MORPHOLOGICAL CHARACTERIZATION AND PRELIMINARY SCREENING FOR ANTIBACTERIAL ACTIVITY OF ACTINOBACTERIA STRAINS ISOLATED FROM TAMANRASSET SOIL AS A HYPER ARID REGION IN SOUTHERN ALGERIA

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Abstract.- *In this study, we aimed to isolate Actinobacteria strains from diverse soil ecosystem of Tamanrasset town, and describe the morphological diversity of the isolates. We applied physical and chemical treatment to enhance the isolation of Actinobacteria strains on different free antibiotics culture media. Actinobacteria isolates were characterized based on macroscopic and microscopic observation. In addition, the preliminary screening for antibacterial activity was carried out by the Cross streak method on three culture media (Glycerol yeast extract "GYE", Glycerol soil extract "GSE" and Mueller Hinton "MH") against six clinical bacteria strains viz.,* Staphylococcus aureus, Escherichia coli, Enterococcus faecal, Enterobacter cloacae *and two strains of* Streptococcus spp*. Ten (10) different Actinobacteria isolates, exhibit a distinct morphology were obtained. Sixty-seven percent (67%) of the tested isolates showed at least one antibacterial activity against indicator clinical bacteria, the tested Actinobacteria A1, A6, and A8 have the highest expression on MH medium, whereas A6 has the largest range of antibacterial activity against all indicator clinical bacteria in MH medium. However, in some cases, the change of media influence positively the antibacterial activity. The present study revealed that Tamanrasset soil is rich in Actinobacteria strains, this finding of Actinobacteria can be of importance for further investigation towards obtaining broad-spectrum bioactive compound for medical purpose.*

Key words: *Actinobacteria, Tamanrasset soil, morphology, antibacterial activity, clinical bacteria.*

CARACTÉRISATION MORPHOLOGIQUE ET SCREENING DE SOUCHES D'ACTINOBACTÉRIES AVEC ACTIVITÉ ANTI-BACTÉRIENNE ISOLÉES DU SOL DE LA RÉGION HYPERARIDE DE TAMANRASSET DU SUD ALGERIEN

Résumé.- *Dans cette étude, nous avons isolé des souches d'Actinobactéries de divers écosystèmes du sol de la ville de Tamanrasset et nous avons décrit la diversité morphologique des isolats. Nous avons appliqué un traitement physique et chimique pour améliorer l'isolement des souches d'Actinobactéries sur différents milieux de culture sans ajout d'antibiotiques. Les isolats d'Actinobactéries ont été caractérisés sur la base des observations macroscopiques et microscopiques. Le criblage préliminaire de l'activité antibactérienne a été réalisé par la méthode des stries croisées sur trois milieux de culture (Glycerol- Extrait de levure «GYE», Glycérol Extrait du Sol «GSE» et Mueller Hinton «MH») contre six souches de bactéries cliniques, en l'occurence* Staphylococcus aureus, Escherichia coli, Enterococcus faecalis. Enterobacter cloacae *et deux souches de* Streptococcus *spp. Dix (10) isolats d'Actinobactéries différents, présentent des morphologies distinctes. Soixante-sept pour cent (67%) des isolats testés, ont montré au moins une activité antibactérienne contre les bactéries cliniques indicatrices testées. Les souches d'Actinobactéries A_1, A_6 et A_8 ont montré l'expression la plus élevée sur le milieu MH, tandis que la souche A_6 a présenté un spectre de plus large gamme d'activité antibactérienne contre toutes les bactéries cliniques indicatrices testées sur le milieu MH. Cependant dans certains cas, le changement de milieu de culture, influence positivement l'activité antibactérienne. La présente*

étude a révélé que le sol de Tamanrasset est riche en souches appartenant à la classe d'Actinobactéries, dont l'intérêt peut être orienté essentiellement à des fins médicales, notamment l'obtention de nouvelles molécules bioactif à large spectre d'action.

Mots-clés: *Actinobactéries, sol de Tamanrasset, morphologie, activité antibactérienne, bactéries clinique.*

Introduction

The phylum Actinobacteria is one of the largest taxonomy unite among bacteria domain. The different genera that are part of this phylum exhibit enormous diversity in terms of their morphology, physiology and metabolic capabilities [1-3]. Actinobacteria are widely distributed in soil, animals, plants seawaters and in ocean sediments [4]. Actinobacteria species are aerobic Gram-positive bacteria with high G+C. Under microscopy, the appearance of Actinobacteria genera exist in the forms of thin filaments, rods, cocci, or rodcocci; the filamentous genera like *Streptomyces* develop two types of mycelium such as substrate or vegetative mycelia and aerial or reproductive mycelia with 1-2µm in diameter [5]. The morphological characterization of the Actinobacteria are very important for their identification and taxonomy. Therefore, the primary identification are based on the macroscopic and microscopic observation to determine their structural characteristic [6].

