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Реализира Македонска Трибина - Скопје
(тираж 500)

IN MEMMORIAM
Dr Vasil Kocevski 1950-2001

ВО СПОМЕН НА
Др Васил Коцевски 1950-2001

На нашиот незаборавен,
Почитуван научен работник, колега, соработник,
Драг другар и пријател - Васил Коцевски.

ЈНУ ИНСТИТУТ ЗА ЈУЖНИ ЗЕМЈОДЕЛСКИ КУЛТУРИ - СТРУМИЦА

To our unforgettable,
Respectful, scientific worker, colleague, collaborator,
Dear companion and friend -Vasil Kocevski.

INSTITUTE OF SOUTHEREN CROPS - STRUMICA

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DEPARTMENT OF PROTECTION OF THE PLANTS FROM DESEASES, PESTS AND WEEDS

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Оригинален научен труд
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RACES OF XANTHOMONAS VESICATORIA ISOLATED FROM PEPPER IN MACEDONIA

MITREV S., KAROV I. AND SPASOV D.

2001, Opatija, Croatia, 37th Croatian Symposium on Agriculture. Summary

Summary

From 1994 till 1997, few hundreds of strains were isolated from different samples of pepper (*Capsicum annuum* L.) collected from commercial fields and home gardens throughout Rep. of Macedonia. Bacteria were isolated on semi selective medium from infected plants and identified by a combination of morphological, pathological, biochemical, nutritional and physiological tests. In this study 50 domestic and 3 control strains (races: E-3; 93-1; 71-21 from Florida, USA) were used. All these properties of Macedonian strains were completely same with characteristics of compared control strains, without any significant differences. Additional characterization of strains showed that all were negative for amylolytic activity and ability to utilize dextrin. Those strains belong to type A of *X. vesicatoria* and none of them belong to type B.

Strains of *X. vesicatoria* were identified to race based on the response to infection of a set of near-isogeneic pepper lines derived from and including Early Californian Wonder (ECW): ECW-10R, ECW-20R and ECW-30R containing the resistance genes bs1, Bs1, Bs2 and Bs3, respectively. The majority of the strains were identified as race P0 - 40%, race P2 - 40%, and the remaining 20% could not be identified to race using these differential pepper lines.

All of investigated strains were sensitive to copper sulfate (200µg/ml) and streptomycin sulfate (100µg/ml).

Key words: pepper, bacteria, *Xanthomonas*, races, characteristics, pathogenicity.

РАСИ НА БАКТЕРИЈАТА *XANTHOMONAS VESICATORIA* ИЗОЛИРАНА ОД ПИПЕРКА ВО МАКЕДОНИЈА

Митрев С., Каров И. и Спасов Д.

2001, ОПАТИЈА, ХРВАТСКА, 37 ХРВАТСКИ СИМПОЗИУМ НА АГРОНОМИТЕ, СТР. 348 SUMMARY

Краток извадок

Од 1994 до 1997 година неколку стотини изолати се добиени од повеќе различни примероци на заболена пиперка (*Цаѝсицум аннуум* Л.) добиена од различни делови во Република Македонија. Бактериите беа изолирани на полуселективни вештачки подлоги и нивната идентификација

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беше извршена со помош на различни морфолошки, патогени, биохемиски, одгледувачки и физиолошки тестови.

Во оваа студија беа користени 50 домашни изолати и 3 контролни изолати од Флорида-САД (раси: Е-3; 93-1; 71-21). Сите испитувани карактеристики на домашните изолати во потполност се идентични со контролните изолати од странство, без некоја сигнификантна разлика. Испитувањата покажаа дека нашите изолати се нгативни при амилолитичката активност и способноста да користат декстрин. Изолатите припаѓаат на типот А од бактериите *X. vesicatoria* и ниедна од нив не припаѓаше на типот Б.

Изолатите на бактеријата *X. vesicatoria* се идентификувани во раси на база на манифестираните реакции кај пиперката од сортата калифорниско чудо (ECW) и блиските изогени линии добиени од неа (ECW-10R, ECW-20R и ECW-30R) кои ги содржат гените за отпорност: bs1, Bs1, Bs2 и Bs3.

Поголемиот дел од испитуваните изолати се идентификувани како раса П0-40%, П2-40% и преостанатите 20% не се детерминирани како некој од познатите раси.

Сите испитувани раси се осетливи на бакар сулфат (200µg/ml) и стрептомицин сулфат (100µg/ml).

