



Identification of *Agrobacterium cucumeris* sp. nov. as the causal agent of crazy roots on hydroponically cultivated cucumber plants in Poland

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Introduction

In April 2019, hydroponically cultivated cucumber plants with characteristic symptoms of crazy root disease were found in two different commercial cucumber production greenhouses in Poland. The roots of the obtained cucumbers were highly branched and covered with root hairs. The aim of our study was to determine the aetiology of the observed symptoms in cucumbers and to characterize their causal agent.

Crazy root disease is caused by bacteria belonging to α -Proteobacteria containing root-inducing plasmids (Ri-plasmids). Transfer and integration of a specific section of the plasmid, called T-DNA (transfer DNA) into host DNA with its subsequent expression within host cells results in development of the disease. Due to that, intensive and uncontrolled root proliferation occurs. Abnormal roots are covered with numerous hairs which are responsible for hindering the absorption of both water and nutrients, which finally leads to significant economic losses in vegetable production.



Fig. 1 Fragments of diseased cucumber plants with overgrown roots

References:

WARABIEDA, Michał, et al. *Agrobacterium cucumeris* sp. nov. isolated from crazy roots on cucumber (*Cucumis sativus*). Systematic and Applied Microbiology (under review)

WARABIEDA, Michał, et al. Identification of the causal agents of crazy root disease on hydroponically cultivated cucumber plants in Poland. European Journal of Plant Pathology, 2021, DOI: 10.1007/s10658-021-02340-6



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Materials and methods

Pathogenicity testing

PCR-based bacterial identification

Bacterial isolation



Fig. 2 Collection of the diseased plant material and pathogenicity testing

Table 1 Overview of isolated and tested bacterial strains

| Strain | Test on sunflower | | | Test on cucumber | 16 s rRNA based identification | GenBank accession number | |
|--------|------------------------|--------------------|-------------------|------------------|---|--------------------------|----------|
| | Multiplex ^a | virD2 ^b | rolE ^c | | | 16S rRNA | recA |
| O1.1.1 | - | + | + | + | <i>Rhizobium metallidurans</i> ; ChmEc512 | MW256431 | MW251507 |
| O1.1.5 | 1 | + | + | + | <i>Agrobacterium radiobacter</i> ; ATCC 19358 | MW256422 | MW222382 |
| O1.2.1 | - | + | + | + | <i>Rhizobium metallidurans</i> ; ChmEc512 | MW256430 | MW251508 |
| O1.2.3 | - | + | + | + | <i>Rhizobium metallidurans</i> ; ChmEc512 | MW256432 | MW251509 |
| O1.3.2 | 1 | + | + | + | <i>Agrobacterium radiobacter</i> ; ATCC 19358 | MW256423 | MW222383 |
| O1.3.3 | - | + | + | + | <i>Pararhizobium lotum</i> ; CCBAU 83011 | MW256433 | MW251510 |
| O1.3.4 | 1 | + | + | + | <i>Agrobacterium radiobacter</i> ; ATCC 19358 | MW256424 | MW222384 |
| O2.1.2 | 1 | + | + | + | <i>Agrobacterium radiobacter</i> ; ATCC 19358 | MW256425 | MW222385 |
| O2.3.1 | 1 | + | + | + | <i>Agrobacterium radiobacter</i> ; ATCC 19358 | MW256426 | MW222386 |
| O2.3.2 | 1 | + | + | + | <i>Agrobacterium radiobacter</i> ; ATCC 19358 | MW256427 | MW222387 |
| O3.4 | 1 | + | + | + | <i>Agrobacterium radiobacter</i> ; ATCC 19358 | MW256428 | MW222388 |
| O4.1 | 1 | + | + | + | <i>Agrobacterium radiobacter</i> ; ATCC 19358 | MW256429 | MW222389 |

^aPCR assay for detection of biovar1strains conducted according to Puławska et al. (2006), 1 - means the result of multiplex PCR shows that bacteria belong to biovar 1 of *Agrobacterium*;“-“ no amplification results

^bPCR assay for detection of virulence region of Ri plasmid fragment conducted according to Haas et al. (1995).