Actinobacteria are of universal occurrence in nature, and have been reported to occur under both cold (artic region) [7] and hot temperature under extreme climate conditions. Such climate regimes dominate in the Algerian desert areas, which are known as arid or hyper-arid regions. Different soils of this Algerian desert regions, were the subject of numerous new Actinobacteria species investigations viz.: the characterization and identification of several isolates, and the assessment of their antimicrobial activity [8-18]. It is well known, that Actinobacteria strains are mostly mesophilic, with optimal growth at temperature range between 25°C and 30°C. However, thermophilic Actinobacteria strains are able to grow at high temperature ranging from 50°C to 60°C [1]. In fact, thermos-halotolerant strains as an extremophiles Actinomycetes were recently isolated by an Algerian teams [19], who reveals the richness and the biodiversity of the Algerian soils in rare sources of Actinobacteria strains. In addition, it has been recognized that numerous of strains species belonging to Actinobacteria phylum are of huge biocontrol importance [20]. Therefore, these strains exhibit an important antagonistic activity against several pathogenic microorganisms in different filed such as medicine, biotechnology, agriculture and ecology [1-2]**.**

Therefore, The Actinobacteria phylum are very interesting because of their capacity to produce secondary metabolites with diversified chemical structures [17]. The most extensively representative studies of this group include soil dwelling *Streptomyces* strains. This later are the major of antibiotic producers [3]. The bioactive molecules are not exclusively on antibiotics, there are also antifungal, antiparasitics, immunostimulants, immunosuppressant and other molecules [4]. According to the emerging infection diseases and multidrug resistance, human pathogens are becoming a major threat to global health therefore there is an urgent need to found a novel bioactive compounds products to combat bacterial resistance [21].

The previous locale Algerian studies, have been covered different region with variable ecosystems (fresh water, marine sediment, forest, oasis, sebkha, endemic desert

plants, desert and palm Grove soil, etc.) and climate (Mediterranean and Saharan arid or hyper arid climate) [2]. In Algerian arid and desert soil, Actinobacteria isolation and antibiotics studies have been ushered by the Algerian researcher SABAOU and *al.* since 1998 [22]. In last decade, the sampling sites regarded for the isolation and investigation of rare Actinobacteria have been essentially focused on the extreme Saharan soil and palm groves such as Adrar, Ghardaïa, and Tamanrasset regions [2]. Subsequently, a large number of new species have been discovered, mostly from Algerian Sahara, namely *Actinomadura adrarensis* [15] *Mzabimyces algeriensis* [23], *Saccharothrix ghardaiensis* [24], *Actinopolyspora righensis* [25], , *Saccharothrix tamanrassetensis* [26] *Saccharothrix hoggarensis* [8], and *Actinoalloteichus hoggarensis* [9]. Besides, a new actinobacterial strain assigned to *Saccharothrix xinjiangensis* was recently isolated from Ahaggar region [27]. Consequently the research contributions to explore microbiological arid soils composition in Actinobacteria field in Tamanrasset soils region can be estimated around 17% [2]. As we know, Tamanrasset is the heart of Ahaggar national park, belonging to the great desert in the world [28]. It is a hyper arid region that located in southern Algeria at a 1370-m on altitude which covered an area of 450.000 km^2 [2]. The temperature are characterized by very high thermal amplitude around 30.5°C [29-30]. These regions have a natural and biological particularity deserve to explore. In the present study, our aim is to highlight the richness and biodiversity of Actinobacteria of Algerian hyper-arid Sahara soil, and the discovery of several interesting bioactive molecules producing by novel Actinobacteria species.

1.- Material and methods

1.1.- Soil samples collection

Five (05) soil samples (S_1, S_2, S_3, S_4 and S_5), of 100 to 150 g each were collected from a different hyper arid area of Tamanrasset town (South of Algeria) (tab. I). Based on previous study [31-33], the soils samples were taken from a variable depth of different plant's rhizosphere ecosystems. The samples, were collected in sterile containers, and were aseptically transported to the research laboratory of University of Tamanghasset for microbiological analysis.

