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Production of antimicrobial agents by *Bacillus* spp. isolated from Al-Khor coast soils, Qatar

Asmaa Missoum* and Roda Al-Thani

Microbiology Laboratory, Department of Biological and Environmental Sciences, College of Arts and Sciences, University of Qatar, Doha, Qatar.

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Bacilli are Gram-positive, sporulating bacteria that can be found in diverse habitats, but mostly in soil. Many recent studies showed the importance of their antimicrobial agents that mainly target other Gram-positive bacteria. This study was conducted to assess the antibacterial activity of Al-Khor coastal soil *Bacillus* strains against four bacterial strains (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) by agar diffusion method as well as investigating best strain antibiotic production in liquid medium. The 25 isolated strains were identified using physical and biochemical tests. Results show that most strains possess significant antibacterial potential against *S. aureus* and *S. epidermis*, but however less toward *E. coli*. Strain 2B-1B optimum activity was achieved using Luria Bertani broth and at 35°C, with pH 9. Therefore, antimicrobial compounds from these strains can be good candidates for future antibiotics production. Further screening for antimicrobial agents should be carried out in search of novel therapeutic compounds.

Key words: Al-Khor, zone of inhibition, *Bacillus*, coastal soil, antimicrobial agent.

INTRODUCTION

Bacillus bacteria belong to the Firmicutes phylum, the Bacillaceae family, and are Gram-positive bacteria that can be found in diverse habitats, but mostly in soil (Slepecky and Hemphill, 2006; Graumann, 2012). Their cells are straight; rod shaped and can be either found in pairs or singles, chains, or even as long filaments as well as peritrichous flagella in some motile species (Baruzzi et al., 2011). Moreover, for each of the cells, an endospore forms to resist adverse and harsh conditions such as radiation, heat, and chemicals such as disinfectants (Tan and Ramamurthi, 2014). Although most species are facultative aerobes, some can be anaerobic (Prieto et al., 2014).

Several studies have revealed that some species such as *Bacillus brevis, Bacillus subtilis, Bacillus circulans, Bacillus polymyxa, Bacillus cereus,* and *Bacillus licheniformis* are capable of producing antibiotics (Hirad et al., 2013; Sawale et al., 2013; Rai et al., 2017). Some of them were extracted and used for many medical treatments, for example Tyrothricidin for sore throat infections and Polymyxin for ear inflammations (Mondol et al., 2013). Antibiotics are produced during the early

*Corresponding author. E-mail: amissoum@live.com.

stages of spore formation, which suggest that this phenomenon is considered as one defense mechanism of these bacterial species against other microorganisms (Sumi et al., 2015). In addition, the antibiotics synthesized by *Bacillus* species can be classified into four categories according to their mechanism of action: cyclic oligopeptides (for example, bacitracin) that hinder cell wall synthesis; linear oligopeptides (for example, gramicidins and polymyxin) that interfere with cell membrane function; basic peptides (for example, edeines) that restrain the initiation complex formation on the small ribosome subunit; and aminoglycoside antibiotics that distress ribosome function (Graumann, 2012).

Various scientific literature have revealed that most *Bacillus* species isolated from marine sediments showed more antagonistic activities against Gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, but however less toward Gram-negative bacteria such as *Pseudomonas aeruginosa* (Kannahi and Eshwari, 2016). This phenomenon is supported by recent studies in India and the Arabian Gulf (Parvathi et al., 2009; Hirad et al., 2013; Sawale et al., 2013). In addition, this case could be also explained due to differences in cell wall composition of tested bacteria. Unlike Gram-positive bacteria which has only one thick layer of peptidoglycan, Gram-negative bacteria has far more complex components, in which the antimicrobial peptides must cross to interfere with its molecules required during cell wall biosynthesis (Malanovic and Lohner, 2016).

Moreover, lantibiotics inhibit growth of the Gram-positive bacterium *S. aureus* via the obstruction of a carrier of peptidoglycan monomers, Lipid II, across the cytoplasm membrane to the exterior side. As a consequence, the incorporation of the growing peptidoglycan network by Lipid II is prevented during cell wall synthesis (Baruzzi et al., 2011; Sumi et al., 2015). Another possible explanation for the antimicrobial peptides' action is that these disrupt the cell cytoplasm by binding to and aggregating with the membrane. As a result, these form pores through which ions can pass and lead to cell components leakage and eventually, cell death (Sumi et al., 2015).

