Endophytic microbial community of Italian Lemon *Citrus limon* Var Eureka

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Introduction. The endophytic microorganisms inhabited within of the plants in a permanent way or in some phase of its lifecycle. Some of these exhibits slowly or fasted growing without causes any symptom or visible harmful to the plant (1). The use of non-culturable methodologies to analysis of these microorganisms in plants has been very limited due a high amount of plant DNA. Current study deal to establish a method to enrichment this microbial DNA fraction in Italian lemon (*Citrus limon* Var. Eureka) due to the importance of this crop in Mexico.

Methods. A microbial DNA enrichment was conducted from sterilized leaves and fruits of Italian lemon by including three centrifugation phases (602, 15,038 and 100,000g) of plants homogenized with different buffers (TEN, CTAB, UREA). Supernatants and pellets obtained were analyzed by FISH using a universal probe (EUB338) and colony enumeration in PDA and NA broths (2). PCR specific amplification (for bacteria; COM1/COM2, and for fungi; NL1/Nl4 primers) also was conducted to confirm microbial (bacteria and fungi) presence. Some PCR products were separated by SSCP and sequenced to identify to the microorganisms.

Results. In general, the amount of DNA obtained regardless buffer extraction ranged of 43 to 91 ng/ml. PCR amplification was positive for bacteria in almost all phases and buffer extractions while that for fungi only was positive in the P1 phase with CTAB and UREA buffers. Colony enumeration shows a bacterial increasing in the P2 phase in fruits while fungus increasing the P3 in fruits and leaves (Table 1).

Table 1. Colony enumeration during DNA enrichment protocol

	Fruit		Leaves	
	Fungus (PDA)	Bacteria (AN)	Fungus (PDA)	Bacteria (AN)
Epiphytic	3	1	5	32
P1(602g)	2	5	5	4
S1(602g)	0	3	0	2
P2(15038g)	0	11	0	2
S2(15038g)	0	3	0	2
P3(1000000g)	5	2	5	1
S3(1000000g)	0	0	0	0

FISH analysis shows a slight presence of bacteria in the P2 phase (Fig. 1) even though it is necessary included a specific probe to confirm it.

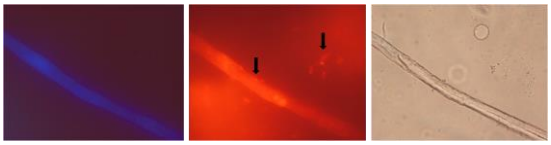

Fig 1. FISH showing specific bacteria (arrows) in P2 phase. Left panel (DAPI), middle panel (EUB338 probe), right panel (visible field) (100X).

SSCP analysis shows a different profile using specific bacteria primers during different phases (Fig. 2). Sequencing of some representative bands allows identify microorganisms of the genus Pantoea, Erwinia and uncultivable bacteria.

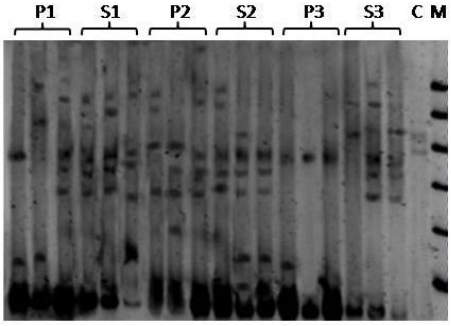

Fig 2. SSCP analysis showing PCR profile of bacterial primes in different phases. C: positive control using *Azospirillum*, M: 100 pb, molecular marker.

Conclusions. This approach including different centrifugation phases reduced the occurrence of chloroplastic sequences, and it is successful to amplify specific microbial DNA. *Pantoea* and *Erwinia* genera were more abundant in SSCP analysis.

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