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Isolation, identification and characterization of soil *Actinobacteria* from Jazan, Saudi Arabia

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ABSTRACT

Actinobacteria are a gram-positive, filamentous subgroup of bacteria most known for antibiotic production. The exploration of new habitats with unusual environment and poorly explored areas of the world has become important and useful to the discovery of novel compounds and actinobacteria. In this study, we have isolated actinobacteria species from 5 different soil samples from different locations (Sabya, Baish Samtah, Alddarb and Abu Arish) in Jazan, Saudi Arabia. The soils were characterized for physicochemical analysis. The comparisons of the actinobacteria abundance with the physicochemical characteristics of these soils were also performed. There were no differences in the soil textures (sandy loam) and organic matter values from all the regions. In general, the physical and chemical characteristics for all the soils analyzed were similar. A total of 270 actinobacteria strains were isolated. All purified isolates grew on yeast starch agar media showing morphology of typical *Streptomyces*. Total bacterial counts were compared between the five collection sites of soil samples. Alddarb exhibited higher microbial counts (10.48 log CFU/g) compared to Baish and Samtah with a minimum of (10.31 log CFU/g). 6 isolates designated: JS3, JS4, JS6, JD7, JA8 and JA10, were selected with effective in terms of colony color and morphology and were subject to cultural, morphological, physiological and biochemical analysis. The present data suggest that the number and diversity of actinobacteria in desertic soils represent a vast unexplored resource for the biotechnology of bioactives production.

Keywords: *Actinobacteria, Jazan, Scan Electron Microscope, Physiological analysis, Biochemical analysis.*

1. INTRODUCTION

Actinomycetes including its physiologically related *Streptomycetes* are ubiquitous Gram-positive filamentous soil bacteria that undergo unique morphological differentiation (Chater, 1993; Paradkar et al., 2003). Actinomycetes were originally thought of as bacteria in the guise of fungi or vice versa. It was indeed a challenge to classify them. Some scientists considered them as Eubacteriales or higher bacteria, while some thought them to belong to Hypomycetes or lower fungi. After much speculation and the amassing of copious volumes of data, currently, Actinomycetes are classified as Actinobacteria. Actinobacteria are one of the most attractive families of industrial bacteria on account of their superior potential for producing valuable secondary metabolites including antibiotics, anti-cancer drugs, immunosuppressors, and enzyme inhibitors (Petkovic et al., 2000; Weber et al., 2003). Almost 80% of the world's antibiotics are known to come from Actinobacteria, mostly from the genera *Streptomyces* and *Micromonospora* (Pandey et al., 2004). They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds (Narendra et al., 2010). Actinobacteria can be isolated from soil and marine sediments. In nature, Actinobacteria play an important role in the cycling of organic compounds and have been associated with soil organic matter production, including production of the black pigments called melanin, which are related to soil humic acid (Pandey et al., 2004). Although soils have been screened by the pharmaceutical industry for about 50 years, only a small fraction of the surface of the globe has been sampled, and only a small fraction of Actinobacteria taxa has been discovered (Baltz 2005, 2007). Therefore, the exploration of new habitats with unusual environment and poorly explored areas of the world has become important and useful to the discovery of novel compounds and actinobacteria (Learn-Han et al., 2012).

Jazan region, located in the south west part of Saudi Arabia (E: 42.0°-43.8° and N: 16.5°-17.0°) have an enormous biodiversity potential. It`s area is 13.500 km^2 (Chapman, 1978). The landforms, developed in Jazan region, are mainly of alluvial nature, formed as a result of the downward transportation of soil material from the highlands by the many valleys and drainage channels that drain out in the sea (Al-Farraj, 2008). Jazan soils have been described as habitats with high biological activity but have not been extensively explored for the search and discovery of novel actinobacteria spp. In this study, isolation of actinobacteria species from 5 distinct regions in Jazan soils: Sabya, Baish, Samtah, Alddarb and Abu Arish and comparisons of the actinobacteria abundance with the physicochemical characteristics of these soils were performed. Some of the isolates that showed promise for use in biotechnology were identified and characterized.

