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ANTIGENIC CHARACTERISTICS AS TAXONOMIC CRITERION OF DIFFERENTIATION OF *ALTERNARIA* SPP., PATHOGENIC FOR CARROT AND PARSLEY

ABSTRACT: Identification of *Alternaria* genus species is a very complicated process which demands broadly designed investigations and studying of great number of properties which together can be considered as satisfying taxonomic criteria. The main objective of these investigations was examining the possibilities of applying the antigenic characteristics of *Alternaria* spp. phytopathogenic fungi as a taxonomic criterion, as well as introducing the serological methods for their identification. Conducting the examination of *Alternaria* spp., pathogenic for Apiaceae plants in Serbia, several isolates were obtained and identified as *Alternaria radicina, A. petroselini, A. dauci* and *A. alternata*, based on the conventional mycological methods and host range, as well as on molecular detection and partial characterization.

The investigation included 12 isolates from plant leaves, seeds and soil, which were pathogenic mainly to carrot and parsley and were identified as *A. radicina, A. petroselini, A. dauci* and *A. alternate*. Investigated isolates were compared with each other, as well as with standard isolates for the mentioned species (a total of 5 isolates, originating from USA and EU).

During the investigation of serological characteristics of *Alternaria* spp., firstly a polyclonal antiserum was prepared against one isolate from Serbia, identified as *A. dauci*. This antiserum was specific to *Alternaria* genus, while there was no reaction with antigens from other phytopathogenic fungi genera (*Fusarium, Rhizoctonia* and *Agaricus*). Antiserum titer, determined by slade agglutination test, was 1/32. Antigenic characteristics of *Alternaria* genus fungi were examined by Electro-Blot-Immunoassay serological method (EBIA, Western blot), i.e. their protein profiles were compared.

Investigated *Alternaria* spp. isolates showed different protein band profiles in gel and on nitrocellulose paper, and the observed differences were in complete correlation with the results of the previous identification. All investigated isolates, both domestic and the standards, were similar to each other, and they could be correctly identified to the species level using EBIA. Besides grouping to the species level, antigenic characteristics indicated similarities and differences among the isolates within the same and different species, showing their complex relationships which properly reflect their diversity in nature.

In all the previous investigations of *Alternaria* genus fungi up to now, there have been no data about their serological characteristics as possible taxonomic criteria. Introduction of this group of characteristics represents an important contribution both to the

taxonomy and implementation of fast and accurate methods of phytopathogenic fungi identification.

KEY WORDS: *Alternaria* spp., antigenic characteristics, EBIA, polyclonal antiserum, taxonomic criterion

INTRODUCTION

Alternaria species are probably the most common fungi that mycologists deal with in different scientific fields. There is a huge number of species names in literature which are, in fact, synonims for undetermined taxonomic accuracy. That is the reason why it is so difficult even to collect any reliable data about how many species belong to this genus. It is estimated that this number varies from 100 up to several hundred species (R o t e m, 1994). Since they are extremly variable and widely distributed, *Alternaria* spp. have been described under different names and that is why their taxonomy, classification, and systematics are very complex and mostly incompletely solved today (S i m m o n s, 1992, P r y o r and M i c h a i l i d e s, 2002). Diagnostic characteristics of *Alternaria* genus include the formation of chains of dark-coloured, multi-celled conidia with beaks of tapering apical cells. However, these characteristics are not common to all the species belonging to this genus, which led to taxonomic confusion among researchers during the history of *Alternaria* classification (R o t e m, 1994).

Because of the complex differentiation of particular species within this genus, as well as the differences among particular isolates of the same species, some authors have suggested and used different criteria for determination of taxonomic relationships among the species (host range, cultural and morphological properties, pigment and crystal formation, etc.). In this way, for example, P r y o r and G i l b e r t s o n (2002) differentiated some isolates of *A. radicina* and *A. petroselini*, which had been previously improperly classified as undetermined nomenclature. The criteria which they used were the growth rate on PDA, yellow crystal formation and host range.

