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Alkaliphilic Actinomycetes
I.Isolation, Identification and Some Characterization

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ABSTRACT

Five strains of alkaliphilic actinomycetes were isolated from Al-Fayoum region soil, Egypt. Four strains A3, A6, A8, and A10 were classified as genus *Kitasatospora* on the basis of morphological and chemotaxonomic characteristics. These strains produced aerial mycelium consisting of chains of 20 or more smooth-surface spores. Whole-cell hydrolysates contained both LL- and meso-DAP (diaminopimilic) acid and galactose. Alkaline media were favored for growth, production of aerial mycelium and soluble yellow-brownish pigments. All these strains may be considered as new species for the genus *Kitasatospora*. The fifth strains contained neither meso-nor-LL-DAP (diaminopimilic acid) also it didn't contain any diagnostic sugar in the whole-cell hydrolysate. The colonial growth of strain A11 was beige beaver yellow-brownish. It may be considered as a new genus. All isolated strains had a temperature range of 25-40°C and pH range (7-12). Lecithinase; protease and lipase enzymes were secreted by all strains on neutral and alkaline egg emulsion agar medium. All strains reduced nitrate degraded gelatin, casein, skimmed milk, starch, tween 60, DNA, Urea, L-tyrosine and hypoxanthine.

INTRODUCTION

Most of the microorganisms live in moderate environments of temperature, hydrogen ion, and salinity, while other organisms live in strange difficult or exotic environments and we cannot imagine that there is life in them but other creatures die in them. These environments are called extreme environments or exotic environments and organisms that live in it are called extremophiles (Krulwish and Guffanti, 1989). These extremophiles microorganisms are called extremist or extremist-loving because it from the human viewpoint can live where other creatures - including the man himself - cannot live or imagine that there is life in these environments. The living in these places is considered a punishment from the Adamian viewpoint (Madigan and Marrs, 1997). Among these organisms are those who can live in more than one extreme environment, biotopes, some of which live for example in very high temperatures and intense salinity thermohalophiles, or intense salinity and excessive alkalinity haloalkalophiles.

Many of these organisms as fungi and yeasts have been isolated and identified (Horikoshi and Grant, 1998). Cellular contents such as DNA and RNA and cell membranes are affected by living in these extreme environments (Rothschild and Mancinelli, 2001). Halophiles or salt lover organisms, some of which live in salt crystals and grow and activate in seas, oceans, and highly salty lakes and pools of water that dried in the sun and salty soil, and some of these environments may also be alkaline due to the presence of a percentage of sodium carbonate in them. Organisms that live in these environments are called haloalkaliphilic microorganisms such as *Natronobacterium magadii*, which live in a salinity ratio of 20% H8.5-12, which adapts to both high salinity and excess alkalinity. It is a salt-loving bacteria *Halobacterium salinarum*and lives in a salinity of 25% g (w / volume), and works to accumulate a high percentage of potassium chloride inside it while the proteins in the cell structure relate to a high percentage of sodium chloride salt with the outer circumference and thus creates the required balance Adapt to High Salinity Outside (Madigan and Marrs, 1997).

Alkaliphilic organisms live in soils with a high content of carbonates as in the soda lakes of Egypt and the Rift Valley in Africa and the western United States (Madigan and Marrs, 1997). Many of the diverse alkaliphiles that display novel physiological features and potential applications were isolated from soda lakes found in various continents and settings (Borkar, 2015). Their properties, in addition to alkaliphily, cover wide tolerance ranges for salinity, temperature, and/or hydrogen levels. Soda lake alkaliphiles also participate in biogeochemical redox cycles of carbon, sulfur, and nitrogen (Sorokin *et al*., 2008, 2011, 2014). Many researchers isolated many alkaliphilic bacteria (Grant *et al.*, 1979; Horikoshi and Akiba, 1982). The first research since about seventy years ago (Downie and Cruickshank 1928) and isolated *streptococcus faecalis* strain resistant to acids and alkalines. Several species have been isolated from true alkaliphilic bacteria such as the *Bacillus alcalophilus, Bacillus sp*. was isolated from alkaline and acidic soils by (Ando *et al.*, 1981); *Bacillus* strain A007 (Vedder, 1934) and WN 13 (Weisser and Truper, 1985); from every acidic springs of *Clostridium sp*. (Souza *et al.*, 1974); from many environments, species such as *Exiguobacterin sp*. (Collins *et al.*, 1983); *Streptococcus sp*. (Graham and Lund, 1983); and *Corynform* bacteria (Nagata, 1988) have been isolated. Studies on alkaliphilic fungi have shown that most fungi isolated from the genera were from the genera of *Fusarium sp., Aspergillus sp.* (Khodair *et al.*, 1991) and then ranked *Cephalosporium* fungus (Kang *et al.*, 1996) as well as *Acrymonium sp.* and fungi such as *Gliocladium cibotii*; *Phialophora geniculata*; *Stachylidium bicolor* and *Stachylidium bicolor annulata*. The *Acrymonium* genus was the fastest growing and predominant on alkaliphilic soil fungi (Nagai *et al.*, 1995). Salama *et al.* (1993) found that the genus Fusarium was the highest fungus in soil followed by the genus *Acremonium* and genus *Aspergillus*.

Actinomycetes are the dominant group of soil populations together with bacteria and fungi. They are Gram-positive bacteria having high G+C (>55%) content in their DNA and they are originally considered as an intermediate group between bacteria and fungi (Naikpatil and Rathod, 2011); filamentous nature found in most environments including terrestrial and aquatic habitats (Strzelczyk *et al.*, 1969). Microbes from extreme environments have attracted considerable attention in recent years. This is primarily due to the secret that they hold about the molecular evolution of life and stability of the macromolecules. The majority of the studies on extremophilic organisms, however, have been confined to extremophilic bacteria and actinomycetes are relatively less explored group (Vasavada *et al.*, 2006). Actinomycetes are an important class of microbial resources, they are important producers of antibiotics and other

important bioactive substances. So far, about two-thirds of the world's antibiotics were secreted by actinomycetes (Liu Zh, 2002). Actinomycetes term are now called for many bacteria. Some scientists include organisms such as *Micrococcus and Cellulomonas, Arthrobactes*. Some species of actinomycetes live anaerobic (e.g. Actinomyces) and some require specific environments and special conditions for growth (e.g. *Frnkia*) (Holt *et al.*, 1994). Filamentous bacteria that show different forms of spores are concerned with the term actinomycete and also includes *Nocardia* form actinomycetes. This type of organism has drawn the attention of researchers to its biotechnological applications because of the ability of these organisms to produce many secondary metabolic metabolites. Among the most important metabolites are antibiotics and enzymes (Willimans *et al.*, 1989). Actinomycetes are defined according to several criteria, including morphology (Cross and Maclver, 1966; Shirling and Gottlieb, 1966 and Gottlieb and Shirling, 1970), production of antibiotics (Holt *et al.*, 1994) and Cell chemistry that depend on presence of Diaminopimilic acid (-L or –meso tpe) (Lechevalier *et al.*, 1971, 1973 and Holt *et al.*, 1994). In *Kitasatospora setae* KM-6054T, there was no fragmentation of the vegetative mycelia, sporangia, motile spores and sclerotia, the aerial mycelia produce more than 20 spores,aerial spores, per chain The cell wall hydrolysates of the *Kitasatospora* strains contain similar amounts of both LL-diaminopimelic acid (DAP) and meso-DAP, as well as glycine and galactose (Takahashi, 2017). In the current study, isolation and identification of some alkalophilic actinomycetes from Al-Fayoum region was investigated.