The main aim of this study was to isolate, for the first time, *Bacillus* species from Qatari coastal soils that exhibit antimicrobial activities against both Gram-positive and Gram-negative bacteria, as well as to investigate their production in liquid media.

MATERIALS AND METHODS

Location and collection of soil samples

A total of 36 soil samples were collected near a coast of Al-Khor at 25.67 north latitude and 51.57 east longitude, at a depth of 10 cm (50 g for each sample). The temperature on collection day was 32°C with humidity of 35%, wind speed of 29 km/h, and 0% precipitation. Each sampling was carried out 5 m apart and only 1 m away from the sea, and three replicates were collected from each site (Figure 1).

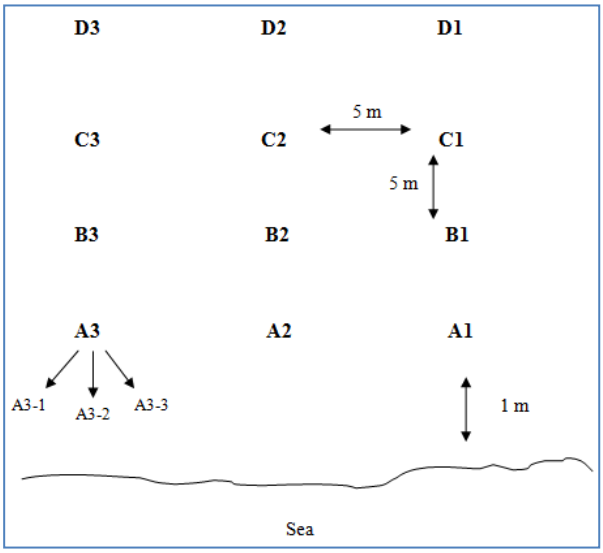

Figure 1. Soil sample collection sites at Al-Khor coast. There were 4 levels and a total of 12 sites and from each three replicates were taken. Sites A1, A2, and A3 are one meter away from the shore.

Culture media and isolates selection

Each 1 g of soil sample was diluted to achieve 10^{-2} and 10^{-3} dilutions. The media used were nutrient agar (NA): 28 g of nutrient agar (HIMEDIA) was dissolved in 1000 ml of distilled water, pH 7.02, and starch casein agar (SCA): 0.3 g Casein (BDH), 10 g starch (Riedel-de Haen), 2.0 g KNO_3 (Scott Science UK), 2.0 g K_2HPO_4 (Riedel-de Haen), 2.0 g NaCl (Eurostar Scientific), 0.005 g $MgSO_4.H_2O$ (Scott Science UK), 0.02 g $CaCO_3$ (Scott Science UK), and 20 g Agar type I (HIMEDIA) were dissolved in 1000 ml of distilled water, pH 7.0. Thereafter, all plates were incubated at 35°C for a total period of 5 days, and isolates which exhibited inhibition zones surrounding their colonies were selected and sub-cultured on nutrient agar.

Identification of Isolates

The identification of *Bacillus* strains was carried out on the basis of morphological (Gram stain and cultural characterization), physical (growth at pH: 3, 5, and 10, temperatures: 35, 45, and 60°C, and NaCl concentration: normal, 5, 10, and 15%), and biochemical characteristics (starch, casein, and tributyrin oil hydrolysis, HIMEDIA). The characteristics of each strain were compared with those described in "*Bergey's Manual of Systematic Bacteriology"* and other scientific literature.

Bacterial strains

Tested bacterial strains including Gram-positive, *S. aureus* and *S. epidermis,* and Gram-negative strains *E. coli* and *P. aeruginosa* were obtained from Department of Biological and Environmental sciences, Qatar University, Doha, Qatar.

Antimicrobial assay using agar well diffusion

For all bacterial test strains, a loopful of each four bacterial culture was inoculated in 9 ml autoclaved distilled water and all four tubes were incubated at 35°C for 10 min. Then, four NA plates were swapped; one for each 'aqueous' culture, using sterile cotton swaps (Eurotubo). Afterwards, 0.6 cm agar wells were prepared by punching sterile Pasteur pipettes in NA medium with test bacteria. The cell free supernatant for each *Bacillus* isolate was prepared by centrifuging 1.5 ml of inoculated Nutrient broth, pH 7 (HIMEDIA) at 6000 rpm for 20 min at RT. Then, 100 µl of each supernatant culture was applied onto wells separately and plates were incubated at 35°C for 24 h. Un-inoculated nutrient broth was used as negative control against test bacterial cultures. All tests were carried out in triplicate. After 24 h incubation, antibacterial effect was observed, measured as zone of inhibition and recorded in cm.

