Immunochemical, Biochemical and Pathogenic Properties of Fluidal and Intermediate Strains of *Clavibacter michiganensis* subsp. *sepedonicus*

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Abstract

Clavibacter michiganensis subsp. *sepedonicus* (*Cms),* causing ring rot of potato, is a quarantine bacterium. According to colony morphology, *Cms* occurs mostly as fluidal (smooth), but sometime as intermediate (rough, i.e. less fluidal) variants, too. Commercial monoclonal antibodies (Agdia, USA) were used for determination of 40 *Cms* strains representing both forms. All *Cms* strains were reliably identified by IFAS, but atypical cells were sometime recorded in population of intermediate strains. The fluidal *Cms* strains were more reliably identified using DAS-ELISA and the Biolog GP MicroPlate System™ than intermediate strains. The intermediate *Cms* strains had decreased metabolic activity compared with fluidal strains and that is why they were identified only to the genera or to the species level or not identified. The differences among fluidal and intermediate *Cms* strains were recorded also in bioassay on eggplants. The intermediate *Cms* strains caused atypical or no symptoms with comparison to fluidal strains.

Keywords: *Clavibacter michiganensis* subsp. *sepedonicus*; ring rot of potato; ELISA; IFAS; Biolog; bioassay

INTRODUCTION

Clavibacter michiganensis subsp. *sepedonicus (Cms),* causing bacterial ring rot of potato, is listed among quarantine pests in many countries all over the world. Detection and determination of *Cms* is performed in conformity with the requirements of EU directive No. 93/85/EEC (SMITH *et al*. 1997). According to colony morphology, *Cms* occurs mostly as fluidal (smooth, mucoid), but some *Cms* strains have been found as intermediate and non-fluidal (rough, non-mucoid) variants, too (SNIEZSKO & BONDE 1943; BISHOP *et al*. 1988). A change in colony morphology takes place spontaneously during *in vitro* and *in vivo* growth (BAER & GUDMESTAD 1993). Non-fluidal strains not produce sufficient exopolysaccharide (EPS) or produce different EPS with comparison to fluidal strains and that is why detection of pathogen by immunochemical methods is complicated sometime (DE BOER 1983; BISHOP *et al*. 1988; BAER & GUDMESTAD 1993). On the other hand, the loss of fluidal colony morphology and lack *in vitro* production of EPS do not affect the ability of *Cms* to infect and develop of bacterial ring rot foliar symptoms in eggplant and potato (WESTRA & SLACK 1992).

MATERIALS AND METHODS

A total of 40 *Cms* isolates (coming from 14 countries) representing fluidal and intermediate colony morphology was tested to determine their immunochemical, biochemical and pathogenic characteristics. Isolates of *Cms* were cultured on nutrient medium according to SNIESZKO and BONDE (1943) at 23°C and used as 5–7 day cultures to all tests. Commercial monoclonal antibodies (Agdia, USA) were used for determination

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of *Cms* by means of DAS-ELISA (double antibody sandwich enzyme-linked immunosorbent assay) and IFAS (indirect fluorescent antibody stain) performed according to the manufacturer's recommendation. Bacterial suspensions were prepared in concentrations 10^7, 10^6 and 10^5 cfu/ml. Positive/negative reactions in DAS-ELISA were recorded with an ELISA reader. Immunofluorescence slides were observed under the microscope with 1 000× magnification using a mercury lamp and suitable filter system. For the Biolog GP MicroPlate System™, bacterial suspensions were prepared according to recommendation of company Biolog Inc. (USA). Evaluation was performed with the naked eye after 24 h and 48 h of incubation. Cultures were identified using the MicroLog™ 2 database for gram-positive (GP) bacteria. The bioassay on egg-plant was carried out according to EU Directive No. 93/85 EEC.

RESULTS AND DISCUSSION

The fluidal *Cms* strains were more reliably identified in DAS-ELISA than intermediate *Cms* strains. The complication with detection of intermediate *Cms* strains could be explained by it, that those strains not produce sufficient exopolysaccharide (EPS) or produce different EPS with comparison to fluidal strains and that is why are undetectable using antibodies made against EPS of fluidal *Cms* strains by means of ELISA (De Boer 1983; Bishop *et al*. 1988; Baer & Gudmestad 1993). The fluidal and intermediate strains were reliably identified by IFAS as *Cms,* but in population of some intermediate *Cms* strains were sometime recorded atypical cells different from fluidal ones. IFAS seems to be the only existing serological technique capable of detecting both fluidal and non-fluidal *Cms* strains with equal sensitivity (Baer & Gudmestad 1993; Henningson & Gudmestad 1993). The fluidal *Cms* strains were identified more reliably than intermediate strains using the Biolog GP MicroPlate System™ (USA). The intermediate *Cms* strains had decreased metabolic activity compared with fluidal strains. It explains why intermediate strains were identified only to the genera level, i.e. *Clavibacter* or to the species level, i.e. *Clavibacter michiganensis* or not identified. It means Biolog GP MicroPlate System™ (MicroLog2 4.01 B version) is not capable to identify reliably all *Cms* strains. The differences among fluidal and intermediate *Cms* strains were recorded also in bioassay on egg-plants. The intermediate *Cms* strains caused more frequently atypical or no symptoms with comparison to fluidal *Cms* strains. Atypical symptoms mostly appeared as plant dwarfing and curling of leaves (Table 1).

In our study was confirmed that the presence of intermediate *Cms* strains could be the cause of false negatives in routine testing of tuber potato samples screened to presence of *Cms*.

Table 1. Reliability of diagnostic techniques as DAS ELISA, IFAS, Biolog and bioassay for fluidal and intermediate strains of *Clavibacter michiganensis* subsp. *sepedonicus*

Population of strains	Number of strains	Result	DAS-ELISA absorbance (405)		IFAS	Biolog		Bioassay[d]		
			OD[a] (0.3)	OD (0.1)	cell shape[b] (T/A)	identification to level	SIM[c]	T	A	N
Fluidal	18	average	0.78	0.88	T	*C. michiganensis*	0.511			
		positives (%)	100	100	100		83.3	51.5	21.8	26.7
Intermediate	22	average	0.41	0.39	T and A	*C. michiganensis*	0.234			
		positives (%)	66.5	62.5	100		54.5	45.4	25.7	28.9

[a] OD = optical density; threshold level: > 0.21 – positive < 0.20 – negative reaction; 100.0% of fluidal, but only 62.5–66.5% of intermediate strains identified as *Clavibacter michiganensis* subsp. *sepedonicus*

[b] T/A – typical/atypical cells with comparison to cells of *Clavibacter michiganensis* subsp. *sepedonicus*

[c] evaluation criterion: SIM (index similarity) > 0.1; 83.3% of uidal strains identified as *Clavibacter michiganensis* with average SIM 0.511; 54.5% of intermediate strains identified as *C. michiganensis* with average SIM 0.234

[d] number of plants with symptoms within 7–14 days after inoculation (%); T – typical, A – atypical, N – none symptoms

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