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Research Article

Screening for Antimicrobial Activities and Enzymatic Activities Production of Some Actinomycetes spp. Isolated from different Soil Samples from Hilla Province

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Abstract

One hundered and fifty soil samples were collected from different city of Babylon/Iraq. Thirty three Actinomycetes isolates were obtained, purified and identified according to morphological properties and biochemical ltests. All isolates were tested for the irantimicrobial activities and screened for production of some important enzymes such as amylase, catalase, protease, cellulose and lipase. There salts obtained that 13 isolated have such potential and all 13 isolates have amylase activity. The most active isolates in production of study enzymes were KO-AG18, HI-SA24 and KO-RS27. Most 13 Actinomycetes isolates were showed antimicrobial against some Gram-positive bacteria (*Staphylococcus albus, Staphylococcus aureus and Streptococcus pyogenes*) and Gram- negative (Klebsella pneumoniae, Escherichia coli, Serratia marcescens, *Pseudomonas aeruginosa* and *Aeromonas hydrophila* due to inhibition zone diameter (mm) ranged from 4-37 against gram negative and from 6-37 against gram positive bacteria.

Keywords: Isolates; Antimicrobial Activity become isolates; antimicrobial; activity

Introduction

Actinomycetes are gram-positive bacteria, and it is adiverse group of heterotrophic prokaryote forming hyphae at some stage of their grow than widely founds in both terrestrial and aquatic environments. Actinomycetes are the major type of soil bacteria that produce huge natural metabolites. The antibiotics and enzymes are the most and important metabolites [1].

They produce some enzymes which Crashing of complex organic materials in sediments or soil as cellulases, protease, gelatinase, ureases, lectinases and amylase [2]. Amylase is the most commonly used enzyme in the starch in- dusty. It has huge Applications in industry such as in the syrup production which made from oligosaccharide and monosaccharide.

Also in industries of textile, amylase is used for resizing of clothing materials [3]. Actinomycetes are good decomposers of organism materials and production amylase was cleared by several strains of Actinomycetes [4].

Cellulases produced by Actinomycete are degrade extracellular enzymes and inducible [5]. Actinomycete can produce cellulase through their growth on Actinomycete materials of cellulose. Thus, introduction of cellulolytic enzymes by microbiology is degraded as beneficial microbiological tool for recovery of bioenergy from degraded cellulose [6] and have gained significant attention due to their wide applicability in various industrial processes including pulp and paper, textile, laundry, biofuel production, food and feed industry, brewing and agriculture [7]. Cellulase is involved in hydrolysis of cellulose, one of abundant organic compound which can be easily converted to new products that have significant commercial-interest. Bioconversion of cellulose to mono- sugars has been studied as researchers seek to produce bioethanol and bio-based products, food and animal feeds, and many valuable chemicals [8]. One of the most important groups of enzymes that produced commercially and for industrial purposes are Proteases [9]. They have important applications in a many of industrial applications and some processes such as food, detergents, leather and pharmaceuticals [10]. In spite of considering Actinomycetes to be among the important- producers of enzymes and antibiotics [11], However, the pres-

ent studies concerning that proteases produced by actinomycetes to be most important of secondary products or metabolites that are exploited [12]. This study aimed to isolate various Actinomycetes species from different soils in Hillah city and screening it for their potential to produce some hydrolytic enzymes (cellulase, amylase, Catalase, protease and Lipase).

Materials and Methods

Collection of Samples

The different soils were collected randomly from different city in Babylon /Iraq. These samples were kept in sterile and dried polyethylene sac and kept until used at room temperature.

Isolation of Actinomycetes

Soil samples were treated with calcium carbonate for 24h, then subjected to serial dilution till 10-6. One milliliter of each dilution cultured on ISP2 media by pour plate method [13]. The plates of bacteria were put in incubator for seven days at 28Co. The colonies were sub cultured several times on the same media to get pure isolated colonies.

