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Isolation of actinomycetes from different soils of Beni Amir Morocco

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Abstract

Actinomycetes strains were isolated for the first time from 11 soil samples from the Beni Amir region of Morocco. A physicochemical characterization of the different soils studied shows that each sample represents a particular ecosystem. Isolation was performed using two culture media Bennett and minimal synthetic media (MMS). Number of bacteria varies from 0.03 × 10^8 UFC/g to 146 × 10^8 UFC/g, from 0.36 × 10^5 UFC/g to 209 × 10^5 UFC/g for the actinomycetes and from 0.51 × 10^5 UFC/g to 2310.33 × 10^5 UFC/g for mushrooms. Thus, a percentage of actinomycetes relative to the total microflora is maximum on the S4 site (20.31%) and minimum (0.001%) on the S11 site. It seems that the distribution of actinomycetes in the studied soils is influenced by the physicochemical factors prevailing there. Indeed, the percentage of actinomycetes shows a positive correlation with organic matter and total organic carbon and negative correlation with other parameters such as pH, moisture, potassium and phosphorus.

1. Introduction

Actinomycetes are aerobic Gram-positive bacteria [1-2-3], with a load factor generally greater than 55%, between 60 and 75% [4-5 -6-7]. They usually grow by filament formation [8]. Past experience proves that actinomycetes are the richest source of secondary metabolites [9]. They are able to produce rare secondary metabolites, which are important not only for the pharmaceutical industry, but also for agriculture. They are ubiquitous bacteria in almost all environments, even those where life is extremely hostile [10-11]. A number of them are able to grow in salt-rich habitats, and so they are halophilic or halotolerant [12].

It has been shown that the presence, distribution and diversity of actinomycetes are associated with their different ecological habitats (marine, soil, etc.). They are universally widespread and constitute an important part of the soil microflora [13-14]. In particular, ecological studies of actinomycetes have been widely conducted in terrestrial environments [15], a component of 10% to 50% of the soil microflora community over a large part of the soil condition [16]. The number and types of actinomycetes present in a particular soil would be greatly influenced by geographic location, and physicochemical conditions such as temperature, type, pH, salinity, organic matter, cultivation, aeration and moisture content. That's why, the ecological study of the environmental habitats of actinomycetes is important for discovery of actinomycetes, that produce new useful bioactive substances.

In view of the absence of microbiological studies in the Beni Amir region, our laboratory is interested in this region, which is characterized by soils with high salinity levels, thus the probability of isolating halophile halotolerant strains. Our study is following a work that we published on the actinomycetes in 2016 [17] .This led us to propose a study on:

-The determination of physico-chemicals parameters of different soils.

-The microbiological analysis of different soil, by the enumeration of three groups (bacteria, actinomycetes and Fungi".
-The effect of soil physicochemical characteristics on the distribution of actinomycetes.

2. Materials and methods

2.1. Sampling

11 samples were collected according to the technique of Pochon Tardieux [18], from the perimeter Tadla , Beni Amir region (figure1). Using a large sterile spatula, the first five centimeters of the surface layer of the soil are removed, then with a small sterile spatula in the layer subjacent (between 5 and 15 cm of depth) 100–150 g of soil were placed in sterile polyethylene bags, closed tightly and transported to the laboratory [19].