MATERIALS AND METHODS

1- Soil Samples:

Several soil samples (five samples) were collected randomly from several different places, represented in agricultural lands in Al-Fayoum Governorate, Egypt. From the surface layer at a depth of 5-15 cm using a sterile inserted metal tube of 30 cm length (Bezbaruah, 1983). Samples were placed in clean plastic bags and kept at a low temperature until transported to the laboratory. The samples taken were well mixed to give one sample. It was passed in a 5.0 mm hole sieve to remove gravel, stones and plant roots, then a 1.0 mm hole sieve. The soil sample was divided into two identical parts. A part was sent to the Desert Research Centre, Cairo, Egypt for chemical and mechanical analysis. The second part was used for the isolation of alkalophelic actinomycetes.

1-1 Soil Chemical Analysis:

The chemical analysis of the soil was done in the Desert Research Centre, Cairo, Egypt according to the method of Chapman and Pratt (1961).

2- Isolation of Alkaliphilic Actinomycetes From Soil.

All media used for isolation of alkalophelic actinomycetes at pH 10.5.

2-1 Media Used In Isolation Of Alkaliphilic Actinomycetes.

A- Media of Alkalophelic Actinomycetes (Sato *et al.*, 1983).

B- Nutrient Agar Medium (American Public Health, 1920).

2-2 Methods of Isolation of alkaliphilic actinomycetes.

Two methods were used for isolation of alkalophelic actinomycetes:

A- Using the Soil Sample Directly:

0.2 g of soil sample was distributed above the surface of Sato *et al,* 1993 and nutrient agar medium. Five replicates were made for each medium. Media were incubated at 32±2 °C for a week and then examined to capture the growing colonies and replant them on the same medium of isolation.

B- Using the Soil Dilution Technique:

One gm of soil sample was to 99.0 mL of sterile distilled water in a 250.0 mL conical flask, using shaker for one hour, then the solution filtrated and the filtrate was used as a source of the microbes. Suitable dilutions 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} were used. One ml of each diluted solution was taken and

placed over the surface of the isolation media. The dishes were incubated for a week at 32±2 °C, then examined to capture the growing colonies and recultured on the same isolated medium; five replicates for each concentration were made. The growing colonies were picked and grown on the same medium used for isolation, and they are frequently moved and purified to ensure their purity.

2-3- Identification of Temperature Range of Isolated Alkaliphilic Actinomycetes:

The alkaliphilic actinomycetes medium (pH10.5) was used to make several replicates and each group was placed in an incubator at a temperature from 20 °C to 50 °C. Five replicates were made for each organism and the dishes were examined after a week and fifteen days later. The growth of these isolates was tested at 4, 10, 37 and 45 °C on the modified Bennett agar medium (Sato *et al.*, 1983).

2-4- Identification of pH Range Of Isolated Alkaliphilic Actinomycetes:

The alkaliphilic actinomycetes medium (Sato *et al*, 1983) was used at different pH range from pH5 to pH13 by adding dilute hydrochloric acid or dilute sodium hydroxide to the medium before sterilization. Fifty ml of the liquid medium were used in 250.0 ml conical flasks. Flasks were sterilized at 1.5 atmospheres for twenty minutes in an autoclave. Confirmation of hydrogen ion concentration was made using pH paper. Three replicates were made for each treatment. Flasks were incubated in an incubator at 32±2 °C for one week. The experiment was performed by using a solid medium, five replicates were made for each organism at each pH concentration. The growth was also tested at pH4.3 using the modified Bennett agar medium.

3- Identification of Isolated Actinomycetes:

Identification of Genera:

Identification of genera includes the identification of the phenotype as well as the identification of the amino acid type in whole-cell hydrolysates (WCH), which the amino acid (DAP) diaminopimelic acid is, and if it is LL-DAP or m-DAP, or both. Also, the type of sugar associated with the amino acid.

3-1 Cell Whole Hydrolysates (WHC):

The total cell extract was used according to the method of (Becker *et al.*, 1964) and (Lechevalier and Lechevalier, 1970 & 1980).

3-1-1- Detection of Diaminopimelic Acid:

20.0 µl is taken from the total cellular extract and dotted on Whatman No. 1 Chromatographic filter paper. Also, 10.0 µl of diaminopimelic acid was dotted next to the cellular extract. The solution of di-aminopimelic acid contains a mixture of meso and L- DAP. The descending liquid chromatography technique is used in it by using a solvent solution consisting of methanol-water – 10N HCl - pyridine (V / V) (10 - 2.5 -80- 17.5) and leave to supply the paper with the solution overnight. Remove the paper and let it air dry. Amino acids were detected after spraying the chromatographic paper with the Acetone-ninhydrine solution (0.1% (W.V)) the papers are left to dry and then placed in the oven at 100 ° C for two minutes. Green olive fading to yellow color (L-DAP acid migrates faster than m-DAP acid and thus can distinguish them). Other amino acids migrate faster than this acid, so it can get out of this Paper and appears by the purple color.

3-1-2 Detection of Diagnostic Sugars In Whole-Cell Hydrolysates:

This was done using paper chromatography Whatman No. 1. Using the descending liquid method, the solvent (V / V) n-butanol-pyridine-water (5: 3: 2) was used. Standard solutions of xylose sugar, arabinose sugar and galactose sugar (separately) were made by dissolving 0.1 mg of the sugar in 0.5 ml of pyridine. A10.0 µl of the total cell extract was used on a Whatman Paper No. 1 and next to it 10.0 µl of the standard solutions of sugars were spotted Papers overnight and provide the solvent solution with the liquid carrier. The paper is removed from the chromatograph

tanks and allowed to dry. Existing sugars are identified by spraying paper with an alkali silver nitrate solution as a reagent.

4- Identification Methods For Morphological Studies:

The phenotype was identified using methods mentioned in (Whitman *et al.*, 2012).

Media Used in The Definition According To (Shirling And Gottlib, 1966):

Tryptone-Yeast extract broth (ISPI), Agar yeast extract - barley extract, Yeast extract - malt extract agar medium (ISP2), Oatmeal agar (ISP3), Inorganic Salts - Starch agar (ISP4), Glycerol - asparagine agar (ISP5), Peptone-Yeast extract - iron agar (ISP6) consisting of g / L (w / v), Tyrosine - agar (ISP7), Trace salts solution (A), Carbon utilization medium (ISP9), Basal mineral salts agar and Pridham and Gottlieb trace salts (B), Czapek- Dox solid medium, Nutrient broth, Glucose-Asparagin Solid medium (Waxman 2), Glycerol - Aspargen Solid (Waxman 3), Oatmeal - Nitrate agar medium, Emerson's solid medium: Emerson's agar (YPSs), Blood agar medium: Blood agar medium, Oatmeal - yeast extract agar medium.

Inoculation of Plates for Morphological Studies:

Newly developed colonies are used either on a liquid medium (ISP1) or solid colonies for the same medium.

1- About 0.05 mL is taken by means of a sterile pipette inserted from the colony to be recognized the apparent shape (or fill a sterile needle from a solid colony) and placed on the surface of the agar.

2- With a sterile needle, five streaks are planned on the surface of the dish from the start of the colony point.

3- The needle is also planning reverse plans on the previous one.

4- Seven dishes are made for each medium of each organism.

5- The dishes were incubated at 32±2 °C. Two dishes were examined for each colony after one, two, three and four weeks.

6- The dishes are compared to the dishes of the positive and negative comparison groups, and the results are recorded.

Pigmentation of Substrate Mycelium (Colony Reverse):

The color of the ground mycelium was determined fourteen days after incubation, according to (Tresner and Backus 1963) and the yellow-brown color was attributed to the isolates that did not have a specific color from the five colors (red-orange-green-blue-violet) (Shirling and Gottlieb, 1966).

