The diversity and pathogenicity of Rahnella species isolated from diseased onion bulbs in the United States and South Africa

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INTRODUCTION

The genus *Rahnella* belongs to the family Yersiniaceae and includes Gram-negative, facultative anaerobic bacteria that have a ubiquitous distribution. *Rahnella* species have been isolated from water, human wounds, oak trees, beetle guts, and recently, from symptomatic onion bulbs and leaves. Onion (*Allium Cepa* L.) is one of the most consumed vegetables worldwide. Ten states in the USA produce an average of 33 million tons of bulbs each year, whereas, in South Africa, approximately 600 000 tons are produced, mainly from the Western Cape province. Onion production is greatly hampered by pre- and post-harvest diseases caused by bacterial pathogens, resulting in up to 100 % yield loss. Isolates used in this study were collected as part of surveys undertaken in the USA and SA in 2021.

AIM AND OBJECTIVES

To aim of this study was to determine the diversity and pathogenicity of *Rahnella* spp. isolated from harvested onion bulbs stored in various facilities in the USA and SA. Objectives were to:

- Identify the isolates collected by sequencing the 16S rRNA gene for genus identification
- Using MLSA of the *rpoB, atpd, infB* and *gyrB* genes for species identification
- Test the pathogenicity of the isolates using foliar, bulb inoculations as well as the red onion necrosis assay

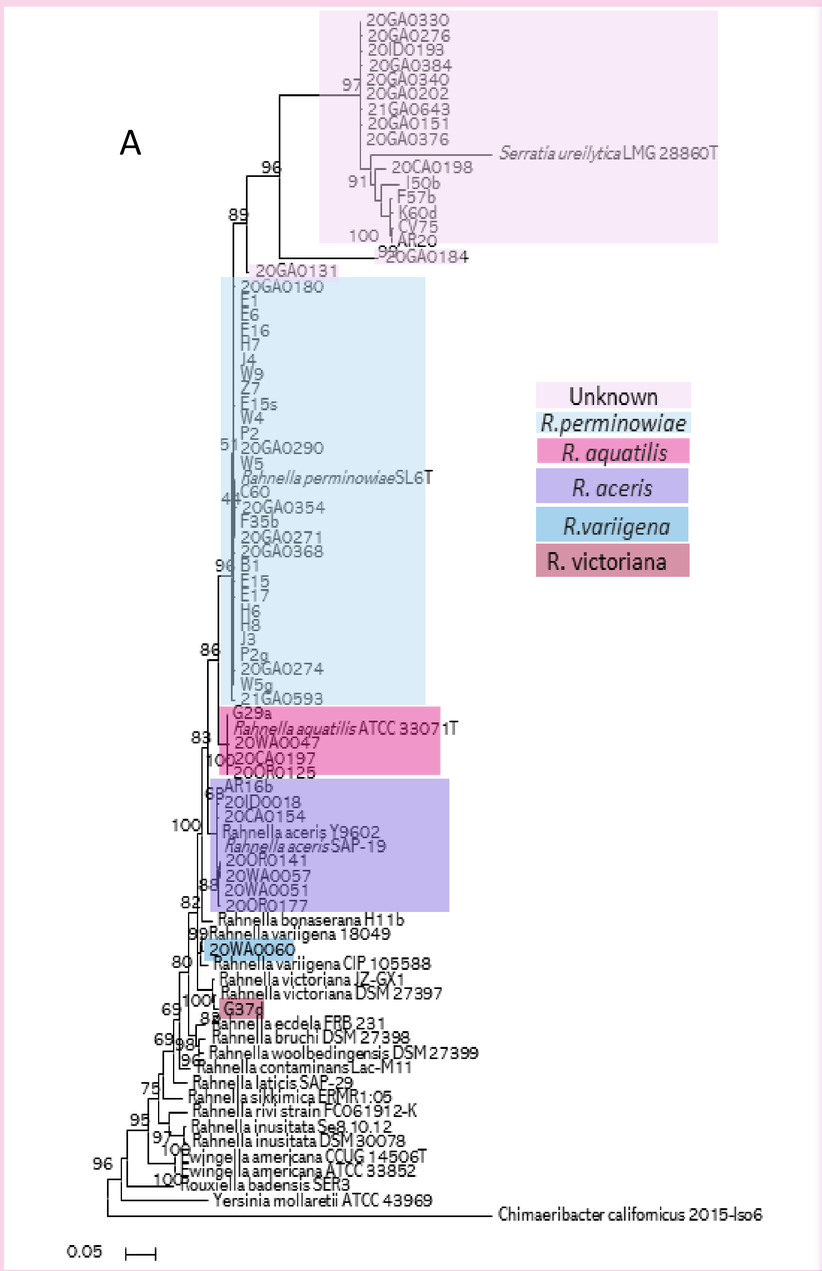
METHOD AND MATERIALS

GENUS AND SPECIES IDENTIFICATION –use flow diagram

- Isolation from SA and USA diseased post-harvest onions.
- Purification of isolates on nutrient agar plates
- DNA extractions using standard protocols
- Genus delineation using 16S rRNA gene sequences and nucleotide NCBI BLAST
- Amplification of four conserved genes regions: *atpD, gyrB, infB, and rpoB*
- Concatenation of a maximum likelihood tree using MegaX

