referred to as rep-PCR) has been efficiently used in studies to characterize the genetic diversity of pectinolytic bacteria (LOUWS et al., 1994). Thus, this study performed a pathological and molecular characterization of strains causing soft rot in vegetables in the municipalities of Belterra, Mojuí dos Campos, and Santarém, located in the western region of the Pará State, Brazil, using artificial inoculation on different hosts and rep-PCR. We collected samples of lettuce (*Lactuca sativa* L.), bell-pepper (*Capsicum annuum* L.), kale (*Brassica oleraceae* var. *acephala*

DC.), tomato (*Solanum lycopersicum* L.), chive (*Allium schoenoprasum* L.), parsley (*Petroselinum crispum* (Mill.) Nym), chicory of Pará (*Eryngium foetidum* L.), and cucumber (*Cucumis sativus* L.) showing soft rot symptoms, from February 2018 to December 2019. Seventy-five bacterial strains were isolated as described by TAKATSU et al. (1981). Typical pectinolytic bacterial colonies were isolated through morphology characteristics described by MARIANO et al. (2005) and the strains were preserved in sterilized distilled water (SDW) (SOUZA et al., 2016).

To the pathological characterization, the strains obtained were cultivated in casamino acid-peptone-glucose (CPG) medium for 48 hours at 29 °C, and the bacterial colonies were touched with sterilized wooden sticks (TAKATSU et al., 1981), which were used to drill the epidermis of bell-pepper fruits, onion cataphylls (*Allium cepa* L.), carrot (*Daucus carota* subsp. *sativus* L.) and potato (*Solanum tuberosum* L.) slices. All these hosts tissues were previously disinfested in 0.5% sodium hypochlorite solution for 5 min and washed in sterile distilled water (SDW). Four different experiments were performed, one for each host. Each experiment consisted of 75 treatments (75 strains), with four replicates per treatment, and each replicate was characterized by an inoculation point. Tissues treated similarly with SDW constituted the negative control treatment. After 48h of incubation at 29 °C, re-isolation was performed to fulfill Koch's postulates. Pathological characterization was qualitative, considering the pathogenicity of each strain in relation to the inoculated hosts.

Thirty-eight strains selected according to pathogenicity to the different hosts were used to evaluate the genetic variability using rep-PCR. The DNA of the strains was extracted according to GAMA et al. (2018), and molecular analysis was performed using rep-PCR as described by LOUWS et al. (1994).

The PCR conditions and electrophoresis were performed according to GAMA et al. (2018). The analysis of the rep-PCR profiles obtained with REP, ERIC, and BOX markers was performed according to the presence (1) or absence (0) of bands and the data were analyzed combined using MVSP 3.1 (Kovach Computing Services, Wales). To determine the genetic relationships among the strains, we used the Jaccard similarity coefficient at 70% similarity and Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) cluster analysis.