Table I.- Soil characterization and sampling

Soil sample code	Location	GPS location	pH	Characteristics	Vegetation	Depth from soil surface (cm)	References for the depth choice
S_1	Tam Univ.	N 22° 48' 21.9" E 5° 30' 22.9	9.92	Brown dark rhizosphere soil Watering by Rainwater	*S. Senegal*	20	[31]
S_2	Tam. Univ.	N 22° 48' 16.4" E 5° 30' 17"	9.48	Watering by tap water Grit light soil	*W. filifera*	15	[32]
S_3	Tam. Univ.	N 22° 48' 16.4" E 5° 30' 17"	8.60	Watering by tap water Grit light soil	*B. villea*	10	[33]
S_4	Town	N 22° 47'	8.70	Dark humid	*O.*		[33]

	centre	25" E 5° 31' 19"		Watering by tap water	*europaea C. aurantifolia*	10	
S_5	Town centre	N 22° 47' 25" E 5° 31' 19"	8.70	Watering by tap water	*O. basilicum associated with an ornamental palm*	10	[33]

1.2.- Soil samples processing

The soil samples were exposed ether to physical treatment which represented in air drying during 1week [31], heating at dry temperature (110°C/10min) [34] or 125°C/15min, heating at dry temperature or/and chemical treatment by the addition of calcium carbonate $CaCO_3$ at 1% coupled with incubation at 30°C during two weeks [31]. Samples treatment methods used in this study are summarized in table II.

Table II.- Soil samples treatment

Samples	Treatment type		References
S_1	Physical	110°C/10min	[66]
S_5		125°C/15 min	[34] Slightly modified in this work
S_1, S_2, S_3, S_4		Air drying	[31]
S_1, S_2, S_3, S_4	Chemical	$CaCO_3$ at 1%	[31]

1.3.- Actinobacteria isolation

The isolation of Actinobacteria strains, was carried out by soil dilution plate technique on the following tow kind of culture media: Glycerol Yeast Extract agar (GYE) g/l: glycerol,10; yeast extract,10; PCA,15; NaCl,5; K_2HPO_4, 1; pH:6 and Glycerol Soil Extract agar (GSE) g/l: media for each soil sample (S_1, pH: 9.92; S_2, pH: 9.48; S_3, pH: 8.6; S_4, pH: 8.7, S_5, pH: 8.7) based on: soil,15; PCAP (Plate Count Agar Powder),15; glycerol,10. The extract soil media considered as a source of humic acid, which have an important role in the activation of Actinobacteria spores [35]. Serial decimal dilutions were performed from stock soil solution which was prepared by dissolving 01g of soil in 09 ml of sterile distillate water [31]. We have spread 500 µl of the previous soils samples dilutions on the surface of the culture media previously indicated. The culture media were incubated at 27°C until appearance of Actinobacteria colonies. The pure colonies were cultured on GYE-agar media by streak plate method for purification. Then, the purified colonies were maintained in glycerol (30%, v/v) at +4°C [36]. The macroscopic and microscopic morphological characterization of the isolates were performed using the pure colony grown on GYE media. Actinobacteria colonies were examined with naked eyes; while, Actinobacteria isolates were observed under light microscope oil immersion (100)x[10] after Gram staining method [37] implementation.

1.4.- Preliminary screening for antibacterial activity

The preliminary screening for antibacterial activity was carried out on three culture media: Glycerol Yeast Extract "GYE", Glycerol Soil Extract "GSE" and Mueller Hinton

"MH" as reference culture media (MH CM0898 Haemophilus Test Media Base: g/l: Hinton agar,38; yeast extract,5 which enhance the growth of Actinobacteria and offer clear results due to its transparency [38-39], against six (06) clinical bacteria strains, viz.: *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Enterobacter cloacae* and tow strains of *Streptococcus* spp. Each of selected Actinobacteria, were streaked as central straight-line on the tested culture media (GYE-agar, GSE and MH) and incubated at 27°C until to get a visible growth. Then, the Actinobcateria isolates, are exposed to the preliminary screening, which was performed by using the Cross Streak method [40-42]. Then, the incubation was achieved at 37°C for 24h to 48h to allow growth of clinical test bacteria. The Cross Streak method, is based on the measuring of the distance inhibition growth between the central straight-line culture of the Actinobacteria isolate and each clinical test bacteria.

