Distribution of *Bacillus subtilis* and *Bacillus cereus* in soils of Damascus zone and its antibacterial activity against *Staphylococci* species

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Received 07/09/2014
Accepted 24/12/2014

ABSTRACT

The current work aimed to study the distribution of *B. subtilis* and *B. cereus* in soils of Damascus zone and focused on the antibacterial activity of *Bacillus* species against *Staphylococci* species which were isolated from pathogen and soil's samples. The study showed that the rate of the distribution of *B. subtilis* was (65.71%) and it was more than the rate of distribution of *B. cereus* which was (35.29%) in the soil, and the rate of distribution of *Staphylococci* were between (10 – 20%). The antibacterial activity was studied for two species of *B. subtilis*, and two species of *B. cereus* against 35 species of *Staphylococci* which isolated from soil and deferent pathogen samples: ear, urine, Bronchitis excretions, Liver's wash, CSF, Blood, Abscess, Furuncle, Pus, CV shant, Wounds. The results showed that *B. subtilis* which isolated from the soil of zone of Al-tall has more antibacterial activity which gives bigger inhibition zone against all strains of *Staphylococci* which were isolated from soil and pathogens (100%), whereas another strain which was isolated from the soil of zone of AL-Rabwah didn't give antibacterial activity against some of these strains of *Staphylococci* (38.86%, 27.27%). In addition, two species of *B. cereus* didn't have any antibacterial activity towards most strains of *Staphylococci*, except six of it (31.42%). With respect to strains of *Staphylococci* which was isolated from soil, *B. subtilis* of Al-tall has more antibacterial activity which give bigger inhibition zone (10-21 mm) against all of strains of *Staphylococci* which isolated from different soil except *S. epidermidis* (0 mm) which isolated from Adawee, while another species which isolated from the soil of AL-Rabwah gave antibacterial activity against some of these strains of *Staphylococci*. In addition, two species of *B. cereus* had antibacterial activity towards three strains of *Staphylococci*, but no activity towards four strains of it, and there is a significant difference between *B. subtilis1* and *Bs2, Bc1, Bc2* in almost strains of *Staphylococci* (P< 0.05).

Key words: Pathogen, Antimicrobials, Antibiotics, well Diffusion, Damascus zone.

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توزع العصويات الرقيقة والشمعية في ترب منطقة دمشق وفاعليتها التصادية تجاه أنواع العنقوديات

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تاريخ الإيداع 2014/09/07

قبل للنشر في24/12/2014

الملخص

هدفتِ الدراسة الحالية إلى دراسة توزع العصوية الرقيقة والعصوية الشمعية في ترب منطقة دمشق، وفاعليتها التصادية ضد العنقوديات المعزولة من عينات مرضية والتربة، وبينت هذه الدراسة أن انتشار العصوية الرقيقة وتوزعها بلغ (65.71%)، وهي أكبر من توزع العصوية الشمعية التي بلغ انتشارها (35.29%)، ونسبة توزع العنقوديات في ترب منطقة دمشق كانت بين (10 – 20%). درس النشاط التضادي لنوعين من العصوية الرقيقة ونوعين من العصوية الشمعية ضد 35 نوعاً من العنقوديات المعزولة من التربة، ومن العينات المرضية المختلفة: الأذن، والبول، ومفرزات قصبية، وغسالة كبدية، والسائل الدماغي الشوكي SF، والدم، وخراج، ودمامل، وقيح، وشانتCV، وجروح. أظهرت النتائج أن العصوية الرقيقة المعزولة من تربة منطقة التل أعطت نشاطاً تصادياً قوياً ضد أنواع العنقوديات المعزولة جميعها سواء من المرضى أو من التربة (100%)، أمّا العصوية الرقيقة المعزولة من تربة منطقة الربوة فقد كان نسبة نشاطها التصادي متوسطاً (38.86%) ضد العنقوديات الذهبية، (27.27%) وضد العنقوديات الجلدية، أما العصوية الشمعية فلم تبدِ نشاطاً كبيراً ضد العنقوديات باستثناء 6 أنواع منها، أمّا العنقوديات المعزولة من التربة فقد أبدت العصوية الرقيقة المعزولة من تربة التل نشاطاً تصادياً قوياً (10– 21 مم) ضد أنواع العنقوديات المعزولة من التربة جميعها ما عدا العنقودية الجلدية (0 مم) المعزولة من تربة العدوي، والعصوية الرقيقة المعزولة من تربة الربوة فقد كان نشاطها التضادي متوسطاً، أمّا العصوية الشمعية فقد أبدت فاعلية جيدة ضد ثلاث أنواع من العنقوديات ولم تبدِ فاعلية ضد أربعة منها، ووجدت فروق معنوية ذات دلالة إحصائية بين العصوية الرقيقة BS1 والعصويات الأخرى BS2, BC1, BC2 في معظم السلالات (P<0.05).

الكلمات المفتاحية: مرضي، مضاد جرثومي، الصادات، الانتشار الحفري، منطقة دمشق.

