Sensitivity and Specificity of Monoclonal Antibody Mn-Cs1 for Detection and Determination of *Clavibacter michiganensis* subsp. *sepedonicus*, the Causal Agent of Bacterial Ring Rot of Potato

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Abstract

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Monoclonal antibody Mn-Cs1 with a high level of sensitivity and specificity for detection and determination of *Clavibacter michiganensis* subsp. *sepedonicus* was prepared. Strain *C. m.* subsp. *sepedonicus* NCPPB 3467 (as whole cell antigen and extracellular polysaccharides) was used for immunisation of four mice Balb/c. After cloning and verifying, two stable hybridoma clones were gained. One monoclonal antibody, designated Mn-Cs1, was used in all tests. It reacted intensely with extracellular polysaccharides from homologous antigen (> 0.5 mg/ml), weakly with proteins from cell walls (> 200 µg/ml) and with whole homologous antigen (concentration 10^4–10^3 cfu/ml) in DAS-ELISA. Monoclonal antibody Mn-Cs1 showed a high level of specificity. It reacted neither with bacterial strains of closely related subspecies of *Clavibacter michiganensis* (*C. m.* subsp. *michiganensis* and *C. m.* subsp. *insidiosus*) nor with the saprophytic bacteria *Pseudomonas fluorescens* and *Pantoea agglomerans*.

Keywords: bacterial ring rot of potato; *Clavibacter michiganensis* subsp. *sepedonicus*; DAS-ELISA

Bacterial ring rot of potato, caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff 1914) Davis et al. 1984 *(Cms)*, is a quarantine disease for the EU and EPPO (European Plant Protection Organisation). A zero tolerance for ring rot exists in all certified seed potato production areas of EPPO (SMITH *et al.* 1997). *Cms* is dangerous because it survives in potato tubers in a latent form and can thus be disseminated over large distances, complicating international trade and introducing the pathogen to areas where it did not occur before (SMITH *et al.* 1997).

In 1996, bacterial ring rot was recorded in the Czech Republic for the first time (KOKOŠKOVÁ & PÁNKOVÁ 1998) and immediately strict phytosanitary measures concerning import and export of potatoes were applied in conformity with the requirements of EU directive No. 93/85/EEC. Systematic and careful control of potato seed with the aim to eliminate *Cms* from seed potato production has been adopted in the Czech Republic. Bacteriological analyses of potato samples have been conducted according to the above mentioned directive of EU, where the immunofluorescence (IF) test and test of pathogenicity on eggplant were used to confirm *Cms* in potato tubers (JANSE & VAN VAERENBERGH 1987).

Serological procedures are of special interest because of their simplicity and sensitivity. Polyclonal and monoclonal antisera for *Cms* are used in immunochemical techniques like IF and ELISA that can be used to detect symptom-less ring rot infections during the growing season (GUDMESTAD *et al*. 1991). The diagnosis of *Cms* has improved considerably with the use of monoclonal antibodies that proved more sensitive than polyclonal antibodies since they are able to detect low concentrations of the bacterium population with a high degree of accuracy (DE BOER *et al*. 1988; WESTRA *et al*. 1994). Monoclonal antibodies are usually more specific than polyclonal anti-

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bodies because they are produced to single determinants (DE BOER *et al.* 1988). They can replace polyclonal antisera in any one of the serological diagnostic procedures. In contrast to data obtained with monoclonal antibodies, those gained with polyclonal antisera were often conflicting and un-convicting due to the occurrence of false positive cross-reactions. The use of monoclonal antibodies, in comparison with polyclonal antibodies, is also complicated by the appearence of cross-reactions with various pathogenic and saprophytic soil bacteria found in potato extracts.

Research institutes and laboratories of plant health services dealing with the diagnosis of the ring rot pathogen buy antibodies for immunochemical diagnosis of *Cms* available on the market and/or prepare their own antibodies (DE BOER *et al.* 1988; WESTRA *et al.* 1994). The use of more than one antibody for detection of the target bacterium in plant samples excludes even more potential cross-reactions.

Individual bacterial pathogens could be more or less heterogeneous in their genetic, pathogenic, immunochemical and biochemical characteristics due to e.g. changing climatic conditions and/or changes in the host plants. It is, therefore, possible that an available antiserum would not identify all strains of one bacterial pathogen. That is why testing new antibodies for *Cms* against a large panel of strains is always significant.

