

*Pseudomonas viridiflava*

**BACTERIAL LEAF SPOT OF *Malvaviscus penduliflorus* INCITED BY LEVAN-POSITIVE STRAIN OF *Pseudomonas viridiflava***

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( // : // : )

(*Malvaviscus penduliflorus*)

King's B

- *Pseudomonas viridiflava*

GenBank

( Millegen )

16S rRNA

DNA

*Pseudomonas viridiflava*

16S rRNA

IS50-PCR

ERIC-PCR REP-PCR, BOX-PCR

rep-PCR

*P.viridiflava*

*Pseudomonas viridiflava*

rep-PCR , rRNA , Malvaviscus :

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DNA  
(Mahillon & Chandler 1998)  
IS50  
*Pseudomonas*  
(Nobel 2008 Weingart & Volksch 1997)  
(ERIC REP)  
IS50  
*Pseudomonas syringae*  
IS50-PCR  
(Weingart & Volksch 1997)  
16S rRNA  
(Stackebrandt & Goebel 1994, Weisburg 1991)  
DNA  
16S rRNA  
*Pseudomonas syringae*  
(genomespecies)  
(Gardan *et al.* 1999)  
(Sleeping hibiscus)  
*Malvaviscus penduliflorus*  
Malvaviscus Malvaceae  
*M. Penduliflorus*  
(*Althea rosea*)  
(*Hibiscus rosa-sinensis*)  
*Pseudomonas*  
*Xanthomonas campestris* pv. *cichorii*  
(Chase 1986) *P. syringae malvacearum*  
*Xanthomonas smithii* pv. *smithii*  
Schaad *et al.* )  
(2005, Huang *et al.* 2008)  
Malvaviscus  
(Rahimian 1989) *P. syringae* pv. *syringae*  
(Insertion sequence) IS  
DNA  
DNA

...

:

( )

°C

(*Malvaviscus penduliflorus*)

(*Hibiscus rosa-sinensis*)

(*Citrus aurantium* L.)

(*C.sinensis* (L.) Osb.)

(Sucrose nutrient agar ,NAS)

NAS

King's B

°C

(King *et al.* 1954)

) cfu/ml

Shams-Bakhsh )

*P.viridiflava*

(

(& Rahimian 1997

cfu/ml

Schaad *et al.* Lelliot & Stead 1987)

(2001

(Ayer)

.(Arabi *et al.* 2006)

(Schaad *et al.* 2001)

/

(Tyndall)

/

- °C

(Nutrient agar,NA)

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(Template) DNA (OD)

(Arabi *et al.* 2006) (PCR) / .

*P.viridiflava* ICMP (Intern. % (SDS)

Collection of Microorganisms from Plants,  
ICMP 3938 Auckland, New Zealand) 2848

DNA *P.s.pv.syringae* (UP200 Hielscher, Germany)

**IS50-PCR rep-PCR DNA** (Supernatant)

DNA

(repetitive extragenic palindromic,rep) rep-PCR

REP1R/REP2 BOXAIR

(insertion sequence)IS50-PCR ERIC1/ERIC2

IS50 (Laemmli 1970)

(5'CAGGACGCTACTTGTGT-3') (Bromphenolblue)

Weingart & Volksch Versalovic *et al.* 1991) /

/ (1997)

MgCl<sub>2</sub> / PCR buffer 10X

dNTP /

/ DNA / %

( ) (Ausubel *et al.* 1991, Ahmadvand & Rahimian 2005)

Applied Biosystems 2720(USA)

DNA

°C

(REP,BOX) °C

°C °C (IS50) (ERIC) NA

°C °C °C °C - °C

°C IS50 ERIC REP BOX / /

(REP) (IS50 ERIC BOX) (KOH)

DNA

...