1. Introduction

Xanthomonas vesicatoria is a causal agent of bacterial spot of pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum*) in many countries around the world. This bacterium present in all regions where these plants are grown, especially under conditions of high temperature and abundant rainfall. The disease reduces plant growth, fruit yield, and quality, bacterial spot of pepper is typically initiated in the field under hot and humid conditions, after infested soil and plant debris are splashed by wind or rain into plants. Bacterium is capable of surviving in artificially infested field soil for at least 18 months in the absence of host plants. Several soil borne xanthomonads, including *X. vesicatoria*, have been implicated in the occurrence of plant disease (O'Garro et al., 1997)

In Macedonia, pepper (*Capsicum annuum* L.) is traditional crop cultivated on about 9.000 ha of open field, plastic tunnels and greenhouses. Bacterial spot was an important pepper disease in many production areas in the country and cause a significant loses in the field (Mitrev et al., 1998). The severity of occurrence of the bacterial spot depends of "pathogen free" seeds and transplants, sanitation, crop rotation, resistant cultivars, and chemical applications (Sahin et al., 1996) .

The losses caused by this bacterium in Macedonia were different every year. There was estimated about 10-20%, but some year (July and August of 1995), the damages were extremely higher as a result of favorable climatic conditions, warm and rainy summer (Mitrev et al, 1998).

The strains of *Xanthomonas vesicatoria* (sin. *Xanthomonas campestris* pv. *vesicatoria* Young et al., 1996) used in this work were isolated from pepper plants (*Capsicum annuum* L.) were identified and characterized according to race, sensitivity to copper sulfate and streptomycin sulfate, pathogenical, morphological, biochemical, and physiological characteristics.

2. Materials and methods

2.1. Isolation of the pathogen

The bacteria were isolated from the spots of pepper plants, surveys in open-field, performed during June - September of 1994-1997 from different production areas in Macedonia. Collected leaves were surface-disinfected, cut small pieces of leaf tissue from margins of spots with sterilized razor blade and comminuted in sterile deionized water (SDW). Suspension was streaked on plate's surface of nutrient agar (NA) medium or yeast dextrose carbonate (YDC) medium (Schaad, 1994). Plates were incubated at 26ºC for 48 h. Representative round, convex, mucoid, yellow colonies on YDC, or small, yellow colonies on NA were selected and purified by repeated restreaking on YDC. These pure cultures were preserved in tube on YDC slope medium on 4ºC for short-term storage and under oil for long-term storage (Schaad, 1994).

2.2. Identification of the strains

Identification of 50 bacterial strains was performed according to the following biochemical and nutritional tests described by Lelliot et al., 1987; Bouzar et al. (1994), Schaad (1994) and Klement et al. (1990): Potassium hydroxide solubility test (3%), Gram's stain; oxidative/fermentative metabolism; sodium chloride tolerance (1%); catalase, oxidase, aminopeptidase and urease activities; nitrate reduction; hydrogen sulfide production from cysteine; tween 80, aesculin, starch, and gelatin hydrolysis; growth at 35°C; triphenyl tetrazolium chloride tolerance (0,1% and 0,02%); and acid production from arabinose, cellobiose, trehalose, sucrose, glucose, lactose, mannose, ramnose, rafinose, fructose, galactose, xylose, dextrin, dulcit and manitol. The tests were repeated at least twice with three replications per test.

2.3. Race determination

X. vesicatoria races were differentiated using the pepper cultivar Early Californian Wonder (ECW) and a set of near-isogeneic pepper lines derived from and including Early Californian Wonder (ECW): ECW-10R, ECW-20R and ECW-30R containing the resistance genes bs1, Bs1, Bs2 and Bs3, respectively (Minsavage et al., 1990). Pepper race 1 (strain 71-21), race 2 (strain E-3), and race 3 (strain 93-1) of *X. vesicatoria* were used as reference cultures.

Investigated strains were suspended in sterile, distilled water, adjusted to a concentration of approximately 10^8 CFU/ml, and infiltrated into fully expanded leaves of the pepper lines. Plants were incubated for 48 h under greenhouse conditions and HR was recorded within 24 to 48 h. Each strain was tested three times (Sahin et al., 1996).

2.4. Physiological characterization

Sensitivity of strains to copper and streptomycin were assayed in sucrose peptone agar (SPA) medium amended with either $CuSO_4 \cdot 5H_2O$ (200µg/ml) or streptomycin sulfate (100µg/ml) as described by Buonaurio et al., (1994). Plates were incubated at 26°C for 48 h and the presence or absence of growth was recorded. Bacterial strains that grew on SPA medium, amended with either copper sulfate or streptomycin sulfate at the concentrations reported above, were considered resistant to copper or streptomycin, respectively.

3. Results

3.1. Field survey and identification of the pathogen

Symptoms of bacterial spot were present on pepper leaves on all of the places were visited in different regions in Macedonia. Disease incidence ranging from 20 to 80% depending of pepper cultivar. Disease symptoms on pepper fruits were found

only in fields located in production area of Kumanovo. Few hundred strains were isolated from more than hundred pepper samples collected in 10 different regions in Macedonia.