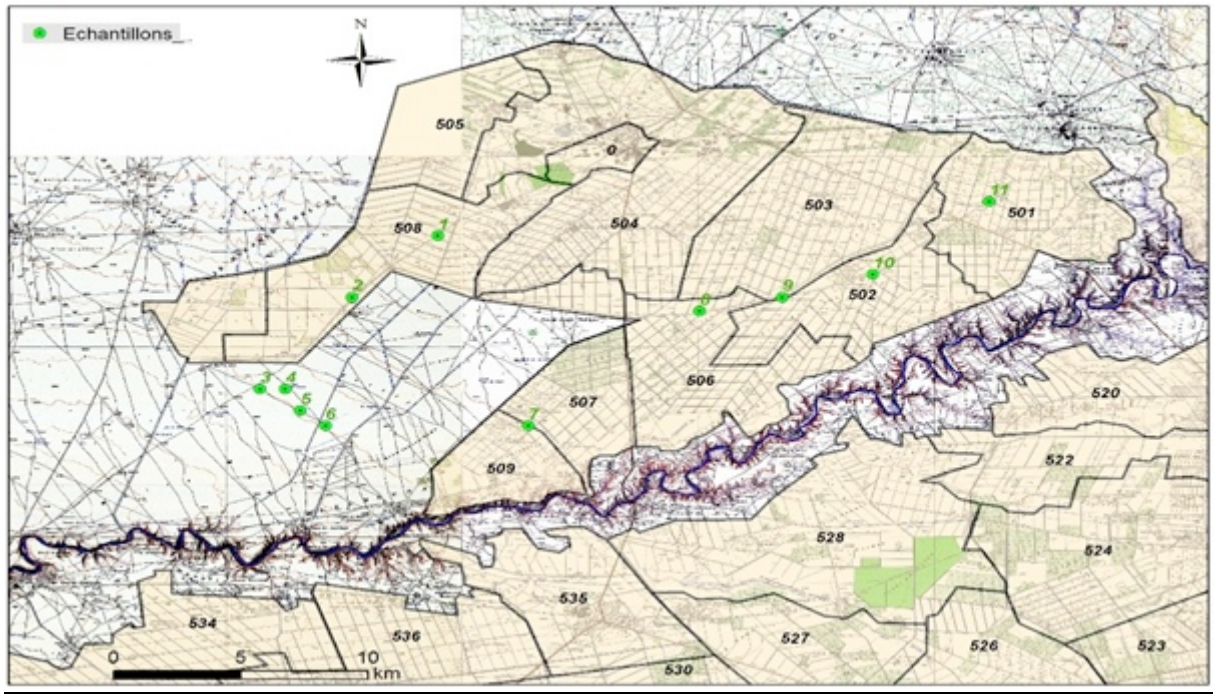

Figure 1: Map shows the areas of sampling in the region Beni Amir Tadla-Azilal

2.2. Soil chemical analysis

The pH was measured using a pH meter according to the technique Pochon Tardieux [18]. Electrical conductivity (EC) was determined, using a saturated paste extract [20]. Total organic C in soil was measured by dichromate potassium [21]. The moisture content was measured, as the subsequent loss of weight to a constant weight, after drying 10 g of air-dried soil sample overnight at 105°C. After measurement of moisture, the soil sample is ashed at 450 ° C for 16 hours; the organic matter is the difference between dry weight and ash weight [22]. The available phosphorus by Bray [23]. The available potassium, in the soil samples, was extracted using neutral normal ammonium acetate as extractant, and determined using flame photometer [24].

2.3. Isolation and culture conditions

Two gram (wet weight) of each soil sample were resuspended in 18 ml of sterile physiological serum (9 g/l NaCl), stirred for five minutes at Vortex.0.1 ml of each dilution is inoculated to the surface of petri dishes containing one of the two culture media Bennett (anhydrous D-glucose: 10g casamino acid: 2g, extractYeast: 2g, meat extract 1g, agar: 20g, distilled water 1000 ml),synthetic minimum medium (SMM) containing 10 g /l glucose, 2 g /l $NaNO_3$,0.5 g /l $MgSO_4\_7H_2O$, 0.5 g /l KCl, 0.01 g/l $FeSO_4\_7H_2O$ and RPK (0.5 g/l, containing approximately 2.2 mM phosphorus). The pH was adjusted to 7 and the medium was sterilized at 121 °C for 20 min. This medium was supplemented with 40 µg/ml actidione and 10 µg/ml nalidixic acid, growth inhibitors of fungi and Gram negative bacteria, respectively. After plating,the agar plates were incubated for 21 days at 28 °C in order to allow growth of the slow growing actinomycetes. Actinomycetes were recognized on the basis of

morphological features following the International Streptomyces Project (ISP) [25]. The observed colonies were isolated, purified and conserved in 20 % Glycerol at -20 °C.

2.4. Enumeration

Microbial flora was enumerated by the conventional method of suspension dilution using sterile petri dishes. Three repetitions are performed by dilution. The incubation temperature is 28 ° C. The culture media used are: synthetic minimum medium (SMM) containing 10 g/l glucose, 2 g/l $NaNO_3$, 0.5 g/l $MgSO_4\_7H_2O$, 0.5 g/l KCl, 0.01 g/l $FeSO_4\_7H_2O$, 0.5 g/l K_2HPO_4 supplemented with 40 µg/l actidione for the enumeration of actinomycetes and nutrient agar containing 50 µg/l cycloheximide for numeration bacteria and Sabouraud agar supplemented with 50 µg/l of chloramphénicol for counting fungi.