2.- Results and discussion

2.1.- Colony and bacterial morphological traits

The study was performed to isolate Actinobacteria strains with antibacterial activity from hyper arid soil of Tamanrasset region located in southern Algeria. In fact, ten (10) different strains were isolated on GYE-agar media from five (05) soils samples of Tamanrasset town. GYE-agar media seems to be selective for Actinobacteria, because it contain Glycerol that most of Actinobacteria use it as a carbon source [43-45]. Besides, 08 strains (A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_{10}) which represent 80% of Actinobacteria isolates, were mostly obtained from Acacia soil samples, as described in table III. The obtained colonies exhibit on GYE-agar media the typically Actinomyces phenotypes with pinpoint, powdery, chalky and dry colonies, whose diameters vary from 02 to 05mm (fig. 1, tab. IV). Such characteristics are already reported by previous several studies [1,43-47]. All colony exhibit an aerial and substrate mycelium with different colors viz: white, beige, orange, light orange and dark green, with mostly, dominance of the white color. In fact, 60% of Actinobateria isolates colony (A_3, A_4, A_5, A_8, A_9 and A_{10}) display white aerial mycelium vs 50% for substrate mycelium. These criterions are well established by the various bibliographic data [1,43,46-48]. On the other hand, all isolates under light microscopic observation are Gram positive and have a similar filamentous morphology typical to *Streptomyces* sp as described by previous study [1,47] which exhibit a branched substrate mycelium with presence of aerial hyphae (fig. 2).

Table III.- Incubation Time and enumeration of Actinobacteria colony isolated from soil samples

Strain code	Vegetation of rhizosphere soil	Treatment procedure	Incubation time (Days number)	Actinobacteria isolates Enumeration CFU/ ml
A_1	*Acacia*	110°C/10 min	7	10^6
A_2	*Acacia*		14	10^6
A_3	*Acacia*		14	10^6
A_4	*Acacia*		14	10^6
A_5	*Acacia*		26	10^6
A_6	*Acacia*		26	10^6
A_7	*Acacia*		26	10^6
A_{10}	*Acacia*		16	10^6
A_9	*Ocimum basilicum and*	125°C/ 15min	31	$2.0X10^7$

	ornamental palm			
A_8	*Bougainvillea*	$CaCO_3$; 37°C/ 2 weeks	25	10^6

Table IV.- Macroscopic and microscopic characteristics of Actinobacteria isolates which are pre-assigned to *Streptomyces* sp.

Strain	Aerial mycelium	Substrate mycelium	Edge	Texture	Diameter (mm)	Gram stain
A_1	Orange	Orange	irregular	rough convex	5	+
A_2	Beige	Beige	regular	Rough Flat	2	+
A_3	White	White	regular	rough convex	2	+
A_4	White	White	regular	rough convex	2	+
A_5	White	White	regular	rough convex	5	+
A_6	Beige	Beige	irregular	rough concave	3	+
A_7	Clear orange	Clear orange	irregular	rough convex	5	+
A_8	White	Dark green	irregular	rough convex	5	+
A_9	White	White	irregular	rough convex	5	+
A_{10}	White	White	irregular	rough convex	5	+

2.2.- Enumeration of the isolated Actinobacteria strains:

For the enumeration of Actinobacteria, the soil samples were pretreated and plated on culture media GYE-agar using soils samples dilution plat technique, and results are expressed as Colony Forming-Unit (CFU) per gram of soil. After incubation at 27°C for several days (7 to 31 days) the colonies with Actinobacteria phenotype were enumerated on GYE-agar. Furthermore, nine (09) isolates (A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8 and A_{10}) are present at the same density 10^6 CFU per gram of soil (10^6/g). However, the density of A_9 isolate was grater and valued to 2. 10^7 CFU per gram of soil (2.10^7/g), which is twenty fold higher than the count of the previous isolates. Our results are in accordance with those obtained by the pervious study, which recoded 10^6 to 10^9 CFU per gram of soil [49-51]. On the other hand, it is well established that the maximum population density of *Sterptomyces* was observed when soil was exposed to the maximum air drying [52]. Besides, the soil microbial community of hot environment are dominated by the Actinobacteria 36.8% as a higher microbial percentage, and this dominance notably increase when mean annual precipitation decrease [53]. Other investigations, have recorded that thermotolerante and thermophilic Actinobacteria were found in higher abundance, exceeding that of the mesophilic forms, as mentioned by KURAPUVA *et al*. (2012) [54]. Also, the distribution of *Streptomyces* in plant of arid or hyper arid region are very significantly considerable, the maximum recorded counts was about 10^7 per gram, and were found in rhizosphere soil of *Rhanterium epapposum* plant [55]. Surprisingly, an important isolate we called "A_9", appeared alone on GSE medium after heat treatment at 125°C/15min, which is greater than