2. MATERIALS AND METHODS

2.1. Soil samples collection

Soil samples were collected from 5 different locations of Jazan region (Sabya, Baish, Samtah, Alddarb and Abu Arish), georeferenced in Table 1 and Fig. 1. The samples were obtained from different depth from the surface of the earth range of 10-15 cm after removing approximately 3 cm of the soil surface. Sampling was carried using sterile tools in clean sterile plastic bags and labeled with date of collection. All the soil samples were air-dried for 1 week at room temperature 25-30°C on filter paper. Then all the dried soils were crushed and sieved to remove gravel and debris, then stored in a plastic bag in room temperature until use (Kumar et al., 2010).

2.2. Physicochemical analysis of soils

Approximately 200 g of each soil sample was subjected to physicochemical analysis using the procedure of the Embrapa (1997). The concentrations of nitrogen (N), potassium (K), phosphorus (P), calcium carbonate ($CaCO_3$), calcium bicarbonate (HCO_3), organic matter (OM), sulphates (SO_4), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), chloride (Cl), total dissolved solids (T.D.S) and electrical conductivity as well as the pH and soil texture were evaluated. Correlations between the Jazan soil samples and the physicochemical soil variables were also done.

2.3. Isolation of Actinobacteria

Soil samples were suspended in sterile water by using standard microbiological method (serial dilution technique) (Kelley and Post, 1982). The suspension was serially diluted to final dilution of 10^{-4}. Aliquots (0.1 ml) of each dilution were spread on tap water agar medium (22g/L), (Winlab, UK). Plates were incubated at 30°C for 7 days. After incubation, the colonies were carefully counted by visual observation and C.F.U. per gram of soil was determined (Jeffrey, 2008). The observed actinobacteria were purified using yeast starch agar meduim (YSA) (2g yeast extract, 10g starch, 15g agar and 1L distilled water) and incubated at 30°C for 7 days (Ara et al., 2012). Mossel ecometric streaking method (Singh and Agrawal, 2003) was used until purity of soil actinobacteria. For long-term preservation, conidial or mycelial suspensions in 25% glycerol were kept at -80°C (Ozgur et al., 2008).

2.4. Characterization of Actinobacteria

2.4.1. Cultural characterization

The actinobacteria strains chosen for this study were characterized morphologically following the methods given in the International Streptomycetes Project (ISP) (Shirling and Gottlieb, 1966; Alharbi et al., 2012). The color of the substrate mycelia and those of the soluble pigment were determined according to ISCC-NBS color chart (Kenneth, 1958).

2.4.2. Morphological characterization

Morphological analysis done using light and scanning electron microscopy (SEM) (JEOL, JSM, 3060) according to the initial fixation and dehydration steps previously published (Moore et al., 1992). The strains chosen for this study were gram stained, shape and size were identified under light microscope. The mycelium structure and arrangement of spore on the mycelium were examined under the light and scanning electron microscope (SEM) (Tamura et al., 1994; Ara et al., 2012).

2.4.3. Physiological and biochemical characterization of actinobacteria

The physiological characteristics of the actinobacteria isolates chosen for this study such as, growth at different pH (4, 7, and 10), temperature (30, 45 and 50°C) and NaCl concentration (7 and 10 g/l) were recorded in YSA medium. The biochemical characterizations of the isolates were also studied by the procedures of ISP (Tresner et al., 1968): the liquefaction of gelatin, hydrolysis of casein, protolytic activity, catalase activity, Urea hydrolysis, lactose and glucose fermentation, H_2S production and citrate utilization.

2.5. Statistical analysis

Experiments were done in triplicate. LSD ALPHA (0.05) was used for statistical analysis using the One-way anova'test. $P<0.05$ was considered statistically significant. ANOVA analyses were done with IBM SPSS Statistics 21 software (Ara et al., 2004).

4. RESULT AND DISCUSSION

4.1. Physicochemical Characteristics of Jazan Soil Samples

The physical and chemical properties of Jazan regions soil samples are shown in Fig. 2. The pH values of the soils were similar and ranged from 7. 52 in Alddarb to 7. 96 in Baish and average of 7.74. These soil samples had moderate alkalinity. No differences in the soil textures (sandy loam) from all the regions were observed. Our results are similar to the values found in Jazan soils that have been reported by Al-Farraj (2008). The mean abundance of water soluble bases decreased in the order $Na^+ \geq Mg^{2+} \geq Ca^{2+} \geq K^+$. Water soluble anions were predominately Cl^- followed by HCO^{3-} then SO_4^{2-}. These results are not in line with the findings of Al-Farraj in 2008, who reported that the water soluble minerals were mainly Ca then Mg and Na salts, and for the anions, the Cl was predominant. The five soil samples analyzed revealed no significant differences in organic matter values. In general, the physical and chemical characteristics for all the soils analyzed were similar. The Jazan soils had moderate acidity, consistent with the values found in Jazan soils that have been reported by the study of Al-Farraj, 2008.

