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ORIGINAL RESEARCH ARTICLE

Screening of Actinomycetes Producing Antibacterial Substances and Indole Acetic Acid (IAA) and Optimization of Growth and IAA Production Conditions in *Streptomyces* sp. SF5

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ABSTRACT

Starch-casein agar medium has allowed isolation of seven actinomycetes strains from a soil of the North of Algeria (Sétif). All the strains were able to produce antibacterial substances. The strain SF5 showed an antibacterial activity against all the pathogenic bacteria tested. The inhibition diameters for *P. aeruginosa*, *B.cereus*, *S. aureus*, *E. coli* and *S. typhimurium* were respectively; 18 mm, 12 mm, 12 mm, 10 mm and 7 mm. Furthermore, it is appeared the most efficient in terms of production of indole acetic acid (55.58 ± 0.7 µg/ml) followed by the strain SF2 (42.28 ± 0.3 µg/ml). Based on cultural and morphological characters, this isolate is designated as *Streptomyces*. Concerning the optimization of *Streptomyces* sp. SF5 growth and IAA production conditions, it was found that growth was maximal and IAA production reached a maximum of 104.76±0.2µg/ml after 5 days when the culture medium was supplemented with 2mg/ml of L-tryptophan at pH 7 and incubated at 30°C. NaCl concentrations of 100, 200, 300 and mainly that of 500 mM had an inhibitory effect on both growth and IAA production.

Key words: isolation, *actinomycetes*, *Streptomyces* sp.SF5, antibacterial activity, indole acetic acid, optimization.

1. INTRODUCTION

Actinomycetes are Gram positive bacteria. They are considered the major group found in the soil and they are widely distributed [1]. Starch-casein medium was used in a lot of researches for the isolation of actinomycetes from soil and especially *Streptomyces* sp. [2]. It appeared the most appropriate medium for development of aerial mycelium and the selection growth of actinomycetes [3]. Great difficulty in actinomycetes classification exists; it is usually based on morphological characters and colour of aerial and vegetative mycelium. The analysis of the chemical composition of the cell wall is also widely studied [4].

Actinomycetes are included in the decomposition of organic matter [5] production of antibiotics, vitamins and enzymes [6]. In addition, some strains are endowed with the ability to solubilize phosphorus [7] and to produce indole acetic acid (IAA) [8]. Relatively, high percentages of actinomycetes isolated from soil synthesize antimicrobial substances; this shows an extremely important antagonist role in the soil. Streptomycetes only produce more than 75% of antibiotics. *Streptomyces* and *Micromonospora* are the most described and studied. *Streptomyces* is actually considered one of the major sources of bioactive natural products [9]. Indole acetic acid is a common natural auxin and is a product of L-tryptophane metabolism of microorganisms. It influences on several parameters in the plant, namely elongation and cell division, apical dormancy and differentiation of vascular tissues [10]. According to [8], in some strains of *Streptomyces*, IAA has been found capable to stimulate the formation of mycelium and thus improve the production of antibiotics.

Our study is aimed to the isolation of actinomycetes from the soil and the screening of the most efficient strain in terms of production of antibacterial substances and indole acetic acid. Furthermore, growth and IAA production were tested under different conditions by studying the effect of L. tryptophan concentrations, pH, temperature and salinity.

2. MATERIAL AND METHODS

2.1. Actinomycetes isolation

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A fertile soil collected from El-Ourissia region, North of Algeria (Sétif) was used for the actinomycetes isolation. Heat treatment was performed by holding the soil sample in a water bath (50°C for 60 min) to prevent growth of other bacteria. Starch-casein agar medium (g/l): starch 10, casein 0.3, KNO_3 2, NaCl 2, K_2HPO_4 2, $MgSO_4$, $7H_2O$ 0.05, $CaCO_3$ 0.02, $FeSO_4$, $7H_2O$ 0.01 and agar 18 was supplemented with griseofulvin (25 µg/ml), nystatin (10 µg/ml) and nalidixic acid (10 µg/ml) and was used for actinomycetes isolation; the inoculated plates were incubated at 30°C for seven days [11].