PATHOGENICITY ASSAYS –use flow diagram

- Culture grown overnight in Nutrient broth and the concentration of the inoculum adjusted to 10^8 CFU/ml
- Foliar seedling assay: leaves of cultivars of White Lisbon and Ranchero were pin-prick inoculated
- Bulb assay: store-bought onions were peeled and inoculated with 0.5 ml of the inoculum using a needle and syringe.
- Disease severity was evaluated five days after incubation at 25°C. The following score was used: 0 (healthy) to 3 (severely diseased. The results were statistical analysed.
- Red scale necrosis assay: This assays is based on the bacterium's ability to degrade anthocyanin. Scales were pin prick inoculated with the bacteria and assessed for changes in colour around the site of inoculation. Scales were incubated for four days at room temperature



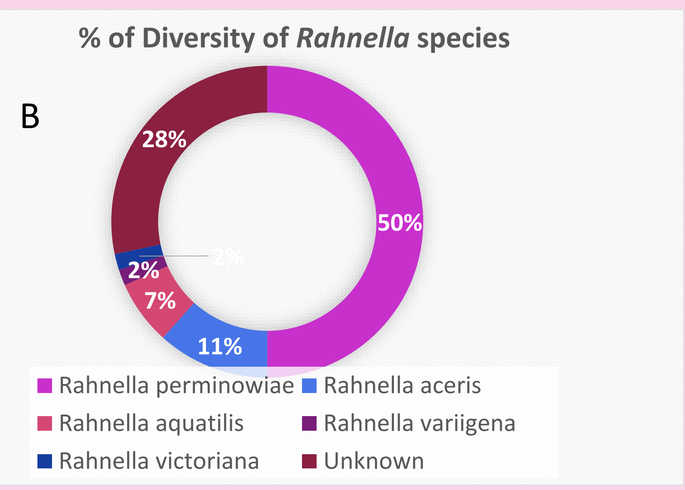


Figure 1 PLEASE REWRITE THIS LEGEND FOR BOTH A AND B
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RESULTS AND DISCUSSION

A concatenated phylogenetic tree of the four housekeeping genes showed that 50% of the *Rahnella* strains primarily belonged to *Rahnella perminowiae*, 28% to *R. aceris*, 7% to *R. aquatilis*, 2% each to *R. variigena* and *R. victoriana* (Fig 2.1). Eleven percent of the isolates did not belong to this genus and were possiblY species of *Serratia*.

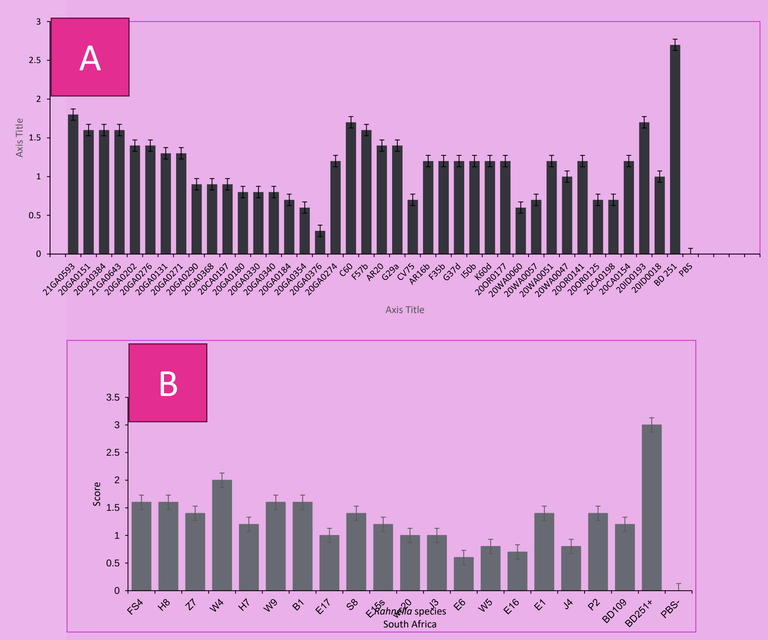

Figure 2: Bar graph fshowing the disease severity of the isolates from both countries with a significance level of 0.05 with a 95% confidence values. (A) USA isolates and (B) SA isolates

Isolates were found to be non-pathogenic using the red scale necrosis assay. Minor discoloration occurred in the region of inoculation in foliage assays. The onion bulb assay revealed that isolates of all species were pathogenic except for those belong to the species *R. variigena* and *R. bonasera* (Fig. 2). The study provides valuable insight into the diversity of *Rahnella* species associated with diseased onion bulbs in the USA and SA and their ability to cause bulb rot in onions.

CONCLUSIONS

A number of *Rahnella* species are capable of causing bulb rot in onions.

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