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Introduction

Soil is highly complex system characterized by a variety of biological, chemical and physical processes, which are markedly influenced by environmental factors, and there are wide varieties of microorganisms. In rich, moist soil, where many nutrients are available, vegetative cells of many genera of bacteria and fungi can flourish (Kolwzan *et al*., 2006, Paul 2007). A wide diversity of physiological abilities of *Bacillus* is exhibited, ranging from psychrophilic to thrmophilic, and acidophilic to alkaliphilic; some strains are salt tolerant and some are halophilic. (Todar 2011).

The natural habitat of *Bacillus* species is soil. In addition, *Bacillus* species also found in water, food and clinical specimens. Most species have little or no pathogenic potential and are rarely associated with disease in humans or other animals; an exception is *Bacillus anthracis* (Madsen 2008).

B. subtilis is rod-shaped, aerobic, Gram-positive, motile, forming ellipsoidal to cylindrical spores which lie centrally, paracentrally and subterminally in unswollen sporangia. Colonies are round to irregular in shape and of moderate (2 – 4 mm) diameter, with margins varying from undulate to fimbriate; they become opaque, with surfaces that are dull and which may become wrinkled; color is whitish, and may become creamy or brown (Lindquist 2009).

B. cereus is facultatively anaerobic, Gram-positive, usually motile rods 1.0–1.2 by 3.0–5.0 μm, occurring singly and in pairs and long chains, and forming ellipsoidal, sometimes cylindrical, subterminal, sometimes paracentral. Colonies are very variable in appearance, they are characteristically large (2–7 mm in diameter) and vary in shape from circular to irregular, with entire to undulate, crenate or fimbriate edges. Colonies are usually whitish to cream in color (De vos *et al*., 2009), it's endospores are very widespread in soil, in milk and other foods, and in many other environments. The vegetative organisms may multiply readily in a variety of foods and may cause diarrheal and emetic food poisoning syndromes. Occasionally causes opportunistic infections in man and other animals (Brooks *et al*., 2007, Steele 2007).

B. cereus is the etiological agent of two distinct food poisoning syndromes: the diarrheal-type, characterized by abdominal pain with diarrhea 8–16 h after, and the second type is emetic-type characterized by nausea and vomiting and abdominal cramps 1– 6 h after eating the offending food and associated with abdominal pain, headache, malaise, prolonged nausea(Bhunia 2008, Mengel & Schwiebert 2009)

Staphylococci is 0.5–1.5 mm in diameter, occurring singly, in pairs, in tetrads, and characteristically dividing in more than one plane to form irregular grape like clusters. (Colledge *et al*., 2010). Natural populations are mainly associated with skin, skin glands, and mucous membranes of warm-blooded animals. Some organisms may be isolated from a variety of animal products (meat, milk, cheese) and environmental sources like soil, sand, dust, air, and natural waters (Prescott 2002).

The coagulase-positive species especially *S. aureus*, are regarded as potentially serious pathogens. *S. aureus* is responsible for a variety of infections. Among the major human infections caused by this species are furuncles, carbuncles, impetigo, toxic epidermal necrolysis (scalded skin syndrome), pneumonia, osteomyelitis, acute ndocarditis, acute and chronic cystitis, prostatitis, cervicitis, cerebritis, meningitis, bacteremia, toxic shock syndrome, conjunctivitis, skin, infection of eyes, ears, wounds, respiratory tract and UTIs in man, women, children (Wolff & Johnson 2009, McKean *et al.*, 2012).

Staphylococci remains the main reported cause of food poisoning in number of countries including Brazil, Egypt, Taiwan, and most of the other developing countries. The coagulase-negative *staphylococcal* species like *epidermidis*, constitute a major component of the normal microflora of the human; their role in causing nosocomial infections has been recognized and well documented over the last two decades. The increase in infections by these organisms has been correlated with the wide medical use of prosthetic and indwelling devices and the growing number of immunocompromised patients in hospitals (Saene *et al*., 2005, Fauci *et al*., 2008). *Bacillus* species produce peptide, polypeptide and lipopeptide antibiotics, so the known antibiotic producers are *B. cereus* (cerexin), *B. subtilis* (bacitracin, polymyxin, difficidin, subtilin, Subtilosin, surfactin, bacilysin), some of these

antibiotics act against a variety of Gram-positive bacteria like bacitracin; and some are anti-Gram-negative activity like gramicidin; and others are broad spectrum like polymyxin (Dutton *et al*. 2002, Barredo 2005, Stein 2005).

