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STUDY OF THE ANTIBACTERIAL ACTIVITY OF BACTERIAL STRAINS FROM GENUS *BACILLUS* AGAINST THE PHYTOPATHOGENIC *XANTHOMONAS VESICATORIA*

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Abstract

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The aim of this study was to determine the activity of 17 newly isolated strains from genus *Bacillus* against the phytopathogenic bacterium *Xanthomonas vesicatoria*. These strains were cultivated in liquid nutrient medium for 36-48 hours to produce antibacterial substances. The antibacterial activity was tested by the agar diffusion method. It was determined that 7 of the tested strains showed antibacterial activity against the test phytopatogen.

Key words: antibacterial activity, *Xanthomonas vesicatoria*, phytopathogenic bacteria

Introduction

Xanthomonas is a typical genus of plant pathogenic bacteria, which occurs in many climatic regions (Kizheva, 2011). *Xanthomonas vesicatoria* is a typical representative of the genus and it is popular phytopathogenic bacterium that causes one of the most serious diseases on vegetables and is distributed via seeds, irrigation water, wounds, residual contamination in soil (Chupp, 2006). This bacterium affects many types of hosts, including citrus, beans, cabbage (Chupp, 2006) grapes, cotton, and rice. *Xanthomonas vesicatoria* causes citrus cankers and black rot, and affects many commercial plants. Typical symptoms of the disease include lesions on the leaves, fruit, and stems as well as twig dieback (Rat et al., 1991).

Host infection by *Xanthomonas vesicatoria* causes V-shaped chlorotic to necrotic foliar lesions, vascular blackening, wilting, stunted growth, and stem rot symptoms (Alvarez, 2000). The darkening of vascular tissues following bacterial invasion gives the black rot disease its name. Black rot caused by the bacterium *Xanthomonas vesicatoria*, is considered the most important and most destructive disease of crucifers, infecting all cultivated varieties of brassicas worldwide (Alvarez, 2000).

Bacterial spot caused by *Xanthomonas vesicatoria* is one of the most devastating diseases of pepper and tomato. The disease occurs worldwide where pepper and tomato are grown in warm, moist areas. Bacterial spot is a serious disease on tomato and pepper in the vegetable producing regions of Bulgaria and Macedonia. It is a major problem on tomato in Bulgaria and on pepper in Macedonia (Bogatzevska, 2007).

Prevention and control of phytopathogen bacterium *Xanthomonas vesicatoria*, which is based on the antagonistic relationship, is extremely important (Jones, 2003)

The aim of the study was to determine the activity of newly isolated strains from genus *Bacillus*: T (1, 2, 3, 4, 5, 10, 11, 12, 14, 15, 16, 17, 18), V (6, 7, 8, 13) and one strain from species *Bacillus pasteurii* against the phytopathogenic bacterium *Xanthomonas vesicatoria*.

Materials and Methods

Microorganisms, cultures and conditions

Seventeen newly isolated strains from genus *Bacillus* (T-1, 2, 3, 4, 5, 10, 11, 12, 14, 15, 16, 17, 18, V- 6, 7, 8 and 13)

and one strain from species *Bacillus pasteurii* were selected for this study. One strain from species *Xanthomonas vesicatoria* (kindly provided from Department of Microbiology, Faculty of Biology, Sofia University) was used as test phytopathogen. All strains were cultivated in Nutrient broth (Difco) at 28°C for 24 hours. Stock cultures in Nutrient broth supplemented with 20% v/v glycerol were stored at –20°C.

Antimicrobial activity determination

Cell-free supernatant (CFS), obtained after 24 h cultivation in Nutrient broth of each studed strain, was assayed for inhibitory activity against a phytopatogenic strain from the species *Xanthomonas vesicatoria*.

Overlay of test strain (10^6 CFU/ml) was prepared on agar plates and was allowed to dry. Wells (8 mm) were made in the agar plates and CFS (100 µl) was placed in the wells and allowed to diffuse through the agar for 20–40 min at room temperature prior to incubation for 24 h. After which inhibitory zones were measured. All experiments were prepared in triple.

Results and Discussion

In this study, the antibacterial activity of selected strains was estimated by the agar diffusion method. The results for the active strains are presented in Figure 1.