According to R o t e m (1994), *Alternaria* spp. taxonomy is very complex because of the variability of their morphological features, which are affected by the environmental conditions and other factors. Whatever the nature of these factors is that variability of *Alternaria* species has created a taxonomic dilemma, and led to the description of some species which have never been confirmed by other researchers. This situation confuses plant pathologists who investigate physiology, epidemiology, or the possibilities of control measures, and expect from taxonomy the proper and correct identification of the concerned organisms. Taking into consideration the possible influence of the environmental conditions, R o t e m (1994) suggests that a researcher should give his opinion only after examining a great number of isolates originating from different localities. S i m m o n s (1992) states that taxonomy and classification of fungi from any genus, including genus *Alternaria*, have to, at least at the beginning, undoubtedly match a species name with its constant morphological characteristics, as well as with its biology, pathogenicity, reproduction, physiology and other properties.

The main objective of this investigation was the comparison of chosen *Alternaria* spp. isolates on the basis of their antigenic characteristics. The isolates were obtained from tissue, soil and seed of host plants from Apiaceae family, and their proper and precise identification and characterization had been previously done up to the species level, using conventional and molecular detection methods. Since serology has not until now been applied in *Alternaria* genus investigations, the obtained results could contribute to a more precise determination of the relationships and taxonomic status of *Alternaria* fungi, pathogenic for hosts from Apiaceae family, as well as to the development of a new group of methods which could be applied in routine detection and identification of fungi.

MATERIAL AND METHODS

Isolates. In the period 2004—06, the inventory of *Alternaria* spp. fungi included diseased leaves and petioles, soil and seed of carrot, parsley and other plants from Apiaceae family in Serbia. A total of 17 isolates were chosen for the investigation, 12 of which from the Republic of Serbia (8 from seeds, 2 from plant tissue and 2 from soil), and 5 standard isolates originating from USA (B. M. Pryor, University of California, Davis) and Europe (J. van Bilsen, Bejo Zaden B. V., Warmenhuizen, Holland). On the basis of examination and identification, using conventional methods and host range, as well as molecular detection and partial characterization, the obtained isolates were classified as *Alternaria radicina*, *A. petroselini*, *A. dauci* and *A. alternata* (B u l a j i ć, 2007).

Antigen preparation. Antigens for rabbit immunization were prepared from isolate 108 mycelium, originating from Serbia, which had been previously identified as *A. dauci*. Antigen was prepared according to the method of A d a m s and B u t l e r (1979). The chosen isolate was first cultured on a solid nutrient media for three times, and then on a liquid synthetic GNA. The obtained mycelium was dried using vacuum pump, frozen at –18 to –20°C, or –80°C and stored until usage. Amount of 8 g of frozen mycelium was homogenized in a pestle and mortar with 40 ml of 0,1 M Tris-HCl buffer pH 8,0 (1,211 g Trizma base, Sigma, Chemical Company, dissolved in 100 ml distilled water, and pH adjusted with concentrated HCl to pH 8,0). After 2 hours of horizontal stirring, the obtained extract was centrifuged for 40 min at 11000 rpm in Eppendorf centrifuge (5804 R). Pellet was eliminated and supernatant was used as an antigen for rabbit immunization.

Antiserum production. Antiserum was produced by rabbit immunization, by daily intramuscular injecting of increasing volume of previously prepared mycelium suspension, for 10 days. Total of 21,25 ml of isolate 108 mycelium suspension was injected. After blood coagulation, the liquid phase was separated from clot, and it was used as antiserum in further investigation of *Alternaria* spp. antigenic properties.

Determination of antiserum titer. Antiserum titer was determined using serological method of agglutination on a glass slide. Antigen dilutions were

prepared in 0,01M phosphate buffer pH 7,0 and antiserum dilutions in physiological solution. Agglutination on a glass slide was carried out according to Noordam (1973) and Krstić and Tošić (1994) method. Formation of precipitates was observed in dark field (DF) using Olympus CX41 microscope (magnification 100x).