Diffusible Pigments:

These dyes were determined when using media such as Glycerol/asparagine agar (ISP5) (Shirling and Gottlieb, 1966) which stimulates pigment production, and uses the same previous colors to determine the resulting color. It also tests the sensitivity of these soluble dyes to acids (using 0.1 N HCl) or alkalis (using 0.1 N NaOH).

Melanin Pigments Production:

These pigments were determined after four days of growth on Peptone - Yeast extract - iron agar and tyrosine agar media (Shirling and Gottlib, 1966). It can be given to one of these media, not necessarily to the two.

5- Identification of Micromorphological Characteristics of The Spore-Bearing Hyphae and Spore Chain Morphology:

The phenotype was identified using direct microscopy by using an optical microscope. At least ten places on the surface of the plate were tested with the lens (x 100). The bacteria are counted in the chain, the terminal end of the chain is recognized, the shape of the bacterial chain and the shape of the spores. To visualize the external appearance of spore chains, a nourishing agar medium or an agar-added water peptone medium was used according to Lechevalier (1989) using the Cover Slip technique. Then use the microscopic Wolfe No: 875802 Circa 2000 Carolina Biological supply company with sample camera. To identify the shape of the spore surface morphology, a transmission electron microscope JEOL JEM- 1200 EX II (Japan)

was used at the Faculty of Science - Ain Shams University. Cairo, Egypt. By using collodion-covered mesh technology which is applied by means of lightly forceps on the growth surface to capture mature spores, spores that adhere to the surface of the Claudian are examined directly without fixation or dye by the transmission electron microscope.

6- Enzyme Activity:

According to Williams *et al.* (1989), enzyme activity was determined by using the egg Yolk medium method. The activity of pectin analysis enzymes, nitrate reduction, degradation activity**,** adenine hydrolysis, gelatin hydrolysis (0.4% w / v) and starch (1.0% w / v) was tested.

7- Amino Acids Utilization By Isolated Strains:

Fifteen nitrogen sources were used to test the growth of strains isolated on them by using the alkaline medium consisting of g% (w / v): D-glucose, 1.0; $MgSO_4$. $7H_2O$, 0.05; NaCl, 0.05; $FeSO_4$. $7H_2O$, 0.001; K_2HPO_4, 0.1 and agar 1.2. The medium is sterilized and poured into dishes. The amino acids used are DL- -amino – n – butyric acid; L-arginine; L-cysteine, L-histidine; L-hydroxy proline, L-methionine, L-phenylalanine; L-serine; L-theronine, L-valine; potassium nitrate; Glycine, alanine; glutamic acid and cystine. Two weeks after incubation, growth is compared to a negative comparison group (which is devoid of a nitrogen source) and a positive comparison group (containing L-asparagine or L-proline.

8- Growth Test in The Presence of Inhibitory Compounds:

Growth in the presence of inhibitory compounds was determined according to Williams *et al.* (1989).

9- Antimicrobial Activity of Isolated Alkaliphilic Actinomycetes:

A number of fungi and bacteria have been used to test the production of antibiotics and the coat layer technique was used to test the ability of isolates to secrete antibiotics (Williams *et al.,* 1989). The antimicrobial activity of strains isolated against several types of bacteria has been tested, such as *Escherichia coli* ATCC 25922 *Pseudomonas fluorescens*; *Serratiamarcescens* ATCC 800; *Staphytococcus aureus* ATCC 25923; *Streptococcus pyogenes* ATCC 19615; *Salmonella typhimurium* ATCC 14028; *Lactobacillus fermentous* and *Proteus vulgaris; Aspergillus niger*; *Penicillium chrysogenum*; *Fusarium* and some types of fungi such as *oxysporum; Aspergillus fumigatus* and *Candida albicans.* The inhibition zone around the colony is determined after 24-hour incubation at 32±2 °C or 4 days in the case of fungi.

10- Sensitivity of Isolated Strains To Some Antibiotics:

The sensitivity of isolated strains to some antibiotics was tested such as Penicillin G (10 µ); gentamicin (100µg); Streptomycin (100µg) and vancomycin (50µg). By using tablets of preloaded filter paper with known doses and subjected to lyophilized drying. Preloaded tablets have been placed on the surface of the modified Bennett's agar medium1which were inoculated by test organisms. Results were recorded after 1, 2, 3 and 7 days from incubation, the isolates sensitivity is a positive result by measuring the inhibition zone around the disc.

RESULTS

A- **Chemical and Mechanical Analysis of The Soil Sample:**

Table (la) shows the chemical analysis of the soil sample, it contains high concentrations of sodium and chloride, as there are many cations and anions such as chlorides, sulfates and bicarbonate, also it contains high amounts of potassium and phosphorous.The soil solution pH A8; hence it is clear that it is an appropriate medium for the growth of many microorganisms, including bacteria and actinomycetes.

Table (1b) shows the mechanical composition of the soil sample used in the isolation of alkaliphilic actinomycetes. It is sandy soil contains 91% sand, 6% clay and 3% silt.

B- Isolation of Alkaliphilic Actinomycetes From Soil:

Isolation of alkaliphilic actinomycetes takes place by using Sato *et al*, 1993 medium and nutrient agar media (supplemented with casein or skimmed milk)at neutral pH7 and alkaline pH10.5.Five isolates of alkaliphilic actinomycetes were obtained at the pH range pH7-12, which indicated that they are alkalitolerant, where they grew in the neutral and the alkaline media. All isolates had high capacity in the production of alkaline protease enzyme where they were able to analyze casein or skim milk in the growth media at pH10.5.

Table (1): Chemical and mechanical analysis of the soil sample.

A-Chemical.

soil reaction	Total soluble salts at 25 C		Soluble anions (meq/L)			Soluble cations (meq/L)			Available (ppm)	
PH	mmol e/cm	Ppm	CO_3+ HCO_3	CL-	SO_4=	Ca2+	Mg2	Na+	PO_4-	K+
8.0	47.9	30656	3.25	435.70	54.95	80.50	134.4	376.5	9.70	312

B-Mechanical.

Texture	Sand %	Clay %	Silt%
Sandy	91	6	3

Identification of Isolated Strains of Alkaliphilic Actinomycetes:

1- Identification of Diaminopimelic Acid (DAP) And Sugars in Total Cell Hydrolysate of Alkaliphilic Actinomycetes:

The whole-cell hydrolysate of cell content of alkaliphilic actinomycetes contains both L-DAP and also m-DAP for isolates A3, A6, A8, and A10, while there is no amino acid in the cellular hydrolysate of isolate A11 as it does not contain L-DAP or m-DAP or any other amino acids. Also, isolate A11 not contain any type of sugars while isolates A3, A6, A8, and A10 contained galactose sugar. This indicated that they (A3, A6, A8, and A10) all belong to the genus *Kitasatospora*, which is characterized by the presence of the two types L-DAP & m-DAP in their cell walls associated with galactose, i.e. they are from the cell wall type III + galactose. As for isolate A11, the genera that do not contain di-aminopimilic acid or any type of sugars are: the genus *Oreskovia* and it consists of ground mycelium only and it breaks into small non-moving units, and this is incompatible with the form of this isolate, and the other sex is *Promicromonospora* which is an aerial mycelium aseptic and terrestrial mycelium breaks down into immobile units, and this also does not represent the shape of this isolate, which may be a new genus (Fig. 5).

