MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF *Monilinia laxa* ISOLATES ORIGINATED FROM STONE FRUITS IN SERBIA

Trkulja Nenad, Milosavljević Anja, Ivanović Žarko, Popović Tatjana, Živković Svetlana,
Oro Violeta, Dolovac Nenad
Institute for Plant Protection and Environment, Belgrade
trkulja_nenad@yahoo.com

Monilinia laxa (Aderhold & Ruhland) Honey is one of the three most important pathogens from the genus *Monilinia* which causes a drying of flowers and twigs and brown rot of pome and stone fruits. In Serbia *M. laxa* regularly appears on stone fruits, i.e. plum (*Prunus domestica* L.), cherry (*Prunus cerasus* L.), peach (*Prunus persica* (L.) Batsch), apricot (*Prunus armeniaca* L.) but also appears on apple (*Malus domestica* Borkh.) and pear (*Pyrus communis* L.) causing severe yield losses (Trkulja et al. 2010).

Symptomatic shoots affected with brown rot disease were collected in 2010 at localities Šabac, Smederevo, Topola, Grocka from orchards of plum, cherry, peach and apricot in order to obtain isolates of *M. laxa*. Isolation of the pathogen was made by transferring piece of infected tissue to potato dextrose agar (PDA) to obtain monosporial isolates. Identification was performed according to the culture and morphological characteristics (De Cal and Melgarejo, 1999). To obtain uniform colonies for each isolate, plugs with actively growing mycelium were removed from the periphery of a 4-day-old colony grown on PDA and were placed in the center of plastic Petri dishes (90mm diameter) containing PDA and MEA media and incubated at 25°C in the dark. After 12 days of incubation, the following morphological characteristics were recorded for each isolate on: colony growth (mm/day), colony color, colony rosette, rosettes with black arcs. Mycelial growth rates were determined by measuring colony diameters (excluding the transfer plug of 5mm) every 2 days and expressed as a millimeter of growth per day. The mean of 3 replicate colonies were used to represent each isolate.

The internal transcribed spacer 1 (ITS 1), 5.8S ribosomal RNA, internal transcribed spacer 2 (ITS 2) and 28S ribosomal RNA regions of the fungal rDNA were amplified using the primers ITS1 and ITS4 by standard protocol (White et al., 1990). Amplified products were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide and visualized under a UV transilluminator. All amplified products were purified using the QIAquick PCR purification kit (QIAGEN) according to the manufacturer's instructions and sequenced using the automated equipment (Macrogen, Korea). Sequences were manually aligned in MEGA5 software by *Clustal*W program (Tamura et al., 2011). The DNA sequences of the ITS region of four analyzed isolates were aligned and compared to each other and with available sequences of the ITS region of *M. laxa* retrieved online from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

Table 1. Morphological characteristics of the isolates of *M. laxa* on PDA medium

PDA	KJ3	SDV2	BRIC	SD1
Colony growth (mm/day)	6.1	4.5	5.2	6.1
Colony colour	White	Green	White	White
Colony rosetted	M[1]	H	S	H
Rosettes with black arcs	No	Yes	No	Yes

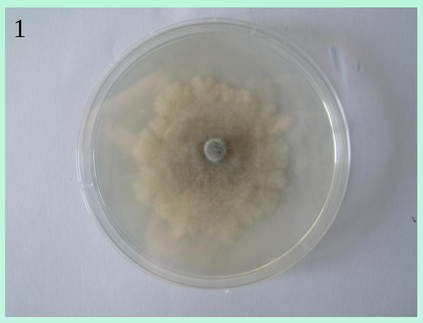
[1] – Colony rosetted: S (slightly); M (moderately); H (highly)

Table 2. Morphological characteristics of the isolates of *M. Laxa* on MEA medium

MEA	KJ3	SDV2	BRIC	SD1
Colony growth (mm/day)	5.1	1.4	2.5	3.7
Colony colour	White	White	White	White
Colony rosetted	H[1]	H	S	H
Rosettes with black arcs	No	Yes	No	Yes

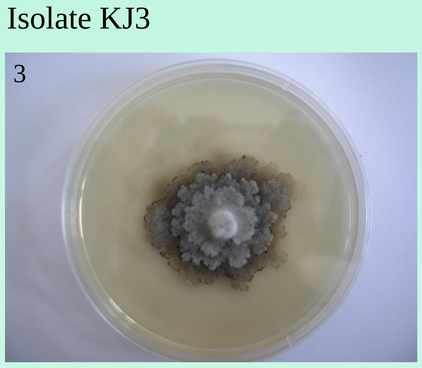
[1] – Colony rosetted: S (slightly); M (moderately); H (highly)

Pictures 1.-4. Uniform colonies of the isolates of *M. laxa* grown on PDA medium



Isolate KJ3

Isolate BRIC



Isolate SDV2

Isolate SD1

Pictures 5.-8. Uniform colonies of the isolates of *M.laxa* grown on MEA medium

Isolate KJ3

Isolate BRIC

Isolate SDV2

Isolate SD1

The ITS region was successfully amplified for all tested isolates in this study. Nucleotide sequences were obtained for ITS 1 partial sequence, 5.8S and ITS 2 complete sequence and 28S partial sequence of *M. laxa* tested isolates. Obtained DNA sequences of the ITS region for isolates KJ3, SDV2 and SD1 were identical among each other showing 100% homology with a reference ITS sequences of *M. laxa* in NCBI base (Acc. No. EF153013.1, EF153014.1, EF153015.1, EF153016.1, EF153017.1). The ITS sequence for isolate BRIC which was obtained from peach, has a single nucleotide mutation compared with other isolates observed in this study collected from apricot, cherry and plum, as well as the reference sequences from NCBI base listed above.

M. laxa causes a brown rot is distributed worldwide in all areas where the stone and pome fruits are grown. Considering its wide distribution and ability to infect a wide range of plant species, morphological and genetic diversity could be excepted for isolates of *M. laxa* originating from different geographical regions and host plants. In our study we revealed differences in culture characteristics such as a colony growth and color. Isolate SDV2 has lower growth on both PDA and MEA nutrition media then other three isolates. Given that the morphological and cultural characteristics vary among isolates that may affect on their ability to adapt to different host plants as well as the expression of pathogenicity (Munoz et al. 2008). Sequences of the *Monilinia* spp. showed a 100% similarity at the 5.8S rDNA, however some diversity was shown at the ITS1 and ITS2 region (Gell et al. 2007). Analysis of the ITS sequences of *M. laxa* revealed almost no intraspecific polymorphism (Fulton et al., 1999; Ioos and Frey, 2000). Isolate BRIC has a single nucleotide change at ITS1 region indicating that this isolate may contain genetic difference at other genes which created some trait characteristics associated with geographic origin or host plant. Fulton et al. (1999) found a variation in *M. laxa* based on RAPD-PCR data, but this difference was not correlated to a geographic origin. They suggest that *M. laxa* is randomly distributed worldwide and appears to have readily adapted to its different hosts. Gril et al. (2008) for the first time used AFLP techniques in diversity analysis of *M. laxa* isolates from different hosts and deduced significant differences between isolates from apple trees and from other host plants. This finding clearly suggests the genetic specialization of *M. laxa* to different host plants.