Determination of antigenic characteristics. *Alternaria* spp. antigenic characteristics were investigated using Electro-Blot-Immunoassay serological method (EBIA, Western blot). For EBIA, fungi samples were prepared by adding 250 µl of loading buffer (Laemmli, 1970) into 500 µl of mycelium suspension prepared in the same way as for the antiserum production. Then, the samples were boiled in water bath for about 10 min, and after cooling stored, at –20°C until usage. Electro-Blot-Immunoassay serological method (EBIA, Western blot) was used for comparing the fungi isolates on the basis of their antigenic characteristics, as well as their protein profiles. This method (O'Donnell et al., 1982; Rybicki and Von Wechmar, 1982) includes: antigen electrophoresis in polyacrylamide gel, adsorption from the gel to nitrocellulose paper, and immunoenzyme detection of antigen on NC paper. SDS polyacrylamide gel electrophoresis was performed by using discontinuous gel, 5% for protein concentration, and 12,5% for protein separation. About 40 µl of antigen sample was added per each slot of the gel. Electrophoresis was carried out by Protean II xi Cell (Bio-Rad) vertical slab gel apparatus with cooling. Components were conducted through the gel for protein concentration at constant 30mA, and through the gel for protein separation at 50mA. After electrophoresis had been completed, proteins were immediately adsorbed on NC paper (Protran, Nitrocellulose Transfer Membrane, pore size 0,45 µm, Whatman, Scheicher and Schuell, EU). Antigen adsorption was performed at room temperature. NC paper was processed using immunosorbent method, according to the catalogue manual No 170-6545 Bio-Rad, as O'Donnell et al. (1982) had previously described, and Shukla et al. (1989) modified. In the investigation of isolate antigenic characteristics, antiserum dilution was 1:250, and Anti-rabbit IgG peroxidase conjugate affinity isolated antibody developed in Goat (Sigma Immuno Chemicals) was diluted 1:2500 in skimmed milk. Paper development was performed in the mixture of 50 ml TBS with 10 ml methanole solution of 20 mg 4-chloro-1-naphthol (Bio-Rad) and 30 µl 30% hydrogen peroxide. Colour development was followed visually. The presence of bluish-purple band was considered as a positive reaction, and its absence as a negative reaction between antigen and antiserum.

RESULTS

Polyclonal antiserum was prepared against isolate 108 which, according to the previously obtained results, belongs to species *A. dauci.* Antiserum was prepared by rabbit intramuscular immunization with the total of 21,25 ml of mycelium suspension. Antiserum, in dilution 1/1, reacted positively in slide agglutination test with homologous antigen, i.e. with prepared antigen of isolate 108. In additional agglutination tests, negative reactions were observed with

the following antigens: MLE isolate of double-nucleate *Rhizoctonia* AG-A (V i c o, 1997), and *Fusarium* sp., both from the Collection of Department of Plant Pathology, Faculty of Agriculture, Belgrade, and *Agaricus* sp. (Basidiomycotina) prepared from fresh carpophora. In this way it was confirmed that antiserum shows specific reactions only with *Alternaria* species. According to the obtained results, the antiserum titer was 1/32, while the antigen titer was 1/256 (Table 1). Reactions were visualized about 2h after mixing the antiserum with the antigen.

Tab. 1 — Determination of polyclonal antiserum titer, prepared against *Alternaria* isolate 108

Antigen dillution	Dillution of polyclonal antiserum prepared against isolate 108							
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	K_f
1/1	++++[a]	++++	+++	++	++	+	—	—
1/2	++++	++++	+++	+	++	+	—	—
1/4	++	+++	+	±	++	±	—	—
1/8	++	+++	++	—	—	—	—	—
1/16	++	+++	+	—	—	—	—	—
1/32	+++	+++	++	—	—	—	—	—
1/64	++	+	—	—	—	—	—	—
1/128	±	+	—	—	—	—	—	—
1/256	±	—	—	—	—	—	—	—
1/512	—	—	—	—	—	—	—	—
K_t	—	—	—	—	—	—	—	—