:

(Manceau & Horvais 1997) °C  
 ( Bionner PCR /  
 °C / TBE )  
 °C DNA (pH ~ / EDTA / /  
 °C % /  
 °C  
 (Manceau & Horvais 1997)  
 (Fermentas ) 1Kb  
 / )  
 (Ausubel (   
 .et al. 1992)

*P. s Pv. Syringae*

(Rahimian 1989)

DNA

( ) (Jaccard)

NTSYS (UPGMA)

2.02

(ooze)

**16S rRNA**

/  
 / MgCl2 / 10X PCR  
 / DNA / dNTP  
 ( )

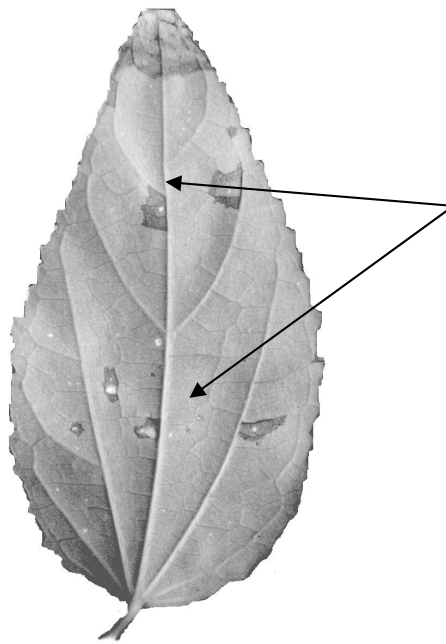
NAS

Kings B

A1(5'-GAGTTTGATCATCATGGCTCAG-3')

B6 (5'-TTGCGGGACTTAACCCAACAT-3')

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*Pseudomonas viridiflava*

**Fig. 1.** Necrotic irregular brown to black spots, surrounded by chlorotic halos on leaves of *Malva viscosa* incited by a levan-positive strains of *Pseudomonas viridiflava*

King B NAS

*P.viridiflava* ICMP2848

*P.viridiflava* ICMP2848

( )

**16S rRNA**

*P.viridiflava*

( )

*P.viridiflava**P.syringae* pv. *syringae***Table 1 . Morphological, biochemical and physiological characteristics of the bacterial strains isolated from *Malvaviscus penduliflorus* compared with those of a citrus isolate, *Pseudomonas viridiflava* and *pseudomonas syringae* pv. *Syringae***

<i>P.syringae</i> pv. <i>syringae</i>	<i>P.viridiflava</i>	(Reaction)		(Tests)
		(Citrus)	(Malvaviscus)	
				( Oxidase )
+	+	+	+	( Catalase )
	+	+	+	( Potato rot )
+		+	+	( Levan formation )
+	+	+	+	( Tobacco Hypersensitivity )
				( Arginine dihydrolase )
+	+	+	+	( Hydrolysis of gelatin )
+	+	+	+	( Hydrolysis of casein )
-	-	-	-	(Hydrolysis of starch)
-	-	-	-	(Urease)
-	-	-	-	( Nitrate reduction )
-	-	-	-	( Production of indole )
-	-	-	-	( Methyl red reaction )
				Acid production from
+	+	+	+	( D-manitol )
-	-	-	-	( Adonitol )
+	+	+	+	( Galactose )
+	+	+	+	( Inositol )
-	-	-	-	( D-sorbitol )
+	+	+	+	( Glycerol )
-	-	-	-	(Cellobiose)
-	-	-	-	(Rhamnose)
-	-	-	-	( D-trehalose )
+	-	-	+	( Sucrose )
+	+	+	+	( meso-Erythritol )
+	+	+	+	( D-Glucose )

+	+	+	+	( D-Fructose )
-	-	-	-	( D-raffinose )
-	-	-	-	( Xylitol )
+	+	+	+	( D-Xylose )
+	+	+	+	( D-Mannose )
				(Utilization of )
+	+	+	+	( Citrate )
+	+	+	+	( Succinate )
+	+	+	+	( Lactate )
+	+	+	+	( Malonate )
-	-	-	-	( L-Tartrate )
-	+	+	+	( D-Tartrate )



Kh1

Fig. 2. Necrotic spots appearing on Washington navel orange leaves, 10 days after inoculation with strain Kh1