to the sterilization temperature. This result show that spores of A_9 isolate seems to be more resistant to dry heat treatment compared to the others nine strains which are isolated during this research work. That mean that A_9 isolate spores are more resistant to dry heating than not only nonfilamentous bacteria but also more than general actinomycetes. The Actinobacteria isolate A_9, seems belonging to *Actinomadura rugatobispora* whose selective isolation is effective only under dry heating at high temperature from (120°C to 130°C) as described by Japanese team since 2000 [34]. Besides, two new species strains belonging to *Actinomadura* genus viz.: *Actinomadura adrarensis* [15] and *Actinomadura algeriensis* [56] were already isolated from Algerian arid soil of Adrar and Hoggar-Tamanrasset region [15]. In fact, our results reinforce those previously indicate that Algerian arid and hyper arid soil as extreme ecosystem are a promising source of new Actinobacteria strains. Namely, strains, which exhibit spores high thermotolerance such as our Actinobacteria, isolate called A_9. This later seems to be very interesting in biotechnology and medical perspectives

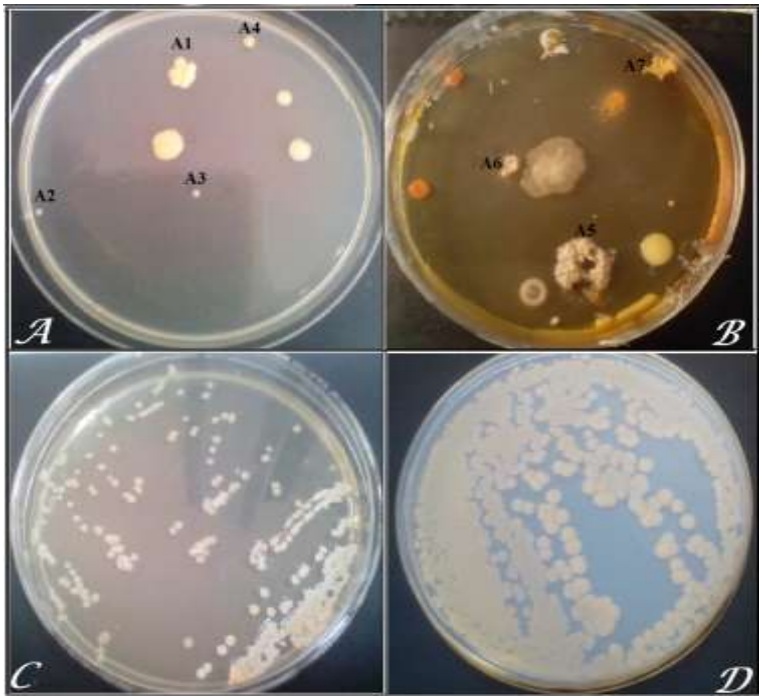

Figure 1.- Isolation (A, B) and purification (C, D) of Actinobacteria strains on free antibiotics GYE-A media

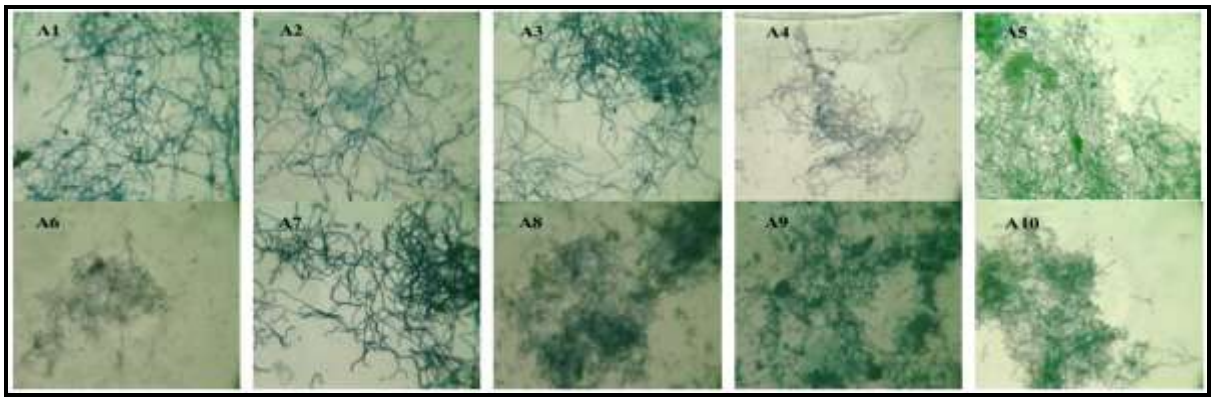

Figure 2.- Microscopic characteristics of the 10 Actinobacteria Isolates strains from A_1 to A_{10} observed after Gram stain under light microscope with oil immersion: (100)x[10]