In this study, we have focused on the preparation of a monoclonal antibody for detection and determination of *Cms,* and on the evaluation of its sensitivity and specificity by DAS-ELISA.

MATERIAL AND METHODS

Bacteria: Bacterial strain *Cms* NCPPB 3467 (National Collection of Plant Pathogenic Bacteria, York, Great Britain) was used as a source of antigen for preparation of monoclonal antibodies. This strain and strain NCPPB 3279 were used as standards in all serological tests together with 20 additional strains of *Cms* from the Czech Republic, isolated in 1998–1999. The specificity of monoclonal antibodies was assessed with strains of related subspecies of *Clavibacter michiganensis (C. m.* subsp. *insidiosus, C. m.* subsp. *michiganensis* and *C. m.* subsp. *nebraskensis*), with reference strains from other genera of plant pathogenic bacteria (*Agrobacterium, Erwinia,*

Table 1. Determination of cross-reactions of monoclonal antibody Mn-Cs1 in DAS-ELISA

Bacteria	Origin	Strain	Readings in DAS-ELISA[a]
Clavibacter michiganensis subsp. *sepedonicus*	Great Britain	NCPPB 3467	3.640
Clavibacter michiganensis subsp. *sepedonicus*	Great Britain	NCPPB 3279	3.520
Agrobacterium tumefaciens	Czech Republic	CCM 2385	0.040
Clavibacter michiganensis subsp. *insidiosus*	Czech Republic	RICP 12/5/98	0.035
Clavibacter michiganensis subsp. *michiganensis*	Czech Republic	CCM 1635	0.150
Clavibacter michiganensis subsp. *nebraskensis*	Czech Republic	CCM 2749	0.060
Curtobacterium flaccumfaciens	Czech Republic	CCM 2103	0.474
Erwinia amylovora	Czech Republic	RICP 8/95	0.090
Erwinia carotovora subsp. *carotovora*	Czech Republic	CCM 1008	0.030
Erwinia chrysanthemi	Czech Republic	CCM 989	0.090
Pantoea agglomerans	Czech Republic	CCM 2406	0.400
Pseudomonas fluorescens	Czech Republic	CCM 2115	0.130
Pseudomonas syringae pv. *syringae*	Czech Republic	CCM 4073	0.017
Ralstonia solanacearum	Great Britain	NCPPB 2505	0.060
Xanthomonas vesicatoria	Czech Republic	CCM 2102	0.030

NCPPB – National Collection of Plant Pathogenic Bacteria, York, Great Britain
CCM – Czech Collection of Microorganisms, Brno, Czech Republic
RICP – Research Institute of Crop Production (Collection of Plant Pathogenic Bacteria of Bacteriology Department), Prague, Czech Republic
[a]Absorbance value (405 nm) – 10^5 cfu/ml
negative reaction: 0.00–0.50
positive reaction: 0.51–4.00
reading value: mean value of 4 repeats

Pseudomonas, *Ralstonia* and *Xanthomonas*) and of saprophytic bacteria (*Pseudomonas fluorescens* and *Pantoea agglomerans*) (Table 1).

Purification of antigen*:* Strain *Cms* NCPPB 3467 was grown on C medium (protease peptone 5 g, casein hydrolysate 3 g, yeast extract 3 g, maltose 2 g, lactose 1 g, agar 18 g, 1 l distilled water, pH 7.0) (SNIESZKO & BONDE 1943). For all kinds of antigen, the culture was incubated for 7 d at 24°C. For whole cell antigen, the culture was harvested in physiological saline (pH 7.2), vortexed for 2 min and centrifuged at 10 000 g for 10 min. The pellet was dissolved in 1% formaldehyde solution, incubated overnight at 24°C, and then the suspension was centrifuged. The concentration of antigen was adjusted for immunization and used for monoclonal antibody evaluation in the range 10^7–10^3 cfu/ml.

For purification of extracellular polysaccharides, the method previously reported by DE BOER *et al*. (1988) was used. For immunisation and evaluation of monoclonal antibody, the concentration of the extracellular polysaccharides was adjusted in a range 0.1–2.0 mg/ml by the phenol-sulphuric acid assay using glucose as the standard (ANONYMOUS 1983).

Cell wall proteins were extracted with LiCl in a procedure described by DE BOER and WIECZOREK (1984) and used for evaluation of monoclonal antibodies in a range 50–400 µg/ml in PBS buffer.

