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Isolation and characterization of endophytic actinomycetes associated with *Thymus saturoides* and *Lavandula multifida* grown in Beni-Mellal region in Morocco

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ABSTRACT

The present study aims to isolate endophytic actinomycetes (EPM) that are able to tolerate abiotic stresses, solubilize tricalcium phosphate (TCP) and produce indole-3-acetic acid (IAA), amylase and cellulose activities, while exhibiting high antagonistic activity against *Fusarium oxysporum.* f. sp. *albedinis*. Endophytic actinomycetes were isolated from roots, stems and leaves of *Thymus saturoides* and *Lavandula multifida* plant grown in Beni Mellal region, Morocco. According to the macro- and micro-morphological characteristics, a total of eleven EPM isolates were isolated and purified. Results indicated that the majority of isolates were able to tolerate pH and salt stresses. Moreover, the majority of the isolates were able to solubilize TCP on Pikovskaya (PVK)-agar medium which ranged from 25% to 458%. Additionally, on PVK-liquid medium, soluble phosphate content varied from 163.81 µg/mL recorded for the isolate LRP1 to 730 µg/mL observed for the isolate TSRP2. For IAA production, the amount produced varied from 1.70 µg/mL recorded for the isolate LRP1 to 14.13 µg/mL recorded for both LTP2' and TSRP2. Endophytic actinomycetes isolated in this work also showed a remarkable capacity to degrade some compounds such as cellulose and starch. In addition, some of them showed a strong inhibitory activity against the fungus *F. albedinis* with a percentage of inhibition ranged from 69.13% observed for LTP2' to 86.6% recorded for the isolate LRP2. Results from this study indicate that the two isolates LTP2' and TSRP2 were the most efficient and that actinomycetes could be used in biocontrol against the fungus *F. albedinis*.

Keywords: Endophytic actinomycetes, *Lavandula multifida*, *Thymus saturoides*, Phosphorus, *Fusarium oxysporum*, Biocontrol.

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INTRODUCTION

Actinomycetes are filamentous bacteria, unicellular, Gram-positive, aerobic with a morphology resembling of a fungus, characterized by the formation of aerial and substrate mycelia and typically having more than 50% GC contents in their genome (Nimnoi *et al.,* 2010; Ahmad *et al.*, 2017; Ansari *et al.,* 2020). They are known for their ability to produce bioactive secondary metabolites including antibiotics, antioxidants, antiviral agents, enzymes etc. (Qin *et al.*, 2009). It has been reported that around 23 000 bioactive compounds are originated from many microorganisms and that about 45% of these bioactive compounds are produced by actinomycetes (Hayat *et al.*, 2020). Actinobacetria are also known to fulfill important functions for plant growth through producing plant growth

hormones, phosphate solubilization, nutrient acquisition and nitrogen (N_2) fixation as well as to reduce disease symptoms through different mechanisms (Qin *et al.*, 2009; Glick, 2012).

Endophytic actinobacteria (EPM) inhabit several parts of plants (roots, stems, leaves, etc.) at their whole stages of life cycle without causing any harmful effects (Hasegawa *et al.*, 2006; Singh *et al.*, 2017, Liu *et al.*, 2019; Musa *et al.*, 2020).They were isolated from various crops including banana, wheat, rice, cucumber, tomatoes, carrots, potatoes, mustard, holi basil, turmeric, cabbage and radish (El-Tarabily *et al.*, 2009; Kaur *et al.*, 2013). Recently, EPM associated with medicinal plants was the subject of several studies and the majority of the investigated studies were focused on the genus *Streptomyces* (Taechowisan *et al.*, 2008; Verma *et al.*, 2009; Qiu *et al.*, 2015; Tanvir *et al.*, 2014; Vinayarani and Prakash, 2018; Musa *et al.*, 2020; Rustamova *et al.*, 2020).

Aromatic and medicinal plants (AMP) are a large group of plants which have played key roles in the lives of tribal peoples living throughout the world by providing products for both food and medicine (Mustafa *et al.*, 2017). In Morocco, the aromatic and medicinal flora is characterized by its richness, diversity and socio-economic value (Fennane and Rejdali, 2016; Taleb, 2017). They are used for several purposes such as traditional medicine, cosmetics, foods and essential oils exports. More than 4 200 spontaneous AMP species have been identified and some 1500 introduced species have been catalogued (El Hilaly *et al.*, 2003; El Houari *et al.*, 2018).