B. subtilis produce antifungal volatiles and antibiotics which are used for biocontrol activity (Leifert *et al*., 1995); Cerein 7 is a peptidic antibiotic (bacteriocin) produced by *B.* cereus Bc7 that shows a broad spectrum of activity (Oscáriz & Pisabarro 2000); isolation, characterization of lipopeptide antibiotics produced by *B. subtilis*, and these compound could function as a biocontrol agent against a large spectrum of pathogens (Chen *et al*., 2008); there are many different support factors like pH, time incubation, effect on growth microbial cells, and production metabolites like enzymes and antibiotics (Awais *et al*., 2010); ability of *Bacillus sp.*to produce biosurfactants, antibacterial and antifungal agents were isolated from Romanian soils (Violeta *et al*., 2011); *B. subtilis* isolated from the soil, and showed activity against *S. aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Sethi *et al*., 2013), so many species such as *Bacillus*, have been studied continuously for searching for new antibiotics from natural resources, and their ability to produce antibiotics. In addition, due to the fact that *Bacillus* species have produced antibiotics, and these antibiotics have been found to be cheaper and more effective in studies conducted to date, and these microorganisms are preferable for commercial production, on another hand, the prevalence of antimicrobial resistance among key microbial pathogens is increasing at an alarming rate worldwide particularly *Staphylococci*. Now current solutions involve development of a more rational approach to antibiotic use and discover of new antimicrobials.

The importance of the current research because of the need of the isolation of novel *B. subtilis*, and *B. cereus* strains, for ability to produce the antibiotics anti- *Staphylococci*, so it aimed to investigate their distribution in Damascus soils, and determine the antibacterial activity against *Staphylococci* which were isolated from soil and pathogens samples.

Materials and Methods

Collection of soil's sample

In summer of 2012, (Table 1.) three different soil samples were taken from every region, and collected in the sterile polypropylene bags from cultivated and barren lands in and around Damascus: Qassyion, Demas, Al-Tall, AL-Rabwah, Adawee, Doma, Meliha, Kafer sousa, Yafour, Jedaidah, Khan-ALsheh. The sample of soils were mixed to form composite sample, and all soils were used in all experiments were collected from the 15-20 cm layer (Lindquist 2009).

Table 1. Sampling of soils in Damascus zone

Date	Region
13/6	Qassyion
	Demas
	AL-Tall
	AL-Rabwah
	Adawee
5/7	Doma
	Meliha
	Kafer sousa
	Jedaidah
6/8	Khan-ALsheh
	Yafour

Isolation and Identification of *B. subtilis, B. cereus*

Each 10 grams of sample was suspended in 90 ml of sterile distilled water and shaken vigorously for 2 min. The samples were heated at 80C for 20 min in a water bath. Then the soil suspensions were diluted in sterile distilled water, and the 1 ml of dilutions from 10^{-1} to 10^{-6} were plated on nutrient agar medium NA (Abtec, England). The plates were incubated at 37C for 24 h. (De vos *et al*., 2009). The identification of bacterial isolates was performed both by microbiological, and biochemical methods, according to the Bergey's Manual of Systematic Bacteriology in second edition 2009, by using API 50 CHB (BioMérieux, France), and results of tests were colored by API web, and these tests including: indole production test, citrate utilization test, oxidase test, catalase test etc.. (Table 2), in addition, the spore morphology, gram characteristics and motility, nitrate

reduction. These tests which were carried out on isolates showed that they are *B. subtilis*, and *B. cereus* (Harley & Prescott 2002).

Table 2. Biochemical tests for isolates of *B.subtilis* and *B.cereus* upon API CHB *Bacillus*

tests	*B.S1**	*B.S2***	*B.C1****	*B.C2*****	tests	*B.S1*	*B.S2*	*B.C1*	*B.C2*
GLY	+	+	+	+	SAL	+	+	+	+
ERY	-	-	-	-	CEL	+	+	+	+
DARA	-	-	-	-	MAL	+	+	+	+
LARA	+	+	-	-	LAC	+	-	-	-
RIB	+	+	+	+	MEL	+	-	-	-
DXYL	+	+	-	-	SAC	+	-	+	+
ADO	-	-	-	-	TRE	-	+	+	+
MDX	-	-	-	-	INU	-	-	-	-
GAL	-	+	-	-	MLZ	-	-	-	-
GLU	+	+	+	+	RAF	+	-	-	-
FRU	+	+	+	+	AMD	-	+	+	+
MNE	+	-	-	-	GLYG	-	+	+	+
SBE	-	-	-	-	XLT	-	-	-	-
RHA	-	-	-	-	GEN	-	-	-	-
DUL	-	-	-	-	TUR	-	-	-	-
INO	+	+	-	-	LYX	-	-	-	-
MAN	+	+	+	-	TAG	-	-	-	-
SOR	+	-	-	-	DFUC	-	-	-	-
MDM	-	-	+	-	LFUC	-	-	-	-
MDG	+	-	-	-	DARL	-	-	-	-
NAG	-	+	+	+	LARL	-	-	-	-
AMY	+	+	+	+	GNT	-	-	-	-
ARB	+	+	+	+	2KG	-	-	-	-
ESC	+	+	+	+	5KG	-	-	-	-

* *B.subtilis1* soil of AL-Tall, ** *B.subtilis 2* soil of AL-Rabwah, *** *B.cereus1* soil of Adawee, **** *B.cereus 2* soil of Qassyion.

Isolation and Identification of *Staphylococci*

From soil

Staphylococci strains were collected from different soils in and around Damascus as above. Each 10 grams of sample was suspended in 90 ml of sterile distilled water and shaken vigorously for 2 min. Then the soil suspensions were serially diluted in sterile distilled water, and 1 ml of dilutions from 10^{-1} to 10^{-6} were plated on NA (Abtec, England), and Baird Parker medium (BP Scharlau, Spain).