^cPCR assay for detection of root oncogenic loci region of Ri plasmid fragment conducted according to Weller and Stead (2002); Bosmans et al. (2016)

Results

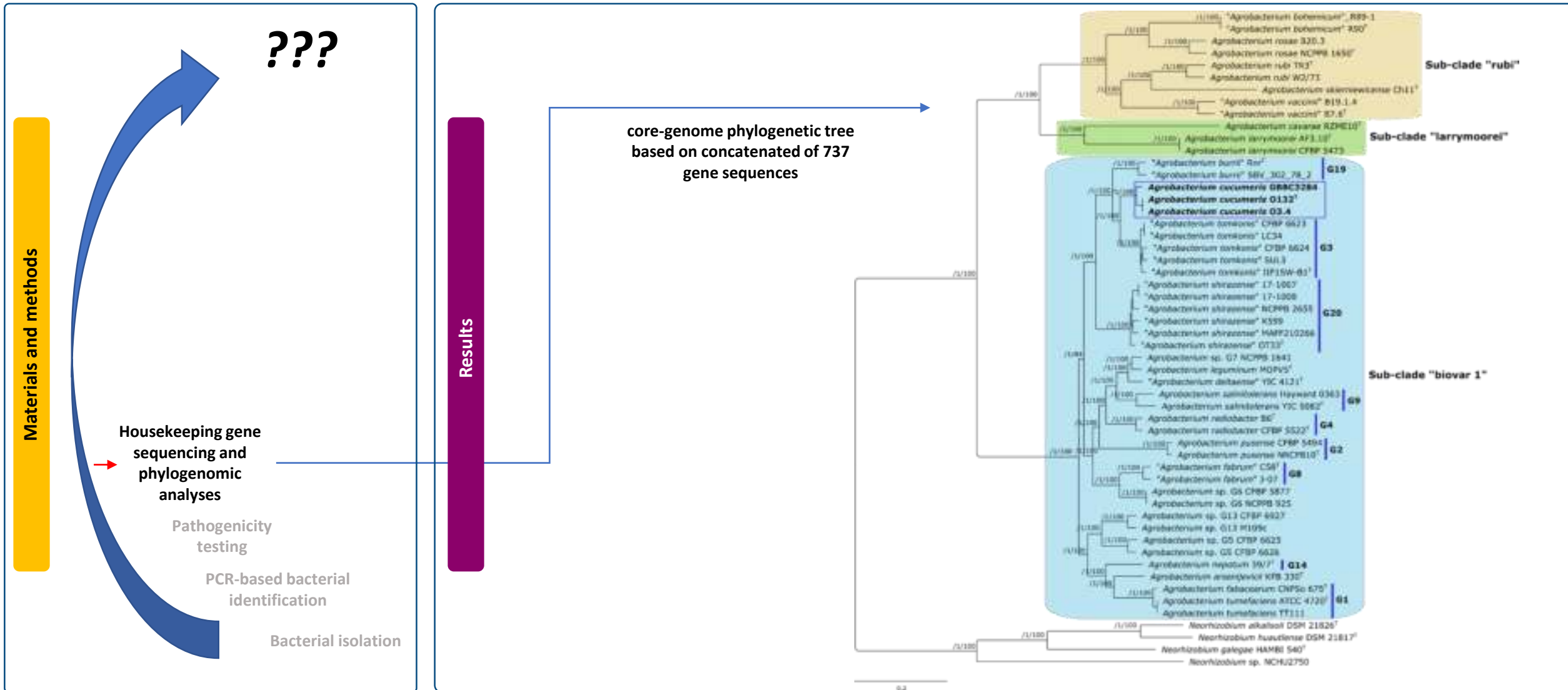
References:

WARABIEDA, Michał, et al. *Agrobacterium cucumeris* sp. nov. isolated from crazy roots on cucumber (*Cucumis sativus*). Systematic and Applied Microbiology (under review)