2.2. Antibacterial activity

The antibacterial activity was performed by using the agar disc method. Actinomycetes strains isolated were grown on GLM medium of the following composition (g/l): yeast extract 5, malt extract 3, peptone 5, glucose 10, agar 20, the pH was adjusted to 7.2. Growth lasted 7 days at 30°C, and then the colonies were removed and placed on Muller-Hinton medium, previously inoculated with strains of *Escherichia coli* ATCC 25922, *Bacillus cereus*, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Salmonella typhimurium*. Cultures were set at 4°C for 4 hours and incubated at 37°C for 18-24 hours, and then the inhibition diameters (mm) were measured [12].

2.3. Indol acetic acid production

To determine the ability of actinomycetes strains isolated to produce indol acetic acid, a production test was performed by using the method of Bano and Musarrat [13]. The cultures were grown in YM medium of the following composition (g/l): malt extract 3, yeast extract 3, peptone 5, glucose 5, agar 18, the pH was adjusted to 7.2. The strain was incubated at 30°C for 3 days. Disks (8-10 mm) in diameter of grown colonies are then transferred into 5 ml of medium (YM) containing 2 mg/ml of L-tryptophan. The culture was then incubated at 30°C on a rotary shaking (125 rpm) for 4 days. After centrifugation (11,000 × g/15 min), 1 ml of the supernatant was mixed with 2 ml of Salkowsky reagent. The solution was vortexed and kept at room temperature for 20 min. The appearance of a pink colour indicates the production of the IAA. Optic density was determined at 530 nm and the levels of IAA are determined in µg /ml.

2.4. Study of optimum conditions for growth and IAA production

To determine the best conditions for growth and IAA production in SF5 strain, different parameters were studied: L-tryptophan concentrations (2, 5 and 7mg/ml), pH levels (5, 6, 7, 8 and 9), temperature (25°C, 30°C and 37°C) and NaCl concentration (100, 200, 300 and 500mM). The cultures were supplemented with 2mg/ml of L-tryptophan and the samples were drawn every 24 hours for 7 days. Bacterial growth was determined at 540 nm.

3. RESULTS

3.1. Actinomycetes isolation

Isolation of actinomycetes from a fertile soil (North of Algeria) using starch-casamino acid has allowed the selection of seven strains (**Fig 1**).

3.2. Antibacterial activity

The antibacterial activity of actinomycete strains isolated from soil showed that isolated actinomycetes had a heterogeneous antagonistic activity against the pathogenic bacteria tested. However, the strain SF5 appeared most efficient (**Table 1**).

3.3. IAA production

All actinomycetes strains produce IAA but with variable rates. The strain SF5 produced high level compared to other strains (55.58 ± 0.7 µg/ml) (**Table 2**).

3.4. Growth and IAA production conditions optimization

3.4.1. Effect of L-tryptophan concentrations

Our results showed that growth was maximal and IAA production reached a maximum of 104.76 ± 0.2 µg/ml when the culture medium was supplemented with 2mg/ml of L-tryptophan (**Fig 2**).

3.4.2. Effect of pH

Acid and alkaline pH had a negative effect on growth and IAA production. The production of IAA is highest (100.02 ± 0.4 µg/l) at pH=7. Low levels of IAA are mainly obtained at pH=5 (53.41 ± 0.4 µg/ml) and pH=9 (37.7 ± 0.2 µg/ml) (**Fig 3**).

3.4.3. Effect of temperature

A temperature of 30°C seems favourable for growth and IAA production which reached a maximum of 104.76±0.6µg/ml at this temperature. At 25°C, growth was relatively similar to that observed at 30°C. For the production of IAA, high level was also obtained (91.27 ± 0.5 µg /ml) (**Fig 4**).

3.4.4. Effect of NaCl concentrations

Concentration of 500mM of NaCl had an inhibitory effect on growth and IAA production. IAA concentrations at 0 and 500mM of NaCl were respectively 104.76 ± 0.6 µg/ml and 47.43 ± 0.2 µg/ml (**Fig 5**).