ISP-2 (International Streptomyces project-2 agar medium) (malt extract Yeast extract agar) [14]

This media was used for growing Actinomycetes isolates. It p prepared from the following chemicals: Bacto-Yeast Extract 4.0g, Bacto-Malt Extract 10.0g, Bacto-Dextrose 4.0go90op-[]\k, Distilled water 1.0 Liter ,then add agar-agar 20.0g. Adjust pH to 7.3. Liquefy agar by steaming at 100°C for 15-20 minutes. Then sterilize by autoclaving at 121°C, for 15minute.

Screening of the antimicrobial agent

Each of the Actinomycetes spp isolates were inculcated in a 500 ml flask containing 250 ml of ISP-2 broth of pH 7.2 and incubated at 28°C for 10 days. After incubation period, the fermented broth is filtered through a what man No.1 filter to separate the cellular components from the culture filtrate, then the broths were centrifuged at 6000 rpm for 15 minutes and the cell free supernatant was taken. Culture supernatants were extracted with an equal volume (1:1 v/v) of appropriate of ethyl acetate before shaking vigorously for one dryness and the residue obtained was weighted [15]. The residue that obtained from evaporated flask in water bath is weighed and used for antibacterial analyses as secondary screening test. This performed by the agar well diffusion method against standard pathogenic organisms organic layer added to the above organic layer [16]. Hour to complete the extraction and formation of two layers (organic layer and aqueous layer). The ethyl acetate phase that contains the antibiotics was separated from the aqueous layer phase, then the ethyl acetate was evaporated in water bath at 80 °C to 90°C.

Enzyme Screening

Production of Amylase: Actinomycetes isolates were screened for amylolytic properties by starch hydrolysis test on starch agar plate. The streaked Actinomycetes isolates were incubated at 28±2°C for 7 days. After the incubation 1% iodine solution (freshly prepared) was flooded on the starch agar plates and a clear zone of hydrolysis were considered as amylase producers.

Cellulase activity

By using CMC agar medium. The bacterial isolates from soils were cultured on CMC agar plates .The plates were incubated at 28±2°C for 7 days. After the incubation apply a clear decomposition zone on the CMC medium after immersing the dish with 1% Congo Red dye were indication for producing cellulose enzymes [17].

Catalase activity: (Slide Test)

Place a small amount of the colony on the surface of a clean glass slide and add 3 drops of 3% hydrogen peroxide H_2O_2 and mix well.

The positive result is the speed of oxygen evolution and bubbles on the slide (within 5-10 sec.) The negative result is that no bubbles or bubbles are small or unclear.

Activity of Lipase

Peptone-tween agar plates (PTA) were prepared according to [18]. Isolates of Actinomycetes were streaked in Peptone-tween agar and plates were cultured for seven days at 28±2°C. Appearance of Opalescent structures around the colonies is evidence of the lipase activity [19].

Activity of Protease

Isolates of Actinomycetes were cultured on Skim milk agar (SMA) and they were incubated for 7 days at 28±2°C. Appearance of a clear zone on plates of SMA is evidence of the of protease activity [20].

Results and Discussion

Actinomycetes a diverse group of heterotrophic procaryotes forming hyphae at some stage of their growth and producing different types of antibiotics of medical and agriculture applications. Actinomycetes spp. Responsible of 80% of total common antibiotics [21]. At the present study all 13 isolates were identified according to their morphological features and biochemical tests. The results showed that all of these isolates return to Streptomyces species, they were giving the characteristic symbolsas showed in Figure (1) and table (1), the antimicrobial activity of different Actinomycetes isolates were tested. The 13 Actinomycetes isolates were showed antimicrobial ability against some bacteria Gram- positive bacteria (Streptococcus pyogenes, Staphylococcus albus, and Staphylococcus aureus) and some Gram- negative (Klebsella pneumoniae, Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa and Aeromonas hydrophila [22] as showed in table (2) & figure (2). Study at the same field, isolated tow antibacterial active Actinomycetes from different soil samples of Hillah.