Production and characterisation of monoclonal antibody: Balb/c mice were immunised by three intraperitoneal injections (on day 1, 21 and 42) of formaldehyde-fixed whole cells and boosted by injection of extracellular polysaccharides antigen of *Cms* NCPPB 3467.

Hybridisation of spleen cells was conducted by a standard procedure (VIKLICKÝ *et al*. 1987), using feeder layers (HARLOW & LANE 1988). Screening for the presence of antibodies was performed in ELISA in a Terasaki system (VIKLICKÝ *et al*. 1987) against the homologous antigen. A reaction was considered positive when PNP

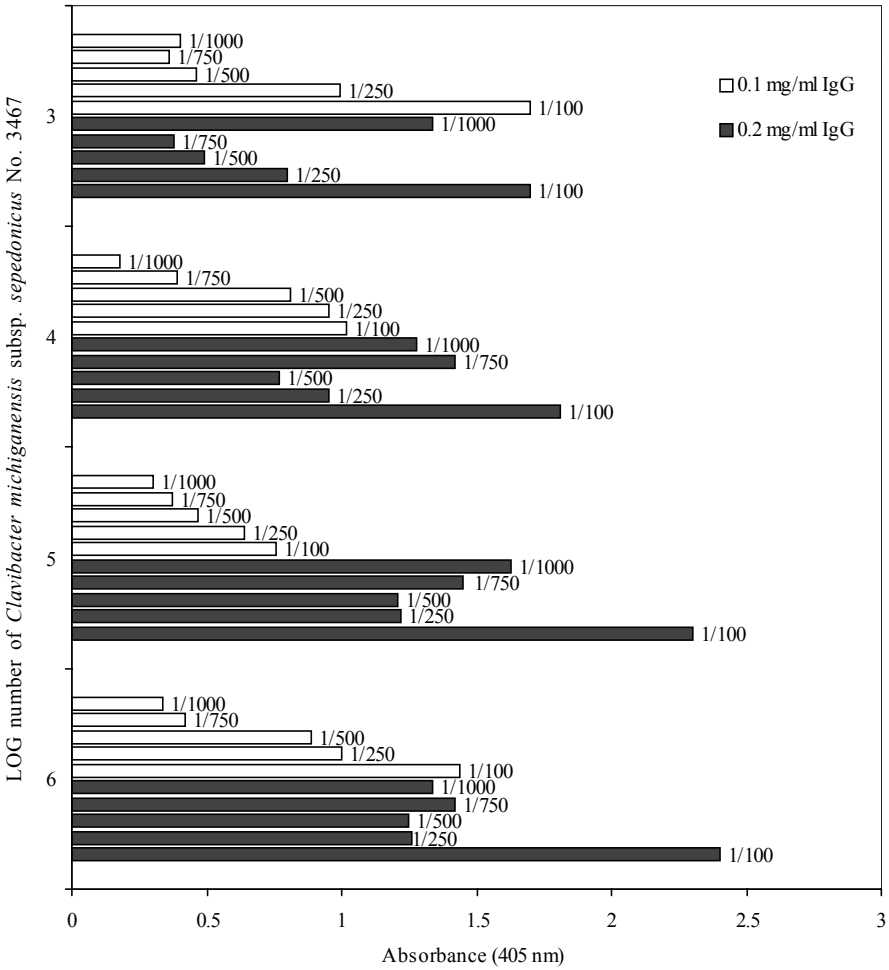

Fig. 1. The properties of new monoclonal antibody Mn-Cs1 in DAS-ELISA. The options for antibody were set with whole cell antigen of *C. m.* subsp. *sepedonicus* NCPPB 3467. The optimal concentration of IgG was set to 0.1 mg/ml, the optimal dilution of IgG-AP was 1:250 and concentration of *C. m.* subsp. *sepedonicus* strain NCPPB 3467 was 10^5–10^4 cfu/ml

(*p*-nitrophenyl phosphate) substrate in wells was stained dark yellow or orange. Specific antibody-secreting hybridomas were cloned under conditions of limited dilution techniques, using feeder layers (HARLOW & LANE 1988). Cloning was repeated three times, and established hybrids were grown in HT (hypoxanthine-thymidine) medium (98 ml of RPMI 1640 medium, 1 ml of hypoxanthine, 1 ml thymidine). Stable hybrids were separated in the following medium (1:4:5 = DMSO:foetal bovine serum:RPMI 1640) to a final concentration 5×10^6–10^7 cells/ml and transferred to a freezer (–135°C). Monoclonal antibodies were purified by ammonium sulphate precipitation (HARLOW & LANE 1988) and by dialysis on a DEAE-cellulose column.