2.5. Purification of actinomycetes

The isolated actinomycete strains were purified by successive subcultures on Bennet medium in the presence of antifungal and stored at 4 °C on inclined agar plates containing the same medium and at 20 °C in suspension in the presence of 20% glycerol (V/V).

2.6. Statistical Analysis

Correlation between Actinomycetes load and soil physicochemical characteristics were carried out using SPSS software.

3. Results and discussion

Ecological study of the environmental habitats of the actinomycetes is important. It is for that, we have found it very useful to determine the physicochemical characteristics of the chosen soils. This study told us to have the results presented in the table 1.

It is interesting to note that the samples represent different physicochemical characters.

- PH:

Soil S3 (7.4) is neutral, while samples S4 (7.91), S5 (7.73) S6 (7.83), S8 (7.7), S9 (7.61), S10 (7.93), S11 (7.75) are soils that tend towards alkalinity, samples S1, S2, S7 whose pH respectively are 8.1, 8.09, 8.1 are slightly alkaline soils.

- Moisture:

Humidity level for different samples ranges from 1.5% to 8% as indicated in the Table 1.

According to lee and Hwang (2002), moisture content is considered low when the moisture percentage is between (2% and 9%) [26]. Then, we can conclude that studied soils have low humidity.

- Organic matter:

Rate of organic matter in the soil is considered low (4% to7%), moderate (7.1% to 9%) and high (9.1% to11%) to refer at lee and Hwang 2002 [26].

Results presented in table1 shows that S7and S10 have low levels of organic matter respectively 4.8% and 4.2%; soil S8 have a moderate rate with a percentage of 7.9%; unlike S1 samples (10.1%), S2 (10.7%), S9 (9.4%) have a high rate ; soils S3 (64.7%), S4(20.1%),S5 (13.2%); S6 (50.3%) have a very high rate; while a very low percentage of soil organic matter is located in sample S11 (2.1%).

- Electrical conductivity:

Electrical conductivity is between 1.29 dS /m and 9.05 dS /m (Table 1). we could classify soil S4 as extremely salty soil (9.05dS/m); soils S1(2.511dS/m),S6 (4.38dS/m), S7(2.529dS/m), S10(2.575dS/m) and S11(2.731dS/m) as very salty soils; the other soils S2 (1.964 dS/m), S3 (1.29 dS/m), S5 (1.625 dS/m), S8 (1.52 dS/m) and S9 (2.23 dS/m) are considered salty soils.

-Mineral elements:

Three elements were the object of this study:

Total organic carbon, whose percentage is minimal in soil S4 (1.21%) and maximum in soil S3 (37.52%) (Table1).

Available phosphorus is between 0.132 in soil S9 and 1.005 in soil S1 (Table1).whereas Potassium ranges from 10.3 in soils S1; S2; S3; S4; S5; S6; S7; S8; S11 to 87.2 in soil s9 (Table1).

Table 1: Physicochemical parameters of the eleven Beni Amir soil samples

Samples	Physicochemical parameters						
	PH	**Moisture**	**%MO**	**Electrical conductivity: (dS/m)**	**% COT**	**Phosphore ppm**	**Potassium ppm**
S1	8.1	4	10.1	2.511	5.85	1.005	10.3
S2	8.09	4	10.7	1.964	6.2	0.216	10.3
S3	7.4	3.5	64.7	1.29	37.52	0.188	10.3
S4	7.91	8	20.1	9.05	1.21	0.394	10.3
S5	7.73	3	13.2	1.625	7.65	0.977	10.3
S6	7.83	3.5	50.3	4.38	29.17	0.195	10.3
S7	8.1	3	4.8	2.529	2.78	0.817	10.3
S8	7.7	4	7.9	1.52	4.58	0.189	10.3
S9	7.61	5.5	9.4	2.23	5.45	0.132	87.2
S10	7.93	2.5	4.2	2.575	2.43	1.31	39.4
S11	7.75	1.5	2.1	2.731	4.29	0.215	10.3

%MO: Percentage of organic matter, dS deciSiemens (Unit), % COT: total organic carbon

Table 2: Quantitative distribution, bacteria, actinomycetes and fungi in soil of Beni Amir

