Antagonistic Activity Of Endophytic Bacteria Isolated From Mentha Rotundifolia L.

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Abstract: This study is implemented for the isolation, purification and identification of endophytic bacteria which produces antifungal substances from the roots of Mentha rotundifolia L. The 59 obtained bacterial isolates were tested for their antagonistic activity by the dual confrontation against the phytopathogenic fungi Fusarium oxysporum, Aspergillus Niger and Botrytis cinerea. Eight bacterial strains were selected for their strong antifungal activity. These are strains M21, M23, M3a, M4, M14d and M3c which belong to the family Bacillaceae, M12 and M3b which belongs to the family of Pseudomonadaceae. Among these, three bacterial strains namely M21, M23 and M12 induce 70% of inhibition of mycelial growth of phytopathogenic fungi Fusarium oxysporum and Aspergillus Niger while the five bacterial strains M3a, M3c, M3b, M4 and M14d have proved to be effective in inhibiting more than 60% of mycelial growth of Botrytis cinerea.

Keywords: Bacteria endophytic, Bacillaceae, Pseudomonadaceae, antagonistic activity, Fusarium oxysporum, Aspergillus niger, Botrytis cinerea

1 INTRODUCTION

More than two hundred species of dicots plants and monocots are likely to be attacked by pathogenic fungi Fusarium oxysporum, Aspergillus Niger and Botrytis cinerea. Responsible for the significant economic losses on cultures before and after the harvest [1, 2, 3]. The biological control is a promising alternative [4]. Indeed, the endophytic bacteria may be used for landing in the misuse of chemicals [5]. The objective of this study is to isolate bacteria endophytes of Mentha rotundifolia L. and to compare them with mycelium phytopathogenic (F.oxysporume. A.niger and B.cinerea).

2 MATERIEL ET METHODES

2.1 Isolation and purification of endophytic bacteria of Mentha rotundifolia L.

The root samples of the plant Mentha rotundifolia L. were taken at Ouled Amar parcels located in the region of Gharb Chrarda Beni Hssen, Morocco.Ten grams of roots were sterilized in the surface with sodium hypochlorite at 1% for 90 seconds, rinsed several times with sterile distilled water, and ground in the physiological water for 60s. Depositing 0.5 ml of the dilution 1/10 then plated on Petri dishes containing the agar medium. The incubation is performed at 28 ° C for 48h. The purity of the strains was checked by successive subculture on agar medium. The purified bacteria are then stocked at -20 ° C in flasks containing nutrient broth of 20% of glycerol.

2.2 The antagonist activity of bacterial isolates in vitro

Bacterial isolates were tested for antagonism against Fusarium oxysporum, Aspergillus Niger and Botrytis cinerea on the PDA by the dual culture technique [6].The bacterial strains are inoculated in rectilinear streaks at opposite ends of the medium. A cylinder of 4 mm in diameter mycelium phytopathogenic is deposited in the center of the Petri dish. The control contains only a phytopathogenic fungi washer. The petri dish were incubated at 28 ° C. the inhibition of mycelial growth was observed after five or seven days. The percentage of the mycelial growth is estimated by the formula:

(%) = Inhibition= $(R_{control} - R_{test})/ R_{control} * 100$ [7]

- $R_{control}$: maximum radial distance fungus growth.
- R_{test} : radial distance on a line towards the antagonist.

2 Biochemical identification of isolated bacteria of Mentha rotundifolia L.

The bacterial isolates were identified based on the characters of the cultural tests, morphological, and biochemical: the Gram reaction, respiratory-type with catalase and oxidase etc. as described in the manual of Bergey's bacteriology 2001 [8].

3 RESULTS AND DISCUSSION

3.1 The antagonistic activity of bacterial isolates in vitro

From the roots of Mentha rotundifolia L, we isolated and purified 59 bacterial strains. Among them 17 induce an inhibition of growth of F.oxysporum, A. Niger and B.cinerea. (Table I).