Microorganisms are the key drivers of biogeochemical processes in the soil. Thus, it is important to evaluate the physicochemical properties of the soil and how these properties could be related to microbial profiles in different soils (Peixoto et al., 2010). Seasonal variations in the moisture and pH of the soil can lead to

changes in the distribution patterns of the microbial species. For example, bacteria prefer neutral to alkaline conditions, whereas yeasts and filamentous fungi prefer acidic conditions. Some microbial species also have preferences for soils with high or low moisture contents (Bresolin et al., 2010). Consequently, understanding the effects of environmental drivers on microbial communities is an important step toward developing an integrated understanding of biodiversity patterns in soil systems, particularly as we increasingly recognize the intimate connection between certain soil functions and the community composition of mediating microorganisms (Geyer et al., 2014).

4.2. Actinobacteria isolation

A total of 270 actinobacteria strains were isolated from Jazan soils using Tap water agar media. Total bacterial counts were compared between the five collection sites of soil samples (Fig. 3). Samples collected from decorations grass in the street (Alddarb) exhibited a little higher microbial counts (10.48 log CFU/g) compared to those collected from cultivated land for sugar-cane production (Baish and Samtah), with a minimum of (10.31 log CFU/g). In a previous study, the number of actinobacteria isolated from Brazilian cerrado soils has an inferior order of magnitude (8.8 log CFU/g) compared to the number isolated from soils of Jazan (Suela Silva, 2013). These findings suggest that Jazan soils represent a large, unexplored environment for the potential isolation of actinobacteria. Actinobacteria are dominant colonizers in soils. Many species produce extracellular enzymes for degradation of macromolecules such as lignin, cellulose, chitin, and, in part, starch. Therefore, actinobacteria often occur in materials where organic materials are degraded (Schafer et al., 2010). This may explain the high presence of actinobacteria in these soils of Jazan.

4.3. Actinobacteria Identification

All purified isolates grew on YSA media showing morphology of typical *Streptomyces*; the colonies were slow growing, aerobic, glabrous or chalky, folded, and with aerial and substrate mycelia of different colors. In addition, all colonies possessed an earthy odor. All of the strains were gram positive and fitted to the description of genus *Streptomyces* in Bergey's Manual of Systemic Bacteriology (Rahman et al., 2011). The isolates were categorized into eight color series according to their color of the mature sporulated substrate mycelium (Fig. 4). The white series isolates were more predominant (48.5% of the total isolates). Our result is the same found by Rizk et al. (2007) who reported that grey and white colour series of actinobacteria are the dominant forms in the soil, compared to yellow, red, violet and green ones. In nature, actinobacteria play an important role in the cycling of organic compounds and have also been associated with soil organic matter production, owing to their black pigments called melanins, which are related, in some respects, to soil humic acid (Schafer et al., 2010; Coelho and Drozdowicz, 1978). In the present work, the soils studied were characterized as being especially rich in the *Streptomyces* genus, as are other soils throughout the world. The *Streptomyces* genus has been the focus of research because of the commercial applicability of substances produced as well as the systematics of this group, which have been modified with advances in molecular biology (Souza et al., 2008).

4.4. Characterization of selected isolates

The systemic screening process resulted in isolation of six actinobacteria from 5 soil samples designated: JS3, JS4, JS6, JD7, JA8 and JA10. The strains grew well on YSA media and produced purple, white and gray colors of aerial mycelium and 4 strains produce soluble pigments in the media. Aerial mycelium is generally white in color with yellow color reverse pigment.