Alcaline phosphatase conjugation: The conjugate of monoclonal antibody to alcaline phosphatase was prepared using a simple, one-step procedure with glutaraldehyde (HARLOW & LANE 1988). The IgG-AP was stored at –20°C with addition of 1% BSA.

Specificity and sensitivity of monoclonal antibodies: Specificities of the monoclonal antibodies were evaluated in DAS-ELISA (CLARK & ADAMS 1977). Polystyrene microplates for ELISA and Terasaki tests were coated with monoclonal antibodies and then with the bacterial suspensions 10^7–10^4 cfu/ml of whole cell antigens of different bacterial species. Specificity was also tested against 20 isolates of members of the saprophytic flora on potato.

The sensitivity of monoclonal antibody was tested with whole cells antigen used in the concentration from 10^7 to 10^3 cfu/ml, extracellular polysaccharides from 2.0–0.1 mg per ml and cell wall proteins from 0.4–0.05 mg/ml of homologous strain *Cms* NCPPB 3467.

The concentration of IgG and IgG-AP conjugate fractions were also evaluated. The specificity and sensitivity of the monoclonal antibody produced was compared with those of a commercially available monoclonal antibody for *Cms* (company Agdia, Elkhart, Indiana, USA).

RESULTS

Using hybridoma technology, we produced 20 stable growing hybridomas. After cloning and screening for the presence of specific monoclonal antibodies against *Cms*, only two hybridomas remained. They showed about the same pattern of reaction with different forms of homologous antigen (strain NCPPB 3467) and with different phytopathogenic and saprophytic bacteria. One monoclonal antibody, designated Mn-Cs 1, was used in all tests.

Characteristics of the monoclonal antibody were verified in DAS-ELISA test. The options for Mn-Cs1 were set

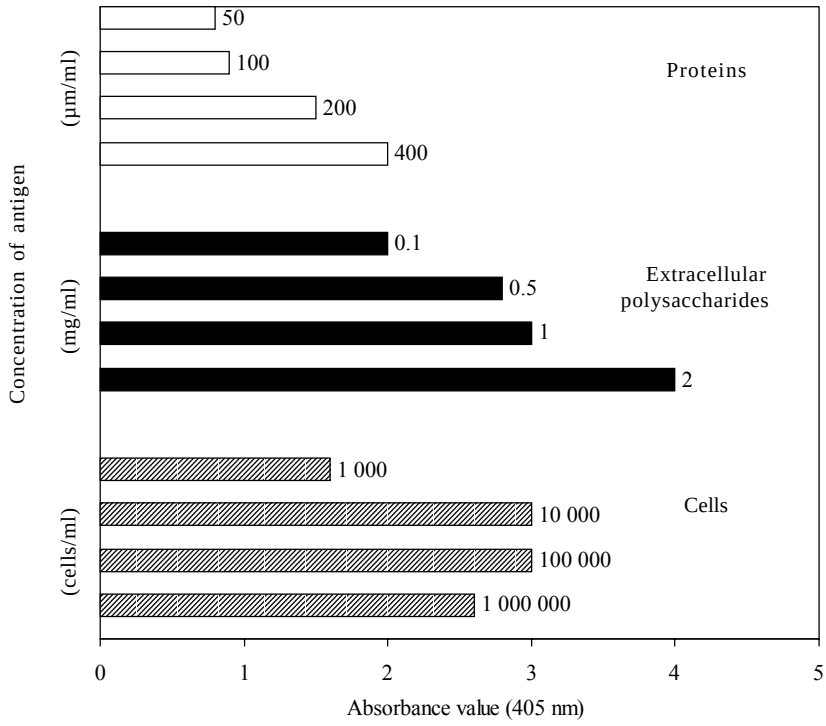

Fig. 2. The comparison of different antigens of *C. m.* subsp. *sepedonicus* in DAS-ELISA. The sensitivity of monoclonal antibody Mn-Cs1 was tested in various concentrations of whole cell antigens, extracellular polysaccharides and cell wall proteins isolated from *C. m.* subsp. *sepedonicus* strains NCPPB 3467 and NCPPB 3279. Concentration of IgG was set to 0.1 mg/ml, dilution of IgG-AP was 1:250. Antibody reacted strongly with extracellular polysaccharides from homologous antigen (> 0.5 mg/ml), weakly with proteins from cell walls (> 200 µg/ml) and with whole antigen (10^4–10^3 cfu/ml) in DAS-ELISA

