

# The Biological Activity of Protein Extracts of *Bacillus* spp. Isolated from Soil against Some Pathogenic Bacteria

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## Abstract

Forty soil samples were collected from different regions in Baghdad city. Among 52 isolates, only 38 (73.1 %) belonged to *Bacillus* spp. All bacterial isolates were submitted to the cultural, microscopical, biochemical examination and VITEK 2 System. *Bacillus cereus* was the predominant (21; 55.3%) followed by *Bacillus subtilis* (11; 28.9%), then *Bacillus amyloliquefaciens* (6;15.8%). *B. cereus* (B1) and *B. subtilis* (B16) isolates showed the highest level of antibacterial activity; therefore, their extracellular proteins were extracted, purified using ammonium sulfate (80%) saturation, and submitted to Sepharose-6B gel filtration column. SDS-polyacrylamide gel electrophoresis was used to determine the molecular weights of the proteins, in which one band of molecular weight 68 and 50kDa was found in *B. cereus* and *B. subtilis* respectively. These proteins revealed high antibacterial activity on Gram positive bacteria, and moderate effect on Gram negative bacteria, except *Pseudomonas aeruginosa* where no effect was reported. The antibiofilm activity of the extract showed increased effect against *Streptococcus pyogenes*, while moderate effect against each of *Staphylococcus aureus*, *Escherichia coli*, *Serratia marcescens*, *Klebsiella* spp with no impact against *P.aeruginosa*. The results of cytotoxicity indicated that the purified extract had a higher cytotoxic effect on the breast cancer cell line MCF7 cells at 1000µg/ml concentration, than the crude extract that showed cytotoxic effect on the same cell line at 100µg/ml.

**Key words:** Soil samples, *Bacillus* spp., Proteins, Antibacterial, Antibiofilm, Cytotoxicity.

## الخلاصة

تم جمع أربعين عينة من التربة من مناطق مختلفة في مدينة بغداد. من بين 52 عذلة، فقط 38 (73,1%) تنتمي إلى *Bacillus* spp. خضعت جميع العزلات البكتيرية للفحص المزرعي والمجهري والكيموحيوي ونظام VITEK 2. كان جنس *Bacillus cereus* هو الغالب (21 بنسبة 55,3%) يليه *Bacillus subtilis* (11؛ 28,9%)، ثم *Bacillus amyloliquefaciens* (6؛ 15,8%). أظهرت العزلات *B. cereus* (B1) و *B. subtilis* (B16) أعلى مستوى من النشاط المضاد للبكتيريا. لذلك، تم استخلاص البروتينات خارج الخلوية، وتنقيتها باستخدام كبريتات الأمونيوم بنسبة تشبع (80%)، وتم استخدامها في عمود الترشيح بهلام Sepharose-6B. تم استخدام هلام البولي أكريلاميد SDS-polyacrylamide gel electrophoresis لتحديد الأوزان الجزيئية للبروتينات، حيث تم العثور على حزمة واحدة بالوزن الجزيئي 68 و 50 كيلو دالتون لكل من *B. subtilis* و *B. cereus* على التوالي. كشفت هذه البروتينات عن نشاط مضاد للجراثيم على البكتيريا الإيجابية لجرام، وتأثير معتدل على البكتيريا السالبة لجرام، باستثناء *Pseudomonas aeruginosa* حيث لم يظهر أي تأثير. أظهر النشاط المضاد للعشاء الحيوي في المستخلص تأثيرًا متزايدًا على *Streptococcus pyogenes*، في حين كان هناك تأثير معتدل ضد كل من المكورات العنقودية الذهبية، الإشريكية القولونية، سيراشيا مارسيسنس، أنواع الكليبيلا مع عدم وجود تأثير ضد *P.aeruginosa*. أشارت نتائج فحص السمية الخلوية بالمستخلص المنقى كان له تأثير سام عالي لخلايا سرطان الثدي MCF7 لخلايا سرطان الثدي بتركيز 1000 ميكروغرام/مل وهو أعلى من المستخلص الخام الذي أظهر تأثير سام للخلايا على نفس خط الخلية عند 100 ميكروغرام/مل.

