Journal of Agricultural, Food and Environmental Sciences Vol 76 No 7 (2022) 35-39
DOI: https://doi.org/10.55302/JAFES22767036v

BASIC CHARACTERISTICS OF *ERWINIA AMYLOVORA* STRAINS ORIGINATING FROM DIFFERENT HOSTS AND AT DIFFERENT ALTITUDES

Milić Vojinović*, Jelica Živić, Sanja Perić, Slađana Golubović, Ivica Stančić, Dušica Ćirković, Dragana Stanisavljević

Toplica Academy Professional Studies, Department of Agricultural and Food Studies, Prokuplje, Serbia
*e-mail: vojinovic63@gmail.com

ABSTRACT

Erwinia amylovora is a causative agent of bacterial blight of plants of the Rosaceae family. The most important hosts of this bacteria are apple fruits and ornamental shrubs. The paper presents comparative characteristics of *Erwinia amylovora* strains originating from different hosts and altitudes in Serbia.The presence of *Erwinia amylovora* was confirmed by classical tests and molecular and serological methods. The tests are: Gram, fluorescence on King B medium, tobacco and nutmeg leaf inoculation, isolation of bacteria on King B medium, presence of bacterial exudate, ELISA test, IF test, BIOLOG test and PCR. PCR can prove the presence of pathogens with a minimal amount of inoculum. Although this method is very reliable, it is necessary to prove it by other methods. A BIOLOG test is used as a very fast, reliable and cheap method for detecting bacteria. The metabolic imprint on the microtiter plate shows the typical characteristics of the bacteria and its connection with the sources of carbon compounds. The studied strains of apple, pear, quince and medlar isolated from different altitudes and different hosts show a negative Gram reaction, do not produce fluorescent pigment on King-B medium, cause hypersensitive reaction to tobacco leaf, cause necrosis of artificially inoculated pears, with the presence of bacterial exudates, react positively with the appropriate serum in the ELISA test, are positive for the IF test, leave a metabolic imprint on the BIOLOG test characteristic of *E. amylovora* and achieve an agglutination reaction by reacting with an antibody from the Express Kit.

Key words: bacterial blight, methods, tests.

INTRODUCTION

Erwinia amylovora is a causative agent of bacterial blight of plants of the Rosaceae family (Arsenijević and Gavrilović, 2007). The most important hosts of this bacteria are apple fruits and ornamental shrubs (Panić and Arsenijević 1996).

Bacterial blight caused by *E. amylovora* belongs to the group of the economically most significant diseases of apple fruits. Since *E. amylovora* parasitizes all the organs of the plant (flower, fruit, skeletal branches, trunk) and causes necrosis and tissue damaging on them, these bacteria are considered as one of the most destructive pathogens (Beer, 1991; Ficher and Richter 1998, Mohan et.al 2001). With further significant spread of the infection in the Republic of Serbia, due to insufficient, superficial, or non implementation of measures to protect sensitive host plants, even more significant economic losses from the quarantine bacteria *E. amylovora*, should be expected in the near future. One of the protection measures in the future could be the use of antibiotics, which are already used and give good results.

MATERIAL AND METHODS

The presence of *Erwinia amylovora* was confirmed by classical tests and molecular and serological methods. The tests are: Gram, fluorescence on King B medium, tobacco and nutmeg leaf inoculation, isolation of bacteria on King B medium, presence of bacterial exudate, ELISA test, IF test, BIOLOG test and PCR. PCR can prove the presence of pathogens with a minimal amount of inoculum (van der Zwet and Keil, 1979; van der Zwet and Beer, 1995; Gavrilović et.al 2014). Although this method is very reliable, it is necessary to prove it by other methods. A BIOLOG test is used as a very fast, reliable and cheap method for detecting bacteria. The metabolic imprint on the microtiter plate shows the typical characteristics of the bacteria and its connection with the sources of carbon compounds.

