Screening of microorganisms for antagonistic activity against pathogenic bacteria *Xanthomonas* spp.

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Introduction

Plant pathogenic bacteria belonging to the genus *Xanthomonas* are spreading rapidly around the world and causing significant crop losses, leading to intensified research in this area worldwide. However, information about these pathogens and their control methods is still lacking in Lithuania.

The **aim** of this study was to **identify** the **endophytic bacteria** with the highest **antagonistic activity** against phytopathogenic ***Xanthomonas***.

Materials and Methods

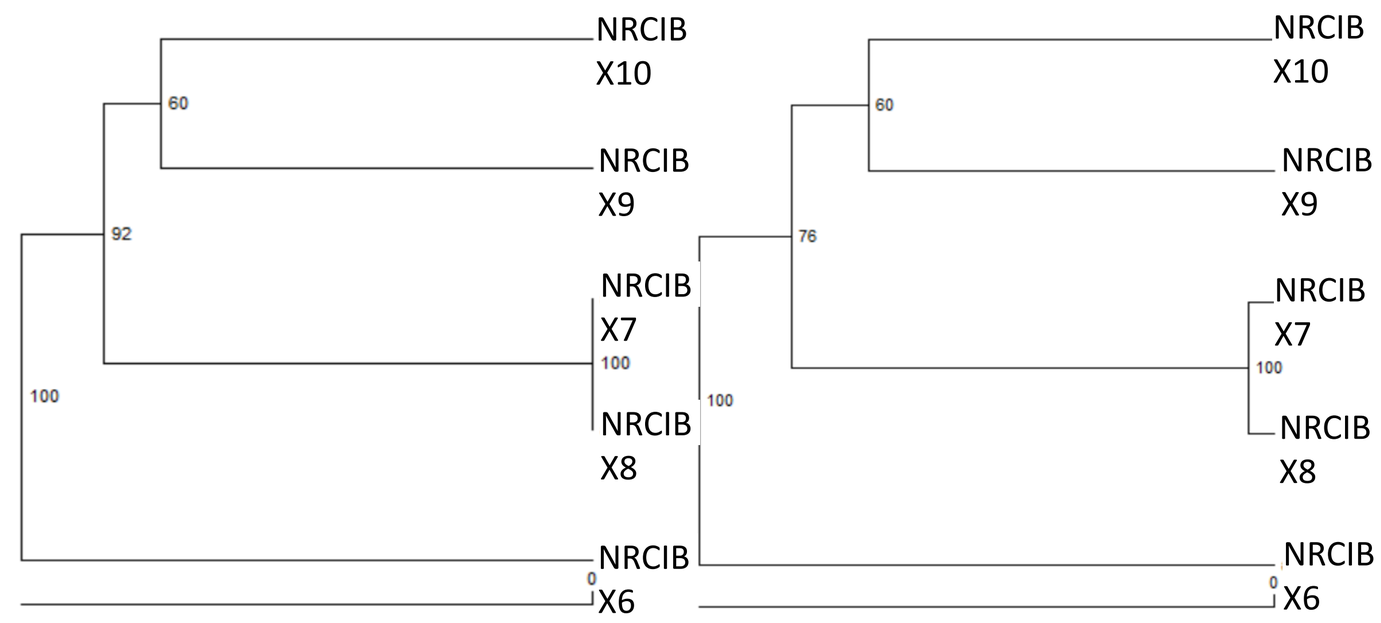
In this study, 295 bacterial isolates were obtained and investigated, where endophytic bacteria were isolated from legume roots and nodules. All strains were tested by Gram staining and hypersensitive reaction (HR) on tobacco and tomato plants (Fig. 1. B.), also the ability to cause rot on potato and pectolytic activity were investigated[1]. All bacterial isolates were selected for further molecular studies.

Bacterial genomic DNA was extracted using CTAB protocol. Yellowish *Xanthomonas*-like bacteria (Fig. 1. A.) were screened by PCR using genus-specific primers X1 and X2. Genetic diversity and phylogenetic analysis of *Xanthomonas* were performed: PCR melting Profile (PCR MP); repetitive PCR (rep-PCR); multilocus sequence analysis (MLSA)[2]; type three effector (T3E)[3] genes identification.

All bacteria used in this study were identified by sequenced 16S rDNA (accession numbers in GenBank: OL505113-OL505122).

Endophytic bacterial strains belonging to *Bacillus*, *Paenibacillus*, and *Pseudomonas* genera were used as antagonists. The antimicrobial activity of tested bacteria was determined by agar diffusion method. Thus, 10 out of 73 endophytic isolates were selected as the most effective isolates against pathogenic *Xanthomonas* strains.

Fig. 1. A – The yellowish bacteria – NRCIB X8; B – Hypersensitive reaction tests on tomato.



A. PCR MP with primers for *NcoI* and *PstI* B. rep-PCR with primers for ERIC and BOX

Fig. 2. A – **PCR MP** and B – **rep-PCR** fingerprints separated our isolates into **two different species**, where one species (as we suspect) is comprising **three different pathovars**.

