

Identification of phytopathogenic *Agrobacterium* spp. in Serbia

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INTRODUCTION

The genus *Agrobacterium* consists of Gram-negative, soil-borne bacteria, both pathogenic and non-pathogenic for plants. Members of the genus *Agrobacterium* are ubiquitous components of the soil microflora, with vast majority of saprophytic bacteria, surviving primarily on decaying organic matter. However, several species of agrobacteria cause neoplastic diseases in plants. Pathogenic strains include bacteria causing crown gall and hairy-roots diseases. Crown gall disease could cause fatal infection of young plants, and it is related to reduction in crop yield. In this study polymerase chain reaction (PCR) has been used for identification and detection of *Agrobacterium* spp. isolated from sour cherry, plum, blackberry, and grapevine.

MATERIAL AND METHODS

The bacteria were isolated from collected samples of diseased sour cherry, plum, blackberry, and grapevine plants using young galls. Pathogenicity of the strains isolated from tumors was tested on sterilized and aseptically cut carrot disks by inoculation with bacterial suspension. The presence of galls was checked after four weeks. Total genomic DNA was prepared by using a modification of the procedure of Ausubel et al. (1992). PCR was conducted using primers specific for detection of tumorigenic agrobacteria (complementary to the *tms2* gene) with the following sequence: *tms2F1* (5' TTT CAG CTG CTA GGG CCA CAT CAG 3') and *tms2R2* (5' TCG CCA TGG AAA CGC CGG AGT AGG 3') where the expected PCR products are 617 base pairs.



Figure 1. Crown gall on plum



Figure 2. Crown gall on sour cherry



Figure 3. Crown gall on grapevine

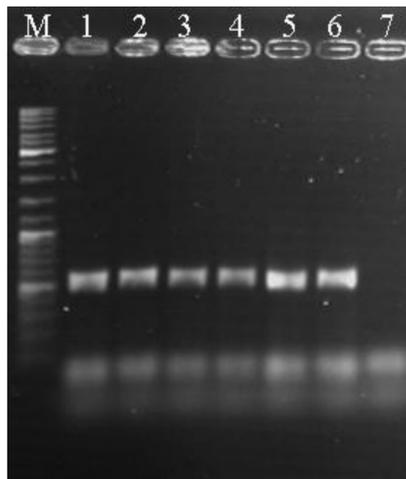


Figure 5. Amplification of 617 bp PCR products with DNA of following *Agrobacterium* strains: Lane 1 Positive control strain CFBP 2621 (*A. vitis*); Lane 2 Positive control strain CFBP 4442 (*A. tumefaciens*); Lane 3 *Agrobacterium* sp. from sour cherry; Lane 4 *Agrobacterium* sp. from plum; Lane 5 *Agrobacterium* sp. from blackberry; Lane 6 *Agrobacterium* sp. from grapevine; Lane 7 Negative control; Lane M Molecular weight marker 100 bp ladder



Figure 4. Crown gall on blackberry

RESULTS

Infected plants had crown on the roots and crowns of diseased plants near the soil surface. In early stages of symptom development, galls were spherical with rough and spongy consistency. With age they become brown, woody knots. The plant may show water stress and nutrient deficiency symptoms since the movement of water and nutrients through the plant is disrupted. All tested strains caused small, green tumors on carrot disks three weeks after inoculation. No changes were observed on carrot slices inoculated with water. The appropriate amplification bands were obtained after amplification with primers for the *tms2* gene. Using *tms2F1* and *tms2R2* primers, 617 bp PCR products specific for tumorigenic *Agrobacterium* strains were detected. Therefore, we confirmed that the strains isolated from tumors in sour cherry, plum, blackberry, and grapevine plants were tumorigenic *Agrobacterium* strains.

DISCUSSION

The purpose of this study was to identify different virulent *Agrobacterium* spp. strains from different hosts using pathogenicity test and PCR identification. The bacteria that induce crown gall are responsible for great losses, first of all in nursery production of fruit trees and grapevines worldwide. *Agrobacterium* spp. were diagnosed using conventional methods based on the isolation on selective media, followed by pathogenicity tests on carrot disks. PCR amplifications were conducted with specific sets of primers and the presence of *Agrobacterium* spp. on sour cherry, plum, blackberry, and grapevine was confirmed in Serbia.