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Research Article

The Study of Bacillus Subtils Antimicrobial Activity on Some of the Pathological Isolates

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ABSTRACT

The study involved the selection of two isolates from *Bacillus subtilis* to investigate their inhibitory activity against some bacterial pathogens. B sub-bacteria were found to have a broad spectrum against test bacteria such as *Staphylococcus aureus and Pseudomonas aeruginosa*. They were about 23-30 mm and less against *Klebsiella sp*. The sensitivity of some antibodies was tested on the test samples. The results showed that the inhibitory ability of bacterial growth in the test samples using *B. subtilis* extract was more effective than the antibiotics used.

Keywords:

INTRODUCTION

Since ancient times, humans have relied on primitive methods of treatment. Raw materials, such as plant or animal materials, which have a loss, have an effect on pathogenic microorganisms. The Greeks used plant resins and various mineral salts to treat some common diseases. Chinese soybeans were used to treat pimples and boils and other injuries[1]. The term antiviral (Antibiotic) was derived from the term antibody (Antibiosis), which was used for the first time in the world by Vuillemin. (1889)[2]. Microbiological microorganisms are widely found in nature, found in soil, water, and the remains of flora and fauna Soil is the main source Many of them are therefore leading many researchers into soil for the purpose of obtaining strains of Microrganisms is the product of new antibiotics[3]. Scientific evidence has been shown on the efficacy of therapeutic reuptake in effect, and it has included many species of bacillus and other types of bacteria[4]. The term Antibiotics was first used in (1942) by the Waksman, who was able to define antimicrobial agents as metabolic substances produced by microorganisms, inhibited the growth of other microorganisms and did not affect the bacteria[5] Antibiotics are classified as one of the secondary metabolic products that are produced by microorganisms after their growth reaches a secondary phase which is also known as the production phase Therefore, some sources pointed to the possibility of naming these substances (Idiolites)[6].

MATERIALS AND METHODS

10 soil samples were collected from 3 different sites in Najaf city by scattering the soil from the surface of the soil and taking a depth of 5-10 cm. The sample was taken from each of the studied sites and placed in a sterile bag the information is recorded (model number, type of soil, type of crop, and location) and brought to the laboratory.

I attended a series of decimal decimals by adding 1 ML of 10^{-2} of serial dilution to the Nutrient agar medium surface and incubation was incubated in temperatures ranged from 15-45 ° C for 24-48 h. Each bacterial colony was then transferred to the same medium that was isolated for purification and was preserved on the center of slant of Nutrient agar and preserved at 4°c until to be used.

The following Bacterial isolates were obtained: *Staphylococcus aureus, Stryptococcus sp., Pseudomonas aeruginosa, Pseudomonas putida, Klebsiella ssp, Escherichia coli* from the Central Health Laboratory / Najaf that isolated from the operating room. The diagnosis was made by doing some physical tests and the addition of biochemical so some have been made special tests for *Staphylococcus aureus* like the growth on the mannitol salt agar and coagulase test, the *Pseudomonas aerogenosa* was cultured on King B and King A medium for the purpose of detecting the ability of isolated organism (*B. subtilis*) to produce inhibitors (agar discs)

Where use of a 6 mm diameter cork borer to make holes in which transfer the wanted bacteria to test their ability to produce antibiotics and growing on the Muller Hanton agar containing one of the wanted bacteria that cultivated on 37°c /24h, the producer organism was detected by inhibition zones on agar[3].

By the swab the bacteria were to be tested for antibiotic sensitivity (isolated bacteria B1 and B2) on the Muller Hanton agar, equally 5 to 7 antibiotics were placed three replicates per experiment and incubated at 37 ° C / 24h , the Inhibition area diameter was measured to determine the resistance and sensitivity of bacteria to the antibiotics[7] as in the table (2).

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Table 1: Biochemical tests of *Bacillus subtilis.*

Test	B1 & B2
Gram stain	+
catalase	+
oxidase	-
citrate	+
urea	-
motility	+
H2S	+
Nitrate	+
Indol	-
MR	+
VP	..
Gelatinase	+
heamolysis	+
Manitol	+
Spore stain	+

B1 & B2= *Bacillus subtilis*

The isolates were distinguished by their effectiveness in antibiotics production and these isolates were diagnosed using physiological and biochemical tests based on the scientific sources used globally to diagnose bacteria[8], the diagnosis has been included the following tests: microscopy and agricultural traits and movement examination[9], catalase, oxidase, coagulation enzyme and ability to blood analysis[10].