Biochemical and nutritional tests presented that all investigated bacterial strains grew on YDC medium and produced round, convex, mucoid, yellow colonies, and they were Gram-negative, aerobic, catalase- and aminopeptidase-positive, oxidase- and urease-negative. They were hydrolyzed aesculin, gelatin, tween 80 but did not starch. Grew at 35°C, produce hydrogen sulfide from cysteine and did not reduce nitrates; produced acid from arabinose, glucose, mannose, sucrose, fructose, galactose and xylose, but did not produced acid from cellobiose, trehalose, lactose, ramnose, rafinose, dextrin, dulcit and manitol. They did not showed tolerance of 1% NaCl, 0,1 and 0,02% TTC.

All bacterial strains were induced a HR on tobacco plants after 24 h and they were pathogenic on pepper cultivar ECW.

On the bases of biochemical, nutritional, and pathogenicity tests, it was confirmed that all bacterial strains belonged to *Xanthomonas vesicatoria.*

3.2. Race, copper and streptomycin sensitivity determination

Strains of *X. vesicatoria* were identified to race based on the response to infection of a set of near-isogeneic pepper lines derived from and including Early Californian Wonder (ECW): ECW-10R, ECW-20R and ECW-30R containing the resistance genes bs1, Bs1, Bs2 and Bs3, respectively. The majority of the strains were identified as race P0 - 40%, race P2 - 40%, and the remaining 20% could not be identified to race using these differential pepper lines.

All investigated bacterial strains did not presented growth on SPA medium amended with 200µg/ml copper sulfate and were therefore considered not resistant to copper sulfate. Also, none of the strains grew on SPA medium containing 100µg/ml of the streptomycin sulfate and were therefore considered not resistant to the antibiotic.

4. Discussion

Pepper (*Capsicum annuum* L.) cultivation has a significant economic meaning in Rep. of Macedonia. Pepper plants were attacked by a great number of pathogens of various nature. Besides of the viruses diseases, which have the greatest meaning, fungi and phytopathogenic bacteria like *Pseudomonas syringae* pv. *syringae*, *Xanthomonas vesicatoria* and *Erwinia carotovora* subsp. *carotovora* attacked the pepper plants, characteristically about this region. From all bacteria, which attacked pepper plants in the open field, *X. vesicatoria* had the greatest meaning (Mitrev, 1998).

Recently this pathogen was mentioned in important pepper production arias in Balkan region, it was noted as very significant parasite and significant factor on ruin the pepper plants (Arsenijević, 1980; Balaž Jelica, 1994; Bogatzevska et al., 1996).

The characteristic symptoms that were appeared in the field on the older plant leaves were suspected to have a bacterial origin. In favorable climate condition, rainy and hot days, the disease was spread very fast and all the date obtained in this study proven that our strains isolated from pepper belonged to *X. vesicatoria*.

A lot of authors cited the bacterium *X. vesicatoria* as a causal agent for pepper plants in the field and on pepper seedlings, but we found less date in our region about the pathogenic changes of pepper seedlings. The most susceptible cultivar in Macedonia on this bacterial disease was *kurtovska kapija* (*Capsicum annuum*).

The morphological, pathological, biochemical and physiological tests were confirmed that the characteristics of our strains were similar with the characteristics of control strains of bacteria *X. vesicatoria* 71-21, E-3 and 93-1. Additional characterization of 50 strains showed that all were negative for amylolytic suggesting and ability to utilize dextrin. Those strains belong to type A of *X. vesicatoria* and none of them belong to type B (Bouzar, et al., 1994; Schaad, 1994; Sahin, et al. 1996; Lelliot et al., 1987; Jones et al., 1986; Arsenijević, 1988).

In this study the bacterial strains of *X. vesicatoria* obtained from diseased pepper plants showed that pepper races P0 and P2 were dominant in Rep. of Macedonia. The predominant pepper races in the world were P1, P2, and P3. The races P0, P2 and P4 are unusual pepper races, which had previously been found only in USA (North Carolina and Mexico and Florida), Australia, Caribbean and Central America (Bouzar, et al., 1994). In Europe the pepper races were determined only in Italy (central and southern part of Italy), and there were found races 1 (39%), 2 (16%) and 3(45%) (Buonario et al., 1994). Previously, there was not any indication for proving the pepper races of *X. vesicatoria* in Rep. of Macedonia.

Streptomycin sulfate and copper sulfate resistant strains were not detected in this study. Two plausible explanations for streptomycin sensitive strains are that the use of them is not permitted in Macedonia, or that the pathogen was introduced on seed, which had been produced in countries where the antibiotics are not used or they are permitted.

The use and deployment of resistant cultivars to the races of *X. vesicatoria* may provide the best disease-management strategy.

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