The plates were incubated at 37C for 24 h. The identification of bacterial isolates was performed both by microbiological, and biochemical methods, according to the Bergey's Manual of Systematic Bacteriology in second edition 2009, by using API Staph and these tests including: VP test, nitrate reduction, esculin, and production of acid from D-glucose, Arabinose etc,.. (Table 3), in addition, the morphology of cultures, gram stain, production of catalase (Benson 2001, Harley & Prescott 2002).

From patients

Staphylococci strains were taken from different source's patients from Al-Mowasat, and Children Hospital in Damascus (ear, urine, Bronchitis excretions, Liver's wash , CSF

Table 3. Biochemical tests for isolates of *Staphylococci* upon API Staph.

Tests	*S.aureus*	*S.epidermidis*	*S.lentus*	*S.xylosus*	*S.warnari*	*S.hominis*
GLU	+	+	+	+	+	+
FRU	+	+	+	+	+	+
MNE	+	-	+	+	+	-
MAL	+	+	+	+	+	+
LAC	+	+	+	-	-	-
TRE	+	-	+	+	+	+
MAN	+	-	+	+	+	-
XLT	-	-	-	-	-	-
MEL	-	-	+	+	-	-
NIT	+	-	+	+	+	+
PAL	+	+	+	+	+	-
VP	+	+	+	+	+	+
RAF	-	-	+	-	-	-
XYL	-	-	+	+	-	-
SAC	+	+	+	+	+	+
MDG	-	-	-	-	-	-
NAG	-	-	+	-	-	+
ADH	+	-	+	-	-	-
URE	-	+	-	-	+	+

Blood, Abscess, Furuncle, Pus, CV shant, Wound) and plated on (NA), and Blood agar (BA Abtec, England), and Mannitol salt agar (MSA Biolife, Italy) to distinguish the *Staphylococci* strains. The plates were incubated at 37^0C for 24 h or then catalase test and API Staph, and these tests including: VP test, nitrate reduction, esculin, and production of acid from D-glucose, Arabinose, etc.. (Table 3), in addition, the morphology of cultures, gram stain, production of

catalase, Coagulase slide test (Sigma) which distinguish *S. aureus* from other strains of *Staphylococci*, and Novobiocin discs (Bio-analyse, Turkey) resistance to distinguish *S. saprophyticus* from *S. aureus*, and *S. epidermidis*. These tests which were carried out on isolates showed that they are *Staphylococci* strains (Fischbach & Dunning 2009, Longo *et al*., 2012).

Microbial strains and culture conditions.

2 *B. subtilis*, 2 *B. cereus* and 35 strains of *Staphylococci*, 8 of them are pathogen, and 7 from soil were used in our experiments (table 5 and 6). Bacterial strains were maintained on NA agar slants at 4^0C.

Inoculum preparation

For *Bacillus* species:

Inoculum was prepared in lactose broth (LB Merck). In all experiments, 50 ml of media was prepared in 100 ml flask and autoclaved at 121°C and 15psi pressure for 20 minutes, the flask was inoculated with a fresh culture of *Bacillus* specie by using the sterilized loop and incubated again at 37°C for 24 hours, then used for antagonistic tests (Goldman & Green 2009).

For *staphylococcus* species:

Inoculum was prepared in nutrient broth (NB SRL India). In all experiments, 50 ml of media was prepared in 100 ml flask and autoclaved at 121°C and 15psi pressure for 20 minutes, the flask was inoculated with a fresh culture of *Staphylococcus* species by using the sterilized loop and incubated again at 37°C for 24 hours, then used for antagonistic tests (Harley & Prescott 2002).

Antimicrobial activity by Agar diffusion assay:

The antimicrobial activity of *Bacillus* isolate was checked by agar well diffusion method. 24 h. fresh cultures of *Staphylococci* strains, and the turbidity of the cultures was adjusted by optical density OD (0.5 OD = 1×10^8 cfu/ml) (WPA CO8000, Biochrome England). A sterilized cotton swab was dipped in the overnight cultures and lawns were prepared over the Müller Hinton agar surface (MHA, Abtec England). Wells were made in the inoculated plates using sterile stainless still borer (external diameter 8mm, internal diameter 6 mm). The liquid culture of bacillus species and the turbidity and

concentration was adjusted by optical density (0.5 OD = 5 × 10^7 cfu/ml). About 50 µl of *Bacillus* species culture were added in the wells and the plates were incubated at 37ºC for 24 hours. After 24 h. inhibition zones were observed. The diameter of the zone of inhibition was measured in mm with well size of 6mm (Tang & Stratton 2006).

Statistical analysis

Statistical analysis (ANOVA) were performed using SPSS program software, version 17 to validate the signification of the results. The data are presented as means (± SD) of three replicates.