2- Morphological Description Of Isolated Strains:

The isolates A3, A6, A8, A10, and A11 have been grown on solid media (Table 2a, b) at neutral pH7 and at alkaline pH10.5 for a period of 3 to 4 weeks at temperature 32ºC±2. All isolates showed abundant to moderate growth in alkaline media, aerial mycelium did not appear in many neutral media, whereas it was well visualized in alkaline ones.

2-1 Morphological Description Of The Isolate*kitasatospora*a3:

This isolate characterized by the presence of a branched and prolific aerial mycelium that is carried on a short conidiophore and chain of straight and sometimes folded ends at the end of the spores in the form of a two-pronged RF (*Rectus flexibilis*) and carries smooth rectangular to cylindrical and coated membranes of more than twenty aerobic spores, often yellow to gray, and ground mycelium, ranging from yellow to brown depending on the medium (Plate 1). This organism grows on neutral and alkaline

media, better growth was atalkaline ones (Table 2). This organism produces soluble pigments ranging from yellow-brown to green according to the type of medium, as well as brown melanin pigment, according to its growth on media of ISP-1,3,6 & 7. The soluble pigments are not affected by the change in the pH, either by acidity or alkalinity.

Plate 1: Photomicrographs of a 21-day old culture of *Kitasatospora sp*. A3 on Oatmeal agar medium (ISP_3) by transmission electron microscopy showing short conidiophore and smooth spores. X 4000.

2-2 Morphological Description Of The Isolate *Kitasatospora*A6:

This isolate characterized by its good growth on all media used (Table 2), and characterized by good aerialmycelium on neutral and heavy on alkaline pH. The aerial mycelium, characterized by a long chain of spores ending by a loop (RA) *Retinaculum apertum*, spores are smooth and enclosed by a film on a short conidiophore (Plate 2). Aerial mycelium is often beige to brown or white to gray in some media. Ground mycelium is mostly brown to yellow. This isolate produces soluble pigments of yellow and brown color, and a brown melanin pigment produced on media ISP1,3,6 & 7 didn't change by acid or Alkali.

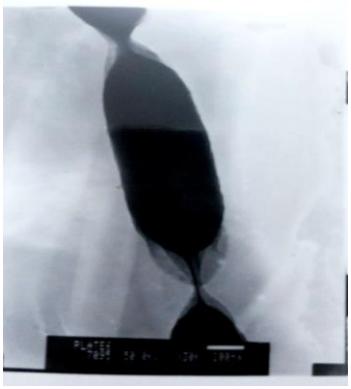

Plate 2: Photomicrographs of a 21- day old culture of *Kitasatospora sp*. A6 on Oatmeal agar medium (ISP-3) by transmission electron microscopy showing smooth spores.

2-3 Morphological Description Of The Isolate *Kitasatospora* A8:

Isolate A8 grows abundantly in all media used for identification and profuse air profuse, especially on alkaline media (Table 2). The aerial mycelium is characterized by a long chain of bacteria that ends with a loop or hook (RA) *Retinaculum apertum* and sometimes it forms at the end of the aerial mycelium The shape of the Solar and the spores grow directly on the ground mycelium are smooth and surrounded by a membrane and their shape is slightly white (Plate 3). The aerial mycelium is characterized by a beige gradient to reddish-brown and sometimes appears in white depending on the medium components and the degree of pH while the ground mycelium is characterized by a yellowish-brown color sometimes (Table 2). This isolate secretes brown or mostly yellow dissolved pigments as it produces yellow melanin pigment based on ISP media 1.3.6 & 7 (Table 2) and the dye color does not change using acid or alkali.

Plate 3: Photomicrographs of a 21-day old culture of *Kitasatospora sp*. A8 on Oatmeal agar medium (ISP-3) by transmission electron microscope showing smooth spores x 15k.

2-4 Morphological Description of The Isolate *Kitasatospora* A10: IsolateA10 grew on most media used, and profuse aerial mycelium appears on alkaline media (Table 2). Aerial mycelium characterized by a large number of branches and the presence of long chains of spores that sometimes end with a hook or take the form of a sickle (RA) (*Retinaculum Apertum*). Spores are smooth, oblong-shaped that appears directly on the branching mycelium, and a very short conidiophore appears (Plate 4). The aerial mycelium is characterized by a reddish-brown color, with yellow appearing on the edges on some media, as well as a light gray color (Table 2). The ground mycelium is brown to light brown or yellow. Isolate A10 produces yellow or brown melting pigments, as the yellow or brown melanin pigment is secreted on media ISP-1,3,6 & 7. The color of the dye does not change using acid or alkali.

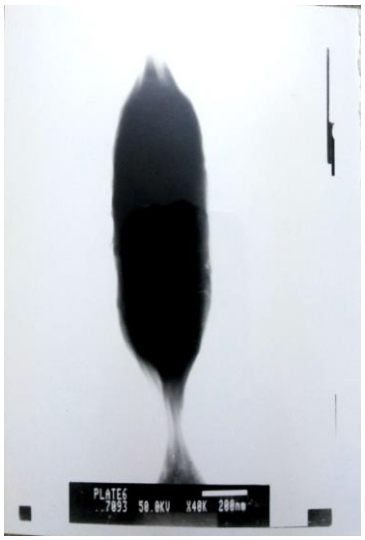

Plate 4: Photomicrographs of a-21–day old culture of *Kitasatospora sp*. A10 on Oatmeal agar medium (ISP-3) by transmission electron microscopy showing smooth spores. X10k–40k.

2-5 Morphological Description Of The Isolate *Kitasatospora* A11:

Abundant growth ofA11on all media used for identification, the aerial mycelium appeared on alkaline media clearly (Table 2). The aerial mycelium is branched and carries long chains of more than twenty spores that sometimes end with a loop or hook (RA) *Retinaculum apertum*, grow on a short conidiophore, conidia are smooth, enclosed in a membrane with an oval shape (Plate 5). The aerial mycelium characterized by a reddish-brown or beige-yellowish brown color, depending on the components of the cultivated media. The ground mycelium is brown or yellow to reddish-brown. This isolate often secretes dissolved pigments with brown color and secrete brown melanin pigment on the ISP-1,3,6 &7 media, the color of the pigment didn't change by changing the pH using acid or alkali (Table 2).

Plate 5: Photomicrographs of a 21–day old culture of unknown strain A11 on Oatmeal agar medium (ISP-3) by transmission electron microscopy showing smooth spores- x 6000 – x50k .

Table (2a): Morphological characteristics of isolated strains of alkaliphilic actinomycetes at pH7.