Petri dishes containing a solid feeder were used to fertilize the bacteria and incubate in 37ºc /24h.

The antibiotic susceptibility also estimated by kirby bauer disk method to following antibiotics amoxicillin-clavulanic acid, cefixime, cefotaxime, ciprofloxacin and gentamicin.

RESULT AND DISCUSSION

The isolation results showed 2 bacterial isolates from 10 soil specimens were able to produce inhibitors. These isolates differed in their ability to produce inhibitors depending on the diameter of the inhibition against the tested bacteria and were selected as efficient bacterial isolates and were observed as a prevalent in the soil environment cultivated with vegetable and fruit crops. Two domestic isolates were given symbol B1and B2 in their high capacity to produce inhibitors from the rest of the isolates were from different locations of the city. These isolates were selected for in vitro tests for the purpose of final diagnosis and identification of genus and species .The results of confirming by culturing, microscopic and biochemical tests of producer isolates were shown and depending on[12] it carrying the *Bacillus subtilis* properties.

The results of the tests on this isolates were positive bacillus for gram stain and found in chains or in pairs, spore forming strict aerobic bacteria with a yellow-light circularly serrated edges on the nutrient agar, urea ,oxidase and indole negative and positive for catalase, motility , methyl red, and reducing nitrates to nitrites, fermenting the glucose and producing acid, H_2S forming and had ability to blood hemolysing table (1).

The isolates B1 and B2 showed a wide spectrum inhibitory capacity for gram positive and gram negative tested bacteria and are considered competent in the production of antibacterial agents against tested bacteria.

It was observed that both isolates had the highest inhibitory efficacy on *Staphylococcus aureus* fig. (1 and 2) and *Pseudomonas aeruginosa*, it was about (23-30)mm as in figure (1,2,3) , followed by *E. coli* (21)mm , *P. putida* (19)mm , *Streptococcous sp*. (19)mm and the minimum inhibitory zone was to *Klebseilla sp.*(17) mm with susceptibility ratio as (97%) .

Inhibition of the *Staphylococcus aureus* and *Pseudomonas aeruginosa*, is particularly important if *Pseudomonas aeruginosa* is one of the most common types of natural resistance and resistance caused by many of the mutations that it constantly causes to antibiotics resistance[15].

Also *Staphylococcus aureus* is one of the most important causes of septicemia, as well as its ability to produce intracellular toxins and its ability to produce rapid resistance to many antibiotics and cause various problems

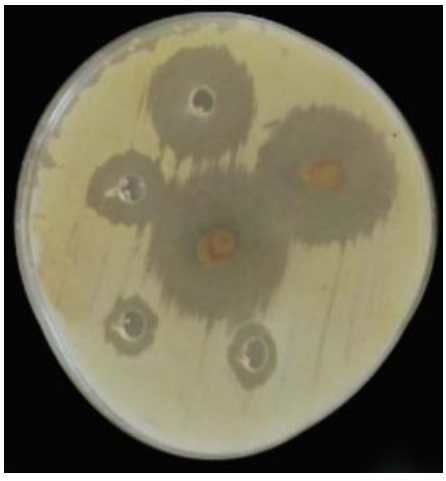

Figure 1: the Bacillus subtilis(B1) inhibition zone against *Staphylococcus aureus* bacteria.

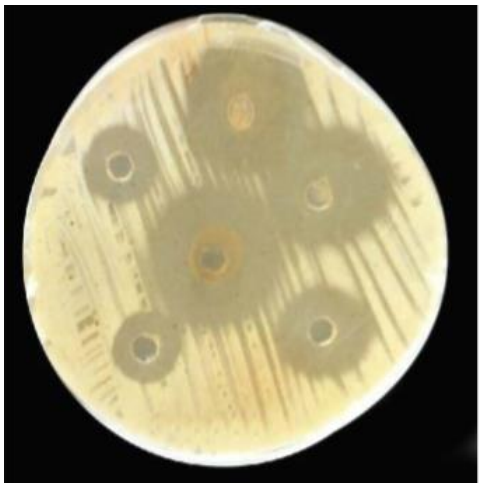

Figure 2: the Bacillus subtilis(B2) inhibition zone against *Staphylococcus aureus* bacteria.

