
Antifungal Activities of Actinomycetes Isolated from a Sample of Iraqi Soils.

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Abstract:

Background & objective: This work aimed to isolate and identify naturally actinomycetes from different samples of Iraqi soils producing antifungal activity on different culture media.

Materials & Methods: Twelve soil samples were collected from different sites of Baghdad (Jadria, Yarmouk, and Kadisa) in Iraq at summer season 2007. The actinomycetes were isolated on Starch – Casein – nitrate at different incubation temperatures and screened for antifungal activity on modified Bennett agar, Yeast extract - malt extract agar and Gaza agar against fungi including yeast (*Candida albicans*) and molds (*Fusarium*, *Penicillium*). All isolates of actinomycetes were tested for sensitivity to commonly used antibacterial drugs employing Kirby – Bauer method. Morphological characterization of these isolates were studied according to ISP (International streptomyces project) media which included color of aerial, substrate mycelium, soluble pigments and their PH sensitivity, form of sporophore. Cell wall analysis was carried out for these isolates.

Results: During a search for antifungal activity, either two actinomycetes strain were isolated from different samples of Iraqi soils. Twenty – Two isolates showed strong activity against yeast, & molds. All isolates were found to be belonging to genus streptomyces. Modified Bennett agar is good medium for antibiotic production at 30C°. These isolate were sensitive to all antibacterial drugs in this study. Their cell wall consists of Peptidoglycan.

Conclusion: The metabolite from Iraqi actinomycetes was active against yeast & molds. However, this needs further investigation using purified form of the active component of antibiotics from local isolates.

Key words: Actinomycetes, antifungal activity Antibiotic.

Introduction:

Actinobacteria or actinomycetes are a group of Gram – Positive bacteria with high G + C ratio. They include some of the most common soil life, Actino bacteria are well known as secondary metabolites producers and hence high pharmacological and commercial interest^[1].

The pioneering work of Waksman showed that actinomycetes are capable of producing medically useful antibiotics^[2]. Actinomycetes are bacteria – Large, diverse group of gram – positive bacilli with a tendency to form chains or filaments^[3]. The filaments may be long or short, depending on the species. They form an aerial mycelium, much smaller than that of fungi and may species produce a sexual spores called conidia,^[4] and a unique group having different morphological cultural, biochemical and physiological characters.

Actinomycetes isolated were found to have potent antifungal activity antimycotic property greater potency than griseofulvin^[5]

However many compounds, polyenes in particular, cannot be used because of their toxicity while therapy, agriculture and industry

Materials & Methods

-Collection of soils samples: Twelve samples were collected from different locations in Baghdad (Jadria, Yarmouk, and Kadsia) of Iraqi soils at summer season in sterile container and maintained a 4 C° until analysis.

-Isolation and screening of antifungal actinomycetes: Actinomycetes were isolated on SCAN (starch – casein nitrate) agar medium supplemented with 40µ/ ml actidion by sterile dilution methods at different incubation, temperatures^[6]. All cultures were purified by streak plate technique and screened for their antifungal activity.

-Characterization of the selected actinomycetes isolates:

Morphological feature of the actinomycetes were studied by inserting sterile coverslips in the solid starch casein medium at angle 45°. Spore suspension of actinomycetes were inoculated at the intersection of the medium and coverslip^[7]. The coverslip were observed under light microscope. Cultural characterization was done on ISP (International streptomyces project) media vize, yeast extract – malt extract (ISP2), Oat meal agar (ISP-3), Inorganic salt starch agar (ISP4), glycerol asparagin agar (ISP- 5)^[8]. Chemotaxonomic studies were done by analyzing the cell wall for 2, 6 – diaminopimelic acid and whole sugar content^[9]

- *Antibacterial sensitivity test:*

Well known Kirby – Bauer technique^[10] is applied for detection of susceptibility of the isolates for the commonly used antimicrobial agent. The results as sensitive or resistant were compared to a standard zone of growth inhibition table (1).

-Antibiotic activity: By using double layer methods⁽¹¹⁾ on modified Bennett agar⁽¹²⁾ YEMA, Guaza

agar ^[13]. Plates were incubated At different temperatures (25 , 30 , 37 , 45 , and 50) C° for 7 days then inverted for 40 min over chloroform in a fumhood. colonies were then covered with 0.6 % agar layer of sabouraud agar for (yeast) and malt extract agar for (molds) these media previously

seeded with test organisms. Incubation temperatures were 37 C° for yeast and 25 C° for mods. inhibiting zone (20mm) or more was recorded after 24 and 48 hours. Strains of yeast and molds were obtained from Central General Public Health Laborabry, Baghdad, Iraq).