RESULTS AND DISCUSSION

Table 1. Basic characteristics of typical strains of E. amylovora originating from different hosts from 0-200 meters above sea level

Ordinal number	Isolate code	Gram	King-B	HR	BEPF		ELISA test	IF test	EKSPRES test
					48^h	72^h			
1.	A-1	-	-	+	+	+	+	+	+
2.	A-2	-	-	+	+	+	+	+	+
3.	A-3	-	-	+	+	+	+	+	+
4.	A-6	-	-	+	+	+	+	+	+
5.	A-7	-	-	+	+	+	+	+	+
6.	Q-1	-	-	+	+	+	+	+	+
7.	Q-2	-	-	+	+	+	+	+	+
8.	Q-3	-	-	+	+	+	+	+	+
9.	Q-7	-	-	+	+	+	+	+	+
10.	M-303	-	-	+	+	+	+	+	+
11.	M-304	-	-	+	+	+	+	+	+
12.	M-305	-	-	+	+	+	+	+	+
13.	CFBP1430*	-	-	+	+	+	+	+	+
14.	NCPPB 595*	-	-	+	+	+	+	+	+
15.	P-101**	-	+	+	-	-	-	-	-

Legend: Gram - Behavior towards Gram, King-B - Fluorescence on King's B substrate, HR - Hypersensitivity reaction, BEPF - Bacterial exudate on pear fruits, ELISA - test, IF - test, Express kit - test, A - apple as host, Q - quince as host, M - medlar as a host, P - pear as a host, H - hawthorn as a host, Sd - service tree (*Sorbus domestica*) as host, * - control strains, **- *Pseudomonas syringae v. syringae*

The studied strains of apple, quince and medlar isolated from an altitude of 0-200 meters show a negative Gram reaction, do not produce fluorescent pigment on King-B medium, cause hypersensitive reaction to tobacco leaf, cause necrosis of artificially inoculated pears, with the presence of bacterial exudates, react positively with the appropriate serum in the ELISA test, are positive for the IF test, leave a metabolic imprint on the BIOLOG test characteristic of *E. amylovora* and achieve an agglutination reaction by reacting with an antibody from the Express Kit. (table 1).

The studied strains of apple, quince, medlar and service tree (*Sorbus domestica*) isolated from an altitude of 200-400 meters show a negative Gram reaction, do not produce fluorescent pigment on King-B medium, cause hypersensitive reaction to tobacco leaf, cause necrosis of artificially inoculated pears, with the presence of bacterial exudates, react positively with the appropriate serum in the ELISA test, are positive for the IF test, leave a metabolic imprint on the BIOLOG test characteristic of *E. amylovora* and achieve an agglutination reaction by reacting with an antibody from the Express Kit. (table 2).

Table 2. Basic characteristics of typical strains of E. amylovora originating from different hosts from 200-400 meters above sea level

Ordinal number	Isolate code	Gram	King-B	HR	BEPF		ELISA test	IF test	EKSPRES test
					48^h	72^h			
1.	Q-4	-	-	+	+	+	+	+	+
2.	Q-5	-	-	+	+	+	+	+	+
3.	Q-6	-	-	+	+	+	+	+	+
4.	Q-13	-	-	+	+	+	+	+	+
5.	Sd-201	-	-	+	+	+	+	+	+
6.	Sd-202	-	-	+	+	+	+	+	+
7.	Sd-203	-	-	+	+	+	+	+	+
8.	Sd-204	-	-	+	+	+	+	+	+
9.	Sd-205	-	-	+	+	+	+	+	+
10.	M-301	-	-	+	+	+	+	+	+
11.	A-5	-	-	+	+	+	+	+	+
12.	A-12	-	-	+	+	+	+	+	+
13.	A-13	-	-	+	+	+	+	+	+
14.	A-14	-	-	+	+	+	+	+	+
15.	CFBP1430*	-	-	+	+	+	+	+	+
16.	NCPPB 595*		-	+	+	+	+	+	+
17.	P-101**	-	+	+	-	-	-	-	-

Table 3. Basic characteristics of typical strains of E. amylovora originating from different hosts from 400-600 meters above sea level