2.3.- Growth characteristics of the isolated Actinobacteria strains

Growth capability of the ten (10) isolates was carried out on two culture media GYE-agar media and extract soil GSE, that contain glycerol as sole carbon source. First, it should be noted that all our ten (10) isolates seems to be able to use the glycerol in both culture media because of their ability to grow ever on GYE-agar or on GSE. In fact, Glycerol as sole carbon source compound in culture media seems to have selective power and thus improve Actinobacteria strains isolation from soil. Our results join those one obtained previously by Oskay and collaborators [43]. Besides, these metabolic properties to know using glycerol by all our ten isolate, will be useful to implement this Actinobacteria isolates bio resources not only in therapeutic domain, but also in biotechnology, notably in renewable energy field in extreme region like Tamanrasset area. In fact, it is well recognized that members of the Actinobacteria phylum, such as *Streptomyces clavuligerus*, *Propionibacterium acidipropionici*, *Propionibacterium freudenreichii*, *Rhodococcus opacus*, *Corynobacterium glumaticum*, *Corynobacterium erythropolis*, and *Corynobacterium kutscheri*, have exhibit their ability to use glycerol as the sole source of carbon, and energy strains, can use Glycerol as substrate and convert it to biodiesel [45].

On the other hand, our results demonstrated that with both physical and chemical treatment (heat dry; $CaCO_3$) and without any antibiotic culture media supplement, no contamination by fungi (yeast or molds) was detected, and bacterial colony out of Actinobacteria were minor. Our results is in accord with the successful plate-method for the preferential isolation of Actinomyces from soils which was already implemented by AGATE and BHAT since 1963 [57]. In fact treatment of soils samples at high temperature of 110°C for 10 min is efficient to inhibit the non-sporulating bacteria and limit the spread growth of spore forming bacteria and fungi by the use of dried plates method [57]. Besides, Absence growth of fungi will be related to the high alkalinity (pH>8) of the Tamanrasset soils samples, with recorded pH values ranging from 8.6 to 9.92 (tab. I). Such soil is called soda soil, and It's well recognized that soda soil is the mean characteristic of the, semi-arid, arid an hyper-arid lands through the world with Sodium (Na^+) as the dominant cation [58]. Consequently, high alkalinity of Tamanrasset soil, where water evaporation because Tamanrasset dryness climate will reinforce the process of soda (Na^+) accumulation, seems to be unfavorable to fungi growth. Therefore, it is well known that fungi are almost living in neutral or acidic abiotic conditions. But, in some arid region fungi with high alkalinity soil tolerance were detected at high density [59]. Taking in a count the pH parameter, our results are not in accordance with bibliographic data, like those obtained by Alotaibi and collaborators. This later have recorded the presence of fungi from alkaline desert sites, like Al-Aushazia area (pH soil = 8.54) and Abqaiq region (pH soil = 8.40) in Saudi Arabia [60]. Besides, our ten (10) Actinobacteria isolates, seems to be halotolerant or halophilic strains, because their capability to growth and living in Tamanrasset soda soil. Hence, Our results is in accordance with those reported by several authors who have already isolated and characterized a numerous of halotolerant or halophilic Actinobacteria strains such as *Actinopolyspora saharensis* [61], *Nocardiopsis algeriensis* [62], *Bounagaea algeriensis* [16], *Prauserella isguenensis* [63], *Actinoalloteichus hoggarensis* [9]. Variation in time incubation at 27°C from 1 week to 4 weeks on GYE-agar media to get a visble and good growth of Unite Forming Colony was recorded as an important parameter in growth characteristics among our ten (10) Actinobacteria isolates. Taking in count the required time for growth, we have grouped our isolates in four class: (i) slightly slow class, which contain only the A1 isolate for which good growth was observed after only 7 days of