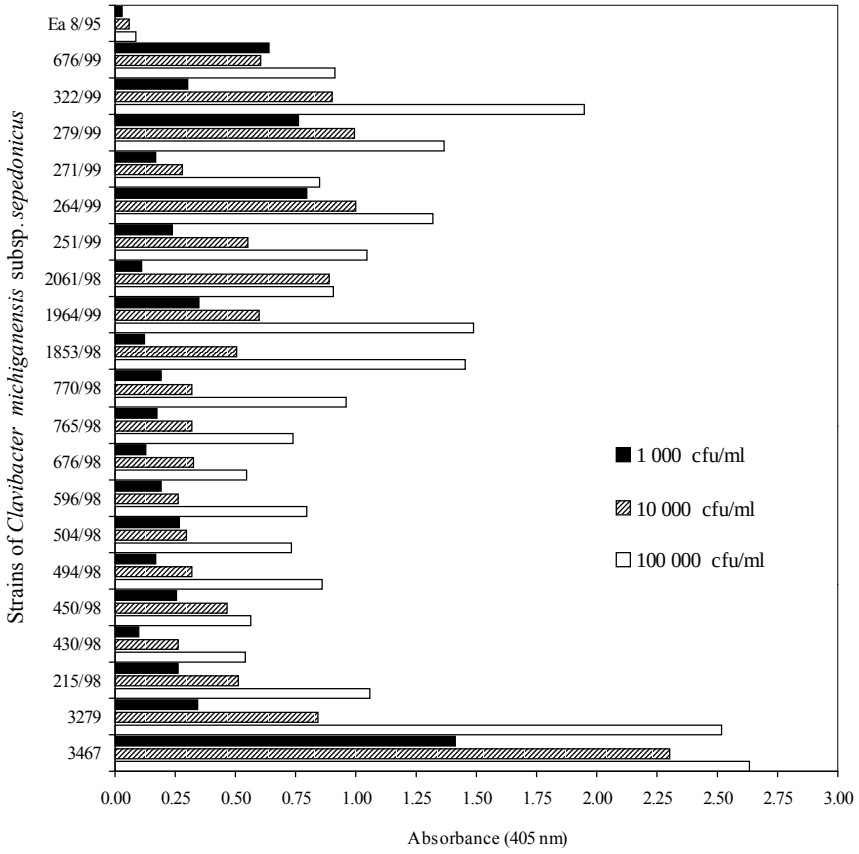

Fig. 3. Determination of *C. m.* subsp. *sepedonicus* with monoclonal antibody Mn-Cs1 in DAS-ELISA. Antibody was used in concentrations of 0.1 mg/ml IgG and 1:250 IgG-AP. The absorbance threshold value A_{405} 0.5 was exceeded with all 20 *C. m.* subsp. *sepedonicus* strains in a concentration of 10^5 cfu/ml and with 50% of strains in a concentration of 10^4 cfu/ml (+++ strongly positive reactions). The absorbance threshold value A_{405} 0.25 was exceeded in a concentration of 10^4 cfu/ml and A_{405} 0.1 in a concentration of 10^3 cfu/ml with the other 50% of *C. m.* subsp. *sepedonicus* strains (++ positive reactions). Negative control – *Erwinia amylovora (Ea)*

with whole cell antigen of *Cms* NCPPB 3467 (Fig. 1). The optimal concentration of IgG was set to 0.1 mg/ml, the optimal dilution of IgG-AP was 1:250 and concentration of *Cms* bacterium 10^5–10^4 cfu/ml (Fig. 1).

The absorbance values for these options were never lower than 0.5. The sensitivity of Mn-Cs1 in DAS-ELISA was tested also with various concentrations of cell wall proteins and extracellular polysaccharides isolated from *Cms* strains NCPPB 3467 and NCPPB 3279 (Fig. 2). Extracellular polysaccharides were tested in range 1–2 mg/ml. Monoclonal antibody Mn-Cs1 reacted strongly with extracellular polysaccharides (> 0.5 mg/ml). The cell wall proteins reacted positively to concentration 200 µg/ml, but the coloured reaction developed very slowly, even at a concentration 400 µg/ml of cell wall proteins. The absorbance values for proteins were lower than for whole cell antigen (Fig. 2).