Samples	Microorganismes			
	Bacteria UFC/g 10^8	**Actinomycetes UFC /g 10^5**	**Fungi UFC/g 10^5**	**%Actinomycetes**
S1	36.20±0.721	117.67±2.516	0.53±0.030	0.32
S2	32.73±2.000	9.97±3.605	600.00±10.000	0.03
S3	146.00±1.250	209.00±0.251	2310.33± 32.145	0.14
S4	0.03±0.003	10.30±0.608	10.40±0.529	20.31
S5	1.44±0.116	60.67±2.081	10.10±0.360	4.01
S6	4.73±0.416	4.00±0.025	0.51±0.020	0.08
S7	1.22±0.0251	1.01±0.010	300.00±20.000	0.06
S8	1.34±0.055	34.67±1.527	100.00±3.000	2.35
S9	1.70±0.030	20.07±0.057	1.07±0.061	1.16
S10	4.27±0.0642	0.36±0.020	91.33±1.527	0.008
S11	36.00±2.645	0.41±0.040	130.67±4.041	0.001

Microbiological studies of Beni Amir soils have been carried out, namely the enumeration of microorganisms. This enumeration concerned 3 types of microorganisms: actinomycetes, other bacteria cultivable under conditions described previously and mushrooms (Table 2).

Results show that number of actinomycetes varies from 0.36×10^5 UFC/g in soil S10 to 209×10^5 UFC/g in soil S3, from 0.03×10^8 UFC/g in soil S4 to 146×10^8 UFC/g in soil S3 for the bacteria and from 0, 51×10^5 UFC/g in soil S6 to 2310.33×10^5 UFC/g in soil S3 for fungi.

Thereby, percentage of actinomycetes, relative to the total microflora, range from 0.001% in soil S11 to 20.31% in soil S4.

In order to keep actinomycetes isolated from these soils for further investigations, we have tried to purify isolates. As well as, 80 different colonies of actinomycetes were isolated and purified. The share of each soil is presented in (Table 3).

These isolates are morphologically identical, that is to say that the colonies are opaque, of rounded shapes, with irregular edges and embedded in the culture medium. But what differentiates the strains is the color of the aerial mycelium, that of the vegetative mycelium, their sizes and the production of pigments.

Table 3: Number of purified actinomycete strains isolated from soil samples.

Samples	Number of actinmycets strains isolated from soils samples
S1	11
S2	2
S3	1
S4	18
S5	6
S6	24
S7	1
S8	3
S9	3
S10	5
S11	6

According to the results presented in Table 2,the percentage of actinomycetes, relative to the total microflora, range from 0.001% in soil S11 to 20.31% in soil S4 (Figure 2).