## Introduction

Antibiotics, naturally formed by microorganisms are responsible for the control and regulation of microbial population in soil, compost and water. The majority of antibiotics

are toxic in nature and only a few are used in medicine for treatment of multiple diseases due to their high safety [1]. Bacterial secondary metabolites or bioactive compounds have structural diversity obtained from natural

sources that enhance the biological activity of the host. The products of the bioactive compounds are used widely in alternative therapies, such as cancer chemotherapy [2]. Important advances have been made to increase studying live bacteria or bacterial products such as proteins, antioxidant, enzymes, secondary metabolites and immune toxins, which specifically target cancer cells and cause tumor decline through growth inhibition and blocking cell proliferation in order to arrest and obstruct apoptosis induction [3]. The genus *Bacillus* is a heterogeneous group of Gram-positive, large, rod-shaped, aerobic or facultative anaerobic, endospore forming bacteria. *Bacillus* isolates have a variety of roles in biotechnology, ecology, industry as well as in clinical microbiology [4]. Many *Bacillus* spp., such as *B. cereus*, *B. mojavensis*, *B. megaterium*, *B. stercorarius*, and *B. subtilis* produce a wide variety of extracellular enzymes, including proteases [5]. Furthermore, *Bacillus* spp. have been found to be a rich source of various bacteriocins, lipopeptides, and other bacteriocin-like inhibitory substances. Bacteriocins are small, ribosomally synthesized antimicrobial peptides which are secreted and produced by bacteria for self-defense against the growth of closely related bacterial spp. Bacteriocins mainly decrease the growth of bacteria by pore production on the cell surface or via interference with cell wall synthesis [6]. The aims of this study were to extract some proteins from *Bacillus* spp. isolated from soil, study their effectiveness against some pathogenic bacteria, and determining their antibiofilm and anticancer activities.

## Materials and Methods

### Sample collection

Forty soil samples were collected from different regions in Baghdad city at a depth of 5-10 cm below the soil surface (approximately 4 g each), and gathered with sterile spatula inside dry, clean and sterile polythene bag along with sterile spatula. All the samples were transferred to the laboratory under sterile conditions, and kept at 4°C [4].

### Isolation of *Bacillus* spp.

One gram of the soil sample was mixed with 9ml of distilled water to make  $10^{-1}$  soil dilution, and then serial dilutions were made till  $10^{-6}$  dilution. Subsequently, 0.1ml was taken from  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  dilutions, and streaked on Nutrient agar, Blood agar, and Tryptic soy agar and incubated at 37°C for 24 hours [7].

### Identification of Bacterial Samples

Diagnosis of the isolates primarily depended on morphological characteristics of the colonies, including shape, color, texture and edges [8], along with microscopic examination, biochemical tests and confirmation by VITEK 2 System.

### Protease production test:

This test was done by inoculating the bacterial isolates on the skim milk agar plates and observing the zone of casein hydrolysis around the colony after incubation for 48hr. [9].

### Pathogenic bacteria used

The pathogenic bacterial isolates used in the study were obtained from the Post-graduate Laboratories in College of Science/ Al-Mustansiriyah University. The isolates were: *S. aureus*, *Klebsiella* spp., *P. aeruginosa*, *Escherichia coli*, *Serratia marcescens*, and *Streptococcus pyogenes*.

### Biofilm formation by pathogenic test bacteria

The quantification of the biofilm was determined by crystal violet assay (CV) according to [10] with some modifications. Colonies obtained from fresh culture were suspended in 5ml of 0.9% (w/v) NaCl solution. Twenty microliters of this solution were transported to each well of the microplate. Then, 180 µl of Brain Heart Infusion Broth (BHB) including 2% sucrose were added to each well of micro plate, which was incubated at 37°C for 24hr. After incubation the plate was washed with 0.9% (w/v) NaCl solution for three times to eliminate non-adherent cells, and 200 µl of 100% ethyl alcohol were added for 20min to fix the adherent cells. The plate was dried at room temperature for 30min and later 200 µl of 0.5% crystal violet dye were added for 15min. After eliminating the dye solution and

washing with sterile distilled water, the attached dye was solubilized with 0.5% (v/v) ethanol and the optical density was determined using a micro-titer plate reader at 560nm. The pathogenic bacteria were tested at three repeats. The results depended on the optical density (O.D) in ELISA reader, which indicated that  $O.D > 0.12$  produced weak biofilm, OD of 0.12-0.24 gave moderate adherent, and  $OD < 0.24$  gave strong adherent (produce strong biofilm) [11].