4.4.1. Culture characteristics

The isolates comprised Gram positive, aerobic organisms with branched hyphae. A non fragmenting substrate mycelium was formed. Morphological observations by light microscope indicated the presence of single or clustered spherical to irregular structures on the substrate mycelium. Observation by Scanning Electron Microscope (SEM) of isolates showed that all six strains appeared to be different from each other spores and mycelium wise; in all six strains the aerial mycelium seems to be branched but some strains branches more than others (Fig. 5). The strain JS3 mycelium appeared to be long chains with spores that are smooth and in tall rods chains, JS4 mycelium was long chains of warty spores, JS6 mycelium is highly branched with single smooth spore, JD7 mycelium seems to be branched with warty spores. For JA8, the mycelium is branched with straight chains of rod smooth spores and finally JA10 contains branched mycelium with smooth single spore. Morphological characteristics are considered important in identification of various genera of actinobacteria (Saxena et al., 2013). Our results confirmed that the 6 isolates belong to the genus *Streptomyces*.

4.4.2. Physiological and biochemical characteristics

Physiological characterization is one of the traditional methods used in classification of bacteria (Shirling and Gottlieb, 1966). The physiological properties of the 6 isolated strains can be summarized as follows. They grew well at 45-50°C except for the strains JS4 and JA10 which showed week growth at 50°C and strain JA10 which showed week growth at 45°C. The ability of the six strains to grow at various pH was also studied. They exhibited good growth at pH 7-10. The strains JS4, JD7 and JA8 showed luxuriant growth at pH 10 indicating their alkalophilic nature. The tolerance to NaCl was studied. Most of the strains have shown no sign of growth on 7% NaCl except two strains (JS4 and JD7) which have shown weak growth. All the six selected strains could not grow on 10% NaCl, (Table 3). Biochemical test results are as follows: catalase is produced for all the strains, except for JS4, urea and gelatin were not hydrolysed for the strains JS3, JA8 and JA10. All the strains were negative for citrate utilization. Production of H_2S was studied using tap water agar. Only JS6 and JA10 showed H_2S production (Table 4). Lactose and glucose were utilized as good carbon sources except for the strain JS3.

5. CONCLUSION

Microorganisms are the key drivers of biogeochemical processes in the soil. Thus, it is important to evaluate the physicochemical properties of the soil and how these properties could be related

to microbial profiles in different soils. The present data suggest that the number and diversity of actinobacteria in desertic soils which is the case of Saudi Arabia represent a vast unexplored resource for the biotechnology of bioactives production. Studies are currently being conducted to produce bioactive compounds from actinobacteria fermentations on different substrates.

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Tables

Table 1. Details of soil samples collected from different locations of Jazan, Saudi Arabia

Collection site	Date of collection	Color of soil sample	Details of geography of collection site
Sabya	25/8/2012	Brown	Wild plants on the hill
Baish	25/8/2012	Deep Bronze	Cultivated land for Sugar-cane production
Samtah	18/7/2012	Brown	Cultivated land for Sugar-cane production
Alddarb	25/8/2012	Espresso	Decorations grass in the street
Abu Arish	22/8/2012	Espresso	Wild plants in the dry valley

Table 2. Color grouping for selected isolates

Isolates	Color of aerial mycelia	Color of substrate mycelia	Diffusible pigment	Growth rate	Mycelium
JS3	purple	Purple	-	+++	-
JS4	White	Brown	+	+++	-
JS6	White	Yellow	+	+++	-
JD7	Gray	Dark brown	+	+++	+
JA8	Beige	Yellow	+	+++	+
JA10	Pale White	White	-	+++	-

Table 3: Physiological characterization of the isolates such as, growth at different pH (4, 7, and 10), temperature (30, 45 and 50°C) and NaCl concentration (7 and 10 g/l) were recorded in yeast starch medium

Isolates	Physiological characterization							
	Temperature			pH			Nacl (%)	
	30°C	45°C	50°C	4	7	10	7	10
JS3	+++	++	++	-	+++	+	-	-
JS4	+++	+++	+	-	+++	+++	+	-
JS6	+++	++	++	-	+++	+	-	-
JD7	+++	+++	+++	-	+++	+++	+	-
JA8	+++	+++	+++	-	+++	+++	-	-
JA10	+++	+	+	-	+++	++	-	-