Antimicrobial agent production in liquid medium

After testing antimicrobial activities of all 25 isolates, strain 2B-1B was selected as best promising strain. Therefore, its antimicrobial activity was further studied against most sensitive strain, *S. epidermis*, in previous screening. First, each loopful of 2B-1B bacterial culture was inoculated into each 50 ml of four media (pH 7): nutrient broth (NB), starch casein broth (SCB), Luria bertani broth (LB), and lactose broth (LacB), each in 250 ml Erlenmeyer flask. After incubation at 35°C in a shaking incubator maintained at 200 rpm for 20 h, the antimicrobial assay was carried out by swapping NA plates with *S. epidermis* aqueous culture (see previous section) using sterile cotton swaps (Eurotubo). Afterwards, 0.6 cm agar wells were prepared by punching sterile Pasteur pipettes in NA medium with test bacteria. The 2B-1B cell free supernatant cultures were prepared by taking 1 ml from each production medium and centrifuging it at 13,300 rpm for 5 min. Consequently, 100 µl of each supernatant culture was applied onto wells separately and plates were incubated at 35°C for 24 h. Un-inoculated media was used as negative control and all tests were carried out in triplicate. After 24 h incubation, antibacterial effect was measured as zone of inhibition and recorded in cm.

Effect of pH and temperature on antimicrobial agent production

Luria Bertani broth (LB) was found to be the best medium for antimicrobial agent production by the isolate 2B-1B; hence LB was used as production medium for further studies. To see the effect of pH, each loopful of 2B-1B bacterial culture was inoculated into each 50 ml of five LB media with different pH levels: 3, 5, 7, 9, and 11, each in 250 ml Erlenmeyer flask. After incubation at 35°C in a shaking incubator maintained at 200 rpm for 20 h, the antimicrobial assay was carried out by swapping NA plates with *S. epidermis* aqueous culture (see previous section) using sterile cotton swaps (Eurotubo). Afterwards, 0.6 cm agar wells were prepared by punching sterile Pasteur pipettes in NA medium with test bacteria. The 2B-1B cell free supernatant cultures were prepared by taking 1 ml from each production medium and centrifuged at 13,300 rpm for 5 min. Un-inoculated media was used as negative control against *S. epidermis* and all tests were carried out in triplicate. After 24 h incubation, antibacterial effect was measured as zone of inhibition and recorded in cm. To see the effect of temperature, the same procedures were carried out except that five LB media (pH 7) were incubated at different temperatures: 30, 35, 40, 45, and 50°C in a shaking incubator.

Statistical analysis

Mean and standard deviation were calculated for all experimental results using Excel. Using Minitab 17, the ANOVA general linear model test was used to compare the inhibition zone diameters due to strain 2B-1B antimicrobial agents produced in different liquid media as well as at different temperatures and pH levels. Tukey test was done to compare that the sample means which were significantly different from each other. Using SPSS, Friedman test was used to compare the diameters of the inhibition zones by the antimicrobial activity of the 25 isolates. Moreover, Kruskal Wallis test was used to compare if treatment on each indicator strain was significantly different from the other. Finally, all statistical tests were carried out at a significance level of $P < 0.05$.

RESULTS

Identification of isolates

The isolates' colonies were either circular (13 isolates) or irregular (12 colonies) but had variations in both margin and elevation. Their diameters ranged from 0.1 to 0.8 cm and most of their characteristic colors were different shades of cream. Moreover, all strains were rod shaped, Gram-positive, and mostly single in arrangement. Five strains were spore forming (Table 1). Most strains hydrolyzed starch and milk agars, but only some hydrolyzed tributyrin oil agar. In addition, all strains grew at all pH levels (3, 7, and 10) and up to 45°C. However, only 7 isolates tolerated 10% NaCl as maximum (Table 2). Using "*Bergey's Manual of Systematic Bacteriology"* and other scientific literature, most strains showed closest characteristics to *B. subtilis, B. lichenoformis, B. coagulans, B. us megaterium, B. polymyxa, and Brevibacillus brevis*. (Arbsuwan et al., 2014; Arumugam et al., 2017; Jianmei et al., 2015; Ramachandran et al., 2014; Malanicheva et al., 2012; Sumi et al., 2015).