Site	*Sample no.*	*Abbreviated*
Al-Mahaweel	16	MA-AG16
Al-Kothar	18	KO-AG18
Al-Hilla	24	HI-SA24
Al-Kothar	27	KO-RS27
Al-Amam	34	AM-AG34
Al-Hamza	28	HA-RS28
Al-Hamza	37	HA-CL37
Al-Kasim	41	KA-SA41
Al-Mashrwia	42	MA-CL42
Al-Mashrwia	43	MA-CL43
Al-Mussiab	46	MU-AG46
Al-Amam	52	AM-AG52
Al-Hamza	55	HA-RA55

Table 1: Sampling sites.

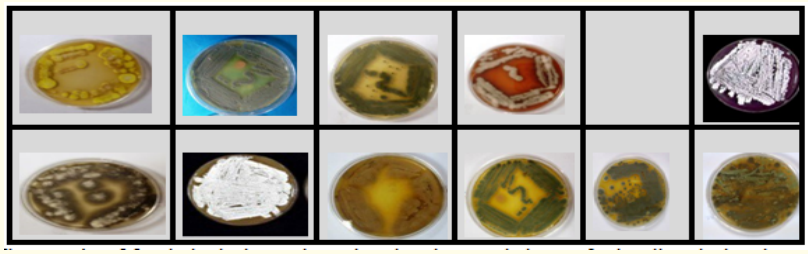

Figure 1: Morphological and cultural characteristics of locally isolated Actinomycetres, growing on ISP2, after 7-14 days at 28±1°C.

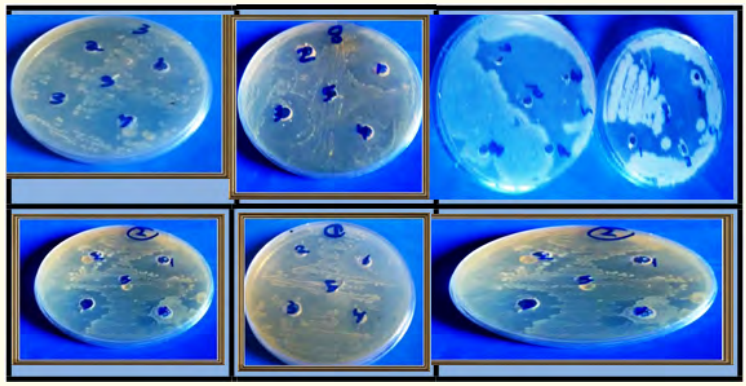

Figure 2: Inhibition zone diameter (mm) of culture filtrate extract of the active isolates against tested organisms on Mueller Hinton agar medium.

Diameters (mm)								
Actino. spp Symbol	***Pathogenic gram positive and negative bacteria***							
	E. coli	***K. pneum.***	***P. aerug.***	***S. typhia.***	***A. hyDro***	***S. aureu.***	***S. pyog***	***S. albus***
MA-Ag16	15	16.8	30.2	19.7	0	28.2	35	17.2
KO-AG18	37	32	18.5	33	33	25	37	32
HI-SA24	33	33	36	0	23	31	24.5	0
KO-RS27	8	15.3	33	12.4	19	28	8	23
HA-RS28	9	6	7	13	32	0	19	9
AM-AG34	20	0	15	33	8	12.4	28	24
HA-CL37	8	9	8	6	33	35		15.3
KA-SA41	15.3	0	8	12.4	0	9	7	23
MA-CL42	9	20	0	19	8	6	13	19
MA-CL43	0	13	7	20	28	24	33	6
MU-AG46	19	4	8	33	8	12.4	0	13
AM-AG52	7	36.3	9	19	8	13	0	0
HA-RA55	8	6	0	23	15.3	8	33	20

Table 2: Inhibition zone diameter (mm) of culture filtrate extract of the active isolates against tested organisms on Mueller Hinton agar medium.

Actinomycetes are important group of soil bacteria that have a main role in some applications such as recycling of most organic matters by their ability to produce of some hydrolytic enzymes [23]. In our study Actinomycetes were tested to produce five hydrolyzing enzymes (catalase amylase, cellulose, protease and lipase), in addition to their antimicrobial activity. All isolates of Actinomycetes were cultured on starch media to test their ability to analyze the starch by immersing the dish with iodine solution (1%).