### **Screening antibacterial activity produced by *Bacillus* spp.**

The agar well diffusion method was used to determine the antibacterial activity of *B. cereus* (B1) and *B. subtilis* (B16) isolates selected based on the protease production, and hemolysis activity. The pathogenic test strains included: *S.aureus*, *Klebsiella* spp., *E.coli*, *S.pyogenes*, *P.aeruginosa* and *S. marcescens* of 24hr old cultures and adjusted to 0.5 McFarland turbidity standards. Then, the bacteria were inoculated onto the surface of Muller Hinton agar plates by sterilized cotton swabs and left to dry for 15min. Wells were made by using sterilized cork- borer (8mm) , into each well 100 $\mu$ l of cell-free culture supernatants of *Bacillus* spp. were transferred. The agar plates were incubated at 37°C for 24hr and the zones of inhibition around each well were measured [1]

### **Determining the optimal conditions for antibacterial activity of *Bacillus* spp.**

#### ***Optimum pH of the medium***

The pH of tryptic soy broth (TSB) medium was adjusted to six different pH values (4, 5, 6, 7, 8 and 9) using 0.1N HCL and 1N NaOH. Then, the medium was inoculated with a fresh culture of the selected isolate. The flasks were incubated at 37°C for 48hr and the antibacterial activity was assayed according to [12].

#### ***Optimal incubation temperature***

The pH of the production medium was adjusted to the optimum pH 7.0. It was inoculated with a fresh culture of the selected isolate and

incubated at different temperatures 30°C, 37°C and 42°C for 48hr to find the optimal temperature of incubation to be assayed for antibacterial activity [12].

#### ***Optimal incubation time***

Different incubation times were examined to determine the optimal incubation period. The flasks of the production medium adjusted to the optimum pH 7.0 were inoculated with 24hr old culture of the selected isolate incubated at 37°C for 48hr or 72hr and evaluated for antibacterial activity [12].

### **Extraction and partial purification of extracellular proteins**

A loop-ful of the selected producing isolate was inoculated into 100ml of TSB with pH 7, and incubated at 30°C for 72hr. Bacterial extracellular protein extraction was done according to [13] with some modifications. The bacterial culture was centrifuged at 7000  $\times$ g for 6min at 4°C. The supernatant was collected and filtered through 0.2 $\mu$ m syringe filter in order to produce sterile cell-free supernatant. A specific weight of ammonium sulfate 80% was added gradually to the supernatant in an ice bath with continuous stirring for 30min, and left for 1hr. at 4°C to precipitate the proteins. Then, the precipitated proteins were pelleted by centrifugation at 10,000  $\times$ g for 30min at 4°C and the process was repeated three times. Finally, the pellet was dissolved in 20 mM phosphate buffered saline (PBS) of pH 7.4, the salt was removed by dialysis using dialyzes tube 3kDa over night against the same buffer at 4°C, and then concentrated with sucrose at 4°C.

### **Gel filtration chromatography**

After the sample partially purified with ammonium sulfate, it was applied to a column (1.5  $\times$  80 cm) of Sepharose –6B equilibrated with PBS. The column was leave about with the same buffer at flow rate of 0.5ml/min. The fraction (5ml) was collected and assessed for absorbance at 280nm to determine protein concentration. The fractions containing pure proteins were pooled and concentrated using

ultrafiltration cell membrane (3kDa) [14] with some modifications.

### ***SDS-polyacrylamide gel electrophoresis***

According to [15] the purified samples were boiled in a water bath for 10min and centrifuged at 10,000 g for 10min at room temperature. Tris glycine electrophoresis buffer was added to the top and bottom of reservoirs, and 15-25  $\mu$ l of the supernatant were loaded into the bottom of the wells. An equal volume of 1x SDS gel buffer was loaded into the other unused wells. Then, the electrophoresis apparatus was prepared, in which positive red electrode was associated to the bottom container and negative black electrode to top one, and the voltage was adjusted to 100V (8V/cm of the gel). The gel was stained for 10min with 0.2% Coomassie-blue G-250. Later, the gel was de-stained with the destaining solution for several times at 10-15min each. The molecular weights of the proteins were estimated by comparing the migration of proteins with standards of known size