Regarding the mountain ecosystems, Beni Mellal-Khenifra region has a rich and varied biological diversity and represents a valuable source of medicinal plants (El Alami *et al.*, 2020). The different ethnobotanical studies and floristic analyses conducted in this region revealed the dominance of the Lamiaceae family (Hachi *et al.*, 2015; El Alami and Chait, 2017; El-Hadri, 2019). This family is represented by 14 different species including *Thymus satureioides* and *Lavandula multifida*, the most studied species (Aneb *et al.*, 2016; El Yaagoubi *et al.*, 2021; Fakchich and Elachouri, 2021). Dried leaves of *Thymus satureioides* have been used in traditional Moroccan medicine to treat different diseases such ascough, bronchitis, diabetes, rheumatism, gastrointestinal, genital, nervous and urinary (Ouhadouetal., 2015; El Yaagoubi *et al.*, 2021). Similarly, *Lavandula multifida* is traditionally used to cure many diseases and inflammatory disorders such as rheumatism (Sosa *et al.*, 2005; Bachiri *et al.*, 2015).

To date, in our knowledge, no study was conducted on the isolation of actinobacteria endophytes associated with *Thymus satureioides and Lavandula multifida* and their potential to plant growth promoting activities and *in vitro* antagonism against fungal phytopathogens. Therefore, this work aims to i) isolate and characterize endophytic actinomycetes from two medicinal plants (*Lavandula multifida* and *Thymus saturoides*) grown in Beni Mellal-Khenifra region (M'Goun Park), ii) study their ability to tolerate abiotic stress in terms of pH and NaCl, iii) determine their plant growth promoting activities in terms of inorganic phosphate solubilization, indole-3-acetic acid (IAA) production, free nitrogen fixation, amylase and cellulase production and iiii) test *in vitro* their antifungal activity against the fungus *Fusarium oxysporum*. f.sp. *albedinis*.

MATERIALS AND METHODS

Collection site and plant materials

The area of interest is located in the GeoParkM'Goun, Ouaouizeght in the Beni-Mellal region of Morocco (32°12'03.8"N 6°18'44.5"W). This region constitutes one of the most agriculture area in Morocco with two independent irrigated perimeters in Beni-Amir and Beni-Moussa of 69 5000 ha and 33 000 ha respectively (Aallam *et al.*, 2021). This region is characterized by a semi-arid climate, an average rainfall generally below 280 mm and an average temperature of 19 ˚C (Barakat *et al.*, 2012; El Baghdadi *et al.*, 2011; Aallam *et al.*, 2021).

Healthy plants of *T. saturoides* and *L. multifida* were carefully detached from the soil, put in

polyethylene bag and stored at 4 °C and brought to the laboratory. Samples were then divided in stems, roots and leaves and used to isolate endophytic actinobacteria within 48h of collection.

Isolation and morphological identification

Samples (stems, roots and leaves) from different plants were washed thoroughly with tap water to remove the adhering soil particles, organic matter and epiphytes. After removing the excess of water, tissues were surface sterilized following the method described in Qin *et al.* (2009). According to morphological characteristics of the colonies, they were selected and successively sub-cultured by single colony streaking on SCA medium supplemented with 50 µg/mL of cycloheximide until a pure and easy to characterize culture was obtained. The purified actinobacteria isolates were stored on SCA medium at 4 °C.

The macro-morphological study allows determining the different characteristics of substrate mycelium (SM) and aerial mycelium (AM). After incubation, the appeared colonies of the actinomycetes were examined with both the naked eye and a binocular magnifier glasses. The shape, size, presence of AM and SM, pigmentation of the mycelia and the presence of diffusible pigments in the agar were observed and noted after 7 to 21 days of incubation on the International Streptomyces Project mediums (ISP2, ISP3, ISP4 and ISP5) at 28 °C (Shirling and Gottlieb, 1966). The microscopic examination is done on thin smears prepared from purified colonies of each isolate obtained on Casein-Starch medium. These smears are stained by Gram staining, after, observation with a light microscope at x1000 magnifications.

Stress tolerance test

The tolerance of all the isolates to some abiotic stresses such as pH (4 and 8) and salinity (8%) was tested in SCA medium. The tolerance of the selected isolate to the applied stresses was indicated by their growth in the medium after 7 to 15-days of incubation at 28 °C (Saurav and Kannabiran, 2009).