[a] — reaction intensity: — = negative reaction, ± = very weak reaction, + = weak reaction, ++ = medium reaction, +++ = strong reaction i ++++ = very strong reaction,
K_f = negative control, physiological solution, K_t = negative control, Tris-HCl buffer

Electro-Blot-Immunoassay serological method (EBIA, Western blot), applied in these investigations, made possible, to a considerable extent, the study of antigenic characteristics and serological relationships of 12 domestic and 5 standard *Alternaria* spp. isolates. Figure 1 shows the presence of bluish-purple bands, which indicates positive reactions between the antigen and the antiserum. It is clear that the prepared antiserum reacted with all *Alternaria* fungi isolates, but not with *Agaricus* spp., which proves its specificity. The presence and intensity of bands on NC paper were estimated visually, and the results were summarized in Table 2.

According to the protein band profiles on NC paper, antigenic characteristics of examined *Alternaria* spp. isolates indicate that they belong to four different species: *A. radicina, A. petroselini, A. dauci* and *A. alternata*. *A. dauci* isolates (68-5, 94 and 108, as well as the standards) reacted by forming the greatest number of bands (9—14, depending on the isolate), which was expected, considering that antiserum was prepared against isolate 108. These five isolates can be clearly separated from the others by their protein band profiles. It should be emphasized that studying the antigenic characteristics under the given conditions revealed some additional differences among the isolates from the same species. Therefore, besides great similarities, some differences were also observed among some isolates within this compact group-species. The

Tab. 2 — Serological reactions of selected *Alternaria* spp. isolates by EBIA with polyclonal antiserum against isolate 108

Band No	Antigen (isolate marks)														
	BMP155	NL2D	108	94	68-5	Agaricus	13	BMP79	89	NL1R	68-1	106	BM P139	95	69
1	++++	++++	++++	++++	++++	—	++++	++++	++++	++++	++++	++	++	++	++
2	++++	++	++++	++++	++++	—	++++	++	++++	++++	++++	++	++	++	++
3	++	++	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	++	++	++++	—	—	—	—	—	—	—	—	—	—
5	—	—	++	++	++++	—	+++	++	++	++	+	+	+	+	+
6	+++	++	+++	+++	++++	—	—	+++	+++	+++	—	—	—	—	—
7	++	+	+++	+	++++	—	+++	+	+	+	+	+	+	+	+
8	+++	+	++	+	+++	—	+	—	—	—	—	—	—	—	—
9	—	—	—	++	++++	—	+++	—	—	—	—	—	—	—	—
10	+++	+	++++	++++	++++	—	++++	++++	++++	++++	+	+	+	+	++
11	++	—	++	++	++	—	+++	+	+	+	+	—	—	—	—
12	—	—	—	—	++++	—	—	—	—	—	—	—	—	—	—
13	++++	++++	++++	++++	++++	—	++	++	++	+++	+++	—	—	—	—
14	—	—	—	—	++	—	+	—	—	—	—	—	—	—	—
15	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—

[a] — reaction intensity: — = negative reaction, + = weak reaction, ++ = medium reaction, +++ = strong reaction i ++++ = very strong reaction

isolate 68-5 has protein bands 14 and 15, which are omitted in the other isolates from this species. Only this isolate forms protein band 12 and shows some similarity with isolate 13, identified as *A. alternata*. Among the all investigated, only these two isolates, 68-5 (*A. dauci*) and 13 (*A. alternata*), although belonging to different species, form protein bands 14 and 15. Nevertheless, isolate 13 (*A. alternata*) clearly differs from all the other isolates included in the investigations, by omitting the protein band 6, which is common to all *A. dauci* and *A. radicina* isolates, except for the isolate 68-1. The group of isolates which belong to species *A. radicina* (68-1, 89, BMP 79 and NL1R), with a few exceptions, showed smaller number of protein bands in profile, i.e. revealed differences between particular isolates. Thus, only isolate 68-1 does not form band 6, which is present in all the others in this group, and does not completely correspond to a common profile. The group of isolates belonging to *A. petroselini* (69, 95, 106 and BMP 139) also exhibited great uniformity of characteristics, but reacted with smaller number of bands, which were of weaker intensity, compared to the other *Alternaria* spp. All the isolates from this group formed five bands each, while variations within this group were not observed under the investigations conditions.

