JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

ISOLATION OF BACTERIAL SPECIES PRODUCING ANTIBIOTICS FROM THE SOIL OF THE CITY OF TIKRIT

Sundus Jassim Muhammad[1], Dunia Kamal Salim[2], Marwa M.Mahdi[3]

[1,2,3,]Department of Biology, Collage of Science, University of Tikrit, Tikrit, Iraq.

Received: 14 March 2020 Revised and Accepted: 8 July 2020

ABSTRACT: Objective This study was conducted with the aim of obtaining different *Bacillus* bacteria isolates from different regions of Salah al-Din Governorate and studying its susceptibility to antibiotic production.

Material and methods:-25 soil samples were collected from different area of Tikrit city and 20 isolated Bacillus bacteria were obtained. Phenotypic and biochemical tests were performed for the purpose of their diagnosis. Seven isolates were obtained belonging to the sex of *Bacillus subtilis*. The susceptibility to inhibit the pathogenic bacterial species were tested and the study included , *Pseudomonas aeruginosa and Escherichia coli , Proteus mirablis,Staphylococcus aureus.*

Results: The results showed that Bacillus bacteria are highly effective in inhibiting the bacterial species *Pseudomonas* and *Staphylococcus*, as they were in the range of 22-31 mm, while their effectiveness was less inhibiting *Proteus mirablis and Staphylococcus aureus* , as the inhibition diameter ranged between 15-22.

The ability of *Bacillus* bacteria isolates to grow at different temperatures has also been tested, as they showed that they grow well at temperatures 40 and 45 ° C, while the growth of isolates 1, 4, 5, and 6 was weak at a temperature of 50 ° C. The rest of the isolates did not show any growth in this degree, and not all the isolates were able to grow. At a temperature of 10 ° C, **Conclusion:**the ability of Bacillus bacteria to grow in high salt concentrations was showed a good growth in high salt concentrations 3% and 7%, while no growth appeared at the concentration of 10%.

KEYWORDS: ***Bacillus,*** **Antibiotics, Antibacterial activity**.

I. INTRODUCTION:

The emergence and spread of multiple bacterial isolates resistant to antibiotics led researchers to try to find new alternatives to familiar antibiotics in an attempt to find solutions to this problem. The soil is the largest reservoir of microorganisms producing many substances with an anti-bacterial effect, which is called Microbial antagonisms [1]. The antagonistic effect between microorganisms plays an important role in biological control, which makes it receive great attention from researchers who are trying to benefit from this effect.

One of the most important types of antagonism is the antagonism caused by the secretion of microbiology of special substances known as antibiotics [2] .other antibiotic-like substances called Bacteriocins [3] and other materials like proteins and enzymes such as protease (Amylase [4,5]. Bacillus subtills genus is one of the important species in this field and it is considered as a safe microorganism and was given the title (Generally Recognized as safe) GRAS by the American Food and Drug Organization [6]. Bacillus bacteria is belong to the Bacillceae family and it is positive for Gram stain in the early stages of growth . The first hours of growth after that dye varies between positive to negative for Gram stain and this is why the gender of Bacillus is known to be heterogeneous to the Gram stain [7]. It is an Pathogenic bacteria that can be found in the soil and helps to stabilize atmospheric nitrogen and is a good source of industrial production proteins because of their large size compared to other organisms [7] and is a solvent of phosphate in the soil [8] and it can produce various sources of carbon such as glucose and lactose [9] and some types are positive for testing for the methylation test and examination of starch, urea and gelatin, it can move and grow at different temperatures and can tolerate salinity and its agricultural characteristics are milky white with a diameter of 1-3 mm [10] There are members of this species widely in various environments where they are found in the soil very broadly and for this (This study was conducted for the purpose of isolating species of this species and studying its inhibitory effect on pathogenic bacteria

II. MATERIALS AND METHODS

25 soil samples were collected from 7 different locations from Salah al-Din Governorate and taken with a depth of 5-10 cm after scraping 1 cm from the soil surface. 100 grams were weighed from each of the sites covered by the study and placed in a nylon bag and recorded the required information and after transferring the samples to the laboratory Decimal fears were conducted for them, and the first three fears were cultured in the Nutrient Agar media to obtain a large number of isolates of the bacillus bacteria. Then the dishes were incubated at temperatures ranging between 15-45 °C for a period of 24-48 hours and then moved each developing colony to the same medium that was planted for a purpose Purification and preserved at the slant Nutrient medium at temperature 4 °C until used to take the necessary tests

* Growing isolates were diagnosed using phenotypic and biochemical examinations and adopting internationally followed scientific sources for diagnosing bacteria [11]. The diagnosis included the following examinations phenotypic and transplant traits and movement test catalase test, indole test, IMVC urea test [12].