Table 4. The biochemical characterizations of the isolates such as liquefaction of gelatin, hydrolysis of casein, protolytic activity, catalase activity, Urea hydrolysis, D-Lactose and glucose Citric acid test

Biochemical characterization	Isolates					
	JS3	JS4	JS6	JD7	JA8	JA10
Citric acid test	-	-	-	-	-	-
D-Lactose fermentation	-	+	+	+	+	+
Glucose fermentation	-	+	+	+	+	+
H_2S Producion	-	-	+	-	-	+
Urea hydrolysis	-	+	+	+	-	-
Catalase test	+++	-	+	+	+	+
Gelatin hydrolysis	-	-	+	+	-	-
Protolytic activity	+	-	-	+	+++	++

Figures

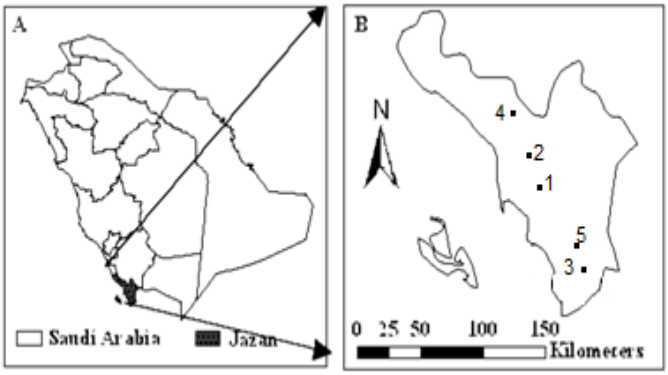

Fig. 1. (A) Map of Saudi Arabia, (B) Map of the study area (Jazan region) and the locations of collection sites of soil samples: (1) Sabya, (2) Baish, (3) Samtah, (4) Alddarb, (5) Abu Arish.