(multiple sequence alignment) Clustal W

*Pseudomonas* (Thompson 1994) GenBank

16S rRNA

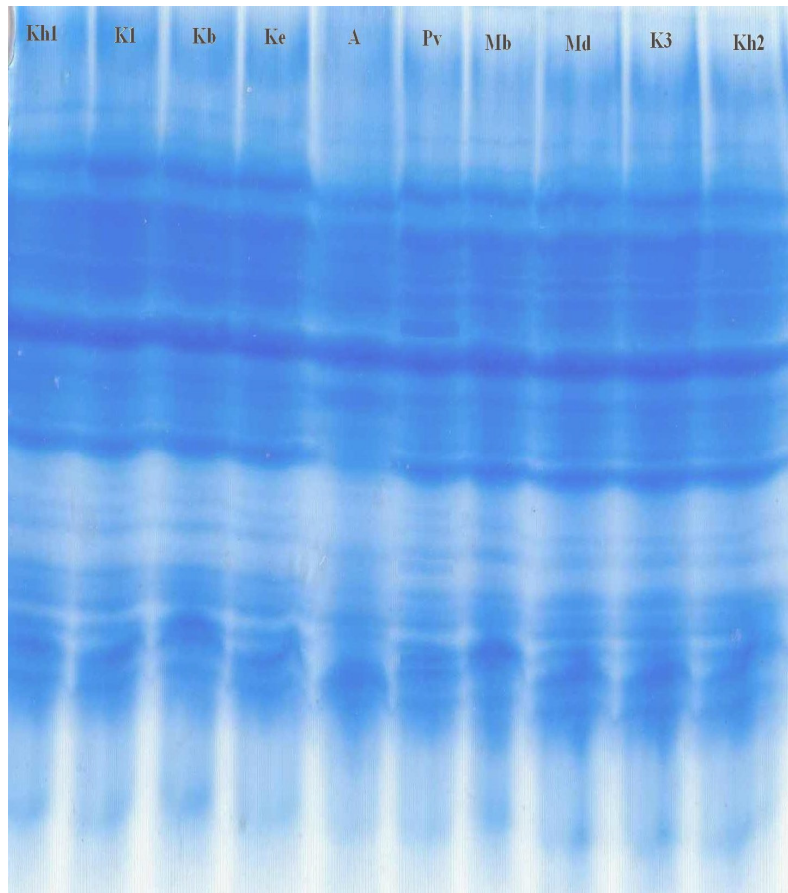
*P.chicorii* *P.viridiflava*

High Pure PCR Product Purification Kit (Roche)

Millegen

16S rRNA





A Kh2 K3 Md Mb Ke Kb K1 Kh1 :

*P. viridiflava*(Pv.)

Fig. 3. Polyacrylamide gel electrophoretic protein profiles of strains inciting *Malvaviscus penduliflorus* leaf spot (Lanes Kh1,K1.Kb,Ke.Mb,Md,K3,Kh2), citrus isolate (Lane A) and *Pseudomonas viridiflava* (Lane Pv).

IS50- rep-PCR

DNA

*P. syringae* pv. *morsprunorum*

PCR

Kb

16S rRNA

rep-PCR

DNA

GenBank JF836019

*P. syringae* pv. *syringae* *P. viridiflava*

(Saitou & Nei 1987) ( Neighbour-joining)

bootstrap MEGA 4

ERIC-PCR

*P. viridiflava*

( )