The isolate 2B-1B, which was selected as best producing strain has rod shaped cells arranged in chains/filaments (Figure 2), as well as off-white, irregular colonies with undulate margins and flat elevation (Figure 3).

Antimicrobial assay using agar well diffusion and statistical analysis

Most isolates were antagonistic against Gram-positive bacteria (88% of the total isolates) compared to Gram-negative bacteria (20% of the total isolates) exhibiting inhibition zones ranging from 0.1 to 0.83 cm. Each strain targeted the test microorganisms differently (Table 3). Most strains, about 36%, inhibited both *S. aureus* and *S. epidermis*, and 32% inhibited *S. aureus* only. However, only 20% showed antimicrobial activity on both Gram-positive and Gram-negative bacteria excluding *P. aeroginosa*. In addition, the strains 2A-1B, 13-2B, and 14-2B (composing about 12% of the total) did not exhibit any antimicrobial activities. Moreover, the null hypothesis states that there is no significant difference between the antimicrobial activities of the 25 strains provided the treatment of each of the indicator strains is the same. The

Table 1. Cultural and cell morphology characteristics for all 25 *Bacillus* isolates.

Site	Code	Whole colony appearance	Margin (Edge)	Elevation	Color	Diameter (cm)	Pigment	Cells Morphology	Arrangement	Gram stain	Spores
B3-3	2A-1B	Circular	Entire	Raised	Light yellow	0.4	No	Bacilli	Single/pairs	+ve	No
B3-3	2B-1B	Irregular	Undulate	Flat	Off-white	0.4 - 0.8	No	Bacilli	Chains/filaments	+ve	Yes
C2-3	3-1B	Circular/ Transparent	Entire	Convex	Cream	0.4	No	Bacilli	Single	+ve	No
D1-1	5-1B	Irregular/Transparent	Lobate	Umbonate	Cream	0.6	No	Bacilli	Single	+ve	No
B2-1	6-1B	Irregular/Transparent	Lobate	Umbonate	Cream	0.4	No	Bacilli	Single	+ve	No
D2-2	8-1B	Irregular	Lobate	Umbonate	Cream	0.4	No	Bacilli	Single	+ve	No
C3-2	10-1B	Irregular	Undulate	Flat	Off-white	0.4 - 0.6	No	Bacilli	Single/filaments	+ve	Yes
B2-2	11-1B	Circular	Entire	Raised	Off-white	0.1	No	Bacilli	Pairs	+ve	Yes
B1-3	12-1B	Irregular	Undulate	Flat	Yellow/Cream	0.4 - 0.5	No	Bacilli	Single	+ve	No
B1-3	13-1B	Irregular	Undulate	Umbonate	Cream	0.3 - 0.4	No	Bacilli	Single	+ve	No
D2-3	14-1B	Irregular	Lobate	Umbonate	Off-White	0.5 - 0.7	No	Bacilli	Single	+ve	Yes
D1-2	15-1B	Irregular	Undulate	Flat	Gray White	0.5 - 0.7	No	Bacilli	Single	+ve	No
D1-2	16-1B	Circular	Entire	Raised	Cream	0.1 - 0.2	No	Bacilli	Diplo	+ve	No
B1-1	1-2B	Circular/shiny	Entire	Convex	Light yellow	0.4 - 0.5	No	Bacilli	Single	+ve	No
B1-1	2-2B	Circular/shiny	Entire	Convex	Cream white	0.6 - 0.7	No	Bacilli	Single	+ve	No
B1-1	3-2B	Circular/shiny	Entire	Raised	Cream white	0.2 - 0.4	No	Bacilli	Single	+ve	No
B1-1	4-2B	Irregular	Undulate	Flat	Gray	0.2 - 0.4	No	Bacilli	Single	+ve	No
B1-3	5-2B	Irregular	Lobate	Umbonate	Cream	0.4 - 0.6	No	Bacilli	Single	+ve	No
B1-3	6-2B	Irregular	lobate	Umbonate	Cream	0.5 - 0.6	No	Bacilli	Pairs	+ve	No
B1-3	8-2B	Circular	Curled	Flat	Gray yellow	0.2 - 0.3	No	Bacilli	Single	+ve	Yes
C2-2	11-2B	Circular	Entire	Raised	Cream	0.4	No	Bacilli	Single/pairs	+ve	No
C2-3	13-2B	Circular/shiny	Entire	Raised	Cream	0.1 - 0.2	No	Bacilli	Single	+ve	No
C2-3	14-2B	Circular/shiny	Entire	Raised	Dark cream	0.3	No	Bacilli	Single	+ve	No
C2-3	15-2B	Circular	Entire	Raised	Dark cream	0.2 - 0.5	No	Bacilli	Single/chains	+ve	No
C3-2	16-2B	Circular/shiny	Entire	Flat	Gray white	0.1	No	Bacilli	Single	+ve	No