Monoclonal antibody Mn-Cs1 showed a high level of specificity. It reacted neither with bacterial strains related subspecies of *Clavibacter michiganensis* such as *C. m.* subsp. *insidiosus*, *C. m.* subsp. *michiganensis and C. m.* subsp. *nebraskensis* nor with saprophytic bacteria like *Pseudomonas fluorescens* and *Pantoea agglomerans*, which are the most frequent cause of cross-reactions of polyclonal antisera (Table 1). The threshold absorbance value A_{405} 0.5 was reached only by strains *Curtobacterium flaccumfaciens* CCM 2103 (0.47) and *Pantoea agglomerans* CCM 2406 (0.40) (Table 1).

A DAS-ELISA kit using Mn-Cs1 antibody with the optimal concentrations of IgG and IgG-AP was used to determine 20 *Cms* strains previously isolated in the Czech Republic during 1998–1999. The absorbance threshold value at the concentration 10^5 cfu/ml A_{405} 0.5 was passed in four repetitions by all strains, with 50% of them even in a concentration of 10^4 cfu/ml (Fig. 3). These strains of *Cms* were marked as strongly positive (+++). The other 50% of the strains were marked as positive (++), because all of them passed the threshold absorbance values A_{405} 0.25 at 10^4 cfu/ml and A_{405} 0.1 at 10^3 cfu/ml (Fig. 3). The development of the colour reaction was rapid and intense even at 10^3 cfu/ml in DAS-ELISA.

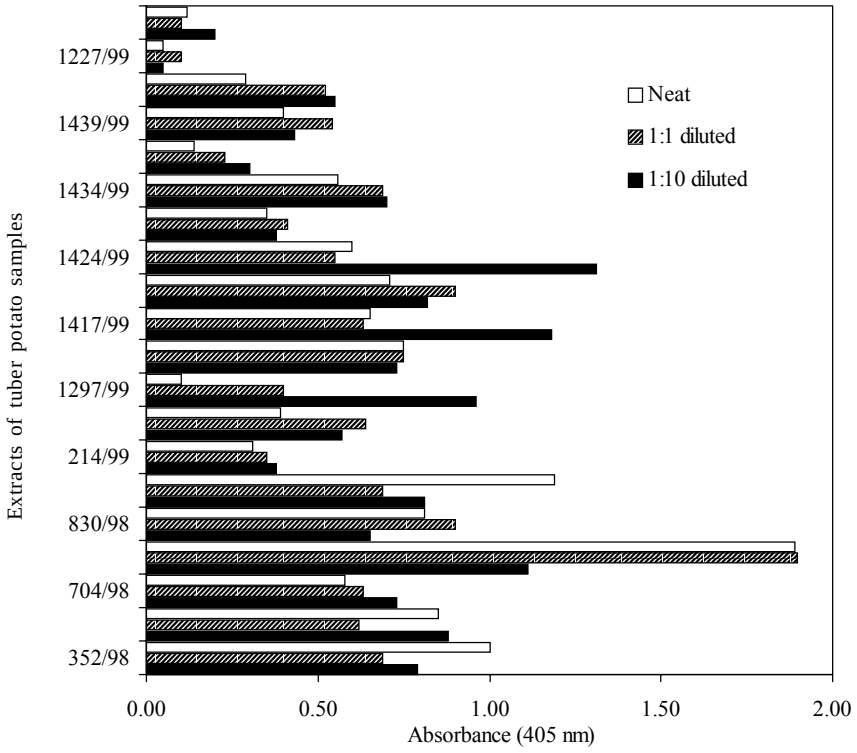

Fig. 4. Detection of *C. m.* subsp. *sepedonicus* with monoclonal antibody Mn-Cs1 in DAS-ELISA. Antibody was used for detection of *C. m.* subsp. *sepedonicus* in twenty tuber extracts of potato samples in concentrations of 0.1 mg/ml IgG and 1:250 IgG-AP. Two extracts (1135/99 and 1227/99) were used as negative controls. The absorbance threshold value $A_{405} = 0.2$ was exceeded with all extracts in dilution 1:1 and 1:10 and in neat extracts with 16 of them, respectively. Extract dilutions of 1:1 and 1:10 were considered to be more convenient for screening of presence ring rot pathogen in potato extracts

The DAS-ELISA kit of Mn-Cs1 antibody with optimal concentrations of IgG and IgG-AP was also used to detect *Cms* in 20 tuber extracts from potato samples of Czech provenience. Altogether 18 extracts were found positive for *Cms* according to IF test, two of them (1227/99 and 1351/99) were negative. The absorbance threshold value $A_{405} = 0.2$ was exceeded with all extracts in dilution 1:1 and 1:10 and in neat extracts with 16 of them, respectively (Fig. 4).

