Antibiotic Resistance of *Clavibacter michiganensis* subsp. *sepedonicus* Georgian Isolates

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Abstract

Clavibacter michiganensis subsp. *sepedonicus* (*Cms*) is a causative pathogen of potato bacterial ring rot. This devastative pathogen poses a constant threat to potato growers worldwide. It was regarded as a disease of cool northen regions but is currently spreading within Europe, including some southern areas (Crete, Cyprus and Spain). The pathogen damages vascular system of tubers, stems and leaves with slow or no development of symptoms on plants. Management of ring rot of potato are especially difficult in storage places, where the pathogen, being in a latent form, may infect almost all tubers. The aim is the study of antibiotic resistance of potato ring rot causative *Cms* geographically and temporarely representative isolates in Geogia. *Cms* were initially detected in collected in different regions of Georgia both symptomatic and asymptomatic potato tubers by specific molecular method. Dozens of pure *Cms* isolates were recovered from these samples and identified by PCR using the pathogen-specific primer set PSA-1 and PSA-R. Relation of 19 virulent strains towards 9 antibiotics: tobramycin, penicillin, trimethoprim, erythromycin, kanamycin, ofloxacin, chloramphenicol, tetracycline and streptomycin by disc-diffusion method have been studied. The isolates demonstrate different susceptibility to the antibiotics, independently they were obtained from symptomatic or asymptomatic tubers.

Keywords: *Clavibacter michiganensis* subsp. *sepedonicus*, potato ring rot, antibiotic resistance

1. Introduction

Clavibacter michiganensis subsp. *sepedonicus* (*Cms*) is a causative pathogen of potato bacterial ring rot. This devastative pathogen poses a constant threat to potato growers worldwide. The pathogen damages vascular system of tubers, stems and leaves with slow or no development of symptoms on plants. The pathogen can latently infect and colonize seed potatoes without expression of symptoms and in this way can survive undetected in high populations over several generations of seed multiplication (Slack, 1987). This pathogen poses a constant threat to potato growers worldwide. Crop losses caused with bacterial ring rot range from 11 to 44% in different countries and it significantly increases during potato storage.

It was regarded as a disease of cool northen regions but is currently spreading within Europe, including some southern areas (Crete, Cyprus and Spain) (Elphinstone, ring_rot_review-pcl_logo-2010.pdf).

Management of the bacterial diseases require complex approaches directed to: host resistance, sanitation and cultural practice. It is especially difficult in storage places, where the pathogen, being in latent form, may infect almost all tubers.

Integration of the above described approaches with the use of chemical bactericides, such as copper compounds and antibiotics as well as biological control agents, when available is important disease management strategy for certain bacterial diseases.

The aim of the article is to study of antibiotic susceptibility of potato ring rot causative *Cms* geographically and temporarily representative isolates in Geogia.

2. Methods

An official EC method for detection and diagnosis of *C. michiganensis* subsp. *sepedonicus* in potatoes (OEPP/EPPO 2006) was used.

Molecular identification of *Cms* isolates was conducted by PCR using pathogen-specific primer set PSA-1 and PSA-R (Pastrik and Rainey 1999).

Bacterial strains applied in the study include: *Cms* isolates from potato tubers collected in potato depots (GE45, 40, 97, 110), agrarian markets (#98, #961) and Georgia's Kazbegi border customs checkpoints. *Cms* Reference strains BPR#527 and NCPPB No1327 were also applied in studies.

In vitro susceptibility of the strains was studied by disk diffusion test (Bauer et al., 1966). The cultured cells $5x10^8$ CFU/ml were evenly spread-plated on 1.5% agar

solidified surface in Petri plates and then allowed to dry for 5 min. Nine specific antibiotics disks were placed on the surface. the plates: tobramycin, penicillin, trimethoprim, erythromycin, kanamycin, ofloxacin, chloramphenicol, tetracycline and streptomycin. After incubation at 28°C for 24 h, the diameters of inhibition zone were evaluated.