Ordinal number	Isolate code	Gram	King-B	HR	BEPF		ELISA test	IF test	EKSPRES test
					48^h	72^h			
1.	P-101	-	-	+	+	+	+	+	+
2.	Q- 8	-	-	+	+	+	+	+	+
3.	Q- 9	-	-	+	+	+	+	+	+
4.	Q- 12	-	-	+	+	+	+	+	+
5.	Q- 16	-	-	+	+	+	+	+	+
6.	M- 302	-	-	+	+	+	+	+	+
7.	M- 307	-	-	+	+	+	+	+	+
8.	CFBP1430*	-	-	+	+	+	+	+	+
9.	NCPPB 595*	-	-	+	+	+	+	+	+
10.	P-101**	-	+	+	-	-	-	-	-

The studied strains of pear, quince and medlar isolated from an altitude of 400-600 meters show a negative Gram reaction, do not produce fluorescent pigment on King-B medium, cause hypersensitive reaction to tobacco leaf, cause necrosis of artificially inoculated pears, with the presence of bacterial exudates, react positively with the appropriate serum in the ELISA test, are positive for the IF test, leave a metabolic imprint on the BIOLOG test characteristic of *E. amylovora* and achieve an agglutination reaction by reacting with an antibody from the Express Kit. (table 3).

The studied strains of apple, quince and medlar isolated from an altitude of 600-800 meters show a negative Gram reaction, do not produce fluorescent pigment on King-B medium, cause hypersensitive reaction to tobacco leaf, cause necrosis of artificially inoculated pears, with the presence of bacterial exudates, react positively with the appropriate serum in the ELISA test, are positive for the IF test, leave a metabolic imprint on the BIOLOG test characteristic of *E. amylovora* and achieve an agglutination reaction by reacting with an antibody from the Express Kit. (table 4).

Table 4. Basic characteristics of typical strains of E. amylovora originating from different hosts from 600-800 meters above sea level

Ordinal number	Isolate code	Gram	King-B	HR	BEPF		ELISA test	IF test	EKSPRES test
					48^h	72^h			
1.	Q- 10	-	-	+	+	+	+	+	+
2.	A-7	-	-	+	+	+	+	+	+
3.	M-308	-	-	+	+	+	+	+	+
4.	CFBP1430*	-	-	+	+	+	+	+	+
5.	NCPPB 595*	-	-	+	+	+	+	+	+
6.	P-101**	-	+	+	-	-	-	-	-

Table 5. Basic characteristics of typical strains of E. amylovora originating from different hosts from 800-1000 meters above sea level

Ordinal number	Isolate code	Gram	King-B	HR	BEPF		ELISA test	IF test	EKSPRES test
					48^h	72^h			
1.	Q-11	-	-	+	+	+	+	+	+
2.	Q-14	-	-	+	+	+	+	+	+
3.	CFBP1430*	-	-	+	+	+	+	+	+
4.	NCPPB 595*	-	-	+	+	+	+	+	+
5.	P-101**	-	+	+	-	-	-	-	-

The studied strains of quince isolated from an altitude of 800-1000 meters show a negative Gram reaction, do not produce fluorescent pigment on King-B medium, cause hypersensitive reaction to tobacco leaf, cause necrosis of artificially inoculated pears, with the presence of bacterial exudates, react positively with the appropriate serum in the ELISA test, are positive for the IF test, leave a metabolic imprint on the BIOLOG test characteristic of *E. amylovora* and achieve an agglutination reaction by reacting with an antibody from the Express Kit. (table 5).