Table 1: Antibacterial activity of isolated actinomycetes against some pathogenic bacteria

Actinomycetes strain type	Zone of inhibition in (mm)				
	E. coli	*B.cereus*	*P. aeruginosa*	*S. aureus*	*S. typhimurium*
SF1	18 mm	12 mm	-	7 mm	9 mm
SF2	10 mm	19 mm	6 mm	-	8 mm
SF3	16 mm	15 mm	-	-	8 mm
SF4	7 mm	8 mm	10 mm	6 mm	4 mm
SF5	10 mm	12 mm	12 mm	18 mm	7 mm
SF6	12 mm	5 mm	6 mm	-	6 mm
SF7	10 mm	4 mm	-	12 mm	4 mm

_ *Indicatesabscence*

Table 2: Indole acetic acid production by the isolated actinomycetes

Actinomycetes strain	Indole acetic acid (µg/ml)
SF1	33.71±0.3
SF2	42.28±0.3
SF3	40.85±0.2
SF4	38.57±0.5
SF5	55.58±0.7
SF6	18.57±0.4
SF7	23.57±0.6

Fig 1: Telluric actinomycetes strains isolated by using starch-casamino acid medium

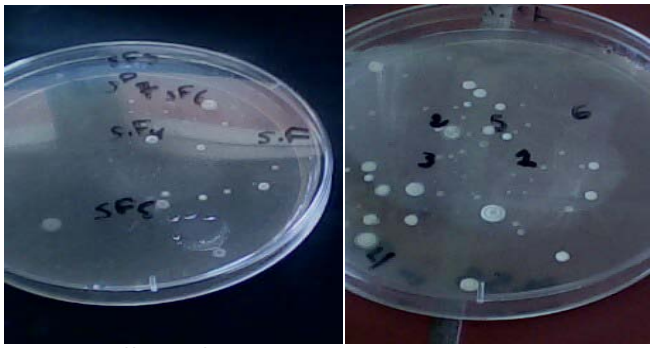


Fig. 2: Effect of L-tryptophan concentrations on SF5 actinomycete strain growth (A) and IAA production (B)

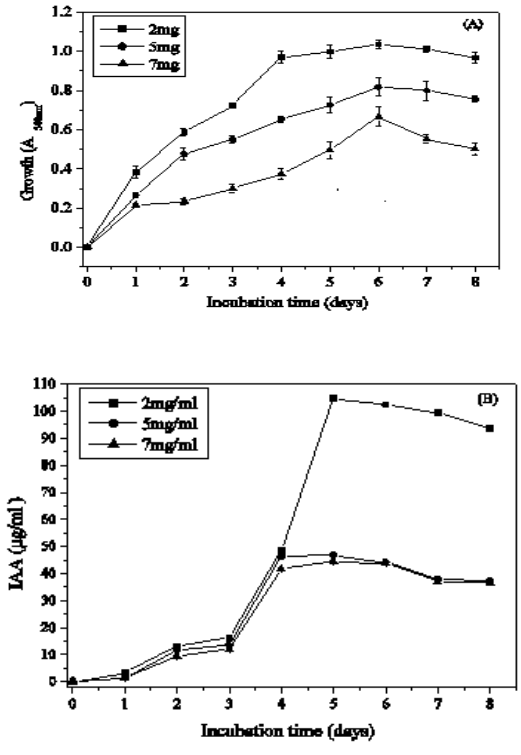


Fig 3: Effect of pH on SF5 actinomycete strain growth (A) and IAA production (B)