REP-PCR

BOX-PCR

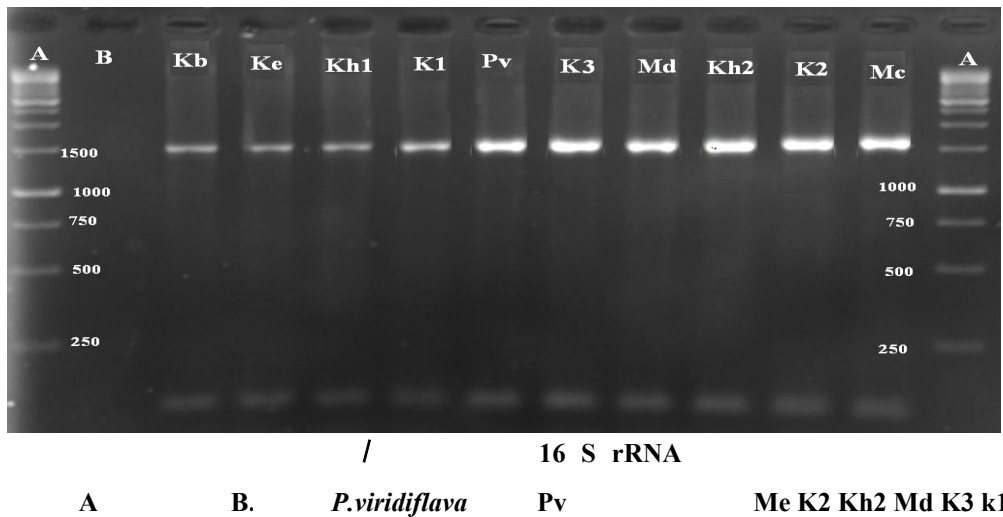


Fig. 4. The 1550 bp fragment from the 16S rRNA gene in 1 % agarose gel stained with etidium bromide. *Malvaviscus penduliflorus* isolates ( Lane Kb,Ke,Kh1,K1,K3,Md,Kh2,K2,Me ). *P.viridiflava* (Lane Pv), B: (Negative control). A: 1kb DNA ladder.

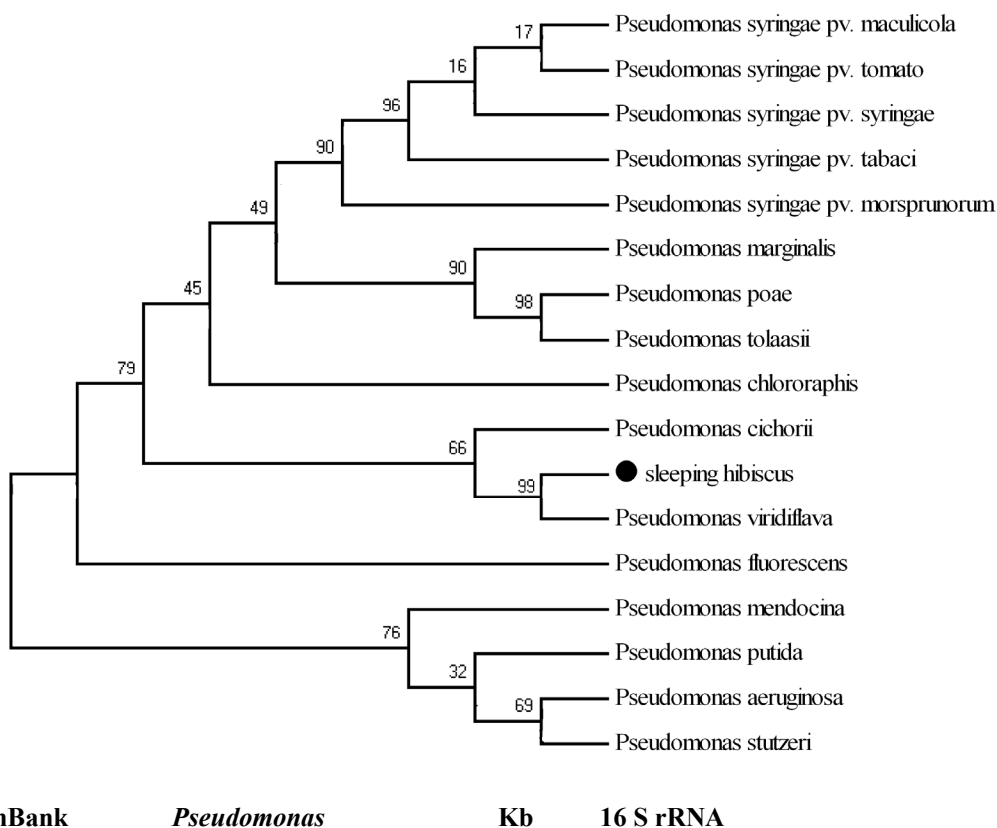


Fig. 5. Phylogenetic tree comprising the 16S rRNA nucleotide sequence data of *Malvaviscus penduliflorus* strain K1 and *Pseudomonas* strains available in GenBank

