AJPS, 2016, Vol. 16, No.2

Date of acceptance: 26-9-2016

Antimicrobial Activity of Extracts Bacillus Species Isolated From Baghdad Soil Against Some Human Pathogenic Microorganisms

Khalida Jhalil Ibraheem
***Department of Sciences, College of Basic Education, Al-Mutansiriyah University*.**
E-mail: kalidaj976@gmail.com

Abstract:

Bacillus species are the predominant soil bacteria because of their resistant endospore formation and can produce many different antimicrobial substances.

The main aim of this study was to isolate *Bacillus* species from soil and investigate their antimicrobial activity against some pathogenic bacteria and fungi isolated from human.

48 soil samples were collected from different region of Baghdad city (Rashidiya, Mahmudiyah, Alkraat and Aldora) during December 2015 and analyzed for the presence of *Bacillus* species. Bacterial isolates were identified by using different microscopical examination, cultural characteristics, biochemical tests and confirmed by VITEK 2 bacterial identification system. The antimicrobial effects of *Bacillus* species extracts against some pathogenic bacteria (Gram-positive, Gram-negative) and fungi were examined. The identified *Bacillus* species included *B. polymyxa, B. cereus, B. licheniformis, B. mycoides, B. firmus* and *B. subtilis*.

The results indicate that the bacterial isolates showed antimicrobial activity against all tested pathogenic bacteria and fungi. *B. polymyxa* showed best activity against most test organisms compare to other *Bacillus* isolates, follow by *B. subtilis, B. cereus, B. licheniformis, B. firmus* and *B. mycoides*.

This study reveals that some *Bacillus* species have the ability to produce antimicrobial compounds that can be used to control microbial infections in future.

Keywords: *Soil Bacteria, Bacillus species, Antimicrobial activity.*

الفعالية المضادة للميكروبات لمستخلصات أنواع الـ *Bacillus* المعزولة من تربة بغداد ضد بعض الأحياء الدقيقة الممرضة للأنسان

خالدة جليل إبراهيم
قسم العلوم، كلية التربية الأساسية، الجامعة المستنصرية.

الخلاصة:

أنواع الـ Bacillus هي بكتريا التربة السائدة بسبب تشكيلها سبورات داخلية مقاومة ويمكنها ان تنتج العديد من المواد المختلفة المضادة للميكروبات. الهدف الرئيسي لهذه الدراسة هو لعزل أنواع الـ Bacillus من التربة والتحقق من فعاليتها المضادة للميكروبات ضد بعض المسببات المرضية البكتيرية والفطرية المعزولة من الانسان.

تم جمع 48 عينة تربة من مناطق مختلفة من مدينة بغداد (الراشدية، المحمودية، الكريعات والدورة) خلال شهر كانون الأول 2015 وتم تحليلها لوجود أنواع الـ Bacillus. شخصت البكتريا المعزولة من خلال استخدام الفحوصات المظهرية، الصفات الزرعية، الاختبارات البايوكيميائية وأكدت بواسطة نظام تحديد البكتريا VITEK2. تم فحص التأثيرات المضادة للميكروبات لمستخلصات أنواع الـ Bacillus ضد بعض المسببات المرضية البكتيرية (الموجبة لصبغة غرام – السالبة لصبغة غرام) والفطرية.

أنواع الـ Bacillus المشخصة تضمنت B. polymyxa، B. cereus ، B. licheniformis ، B. mycoides ، B. firmus و B. subtilis . تشير النتائج الى ان البكتريا المعزولة أظهرت فعالية مضادة للميكروبات ضد كل المسببات المرضية البكتيرية والفطرية قيد الاختبار. B. polymyxa أظهرت افضل فعالية ضد معظم الاحياء قيد الاختبار بالمقارنة

Date of acceptance: 26-9-2016

بعزلات الـ Bacillus الأخرى ثم تليها بكتريا B. Subtilis، B. cereus، B. licheniformis، B. firmus، B.mycoides.

تكشف هذه الدراسة الى ان بعض أنواع الـ Bacillus لديها القدرة على انتاج مركبات مضادة للميكروبات والتي يمكن استخدامه للسيطرة على الإصابات الميكروبية في المستقبل.

الكلمات المفتاحية: بكتريا التربة، أنواع الـ Bacillus ، الفعالية المضادة للميكروبات.

Introduction:

Bacillus species are Gram-positive, aerobic or facultative anaerobic, endospore-forming, chemoheterotrophic, rod-shaped bacteria which are usually motile with peritrichous flagella[1]. Members of the *Bacillus* genus are generally found in soil and represent a wide range of physiological abilities that allow the organisms to flourish in every environment and compete favorably with other organisms within the environment, due to its ability to form extremely resistant spores and produce metabolites that have antagonistic effects on other microorganisms[2,3].