Friedman (related samples) test was used to compare antimicrobial activities expressed as inhibition zones diameters between the 25 *Bacillus* strains. The obtained p value was found to be 0.00, which is less than 0.05, meaning that the null hypothesis is rejected and that the antimicrobial activities on each indicator strain from each of the isolates differ from each other significantly.

In addition, the Kruskal Wallis (independent samples) test was used to compare if the antimicrobial activities (expressed as inhibition zones diameters) of each isolate affects the indicator strains differently or not. The obtained *p* values were found to be less than 0.05; meaning that the null hypothesis is rejected as the antimicrobial activities of each of these strains affects the indicator strains differently. In all tests, sample means were compared to note if they differ significantly from each other at a significance level of $P < 0.05$. The main reason why Friedman test and Kruskal Wallis were used instead of Student's *t*-test, is that our data (Table 3) are not normalized, and these tests are commonly used for small sample size non-normalized data.

Production of antimicrobial agent in liquid medium

Strain 2B-1B isolated from site B3-3, which is closer to the coast, was selected to be the best producing strain and was found to have wide range antimicrobial activities on both Gram-positive and Gram-negative bacteria. Using agar

Table 2. Physical and biochemical characteristics for all 25 Bacilli isolates.

Site	Code	pH			Temperature			NaCl				Biochemical tests		
		3	7	10	35°C	45°C	65°C	Normal	5%	10%	15%	Starch hydrolysis	Lipid hydrolysis	Casein hydrolysis
B3-3	2A-1B	-	++	++	++	+	-	++	++	-	-	+	-	-
B3-3	2B-1B	+	+	++	++	+++	-	++	+	-	-	++	+	++
C2-3	3-1B	+	+	++	++	+++	-	++	++	-	-	+	-	++
D1-1	5-1B	+	+	++	++	+++	-	++	++	-	-	++	+	+
B2-1	6-1B	+	+	++	++	+++	-	++	+	-	-	++	-	+
D2-2	8-1B	+	++	++	++	+++	-	++	++	++	-	+	-	+
C3-2	10-1B	+	+	++	++	+++	-	++	++	++	-	++	+	-
B2-2	11-1B	+	++	+	++	-	-	++	++	-	-	++	+	++
B1-3	12-1B	+	+	++	++	+++	-	++	++	++	-	++	-	+
B1-3	13-1B	+	+	++	++	++	-	++	++	++	-	++	-	+
D2-3	14-1B	+	+	++	++	+++	-	++	++	-	-	-	-	-
D1-2	15-1B	+	+	+	++	+++	-	++	++	+	-	+	-	++
D1-2	16-1B	+	+	++	++	+	-	++	++	-	-	+	+	+
B1-1	1-2B	+	+	+ +	++	+++	-	++	++	-	-	++	+	-
B1-1	2-2B	+	+	++	++	+++	-	++	+	+	-	+	-	-
B1-1	3-2B	+	+	++	++	+++	-	++	-	-	-	++	+	-
B1-1	4-2B	+	+	++	++	+++	-	++	++	-	-	+	-	-
B1-3	5-2B	+	+	++	++	+++	-	++	-	-	-	+	-	+
B1-3	6-2B	+	++	++	++	+++	-	++	+	+	-	++	+	+
B1-3	8-2B	+	+	++	++	+++	-	++	-	-	-	+	+	++
C2-2	11-2B	+	+	++	++	+++	-	++	-	-	-	+	-	+
C2-3	13-2B	+	+	+	++	++	-	++	+	-	-	+	-	-
C2-3	14-2B	+	+	++	++	++	-	++	++	-	-	-	-	+
C2-3	15-2B	+	+	++	++	++	-	++	-	-	-	+	+	+
C3-2	16-2B	+	+	++	++	++	-	++	+	-	-	+	-	-