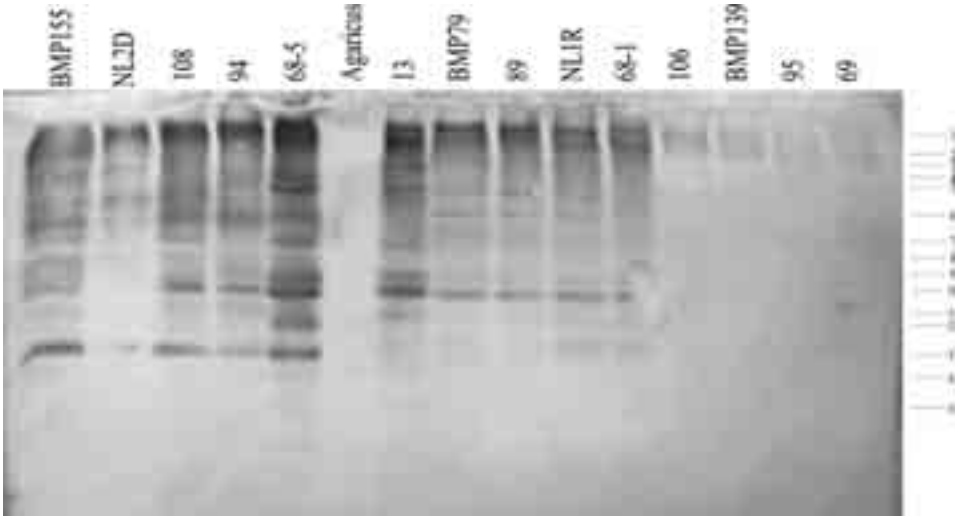

Fig. 1 — Serological reaction of *Alternaria* spp. isolates using EBIA method

BMP 155 and NL2D standards for *A. dauci*, 108, 94 i 68-5 (*A. dauci*), *Agaricus* sp. (-) control, 13 (*A. alternata*), BMP79 and NL1R standards for *A. radicina*, 89 i 68-1 (*A. radicina*), 106, 95 i 69 (*A. petroselini*) and BMP 139, standard for *A. petroselini*

DISCUSSION

In comparison to other features, serological characteristics in mycology are investigated and used less frequently. Nevertheless, there are examples of a very successful application in distinguishing or diagnostics of particular plant pathogenic species of fungi, even the categories lower than species level (Mohan, 1989; Mathew and Brooker, 1991; Benson, 1992; Bowen et al., 1996; Dewey and Cole, 1996; Hahn and Werres, 1996; Heppner and Heitefuss, 1996; Stcherbaukova and Umnov, 1996; Ueli and Walsh, 1996; Srivastava and Arora, 1997; Vico, 1997; Williams and Fitt, 1999; Kesari et al., 2005). Antigenic characteristics of particular fungi have been successfully used even for detection of the presence of some mycotoxins in different substrates (Szurdoki et al., 1996). This experience shows that studying of phytopathogenic fungi serological characteristics could lead to the development of very powerful and fast methods for detection or diagnostics, which could be routinely used.