Treatment of infectious disease with multidrug resistant strain of bacteria and fungi are becoming a major problem in the whole world[4]. Screening for new antibiotics from natural products produced by microorganisms is becoming more important for the pharmaceutical industry. The genuses of Bacillus are good sources for the production of biologically active secondary metabolites. The potential of *Bacillus* species to synthesize a wide variety of metabolites with antimicrobial activity has been widely used in medicine and pharmaceutical industry[5,6].

In recent years, many investigations have been carried out to isolate different strains of terrestrial *Bacillus* and identify their inhibitory compounds[7,8].

The present study is an attempt to isolate and identify *Bacillus* species from soil and investigate their antimicrobial activity against some pathogenic bacteria and fungi isolated from human.

Materials and Methods:

Collection and preparation of soil sample:

48 soil sample collected from different regions of the Baghdad city (Rashidiya, Mahmudiyah, Alkraat and Aldora) during December 2015. All the samples were collected in sterile containers from the surface of the soil to depth of 4 inches or/and 10 centimeters, then transferred directly to the laboratory at the Department of Sciences in Collage of Basic Education under sterile conditions. Approximately 1 gram of soil samples was suspended in 9 ml sterile distilled water and shaken vigorously for 2 minutes. The samples were heated at 60˚C for 60 minutes in water bath (Gallen Kamp, England). Then the soil suspensions were serially diluted and then streaked on to nutrient agar (Oxoid, England) plates and incubated at 37˚C for 24 hours.

Identification of the *Bacillus* isolates:

After the incubation period, isolates identified by their morphological and biochemical characteristic [9] and confirmed by VITEC 2 bacterial identification system (bioMe'rieux, France).

Test Microorganisms (bacteria and fungi):

The pathogenic test bacteria and fungi used in this study were obtained from culture collections of the Educational Laboratories in Medicine City and Central Public Health Laboratory in Baghdad City during January 2016.

The bacterial isolates were re-diagnosed using different microscopical examination, cultural characteristics and biochemical tests[9,10]. Morphological examination and other identification tests were used for identified fungal isolates[11,12].

Date of acceptance: 26-9-2016

Extraction of antimicrobial compounds from *Bacillus* species:

Each isolated *Bacillus* were inoculated in to 100 ml glucose nutrient broth (Oxoid, England) each only and incubated at 37 ˚C for 24 hours. After incubation, 10 ml of culture broth was transferred to another 100 ml of sterile broth in conical flask and incubated for 3 days at 37˚C with shaking (120 rpm). After 3 days incubation the broth culture was centrifuged at 10.000 rpm at 5 minutes. The culture supernatant was collected and mixed with equal volume of ethyl acetate solvent in a separating funnel and then shake gently for 2 hours. The ethyl acetate layer was then collected and dried at room temperature. The yield from extract was dissolved in to ethanol for determination of antimicrobial activity[13].

Determination of antimicrobial activity:

Agar diffusion assay was used for the assessment of antimicrobial activity. This method is based on the observation the inhibition of growth of microorganisms in plates[14]. Gram–positive, Gram–negative bacteria and fungi were used in this test.

Activated bacterial and fungal isolates by re-cultured on nutrient agar and Sabouraud dextrose agar (SDA) (Oxoid, England) plates, respectively. After 18–24 h. incubation at 37 °C (for bacterial species) and 27˚C for week (for fungal species), a loopful of each test species was added to nutrient broth or Sabouraud dextrose broth (SDB) (Oxoid, England) for bacterial and fungal species, respectively and incubated at optimum temperature to reach the turbidity at concentration 6×10^8 by using McFarland scale. Spreading bacterial and fungal isolates suspension on Muller-Hinton agar and SDA, respectively. Holes were made in the media by cork-borere (5 mm). 100 µl of the obtained extracts of six species of *Bacillus* were loaded in to the plates hole and blank well (fill with solvent only) were used as a negative control. The Petri dishes were kept in room temperature for 1 hour. The Muller-Hinton agar (Oxoid, England) plates were incubated at 37 °C for 24 h., while the SDA plates were incubated at 28 ºC for about 7 days. After incubation period, the inhibitory zones (in mm) were measured. In a separate trial, the inhibition zone of ciprofloxacin (abroad range antibacterial agent) and ketoconazole (atypical antifungal agent) were determined as positive control.