### ***Applications of purified extracts as antibacterial, antibiofilm and anticancer***

#### ***Antibacterial activity of the purified extract***

Agar well diffusion assay, described previously was used for this experiment. The derived extracts (100  $\mu$ l) were loaded into the wells of agar plates inoculated with the test microorganisms and then discs of antibiotics were used as control. The plates were conserved at room temperature for 1hr., and then they were incubated at optimal cultural conditions. After the incubation period, the inhibitory zones (mm) were measured [1]

#### ***Antibiofilm analysis of the extracts***

The biofilm inhibition assay was described by [16]. Following incubation for 24hr., the biofilm was allowed to be formed. Then, the plate was washed with sterile distilled water to eliminate non-adherent cells, and 100  $\mu$ l of the purified extract solution was added to each well. Equal volumes of sterile distilled water were used as a negative control. Subsequently, the plates were incubated for 24hr, and the next day, the biofilm was assessed using the crystal

violet assay as described by [10], and the absorbance of the plate was measured at 450nm in an ELISA reader. The % inhibition and disruption were calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{control OD} - \text{sample OD}}{\text{Control OD}} \times 100$$

### ***Cytotoxicity effect of the extracts in vitro***

#### ***Preparation and maintenance of the cell lines***

According to [17], the cancer cell-lines were routinely cultured in 25ml flask and incubated under standard conditions (37°C). The medium used for growing of the cells was RPMI-1640. The cells were monitored daily to form a confluent monolayer. Subculturing was established by discarding the old medium, followed by washing the cells with sterile PBS under aseptic conditions. Trypsin-versine solution (2-3ml) was added to the confluent monolayer, and incubated at 37°C for 2-3min till the cells became rounded and separated into single cells by gentle shaking. Then, 20ml of the culture medium supplemented with 10% fetal calf serum were added to the flask to make up cell suspension. While for cell line maintenance, the flask containing cell suspension was incubated horizontally at 37 °C in the presence of 5% CO<sub>2</sub>.

#### ***Viable cell counting***

Viable cell counting was performed according to [18] using the trypan blue exclusion method. Dead cells take up the stain within a few seconds, making them easily distinguished from viable cells. The following protocol was done: One part of cell suspension (0.2ml) was mixed with an equal volume of diluted trypan blue (0.2ml trypan blue in 1.6ml of PBS). Then, 20  $\mu$ l of the cell suspension were transferred immediately to the edge of haemocytometer chamber, and the slide was left for 2min. Eventually, the number of cells was counted in one large center square (1mm<sup>2</sup>) using a light microscope under 20x objective lens. Cell concentration (cells/ml), total cell count and cell viability (%) were calculated from the following equation (Freshney, 2010):

$$C = n \times d \times 10^4, \text{ where:}$$

C = Cell concentration (cells/ml), n = number of counting cells

d = dilution factor= 2

$10^4$  = inversions of volume index

Total cell count = C (cell/ml) × the original volume of fluid from which the cell sample was removed. The percent of cell viability was calculated as follows:-

Cell viability % = (No. of unstained cells / Total No. of cells) × 100

In order to study the cytotoxic effect of the extract on the cancer cell line, the cell viability should be determined. The cell line must contain more than 95% viable cells to be ready for the test.

#### **Addition of MTT and cytotoxicity assay**

MTT solution (yellow in color) was made by dissolving 5mg MTT crystals in 1ml of PBS solution. The MTT solution (10 µl) was added to each well of 96-well plate, then incubated for 4hr at 37 °C After that all the solution added to the 96-well plate, including media was removed. Then, 50 µl of di-methyl-sulfoxide (DMSO) were added to each well and shaken for 5min (The colorless DMSO solution became purple). After complete solubilization of the dye, the absorbance of the colored solution derived from living cells was read at 570nm with an ELISA reader. MTT can be decreased by mitochondrial dehydrogenases to form water insoluble pink compound called formazan, based on the viability of cells [19].