Table 6. Basic characteristics of typical strains of E. amylovora originating from different hosts from 1000-1200 meters above sea level

Ordinal number	Isolate code	Gram	King-B	HR	BEPF		ELISA test	IF test	EKSPRES test
					48^h	72^h			
1.	A-9	-	-	+	+	+	+	+	+
2.	H-1	-	-	+	+	+	+	+	+
3.	H-2	-	-	+	+	+	+	+	+
4.	H-3	-	-	+	+	+	+	+	+
5.	H-4	-	-	+	+	+	+	+	+
6.	H-5	-	-	+	+	+	+	+	+
7.	CFBP1430*	-	-	+	+	+	+	+	+
8.	NCPPB 595*	-	-	+	+	+	+	+	+
9.	P-101**	-	+	+	-	-	-	-	-

The studied strains of apple and hawthorn isolated from an altitude of 1000-1200 meters show a negative Gram reaction, do not produce fluorescent pigment on King-B medium, cause hypersensitive reaction to tobacco leaf, cause necrosis of artificially inoculated pears, with the presence of bacterial exudates, react positively with the appropriate serum in the ELISA test, are positive for the IF test, leave a metabolic imprint on the BIOLOG test characteristic of *E. amylovora* and achieve an agglutination reaction by reacting with an antibody from the Express Kit. (table 6).

CONCLUSION

The studied strains of apple, pear, quince, medlar, service tree and hawthorn isolated from different altitudes and different hosts show a negative Gram reaction, do not produce fluorescent pigment on King-B medium, cause hypersensitive reaction to tobacco leaf, cause necrosis of artificially inoculated pears, with the presence of bacterial exudates, react positively with the appropriate serum in the ELISA test, are positive for the IF test, leave a metabolic imprint on the BIOLOG test characteristic of *E. amylovora* and achieve an agglutination reaction by reacting with an antibody from the Express Kit (Vojinović, 2010).

REFERENCES

Arsenijević, M., & Gavrilović, V. (2007): A practical handbook on bacterial blight of apple fruit trees. Institute for Plant Protection and Environment, Belgrade, pp 1-80.

Beer, S. V. (1991): Fire blight, pp 61-63. In: Compendium of Apple and Pear Diseases, by A.L. Jones and H.S. Aldwinckle. APS Pres, St. Paul.

Ficher, C., & Richter, K. (1998): Results of fire blight resistance within the Pillnity apple breeding program. 8th International Workshop on Fire blight. Kuşadasi, Turkey, 12-15 October, 1998. Book of Abstract pp. 56.

Gavrilović, V., Živković, S., Ivanović, Ž., & Vojinović, M. (2008): Sorbus domestica and Sorbus torminalis new hosts of *Erwinia amylovora* in Serbia. "Plant Protection" Vol 58 (1-4) No.263-266, 69-79. Belgrade

Gavrilović, V.,Stanisavljević, R., Stošić S., Stevanović m., Aleksić G., Stajić M., & Dolovac N. (2014): Examination of resistance of pear varietes to Erwinia amylovora by inoculation of immature fruits. "Plant Protection" Vol 65 No.289,117-123. Belgrade

Mohan, S., Fallahi, E., & Bijman, V. P, (2001): Evaluation of apple varietes for susceptibility to *Erwinia amylovora* by artificial inoculation under field conditions. 9th international Workshop on Fire Blight. Hawke Bay, New Zealand, 8-12 October 2001. Book of abstrakt P52.

Panić, M., & Arsenijević, M. (1996): Bacterial blight of fruit trees and ornamental plants - *Erwinia amylovara.* Monographic study. Community for fruits and vegetables, Belgrade D.D. and Faculty of Agriculture, Novi Sad, p.413.

van der Zwet, T., & Keil, H.L. (1979): Fire Blight – A Bacterial Disease of Rosaceous plants. U.S. Department of Agriculture, Agriculturae Handbook 510, Washington, D.C., pp., 200.

van der Zwet, T., & Beer, S. V. (1995): Fire blight - Its Nature, Prevention and Control. A Practical Guide to Integrated Disease Menagment U.S. Department of Agriculture. Agricultural Information Bulletin No.631, 97 pp.

Vojinović, M., (2010): Comparative characteristics of *Erwinia amylovora* strains obtained from diferent hosts, areas and altitudes in Serbia, Ph thesis, Faculty of Agriculture in Kosovska Mitrovica, 2010