Results

For isolation

The table 4 shows the characteristics of soil like color, humidity, and its sources, in addition, the results showed that the number of *Bacillus subtilis* isolated were more than *Bacillus cereus*, and we didn't isolate any *B. subtilis*, and *B. cereus* from Yafour 'soil (table 4), and for results of *Staphylococci* strains, it showed that the number of isolates are little, where *S.xylosus* is 5, then *S.warnari* is 3, *S.aureus* is 1, and *S.epidermidis* is 1, at the same time we didn't isolate any *staphylococci* from some soils (table 5).

Table 4. Characteristics of soils and the isolates of *B. subtilis* and *B. cereus* and it's percent.

Soil's sample	Source's sample	Color	Humidity	B. subtilis	B. cereus
Qassyion	mountain	white	dry	2	1
Demas	fruit garden	black	little wet	1	0
AL-Tall	Wheat garden	black	little wet	1	1
AL-Rabwah	Grass soil	black	wet	1	2
Adawee	garden	black	wet	1	1
Doma	garden	black	wet	1	0
Meliha	garden	black	wet	1	0
Kafer sousa	fruit garden	black	wet	1	1
Jedaidah	soil	black	dry	1	0
Khan-ALsheh	soil	white	dry	1	0
Yafour	garden	red	little wet	0	0
collection				11 65.71%	6 35.29%

Table 5. Characteristics of soils and *Staphylococci* isolates.

Staphylococci	1	2	3	4	5	6	7	8	9	10	11
S.aureus	0	0	0	0	1	0	0	0	0	0	0
S.epidermidis	0	0	0	0	1	0	0	0	0	0	0
S.xylosus	1	1	0	1	0	0	0	2	0	0	0
S.warnari	2	0	0	1	0	0	0	0	0	0	0
Source's sample	m	f. g	Wh.g	gr	g	g	g	f. g	s	s	g
Color	wi	b	b	b	b	b	b	b	b	b	r
Humidity	d	l. w	l. w	w	w	w	w	w	d	d	l.w

b: black, d: dry, f: fruit, g: garden, gr: grass soil, l: little, m: mountain, s: soil, r: red, w: wet, wh: wheat, wi: white.

1: Qassyion, 2: Demas, 3: AL-Tall, 4: AL-Rabwah, 5: Adawee, 6: Doma, 7: Meliha, 8: Kafer- sousa, 9: Jed-aidah, 10: Khan-ALsheh, 11: Yafour.

Antimicrobial activity

The (Table 6), shows the diameters of inhibition zone of *B. subtilis* and *B. cereus* against isolated *staphylococci* strains which were isolated from patients. It shows that *B. subtilis* which were isolated from the soil of Al-tall has more antibacterial activity which gives bigger zone against all of strains of *staphylococci* which were isolated from deferent samples: (ear, urine, Bronchitis excretions, Liver's wash, CSF, Blood, Abscess, Furuncle, Pus, CV shant, Wound), whereas another strain which were isolated from the soil of AL-Rabwah don't give antibacterial activity against some of these strains of *staphylococci*. In addition, two species of *B. cereus* don't have any antibacterial activity towards most strains of *staphylococci*, except seven of them. With respect (Table 7) to strains of *staphylococci* which isolated from soil, *B. subtilis* which were isolated from the soil of Al-tall has more antibacterial activity which gives bigger zone against all of strains of *staphylococci* which was isolated from different soil except *S.epidermidis* which isolated from Adawee, while another species which isolated from the soil of AL-Rabwah gives antibacterial activity against some of these strains of *staphylococci*. In addition, two species of *B. cereus* have antibacterial activity towards three strains of *staphylococci*, but no activity towards four strains of them.

Table 6. The mean of the diameters Diameter of inhibition zone of *B. subtilis* and *B. cereus* against *staphylococci* strains which isolated from patients as test organisms.

No.	*Staph.* strains	Source's sample	*B.subtilis* 1	*B.subtilis* 2	*B.cereus 1*	*B.cereus 2*
S1	*S.epidermidis*	Liver's wash	21.66 ±2.88	12 ±3.46	0	0
S2	*S.aureus*	CSF	23.67 ±1.52	21 ±1	18.67±1.15	17.67 ±2.51
S3	*S.aureus*	CSF	13 ±1.73	0	0	0
S4	*S.aureus*	CSF	38.33 ±2.88	22.67 ±1.15	6.67±1.15	14.67 ±1.15
S5	*S.aureus*	Bronchitis excretions	24.67±0.5	10.67±1.15	0	12.67 ±1.15
S6	*S.epidermidis*	Blood	25.33 ± 0.5	12.67±1.15	0	0
S7	*S.aureus*	C.V shant	14.67±4.16	0	0	10 ±0.0
S8	*S.hominis*	CSF	0	0	0	0
S9	*S. aureus*	Abscess	0	0	0	0
S10	*S. epidermidis*	CSF	16 ±1.73	0	0	10.67 ±1.15
S11	*S.epidermidis*	Bronchitis excretions	21.67 ±0.5	12.67 ±1.15	10 ± 0.0	12 ± 2.0
S12	*S. epidermidis*	Ear	27 ±1.7	0	0	12.67 ±1.15
S13	*S. aureus*	Ear	15.67±0.57	0	0	0
S14	*S.aureus*	Eye	21.33 ±3.0	11 ±1	14.67 ±0.5	15.67±2.0
S15	*S.aureus*	Ear	19 ±1.0	0	0	0
S16	*S.aureus*	Pus	17.66 ±2.51	0	0	0
S17	*S.aureus*	Pus	22 ±1	0	0	0
S18	*S.aureus*	Bronchitis excretions	21.67 ±2.0	10 ±0.0	0	0
S19	*S.aureus*	Ear	16.33±1.52	9.33±1.154	0	0
S20	*S.aureus*	Ear	16 ±4.0	0	0	0
S21	*S.aureus*	Pus	19 ±1.0	0	0	0
S22	*S.aureus*	furuncles	19.67 ±0.57	0	10 ±0.0	9.67 ±0.5
S23	*S. epidermidis*	urine	15.66 ±2	0	0	0
S24	*S.epidermidis*	Bronchitis excretions	12.66 ±1.15	0	0	0
S25	*S.epidermidis*	Ear	16.33 ±1.52	0	9.33 ±1.15	10.66 ±1.15
S26	*S.epidermidis*	Abscess	14.66 ±1.15	0	0	0
S27	*S.epidermidis*	Wound	28 ±2.0	0	10.66 ±1.15	13.33 ±2.3
S28	*S.aureus*	pus	35.67±3.0	11.33±1.15	10 ±2.0	13.33 ±1.15