For physical tests, the sign "-" indicates no growth, the sign "+" indicates that there is slight growth, "++" intermediate growth, and "+++" heavy growth. Regarding biochemical tests, the sign "+" indicates that there is a small/medium hydrolysis zone (Positive test), "++" large hydrolysis zone and the sign "-" indicates no hydrolysis zone.

well diffusion method (Figure 4), the antimicrobial activities of the strain 2B-1B were investigated against *S. epidermidis* after producing in liquid media at different conditions. All controls showed no inhibition zones. This antimicrobial production of this strain was optimized at various conditions using most sensitive strain *S. epidermis*. The best production was at pH 9.0 and temperature 35°C using LB broth (Figures 5, 6, and 7).

One-way ANOVA was used to compare the effect of using different liquid media, pH levels, and temperatures on the antimicrobial agents' production by strain 2B-1B at significance level of $P < 0.05$. The calculated p values for the effect of production using different broths was found to be 0.001, and those of the pH levels effect as well as temperatures were both 0.000. Thus, each of three growth conditions had a significant effect on the production of 2B-1B antimicrobial activity. Moreover, Tukey test was done to compare the sample means if they were significantly different from each other at a significance level of $P < 0.05$. Means that do not share letter differ significantly.

DISCUSSION

In the present investigation, the antibacterial activity of 25 *Bacillus* isolates against wide range of Gram-positive and Gram-negative bacteria were demonstrated. These isolates were identified based on their morphological, physical, and biochemical characteristics using Bergey's manual and other scientific literature (Arbsuwan et al., 2014; Arumugam et al., 2017; Ramachandran et al., 2014; Malanicheva et al., 2012). It has been also observed that highest antibacterial activity was demonstrated against *S. aureus* and *S. epidermis* than *E. coli.* This can be explained by the fact that Gram-positive bacteria are more likely to inhibit other Gram-positive bacteria as they are reported to excrete various types of antimicrobial compounds such as bacteriocins (Sumi et al., 2015).

Example of these compounds include coagulin produced by *B. coagulans*, lichenin produced by strain 26-103RA of *B. licheniformis*, cerein produced by *B. cereus* strains, and megacin produced by many *B.*

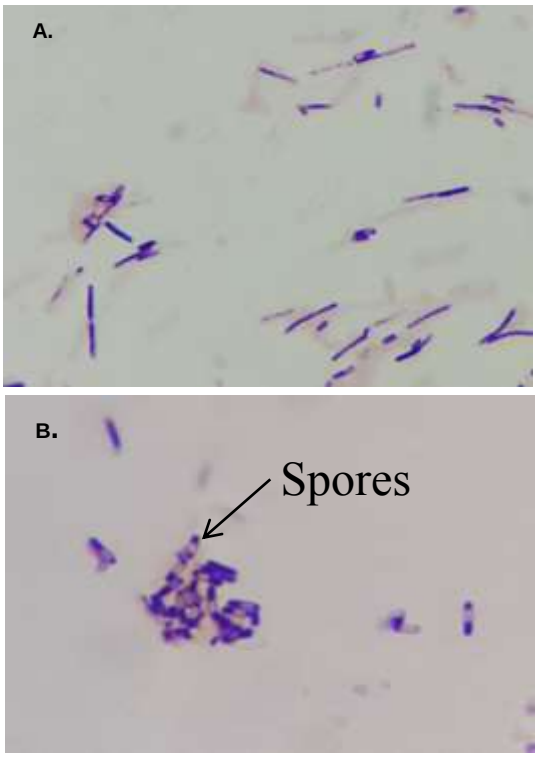

Figure 2. A. 2B-1B cell morphology. **B.** Spores formed from 2B-1B (indicated by arrows) strains using Gram strain and light microscope at ×1000 magnification.