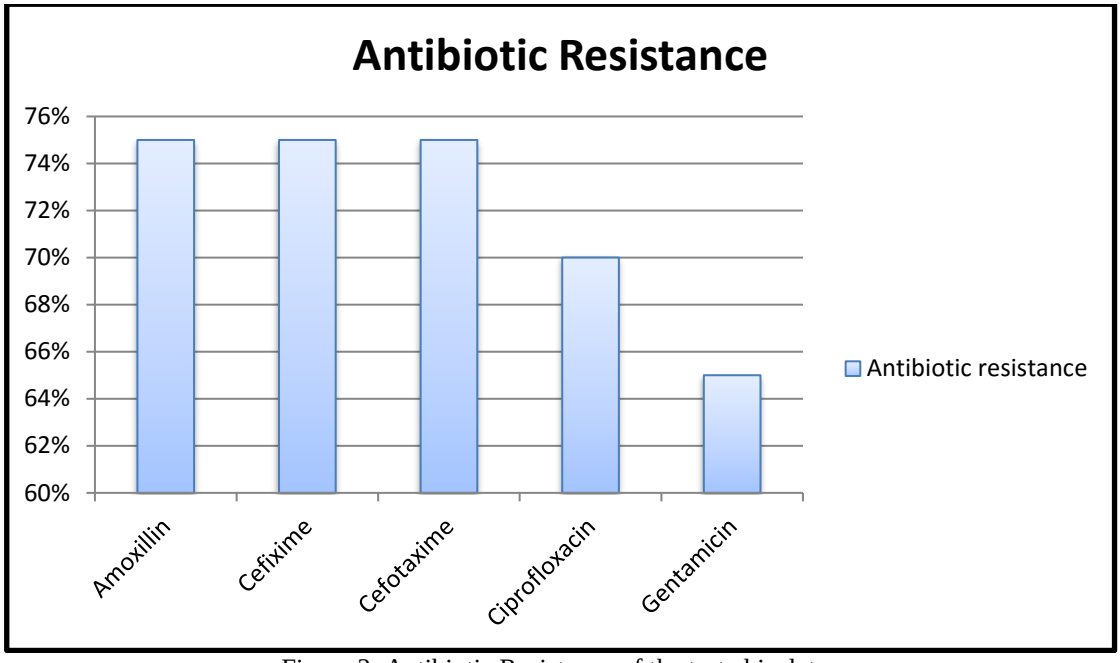

Figure 3: Antibiotic Resistance of the tested isolates.

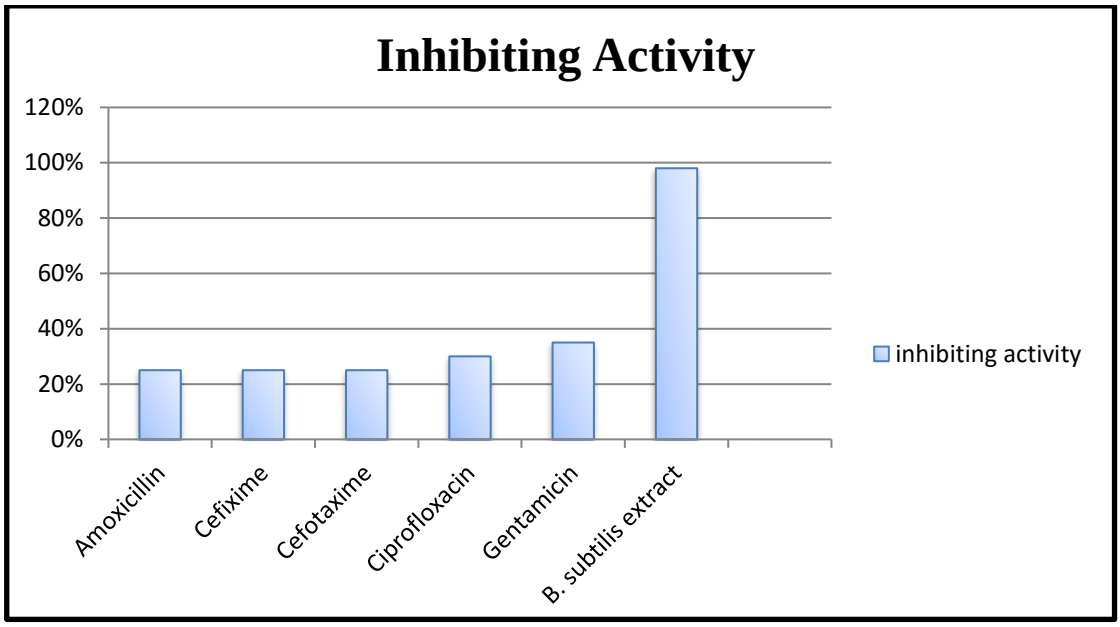

Figure 4: Inhibiting Activity differences between some antibiotics and *B. subtilis* extract.

as a result of this resistance[16].