Bacterial isolates used in the study

Diagnostic bacterial isolates were obtained from the laboratory of graduate studies, Faculty of Science, Tikrit University, and biochemical and diagnostic tests were performed according to the source [13,14] for the purpose of ascertaining their types. The following types were used.

Staphylococcus aureus

Psedomonas aeruginosa

Escherichia Coli

Proteus mirabilus

Bacillus subtilis inhibition test against bacterial isolates

To study the inhibitory efficacy *Bacillus subtilis* against bacterial isolates under study mentioned in the previous paragraph above, the Well diffusion method was used according to[15].

filtrate of the liquid cultures of *bacillus* attended by the development of the *bacillus* bacteria in the Nutrient broth medium pH 7 and incubated the tubes at 37 °C for four days.

* Perform centrifugation (5000 course/ min) for 10 minutes, then take the filtrate, discard the precipitate, then mix the filtrate with an equal volume of Ethyl acetate. The filtrate was concentrated with an Evaporatory device on 50 °C (Iwaki, shzuoka Japan) and pathogenic bacterial isolates were cultured over the Nutrint Broth medium and incubated tubes at 37 °C for 24 hours. Acidity was stuck compared to the standard MacFarland tube and transported (0.1 mL) of suspension to each bacterial species. It was spread on the surface of the Nutrient agar medium and then the dishes were left to dry at laboratory temperature

Holes was done on the surface of the solid agar with a diameter of 6 mm, then the holes were filled with 100 µl of the leaky liquid cultures for *Bacillus subtills*. Then the plates were left to dry at room temperature, and then incubated at 37 °C for 24 hours, and then measured the diameters of the inhibition areas around each pit

Bacillus subtills tolerance test for high temperatures and high salt concentrations

The isolates that showed good ability to produce antibiotics were elected and the necessary tests were carried out to withstand high temperatures and salt concentrations, as follows: test tubes containing slant Nutrint agar were used where they were inoculated with bacteria and incubated in two groups, the first at temperatures 40-45-50°C for two days and the second in degrees 5-10-20°C for a period of 2-7 days [16].

* 3%, 7% and 10% of sodium chloride were added to the Nutrient Brouth media, and the tubes were inoculated with the bacterial culture, then incubated for 2-4 days. The result is considered positive with the appearance of turbidity in the medium [17].

III. RESULTS AND DISCUSSION

The results of isolation showed that 7 bacterial isolates producing antibiotics were obtained from a total of 20 *Bacillus* isolates obtained from collecting 25 samples of soil from different locations in Tikrit and its suburbs.

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

The isolates varied in their ability to produce antimatter depending on the diameter of the inhibiting zone towards the test bacteria It was noted that the most efficient isolates were isolated from soils planted with vegetable and fruit crops. The isolates that showed high efficiency were chosen from among the 20 obtained isolates, its species and genus were confirmed through agricultural and biochemical tests for the purpose of their final diagnosis. Tests The cultures and biochemical conditions of the bacterial isolates produced, depending on [18] that they carry the qualities of the genus *Bacillus*, as shown in the table (1). where 7 isolates bearing the characteristics of the bacteria of *Bacillus subtilius* were obtained, and the results of the tests on these isolates showed that they are positive bacilli and are found in chains . Casual pairs consisting of Obligate aerobic bacteria, its colony circles with a light yellow color, serrated edges on Nutrient Agar, negative for oxidase and indole, positive for catalyzes and movement, methyl red, negative for urea testing, nitrate reduction, produced for acid, H2S, gelatin fermentation, sugars fermentation (glucose), blood analyzed , After diagnose the species and genus of bacterial isolates. An experiment was conducted to detect the inhibitory activity of the local *Bacillus subtillus* against the pathogenic bacterial species under study. The results showed, through Table (2), the increased inhibitory efficacy of the *Bacillus subtillus* against the bacterium- species both *Staphylococcus aureus* and *Pseudomonas aeruginosa augosa* Isolation 3 was recorded as the highest inhibition zone

It was (32 and 30) for both *Staphylococcus aureus* and *Psedomonas aerugenosa*, respectively, while isolation number 5 recorded the lowest inhibition region for both previous types, it was (21 and 20) mm, respectively, while the inhibitory capacity of isolates was Localities are lower for negative bacterial species, as diameters of inhibition regions ranged between 14-21 mm for *E. coli* and *Protius* bacterial species.