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*Pseudomonas*

(Schaad *et al.* 2001, Palleroni 1973)

rep-PCR

*P. viridiflava*

*P. syringae* pv. *syringae*

( )

*P. viridiflava* ICMP 3938

DNA

IS50-PCR

*P. viridiflava*

*P. viridiflava*

*P. syringae* pv. *syringae*

*P. syringae* pv. *syringae*

*P. viridiflava*

( )

BOX-PCR

( ) IS50-PCR ( )

REP

BOX ERIC

(Louws 1994)

*P. syringae* pv. *syringae*

( )

DNA

(Rahimian 1989)

(Weingart & Volksch 1997, Little 1997)

rep-PCR

DNA

*P. viridiflava*

IS50-PCR rep-PCR

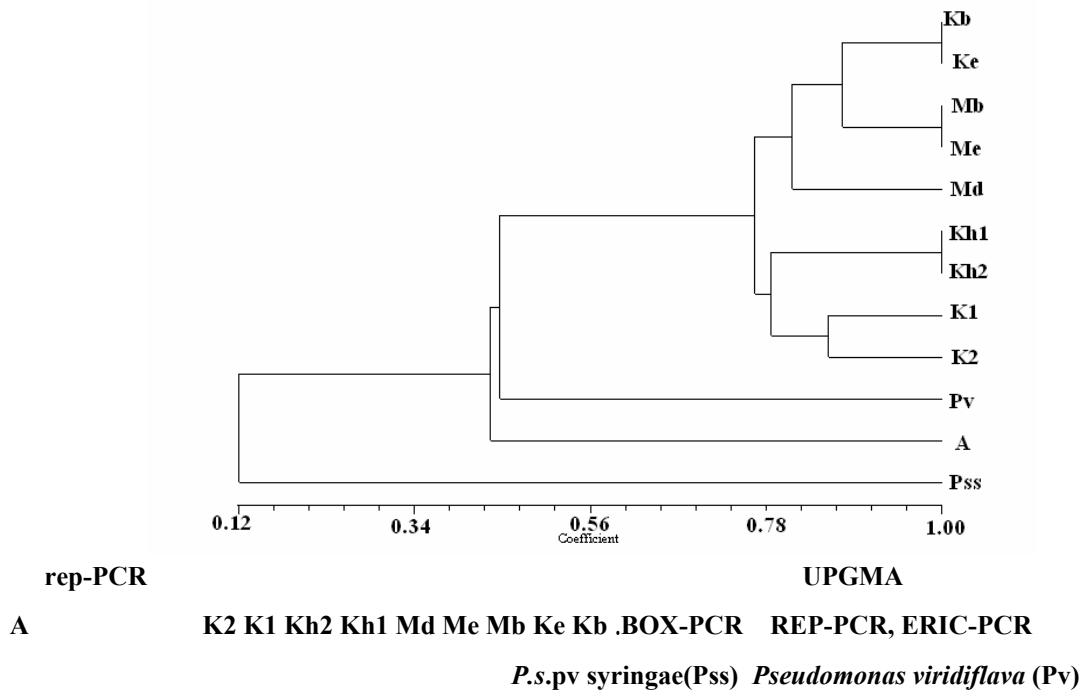


Fig. 6. Dendrogram obtained by comparison of strains isolated from *Malvaviscus penduliflorus* (lanes Kb,Ke,Mb,Me,Md,Kh1,Kh2,K1,K2),citrus isolate (lane A), *P. syringae* pv *syringae* (lane Pss) and *P. viridiflava* (Pv).