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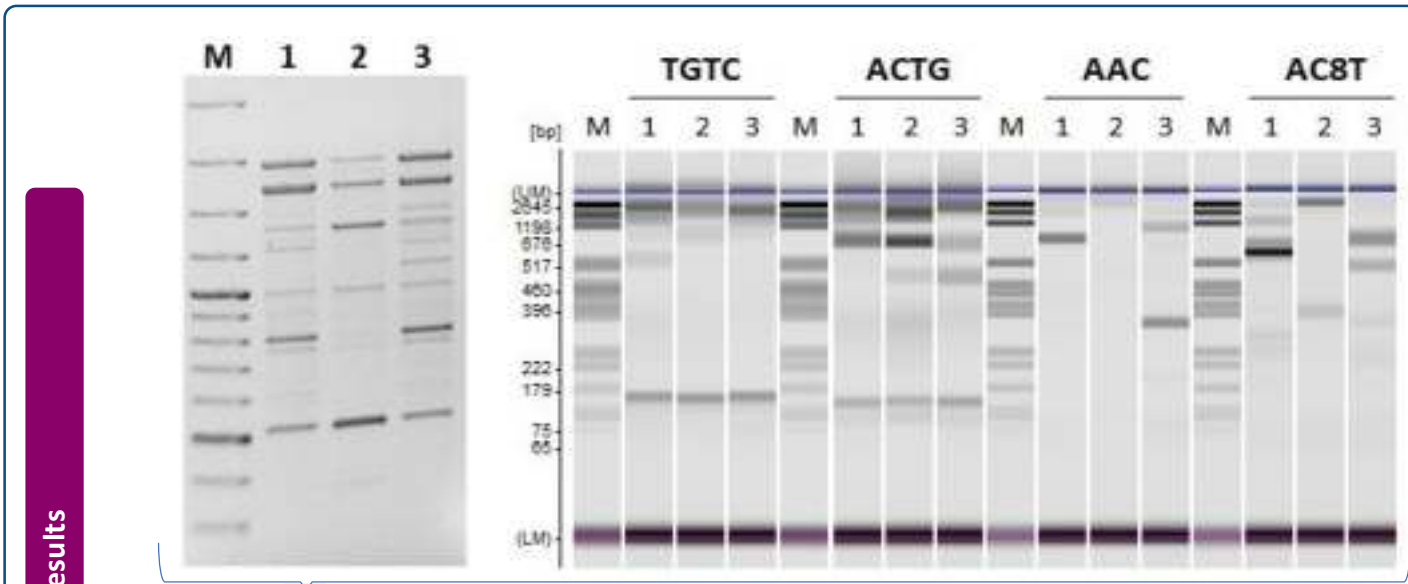
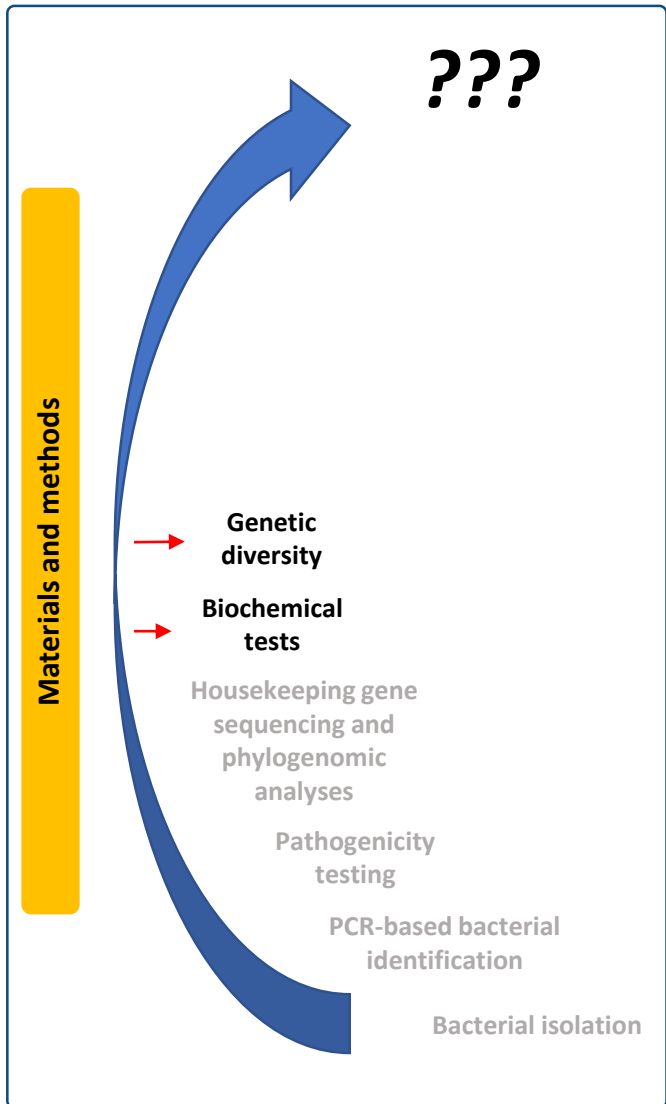