Serological features of *Alternaria* species have not been studied almost at all. These fungi have drawn the greatest attention as powerful allergens, and the greatest number of published papers is related to studying of their antigenic properties in human medicine (Scumacher et al., 1975; Vijay et al., 1997; Bush et al., 1983; Chang et al., 1989; Weber, 2001). Guilong (1995) studied the possibility of application of standard serological test ELISA, which is routinely used in diagnosis of plant pathogen categories other than fungi, for *A. alternata*, detection on tobacco seed. Antiserum used in his investigation was highly specific to *A. alternata* and it did not react with fungi from other genera, which is in complete accordance with the results obtained in this investigation. Our results, obtained by using EBIA, showed the presence of bands in protein profiles, which could be specific for particular species. Specificity of the produced antiserum, if necessary, could be improved for diagnostic purposes. However, since the objective of this investigation was comparison of taxonomic relationships among *Alternaria* isolates, the fact that the produced antiserum reacted with all the examined isolates made the comparison of their protein profiles possible, so that their common or specific features could be observed.

Study on the antigenic characteristics of the examined *Alternaria* isolates, pathogenic for Apiaceae plant hosts, provided a new insight into their taxonomic relationships. Serology, as a taxonomic criterion, succeeded in grouping the isolates based on the appearance of their protein profiles in the way which completely corresponds to all other criteria, used in this investigation. Except the grouping to an assumed species level, antigenic properties indicated both similarities and differences among the isolates within the same and different groups-species, revealing their extremely complex relationships which genuinely reflect the diversity of these fungi in nature.

LITERATURE

Adams, G. C. and Butler, E. E. (1979): *Serological relationships among anastomosis groups of Rhizoctonia solani*, Phytopathology 69: 629—633.

Benson, D. M. (1992): *Detection by enzyme-linked immunosorbent assay of Rhizoctonia species on poinsettia stem cuttings*, Plant disease 76: 578—581.

Bowen, J. K., Peart, J., Lewis, B. G., Cooper, C. and Matthews, P. (1996): *Development of monoclonal antibodies against the fungi of the 'Ascochyta complex'*, Plant Pathology 45: 393—406.

Bulajić, A. (2007): *Identifikacija i taksonomski međuodnos vrsta roda Alternaria Ness patogenih za povrtarske i začinske biljke familije Apiaceae u Srbiji*, doktorska disertacija, Poljoprivredni fakultet, Univerzitet u Beogradu, Beograd—Zemun, pp.

Bush, R. K., Voss, M. J. and Bashirian, S. (1983): *Detection of Alternaria allergens by crossed radioimmunoelectrophoresis*, J. Allergy Clin. Immunol. 71: 239—244 (Abstr.).

Chang, Z. N., Wang, M. C., Shen, H. D. and Han, S. H. (1989): *Using anti--sera and monoclonal antibodies against Aspergillus fumigatus to study its cross--reactions with Alternaria alternata and Penicillium sp*., Zhonghua Yi Xue Za Zhi 43: 75—80. (Abstr.).

Dewey, F. and Cole, L. (1996): *Monoclonal antibody based assays for detection and quantification of Botrytis cinerea. Diagnosis and identification of plant pathogens: absracts of papers and list of participants*, 4th International EFPP Symposium, September 9—12. 1996, Bonn, Germany.

Guilong, M. (1995): [*Study on ELISA for detecting Alternaria alternata on the seed of tobacco*]. Journal of Jilin Agricultural University **17**: 21—25 (Ch). Department of Agronomy, Jilin Agricultural University, Changchun 130118, China. Rev. Plant Pathol. 1997 76: 639 (Abstr.).

Hahn, R. and Werres, S. (1996): *Development of a dot-immunobinding assay for the detection of Phytophthora spp. in roots. Diagnosis and identification of plant pathogens: absracts of papers and list of participants*, 4th International EFPP Symposium, September 9—12. 1996, Bonn, Germany.

Heppner, C. and Heitefuss, R. (1996): *Development of an enzyme-linked immunosorbent assay (ELISA) for Verticillium dahliae detection in soil. Diagnosis and identification of plant pathogens: abstracts of papers and list of participants*, 4th International EFPP Symposium, September 9—12. 1996, Bonn, Germany.

Kessari, R., Mishra, D. P., Garg, G. K. and Kumar, A. (2005): *Suitability of dyed latex bead agglutination test for immunodiagnosis of Karnal bunt (Tilletia indica) teliospores in a single seed of wheat*, Food and Agricultural Immunology, 16:73—80 (Abstr.).