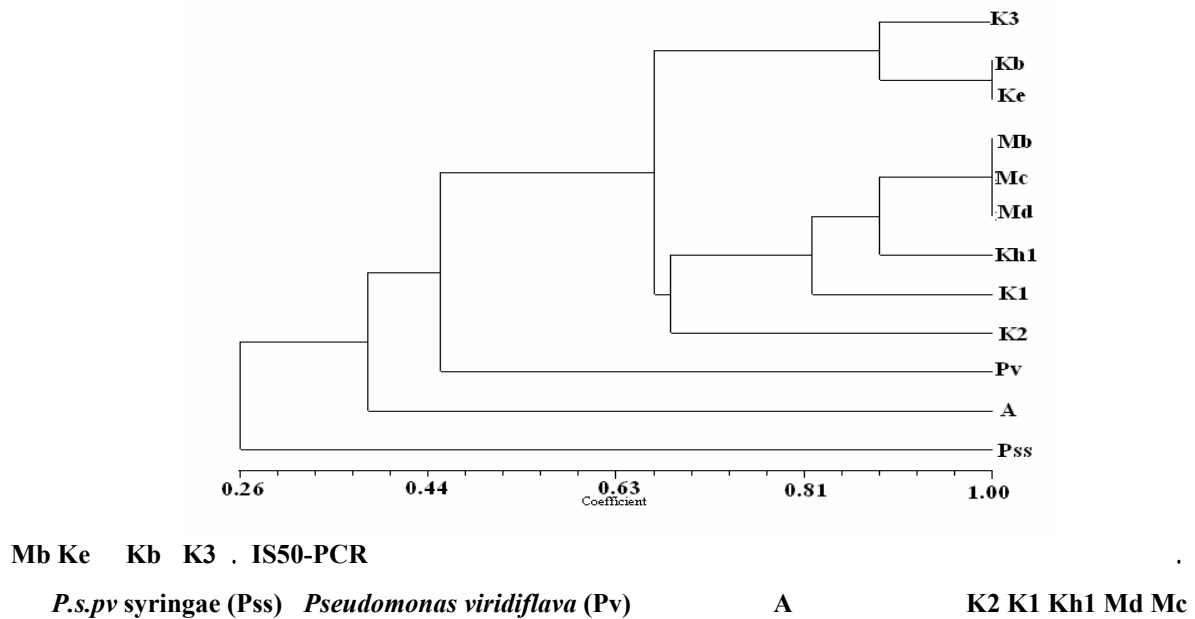
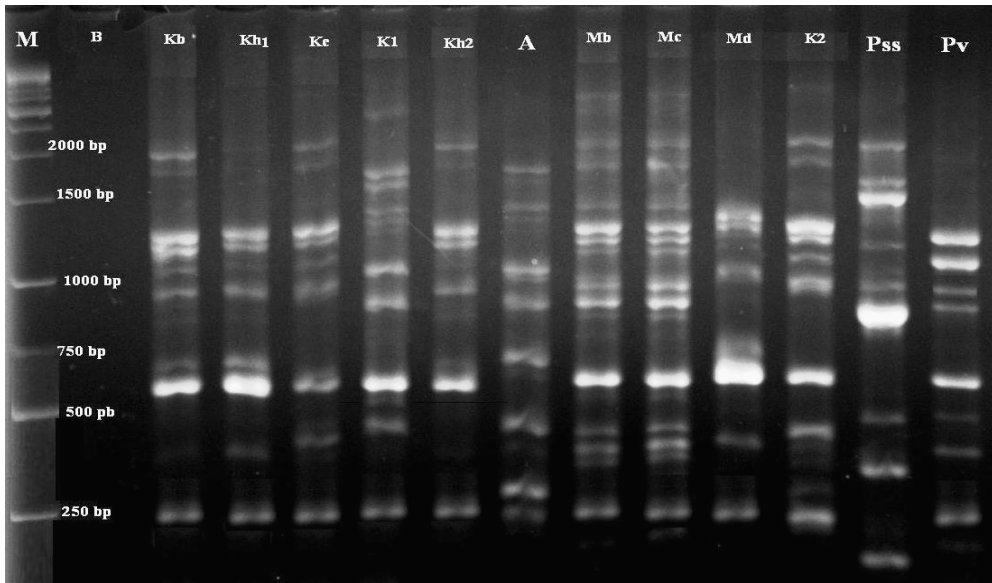
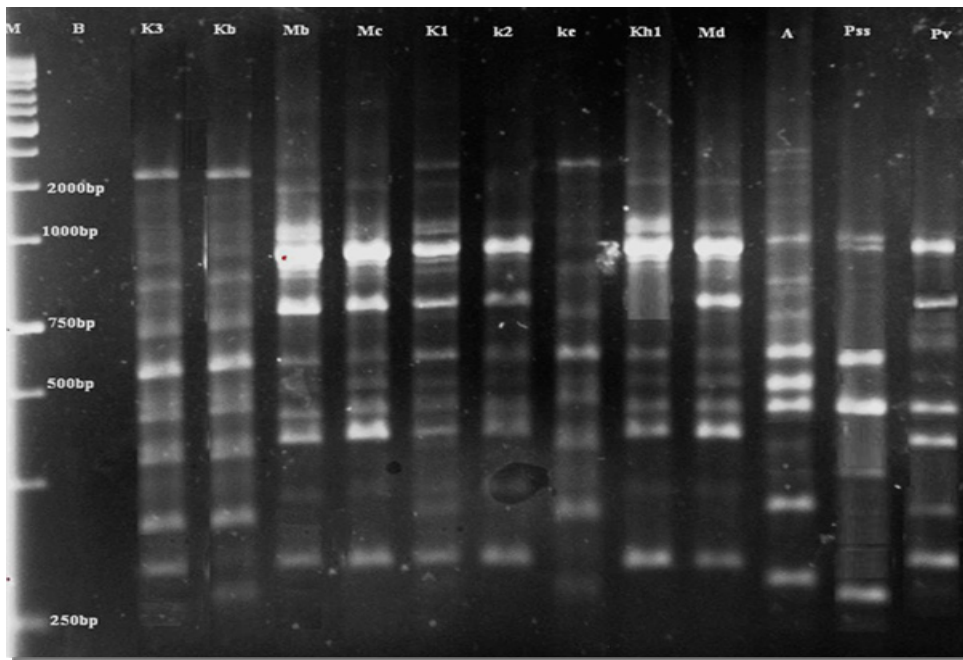