incubation (1 week); (ii) slow class, which regroup four isolates viz.: A_2, A_3, A_4 and A_{10}, this isolates have required 14 to 16 days (2 weeks) to grow well on culture media; (iii) very slow class, this later contain the four following isolates A5, A6, A7 and A8 which need 25 to 26 days (03 weeks) to reach good growth; (iiii) high slowly class with only A9 isolates as member which exhibit a good growth after 31 days (04 weeks) of incubation. It well known, that filamentous Actinobacteria such as *Streptomyces* isolates are recognize as slow growing bacteria as described by several research works [27,31,34,51]. On the other hand, and after several streak on GYE-agar media, we have observed that among the ten (10) Actinobacteria isolates, only six (06) strains: A_1, A_5, A_6, A_7, A_8 and A_{10} were able to grow on soil extract culture media (GSE-Agar) as well as on synthetic ones (GYE-Agar and MH). However, A_2, A_3, A_4 and A_9 Actinobacteria strains were omitted from the study because they have lost the ability to grow again on at least one of the culture media used in this study. This phenotype can be related to the physiological [7] and genetic characteristics of the Actinobacteria strains. In fact, in the case of *Streptomyces* genus, fluctuation in growth is frequently reported. This growth issue will be related to several parameters, such as the inner-culture variability [64]. In addition, several genetic, germling agglomeration which is related to spore kind and environmental effectors such as media composition, dissolved oxygen concentration, surface tension and energy input have been identified over the years, known to contribute to the observed cultivation difficulties [65]

2.4.- Screening of Antibacterial activity of the isolates

Based on the growing capability criteria as describe above, A_1, A_5, A_6, A_7, A_8 and A_{10} Actinobacteria strains were screened for their antibacterial activity, which was evaluated on the three culture media MH, GYE-A and GSE-A by inhibitory growth of clinical test bacteria. Antibacterial activity was assessed against six (06) clinical test bacteria that recorded previously. The results show that only four (04) isolates A_1, A_6, A_8 and A_{10} have exhibit inhibitory growth against at least two clinical test bacteria and at least on one of the tested culture media as summarized in table V. An example of antibacterial activity is shown in figure 3. Our results are similar to those obtained by Pinney and Kalil since 1975 [42]. These later, have shown that not all Actinobacteria strains will produce antibiotics that inhibit growth of test bacteria, but some do. The same authors, add that poor or no growth of the clinical test bacteria are related to their susceptibility to some diffusible substances produced by the Actinobacteria strains [42]. Besides, is the first time that antibacterial activity of at least one of the Actinobacteria isolate called "A_1" strains, is detected on the "GSE" culture media. This later was performed using soil extract which supplemented with Glycerol. We have demonstrate that "GSE" culture media, allow not only to Actinobacteria isolates growth but also it seem be convenient for A_1 strains to the expression and diffusion of the inhibitory substance. Therefore, "GSE" culture media will be an efficient and low coast alternative to implement the preliminary screening of Actinobacteria strains for antibacterial activity. Based on the previous knowledge, soil extract media was only used to improve the selective isolation of certain Actinobacteria species, namely *Actinomadura rugatobispora* [66]. Several other new species of the genus of *Actinomadura* were already isolated by several Algerian teams from Algerian Saharan area with an antibacterial and or antifungal activity [14,15,56]. Such new species, have shown their importance in the new antibiotics molecules production filed. For example, the new species *Actinomadura* sp. called ACD1 and isolated from Hoggar region, has proven its ability to produce an inserting antibiotic named S9 because it no polyenic in nature. Consequently, this antibiotics kind are recognized as not toxic bioactive molecules with high stability, and have exhibit strong antifungal and antibacterial activity [56]. Beside, many others inserting new antibiotics molecules were produced by other new *Streptomyces*

spices isolated from several desert and extreme area of Algerian desert soil. In fact, it was well demonstrated that tow novel intracellular antibiotics produced by *Streptomyces griseoflavus* called mzabimycins A and B that belonging to angucycline family are able to inhibit growth of Gram positive bacteria with multiple antibiotic resistance [17]. In addition, Lahoum and collaborators, reported that for the fisrt time members of *Saccharotriix* genus isolated from Algeria desert are able to produce tow new cyanogriside antibiotics named cyanogriside I and cyanogrise J [67].

On the other hand, our results demonstrate that Glycerol and Saharan soil media appear to play too main roles as modulators and regulators in biosynthesis of inhibitory substances in some *Streptomyces* sp. (A_1, A_8 and A_{10}). In fact, it well known that the Glycerol utilization involved in clavulanic acid production, produced by *Streptomyces clavuligerus* [68]. And possibly supplies glyceraldehyde-3-phosphat precursor of clavulanic acid biosynthesis, which inters in three β-lactam antibiotics: CA, 5S clavams, and cephamycin C [69]**.** The antibacterial activity of the isolates were dissimilar between Gram positive which have only an outer peptidoglycan layer which is not an effective permeability barrier [33] make them more sensible to the secreted biomolecules then Gram negative which have a second polysaccharide membrane make the cell impermeable to the lipophilic solutes [70]. In addition, the type of media seem to have positive or negative effect on biosynthesis of bioactive molecules as showed in table V.