Cytotoxicity effect of various concentrations of the extract on the proliferation of the adherent cells was studied in 96-well Microtiter according to the method of [20]. The extract was sterilized by filtration through 0.22 µm membrane filter and diluted starting with (1000,100,10, and 1.0µg/ml) under aseptic conditions. The cell suspension of the cancer cell line was prepared as mentioned above. A cell suspension of 200 µl and containing  $1 \times 10^5$  cell/ml was added to each well, and the plate was sealed with adhesive film and incubated at 37°C for 24hr. until the cells reached 60 to 70 % confluence. The following day, the medium was removed and 200 µl of

various concentrations of purified extract were added to the cells with the use of 3 well replicates for each concentration. The control involved cancer cells without extract treatment were treated with 200 µl of serum free medium in 6-well replicates, and the plate sealed with adhesive film and incubated at 37°C for 24hr. At the end of the incubation time, the extract and medium were eliminated from the plate and washed with PBS to eliminate unattached (dead) cells.

#### **Statistical Analysis**

The data obtained were subjected to analysis of variance (ANOVA) test, followed by student's t-test to compare various groups with each other. Results were expressed as mean standard error and values of  $p > 0.05$  were considered statically non-significant. While  $p < 0.05$  and  $< 0.01$ , 0.001, 0.000 were considered significantly different and highly significantly different respectively. LSD was carried out by SPSS (20).

## **Results and Discussion**

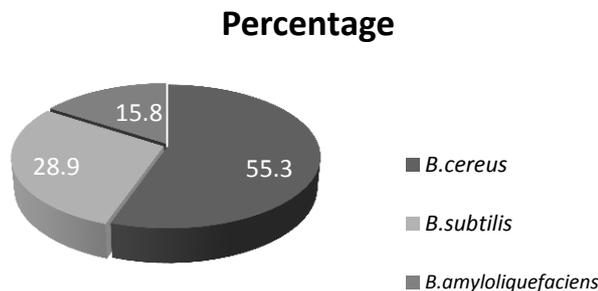
#### **Isolation and identification of bacteria**

Among 52 isolate, only 38 isolates (73.1 %) of *Bacillus* spp. were isolated. It was observed that among the 38 isolates of *Bacillus* spp., *B. cereus* was predominantly found and accounted for 21 (55.3%) followed by *B. subtilis* 11 (28.9%), and *B. amyloliquefaciens* 6 (15.8%), (Figure 1). The identification of *Bacillus* spp. was done by cultural characteristics, microscopic identification, and biochemical tests, then confirmed by using the Vitek 2 system. *Bacillus* which is the most abundant genus in the rhizosphere of soil, are naturally occurring soil bacteria that aggressively colonize plant roots, and benefit plants by providing growth promotion [21].

#### **Screening for protease production**

Bacterial isolates showed protease production on the skim milk agar, and different results are shown in Table 1. These isolates were positive to protease test also they were positive to hemolysis test on Blood agar, so they were

chosen for next experiments. The tested bacteria were incubated for 48hr to observe the maximum protease production as described by [22] and [23].



**Figure 1:** The percentage of *Bacillus* sp. isolated from soil

### Screening for antibacterial activity of filtrate produced by *Bacillus* spp.

The results in Table 2 show that (B1) and (B16) isolates, which produced proteins have antibacterial activity against both Gram positive bacteria (*S. aureus* and *S. pyogenes*) and Gram negative (*E. coli*, *Klebsiella* spp and *S. marcescens*) however the impact on Gram positive was higher than on Gram negative. *Bacillus* spp. have been considered as extremely useful microorganisms for producing antibacterial agents [1]. Several studies reported that secondary metabolites produced from *Bacillus* spp. were more effective against Gram positive bacteria. Resistance of Gram negative bacteria to these secondary metabolites may be attributed to the low permeability of the outer membrane and the lipopolysaccharide barrier for these organisms [24].

### Effect of temperature on antibacterial activity

*B. cereus* (B1) isolate and *B. subtilis* (B16) isolate incubated at different incubation temperatures (30°C, 37°C and 45°C) and tested for their antibacterial effects revealed that the optimal temperature for protein production was 30°C (Table 3). These results agree with [25] who reported that proteins produced by *B. cereus* strains showed stability in a wide range of pH values (3.0–10.0) at 30 °C, which was the most suitable temperature for protein production.

### Effect of pH on antibacterial activity

The optimal pH for protein production was pH 7.0 (Table 4). This result agrees with that of [12] who found pH 7.0 was the best for the protein production from *B. subtilis*.