Sensitive: ≥14 mm, middle sensitive: 10 – 13 mm, resistant: ≤ 9 mm.

Table 7. The mean of the Diameters of inhibition zone (mm) of *B. subtilis* and *B. cereus* against *staphylococci* strains as test organisms.

No.	**Staph. strains**	**Source's soil**	***B.subtilis* 1**	***B. subtilis* 2**	***B. cereus* 1**	***B.cereus* 2**
S29	*S.warnari*	Qasion	18.66 ±1.15	0	0	0
S30	*S.xylosus*	Qasion	18.66 ±1.15	10 ±0.0	10.66 ±1.15	10 ±0.0
S31	*S.warnari*	Qasion	14.66 ±0.57	10.33 ±0.57	11.33 ±1.15	0
S32	*S.aureus*	Adawee	21.33 ±0.57	0	0	0
S33	*S.epidermidis*	Adawee	0	0	0	0
S34	*S.xylosus*	Kafer sousa	17.66 ±0.57	9.66 ±0.0	0	0
S35	*S.xylosus*	Kafer sousa	17.66 ±0.57	13 ±1.7	13 ±1.7	0

Sensitive: ≥14 mm, middle sensitive: 10 – 13 mm, resistant: ≤ 9 mm.

Statistic results

By the statistic results the differences between the antibacterial activity against *Staphylococci* were subjected to One Way Anova statistical test and they showed that there is a significant difference between *Bacillus subtilis1* and *Bs2*,*Bc1*,*Bc2* in almost all strains of Staphylococci except S2,S8,S9, S30. S31, S34 ($P<0.05$), and there is no significant difference between *Bc1*,*Bc2* except *S5, S7,S11,S35* ($P>0.05$), as to *Bacillus subtilis1* and *Bs2* there is a significant difference between them in almost all strains of *Staphylococci* except *S1, S2, S8, S9, S11, S14, S18, S19, S30, S31, S33, S34, S35* ($P<0.05$).

Discussion

The present work showed that the distribution of *Staphylococci* in soils of Damascus are little (1-10), where the seven strains were isolated, which identified as *S. aureus*, *S epidermidis*, *S.warnari*, *S.xylosus* (Table 5).

The present study was based on previous studies, which have shown that the *B. subtilis* produces antimicrobial, which can be used against animal and human pathogens (Risoen *et al*. 2004; Ouoba *et al*., 2007; Chen *et al.* 2008; Degering *et al*. 2010; Fuchs *et al.* 2011; Wu *et al.* 2013). In the present study, as shown above (Table 6 and 7) *B. subtilis1* shows high antibacterial activity against *Staphylococcus aureus* and the diameters of inhibition zone are between (18-40 mm), with 100%, and against *S. epidermidis* between (15–40 mm), with 100%. While percentage of *B. subtilis2* against *S. aureus* is 38.88%, and against *S. epidermidis* is 27.27%, so these results are similar to some results in previous studies, and unlike the other results, where in study of (Kuta *et al.*, 2009) the diameters of inhibition zone against *S. aureus* was 19mm, and study of (Moshafi *et al.*, 2011) noted the diameters of inhibition zone against *S. aureus* was 21mm, and against *S. epidermidis* was 22mm, while as reported from (Sethi *et al.*, 2013) the inhibition zone against *S. aureus* was 13.4mm, two studies of (Moore *et al.*, 2013) showed the diameters of inhibition zone against *S. aureus* were (28.3, 14.6, 20.3, 19.7, 34.1, 17.3 mm). But by *B. cereus1* which were isolated from Adawee give a middle antibacterial activity (8–16 mm) against 11 of 35 strains of *staphylococci* with

31.42% generally, so the percentage of antibacterial activity against *S. aureus* was 27.77%, and against *S. epidermidis* was 27.27% , while *B. cereus2* which were isolated from Qassyion give a middle antibacterial activity (8–18 mm) against 13 strains of *staphylococci* with 31.42% in general, where the percentage of antibacterial activity against *S. aureus* was 44.44%, and against *S. epidermidis* was 45.45%, these results are similar to the study of (Kuta *et al*., 2009) where the diameters of inhibition zone against *S. aureus* were zero or not available.