Media used	Characters	Isolated strains of alkaliphilic actinomycetes				
		A3	A6	A8	A10	A11
ISP1	A	Moderate	Moderate	Moderate	Moderate	Moderate
	B	Yellow tint	Beige brown	Yellow tint	Yellow	---
	C	Dark brown	Yellow	Yellow	Yellow	Natural
	D	Yellow	Brownish	Brownish	"	---
ISP2	A	Abundant	Scarce	Moderate	Moderate	Abundant
	B	Parchment	---	Beige brown	Beige gray	Beige brown
	C	Dark brown	Yellow	Yellow	Yellow	Brownish
	D	Brownish	Yellowish	Brownish	---	Brownish
ISP3	A	Abundant	Abundant	Abundant	Abundant	Abundant
	B	Beaver	Beaver	Beige brown	Brown	Beige brown
	C	Brownish	Brownish	Brownish	Brownish	Brownish
	D	Greenish	"	"	"	"
ISP4	A	Scarce	Moderate	Moderate	Scarce	Moderate
	B	Yellow tint	Scant	---	White	Pastel Yellow
	C	Yellow	Natural	Natural	Yellow	Natural
	D	--	Brownish	Yellowish	Yellowish	--
ISP5	A	Moderate	Scarce	Moderate	Moderate	Moderate
	B	Pastel yellow	White	---	---	Yellow tint
	C	Yellow tint	Yellow	Natural	Natural	Beige brown
	D	Brownish	---	Brownish	---	Brownish
ISP6	A	Moderate	Moderate	Moderate	Moderate	Moderate
	B	Beige brown	White	---	Beige gray	Beige brown
	C	Yellowish	Fawn	Natural	Yellow	Fawn
	D	Brownish	Brownish	Yellowish	Yellowish	Brownish
ISP7	A	Moderate	Scarce	Moderate	Moderate	Moderate
	B	Pastel yellow	Yellowish	---	---	Yellow tint
	C	Brownish	Brownish	Beaver	Yellow	Beige brown
	D	Brownish	Yellowish	---	Brownish	Brownish
Dox's	A	Scarce	Scarce	Moderate	Scarce	Moderate
	B	Yellow tint	White	Scant	White	Yellow tint
	C	Brownish	Beaver	Natural	Beige brown	Natural
	D	--	---	---	---	---
Waksman2	A	Abundant	Abundant	Abundant	Abundant	Abundant
	B	Lemon	---	---	Beige brown	Beaver
	C	Olive	Natural	Brownish	Yellow tint	Natural
	D	Brownish	Yellowish	Brownish	Brownish	Brownish

Cont. Table (2a):

Waksman3	A	Abundant	Abundant	Abundant	Abundant	Abundant
	B	---	Scant	---	Beaver	---
	C	Natural	Natural	Natural	Brownish	Natural
	D	Brownish	Brownish	---	---	---
Oatmeal-No3	A	Abundant	Moderate	Scarce	Moderate	Moderate
	B	Yellow	---	---	Beige gray	Beaver
	C	Natural	White	Natural	Natural	Natural
	D	Brownish	Brownish	---	Brownish	Brownish
Bennett's agar	A	Moderate	---	Scarce	Scarce	Moderate
	B	Olive gray	---	---	---	---
	C	Yellow	---	Natural	Natural	Natural
	D	Brownish	---	---	---	---
Potato dextrose agar	A	Moderate	Scarce	Scarce	Scarce	Scarce
	B	Greenish	---	---	---	---
	C	Parchment	Natural	Natural	Natural	Natural
	D	---	---	---	---	---
Emerson's agar	A	---	---	---	---	Scarce
	B	---	---	---	---	---
	C	---	---	---	---	Natural
	D	---	---	---	---	---
Dox.s peptone agar	A	Scarce	Moderate	Moderate	Moderate	Moderate
	B	Yellow tint	White	White	---	---
	C	White	Natural	Natural	Natural	Brownish
	D	---	---	---	---	---
Glucose asparagine agar	A	Moderate	Scarce	Moderate	---	Moderate
	B	Yellow	---	---	---	---
	C	Olive gray	Natural	Natural	---	Natural
	D	Yellowish	---	---	---	---
Oatmeal yeast agar	A	Moderate	Moderate	Moderate	Scarce	Scarce
	B	Yellow tint	White	Yellow	---	---
	C	" "	Natural	Natural	Natural	Natural
	D	Yellowish	Yellowish	Yellowish	---	---
Starch yeast agar	A	Moderate	Moderate	Moderate	Moderate	Moderate
	B	Parchment	Beaver	Beaver	White	Gray
	C	Pastel yellow	Natural	Natural	Natural	Natural
	D	---	Brownish	Yellowish	---	---

A-Growth; B-Aerial mycelium; C-Substrate mycelium & D-Soluble pigments

Table (2b): Morphological characteristics of isolated strains of alkaliphilic actinomycetes at pH10.5.

Media used	Characters	Isolated strains of alkaliphilic actinomycetes				
		A3	A6	A8	A10	A11
ISP1	A	Moderate	Moderate	Moderate	Moderate	Moderate
	B	Yellow tint	Beige brown	Yellow tint	Yellow	---
	C	Dark brown	Yellow	Yellow	Yellow	Natural
	D	Yellow	Brownish	Brownish	"	---
ISP2	A	Abundant	Scarce	Moderate	Moderate	Abundant
	B	Parchment	---	Beige brown	Beige gray	Beige brown
	C	Dark brown	Yellow	Yellow	Yellow	Brownish
	D	Brownish	Yellowish	Brownish	---	Brownish
ISP3	A	Abundant	Abundant	Abundant	Abundant	Abundant
	B	Beaver	Beaver	Beige brown	Brown	Beige brown
	C	Brownish	Brownish	Brownish	Brownish	Brownish
	D	Greenish	"	"	"	"
ISP4	A	Scarce	Moderate	Moderate	Scarce	Moderate
	B	Yellow tint	Scant	---	White	Pastel Yellow
	C	Yellow	Natural	Natural	Yellow	Natural
	D	--	Brownish	Yellowish	Yellowish	--
ISP5	A	Moderate	Scarce	Moderate	Moderate	Moderate
	B	Pastel yellow	White	---	---	Yellow tint
	C	Yellow tint	Yellow	Natural	Natural	Beige brown
	D	Brownish	---	Brownish	---	Brownish
ISP6	A	Moderate	Moderate	Moderate	Moderate	Moderate
	B	Beige brown	White	---	Beige gray	Beige brown
	C	Yellowish	Fawn	Natural	Yellow	Fawn
	D	Brownish	Brownish	Yellowish	Yellowish	Brownish
ISP7	A	Moderate	Scarce	Moderate	Moderate	Moderate
	B	Pastel yellow	Yellowish	---	---	Yellow tint
	C	Brownish	Brownish	Beaver	Yellow	Beige brown
	D	Brownish	Yellowish	---	Brownish	Brownish
Dox's	A	Scarce	Scarce	Moderate	Scarce	Moderate
	B	Yellow tint	White	Scant	White	Yellow tint
	C	Brownish	Beaver	Natural	Beige brown	Natural
	D	--	---	---	---	---
Waksman2	A	Abundant	Abundant	Abundant	Abundant	Abundant
	B	Lemon	---	---	Beige brown	Beaver
	C	Olive	Natural	Brownish	Yellow tint	Natural
	D	Brownish	Yellowish	Brownish	Brownish	Brownish

Con. Table (2b):

Waksman3	A	Abundant	Abundant	Abundant	Abundant	Abundant
	B	---	Scant	---	Beaver	---
	C	Natural	Natural	Natural	Brownish	Natural
	D	Brownish	Brownish	---	---	---
Oatmeal-No3	A	Abundant	Moderate	Scarce	Moderate	Moderate
	B	Yellow	---	---	Beige gray	Beaver
	C	Natural	White	Natural	Natural	Natural
	D	Brownish	Brownish	---	Brownish	Brownish
Bennett's agar	A	Moderate	---	Scarce	Scarce	Moderate
	B	Olive gray	---	---	---	---
	C	Yellow	---	Natural	Natural	Natural
	D	Brownish	---	---	---	---
Potato dextrose agar	A	Moderate	Scarce	Scarce	Scarce	Scarce
	B	Greenish	---	---	---	---
	C	Parchment	Natural	Natural	Natural	Natural
	D	---	---	---	---	---
Emerson's agar	A	---	---	---	---	Scarce
	B	---	---	---	---	---
	C	---	---	---	---	Natural
	D	---	---	---	---	---
Dox.s peptone agar	A	Scarce	Moderate	Moderate	Moderate	Moderate
	B	Yellow tint	White	White	---	---
	C	White	Natural	Natural	Natural	Brownish
	D	---	---	---	---	---
Glucose asparagine agar	A	Moderate	Scarce	Moderate	---	Moderate
	B	Yellow	---	---	---	---
	C	Olive gray	Natural	Natural	---	Natural
	D	Yellowish	---	---	---	---
Oatmeal yeast agar	A	Moderate	Moderate	Moderate	Scarce	Scarce
	B	Yellow tint	White	Yellow	---	---
	C	" "	Natural	Natural	Natural	Natural
	D	Yellowish	Yellowish	Yellowish	---	---
Starch yeast agar	A	Moderate	Moderate	Moderate	Moderate	Moderate
	B	Parchment	Beaver	Beaver	White	Gray
	C	Pastel yellow	Natural	Natural	Natural	Natural
	D	---	Brownish	Yellowish	---	---