### Effect of incubation time on antibacterial activity

Optimal incubation time was observed at 72hr, (Table 5). Whereas after 96hr of incubation the specific activity decreased with increasing the incubation, this may be due to the change in the conditions of culture along this period, such as diminishing of oxygen, nutrients and accumulation of toxic metabolites that inhibit the bacterial growth. Some studies have demonstrated that the production of the enzyme starts in the early stages of microbial growth [26] and [27].

### Purification of the extract

#### Ammonium sulfate precipitation

In this study, the proteins were precipitated with 80% ammonium sulfate saturation for 3 repeats and the salt was removed using a 3-kDa ultra membrane filter, in which the fraction containing the protein did not pass through the filter. This is similar to the procedure of [28] who reported that the saturation ratio of ammonium sulfate for precipitating proteins produced by *B. subtilis* was 80%.

#### Gel filtration chromatography

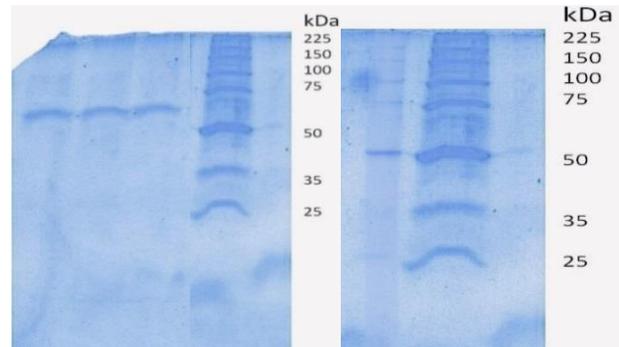
Four peaks appeared for each tested sample subjected to purification by using gel filtration chromatography (Figure 2).

#### SDS-polyacrylamide-gel electrophoresis

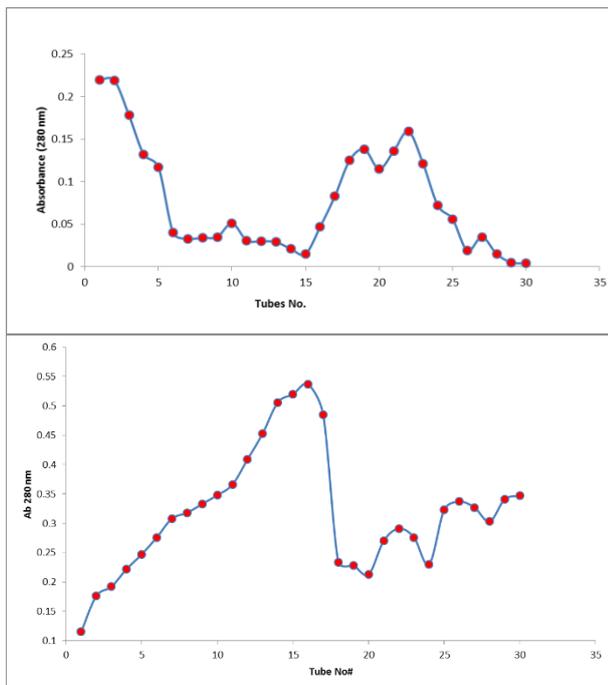
The molecular mass of the purified proteins produced by *B. cereus* and *B. subtilis* determined by using SDS-PAGE was ~68kDa and 50kDa respectively. The protein of 50kDa is considered as a new protein, which might be in the Class III bacteriocins, a group of heat-labile proteins produced by *B. subtilis* as described by [29] who showed similar results with a protein sample of 51.36kDa. Likewise, [30] observed 50kDa protease enzyme partially purified from *B. subtilis* by using 75%

ammonium sulfate precipitation. The protein with ~68kDa produced by *B. cereus* might be similar to ExsD exosporium protein described by [31] who reported a protein of approximately 66kDa that is compatible with an ORF of 154 amino acids, with five cysteine residues near its C terminus in *B. cereus*. While [32] found that the protease fragments produced by *B. cereus* were  $70 \pm 10$ kDa, which are similar to keratinolytic protease. Another study by [28] stated that three new extracellular protein bands appeared on SDS-PAGE with approximate sizes of 30kDa, 22kDa and 23kDa, respectively, secreted by *B. subtilis* ATCC21332.

polysaccharides and nucleic acids, so that it allowed to measure the whole biofilm [33].