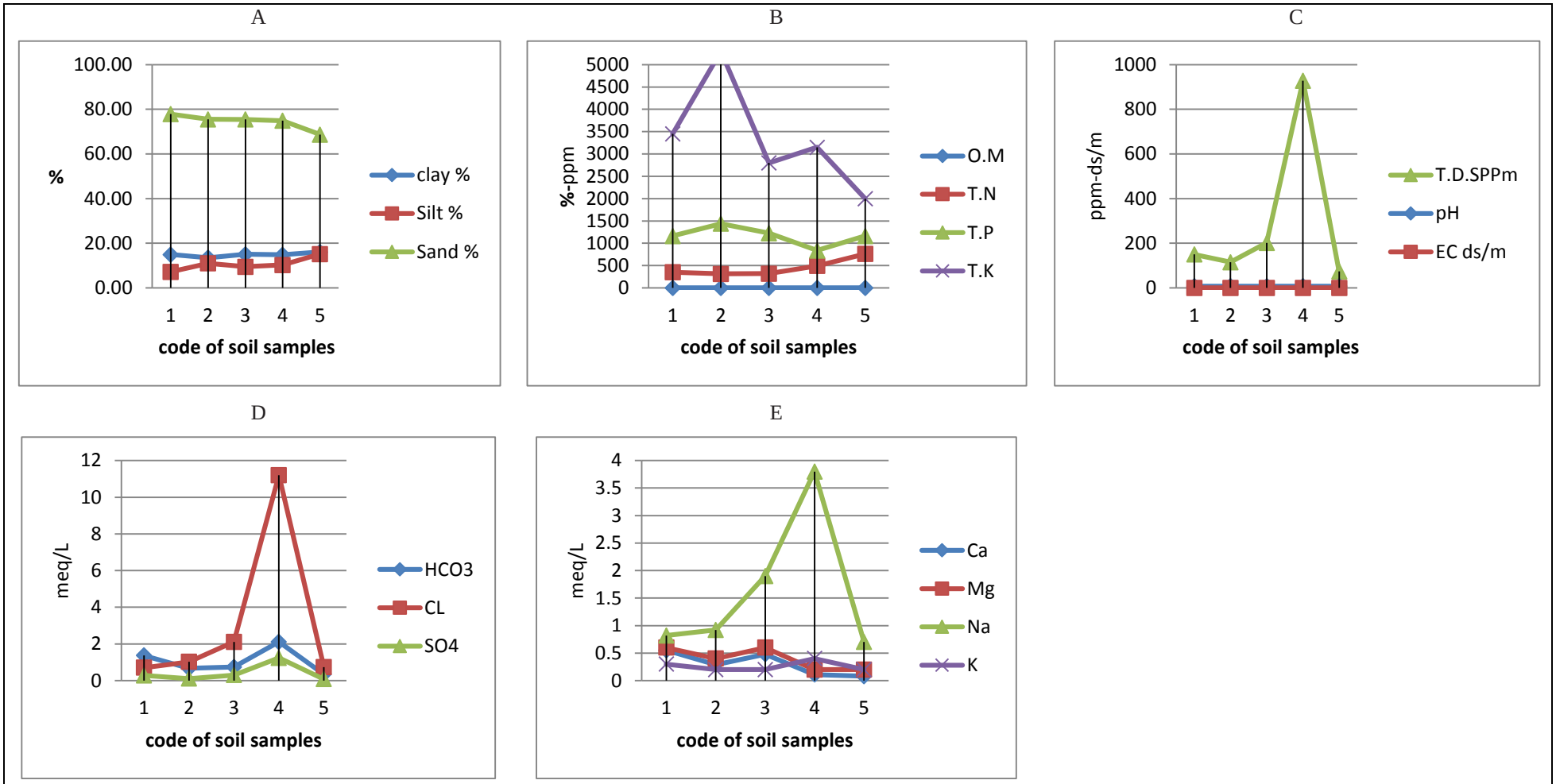

Fig. 2: Physicochemical properties of soil samples. (A): Particle size distribution and texture soil, (B): Determination of Total Soluble Salts, (C): Chemical analysis of soil samples, (D): Chemical analysis of soluble anions of soil samples, (E): Chemical analysis of soluble cations of soil samples.