The quality of monoclonal antibody Mn-Cs1 for the detection and determination of *Cms* was comparable to that of the monoclonal antibody for *Cms* from Agdia (USA) – data are not shown.

DISCUSSION

The quality of polyclonal antibodies for *Cms* prepared in our lab during past years was not sufficient due to a low titre and cross-reactions with saprophytic bacteria (KOKOŠKOVÁ & PÁNKOVÁ 1998). We hoped to prepare monoclonal antibodies with higher specificity and sensitivity. The experience of other research workers in this field was promising (DE BOER & WIECZOREK 1984; DE BOER *et al.* 1988).

According to our previous experience, the preparation of polyclonal antibodies against *Clavibacter* subspecies including *Cms* is very difficult, because the bacterial cells produce rather large amounts of extracellular polysaccharides and the immune system of rabbits forms antibodies to them very slowly (VIDAVER 1982; KOKOŠKOVÁ *et al.* 2000). A higher concentration of antigens, e.g. up to 10^8 cfu/ml, is toxic for rabbits also after complementing the antigen with adjuvant. The animals often die of toxicity. Mice tolerate *Clavibacter* antigens well (DE BOER *et al.* 1988) and also in our case there were no problems with toxicity to mice by *Clavibacter* antigens, neither with whole cells nor with extracellular polysaccharides. Mice were sensitive to lower concentration of *Cms* antigens (5×10^5 cfu/ml) and formed against them monoclonal antibodies with a higher concentration of the IgG fraction (more than 5 mg/ml) than the IgG fraction from rabbits (less than 3.5 mg/ml). When lower concentrations of antigen are used for rabbits, the immune schedule has to be longer in order to obtain antisera with at least an average titre. The frequency of cross-reactions mostly arises during extension of the immune schedule, and the cross-reactivity has to be avoided by absorption with cross-reacting

bacteria. This need not be done for monoclonal antibodies.

We tried to improve the detection limits of our polyclonal antibodies against *Cms* without any success. Better antibodies and prior treatment of antigen did not result in a stronger ELISA reaction, and a more reliable determination and detection of *Cms* by polyclonal antibodies in ELISA did not seem feasible (KOKOŠKOVÁ *et al*. 2000). The preparation of monoclonal antibodies rather than polyclonal antibodies for plant pathogenic bacteria of the genus *Clavibacter* has been definitely preferred in the last 10 years (ERWING 1990).

Previous studies by DE BOER and WIECZOREK (1984) and DE BOER *et al.* (1988) with polyclonal antibodies showed that *Cms* possesses a soluble antigen of diagnostic value that may perform better than cell wall antigens in some serological tests. They produced a monoclonal antibody to that soluble antigen of *Cms* and evaluated its usefulness in several serological procedures. Extracellular polysaccharides that act as specific determinants belong to those soluble antigens and they are particularly useful for serological detection of *Cms*, mainly for ELISA. That is why we decided to use as antigens for immunisation of mice not only whole bacterial cells, but also extracellular polysaccharides from cell walls of strain *Cms* NCPPB 3467.

We obtained 20 growing hybridomas, i.e. approximately 10% of the whole number, which were gradually cloned. The two best hybridomas produced monoclonal antibodies with the same properties. The difficulty of selecting hybridomas producing monoclonal antibodies to an antigen unique to the target bacterium is clear from the large number of hybridomas that had to be screened to obtain antibodies with a certain degree of specificity to *Cms* (DE BOER *et al*. 1988).

The monoclonal antibody Mn-Cs1 showed a high level of sensitivity when it determined homologous antigen to a concentration of 10^3 cfu/ml, which was 1000 times more sensitive in ELISA than polyclonal antibodies for *Clavibacter michiganensis* subspecies (MILLER 1984; KOKOŠKOVÁ *et al*. 2000).