The reason for this may be attributed to the nature and structure of the cellular wall of bacteria, where it was found that the thickness of the cell wall plays an important role in the bacterial cell being resistant or sensitive to antimicrobial material [19]. The bacteria that are positive for the gram stain, due to its possessing the internal resistance represented by the outer layers (which are included in the composition of Lipopolysacchaides (LPS), which lacks the mechanism of bacteria that are positive for the gram stain [20], as it was found that this layer acts as a permeability barrier, which limits the entry of many chemicals, including antimicrobial substances, while the cell wall of bacteria that are positive for a gram stain consists of peptidoglycan and tochic acid [21] These substances do not prevent the entry of antimicrobial substances into the bacterial cell. It was found that chemicals with high molecular weights can easily pass the cell wall of *Staphylococcus aureus*, and this explains the sensitivity of these microorganisms to many antimicrobial substances such as antibiotics, chemical disinfectants and other materials [22]. Inhibition of the pathogenic bacterium *Psedomonas aerugenosa* is of particular importance, as *Psedomonas aerugenosa* is one of the most productive species for natural resistance and resistance resulting from many mutations that constantly occur towards antibiotic [23].

The solid feed media was also used in testing the ability of local isolates of *Bacillus subtillus* to grow at different temperatures and included (5,10, 20, 40, 45, 50) °C. The results shown in Table (3) showed that the isolates managed to grow well in degrees of The high thermal (40,45) °C while the growth was average at a temperature of 50 °C for most isolates while the isolates did not show 7,4,3 any Growth in this temperature of 50 °C, which is consistent with what has been reached [24].

Likewise, not all isolates showed the ability to grow at a low temperature of 5°C and there was growth of varying degrees between the temperatures between the highest and the lowest. The change in thermal temperatures has an effect on the phenotypic and physiological characteristics, and that any change in the temperature values from the optimal values for the growth of microorganisms affects growth and reduces their production of secondary metabolic compounds by affecting the effectiveness of enzymes, and this applies to what has been reached by mechanism [25] The feeding medium was also used with the addition of NaCl salt in testing the susceptibility of bacterial isolates to growth with different salt concentrations and included 3%, 7% and 10%. The results shown in Table No. (4) showed that all isolates grow well and through the appearance of turbidity.

At a minimum concentration of 3%, and by estimating it with a spectrometer, none of the isolates showed growth at a concentration of 10%, while the isolates varied in its ability to grow at a concentration of 7%.

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

Table (1) Biochemical test results for *Bacillus subtills* bacteria isolates

	Test	Result
1	Catalase	+
2	Oxidase	-
3	Motility	+
4	Starch hydrolysis	+
5	Indole	-
6	Voges-proskauer	+
7	Citrate utilization	+
8	Glucose with Gas	+
9	Lactose	-
10	Galactose	+
11	Glucose	+
12	Mannitol	+
13	Sucrose	+
14	Anaerobic Growth	-
15	Fructose	+
16	Xylose	+

Table 2. Antibacterial activity of *Bacillus subtills* zone of inhibition (mm),

	1	2	3	4	5	6	7
Staphylococcus areus	22	25	32	22	21	20	20
Pseudomonas aeruginosa	24	32	30	27	20	25	24
Escherichia coli	17	20	21	18	15	18	17
Proteus	16	16	22	17	14	15	17

Table (3) Results of insulation growth test at different temperatures

	5	10	20	40	45	50
1	-	+	++	++	++	+
2	-	+	+	++	++	-
3	-	+	++	++	++	-
4	-	+	++	++	++	+
5	-	+	+	++	++	+

6	-	+	+	++	+	+
	-	+	+	+	+	-

++ Good growth, + medium growth, + - weak growth, - no growth

Table (4) Results of the growth test for *Bacillus subtills* bacteria isolates in three saline concentrations

	Nacl%3	Nacl%7	Nacl%10
1	+	+	-
2	+	+	-
3	+	+	-
4	+	+	-
5	+	+	-
6	+	+	-
7	+	-	-

IV. REFERENCES

[1] Pagmadulam, B., Tserendulam, D., Rentsenkhand, T., Igarashi, M., Sawa, R., Nihei, C. I., & Nishikawa, Y. (2020). Isolation and characterization of antiprotozoal compound-producing Streptomyces species from Mongolian soils. Parasitology international, 74, 101961.

[2] Barus, T., Wati, L., & Suwanto, A. (2017). Diversity of protease-producing Bacillus spp. from fresh Indonesian tempeh based on 16S rRNA gene sequence. HAYATI Journal of Biosciences, 24(1), 35-40 .

[3] Güllüce, M., Karadayı, M., & Barış, Ö. (2013). Bacteriocins: promising naturalantimicrobials Localenvironment, 3, 6.

[4] Awda, j. m., & fayyadh, a. h. isolation and identification of bacteria that produce amylase.1(10),134-142., .