Figure 3. Isolate 2B-1B colony morphology shown under ×10 stereo microscope.

megaterium strains (Sansinenea and Ortiz, 2011), which are some of the possible identified isolates. These exhibit a high degree of target specificity against closely related bacteria, even though some of them have a wider spectrum of activity (Baruzzi et al., 2011).

Type A antibiotics are peptides belonging to the bacteriocins family, kill Gram-positive cells through the formation of voltage-dependent pores into their cytoplasmic membranes after binding to and aggregating within the membrane. These pores lead to internal cell components leakage and as a result cell death. In case of Gram-negative bacteria, these peptides must cross the cell wall which is negatively charged and contains lipopolysaccharides (Sumi et al., 2015). Another class of lantibiotics include type B mercacidins, which mainly inhibit cell wall formation in Gram-positive bacteria by forming complexes with lipid II, a peptidoglycan precursor, which carries peptidoglycan monomers through the cytoplasmic membrane (Münch and Sahl, 2015).

On the other hand, isolates which inhibited Gram-negative indicator strains, which are resistant to bacteriocins (Sansinenea and Ortiz, 2011), might have excreted specialized peptides or proteins having different action mechanism (Qureshi et al., 2016). One of these are reported by few studies on *Bacillus polymyxa* strains (another possible identified species for 11-1B), which produce positively charged, cyclic peptide that in turn have high affinity to lipopolysaccharides' lipids, and consequently lead to disruptive effect on membrane integrity. Example of these includes polimixins that have been used against Gram-negative bacteria (Baruzzi et al., 2011).

In case of *P. aeruginosa,* it seemed to show resistance that could be caused by several mechanisms such as the formation of thick biofilms, which contain a high level of the poly-saccharides alginate and thus altering its structure. These contributes to conformational changes in invading antimicrobial peptides when binding to them, which then oligomerizes, and this consequently hinders their ability to enter the biofilm (Band and Weiss, 2014).

Based on mean inhibition zones diameter, LB broth was found to be the best for 2B-1B antimicrobial agents' production and the best temperature and pH were 35°C and 9.0, respectively. These findings correlate with recent scientific work regarding the antimicrobial activities of *Brevibacillus brevis*, which initially classified within the *Bacillus* genus as *Bacillus brevis* (Jianmei et al., 2015). Interestingly, this strain also exhibited similar antimicrobial activities against *S. aureus, S. epidermis*, and *E. coli*. Nevertheless, this was found to be the closest possible species that shares physical and biochemical characteristics with strain 2B-1B (Bergey et al., 2012).

Finally, for future perspectives, *Bacillus* strains may be considered a promising source for isolation of antimicrobial agents with potential application in pharmaceutical industry. Therefore, more locations in Qatar should be explored to isolate *Bacillus* strains, as well as traditional identification methods accompanied by 16S rRNA sequencing and comparing within phylogeny

Table 3. Mean and SD of antimicrobial activities for all 25 Bacilli isolates as inhibition zones in cm.

Site	Code	Bacterial strains diameter of zone of inhibition (cm ± SD)			
		S. aureus	*S. epidermis*	*E. coli*	*P. aeruginosa*
	Control	-	-	-	-
B3-3	2A-1B	-	-	-	-
B3-3	2B-1B	0.80 ± 0.00	0.83 ± 0.06	0.40 ± 0.00	-
C2-3	3-1B	0.70 ± 0.00	0.72 ± 0.03	0.50 ± 0.00	-
D1-1	5-1B	0.43 ± 0.06	0.12 ± 0.03	-	-
B2-1	6-1B	0.07 ± 0.06	_	-	-
D2-2	8-1B	0.53 ± 0.06	0.32 ± 0.08		-
C3-2	10-1B	0.12 ± 0.03	_	-	-
B2-2	11-1B	0.43 ± 0.06	0.43 ± 0.03	0.47 ± 0.06	-
B1-3	12-1B	0.15 ± 0.05	0.28 ± 0.03	-	-
B1-3	13-1B	0.20 ± 0.00	0.13 ± 0.06	-	-
D2-3	14-1B	0.10 ± 0.00	_	-	-
D1-2	15-1B	0.70 ± 0.00	0.80 ± 0.00	0.37 ± 0.06	-
D1-2	16-1B	0.17 ± 0.06	_	-	-
B1-1	1-2B	0.08 ± 0.03	_	-	-
B1-1	2-2B	0.47 ± 0.06	0.10 ± 0.00	-	-
B1-1	3-2B	0.23 ± 0.06	0.33 ± 0.06	-	-
B1-1	4-2B	0.50 ± 0.00	0.40 ± 0.00	-	-
B1-3	5-2B	0.40 ± 0.00	0.22 ± 0.03	-	-
B1-3	6-2B	0.47 ± 0.06	0.10 ± 0.00	-	-
B1-3	8-2B	0.78 ± 0.03	0.70 ± 0.00	0.42 ± 0.03	-
C2-2	11-2B	0.28 ± 0.03	-	-	-
C2-3	13-2B	-	-	-	-
C2-3	14-2B	-	-	-	-
C2-3	15-2B	0.17 ± 0.03	-	-	-
C3-2	16-2B	0.18 ± 0.03	-	-	-