Results and Discussion

Xanthomonas-like isolates showing pathogenicity to tomato plants and having pectolytic activity were identified by 480-bp band produced in PCR using genus-specific primers X1 and X2. The approximately 1300-bp PCR product of the 16S DNA fragment was then sequenced. Two genes ***gyrB*** and ***rpoD*** out of three analyzed housekeeping genes were present in 5 *Xanthomonas* strains but ***fyuA*** gene only in 4 of *Xanthomonas* strains was detected.

Our studies revealed that only five isolates belong to the genus *Xanthomonas*: one strain was classified as *X. translucens* (Xt) (NRCIB X6) and four as *X. arboricola* (Xa) (NRCIB X7, NRCIB X8, NRCIB X9, and NRCIB X10).

Endophytic bacterial strains belonging to *Bacillus*, *Paenibacillus*, and *Pseudomonas* genera were used: *Bacillus* sp. strains NRCIB B1, NRCIB B2, NRCIB B3, NRCIB B4, NRCIB B5, NRCIB B6, *Pseudomonas* sp. strains NRCIB P1, NRCIB P2, NRCIB P3, and *Paenibacillus* sp. NRCIB PB1.

However, *Pseudomonas* spp. bacteria, especially *Pseudomonas* sp. strain NRCIB P2 (average ZOI ranged from 23.00 ± 0.47 mm to 33.00 ± 0 mm), had a stronger inhibitory effect on pathogenic *Xanthomonas* spp. than other endophytic bacteria. The efficacy of some bacterial strains has been found to depend on the species of pathogenic microorganiism . *Paenibacillus* sp. NRCIB PB1 showed significantly greater inhibition against *X. translucens* NRCIB X6 (average ZOI 30.28 ± 0.25 mm) compared to *X. arboricola* strains (average ZOI ranged from 16.43 ± 0.58 mm to 20.68 ± 0.33 mm), but *Bacillus* sp. NRCIB B2 was not effective against *X. translucens* NRCIB X6 and inhibited colony growth of *X. arboricola* strains (average ZOI ranged from 21.33 ± 0.24 mm to 30.3 ± 0.46 mm).

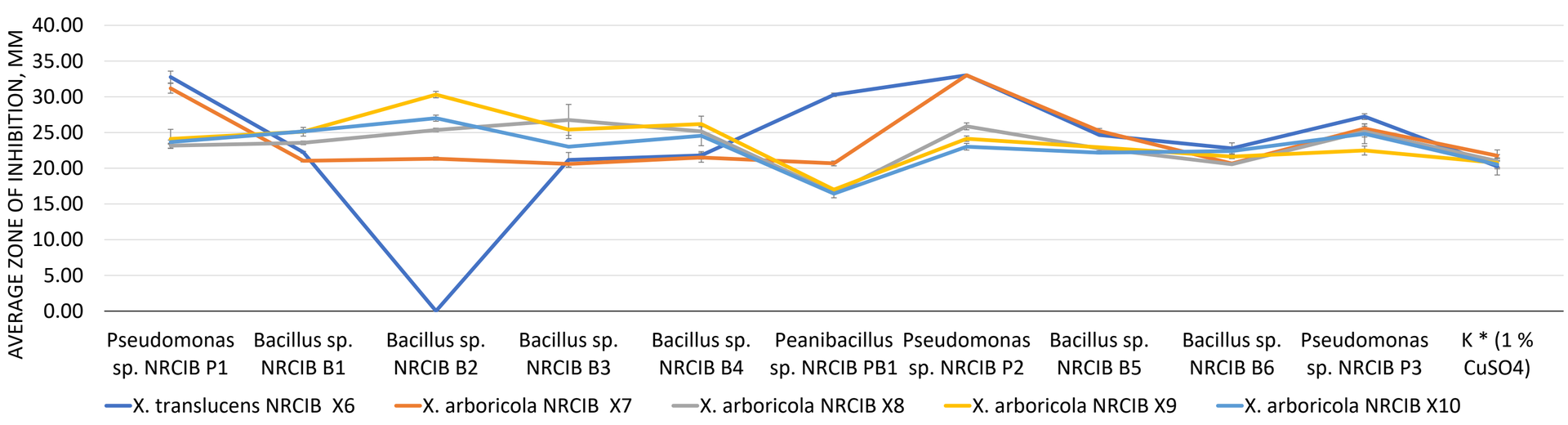


Fig. 3. Antibacterial activity of endofitic bacterial strains against *Xanthomonas* spp. strains

Fig. 4. The agar well diffusion method

Acknowledgements

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Literature:

[1] Schaad N. W. *et al.*, 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd edition, APS, Saint Paul, Minnesota, USA. [2] Young J. M. *et al.,* 2008. Syst. Appl. Microbiol., Vol. 31(5), p. 366-377. [3] Hajri A. *et al.,* 2012. Appl. Environ. Microbiol., Vol. 78, p. 371-384.