A-Growth; B-Aerial mycelium; C-Substrate mycelium & D-Soluble pigments

3- Physiological Characteristics and Growth Criteria of Isolated Strains of Alkaliphilic Actinomycetes:

Physiological properties of isolates A3, A6, A8, A10, and A11 are described in Table (3).It is clear from the table that, all isolates grow in a temperature range of 25-40 ° C and a pH7-12. Table (3) shows that all isolates have high enzyme capabilities as it can secrete Lecithinase, protease, and lipase enzymes by growing on the solid medium of egg emulsion and forming a clear zone around the growth in case of secretion of the enzymes, they reduce nitrate especially on alkaline media and the isolates A6, A8, A10, and A11 secreted pectinase enzyme. While isolate A3 produced hydrogen sulfide gas on the neutral medium which produced by isolates A6, A8 and A10 on the alkaline media only. IsolateA11 fails to grow on this medium. The five isolates A3, A6, A8, A10 and A11 have the ability to analyze gelatin in

alkaline medium. While this character disappears on neutral medium. All isolates analyze casein in the neutral and alkaline medium. They grow abundantly on skim milk and dissolve it and create a clear halo around growth. All isolates can analyze starch on a neutral and alkaline media. All isolates can reduce iron in red blood cells (on the blood agar medium) giving green color, i.e. degradation of the type α-hemolytic. All isolates grew on urea to a varying degree, and while aerial mycelium appears on the alkaline pH medium it does not appear on the neutral medium, A3 appeared good growth on urea medium.

All isolates degrade Tween 60 on the alkaline medium while they didn't grow on neutral medium containing Tween 60. They analyze DNA, xanthine, adenine and tyrosine (Table 3). All isolates grow on Dox's medium, nutrient broth (except A3), nutrient broth + 10% glucose (except A3) and Horikoshi-I medium containing (5.0% g sodium chloride (w / v) while no growth was observed on Horikoshi-I medium containing (10% g of sodium chloride (w / v) except that ofA6 andA11.

From Table (3), it is clear that the isolates A3, A6, A8, A10, and A11 can grow on the alkaline medium in the presence of (5.0% g sodium chloride (w / v) while all isolates can grow on the medium containing 6.0% g sodium chloride (w / v), the isolate A8 cannot grow on this medium at neutral pH and grow well on the alkaline pH. All isolates failed to grow on the primary medium containing 10.0% g of sodium chloride (w / v).

All isolates can't grow anaerobically. All isolates grew on the Fogs Proscar (VP) medium, while not all were able to grow on the primary medium (Shirling and Gottlieb, 1966)at pH4.3.

IsolateA3 grew on the alkaline neutral medium while it didn't grow on alkaline medium at 37 °C. The rest of isolates A6, A8, A10, and A11 were unable to grow on this medium at 37 °C at neutral pH while they grew well at alkaline medium, no growth on temperatures4, 10 and 45 °C.

4- Utilization of Carbon Sources by Isolated Alkaliphilic Actinomycetes:

Table (4) shows the utilization of alkaliphilic actinomycetes to different carbon sources. It is clear from the table that all isolates were not able to grow in the alkaline medium except isolateA10 showed average growth at pH10.5. All isolates grew to various degrees on glucose on alkaline medium pH10.5 (A3<A6<A8 = A10 = A11, respectively) while none of them grew on a neutral medium containing glucose. All isolates grew on all carbon sources, especially on alkaline media, while growth on neutral media was zero or terrestrial mycelium only. All isolates were grown in abundance in case of the neutral or alkaline medium upon using pyruvate as a carbon source. A3 grew on many sugars and carbon sources whereas its growth inhibited in presence of inulin and growth was questionable in the case of using mesoenositol, salicin, adonitol, sorbitol and dolcitol, while its growth was abundant when using glycerol (Table 4). Strain A6 showed abundant growth in the alkaline medium containing mannose sugar, sodium malonate, and pyruvate, but with average growth on glucose, fructose, mannitol, xylose, lactose, maltose, ribose, and sorbitol. This strain was unable to grow on dolcitol and inulin, also it wasn't able to grow on neutral media containing all carbon sources used except for minimal growth on meso-inositol, salicin, maltose, and adonitol. However, medium growth on xylose and sorbitol and abundantly grown on pyruvate and sodium malonate(Table 4). From (Table 4) it is clear that strains A8, A10, and A11 were unable to grow on sugars and carbon sources in the neutral media used, some of them showed very small growths, these strains showed abundant to moderate growths on all used carbon sources on the alkaline medium except for inulin and sodium malonate, A10 could not grow on dolcitol.

Table (3): Physiological characters and growth criteria of isolated strains of alkaliphilic actinomycetes.

Character	Isolated strains of alkaliphilic actinomycetes									
	A3		A6		A8		A10		A11	
	7	10.5	7	10.5	7	10.5	7	10.5	7	10.5
Criteria:										
Gram stain	+Ve		+Ve		+Ve		+Ve		+Ve	
Acid fast stain	-Ve		-Ve		-Ve		-Ve		-Ve	
Temp.range	25 - 40		25 - 40		25 - 40		25 - 40		25 - 40	
pH range	7 - 12		7 - 12		7 - 12		7 - 12		7 - 12	
Enzymes:										
*Lecithinase	++	+++	+	++	++	++	++	++	++	++
*Protease	++	+++	+	++	++	++	++	++	++	++
*Lipase	++	+++	+	++	++	++	++	++	++	++
Pectinase	-	-		+-	-	++	-	++	-	+
NO_3reductase	+	±	-	++	-	++	-	++	-	++
• H_2S production	+	-	-	+	-	+	-	+	-	-
Degradation:										
Gelatin	-	+	-	+	-	+	-	+	-	+
Casein	+	+	+	+	+	+	+	+	+	+
Skimmed milk	+++	+++	++	+++	+++	+++	++	+++	+++	+++
Starch	+	+	+	+	+	+	+	+	+	+
Blood	α-hemolytic		α-hemolytic		α-hemolytic		α-hemolytic		α-hemolytic	
Urea	+s	+++	+s	±	+s	++	+s	+	+s	++
Tween60	+	-	+	-	+	-	+	-	+	-
DNA	++	++	++	++	++	++	++	++	++	++
Hypoxanthine	++	++	+	++	++	++	+	++	+	++
Adenine	+	++	+	++	+	++	+	++	+	+++
L-tyrosine	++	+++	++	+++	++	+++	+	++	+	+++
Growth on:										
Dox's broth	++	+	++	++	++	++	++	++	++	++
Nutrient broth	-	-	+	+	+	+	+	+	+	+
Nutrient broth +10%glucose	-	-	+	+	+	+	+	+	+	+
Horikoshi I	++	+++	++	+++	++	+++	++	+++	++	+++
"+5% NaCL	+	+	++	+++	+	+++	++	+++	++	+++
"+10% "	-	-	+	+	-	-	-	-	+	+
5% + NaCL	+	++	+	++	±	++	+	++	+	++
6% " "	+	++	+	++	-	++	+	++	+	++
7% " "	+	++	+	++	-	+	-	++	-	+
10% " "	-	-	-	-	-	-	-	-	-	-
V P	+	+	+	+	+	+	+	+	+	+
Anaerobic	-	-	-	-	-	-	-	-	-	-
Growth at :										
4C°	-	-	-	-	-	-	-	-	-	-
10C°	-	-	-	-	-	-	-	-	-	-
37C°	+	-	-	++	-	++	-	++	-	++
45C°	-	-	-	-	-	-	-	-	-	-
$pH_{4.3}$	-	-	-	-	-	-	-	-	-	-

*By using egg emulsion agar medium.•By using lead acetate strip.- No growth;± Doubtful; +Scarce; ++Moderate; +++Abundant &s substrate mycelium only.Horikoshi-I medium:(starch, 2%; polypeptone, 1.0 %; yeast extract, 0. 5%; K_2HPO_4, 0. 1%; $MgSO_4$ 7H2O, 0.05%). VP- Vogaes-Proskauer reaction.