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Table 1: *Percentage of inhibition of Fusarium oxysporum (F.o), Aspergillus Niger (A.n) and Botrytis cinerea (B.c) by the isolated bacteria of Mentha rotundifolia L.*

strains	% inhibition of mycelial growth		
	F.o	A.n	B.c
control	0	0	0
M6a	39,47	37,5	11,11
M16c	40,78	31,25	22,22
M14e	57,89	68,75	57,77
M14c	60,52	75	56,36
M7a	64,47	66,25	45,45
M3a	67,10	65	67,27
M3c	63,15	62,50	63,63
M4	67,10	65	60
M14d	53,94	66,25	63,63
M21	75	76,25	56,36
M23	73,68	75	52,72
M12	70,52	77,5	54,54
M3b	67,10	68,75	60
M5b	67,10	57,5	36,36
M2b	60,52	66,25	16,36
M9b	47,36	50	18,18
M3d	30,26	12,25	7,27

We find that 17, 16 and 12 isolated strains have induce an inhibition of F.oxysporum, A. Niger and B.cinerea respectively with a percentage of inhibition higher than 30%. The strains M21, M23 and M12 are remarkably effective. They have trained the inhibition of mycelial growth F.oxysporum and A. Niger with a higher percentage of 70% (Figure 1 and Figure 2). while the five other isolates M3a, M3c, M3b, M4 and M14d shown their effectiveness by inhibiting superior 60% of mycelial growth of B.cinerea (Figure 3)

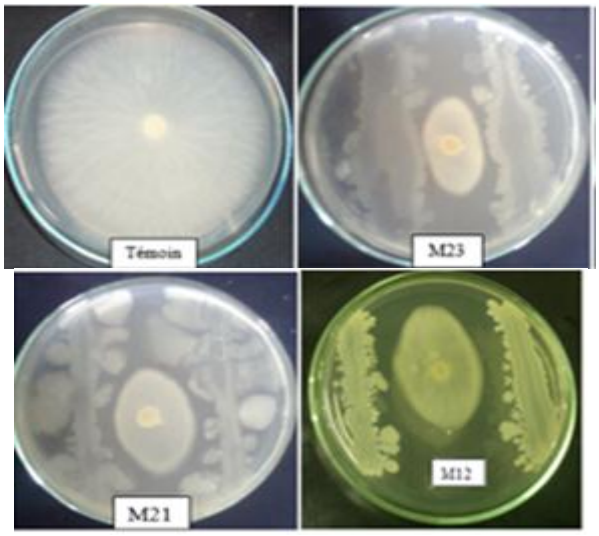


Figure 1: Effects of bacterial isolates M23, M21 and M12 on mycelium growth of Fusarium oxysporum.

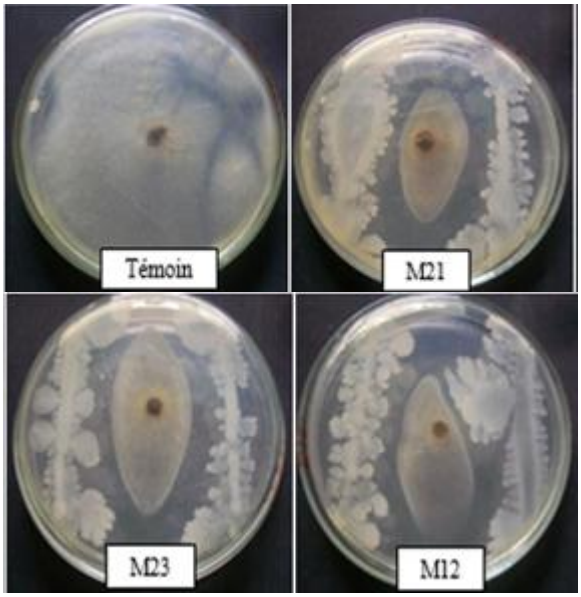
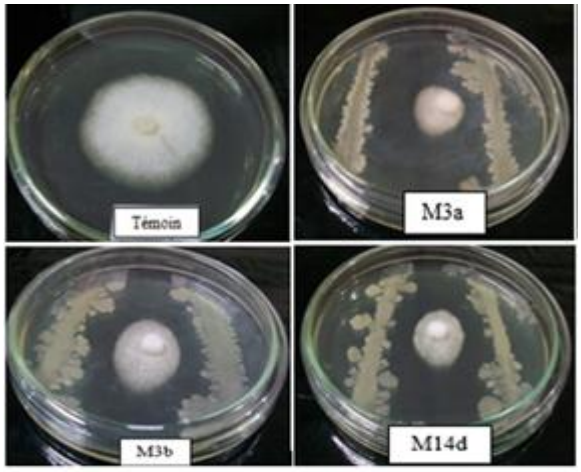


Figure 2: Effects of bacterial isolates M23, M21 and the M12 on the mycelium growth of Aspergillus Niger