Results and Discussion:

The present study was carried out to evaluate the production of antibiotic from newly isolated *Bacillus* species from soil. The obtained results showed that six species of *Bacillus* were identified on basis of morphological and biochemical characteristic and confirmed by VITEC 2 bacterial identification system. The identified *Bacillus* species included *B. polymyxa, B.cereus, B. licheniformis, B. mycoides, B. firmus* and *B. subtilis*. Among terrestrial bacterial strains, the genus of *Bacillus*, considered one of the most abundant bacterial isolates found in soil and can produced different structure of inhibitory compounds [15].

The test microorganisms used in this study included three isolates of Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium diphtheria*), six isolates of Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Klebsiella spp., Serratia marcessens*) and three fungal isolates (*Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus*).

The diameter of inhibition zones of *Bacillus* species extracts of each isolate and also ciprofloxacin and ketoconazole (as antibacterial and antifungal agents, respectively) is summarized in table (1, 2, 3).

Date of acceptance: 26-9-2016

Table-1: Antibacterial activity of *Bacillus* species extracts against test Gram-positive bacteria.

***Bacillus* isolates extracts**	**Gram-positive bacteria [Inhibition zones in mm]**		
	S. aureus	*S. pyogenes*	*C. diphtheriae*
B. polymyxa	28	20	21
B. firmus	8	5	-
B. cereus	18	13	19
B.licheniformis	10	12	-
B. mycoids	-	11	-
B. subtilis	20	25	16
Cip.	25	23	22

Cip: Ciprofloxacin was used as standard antibiotic at concentration of 6 µg/ml.

Table-2: Antibacterial activity of *Bacillus* species extracts against test Gram-negative bacteria.

***Bacillus* isolates extracts**	**Gram-negative bacteria [Inhibition zones in mm]**					
	E. coli	***P.aeruginosa***	***S. typhi***	***V. cholerae***	***S.marcessens***	***Klebsilla spp.***
B. polymyxa	21	15	18	20	17	16
B. firmus	6	_	_	_	9	8
B. cereus	10	_	12	7	11	13
B. licheniformis	_	8	10	6	5	9
B. mycoids	5	_	_	_	6	7
B. subtilis	15	19	13	17	11	12
Cip.	22	21	19	21	20	18

Cip: Ciprofloxacin was used as standard antibiotic at concentration of 6µg/ml.

Table-3: Antifungal activity of *Bacillus* species extracts against test fungi.

***Bacillus* isolates extracts**	**Test Fungi [Inhibition zone in mm]**		
	Candida albicans	***Cryptococcus neoformans***	**S *Aspergillus fumigatu***
B. polymyxa	26	19	18
B. firmus	7	-	-
B. cereus	13	9	10
B. licheniformis	9	-	8
B. mycoides	6	-	-
B. subtilis	12	13	23
Ket.	28	23	21

Ket: Ketoconazole was used as standard antifungal of 500 µg /ml.

The extract of bacterial isolates showed antimicrobial activity against all tested pathogenic bacteria and fungi. The secondary metabolite could produce by *Bacillus spp.* and showed antimicrobial activity against different pathogenic bacteria and fungi [16]. These secondary metabolites produced by different species of *Bacillus*, having a diverse in chemical structure and biological activity [17].

B. polymyxa showed best activity against most test organisms compare to other bacillus isolates, follow by *B. subtilis, B. cereus, B. licheniformis, B. firmus* and *B. mycoides*. The highest zone of inhibition was 28, 26 found against *Staphylococcus aureus*

and *Candida albicans*, respectively by *B. polymyxa* (Table 1 and 3) may be this activity because this isolate can production polymyxin, bacitracin, colistin, gatavalin and jolipeptin which considered peptide antibiotics cyclic there may be make unusual linkage with component of cell wall for bacteria and fungi and prevent formation cell wall [18], while *B. subtilis* the highest zone of inhibition was 25, 23 against *Streptococcus pyogen* and *Aspergillus fumigatus*, respectively (Table 1 and 3).

The obtained results was agreed with many researches, in a study by Basurto-Candena and his team (2012), recorded that *Bacillus subtilis* isolated from soil showed good activity against pathogenic bacteria and fungi[19]. Sertori *et al*., (2008) reported that the extracted of *Bacillus subtilis* have good activity against *Listeria monocytogens* and *Enterococcus fecalis*[20].

Conclusions:

The results of this study indicate that the six *Bacillus* isolates from soil samples possessed antibacterial and antifungal activity. This study reveals that some *Bacillus* species have the potential to produce antimicrobial compounds; therefore it is necessary for more screening, isolation and purification of these antimicrobial compounds that can be used to control microbial infections in future.