Table (4): Carbon sources utilization and growth of isolated strains of alkaliphilic actinomycetes.

Carbon sources in agar medium pH	Growth of alkaliphilic actinomycetes strains									
	A3		A6		A8		A10		A11	
	7	10.5	7	10.5	7	10.5	7	10.5	7	10.5
Basal medium	-	-	-	±	-	-	-	++	-	-
Glucose	-	+	-	++	-	+++	±	+++	-	+++
Arabinose	+	+	-	±	+s	+s	-	±	-	±
Fructose	-	+	-	++	-	+++	-	+	-	++
Galactose	±	+	-	+	-	++	+	++	-	++
meso-inositol	±	±	+s	+	+s	+	-	++	±	±
Manitol	+s	+s	-	++	-	++	-	+++	-	++
Raffinose	-	++	-	±	-	++	-	++s	-	+s
Rhmnose	-	++	-	±	-	+	-	+++	-	++
Salicin	±	±	±	±	-	++	+s	++	+	+
Sucrose	-	+	-	+	±	±	+	++	-	++
Xylose	+++s	++	++	++	+	++	-	±	-	±
Lactose	+s	++	-	++	-	+	-	++	-	++
Mannose	++s	++	-	+++	-	+	-	++	-	+
Maltose	+s	+s	±	++	-	++	+	+	+	++
Ribose	-	++	-	++	-	++	-	++	-	++
Sorbitol	±	±	++	++	-	++	-	+	-	+++
Dulcitol	±	±	-	-	+s	+	-	-	+	+
Aniline	+	+	+	±	±	±	-	+s	-	±
Adonitol	±	±	±	±	-	±	-	±	-	-
Glycerol	-	+++	-	+	-	+	-	+++	-	+++
Inulin	-	-	-	-	-	-	-	-	-	-
Sodium acetate	-	++	-	+s	±	++	-	++	-	+
" citrate	-	++	-	+s	-	+	-	++	-	++
" malate	-	++	-	+s	-	++	-	++	-	+
" pyrovate	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
" malonate	+++	-	+++	+++	+++	-	++	-	++	-

- not utilized; ± doubtful growth; + scarce growth; ++ moderate growth; +++ abundant &s:substrate mycelium only.

5- Amino Acids Utilization and Growth of Isolated Strains of Alkaliphilic Actinomycetes:

The alkaline medium was used to test the ability of isolates to use amino acids. Table (5) shows the growth of alkaliphilic actinomycetes on different amino acids at neutral pH. All isolates used glycine, serine, alanine, proline, phenylalanine, and methionine and grew in abundance. IsolateA3 grew on all amino acids used in the study and growth was minimal in the case of valine and glutamic acid, while isolateA6grew in abundance and growth was minimal on cysteine, cysteine, arginine, and alpha-amino butyric acid. Growth of isolateA11 was superior on most of the amino acids used, while it was not able to grow in the presence of alpha-aminobutyric acid only, while A10 was able to grow in abundance with all amino acids and sparse growth on the amino acids cysteine, asbestine cystine, and arginine (Table 5). Abundant growth of the isolate A8was observed on all amino acids used, and its growth was negligible in the case of histidine, cysteine and cysteine, as well as alpha-amino butyric acid, and grew well on the arginine, but the aerial mycelium did not appear.

Table (5): Amino acids utilization by isolated strains of alkaliphilicactinomycetes.

Amino acids	Growth of alkaliphilic actinomycetes strains at pH7				
	A3	A6	A8	A10	A11
Amino acid free medium	+++	+++	+++	+++	+++
Glycine	+++	+++	++	+++	+++
Serine	++	+++	+++	+++	++
Valine	+	+++	+++	++	++
Histidine	++	+++	+	++	+++
Alanine	+++	+++	+++	++	+++
Glutamic acid	+	+++	+++	++	+++
Proline	++	++	+++	+++	+++
Phenyl alanine	++	++	++	++	+++
Methionine	+++	++	+++	++	++
Cysteine	+++	+	±	+	++
Cystine	+++	+	±	+	++
Arginine	++s	+s	++s	+s	++s
Asparagine	++	+++	++	+	+++
α-iminobutyric acid	++	±	±	+++	

Growth: - no growth; ± doubtful; + scarce; ++ moderate; +++ abundant and s: substrate mycelium only.

6- Sensitivity of Isolated Strains of Alkaliphilic Actinomycetes To Antibiotics and Some Inhibitory Compounds:

The sensitivity of isolated strains of alkaliphilic actinomycetes was tested for some antibiotics such as gentamicin (100 µg / ml), penicillin C (10.0 µg), streptomycin (100 µ / ml) and vancomycin (50 µg / ml) by using tablets in specific doses. Some growth inhibitors were tested by adding them to the growth medium of these strains in doses, as shown in Table (6).

Table (6) shows that all strains are sensitive to gentamicin (100 µg / ml), streptomycin (100 µg / ml) and vancomycin (50 µg / ml) on the neutral medium, all of which are insensitive to a penicillin (10 µ).

A11 showed sensitivity to the neutral medium and did not show it on the alkaline medium. A6 showed sensitivity to streptomycin on the alkaline medium as well as A10 and A11 while A8 showed sensitivity to the antibiotic vancomycin (50 µg / ml) on the alkaline medium. The growth of the isolated strains was inhibited by crystal violet (10^{-4}) and phenol (10^{-1}). Phenyl ethanol with its two concentrations inhibited the growth of strains A3, A6, and A8, on both neutral and alkaline media, while strains A10 and A11 were able to grow in abundance with the first concentration on the alkaline medium and couldn't grow on the higher concentration of phenyl ethanol 3 $x10^{-1}$ (Table 6).

All strains were able to grow in abundance on the used concentrations of potassium tellurite. The strains A3, A6, A8, and A11 were able to grow abundantly on sodium azide with a concentration of (10^{-2}) and the growth of A3, A6 and A11 were abundant to medium on the highest concentration (2×10^{-2}).

It is clear from Table (6) that all strains grew will on the concentration of 4.0% g (w/v) of sodium chloride on the neutral media, while the growth was weak, on the alkaline media except in the case of strain A6, A11. At a concentration of 7% g (w / v) sodium chloride the growth of all strains was weak except for the strain A10 the growth was abundant and the growth ofA11 was medium on the neutral medium and never grew on the alkaline medium with salt. Upon using concentrations of 10% gm and 13% gm (w / v) of sodium chloride, none of the strains were able to grow except the isolate A10 had medium growth at a concentration of 10.0% g (w / v) and the rest of the strains completely failed to grow or were their growth doubtful.