Fig. 3 The results of the RAPD analysis with primer AR03 (on the left) and ISSR analysis with four different sets of primers TGTC, ACTG, AAC, AC8T (on the right). M - molecular DNA ladder; 1 - O132^T; 2 - O115; 3 - O34.

Results

Obtained results of DNA fingerprint analysis confirmed that studied strains are not of clonal origin

Table 2 BIOLOG assay test results concerning the determination of differences and similarities in the ability to carbon sources utilization and sensitivity to chemical compounds between the *Agrobacterium cucumeris* sp. nov. O132^T and related species of the genus

| Results after 48h of incubation | Utilization of: | <i>A. cucumeris</i> | <i>A. nepotum</i> | <i>A. "fabrum"</i> | <i>A. radiobacter</i> | <i>A. tomkonis</i> |
|---------------------------------|--------------------------|---------------------|-------------------|--------------------|-----------------------|--------------------|
| | | α-D-Glucose | + | - | + | + |
| | Citric Acid | + | + | - | - | - |
| | α-Keto-Glutaric Acid | + | + | + | - | - |
| | Sensitivity to Aztreonam | + | + | - | + | + |

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Agrobacterium cucumeris

Overall genome relatedness indices

Whole-genome sequencing, assembly and annotation

Genetic diversity

Biochemical tests

Housekeeping gene sequencing and phylogenomic analyses

Pathogenicity testing

PCR-based bacterial identification

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Materials and methods

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Table 3 Pairwise OGRI comparisons between strains *Agrobacterium cucumeris* O132^T, O34, GBBC3284 and related *Agrobacterium* strains (ANiB comparisons).

| | O132 ^T | O34 | GBBC3284 |
|---|-------------------|-------|----------|
| <i>Agrobacterium cucumeris</i> O132 ^T | 100.0 | 100.0 | 98.0 |
| <i>Agrobacterium cucumeris</i> O34 | 100.0 | 100.0 | 98.0 |
| <i>Agrobacterium cucumeris</i> GBBC3284 | 98.0 | 98.0 | 100.0 |
| <i>Agrobacterium tomkonis</i> CFBP 6624 | 93.5 | 93.4 | 93.4 |
| <i>Agrobacterium tomkonis</i> CFBP 6623 | 93.5 | 93.5 | 93.4 |
| <i>Agrobacterium tomkonis</i> LC34 | 93.2 | 93.2 | 93.2 |
| <i>Agrobacterium tomkonis</i> SUL3 | 93.1 | 93.1 | 93.0 |
| <i>Agrobacterium tomkonis</i> IIF1SW-B1 ^T | 93.1 | 93.1 | 93.0 |
| " <i>Agrobacterium burrii</i> " SBV_302_78_2 | 92.1 | 92.1 | 92.1 |
| " <i>Agrobacterium burrii</i> " Rnr ^T | 91.9 | 91.9 | 91.9 |
| " <i>Agrobacterium shirazense</i> " K599 | 91.8 | 91.8 | 91.8 |
| " <i>Agrobacterium shirazense</i> " NCPPB 2655 | 91.8 | 91.9 | 91.7 |
| " <i>Agrobacterium shirazense</i> " MAFF210266 | 91.8 | 91.8 | 91.5 |
| " <i>Agrobacterium shirazense</i> " OT33 ^T | 91.3 | 91.3 | 91.2 |
| " <i>Agrobacterium shirazense</i> " 17-1008 | 91.1 | 91.1 | 91.0 |
| " <i>Agrobacterium shirazense</i> " 17-1007 | 91.0 | 91.0 | 91.0 |

Conclusions

Based on phylogenetic and genomic data, as well as biochemical tests it was possible to acknowledge the three strains studied O132^T, O115 and O34 as the novel species ***Agrobacterium cucumeris* sp. nov.**

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