The results showed that the higher resistance ratio to amoxicillin-clavulanic acid, cefixime and cefotaxime (75%) followed by ciprofloxacin (70%) and gentamicin (65%) fig.(3).

We can distinguish from the results that the inhibitory activity of the *Bacillus* bacteria was more effective on the tested samples in inhibiting its growth.

The antibacterial activity of *B. subtilis* is due to bacteriocin-producing *Bacillus subtilis*[10] or antagonism[13] or due to microbial food competition between *Bacillus subtilis* and the neighboring pathogenic bacteria[18].

REFERENCES

1. Talaro, K. and Talaro, A. (1996). Fundations in microbiology, basic principles, Times mirror higher education group, Wm. C. Brown Publishers. Chicago.
2. R. Ramachandran, A. G. Chalasani, Ram Lal,and U.Roy.(2014). A Broad-Spectrum Antimicrobial Activity of Bacillus subtilis RLID 12.1. The Scientific World Journal Volume 2014 (2014), Article ID 968487, 10 pages.
3. Egorov, N. S. (1985). Antibiotics ascientific approach. Mir publishers, Moscow.
4. Yilmaz,M. ;Soran,H. and Beyatli,Y. (2006). Antimicrobial activities of some Bacillus spp. strains isolated from the soil. Microbiological Research, Volume 161, Issue 2, 13 February 2006, Pages 127-131.

5. Waksman, S. A. (1967). The actinomycetes: a summary of current knowledge. The Ronald press Co. New york.
6. Hopwood, D. A. (1988). Extracgromosomally determined antibiotic production. Ann. Rev. Microbiol. 32: 373 – 392.
7. Bauer,A.W.;Kirby ,W.M.M.; aherris.J.C.; and Turek,m.(1966) Antibiotic susceptibility testing by astandardized single disk method .J. clin .path,44: 493-496.
8. Baron, E.J; and Finegold, S.M. and Baily Scott.(1990). Diagnostic Microbiology;C.V mosby company Toronto.
9. 9-S. Steller, D. Vollenbroich, F. Leenders, T. Stein, B. Conrad, J. Hofemeisterr, P. Jaques, P. Thonart, J. Vater. (1999) Structural and functional organization of the fengycin synthease multienzyme system from Bacillus subtilis b213 and A1/3 Chem. Biol., 6 (1) (1999), pp. 31-41
10. N. Khochamit ; S. Siripornadulsil ; P.Sukon and W.Siripornadulsil . (2015). Antibacterial activity and genotypic–phenotypic characteristics of bacteriocin-producing Bacillus subtilis KKU213: Potential as a probiotic strain. Microbiological Research,Volume 170, January 2015, Pages 36-50.
11. Holt, J. C.; Krieg, N. R.; Sueath, P. H.; Staley, J. T.; and Williams, S. T. (1994). Bergy`s manual of determinative bacteriology, 9th ed. Williams and Wilkins Co. Baltimor. U.S.A.
12. T. Beric; M. Kojic; S.Stankovic ; L. Topisirovic; V. Venturi and D. Fira.(2012). Antimicrobial Activity of Bacillus sp. Natural Isolates and Their Potential Use in the Biocontrol of Phytopathogenic Bacteria., Food Technol. Biotechnol. 50 (1) 25–31 (2012).
13. Chater, K. F. and Hopwood, D. A. (1993). In A. L. Sanenshein; J. A. Hoch, and R. Losich. (eds.), Bacillus Subtilis and other gram positive bacteria, Biochem. physiol, and Mol. genet. American Society for Microbiology, Washington D. C.
14. Moor, R. A.; Bates, N.C. and hancock, R.E.W(1986). interaction of polycationic antibiotics With Pseudomonas aeruginosa lipopoly saccharide and lipid astudied by using dansyl. polymyxin Antimicrobial Agents, chemoth .29: 496500.
15. Jawets, M.; Adeiberg: E.A.; Brooks, G.f; Butel, J.S; melinck, J. and ornston ,L.N (1995) .Medical microbiology .zoed Appleton and Lange .
16. 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.
17. D. J. Gonzalez, N. M. Haste, A. Hollands, T. C. Fleming, M. Hamby, K.Pogliano, V.Nizet, and P.C. Dorrestein .(2011). Microbial competition between Bacillus subtilis and Staphylococcus aureus monitored by imaging mass spectrometry. Microbiology. 2011 Sep; 157(Pt 9): 2485–2492.