**Figure 3:** SDS-PAGE analysis of proteins produced by *B. cereus* and *B. subtilis*: a-68kDa protein; b- 50kDa protein ; Lane 1: molecular marker;



**Figure 2:** Sepharose chromatography of *B. subtilis* (above) and *B. cereus* (below) extracellular proteins

#### Biofilm formation assay by tested bacteria

The results showed that the biofilm formation differs between the pathogenic bacteria. Both *S. pyogenes* and *P. aeruginosa* had high biofilm formation values, while *Klebsilla spp*, *E. coli* and *S. aureus* had moderate biofilm formation values, but *S. marcescens* showed weak biofilm formation value (Table 6).

Crystal violet was known to bind negatively charged molecules on the cell surface,

#### Application of purified extracts

##### Antibacterial activity

The activity of the purified extracts of *B. subtilis* (B16) and *B. cereus* (B1) isolates measured on some pathogenic bacteria, showed high activity against Gram positive bacteria (*S. aureus* and *S. pyogenes*), moderate activity against Gram negative bacteria (*Klebsilla spp*, *E. coli*, and *S. marcescens*.), and no activity against *P. aeruginosa* (Table 7, Figure 4).

[34] suggested that the outer membrane of Gram-negative bacteria keep on its inner membrane, and so higher doses of proteins are required for inhibition of their growth. The variation in antimicrobial activity has been attributed to the genetic profile of different bacterial strains [6].



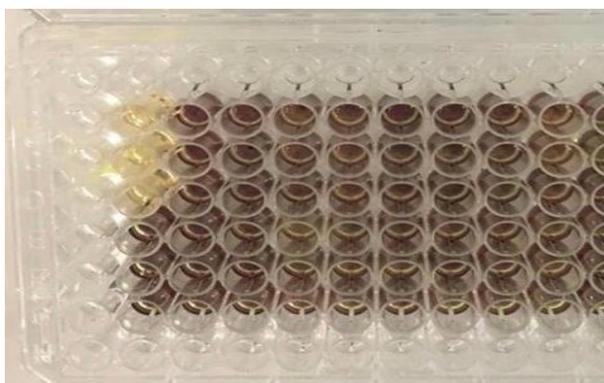
**Figure 4:** Antibacterial activity of *B. cereuse* proteins against *S. aureus*

### Antibiofilm activity of extracts

The ratio of biofilm inhibition of purified extracts differs between the pathogenic bacteria as shown in this study. High inhibition was showed on *S. pyogenes* and moderate on (*S. aureus*, *E. coli* and *Klebsiella* spp.). While low effect on biofilm formation was showed by *S. marcescens*, and no effect on biofilm formation by *P. aeruginosa* (Table 8). The decrease in cell metabolism occurs because most antimicrobial peptides work by interacting with the bacterial cell surface, followed by disruption of cellular integrity [35].

### Cytotoxicity effects of purified extracts on breast cancer MCF7 cell line

The purified extracts produced by *B.cereus* and *B.subtilis* were used to evaluate their anticancer activity against the breast cancer cell line MCF7 (Figure 4). The purified extracts were effective against MCF7 cells in a high concentration of 1000 µg for each protein (p3 and p4) of *B.cereus* which showed a significant rate for residual survival of cancer cells of 33.8 and 34.5 respectively. While the proteins (p1, p2) of *B. subtilis* revealed a significant rate of residual survival of 48.5 and 48.33 respectively, for the cancer cell line (Table 9). Probiotic bacteria are capable to decrease the level of some dangerous enzymes in the human body, such as β-glucuronidase, glycosidase, azoreductase, and nitroreductase which convert the precarcinogens into active carcinogens [36].



**Figure 5:** Cytotoxicity assay for the breast cancer cell line MCF7

### Conclusions

*Bacillus cereus* constitutes the highest percentage among the *Bacillus* spp. isolated from soil, followed by *B. subtilis*. Four peaks of purified proteins were produced by *B. cereus* and *B. subtilis* by using gel filtration chromatography. The molecular weight of these proteins was 68kDa and 50kDa as determined by SDS-polyacrylamide gel electrophoresis. The purified extracts were more active against Gram positive than Gram negative pathogenic bacteria. Also, these extracts had the highest effect on the breast cell line MCF7. Therefore, from these results, it is possible to apply these purified proteins for antibacterial, antifungal and antiviral activities in different fields, along with their anti-cancer effects against one of the cancer cell lines

### Acknowledgment

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