Bulgarian Journal of Agricultural Science, 19 (2) 2013, 80–82
Agricultural Academy

IDENTIFICATION OF *XANTHOMONAS* STRAINS FROM TOMATO AND PEPPER AND THEIR SENSITIVITY TO ANTIBIOTICS AND COPPER

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Abstract

KIZHEVA, Y., T. VANCHEVA, P. HRISTOVA, M. STOYANOVA, M. STOJANOVSKA, P. MONCHEVA and N. BOGATZEVSKA, 2013. Identification of *Xanthomonas* strains from tomato and pepper and their sensitivity to antibiotics and copper. *Bulg. J. Agric. Sci.,* Supplement 2, 19: 80–82

This study aims to characterize 136 *Xanthomonas* strains causing bacterial spot of tomato and pepper from different regions in Bulgaria and Macedonia. Three species were identified by PCR with species-specific primers. *Xanthomonas vesicatoria* affects tomato and pepper and is the dominant species in Bulgaria on tomato while *Xanthomonas euvesicatoria* affects only pepper. This is the first report of *Xanthomonas gardneri* of tomato and *X. euvesicatoria* of pepper in Bulgaria. Amylase activity and ability to utilize *cis*-aconitate of the strains vary and cannot be used for species differentiation. The tested xanthomonads were sensitive to streptomycin. Copper ions in generally used concentrations in practice were effective only against pepper strains.

Key words: Xanthomonas vesicatoria, Xanthomonas euvesicatoria, Xanthomonas gardneri, bacterial spot, tomato, pepper

Introduction

Bacterial spot of pepper and tomato plants was first described of tomato in 1936 in Bulgaria (Kovachevski, 1936) and in 1999 in Macedonia (Mitrev and Pejcinovski, 1999) and is one of the economically most important diseases in these two countries (Mitrev and Pejcinovski, 1999; Bogatzevska et al., 2007). The taxonomy of the causal agents was subjected to many reclassifications but now the pathogens belong to four species *Xanthomonas euvesicatoria*, *Xanthomonas vesicatoria,* and *Xanthomonas gardneri* and *Xanthomonas perforans* (Jones et al., 2004). Disease control is hard and mainly based on agricultural practices and copper or streptomycin-based chemicals. However, the occurrence of resistant strains significantly reduces their effectiveness (Obradovic et al., 2004). Clarifying the species structure in the populations causing bacterial spot in Bulgaria and Macedonia is important for effective disease control.

The aim of this study is identification of the *Xanthomonas* species causing bacterial spot on tomato and pepper plants in Bulgaria and Macedonia according to the current classification and testing their sensitivity to antibiotics and copper as means of control.

Materials and Methods

Bacterial strains. 136 pathogenic *Xanthomonas* strains isolated in the period 1987-2010 were used in this study. 89 strains were isolated from tomatoes with bacterial spot (62) and asymptomatic weeds in tomato fields (27) in Bulgaria. 47 strains originated from diseased pepper plants from Bulgaria (30) and Macedonia (16). The type cultures (NBIMCC) *X. vesicatoria* 2427*, X. euvesicatoria* 8731, *X. perforans* 8729 and *X. gardneri* 8730 were used.

Identification. Genomic DNA was extracted by a DNA isolation kit (STS Ltd). PCR was performed in a thermal cycler Techne TC-312 using species-specific primers for *X. euvesicatoria* (Bs-XeF/Bs-XeR), *X. vesicatoria* (Bs-XvF/Bs-XvR),

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X. gardneri (Bs-XgF/Bs-XgR), and *X. perforans* (Bs-XpF/Bs-XpR) designed by Koenraadt et al. (2009). Amplification was carried out in a total volume of 25 µl containing (final concentrations) 2.5 µl of 10x PCR buffer; 1.5 mM $MgCl_2$; 0.04 mM of each dNTP; 0.4 U *Taq* ДНК polymerase; 10 pmol of each primer, 1 µl of template DNA, under the following reaction conditions: a denaturation step at 94°C for 5 min, followed by 25 cycles at 94°C for 30 s, 64°C for 30 s, and 72°C for 30 s, and a final step at 72°C for 7 min (Koenraadt et al., 2009). The PCR products were separated electrophoretically in 1.5% agarose gel in TBE buffer for 30 min at 100 V, stained with EtBr and visualized under UV light. DNA 100 bp marker (Fermentas) was used.

Characteristics. Identification results were compared to amylase activity test and utilization of *cis*-aconitate data from a previous research (Kizheva et al., 2011).