[5] Abd-Elhalem, B. T., El-Sawy, M., Gamal, R. F., & Abou-Taleb, K. A. (2015). Production of amylases from Bacillus amyloliquefaciens under submerged fermentation using some agro industrial by products. Annals of Agricultural Sciences, 60(2), 193-202.,

[6] Tallent, S. M., Kotewicz, K. M., Strain, E. A., & Bennett, R. W. (2012). Efficient isolation and identification of Bacillus cereus group. Journal of AOAC International, 95(2), 446-451.

[7] Shahid. W and A.Naheed. 2017. Isolation and Study of cellular Components of Aerobacillus polymyxa along with its comparison in soil Layers. RADS J. Biol.Res. Appl. sci. Vol 8 (1), ISSN:2305-8722 .

[8] Radhi, H. A. S., Abdul-Ratha, H. A., & Hadown, H. A. (2018). Isolation and diagnosis of Bacillus megaterium from soil Rhizo-sphere of different crops and test its Efficiencey on dissolving phosphate in broth and Solid media and its Growth with Bacillus Subtilis and Bacillus mucilaginous as combination of biofertilizer. iraqi journal of soil science, 1(1), 214-223.

[9] Narendra. Gondane Sampada. 2014. Effect of liquid Dormulation of Bacillus megaterium on phosphate solubilization in onion (Allium Cepa L.)Master of Science (Agriculture) in Agriculture Microbiology. Maharashtra State, india.

[10] Andriani. Yuli, Rochima. Emma, Safitri. Ratue, Rahayuningsih. Sri Rejeki. 2017. Characterization of Bacillus megaterium and Bacillus mycoides Bacteria as probiotic Bacteria in Fish and Shrimp Feed. in 2nd international Conference on Sustainable Agriculture and food. pag 127-135. Dol 10. 18502b/kls. V2i6. 1029

[11] Harrigan, W. F., & McCance, M. E. (2014). Laboratory methods in microbiology. Academic press

[12] Willey, J. M., Sherwood, L., & Woolverton, C. J. (2009). Prescott's principles of microbiology. McGraw-Hill Higher Education.

[13] Carter, G. R., & Cole Jr, J. R. (Eds.). (2012). Diagnostic procedure in veterinary bacteriology and mycology. Academic Press.

[14] Schwable,R.;Moor,L.S.and Good,A.C.(2007).Antimicrobial susceptibility testing protocols .Taylorr and Frams Group.

[15] Mansouripour S,Esfaudiari,Z,Natelghi L (2013).The effect of heat process on the survival and increased viabillty of probiotc by microcapsulation:areview. Ann.Biolong.Res.4:83-87 .

[16] Claus , D. and Berkeley , R.C. W. (1984) . Genus Bacillus . In P. H. A.Senath ; N. S. air ; E. Sharpe , and J. G. Holt(eds.) Bergy`s manual of systematic Bacteriology .Vol . 11 P. 105 – 139 .Williams and Wilkins Co., Baltimore .

[17] Daniels, R. (2014). Delmar's guide to laboratory and diagnostic tests. Cengage Learning.

[18] Zweers, J.C., Baràk, I., Becher, D., Driessen, A.J.M., Hecker, M., Kontinen, V.P., Saller, M.J., Vavrova, L. and Dijl, J.M.V. (2008). Towards the development of Bacillus subtilis as a cell factory for membrane proteins and protein complexes. Microbial cell factories, 7:10.mk

[19] Gillbert ,P.and Brown,M.R.W.(1995).Some perspectives on preservation and disinfection in the present day .Int.Biodeterior.Biodegard .36:219-226.

[20] Brown, S., Santa Maria Jr, J. P., & Walker, S. (2013). Wall teichoic acids of gram-positive bacteria. Annual review of microbiology, 67, 313-336.

[21] Talaro, K. P., & Chess, B. (2018). Foundations in microbiology. McGraw-Hill.

[22] Potron, A., Poirel, L., & Nordmann, P. (2015). Emerging broad-spectrum resistance in Pseudomonas aeruginosa and Acinetobacter baumannii: mechanisms and epidemiology. International journal of antimicrobial agents, 45(6), 568-585.

[23] Lakshmi, B. K. M., Ratna, P. V., Devi, K. A., & Hemalatha, K. P. J. (2014). Screening, optimization of production and partial characterization of alkaline protease from haloalkaliphilic Bacillus sp. International Journal of Research in Engineering and Technology. 3(2), 435-443

[24] James , P.D.A. and Edwards , C.(1990) . The effects of temprature on growth and production of antibiotic granaticin by athermotolerant bacteria . J. Gen . Microbiol . 135 : 1997 – 2003 .