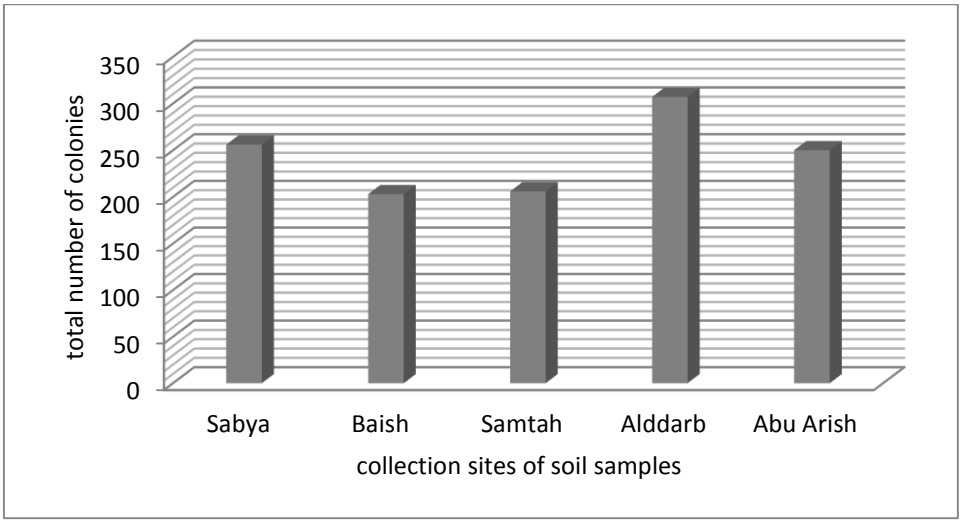

Fig. 3. Total number of colonies for all five soil samples

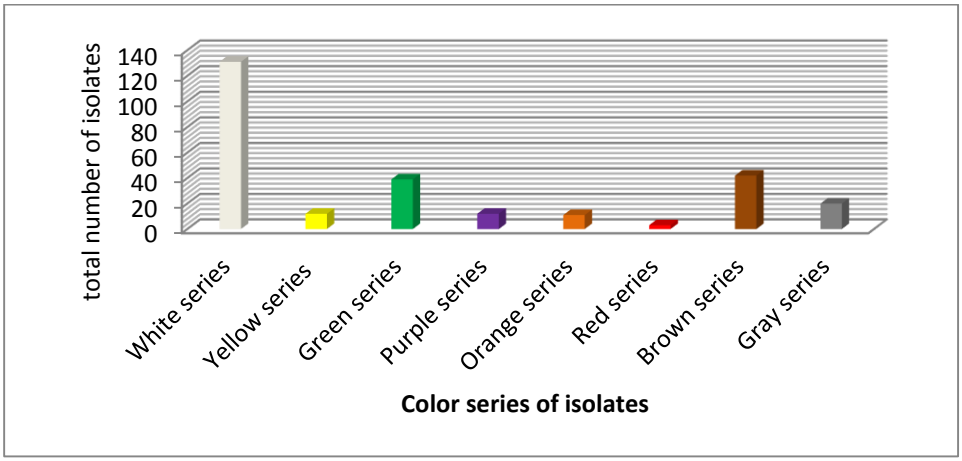

Fig. 4. Color grouping

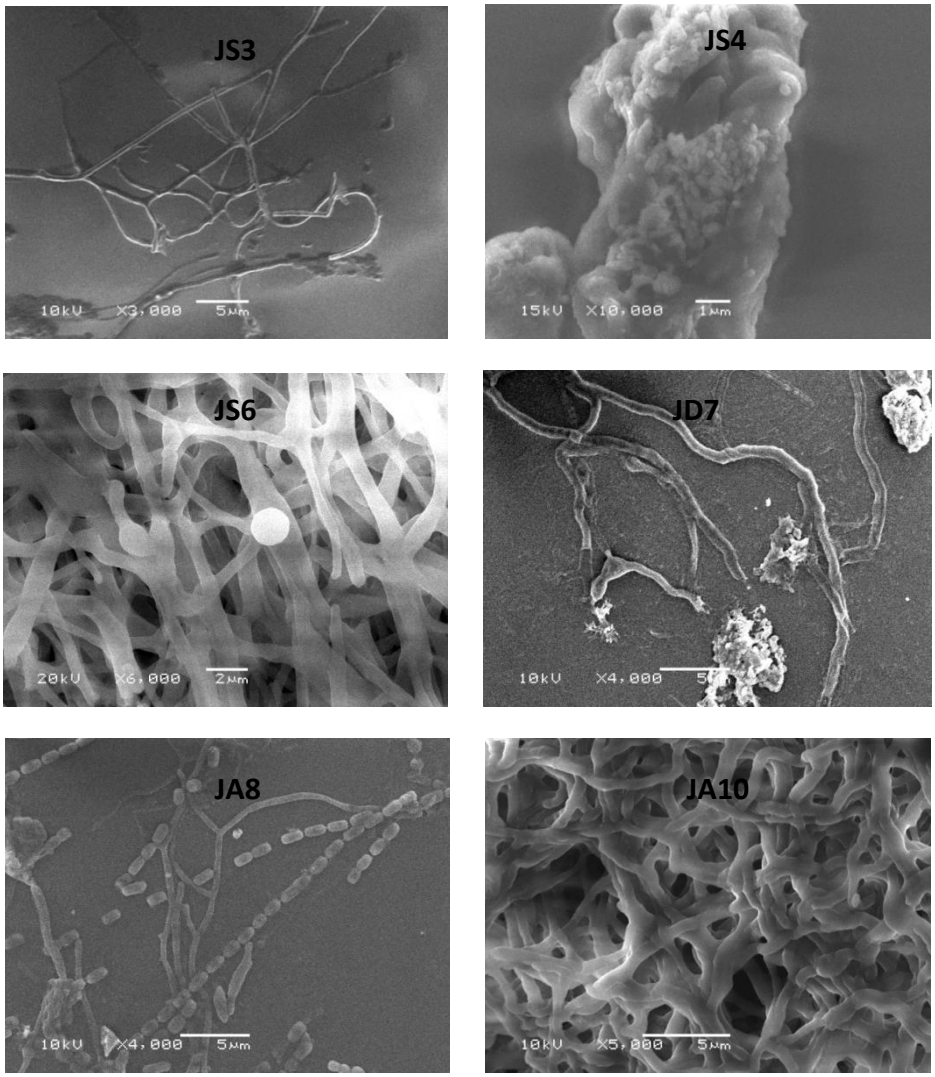

Fig. 5. Scanning Electron Microscope images for the 6 isolated actinobacteria strains.