Phosphate solubilization assay

The ability of the selected isolates to solubilize tricalcium phosphate ($Ca_3(PO_4)_2$) was first determined qualitatively using Pikovskaya (PVK) agar medium (g L^{-1}; Glucose 10, NaCl 0.2, KCl 0.2, $FeSO_4$ 0.002, $MnSO_4$ 0.002, $NH_4(SO_4)$ 0.5, $MgSO_4$ 0.1, yeast extract 0.5, agar 20 and pH 7) containing 5 g $Ca_3(PO_4)_2$ L^{-1} as the only source of P. Thus, purified colony was spot inoculated in PVK medium and the ability of isolates to solubilize phosphate in the medium is determined by the appearance of a clear halo around the colony after incubation at 28 °C for 15 days (Passari, 2015). For each isolate, three replicates were performed. The measurement of the solubilization efficiency in % was performed according to the following formula:

$$Solubilization\ efficiency\ in\ \% = \frac{Diameter\ of\ the\ halo}{Diameter\ of\ the\ colony} x100$$

With, diameter of the Halo = Total Diameter - Diameter of the colony.

Quantitative estimation of soluble phosphate was assessed on liquid PVK medium (Nautiyal, 1999). The tested isolates were inoculated in PVK broth (25 mL) and incubated at 28 °C under continuous agitation at 125 rpm. After 15 days of incubation, 2 mL of each culture was collected, centrifuged at 6,000 rpm for 10 min at 4 °C and the supernatant was used to estimate soluble P (Nautiyal, 1999). Non-inoculated broth was used as control.

For the quantification of P, 0.5 mL of supernatant was added to 2 mL of distilled water and 2.5 mL of molybdate reagent containing 2.5% sodium molybdate prepared in 10N sulfuric acid and 0.15% hydrazine sulfate prepared in water (Watanabe and Olsen, 1965). The mixture was placed in a water bath at 95 °C for 10 min then the optical density was determined at 820 nm. P concentration was estimated using standard curve prepared with known concentration of KH_2PO_4. For each isolate, three replicates were considered and the results were expressed as µg soluble P per mL of the extract.

Indole acetic acid (IAA) production

All the actinomycete isolates were screened for the production of IAA using SCA broth supplemented

with 2 mg/mL of L-tryptophan (Gordon, 1951). For this purpose, tubes containing the SCA medium supplemented with L-tryptophan were inoculated with the isolate and incubated at 28 °C under agitation (125 rpm) for 15 days. Bacterial cells were removed from the medium by centrifugation at 6000 rpm for 15 min, then to 1 mL of the supernatant culture, 2 mL of Salkowski reagent (mixture of 0.5 M ferric chloride ($FeCl_3$) and 35% perchloric acid ($HClO_4$)) was added. After 30 min of incubation in the dark, the intensity of developed pink color was read at 530 nm. IAA concentration was calculated using a standard curve of known concentration of the commercial IAA, and the results were expressed as µg of IAA per mL of the extract (Barra *et al.*, 2016).

Free nitrogen (N_2) fixation

The ability of the actinomycete isolates to fix atmospheric nitrogen was confirmed in N-free medium composed of (per l): glucose 20 g, $CaCO_3$ 20 g, KH_2PO_4 1 g, $MgSO_4$.7H2O 0.5 g, agar 15 g (Bechtaoui *et al.*, 2019). The pH of the medium was adjusted to 7.4 before autoclaving. After 7 to 14 days of incubation at 28 °C, actinomycete isolates grown on the N-free medium were considered as free N-fixer isolates (Okazaki *et al.*, 1995).

Cellulase and amylase production

Cellulase production was revealed on ISP_9 medium supplemented with 1% of carboxymethyl cellulose as a substrate. The isolates were spot inoculated on the medium and incubated at 28 °C. After 14 days of incubation, the plates were flooded with 1% Congo red solution for 15 to 20 min (Saini *et al.*, 2016). An isolate was considered as cellulose producer if a clear zone was observed around the colony (Saini *et al.*, 2016). Cellulosic activity index (CAI) was calculated using formula below:

$$CAI = \frac{Diameter\ of\ halo - Diameter\ of\ colony}{Diameter\ of\ halo}$$

The ability of the isolate to produce amylase was determined on Starch Agar medium supplemented with 1% of starch. The isolates were spot inoculated on the medium and incubated at 28 °C for 5 to 7 days. The isolates which showed a zone of hydrolysis after flooded the plates with lugol's solution indicated the production of α-amylase. Amylolytic index activity was calculated in the same way (Gordon and Smith, 1953).