The specificity of monoclonal antibody Mn-Cs1 was superior to that of polyclonal antibodies. Mn-Cs1 neither reacted to related subspecies of *Clavibacter michiganensis* nor with saprophytic bacteria like *Pseudomonas fluorescens* and *Pantoea agglomerans* which often complicate detection and determination of plant pathogenic bacteria when polyclonal antisera are used. According to MILLER (1984) and also from our experience, *P. fluorescens* is the most frequently cross-reacting bacterium in serological tests (KOKOŠKOVÁ & PÁNKOVÁ 1998). DE BOER (1982) and DE BOER *et al.* (1988) mention that antibodies for *Cms* cross-reacted with other bacteria of *Clavibacter* genus, such as *C. m.* subsp. *michiganensis* and *C. m.* subsp. *insidiosus*. This cross-reactivity confirms a strong serological relationship between these two subspecies and *Cms*.

Cross-reactivity is a problem of all serological diagnostic tests, but the specificity of tests with monoclonal antibodies that react with a single antigenic determinant is potentially much better than tests with polyclonal antibodies that react with a large number of different determinants. The specificity of monoclonal antibodies for one particular bacterial pathogen depends on how frequently the antigenic determinant occurs in other bacteria (DE BOER 1982; DE BOER *et al*. 1988). The possibility of false-positive serological tests remains real, even when highly specific monoclonal antibodies are used. However, a diagnosis based on the reaction with two monoclonal antibodies, each reacting with a different antigenic determinant, enhances test validity. It is less likely that a cross-reacting organism has two antigenic determinants in common with the target bacterium than a single determinant (DE BOER *et al*. 1988). These are probably also the reasons why the use of more than one monoclonal antibody for detection of *Cms* is recommended by EU directive No. 93/85/EEC.

ELISA appeared to be sensitive, since it identified homologous antigen to 10^3 cfu/ml (Fig. 3). Pure cultures of *Cms* reacted strongly in the DAS-ELISA test also in concentration 10^4–10^3 cfu/ml. At all concentrations of antigens, the difference between positive and negative reaction was remarkable. In preliminary studies, the presence of *C. m.* subsp. *sepedonicus* in tuber extracts of potato samples was detected by DAS-ELISA in neat extracts and even better in extracts diluted 1:1 and 1:10. The mean absorbance ratio of positive reactions in DAS-ELISA for pure cultures of *Cms* and for tuber extracts of potato samples corresponded with data from the literature (LELLIOTT & SELAR 1976; GUDMESTAD *et al*. 1991; SLACK *et al*. 1996; DE BOER *et al*. 1996).

Isolation and subsequent identification of the causal agent of bacterial ring of potato is difficult and laborious. Therefore, it is far more practical to detect the pathogen directly in extracts prepared from host plants and/or tubers using monoclonal antibodies.

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Souhrn

PÁNKOVÁ I., KOKOŠKOVÁ B. (2002): **Citlivost a specifičnost monoklonální protilátky Mn-Cs1 pro detekci a determinaci** *Clavibacter michiganensis* subsp. *sepedonicus***, původce kroužkovitosti bramboru**. Plant Protect. Sci., **38**: 117–124.

Pro detekci a determinaci původce kroužkovitosti, bakterie *Clavibacter michiganensis* subsp. *sepedonicus,* byla připravena velmi kvalitní monoklonální protilátka Mn-Cs1. K imunizaci myší Balb/c byl jako celý antigen použit zahraniční sbírkový kmen *C. m.* subsp. *sepedonicus* NCPPB 3467 a extracelulární polysacharidy z něj získané. Byly připraveny dva hybridomy produkující monoklonální protilátku stabilních vlastností. Tato protilátka reagovala v DAS-ELISA testu velmi intenzivně s extracelulárními polysacharidy z homologního antigenu (> 0,5 mg/ml), slaběji s proteiny z buněčné stěny (> 200 µg/ml) a s celobuněčným antigenem (koncentrace 10^4–10^3 cfu/ml). Monoklonální protilátka Mn-Cs1 byla vysoce specifická. Nereagovala s žádnými bakteriemi náležejícími do poddruhu *Clavibacter michiganensis* (*C. m.* subsp. *michiganensis* a *C. m.* subsp. *insidiosus*) ani se saprofytickými bakteriemi *Pseudomonas fluorescens* a *Pantoea agglomerans.*

Klíčová slova: bakteriální kroužkovitost bramboru; *Clavibacter michiganensis* subsp. *sepedonicus*; DAS-ELISA

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