Krstić, B. i Tošić, M. (1994): *Biljni virusi — neke osobine i dijagnoza*, Poljoprivredni fakultet, Univerzitet u Beogradu, pp: 6—137.

Laemmli, V. K. (1970): *Cleavage of structural proteins during the assembly of the head of bacteriophage t4*. Nature, London, 227: 680—685.

Mathew, J. S. and Brooker, J. D. (1991): *The isolation and characterization of polyclonal and monoclonal antibodies to AG 8 of Rhizoctonia solani*, Plant Path. 40: 67—77.

Mohan, S. (1989): *Analysis of cross-reactive antigens of Phytophthora fragariae and strawberry and their relation to resistance and susceptibility*, J. Phytopathology 127: 316—330.

Noordam, D. (1973): *Identification of plant viruses: Methods and experiments*, PUDOC Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands., pp: 13—207.

O'Donnell, I. J., Shukla, D. D. and Gough, K. H. (1982): *Electro-blot radio immunosorbent of virus-infected plant sap — a powerful new technique for detecting plant viruses*, J. Virol. Meth. 4: 19—26.

Pryor, B. M. and Gilbertson, R. L. (2002): *Relationships and taxonomic status of Alternaria radicina, A. carotiincultae and A. petroselini based upon morphological, biochemical and molecular characteristics*, Mycologia 94 (1): 49—61.

Pryor, B. M. and Michailides, T. J. (2002): *Morphological, pathogenic and molecular characterization of Alternaria isolates associated with alternaria late blight of pistachio*, Phytopathology 92: 406—416.

Rotem, J. (1994): *The genus Alternaria, Biology, Epidemiology and Pathogenicity*, APS Press, St Paul, Minnesota, pp. 1—325.

Rybicki, E. P. and Von Wechmar, B. (1982): *Enzyme — assisted immune detection of plant virus proteins electroblotted onto nitrocellulose paper*, J. Virol. Meth. 5: 267—278.

Schumacher, M. J., McClatchy, J. K., Farr, R. S. and Minden, P. (1975): *Primary interactions between antibody and components of Alternaria. I. Immunology and chemical characteristics of labeled antigens*, J. Allergy Clin. Immunol. 56: 39—53 (Abstr.).

Shukla, D. D., Jilka, J., Tošić, M. and Ford, R. E. (1989): *A novel approach to the serology of potyviruses involving affinity purified polyclonal antibodies direct towards virus — specific N termini of coat proteins*, J. Gen. Virol. 70: 13—23.

Simmons, E. G. (1992): *Alternaria Taxonomy: Current Status, Viewpoint, Challenge*. In: *Alternaria Biology, Plant Diseases and Metabolites, Topics in Secondary Metabolism* — Volume 3, ed. by Chelkowski, J. and Visconti, A., Elsevier Science Publishers B. V., Amsterdam, pp: 1—35.

Srivastava, A. K. and Arora, D. K. (1997): *Evaluation of a polyclonal antibody immunoassay for detection and quantification of Macrophomina phaseolina*, Plant Pathology 46: 785—794.

Stcherbakova, L. A. and Umnov, A. M. (1996): *Detection of some phytopathogenic fungi in host plant tissues by means of direct and indirect ELISA. Diagnosis and identification of plant pathogens: absracts of papers and list of participants*, 4th International EFPP Symposium, September 9—12. 1996, Bonn, Germany.

Szurdoki, F., Trousdale, E., Ward, B., Gee, S. J., Hammock, B. D. and Gilchrist, D. G. (1996): *Synthesis of Protein Conjugates and Development of Immunoassays for AAL Toxins*, J. Agric. Food Chem. 44: 1796—1803.

Ueli, M. and Walsh, J. (1996): *Serological detection of Spongospora subterranea f.sp. subterranea in soil. Diagnosis and identification of plant pathogens: absracts of papers and list of participants*, 4th International EFPP Symposium, September 9—12. 1996, Bonn, Germany.