In vitro antagonistic bioassay

For the in vitro antagonistic test, a strain of the fungal *Fusarium oxysporum* f. sp. *albedinis* AMR provided by "Protection and Valorization of Plants team", Faculty of Science Semlalia, Cadi Ayyad University Marrakech was used (El Hassni *et al.*, 2021). Agar disk method was used to detect the antagonistic activity of actinobacteria isolates against the fungal strain (Aghighi *et al.*, 2004). Briefly, a fungal disk of 6 mm in diameter containing 7-day-old mycelial growth was placed at the center of potato dextrose agar (PDA) plates. The four actinobacteria disks cultured on SCA medium for 14 days at 28 °C were placed onto the agar surface at four equidistant points, 3 cm from each actinomycete colony. Plates with pathogenic fungi alone served as a control. All the plates were placed in a refrigerator at 4 °C for 2 hours then incubated at 28 °C for 5 to 7 days and the colony growth inhibition ratio (I) (%) was calculated using Mohamad *et al.* (2018) equation.

Data Analysis

All experiments were conducted in triplicate. Statistical analysis was made by one-way ANOVA using SPSS statistical Software 21.0. Means were compared using Post-hoc test at $p < 0.05$. Data are presented as means ± standard deviation.

RESULTS

Recovery of endophytic actinomycetes

A total of 11 EPM were isolated using Starch-Casein Agar (SCA) medium from different parts of plants (Table 1). Six isolates were obtained from the roots and 3 isolates from the stems of *L. multifida* designated respectively as follows LRP1', LRP1, LRP2, LRP1'', LRP2', LRP2'', LTP2, LTP2', LTP1' (Table 1). Two isolates (TSRP1 and TSRP2) were obtained from the roots of *T. saturoides*. The root segments were predominantly inhabited with the endophytes (Table 1).

Morphological and microscopic characteristics

Morphological characterizations of all isolates were given in Fig. 1. Results revealed that most of the isolates showed abundant growth on the different used media except for LRP2 on ISP4 and TSRP2 on ISP2, which show moderate growth, and different shapes, colors and aspects (Fig. 2).

Table 1. Different endophyticactinomycetes isolates isolated from stem and root of *L. multifida* and *T. saturoides*

Isolate number	Isolate code	Plant species	Plant tissue
1	LTP1'	*Lavandula multifida*	Stem
2	LTP2		
3	LTP2'		
4	LRP1		Root
5	LRP1'		
6	LRP1''		
7	LRP2		
8	LRP2'		
9	LRP2''		
10	TSRP1	*Thymus saturoides*	
11	TSRP2		

Most of them, formed different colors including yellow, brown, ivory, white, grey or beige with the powdery colonies. The production of diffusible pigments was observed on ISP_2 and ISP_4 medium for the isolate LTP2, on ISP_4 and ISP_5 medium for the isolate LRP2, on ISP_4 medium for the isolates LTP2', LRP2' and LRP2" and on ISP_2 and ISP_4 medium for the isolate LTP2. Other isolates did not elaborate any pigment. Microscopic observations showed that all isolates tested are Gram positive, with thin, branched and non-septate mycelia.

***In vitro* evaluation of actinomycetes for their physiological traits**

For salinity tolerance, only isolates LTP2, LTP2' and LTP1' showed high tolerance to NaCl (8%) (Table 2). Additionally, for the pH, except LRP2 and TSRP1 isolates, the majority of actinomycete isolates were able to grow in a slightly acidic (pH=4) and slightly alkaline (pH=8) pH. However, LRP1 and TSRP2 isolates were unable to grow in alkaline pH (Table 2).

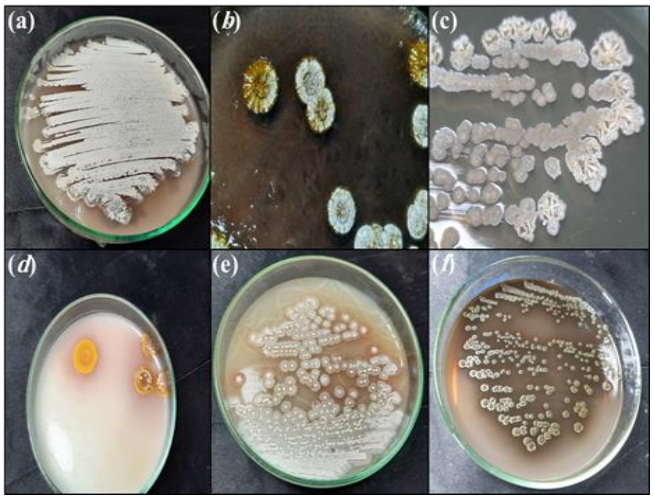

Fig. 1. Different morphological characteristics of the endophytic actinomycetes isolates. a: LTP2' on ISP4; b: LRP2 on ISP2; c: LRP2'' on ISP2; d: LRP2 on ISP4; e: LTP2 on ISP4 and f; LRP2 on ISP5