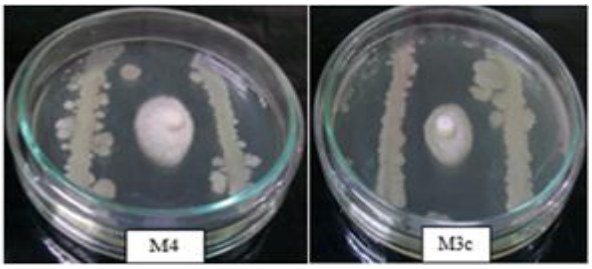


Figure 3: Effects of bacterial isolates M3b, M3a, M3c, M14D and M4 on the mycelium growth of Botrytis cinerea

3 Biochemical identification of endophytic bacteria isolated from Mentha rotundifolia L.

The 17 strains induced an inhibition of mycelial growth of phytopathogenic fungi three F. oxysporum, A. Niger and B.cinerea they are divided in two groups according to their Gram stain (Tables 2 and 3). They are strictly aerobic bacilli of oxidase and catalase positive, and they are all capable of reducing the nitrate to nitrite or ammonia but unable to produce H2S.

Tableaux 2: biochemical and physiological characters of Gram positive isolates isolated from de Mentha rotundifolia L.

Test \ Strains	M3a	M4	M6a	M7a	M14c	M14e	M14d	M3c	M16c	M21	M23
Mobility	+	+	+	+	+	-	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	-	-	-
Glucose	-	-	-	-	-	-	-	-	-	-	-
Lactose	+	+	-	+	+	+	+	+	+	-	+
Methyl red	-	-	+	-	-	-	-	-	+	-	-
Voges Proskauer	+	+	-	+	+	+	+	+	-	+	+
Gelatinase	+	+	-	+	+	+	+	+	-	+	+
casein hydrolysate	+	+	-	-	+	-	+	-	+	+	+
starch hydrolysate	+	+	-	-	+	+	+	+	-	+	+

Among the 11 Gram positive strains, nine are sporulation. They belong to Bacillaceae family. The two other bacterial strains are not acid-resistant and are non-sporulation. They are unable hydrolyze the starch. Which specifies as Corynebacterium xerosis.

Tables 3: biochemical and physiological characters of Gram-negative isolates isolated from de Mentha rotundifolia L.

Test \ strains	M5b	M9b	M2b	M3b	M12	M3d
Citrate	+	+	+	-	+	-
Mobility	+	+	-	+	+	+
Mannitol	-	+	-	+	+	-
Lactose	-	-	-	+	-	-
Methyl red	+	+	+	-	+	-
Voges Proskauer	-	-	-	+	-	+
Gelatinase	+	+	-	+	+	-
casein hydrolysate	-	+	-	+	-	-
starch hydrolysate	-	-	-	+	+	-

The six bacterial Gram-negative isolates belong to Pseudomonadaceae family. The bacterial isolates M21, M23, M3a, M4, M14d and M3c belong to the Bacillaceae family. Among these strains, M21 and M23 induced an inhibition of mycelial growth greater than 70% of F.oxysporum and A. Niger while the other strains M3a, M4, M3c and M14d inhibited 60% of the mycelium growth B.cinerea. These results are in agreement with those left by

Nourozian [9], Munimbazi and Bullerman [10] who showed that the Bacillus sp have a strong potential of inhibition of mycelium growth of many Aspergillus and Fusarium species Also Sadfi-Zouaoui [11] found that the Bacillus sp. were antagonistic to B.cinerea. In previous researches, some strains of Bacillus sp, such as Bacillus subtilis, played an important role in the biological control of fungal diseases of post-harvest and the production of antibiotics [12]. M12 and M3b are Pseudomonadaceae which induce an inhibition rate higher than 70 % against the phytopathogenic fungi F.oxysporum and A.niger for M12. Thus, the strain M3b induced an inhibition superior than 60% against B.cinerea. This is in agreement with a description that which was made by several authors who described the successful control of the Aspergillus flavus by the antagonistic Pseudomonas fluorescens [13, 14]. Srivastava and Shalni [15] also reported the antifungal potential of Pseudomonas fluorescens against the pathogenic fungus, Fusarium sp. Paez et al [16] have indicated that Pseudomonas putida and Pseudomonas aeruginosa had great antagonistic effects on B.cinerea.

4 CONCLUSION

From these results, we can conclude that the bacterial strains M21, M23, M3a, M4, M14d and M3c belong to the Bacillaceae family, M12 and M3b belong to the Pseudomondasea family have an inhibitory effect on the phytopathogenic fungi Fusarium oxysporum, Niger Aspergillus and Botrytis cinerea, and therefore can serve as a biological control agent.

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