The appearance of clear zone around the colonies surrounded by purple color background refer to positive result. 13 isolates were positive (Figure.3, Table.3). There are five isolates of Actinomycetes were positive for protease production which were KO-AG18, HI-SA24, KO-RS27, HA-CL37and MA-Ag16 that grown on skim milk ,while the rest isolates were negative as showed in table 3.

To test the susceptibility of bacteria to the production of cellulose enzyme was developed on the center of CMC for 5 days and then colonies were flooded with the dye of Congo red means positive result; the 8 isolates KO-AG18, HI-SA24, KO-RS27, KA-SA41, MA-CL42, MU-AG46, MA-Ag16 and MA-CL43 [24] while the rest isolates were negative as showed in table 3 and figure 4. [25] Cleared that the Actinomycetes degraded as one of the major bacteria communities that have ability to hydrolysis cellulose the plant cell walls. Actinomycete cellulases degraded as inducible enzymes which produce as extracellular enzyme [26]. The 6 positive lipase strain were confirmed by the production of clear zones (opalescent) around the Actinomycetes [27] as results showed in table (3) some of Actinomycetes isolates have ability to produce catalase [28] as show in figure 5 and table 3. The environmental conditions affecting the on enzyme activities such as (pH, salinity and temperature) are major factors that effect on enzymes production.

Actinomycetes isolates	***Enzymes activity***				
	Amylase	***Protease***	***Lipase***	***Celluloase***	***Catalase***
MA-Ag16	+	+	-	+	+
KO-AG18	+	+	+	+	+
HI-SA24	+	+	+	+	+
KO-RS27	+	+	+	+	+
HA-RS28	+	-	+	-	+
AM-AG34	+	-	-	-	-
HA-CL37	+	+	-	-	-
KA-SA41	+	-	-	+	-
MA-CL42	+	-	+	+	+
MA-CL43	+	-	+	+	-
MU-AG46	+	-	-	+	+
AM-AG52	+	-	-	-	-
HA-RA55	+	-	-	-	-

Table 3: Enzyme activity of Actinomycetes Isolates.

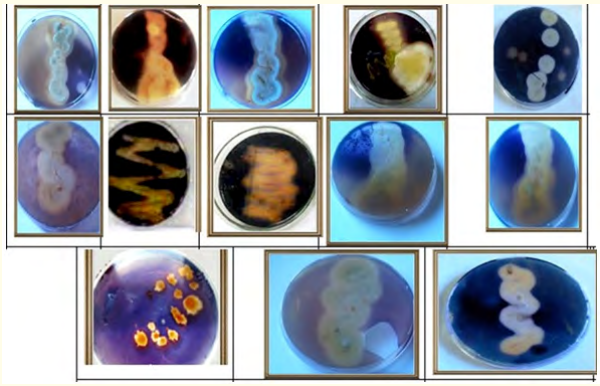

Figure 3: positive amylase isolates.

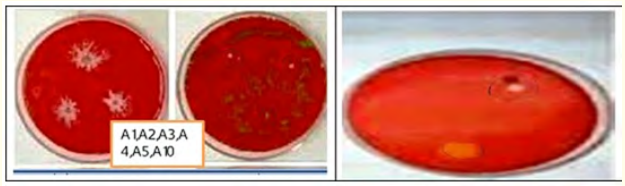

Figure 4: positive cellulase isolates.

Conclusion

Actinomycetes are important group of soil bacteria that have a main role in some applications such as recycling of most organic matters by their ability to produce of some hydrolytic enzymes [23]. Actinomycetes are the major type of soil bacteria that produce huge natural metabolites. The antibiotics and enzymes are the most and important metabolites [1].

They produce some enzymes which Crashing of complex organic materials in sediments or soil as celluloses, protease, gelatinase, ureases, lectinases and amylase [2].

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