Resistance to copper and antibiotics. Resistance to copper and antibiotics was studied by the disk-diffusion method. Copper ions (Cu^{2+}) were used as 50 µl solution in concentrations ($g.kg^{-1}$) 1, 2, 3, 4, 5. Antibiotics from different groups were used as impregnated filter discs (µg/ml, water solutions) as follows: gentamycin (50), polymixin B sulphate (50), streptomycin sulphate (50), lincomycin hydrochloride (10), vancomycin (50), kanamycin (50), bacitracin (50), ampicillin (50), cephazoline (10), tetracycline (30), sulfamethoxazole-trimethoprim (23.75/1.25) and chloramphenicol 20 µg/ml, absolute alcohol solution.

Results

Identification. According to the species-specific PCR 32 strains from pepper (20 from Bulgaria and 12 from Macedonia) were identified as *X. euvesicatoria*, 97 strains (83 from tomato and 14 from pepper) – as *X. vesicatoria*, and 5 strains from tomato – as *X. gardneri* (Figure 1).

Characteristics. All *X. euvesicatoria* strains were amylase negative and all *X. vesicatoria* were amylase positive. Their ability to utilize *cis*-aconitate varied. The *X. gardneri* strains were negative for both tests.

Resistance to antibiotics. All strains were resistant to lincomycin but sensitive to tetracycline and with few exceptions to kanamycin and cephazoline. Generally, gentamycin and streptomycin are also effective against the pathogens with the isolates from pepper being completely sensitive to streptomycin (Figure 2). The differences between the strains isolated from tomato and weeds, as well as between the Bulgarian and Macedonian isolates from pepper were not statistically significant.

Resistance to copper. With the increase of the concentration of Cu^{2+} the sensitivity of the strains increases (Figure 3).

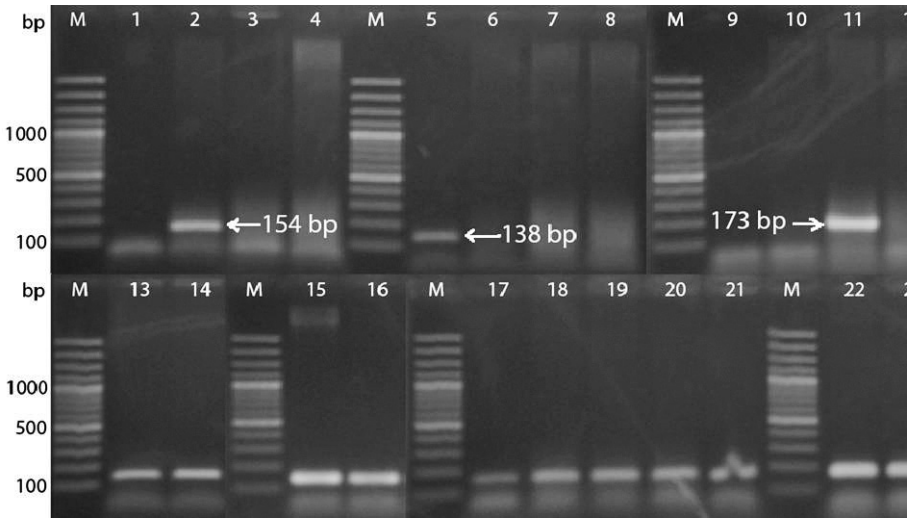


Fig. 1. PCR with species-specific primers for *X. vesicatoria*, *X. euvesicatoria*, and *X. gardneri*. Lines (primers are shown in brackets): M - DNA marker; 1-*X. vesicatoria* NBIMCC 2427 (Bs-XgF/Bs-XgR); 2-*X. gardneri* NBIMCC 8730 (Bs-XgF/Bs-XgR); 3-*X. euvesicatoria* NBIMCC 8731 (Bs-XgF/Bs-XgR); 4-*X. perforans* NBIMCC8729 (Bs-XgF/Bs-XgR); 5-*X. vesicatoria* NBIMCC 2427 (Bs-XvF/Bs-XvR); 6-*X. gardneri* NBIMCC 8730 (Bs-XvF/Bs-XvR); 7-*X. euvesicatoria* NBIMCC 8731 (Bs-XvF/Bs-XvR); 8-*X. perforans* NBIMCC 8729 (Bs-XvF/Bs-XvR); 9-*X. vesicatoria* NBIMCC 2427 (Bs-XeF/Bs-XeR); 10-*X. gardneri* NBIMCC 8730 (Bs-XeF/Bs-XeR); 11-*X. euvesicatoria* NBIMCC 8731 (Bs-XeF/Bs-XeR); 10-*X. perforans* NBIMCC 8729 (Bs-XeF/Bs-XeR); 13,14-*X. vesicatoria* strains from tomato (Bs-XvF/Bs-XvR); 15,16-*X. vesicatoria* strains from pepper (Bs-XvF/Bs-XvR); 17-21-*X. gardneri* strains from tomato (Bs-XgF/Bs-XgR); 22,23-*X. euvesicatoria* strains from pepper (Bs-XeF/Bs-XeR)