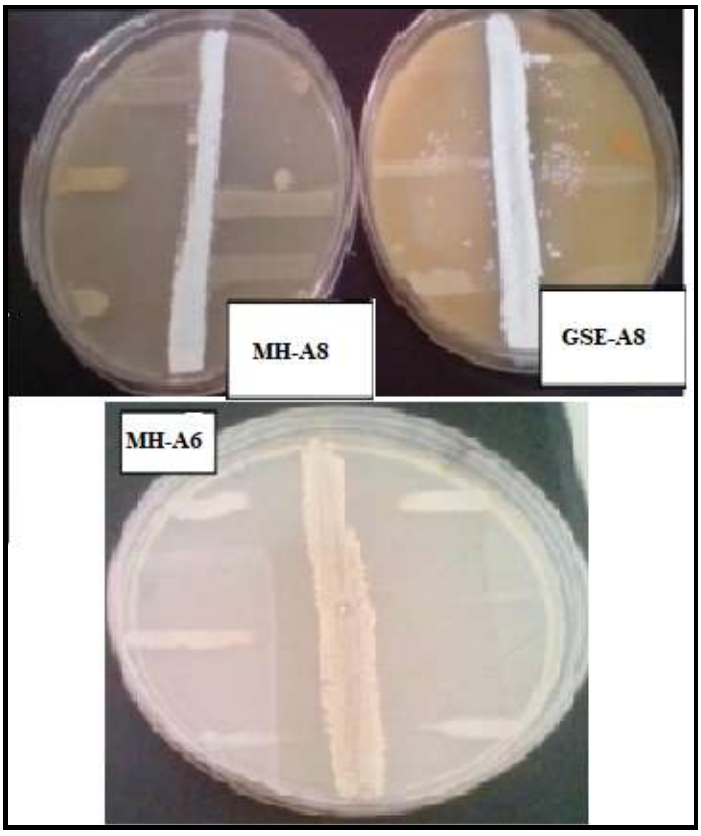

Figure 3.- Cross streak Test for antibacterial activity against six (06) clinical test bacteria
Positive antibacterial activity only on MH culture media of A_6 Actinobacteria isolate
Positive antibacterial Activity on both MH or Glycerol Soil Extract (GSE) culture media of A_8 Actinobacteria isolates
Upper right, 1-*Staphylococcus aureus* (resistant); lower right, 2-*Escherichia coli (*susceptible), 3-*Enterococcus faecalis* upper left, 4-*Enterobacter cloacae* (susceptible), 5-*streptococcus* sp. lower left, 6-*streptococcus* sp. (susceptible)

Conclusion

The choice of rhizosphere soil due to enhanced the possibility to find Actinobacteria, as most of Actinobacteria are abundantly present in soil and represent a high portion of the microbial flora of the rhizosphere. Actinobacteria are capable to colonize rhizosphere through their antagonist and competitive characteristics concerning of the soil microorganisms**.**

A great range of divers new Actinobacteria, have been isolated from Tamanrasset as hyper-arid soil, at least ten species were obtained which exhibit a varied macroscopic morphology on GYE and similar microscopic branching morphology. Six (06) from them (A_1, A_5, A_6, A_7, A_8 and A_{10}) were investigated preliminary in their antibacterial activity, whereas; A1 and A8 have exhibited a large antagonist activity and seem to display a wide spectrum antibacterial agents. Therefore, the adaptation of Actinobacteria to various condition of hard Saharan ecosystem has developed amazing physiological and metabolic capacity to survive, then to classify the several species as prodigious producers of bioactive compounds, which are researched to promote various biological sectors.

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Table V.- Preliminary screening of Actinobacteria isolates using cross streak method against different pathogenic clinical bacteria

	A_1			A_5			A_6			A_7			A_8			1		
	MH	**GYE**	**GSE**	**MH**	**GYE**	**GSE**	**MH**	**GYE**	**GSE**	**MH**	**GYE**	**GSE1**	**MH**	**GYE**	**GSE**	**MH**	**GYE**	**GSE**
***Streptococcus* sp.**	-	++++	-	-	-	-	+++	-	-	-	-	-	+++	-	+++	-	-	-
***Streptococcus* sp.**	++	++	+++	-	-	-	++++	-	-	-	-	-	+++	-	-	-	-	-
Enterobacter cloacae	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	++	-
Staphylococcus aureus	++	+	++++	-	-	-	+++	-	-	-	-	-	+++	-	+++	-	++	-

++++: higher activity; +++: Good activity; ++: Moderate activity; +: Weak activity; -: No activity.