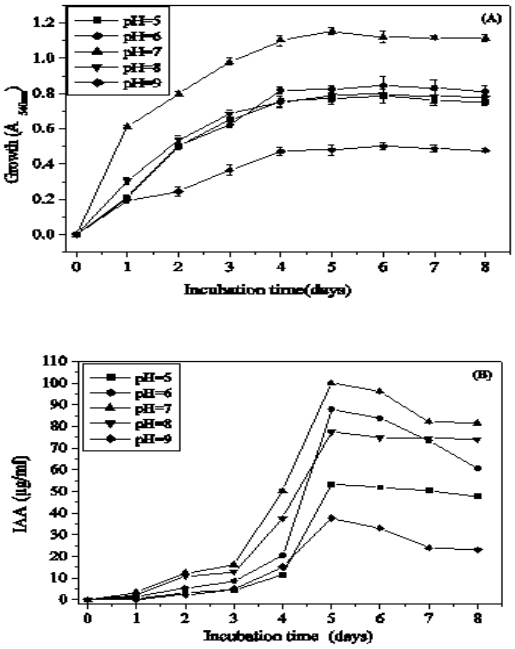


Fig 4: Effect of temperature on SF5 actinomycete strain growth (A) and IAA production (B)

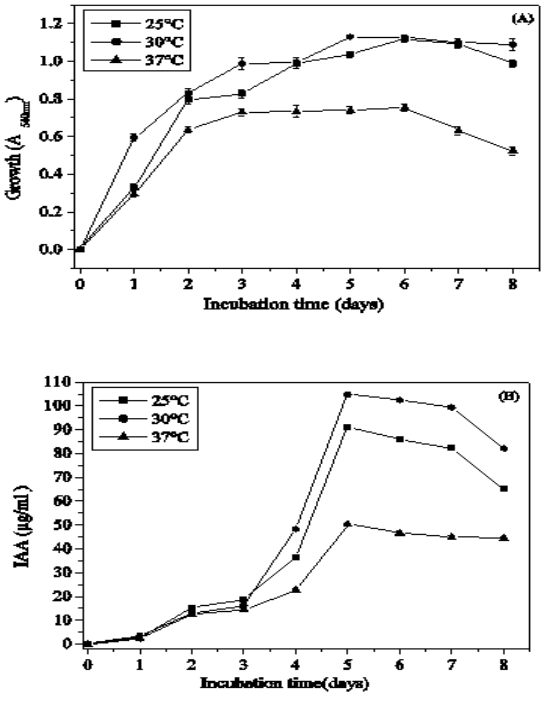
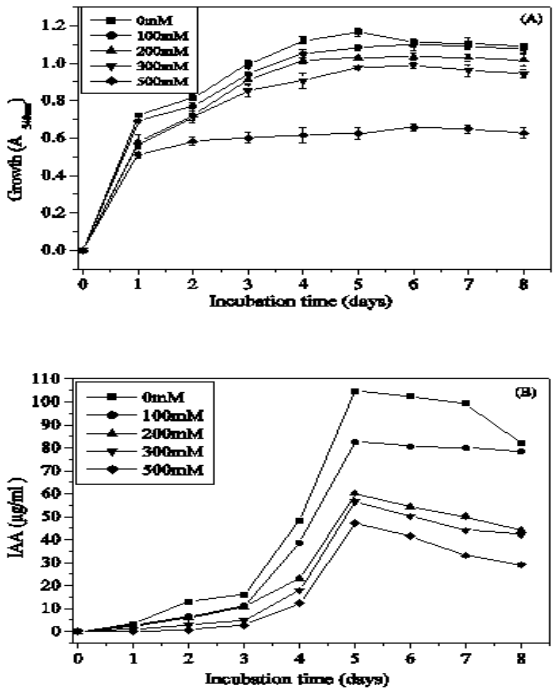


Fig 5: Effect of NaCl concentrations on SF5 actinomycete strain growth (A) and IAA production (B)



4. Discussion

In our study, heat treatment of soil (50°C for 60 min) reduced the number of Gram-negative bacteria [11]. All isolated bacteria are Gram-positive having a mycelian cellular aspect. The number of actinomycetes was $2.5.10^5$ CFU/ml and seven strains of actinomycetes were isolated. According to [14], four different pretreatments were used to isolate the maximum of actinomycetes. Treatment of 70°C for 15 min gave $2.2.10^7$ CFU/ml, while treatment of 100°C for 60 min allowed the isolation of 4.10^6 CFU/ml and that of 55°C for 60 min gave $1.6.10^7$ CFU/ml. The heat treatment had probably participated in the selection of actinomycetes and inhibition of Gram-negative bacteria [15], the effect of nalidixic acid was also involved. [16] Reported that the use of antibiotics is extremely useful for inhibition of bacteria and promotion of actinomycetes growth. According to [3], antifongics supplemented to the growth medium inhibited growth of most fungi, while they had no adverse effect on the growth of actinomycetes despite the increase in their concentrations (50 µg/ml).