3. Results and Discussion

Cms has been detected in collected potato tubers samples either symptomatic or asymptomatic. Pure *Cms* isolates were recovered from samples and confirmed by PCR with the pathogen-specific primer set PSA-1 and PSA-R.

Fig. and Table represents results of *in vitro* susceptibility test for some of *Cms* Georgian strains.

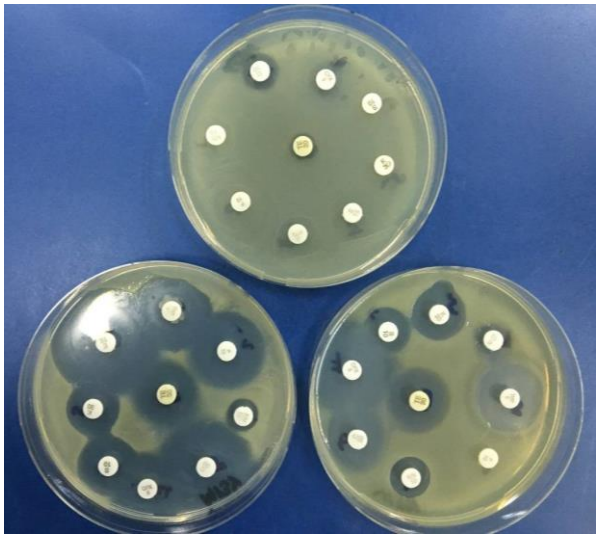

Figure. *In vitro* susceptibility test of *Cms* isolates: GE45(above), GE97 (left) and Ref BRIOR#527 (right).

As seen, *Cms* GE45 differs from other isolates expressing resistance to 7 antibiotics and very weak sensitivity to ofloxacin and chloramphenicol. On the contrary isolate GE97, obtained from asymptomatic, latently infected tubers is susceptible to almost all studied antibiotics, with the least sensitivity to Tobramycin. Reference strain BPR#527 exhibits different susceptibility to studied antibiotics and is resistant to penicillin (Fig). Another Ref. strain NCPPB #2137 is susceptible to all nine antibiotics (Table). *Cms* GE98 is also susceptible to tested antibiotics but to lesser extent. Others, show different susceptibility/resistance spectra towards the nine antibiotics.

Table. Antibiotic susceptibility of *Cms* isolates

Antibiotic	*Cms* GE40	*Cms* GE98	*Cms* GE109	*Cms* 961	*Cms* 906	*Cms* NCPPB #2137
Tobramycin N10	+	2+	+	2+	1+	2+
Penicillin P10	+	+	0	+	0	+
Trimethoprim TMP 55	0	+	0	0	0	2+
Erythromycin T 15	0	+	0	0	0	2+
Kanamycin K30	0	+	0	0	0	2+
Streptomycin S10	0	+	0	0	0	2+
Ofloxacin OFX5	2+	3+	2+	2+	3+	4+
Chloramphenicol C30	2+	3+	2+	2+	3+	3+
Tetracyclin Te30	0	2+	+	1+	1+	3+

4. Conclusion

Cms geographically and temporarely representative isolates in Geogia demonstrate different susceptibility to nine antibiotics, independently they were obtained from diseased or latently infected potato tubers. Most *Cms* strains expressed resistance to trimethoprim erythromycin, streptomycin and kanamycyn.

References

Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. (1966), Antibiotic susceptibility testing by a standardized single disk method, *Am. J. Clin. Pathol*. **45,** 439-496.

Elphinstone, J.G. Bacterial Ring Rot of Potato - The Facts. https://potatoes.ahdb.org.uk/sites/default/files/publication_ upload/ring_rot_review-pcl_logo-2010.pdf.

OEPP/EPPO, Bulletin OEPP/EPPO (2006), **36**, 99–109.

Pastrik K.H., Rainey F.A. (1999). Identification and differentiation of *Clavibacter michiganensis* subspecies by polymerase chain reaction-based techniques. *Journal of Phytopathology* **147,** 687-693.

Slack S. A. (1987), Biology and ecology of *Corynebacterium* sepedonicum, *American Potato Journal*, **64,** 665-670.