Table 2. The ability of the different endophytic actinomycetes isolates to grow under NaCl and pH stress and in N-free medium and to inhibit the fungal *Fusarium oxysporum*. f.sp.

	LTP1'	LTP2	LTP2'	LRP1	LRP1'	LRP1''	LRP2	LRP2'	LRP2''	TSRP1	TSRP2
NaCl (8%)	-	+	+	-	+	-	-	-	-	-	-
pH (4)	-	++	++	++	++	++	-	++	++	-	+
pH (8)	+	++	++	-	++	++	-	++	+	-	-
Nitrogen fixation	-	+	+	-	+	+	+	+	+	-	+
Inhibition of foa (%)	0.00	70.02	69.12	0.00	0.00	86.12	86.6	71.05	78.94	0.00	0.00

Plant growth promoting traits

Phosphate solubilization

Out of 11 isolates, only 7 (LRP1', LRP1, LRP2', LRP1'', LRP2'', LTP2' and LTP2) isolates have showed significant phosphate solubilizing ability under *in vitro* conditions as indicated by the prominent clear halo zones around colonies grown on Pikovskay's agar media. The LRP1' showed the highest phosphate solubilization index (458%) followed by LTP2 and LRP2' (Fig. 2). The

statistical test showed that there is a significant difference between the isolates (P<0.05).

The amount of phosphate solubilized by EPM isolated from the studied medicinal plants was between 730.81 µg/mL recorded for the isolate TSRP2 and 163.81 µg/mL recorded for LRP1 (Fig. 3). Statistical analysis showed significant difference between TSRP2 and LTP1' (P<0.05).

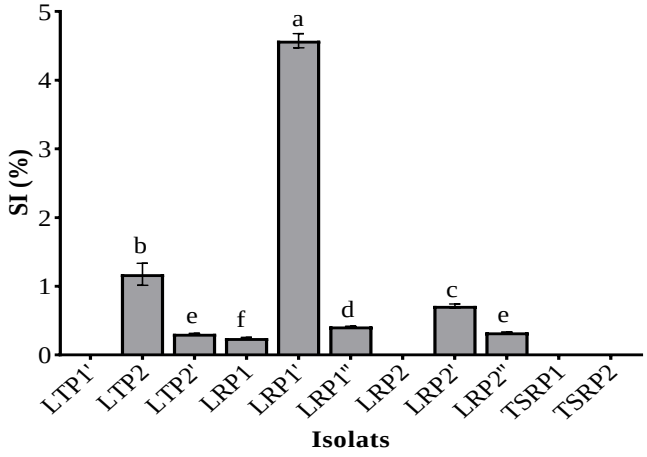

Fig. 2. Phosphate solubilization index (SI) of the endophytic actinobacteria isolates. Values are the mean of three replicates ± standard deviation. The letters above bars indicate statistically significant values

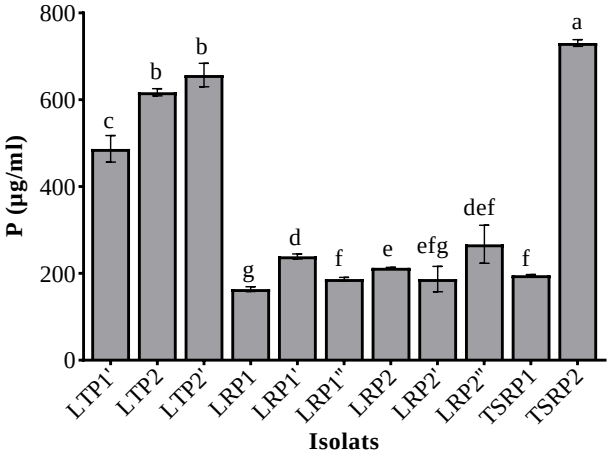

Fig. 3. Concentration of soluble P released from TriCalcium phosphate (TCP) in the inoculated Pikovskaya medium with the isolated endophyticactinobacteria. Values are the mean of three replicates ± standard deviation. The letters above bars indicate statistically significant values