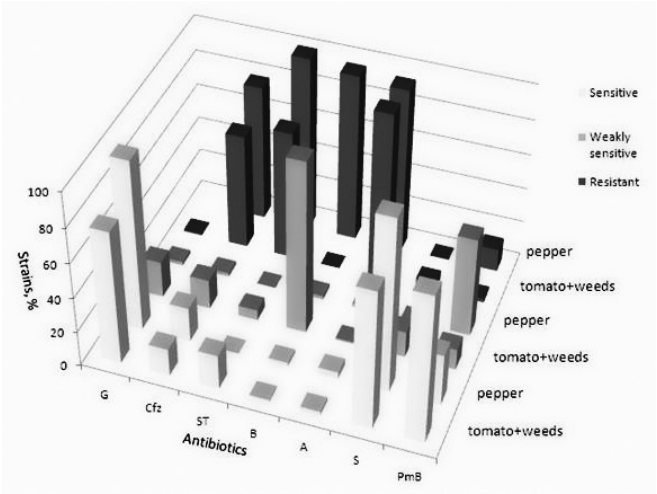


Fig. 2. Sensitivity to antibiotics of *Xanthomonas* strains isolated from weeds

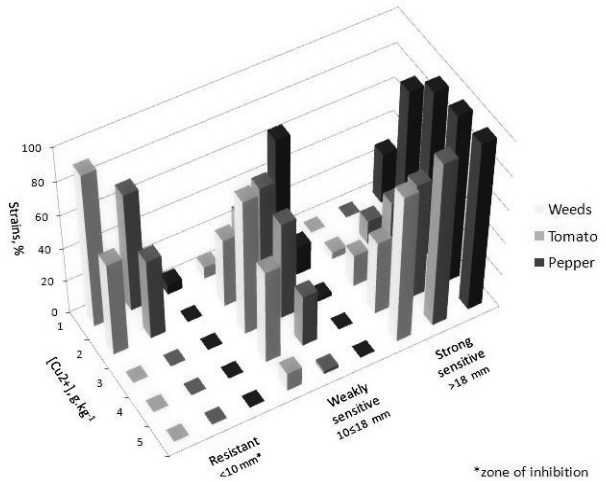


Fig. 3. Sensitivity to Cu^{2+} of *Xanthomonas* strains isolated from weeds, tomato and pepper

No great difference exists between the strains of Bulgarian and Macedonian origin. Resistant strains can be observed by the use of Cu^{2+} up to 2 $g.kg^{-1}$. Strains from pepper are significantly more sensitive than the tomato strains. Resistant pepper strains are only 6% at the minimal Cu^{2+} concentration used compared to 71% of resistant tomato strains. At that concentration also 30% of all pepper strains show strong sensitivity compared to 0% of the other strains. Least sensitive are the strains from weeds. Differences among the species were not observed.

Discussion

Bacterial spot of tomato in Bulgaria is caused by two species – *X. vesicatoria* and *X. gardneri.* For the tested period 1987-2010 *X. vesicatoria* is the dominant species, *X. gardneri* emerges as a pathogen of tomato in the country in the last years. Bacterial spot of pepper in Bulgaria and Macedonia is caused by *X. vesicatoria* and *X. euvesicatoria.* This study is the first report of *X. gardneri* of tomato and *X. euvesicatoria* of pepper in Bulgaria. Amylase activity and ability to utilize *cis*-aconitate of the strains vary and cannot be used for species differentiation on phenotypic level. The strains are sensitive to streptomycin as well as to some other broad-spectrum antibiotics. Copper ions in the generally used concentrations in practice were effective only against pepper strains. These results can be explained by the expansive use of copper-based chemicals in tomato fields and by the regulated use of streptomycin for agricultural purpose under Regulation (EC) № 1490/2002 of EU, p. 16.

Conclusion

The population causing bacterial spot of tomato and pepper consists of *X. vesicatoria, X. euvesicatoria,* and *X. gardneri* in Bulgaria and of *X. vesicatoria* and *X. euvesicatoria* in Macedonia. Copper-based chemicals can be effectively used only for control of bacterial spot of pepper.

Acknowledgements

This study was supported by the Scientific Foundation of Sofia University, Project No 059/05.04.2012.

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