References:

1 - Lavanya A, Aparna D. Characteri-zation of *Bacillus* species isolated from natural sources for probiotic properties. Int J Curr Biotechnol. 2015; 3(8): 22–27.

2 - Violeta O, Oana S, Matilda C, Maria CD, Catalina V, Production of bio-surfactants and antifungal compounds by new strains of *Bacillus* spp. isolated from different sourcers. Rom Biotech Lett. 2011; 16 (1): 84-91.

Date of acceptance: 26-9-2016

3 - Issazadeh K, Karimi–Rad S, Zarrabi S, Rahimibashar MR. Antagonism of *Bacillus* species against *Xanthomonas Compestris* and *Pectobacterium Cartovorum* subsp *Cartovorum*. Afr J Microbiol Res. 2012; 6(7): 1615-20.

4 - Blomberg B. Antimicrobial resistance in developing countries. Tidsskr Nor Laegeforen. 2008; 128(21): 2462– 66.

5 - Kumar SN, Siji JV, Ramya B, Mohandas C. Improvement of anti-microbial activity of compounds produced by *Bacillus* sp. J Microbiol Biotechnol Food Sci. 2012; 1(6): 1424-38.

6 - Al-Ajlani M, Hasnain S. Bacteria exhibiting antimicrobial activity; screening for antibiotics and the associated genetic studies. Open Conf Proccedings J. 2010; Pp: 230-8.

7 - Ayed HB, Hmidet N, Béchet M, Chollet M, Chataigné G, Leclère V, *et al,*. Identification and biochemical characteristics of lipo-peptides from *Bacillus mojavensis A21*. Process Biochem. 2014; 49(10): 1699–1707.

8 - Sumi CD, Yang BW, Yeo IC, Hahm YT. Antimicrobial peptides of the genus *Bacillus*. Canad J Microbiol. 2015; 61(2): 93–103.

9 - Forbes BA, Sahm DF, Welssfeld AS. Baily and Scott's diagnostic microbiology. 12th ed. Louis: Mosby Inc; 2007.

10 - Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH. Manual of clinical microbiology, 8th ed. ASM Press, Washonton DC. 2003.

11 - Freydiere AM, Guinet R, Bioron P. Yeast identification in the clinical microbiology laboratory: Phenotypical method J Med Mycol. 2001; 39(1): 9–33.

12 - Ellis DH. Clinical mycology: The human opportunistic mycosis. Gilling-ham Printers pty Ltd. Austeralia. 1994; Pp: 166.

Date of acceptance: 26-9-2016

13 - Sharma SCD, Shovon MS, Jahan MS, Asaduzzaman A, Rahman MA, Biswas KK, *et al,*. Antibacterial and cytotoxic activity of *Bacillus methylotrophicus*-SCS2012 isolated from soil. J Microbiol Biotechnol Food Sci 2013; 2(4): 2293–2307

14 - Colle J, Fraser A, Marmion B, Simon A. Makie and McCartney: Practical medical microbiology, 14th ed. Churchill Liverstone. New York. 1996; Pp: 978.

15 - Beric T, Stankovic S, Draganic M, Kojic M, Lozo J, Fira D. Novel antilisterial bacteriocin licheniocin 50.2 from *Bacillus licheniformis* VPS50.2 isolated from soil sample. J Appl Microbiol. 2013; 116(3): 502–10.

16 - Mansour A, Zeinab R, Manollah Z. Isolation and identification of *Bacillus* species from soil and evaluation of their antibacterial properties. Avicenna J Clin Microb Infec. 2015; 2 (1): e 23233.

17 - Sandhya MVS, Ramyakrishna E, Divya P, Anoor Pawan Kumar, Karthik Rajkumar, Yazein E. Isolation of antibiotic producing bacteria from soil. Int J Appl Biol and Pharma Tech. 2015; 6(1): 46-51.

18 - Awais M, Ali Shah A, Hammed A, Hasan F. Isolation, identification and optimization of bacitracin produced by *Bacillus sp.* Pak J Bot. 2007; 39(4): 1303–12.

19 - Basurto-Cadena MG, Vázquez-Arista M, García-Jiménez J, Salcedo-Hernández R, Bideshi DK, Barboza-Corona JE. Isolation of a new Mexican strain of *Bacillus subtilis* with antifungal and antibacterial activities. Scientific world Journal. 2012; 20(1): 38–49.

20 - Sirtori LR, Motta Ade S, Brandelli A. Mode of action of antimicrobial peptide p45 on *Listeria monocytogens*. J Basic Microb. 2008; 48(5): 393-400.