These differences between the antibacterial activity of *Bacillus* species may be due to the production of antibiotics of each specie, or due to the environment which are they live in it, or may be due to the genetic differences which are important factor for production of the antibiotics, and the biophysical and chemical factors in each soil.

References

1. Awais M., Pervez A., Yaqub Asim, Shah M. M. (2010). Production of antimicrobial metabolitics by *Bacillus subtilis* Immobilized in Polyacrylamide Gel, Pakistan journal Zoology, Zoological Society of Pakistan. 42(3): 267 – 275.
2. Barredo Jose Luis. (2005). Microbial Processes and Products, firth edition, Humana Press, New Jersey, U.S.A.
3. Benson (2001). Microbiological Applications Laboratory Manual in General Microbiology, Eighth edition, Mc Graw Hills companies, USA. 78: 257– 262.
4. Bhunia Arun K. (2008). Foodborne Microbial Pathogens: mechanisms and pathogenesis, Springer science business media, U.S.A.
5. Brooks F. Geo., Carroll C. Karen, Butel S. Janet, Morse A. Stephen. (2007). Medical Microbiology, ch. 9, 10, 12, 14, Lange, 24th Edition, McGraw-Hill medical.
6. Chen H., Wang L., Su C. X, Gong G. H., Wang P., Yu Z. L (2008). Letters in applied microbiology, the society for applied microbiology. (47):180 – 186.
7. Colledge Nicki R., Walker Brian R., Ralston Stuart H.(2010). Davidson's Principles & Practice of medicine, ch.13, 17, 27, 21st edition, Churchill Livingstone, Elsevier.
8. Degering Christian, Eggert Thorsten, Puls Michael, Bongaerts Johannes, Evers Stefan, Maurer Karl-Heinz, Jaeger Karl-Erich. (2010). Optimization of Protease Secretion in *Bacillus Subtilis* and *Bacillus Licheniformis* by Screening of Homologous and heterologous signal peptides, Applied and Environmental Microbiology, American Society for microbiology. 76(19): 6370 – 6376,
9. De vos Paul, Garrity George M., Jones Dorothy, Krieg Noel R., Ludwig Wolfgang, Rainey Fred A., Schleifer Karl H., Whitman William B. (2009). Bergey's manual of systematic Bacteriology, the firmicutes, second edition, Springer, U.S.A. Vol. (3).
10. Dutton J. Christopher, Haxell A. Mark, McArthur A. I. Hamish, Wax G. Richard. (2002). Peptide Antibiotics, U.S.A. pp 1 – 50,
11. Fauci S. anthony, Kasper L. Dennis, Lorgo Danl Braunwald Eugene, Hauser L. Stephen, Jameson Larry, Loscalso Joseph, Carolblack Dame, Funder John, Metc-alf. Donald, Ramires F. Antonio, Skorecki Karl, White J. Nicholas (2008). Harrisons principles of internal medicine, Chapter 14, 17th edition, Mc Graw Hills companies, USA.
12. Fischbach Frances, Dunning B. Marshall (2009). A manual of Laboratory and Diagnostic Tests, ch. 7, 8th edition, Lippincott Williams & Wilkins. pp 484 – 560,
13. Fuchs W. Sebastian, Jaskolla W. Thorsten, Bochmann Sophie, Kötter Peter, Wichelhaus Thomas, Karas Michael, Stein Torsten, Entian Karl-Dieter (2011). Entianin, a novel Subtilin-Like Lantibiotic from *Bacillus subtilis subsp.* Spizizenii DSM 15029 with high antimicrobial activity, Applied and Environmental Microbiology, American Society for microbiology. 77(5): 1698 – 1707.
14. Goldman Emanuel, Green H. Lorrence. (2009). Practical Handbook of Microbiology, secoth edition, CRS press, Taylor & Francis Group.

15. Harley J. P., Prescott M. Lansing. (2002). Laboratory exercises in microbiology, fifth edition, the McGraw-Hill companies.

16. Kolwzan Barbara, Adamiak Waldemar, Grabas Kazimierz, Pawelczyk Adam. (2006). Introduction to Environmental Microbiology, ch.1, Oficyna Wydawnicza Politechniki Wroclawskiej, Wroclaw, Poland.

17. Kuta A. Faruk, Nimzing Lohya, Orkaa Y. Priscilla (2009). Screening of *Bacillus* species with potentials of antibiotics production, Applied medical information, 24(1-2): 42- 46.