On the other hand, starch-casein medium was used as a selective medium for actinomycetes isolation; it contains selective substrates (starch and casein) which favourite actinomycetes growth, *Streptomyces* sp. for example, has been isolated from arid soils [2]. Mature colonies of SF5 strain are dry, dusty and flat edges and do not easily lift from the agar, they are characterized by an "earthy" odour, and this form characterizes *Streptomyces* genus colonies [17]. *Streptomyces* colonies can be easily identified by their opaque, rough, non-spreading morphology and are usually embedded resulting in adherence to agar medium. The colour of substrate and aerial mycelia was variable; however, almost any colony gave an earthy odour that is characteristic of *Streptomyces* [18]. In addition, the strain SF5 had good growth on starch-casein medium; colour of aerial mycelium was white, while the vegetative mycelium was yellow. Based on cultural and morphological characters [19], this isolate is designated as *Streptomyces*. In soil, the streptomycetes are considered the most common bacteria, and they often constitute the major proportion of the actinomycetes population. When the conventional techniques of isolation are applied, most isolates of actinomycetes identified as *Streptomyces*, which is the most common genus found in the soil [20].

Actinomycetes are known to produce more than 70% of the natural antibiotics in the world, mainly those belonging to the genus *Streptomyces*, which is considered the most important type of soil actinobacteria [21]. Our study showed that actinomycete strains were active against the pathogenic bacteria tested with different degree. The strain SF5 identified as *Streptomyces* sp. was the most efficient, it shown an antibacterial activity towards all the bacterial strains beside SF4 strain (Table 1). *Streptomyces* sp. constitutes 50% of the population of terrestrial actinomycetes and 75% of antibiotics are produced by this genus [22]. *Streptomyces* sp. MADO2 and *Nocardiopsis* sp. MADO3 were screened for their antimicrobial activities. *Streptomyces* sp. showed a significant antimicrobial activity against C. *albicans* PC1 (23 mm), *E. coli* PC2 (16 mm), *P. mirabilis* PC3 (26 mm), *M. luteus* PC6 (27 mm) and *S. aureus* PC11 (26 mm). It was more active than *Nocardiopsis* sp. showed activity against *C. albicans* PC1 (14 mm), *E. coli* PC2 (00 mm), *P. mirabilis* PC3 (12 mm), *M. luteus* PC6 (23 mm) and *S. aureus* PC11 (19 mm) [23].

Indole acetic acid (IAA) is considered as the most active compound among the auxins. It is issue from the metabolism of L-tryptophan in many microorganisms (24), including fungi and bacteria [25]. Studies on the screening of actinomycetes producing IAA were shown that *Streptomyces* sp. is the genus the most involved in the IAA production [26].

The results of the screening of actinomycetes producing high levels of IAA showed that

Streptomyces sp. SF5 produce 55.58±0.7 µg/ml comparatively to the other strains. According to [27], actinomycetes isolated from the rhizosphere of some medicinal plants produce IAA with concentrations ranging from 11-144µg/ml. For certain strains of *Pseudomonas* sp. and *Azotobacter* sp, IAA is produced in a range of 20-36 µg/ml and 5-24 µg/ml when the medium was supplemented with 2 mg/ml of L-tryptophan [28].

The addition of various concentrations of L-tryptophan showed a heterogeneous effect on growth and IAA production of *Streptomyces* sp. SF5. A concentration of 2 mg/ml seems favourable for IAA production; it allowed production of 104.76±0.2 µg/ml of IAA after 5 days of incubation. The levels of IAA obtained in the presence of 5mg/ml 7mg/ml and L-tryptophan were respectively 46.93± 0.4 µg/ml and 44.38±0.4 µg/ml.