Table 1- Interpretation of zone inhibition by using Kirby & Bauer method (disc diffusion methods)

<i>Antimicrobial agent</i>	<i>Code</i>	<i>Disc potency Mcg \ Disc</i>	<i>Diameter of zone Inhibition</i>		
			<i>Resistant</i>	<i>Intermediate</i>	<i>Sensitive</i>
Ampicillin	AM	10	≤11	12-13	≥20
Cefotaxime	CTX	30	≤14	15-22	≥23
Cephalexin	KF	20	≤14	15-17	≥18
Chloramphenicol	C	30	≤12	13-17	≥18
Ciprofloxacin	CIP	10	≤15	16-20	≥21
Clindamycin	CN	2	≤12	13-17	≥18
Tobramycin	TM	10	≤13	13-14	≥15
Erythromycin	E	15	≤13	14-17	≥18
Ampiclox	AMP	30	≤14	15-16	≥17
Gentamycin	GN	10	≤12	13-14	≥15
Nalidixic acid	NAL	30	≤13	14-18	≥19
Pencillin – G	PG	6	≤20	21-28	≥20
Rifampicin	RA	5	≤16	17-19	≥22
Co –Trimoxazole	SXT	25	≤18	19-23	≥24-32
Amoxicillin	AMX	10	≤19	-	≥29
Amikacin	AN	30	≤14	15-16	≥17

Results:

As shown in table (2) 82 local actinomycetes isolated were isolates from different site of Iraqi soil's on starch casein medium which was found its transparency facilitates colony observation. In this table (2) the temperature of isolation of 82 strains and the number of isolates with antifungal activity is shown.

The selected isolates could grow well on all ISP media and produced water soluble brown pigment, the aerial mycelium was grey on all media. The spore chain were spiral type each had more than 12 spores per chain, the cell wall of the strains contained 2, 6,

diaminopimelic acid based on these data, the strains was designolt as streptomyces sp. all these iaoltes were sensitive to antibacterial drugs according to Kirby – bauer Methods.

Antibiotic production, table (3) show the antifungal activity of 22 of 82 strains obtained from Iraqi soil's an three different media and four different incubation temperatures against mycetes test microbes yeast and molds.

The antifungal activity of 22 isolates at 30 C° on modified Bennett Medium which was shown in table (4)

Table 2 : Total number of actinomycetes and of isolated with antifungal activity isolated at different temperature

Isolation temperature	No of Isolates	strains with antifungal Activity
25 C°	16	4
30 C°	24	6
37 C°	30	8
45 C°	10	4
50 C°	2	0
Total	82	22

Table 3 : Percentage of active strains on different media

Medium	Temperatures				
	25 C°	30 C°	37 C°	45 C°	50 C°
Yeast Extract – malt Extract agar	63	63	59	31	-
Modified Bennett	54	64	45	27	-
Gaza agar	22	36	18	-	-

Table 4 : The activity of 22 actinomycetes on Modified Bennett agar at 30 c°

No Isolate	Isolation temperature	Activity against		
		<u>C . albicans</u>	Fusarium	Penicillium
1	25 c	-	+	+
2	25 c	-	+	+
3	25 c	-	+	+
4	25 c	-	-	-
5	30 c	+	+	+
6	30 c	+	-	+
7	30 c	+	+	+
8	30 c	-	+	-
9	30 c	+	+	-
10	30 c	+	+	-
11	37 c	-	-	+
12	37 c	+	+	+
13	37 c	-	-	-
14	37 c	-	-	-
15	37 c	+	+	+
16	37 c	+	+	+
17	37 c	-	-	-
18	37 c	+	+	+
19	45 c	-	-	-
20	45 c	-	-	-
21	45 c	+	+	+
22	45 c	-	+	+

Discussion :

In ascreening program aimed the discovery of antifungal substance Iraqi isolates of actinomycetes on isolation medium starch –casein which was found to be good medium for the isolation and inducing antifungal activity in these isolates of actinomycetes, table (2). Other factors such as temperature may play important role in isolation and antibiotic production which agrees with ^[15].

All local isolates were characterized as bacteria of actinomycetes to be belonging to genus streptomycetes . This finding which agrees with ^[16] that showed that actinomycetes are a broad group of bacteria that thread – like filaments in the soil . In our study is also seen the resemblance of actinomycetes species contain peptidoglycan in their cell walls and are sensitive to antibacterial antibiotics which agrees with ^[4].

The antifungal activity of 22 isolates obtained from different sites of Iraqi soil at 30 c on a modified Bennett medium which appear the best for antibiotic production

There are relationships could be detected between isolation temperature & temperature of antibiotic production .

From the collected data it was well conclusive that the result indicate that the active isolates were found to produce antifungal activity at different temperatures against yeast (Candida albicans) and molds (Fusarium and penicillium). This may be due to their extracellular metabolites of altering its permeability or due to the suppression of spore germination or the diffusion of antibiotic produced by local isolates into the medium which effects fungi & result in their growth inhibition which agrees with

[17-19], who show the active isolates of actinomycetes can be investigated for use as biofungicides.

However, these strains in our study need further investigation using purified powdered form of active component of antibiotic.

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