It can be observed (Figure 1) that the *Bacillus* strains T 2, T 10 and T 18 have the greatest activity of all tested strains (T 2 – 22 mm, T 10 – 22 mm and T 18 – 17 mm) against the phytopathogenic bacteria. Strains T 4, T 5, T 17 and V8 (T 4 – 10 mm, T 5 – 5 mm and T 17- 3 mm, V8 – 8 mm) are less active and strains T 1, 3, 11, 12, 14, 15, 16 do not show any antibacterial activity against

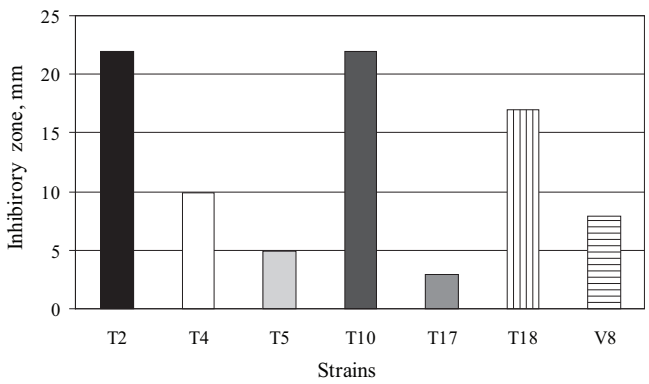

Fig. 1. Antibacterial activity of CFS of active bacterial strains T 2, T 4, T 5, T 10, T 17, T 18, V8 against *Xanthomonas vesicatoria*

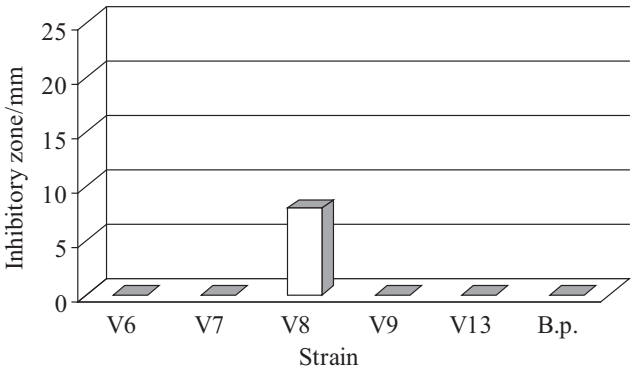

Fig 2. Antibacterial activity of CFS of bacterial strains from V6 to V13 and Bacillus pasteurii against *Xanthomonas vesicatoria*

the phytopathogen *Xanthomonas vesicatoria* (Figure 2). From the obtained results can be concluded that among all tested strains T 2, T 10 and T 18 show the strongest activity against phytopathogenic *Xanthomonas vesicatoria*. Therefore, the biochemical characterization and identification of these newly isolated strains T 2, 10 and 18 was important and necessary as a prerequisite for their potential applications in the biological farming. According to the biochemical tests (BIOLOG®) and the molecular characterization they belong to the species *Bacillus subtilis* and *Bacillus licheniformis* (unpublished data). The effect of these strains on the phytopathogenes is probably because some strains from the *Bacillus* genus produce substances with antimicrobial activity including antibiotics (Chung et al., 2008). Some of the substances produced by the genus *Bacillus* show antifungal and/or antibacterial activity against a great number of phytopathogenic microorganisms (Tabbene et al., 2009).

The high proportion of antimicrobial compound producing strains may be associated with ecological role, playing a defensive action to strains into an established microbial community (Strahl et al., 2002)

Conclusion

In seven of the studied strains (T 2, T 4, T 5, T 10, T 17, T 18 and V8) was detected antibacterial activity against the test phytopathogen. Three of them showed strongest activity (T 2 – 22 mm, T 10 – 22 mm and T 18 – 17 mm).

Until now there was little information available concerning the action of the genus *Bacillus* against the phytopathogenic bacteria *Xanthomonas vesicatoria*. The obtained data on the antimicrobial activity of strains

of the genus *Bacillis* against phytopathogenic bacteria *Xanthomonas vesicatoria* are interesting both from scientific and practical point of view. These strains can be used as agents for the treatment of infectious symptoms in different plants and may have wide application in plant production and agriculture.

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