Indole acetic acid (IAA) production

Data related to the ability of the isolates to produce IAA in vitro was presented in Fig. 4. Results indicated that among the eleven isolated bacteria, 8 isolates revealed high ability to produce IAA with a concentration ranged from 1.70 µg/mL to 14.13 µg/mL in the presence of L-tryptophan. The highest IAA value was recorded for LTP2' (14.13 µg/mL), followed by TSRP2 (12.94 µg/mL) and LRP1 (10.83 µg/mL). The isolates LRP2, LTP2 and LTP1' showed no significant difference (P>0.05).

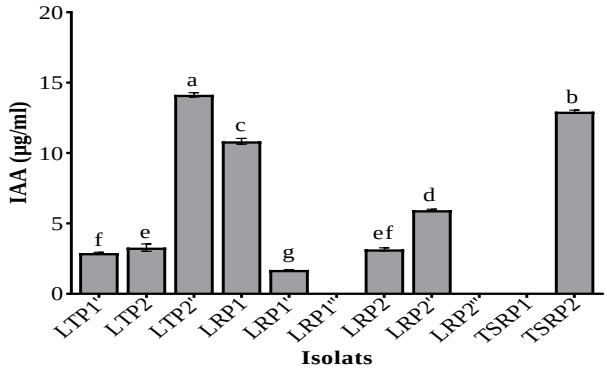

Fig. 4. Quantitative estimation of IAA production in different endophytic actinobacteria isolates. Values are the mean of three replicates ± standard deviation. The letters above bars indicate statistically significant values

Free nitrogen (N_2) fixation

Among the 11 isolates, 8 of them (LRP2, LRP2'', LRP2', TSRP2, LRP1', LRP1'', LTP2' and LTP2) were assessed to be N_2-fixersas indicated by their ability to grow-up in N-free medium agar (Table 2).

Cellulase and Amylase production assays

Results related to the ability of isolates to produce cellulase were presented in Fig. 6a. Of the eleven isolates examined, ten isolates were able to produce cellulose as indicated by the clear halo zones surrounding the colonies. Among all the positive isolates, LRP2 showed the highest ability to produce cellulase with a cellulosic index of 0.87 followed by TSRP2 and LTP1' (Fig. 5a). LRP2' showed a significant difference from the other isolates (P<0.05).

For amylase screening, 6 isolates were appeared as amylase producers with an amylolytic index activity ranging from 0.98-0.79. The isolate LRP2 showed the highest amylolytic index activity followed by LRP1'and LRP1 with an amylolytic index activity of 0.96 and 0.83 respectively (Fig. 5b). No significant difference was noted among the tested isolates.

Antifungal activity assay

All the eleven isolates were tested for their antifungal activity against the fungal phytopathogen *F.. albedinis* AMR strain. Among the 11 isolates, six of them exhibited higher antagonistic activity against the used fungal (Table 2; Fig. 6). LRP2 and LRP1'' were found to exhibit maximum growth inhibition against *F. albedinis* (Table 2).

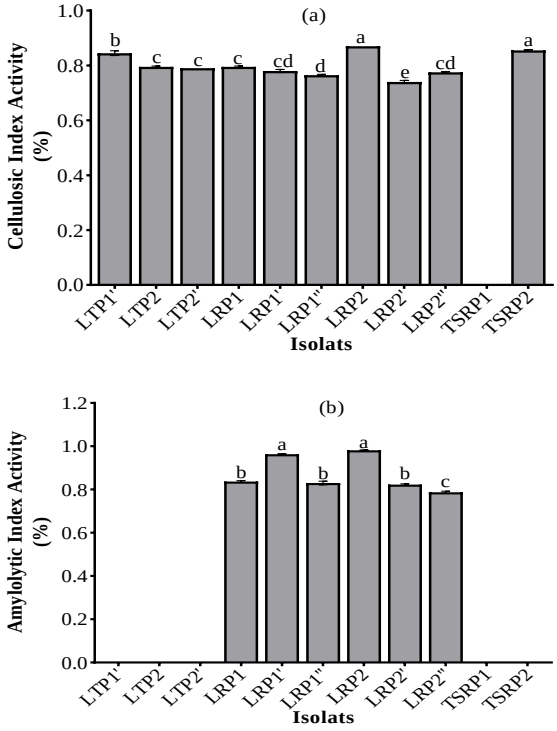

Fig. 5. Hydrolytic activities of the actinomycetes isolated from *L. multifida* and *T. saturoides.* a and b indicated cellulase and amylase activities respectively. Values are the mean of three replicates ± standard deviation. The letters above bars indicate statistically significant values