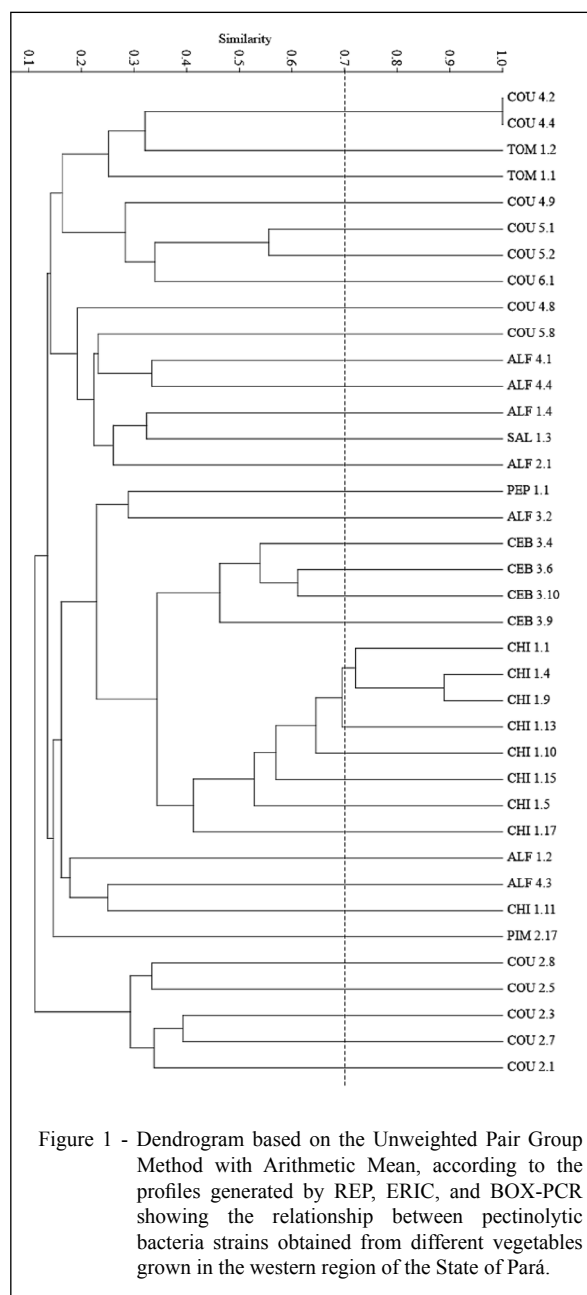
The 75 strains obtained from the different vegetables were divided in five groups according to pathogenicity to the evaluated hosts, namely: group I harbored 58 strains, which were pathogenic to all hosts; group II harbored nine strains, which were pathogenic to potato, pepper, and onion; group III harbored five strains, which were pathogenic to potato and pepper; group IV harbored two strains, which were pathogenic to potato; and group V harbored one strain, which was pathogenic to potato, pepper, and carrot (Table 1). These results demonstrated that the strains obtained from the western region of the Pará state showed high pathogenic variability, corroborating with previous studies (MARIANO et al., 2005; AKBAR et al., 2015; MORAES, 2018).

We observed the amplification of 33, 35, and 34 reproducible bands between 250 and 10,000 bp for the ERIC, REP, and BOX-PCR, indicating a high degree of polymorphism among the strains. Thirty-five groups were formed at 70% similarity by the cluster analysis performed with the three markers (Figure 1), indicating that probably different genera and species may be associated with soft rot of vegetables in the western region of the Pará state. In this context, different genera and species of pectinolytic bacteria have been associated with the soft rot of vegetables, and it is common to isolate different bacteria from a single host, as well as from several hosts. For instance, *Dickeya dadantii*

Table 1 - Pathogenicity groups of pectinolytic bacteria strains according to the reaction to different hosts.

Patogenicity group	-----Host-----				Percentage of strains
	Potato	Bell-pepper	Onion	Carot	
I	+ <sup>1</sup>	+	+	+	77
II	+	+	+	-	12
III	+	+	-	-	7
IV	+	-	-	-	3
V	+	+	-	+	1

<sup>1</sup> + positive reaction; - negative reaction.



subsp. *dantii*, *P. carotovorum* subsp. *brasiliensis*, *P. carotovorum* subsp. *carotovorum*, and *P. atrosepticum* have been isolated from potato tubers in South Africa and Zimbabwe (NGADZE et al., 2012), while different species of *Pectobacterium*, *Dickeya*, *Enterobacter*, *Kluyvera*, *Klebsiella*, *Morganella*, *Providencia*, *Rahnella* and *Raoutella* have been commonly isolated from squash, zucchini, lettuce, eggplant, broccoli, onion, Chinese cabbage, kale, peppers, radishes,

cabbage, arugula, and parsley in Pernambuco state (MORAES, 2018). In addition, we did not observe correlation among genetic groups, pathogenicity groups, geographic location, or the original hosts, similarly to the previous studies (MAISURIA & NERUKAR, 2013; ALVARADO et al., 2011).

The results obtained in this study demonstrated a high pathological and genetic variability of pectinolytic bacteria associated with

soft rot of the vegetables in the western region of Pará state. In addition, these results indicate that probably there are different genera and species of pectinolytic bacteria associated with soft rot in this region. In the future, representative strains from this region will be identified at the genus and species level using phylogenetic analysis of housekeeping genes, which will help in the assessment of control techniques that may be integrated into disease management aiming to decrease the loss caused by the soft rot of the vegetables in the western region of the Pará state.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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