Vico, I. M. (1997): *Prilog taksonomiji fitopatogenih gljiva roda Rhizoctonia DC. ex Fr.*, doktorska disertacija, Poljoprivredni fakultet, Beograd—Zemun, pp: 1—133.
Vijay, H. M., Huang, H., Young, N. M. and Bernstein, I. L. (1997): *Studies of Alternaria allergenes. I. Isolation of alergens from Alternaria tenuis and Alternaria solani*, Int. Arch. Allergy Appl. Immunol. 60: 229—239. (Abstr).
Weber, R. W. (2001): *Cross-Reactivity of Plant and Animal Allergens*, Clinical Reviews in Allergy and Immunology, 21: 153—202 (Abstr).
Williams, R. H. and Fitt, B. D. L. (1999): *Differentiating A and B groups of Leptosphaeria maculans, causal agent of stem cander (blackleg) of oilseed rape*, Plant Pathology 48: 161—175.

АНТИГЕНЕ ОСОБИНЕ КАО ТАКСОНОМСКИ КРИТЕРИЈУМ ЗА РАЗЛИКОВАЊЕ *ALTERNARIA* SPP. ПАТОГЕНИХ ЗА МРКВУ И ПЕРШУН

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Резиме

Идентификација врста у оквиру рода *Alternaria* је веома сложен процес који захтева широко постављена испитивања и проучавање већег броја особина које заједно представљају задовољавајуће таксономске критеријуме. Основни циљ овог рада је био да се испита могућност примене антигених особина фитопатогених гљива из рода *Alternaria* као таксономског критеријума, као и увођење серолошких метода за њихову идентификацију. Проучавајући гљиве из рода *Alternaria* које су патогене за гајене биљке из фам. *Apiaceae* у нашој земљи, добијено је више изолата који су, на основу проучавања конвенционалним методама и круга домаћина, као и молекуларне детекције и делимичне карактеризације, разврстани у четири врсте: *Alternaria radicina*, *A. petroselini*, *A. dauci* и *A. alternata*.

У испитивања је било укључено 12 изолата пореклом са листа, семена или из земље, који су испољавали патогеност првенствено према мркви и першуну и идентификовани да припадају врстама *A. radicina*, *A. petroselini*, *A. dauci* и *A. alternata*. Испитивани изолати су међусобно упоређивани као и са стандардима за наведене врсте (укупно 5 изолата, пореклом из САД и ЕУ).

Приликом испитивања серолошких особина *Alternaria* spp., прво је припремљен поликлонални антисерум на изолат из Србије, који је идентификован као *A. dauci*. Антисерум је испољио специфичност за род *Alternaria*, док није реаговао са антигенима из других родова фитопатогених гљива (*Fusarium*, *Rhizoctonia*, *Agaricus*). Титар антисерума, испитиван методом аглутинације на плочици, био је 1/32. Антигене особине гљива из рода *Alternaria* проучаване су применом електрофоретско-адсорпционо-имуноензимске серолошке методе (EBIA, Western blot), односно упоређивани су њихови протеински профили.

Испитивани изолати *Alternaria* spp. испољили су различите профиле протеинских трака у гелу и на нитроцелулозном папиру, а уочене разлике су, у потпуности, одговарале резултатима претходне идентификације. Наведени изолати, домаћи као и стандарди, испољили су међусобне сличности и могли су бити правилно идентификоване до нивоа врсте, применом ЕБИА. Поред груписања до нивоа врста, антигене особине су указале на постојање сличности и разлика

између изолата унутар истих, односно различитих врста, указујући на њихове веома сложене међуодносе који верно осликавају диверзитет постојања ових гљива у природи.

У досадашњим проучавањима гљива из рода *Alternaria*, не постоје подаци о њиховим серолошким особинама као могућим таксономским критеријумима. Увођење ове групе особина, представља значајан допринос како у таксономији, тако и у примени брзих и тачних метода идентификације фитопатогених гљива.