DISCUSSION

Different studies have focused on the importance of endophytic actinobacteria as biocontrol agent in disease management and growth promotion (Hasegawa *et al.,* 2006; El Tarabily *et al.,* 2009; Kaur *et al.,* 2013; Palaniyandi *et al.,* 2013; Musa *et al.,* 2020). In this case and with the objective of the discovery of new unexplored strains, for the first time ever, we isolate and characterize endophytic actinobateria associated with two aromatic medicinal plants *Thymus saturoides* and *Lavandula multifida* originated from the Beni-Mellal region in Morocco.

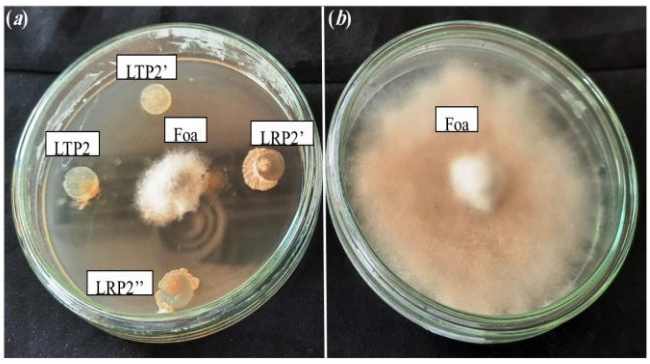

Fig. 6. In vitro antifungal test of the actinomycetes isolates against the phytopathogens fungul *Fusarium oxysporum* f. sp. *albedinis*. a, fungus with actinomycetes isolates. b, control

A total of eleven isolates were isolated from roots and stems. However, no isolate was obtained from the leaves parts. Our results showed that actinobacteria were most prevalent in the root tissue of the host plant. These results were in accordance with the previous investigation of Pratiwi *et al.* (2017) who could not isolate EPM from the leaves of *Neesia altissina*. Additionally, Priya (2012) reported in his work that the most isolates were obtained from stems and roots. Indeed, the maximum number of endophytic actinobacteria was recovered in the roots then in the stems, and less in the leaves (Qin *et al.*, 2009). The result could be explained by the fact that the roots are a good habitat for EPM (Taechowisan *et al.,* 2003). Indeed, actinomycetes reside in abundance in the rhizosphere, penetrate plant tissues through the root system and propagate in the intercellular spaces and the vascular system (Shimizu, 2011). On the other hand, the absence of actinomycetes on leaf samples may be due to the

penetration of ethanol and sodium hypochlorite into the thin leaf tissue during the surface sterilization process (Sunaryanto and Herliyani Mahsunah, 2014). Moreover, Musa *et al.*, (2020) isolated a total of 126 endophytic actinobacteria belonging to two classes, eight orders, fourteen families, and twenty-four genera from different organs of *Thymus roseus* collected from different geographical localization in China.

Our results indicated that the majority of the morphological characteristics observed from our strains are the characteristics of the genus *Streptomycetes* (Shirling and Gottlie, 1966). LTP2, LRP2, LTP2', LRP2'', LRP2' exhibited melanin constitution which is a primary characteristic feature of *Streptomyces sp*. These findings are in agreement with those of Mondal and Ravishankar, (2021) who find that the isolates MI22, MI04, MI24 and MI29 isolated from *Madhuca insignis* exhibited melanin production which is a primary characteristic feature of *Streptomyces* sp.

The ability of actinomycetes isolates to grow on SCA supplemented with 8% NaCl reflected their high tolerance to salt stress (Shariffah-Muzaimah *et al.,* 2018). Similarly, the ability of isolates to tolerate ranges of pH and salinity may favor their competitiveness in the rhizosphere which allows them to play an important role in plant protection under various environmental conditions (Sousa *et al.,* 2008). Therefore, tolerance to high level of salinity and pH could be good criteria for the selection of beneficial microorganisms (Drozdowic, 2016).