Some *Rhizobium* strains isolated from the roots of *Sesbania procumbens* produce IAA with a rate of 30.2µg/ml after three days of incubation in the presence 3 mg/ml of L-tryptophan [29]. According to [28], concentrations of 2 and 5mg/ml of L-tryptophan seem to have different effects on the production of this phytohormone in different strains of *Pesudomonas* sp. and *Azotobacter* sp., 5mg/ml of L-tryptophan appeared favourable for IAA production.

As to IAA production, growth of *Streptomyces* sp. SF5 was optimal in the presence of 2mg/ml L-tryptophan, 5mg/ml and 7mg/ml seem to have adverse effects on bacterial growth.

Our results showed that a pH of 7 appeared favourable for the growth and the production of IAA. The acidic or basic pH are not suitable for IAA production because *Streptomyces* and other actinomycetes have slow growth under these conditions [30]. According to [31], some isolates of *Rhizobium* showed absence or slight production of IAA at pH=5, however, an optimum of production is observed at pH=7, and the production is decreased at pH=9. Growth of *Streptomyces* sp. SF5 is reduced by 1.46 times, (pH=5) and 2.40 times (pH=9). Production of IAA is reduced by 1.87 times (pH=5) and 2.65 times (pH=9). Based on these results, it is important to note that there is a close relationship between growth and IAA production. The pH affects the function of enzyme systems as well as the solubility of many substances that are important for bacterial growth. [32] Reported that the synthesis of high levels of IAA was determined in cultures with alkaline pH (7.5). It was found that growth and production decreased in the sixth day. The production decrease is may be due to the release of degrading IAA enzymes, such as IAA oxidase and IAA peroxidase as has been shown in *Rhizobium* sp. isolated from *Cajanus cajan* [33].

Concerning temperature effect, it is noteworthy that a parallelism exists between growth and IAA production. It was observed that a temperature of 30°C was favourable for the two parameters studied. However, at 25°C there was no significant difference in growth of *Streptomyces* compared to that observed at 30°C. Growth of *Streptomyces* sp. SF5 and IAA production are decreased by 1.49 times and 2.08 times at 37°C respectively. [34] Have found that a temperature ranging from 25°C and 30°C was favourable for growth and production. *Streptomyces* CMU H009 produces a maximum of IAA when it grown at a temperature of 30°C [27]. *Streptomyces albidoflavus* showed a maximum of IAA production at 35°C and pH=7 [26]. [8] reported that some species of *Streptomyces* such as *S. purpurascens*, *S. coelicolor*, *S. olivaceus* and *S. kasugaensis* produce IAA with levels of 28.4, 21.8, 14.2 and 51.5µg/ml respectively under conditions of pH=7 and temperature of 35°C. In *Aspergillus niger* for example, a temperature of 25°C at pH=6 was optimal for AIA production. Furthermore, gibberellic acid was produced under conditions of pH = 5 and temperature of 30°C [35].

Addition of NaCl at different concentrations showed that growth of *Streptomyces* sp. SF5 was inhibited in the presence of increasing concentrations of NaCl, mainly at 500mM where its growth is reduced by factors of 1.77 (500mM) and 1.17 (300mM). According to [23], *Streptomyces* sp.MADO2 appeared more sensitive to salt stress mainly at 500mM compared to *Nocardiopsis* sp. MADO3 which showed an optimum growth at the same concentration.

On the other hand, IAA production is reduced by 1.84 times, 1.74 times and 2.20 times respectively at 200, 300 and 500mM. There is little information on the effect of NaCl on the production of the AIA, those that exist in literature study the effects of pH, temperature, concentrations of L-tryptophan, the carbon and nitrogen sources...Salinity effect is very important to study mainly in saline soils where different cellular processes are inhibited by excessive NaCl concentrations such as growth, metabolism, nutrients uptake and photosynthesis [36]. Our results have showed the negative effect of NaCl on bacterial growth and IAA production and this

can affect and limit without doubt plant productivity.

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