7- Antimicrobial Activity of Isolated Alkaliphilic Actinomycetes:

Antimicrobial activity was tested for strains isolated against several bacteria such as *Escherichia coli* ATCC25922; *Pseudomonas fluorescens*; *Serratia marcescens* ATCC 800; *Staphylococcus aureus* ATCC25923; *Streptococcus pyogenes* ATCC 19615; *Salmonella typhimurium* ATCC14028; *Lactobacillus fermentous* and *Proteus Vulgaris* and fungi such as *Aspergillus niger*; *Penicillium chrysogenum*; *Fusarium oxysporum*; *Aspergillus fumigatus* and *Candida albicans*. The isolated strains did not have any inhibitory activity on the growth of these bacteria as well as the fungi used and their antimicrobial ability was tested on neutral and alkaline media on the fourth and seventh day of the growth of isolated strains.

Table (6): Resistance of isolated strains of alkaliphilic actinomycetes tosome antibiotics and some inhibitory compounds.

Antibiotic and inhibitory compound (µ g /ml)	Growth and inhibition of isolated strains of alkaliphilic actinomycetes									
	A3		A6		A8		A10		A11	
	7	10.5	7	10.5	7	10.5	7	10.5	7	10.5
***Antibiotics:** Gentamycin (100)	15	-	31	-	14	-	2	-	22	-
Penicillin G(10iµ)	-	-	-	-	-	-	-	-	25	-
Streptomycin(100)	20	-	25	25	19	-	22	18	26	24
Vancomycin (50)	20	-	30	-	25	14	36	-	35	-
Compounds: Crystal violet 10^{-4}	-	-	-	-	-	-	-	-	-	-
Phenol 10^{-1}	-	-	-	-	-	-	-	-	-	-
Phenyl ethanol 10^{-1}	-	-	-	-	-	±	±	+++	-	+++
3×10^{-1}	-	-	-	-	-	-	-	-	-	±
Potassium tellurit10^{-3}	++	+++	+++	+++	+++	+++	+++	+++	++	+++
10^{-1}	+	+++	+++	+++	+++	+++	+++	+++	++	+++
Sodium azide 10^{-2}	±	+++	±	+++s	-	++	-	±	-	+++
2×10^{-2}	±	+++	-	+++s	-	+s	-	±	-	++
Sodium chloride 4%	++	+	+++	+++	+++	+	+++	+	++	+++
7%	+	±	±	±	±	±	+++	+	++	-
10%	±	-	±	-	±	±	++	-	±	-
13%	-	-	-	-	-	-	-	-	-	-

*By inhibition zone (mm).Growth:- No growth; + Scarce; ++ Moderate; +++ Abundant &**s** substrate mycelium only

DISCUSSIONS

Soil from the Al-Fayoum region has been used to isolate the alkalophilic actinomycetes which produce the alkaline protease enzyme. These isolates were all found to be alkalo tolerant optional alkalis and were all able to degrade casein and skimmed milk in the growth medium.

Four isolates were characterized by the presence of two types of amino acid, di aminobemelic DAP meso- & L- (Diaminopimelic acid) as well as the presence of galactose sugar in the total cellular content of cells. Also, it was characterized by the presence of terrestrial mycelium and branched aerial mycelium that carries chains of spores more than twenty bacteria in the chain and all these characteristics indicate that these four isolates belong to the genus *Kitasatospora* (Omura *et al.*, 1982; Williams *et al.*, 1989; Holt *et al.*, 1994, Takahashi, 1999, 2017). Likewise, some authors (Labeda, 1988; Wellington *et al.*, 1992; Zhang *et al.*, 1997; Chung *et al.*, 1999; Tajima *et al.*, 2001) explained that this alkaline species has a high rate of meso- & L-DAP-like amino acids and the presence of terrestrial and antimicrobial mycelium more than 20 bacteria in the chain.

The fifth isolate was characterized by the absence of D-aminopimelic acid in possessing either L-or-meso in the total cellular extract and also there is no any type of sugar, and on this basis, there is no type of actinomycetes known to have such characteristics as it has a morphological form (it is a terrestrial mycelium and a branched aerial mycelium that contains chains of bacteria, more than twenty bacteria in the chain). Looking at the identification tables in all the references mentioned above, we find that these four types A3, A6, A8 and A10 of the genus *Kitasatospora* do not apply their characteristics to any existing species. Labeda (1988) explained that *Kitasatospora mediocidica* produces an antifungal, does not reduce nitrates, grows on fructose and sucrose and does not grow on raffinose or rhamnose and does not secrete soluble pigments in the growth medium while all isolates are A3, A6, A8, and A10, do not produce any kind of antagonists, do not grow on fructose sugar, Sucrose, raffinose or rhamnose while secreted pigments in the growth medium and all of them reduce nitrates in the alkaline medium while A3 reduce them in the alkaline and neutral medium.

As for *K. setae*, secrete setamycin as an antibiotic and produces yellow-brown pigments in the growth medium and thus does not resemble any of the isolated strains. As these isolates do not produce any antibiotics or fungi (Takahaashi *et al.* 1984; Labada, 1988). As for *K. phosalacimea* they secrete phosalacine (the anti-fungi), they reduce nitrates and grow on ravinose sugar, rhamnose, and sucrose and do not grow on the fructose and *K. griseola* secrets antibiotic setamycim as well as it grows on raffinose and does not grow on other sugars and produces cochineal pigments (Tahakashi *et al.*, 1984; Labeda, 1988).

By studying all known species of this genus such as *K. cystarginea* (Kusakabe and Isono, 1988); *K. cochleata* and *K. paracochleata* (Nakagaito *et al.*, 1992; Zhang *et al.*, 1997); *Kitasatospora kimorexae* 90-GT-302 (yeo *et al.*, 1994) *K. cheerisanensis* (Chung *et al.*, 1999); *K. cineracea* and *K. Niigatensis* (Tajima *et al.*, 2001) and *Kitasatospora sp.* MBT66 (Wu *et al.*, 2015) and Takahashi (2017) confirmed that the four isolates differ in many physiological and phenotypic properties. The most important characteristic of these species is that they grow on alkaline media better than their growth on neutral media and that their physiological properties appear better on alkaline media than in neutral media and therefore can be considered new species of the *Kitasatospora*. Zhang *et al.* (1997) laid the foundations for the definition of the genus *Kitasatospora*, namely that this genus may be separated from the genus *Streptomyces* by the presence of a high percentage of meso-DAP / L-DAP in the total cellular extract of cells, and this ratio

ranges between 49-89% in the genus of strains of *Kitasatospora* and 1-16% of m-DAP in the *Streptomyces* genus strains. Galactose sugar is present in the total cellular extract of cells of the genus *Kitasatospora* and it is not found in the genus *Streptomyces*.

A tight clade *Kitasatospora* in the process of 16s rRNA analysis to identify the sequence of nitrogenous bases compared to all actinomycetes, which completely excludes the types of *Streptomyces* genus from the genus *Kitasatospora*, and in the process of determining the gene distance 16s-23s rRNA *Kitasatospora* & *Streptomyces* distinct clades genera. The genus *Kitasatospora* can also be identified by the presence of specific nitrogen bases in the sequence of 16s rRNA (fingerprint) nucleotide signatures as well as in the distance between rRNA 23s - 16s and determine the distinct type of this genus *Kitasatospora setae* (Omura *et al.*, 1982).

Recommendations:

It is recognized that all these isolates had high alkaline proteolytic activity so, it is important to identify the alkaline protease(s) in those isolates because this enzyme has many industrial applications especially in alkaline conditions.

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