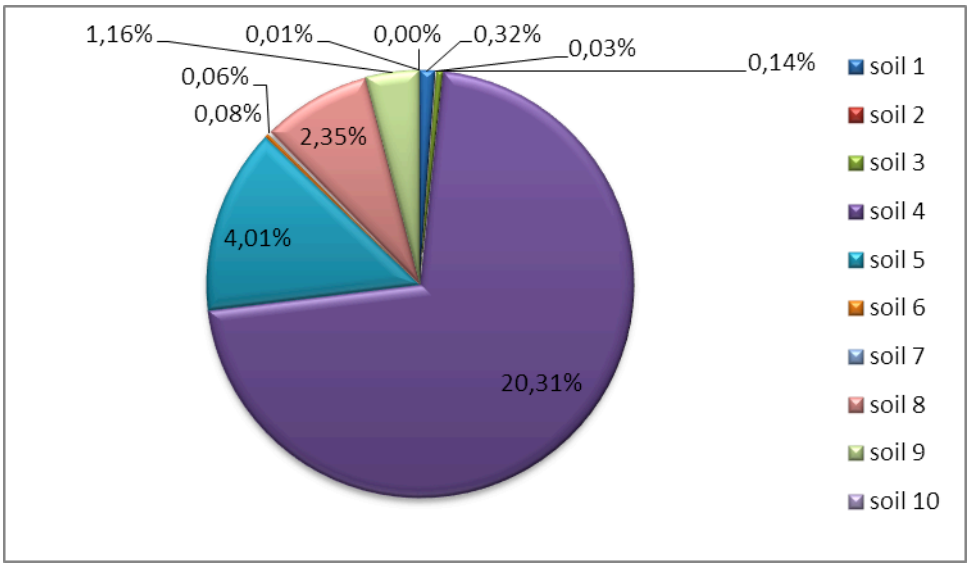

Figure 2: Percentage of Actinomycetes isolated in each soil sample

These results show a significant variation in the number of actinomycetales strains isolated from each sample. It also appears that the number of actinomycetes isolated from the S4 soil is greater than that obtained from the other samples. The maximum percentage was recorded in the S4 (20.31%) soil followed by soils S5 (4.01%), S8 (2.35%), S9 (1.16%) while the other soils such as S1 (0.32), S3 (0.14), S6 (0.08%), S7 (0.06%), S2 (0.03%), S10 (0.008%), S11 (0.001%) recorded a small percentage respectively (Figure 2).

To understand the influence of ecological conditions on the distribution of actinomycetes in different environments, we carried out a statistical analysis which allows to determine the correlation of each physicochemical parameters of the studied soils and the percentage of actinomycetes isolated from each soils. Results are given in table 4.

This results showed that the actinomycete load and soil moisture were negatively correlated, confirming that actinomycetes prefer dry soils than wet soils [27-28]. The pH is alkaline, the correlation between the pH and the charge of the actinomycetes is negative. Basilio *et al*., (2003) [29] showed that the number of actinomycetes obtained in the range pH 7 and 11. The organic matter and the organic carbon have a positive correlation [30]. Potassium and phosphorus have a negative correlation with actinomycetes. In this study, the organic matter was found to have a significant influence on the biological and physical properties of the soil.

The heterogeneous distribution of microorganisms in surface soils can be explained by the difference in distribution of organic matter. Organic matter content is an important indicator of soil quality degradation through its contribution to soil stability, increased soil water retention capacity, fixation of mineral elements, and substrate for the microorganisms of the soil [31]. (Henis, 1986) showed that actinomycetes generally increased in number increase in soil organic matter content [32]. Also Hayakawa *et al*., (1988) demonstrated that actinomycetes were more abundant in the soil content of matter [33]. In our studies too, actinomycetes were found in soils containing quantities of matter. Also pH can be an element influencing the distribution of soil actinomycetes. According to our results, the actinomycetes are distributed in soils with pH varies from 7.4 to 8.1. These results were similar to those of Hayakawa *et al*., (1988) [33] which showed that Streptomyces and Micromonospora were abundant in soils with pH values ranging from 6.5 to 8.0. In this study, all actinomycetes were found in soils with low moisture ranging from 1.5% to 8%. These results are not in agreement with those resented by Hayakawa *et al*., (1988) [33]. Also it is demonstrated that actinomycetes were distributed in vegetative soils with a water content of 6.2 and 7.6%, indicating that moderate moisture is favorable for actinomycete colonization. Therefore, soil pH, moisture content and organic matter content may be important factors influencing the ecology and diversity of actinomycetes.

Table 4: Effect of soil physicochemical characteristics on the distribution of actinomycetes

	pH	Moisture	MO	Electrical conductivity:	COT	Phosphore	Potassium	Actinomycetes
pH	1							
Moisture	0 .023	1						
MO	-0.561	-0.150	1					
Conductivité	0.240	0.705*	-0.172	1				
COT	-0.561	-0.150	1**	-0.172	1			
Phosphore	0.473	-0.267	-0.372	-0.086	-0.372	1		
Potassium	-0.266	0.211	-0.189	-0.126	-0.189	-0.059	1	
Actinomycetes	-0.480	-0.031	0.624	-0.336	0.624*	-0.020	-0.188	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Conclusion

This study allowed us to have interesting data on the distribution of actinomycetes in soils of the Beni Amir region. It has been shown that the distribution of these bacteria varies from one soil to another, the diversity and richness of actinomycetes being associated with the environmental factors of the soil, namely organic matter, humidity, pH, salinity.

From the results found the maximum percentage of actinomycetes 20.31% was also recorded in the S4 site, while the minimum percentage 0.001% was recorded in site S11 and the remaining percentage contributed by the rest of the sites. This can be explained by the fact that on the one hand the sample S4 is richer in organic matter than the sample S11, although the electrical conductivity of S4 is greater than that of the preceding sample. Also for the pH the results showed that the 11 soil samples have a different pH between neutral and alkaline. The moisture content for all soils is considered low.

Therefore, further studies are needed to know a physicochemical characterization of the surface of these bacteria also perform molecular identification up to species level.

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