Phosphorus is one of the most important nutrients required for plant growth and development. In soil, it is mostly available as insoluble phosphorous which make it a limiting factor for plant growth (Pradhan and Sukla, 2006). Previous investigations have stated that phosphate-solubilizing bacteria including actinobacteria could be a promising way to enhance plant growth by increasing organic phosphorus uptake (Passari *et al.,* 2017). In the present study, seven isolates revealed a clear halo zone surrounding the colonies indicating their phosphate solubilization effectiveness with a maximum solubilization activity in the liquid medium recorded for the isolate TSRP2. This is in concurrence with Madhurama *et al.* (2014) who mentioned out of 40 isolates in which twelve were able to solubilize phosphate as they formed a clear zone around the colony on Pikovskaya medium. Similarly, Passari *et al.* (2015) reported that most of the *Streptomyces* isolated from medicinal plants were phosphate solubilizing bacteria. Hamdali *et al.,* (2008) reported that the highest level of phosphate solubilization was exhibited by *Streptomyces cavourensis*, *Streptomyces griseus* and *Micromonospora aurantiaca*. It has been reported that the solubilization of phosphate by the strains is related to their ability to acidificate the medium, and to reduce pH (Chen *et al.,* 2006). This may be either due to the acidification of external medium by producing low molecular weight organic acids like gluconic acid or the production of chelating substances that increase phosphate solubilization (Oteino *et al.,* 2015).

The ability of bacteria including actinomycete to produce IAA was demonstrated to influence positively plant growth, an improvement that may be related to the fact that IAA production helps the plants to grow root parts, which increases the plants water and nutrient uptake (Ribeiro and Cardoso, 2012; Shutsrirung *et al.,* 2013). In this study, 72.72% of the isolates were IAA producers with production ranging from 1.70 to 14.13 µg/mL. These results are in accordance with those of Passari *et al.,* (2017) who stated that most of actinomycetes are IAA producers. Additionally, our work revealed that the majority of the EPM isolates (8 isolates) were able to grow in the N-free medium, suggesting that they could be nitrogen-fixing bacteria. The ability of the EPM to fix atmospheric nitrogen has also been reported on some isolates isolated from *Glycine max* (Fatmawati *et al.,* 2019), *Solanum tuberosum* (Moscol *et al.,* 2020) and *Simmondsia chinensis* (Perez-Rosales *et al.,* 2017).

Microorganisms with cellulase and amylase activity do not merely help in organic matter decomposition and plant growth promotion, but they showed a critical role in disease suppression

by inhibiting soil borne pathogens (Kavamura *et al.*, 2013). Cellulase is an important industrial enzyme used in depolymerization of cellulose into fermentable sugar (Li *et al.*, 2009). In our study, out of eleven isolates, ten formed a clear zone around colonies on CMCase agar media indicating their ability to produce cellulase. These results corroborate with those of Sirisha *et al.*, (2013) and Passari *et al.* (2017). Moreover, six isolates were positive for amylase production. Similar findings were reported by Sirisha *et al.* (2013) who stated that 81% of the isolates were amylase producers.

The antagonistic activity of the EPM community associated with ethnomedicinal plants were screened *in vitro* condition by using a dual culture technique against *Fusarium oxysporum* f.sp. *albedinis*, the causal agent of Bayoud disease in date palm (Djerbi, 1982). This telluric fungusis the main reason of more than two-thirds of Moroccan date palm groves losses (Saaidi, 1992; Fernandez *et al.*, 1995). In the present work, a total of six isolates were showed significant antifungal activity with a maximum inhibition percentage of 86.6% recorded for LRP2. Our findings are comparable with those of Passari *et al.*, (2017) where the maximum inhibition percentage was 72%. In addition, Debananda *et al.* (2009) reported that *S. vinaceusdrappus* having greater antagonistic potency against rice fungal pathogens *F. oxysporum* with plant growth promoting properties. However, Musa *et al.* (2020) revealed that among 126 endophytic actinobacteria isolated from *Thymus roseus*, 54 of them were exhibited moderate and weak antifungal activities against *Fusarium oxysporum* f.sp. and *Fusarium oxysporum*.

Among the phylum actinobacteria, the genus *Streptomyces* has an outstanding record for the production of bioactive metabolites and natural antibiotics (Mingma *et al.*, 2014). Metabolites produced by microbe plays an active role in resistance development by functioning as signals to mediate cross-talk between the endophytes and their host (Graner *et al.*, 2003). Since, the EPM isolated from medicinal plants produce a wide variety of antifungal and plant growth regulatory bioactive metabolites, they can be explored as novel sources of natural products as well as novel biocontrol agents (Li *et al.*, 2012).

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