Fig. 7. IS50-PCR dendrogram obtained by comparison of strains isolated from *Malvaviscus penduliflorus* (lanes Kb,Ke,Mb,Mc,Md,Kh1,K3,K1,K2),citrus isolate (lane A), *P. syringae* pv *syringae* (lane Pss) and *P. viridiflava* (Pv).



( K2 Md Me Mb Kh2 K1 Ke Kh1 Kb..).BOX- PCR DNA  
 A B .P.s.pv syringae (Pss) Pseudomonas viridiflava (Pv) A

Fig. 8. BOX – PCR fingerprints of bacterial strains isolated from *Malvaviscus penduliflorus* (lanes Kb,Kh1,Ke,K1,Kh2,Mb,Me,Md,K2), Citrus isolate (Lane A) , *P.viridiflava* (Lane Pv) and *P.s .pv.syringae* ( Lane Pss). B: (Negative control). M: 1Kb DNA ladder



A Md Kh1 Ke K2 K1 Mc Mb Kb K3 ..IS50- PCR DNA  
 M B . P.s.pv syringae (Pss) Pseudomonas viridiflava (Pv)

Fig. 9. IS50 – PCR fingerprints of bacterial strains isolated from *Malvaviscus penduliflorus* (Lanes Kb,Kh1,Ke,K1, ,Mb,Mc,Md,K1,K2), Citrus isolate (Lane A) , *P.viridiflava* (Lane Pv) and *P.s .pv.syringae* (Lane Pss). B: (Negative control). M:1Kb DNA ladder

(Goumans & Chatzaki 1998) *P.viridiflava*  
16S rRNA  
(Young *et al.* 1988)  
(Shams-bakhsh & Rahimian 1997) *P.viridiflava*  
(Sahragard *et al.* 1997) ME3.1b RMX23 GenBank  
(Rahimian & Goharzadeh 2000) UASWS0038 LPPA 139  
(Razinataj & Taghavi 2004)  
(Stackebrandt & Goebel 1994)

*P.viridiflava* *P.viridiflava*

*P.viridiflava*

(89-91)