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Antimicrobial activity of some *Bacillus spp.* Separated from the soil

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ABSTRACT

In this study select two active isolate from nine isolates of local agricultural soil of Mosul, were collected from 10 sample of 6 different region depending on antimicrobial activity to some test bacteria and fungi, Identified as gram-positive rod-shaped bacteria and, biochemical test, the selected isolates belonged to *Bacillus* genus, gave signal B1 and B2 were effective against both pathogenic bacteria Gram-positive and Gram-negative, two isolates gave high inhibition zone about 23mm against *Staphylococcus aureus* whereas gave less inhibition zone against *Escherichia coli*, *Klebsiella spp.*, *Aspergillus niger* and *Candida spp.*, 20, 16, Furthermore studied the effect of temperature on the growth of two isolates which gave good growth at 40-45 degree whereas there was no growth at low temperature 5°C. Furthermore, studied the effect of salt on the growth of two isolates that gave good growth at concentration 3% NaCl; the two strains were analysed utilizing agar publishing way and cross streak method.



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INTRODUCTION

The *Bacillus types* are Gram-positive, facultatively anaerobic, endospore-forming, motile, rod-shaped bacteria, spread into the environment primary habitat is soil, and catalase positive.

One of the basic features amongst *Bacillus breeds* is the capacity to produce a varied collection of active antimicrobial compounds contra bacteria and fungus, that procedure has been utilized in medicine and pharmaceutical industry and use its capabilities to observation different illnesses in humans, animals and plants as a biological control factor (Eliud *et al.*, 2014). Antibacterial peptide substance produced by *Bacillus spp* were identified as bacteriocin, Bacteriocins are a metabolic substance which prevent or kill microorganisms that are very connected to the producer breed (Bizani and

Brandelli, 2002). It was effective against pathogenic bacteria both Gram-positive and Gram-negative, bacteriocin safe for human consumption and target specific bacterial pathogens while broad-spectrum of chemical antibiotics kill both pathogen and commensal bacteria, Due to bacteriocin from *Bacillus* have attracted attention, production of bacteriocin or bacteriocin-like materials have characterized for *B. subtilis*, *B. cereus* and other *Bacillus* species. There are lots of methods for discovering antimicrobial activity-based diffusion antimicrobial agents through media culture to prevent the increase of sentient microorganisms (Mirac *et al.*, 2006).

The aim of this research was the isolation of local bacteria from rhizosphere of agriculture soil in Mosul city, detectability to produce antimicrobial material against test pathogenic bacteria and fungi, study their ability of growth on different condition of temperature and salt). Nearly 95% of Gram-positive soil bacilli belong to species *Bacillus* (Garbeva *et al.*, 2003).

MATERIALS AND METHODS

Isolation of microorganisms: Soil samples were collected from six different agricultural regions of Rhizosphere of Mosul in Iraq from September 2017 till March 2018, models were gathered to a profundity of 20 cm after tear out nearly 3 cm of

the soil surface, utilizing some neat, arid and sterile polythene pouch together with spoon antiseptic (Al-Grawi and Al-Awsi, 2018). All models were relocated to the laboratory; beneath disinfected statuses, 1 g for every soil models were combined to CaCO₃ (10:1 w / w) and improved at 37°C in 4 days afterwards, every 1 g of the model was suspended in 9ml disinfected water and jolt strongly for 2 min. The model was preheated at 60 C for 60 min in a water tub. After that, the liquid was diluted serially in disinfected water, and successive alleviation was made up to 10⁻⁴. An aliquot (0.5 ml) of suspension from final alleviation experiment pipe was expansion everywhere on alimentary agar medium. Procedures were improved at 37°C for 24–48 h (Chilcott and Wigley, 1993). After the incubation stage, procedures were tested for idealistic colonies of *Bacillus* and doubtful colonies were soiled by Gram smearing way. The Gram-positive, rod-shaped, spore-forming bacilli were choosing for extra match experiments, containing sensitivity experiment to motility, and Catalase. All bacterial colonies were purified and installing on alimentary agar plates, individual colonies were separated and checked up for antimicrobial action, stocked for further assay. Which produces a yellowish white colony on a nutrient agar plate (Eqbal and Al-Awsi., 2019).

Cross Streak Method: Mueller-Hinton agar, Nutrient agar plates were prepared and vaccinated with types of *Bacillus* through individual series of vaccination in the middle of the petri dish (plate). After 2 days of vaccination at 37°C dish was cultivated with signals bacteria by individual series at 90° nook to the *Bacillus* types not influential each other, and embracing 37°C for 24 h in situation of bacteria and 28°C for 48 h in situation of fungus, microbial interactions were illustrated through oversight of extent of the restraint area, bacterial experiment (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp*) were given from the hospital of Mosul city, while (*Aspergillus niger* and *Candida spp*) were taken from the College of Science of Mosul University (Nasser and Samar, 2016).