18. Leifert C., H. Li, Chidburee Siripun, Hampson S., Workman Suzanne, Sigee D., Epton H.A.S., Harbour Agnes. (1995). Antibiotic production and activity by *Bacillus subtilis CL27* and *Bacillus pumilus CL45*, Journal of applied Bacteriology, Society for applied Microbiology, UK., 78: 97-108.

19. Lindquist john (2009). Isolation of *Bacillus*, Bacteriology course, Department of bacteriology, university of Wisconsin-Madison.

20. Longo L. Dan, Fauci S. Anthony, Kasper L. Dennis, Hauser L. Stephen, Jameson J. Larry, Loscalzo Joseph (2012). Harrison's principles of Internal Medicine, eighth edition, The McGraw-Hill companies, U.S.A. vol.2, ch.(134, 135).

21. Madsen Eugene L. (2008). Environmental Microbiology (from genomes to biogeochemistry, ch.(1), Blackwell publishing, U.S.A, U.K.

22. Mangel B. Mark, Schwiebert L. Peter (2009). Family Medicine, Fifth edition, The McGraw-Hill companies, a Lange clinical manual, U.S.A. section 1, pp 48 – 60,

23. McKean C. Sylvia, Ross J. John, Dressler D. Daniel, Brotman J. Daniel, Ginsberg S. Jeffrey (2012). Principles and Practice of Hospital Medicine, first edition, The McGraw-Hill companies, U.S.A. section 10, pp1551–1708,

24. Moore T., Globa L., Barbaree J., Vodyanoy V., Sorokulova I. (2013). Antagonistic activity of *Bacillus* bacteria against Food-Borne Pathogens, Journal of Probiotics & Health, 1(3): 1 – 6.

25. Moshafi H. Mohammad, Forootanfar Hamid, Ameri Alieh, Shakibaie Mojtaba, Noudeh D. Gholamreza, Razavi Mojdeh (2011). Antimicrobial activity of *Bacillus sp.* Strain Fas_1 isolated from soil, Pakistan Journal Pharmaceutical Science, 24(3): 269 – 275.

26. Oscáriz C. J., Pisabarro G. A. (2000). Characterization and mechanism of action of Cerein 7, a bacteriocin production by *Bacillus cereus Bc7*, Journal of Applied Microbiology, 89: 361 – 369.

27. Ouoba I.I.L., Diawara B., Jespersen L., Jakobsen M. (2007). Antimicrobial activity of *Bacillus subtilis* and *Bacillus pumilus* during the fermentation of African locust bean (parkia biglobosa) for soumbala production, Journal of Applied Microbiology, 102: 963 – 970.

28. Paul A. Eldor. (2007). Soil microbiology ecology and biochemistry, third edition, Elsevier, academic press is an imprinted Elsevier, U.S.A.

29. Prescott M. Lancing, Harley P. John, Klein A. Donald (2002). Microbiology, fifth edition, The McGraw-Hill companies.

30. Risoen P. A., Ronning P., Hegna I. K., Kolsto A. B. (2004). Characterization of a broad range antimicrobial substance from *Bacillus cereus*, Journal of Applied Microbiology, 96: 648 – 655.

31. Saen H.K.F. Van, Silvestri L., La Cal M. A. (2005). Infection Control in the Intensive Care Unit, ch.7,8,9,10,11,12,13,14, secoth edition, Springer, printed in Italy. pp 91- 297,

32. Sethi Sonia, Kumar Ravi, Gupta Saksham. (2013). Antibiotic production by Microbes Isolated from Soil, IJPSR International Journal of Pharmaceutical Sciences and Research, 4: 2967 – 2973.

33. Steele Russell W. (2007). Clinical Handbook of Pediatric Infectious Disease, third edition, Informa healthcare, U.S.A.

34. Stein Torsten (2005). *Bacillus subtilis* antibiotics: structures, syntheses and specific functions, Molecular Microbiology, Blackwell Publishing Ltd. 56(4):845 – 857.

35. Tang Yi-Wei, Stratton, W. Charles. (2006). Advanced Techniques in Diagnostic Microbiology, Springer Science, U.S.A. pp 84,

36. Todar Kenneth (2011). University of Wisconsin-Madison Department of Bacteriology, www.textbook of bacteriology.net, Todar's Online Textbook of Bacteriology.

37. Violeta Olteanu, Oana Sicuia, Matilda Ciuca, Maria D. Carstea, Catalina Voaides, Gheorghe Campeanu, Petruta C. Cornea. (2011). Production of Biosurfactants and antifungal compounds by new strains of *Bacillus spp.* isolated from different sources, Romanian Biotechnological Letters, 16(1).

38. Wolff K., Johnson Allen Richard. (2009). Fitzpatrick's Color Atlas and Synopsis of Clinical Dermatology, sixth edition, McGraw-Hill medical companies, U.S.A. section 24, pp 590 – 631,

40. Wu Zhuoying, Ye Chengsong, Guo Feng, Zhang Shenghua, Yu Xin. (2013). Evidence for Broad-Spectrum Biofilm Inhibition by the Bacterium *Bacillus sp.* Strain SW9, Applied and Environmental Microbiology, American Society for microbiology. 79(5): 1735 – 1738.