Method of the spread of the disk agar: Bacteria index were cultivated on Nutrient agar, and embracing at 37°C for 24h, while indicator fungi cultured on potato dextrose agar and embracing at 28°C for 48 h, after growth disks (6 mm) were taken of *Bacillus* colony by using sterile cork borer which was a metal tool for cutting a hole(8g), dipped in alcohol and flamed, putting the cylinders on an agar dish which has been vaccinated by an indicator creature. Antimicrobial activity going to originate an area of bacterial repression as it spreads over the agar, the diameter of clear inhibition zones was measured in millimetres. Date of

disk deployment methods to 1940. Pure area of repression probably noticed, by higher areas noticed contra *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella*, *Aspergillus niger*, and *Candida spp* (Mirac *et al.*, 2014).

Used tubes contain sterile slant nutrient agar, then inoculated with the culture of *Bacillus* on nutrient broth, incubate at 40, 45, 5°C for 24-48 h, addition 3%, 2%, 1% NaCl to Nutrient broth then inoculate with the culture of *Bacillus* incubate for 24-48h, result positive if there was turbidity in broth (Eqbal *et al.*, 2019).

RESULTS AND DISCUSSION

There has been an increase of antibiotic resistance in human pathogenic microorganisms, due to use of chemical antimicrobial drugs in the treatment of diseases. This has forced scientists to study for new antimicrobial substances from various sources. (Eqbal *et al.*, 2019), Most species of *Bacillus* produce high-quality antibiotics. "Bacteriocin", *Bacillus* species commonly found in the rhizosphere of soil, naturally occurring soil bacteria that benefit plants through increasing availability, (Al-Awsi *et al.*, 2019), The ability to produce endospores and output metabolites which have hostile influences on other microorganisms allows *Bacillus* to resist well at both high and low-temperature condition (Al-Awsi *et al.*, 2019).

The current study was accomplished to correct output of antibiotic from separated *Bacillus* type from the soil, acquired outcomes appeared that two separated breeds of *B.* types. have the ability to creating antimicrobial materials two *Bacillus* species were tested against all the target bacteria. 3 indicator bacteria and 2 indicator fungi examined the antimicrobial activity by the cross line way and agar disk extension. Both. Antagonistic effects method show clearly zone (Table 2). Table 2 shows *S. aureus* was more sensitive than *E. coli* and *Klebsiella*. *Bacillus* breeds were active more contra Gram-positive than Gram-negative bacteria while shown moderate antifungal activity against test fungi, which agree with (Chillab *et al.*, 2019). In both methods cross line and agar disk expansion, a special characteristic of gram-negative bacteria is the existence of a multilayered structure of cell wall, whereas in Gram-positive bacteria is a single layer, even though whole bacteria have an internal cell membrane, gram-negative bacteria have a superb external membrane (Joshua and Keshu, 2015). This external membrane excepts some medicine and antibiotics from piercing the cell, that explains why gram-negative bacteria more resistant to antibiotics than other gram-positive bacteria. Two bacterial segregate was established to produce restrained areas about their colonies on plates through signal bacterial and fungus.

(Shamran *et al.*, 2018). The isolate was also inhibited three bacterial (*S. Aureus*, *E. coli*, and *Klebsiella*) by 23, 20 and 16 mm repression areas, and 2 fungus breeds (*Candida spp*, *Aspergillus niger*, by 16, 12 mm inhibition zones. Whereas the result of the present study has shown moderate antifungal activity against test fungal human pathogen, informed that *B. wax torsion* separated from soil was energetic contra most Gram-positive, but not Gram-negative bacteria (Nitha *et al.*, 2012).

A slim demonstrated that *Bacillus* strains had greater effects on Gram-positive bacteria than on Gram-negative bacteria. In cross line, the *Bacillus* had to contend with other microorganisms. In research by Basurto-Cadena and his group, *B. subtilis* separated from soil appeared restrictive action in the laboratory and under greenhouse situations contra various fungus. Morphological searches appeared that isolate B1 was a Gram-positive, rod-shaped strain qualified of figuration endospore which produces a yellow-white colony on alimentary agar dish. One bacterial separate (*Bacillus* sp. B1) offered a favourable bioactivity contra examined bacterial breeds. While *Bacillus* sp. B2 created rotating fatty yellow colonies on an alimentary agar plate. It is a Gram-positive, oxidase negative, catalase positive, and is capable of decreasing nitrate to nitrite, influences of medium components a significant phase to progress metabolizer production of type *Bacillus*. (Lateef *et al.*, 2018).

CONCLUSION

We inform that soil bacterial separate, recognised as *Bacillus* sp., qualified of preventing the growth of bacteria and fungus sp. The current study indicates the significance of soil bacterial separate like the origin of biologically energetic contra both Gram-positive bacteria and Gram-negative strains. Inspection spread plate for discovery the presence of antibiotics, its ease of use, low cost, and enable us to compare outcomes for antibiotic activity with outcomes of other researches. While cross streak method is practical and suitable technique. Inspection spread plate continues an economical and unpretentious method and widely used.

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