The sign ‘‘-’’ indicates no inhibition. Values are in terms of mean ±SD after triplicate analysis.

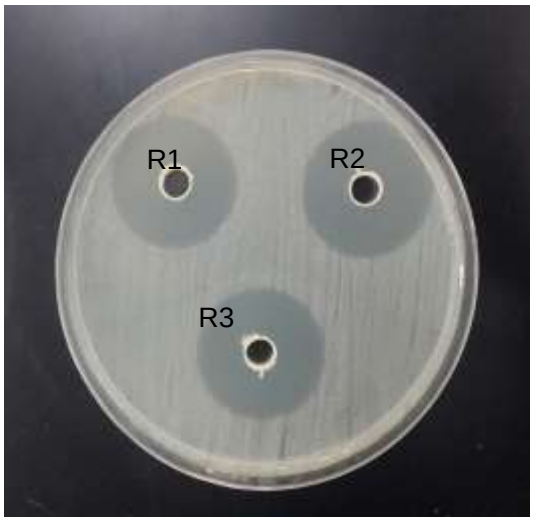

Figure 4. Inhibition zones (Mean 1.07 cm) against *S. epidermis* by strain 2B-1B at 35°C and pH 7, produced using Luria Bertani Broth with replicates (R1, R2, and R3).

tree of closely related species. Moreover, antimicrobial peptides and proteins can be extracted and their structure studied using various methods. In addition, further studies using electron scanning and transmission microscope can be carried out in order to explore mechanisms of action by antimicrobial peptides and proteins that either interferes with cell wall and membrane or other important cell components.

Last but not the least, deriving antibiotics agents from *Bacillus* species and bringing them into a practical use using cost effective methods have more advantages due to their specificity against target bacteria that is mainly caused by unexplored physiological and molecular mechanisms. Although drug discovery from natural sources presents several challenges, these can be overcome through the help of intensive efforts from other biological fields.

Conclusions

In this present study, we have managed to isolate and

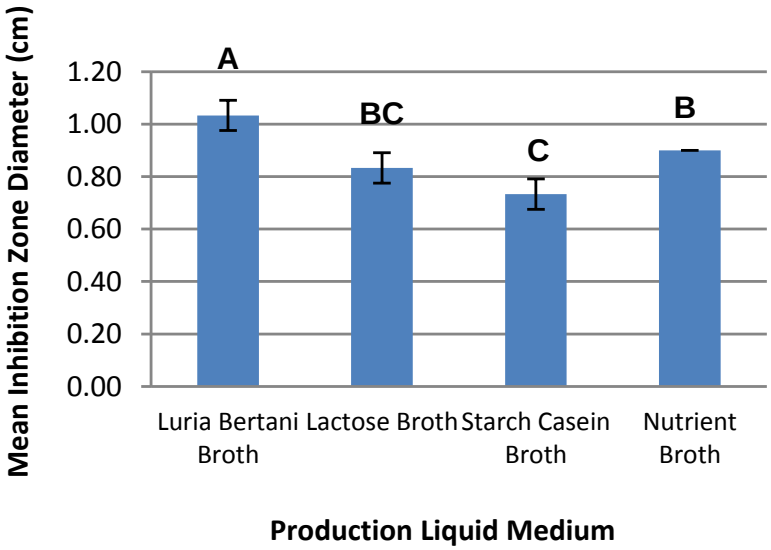

Figure 5. The effect of using different liquid media on the production of 2B-1B antimicrobial agents after 20 h of incubation at 35°C and 200 rpm. The error bars refer to standard deviations.

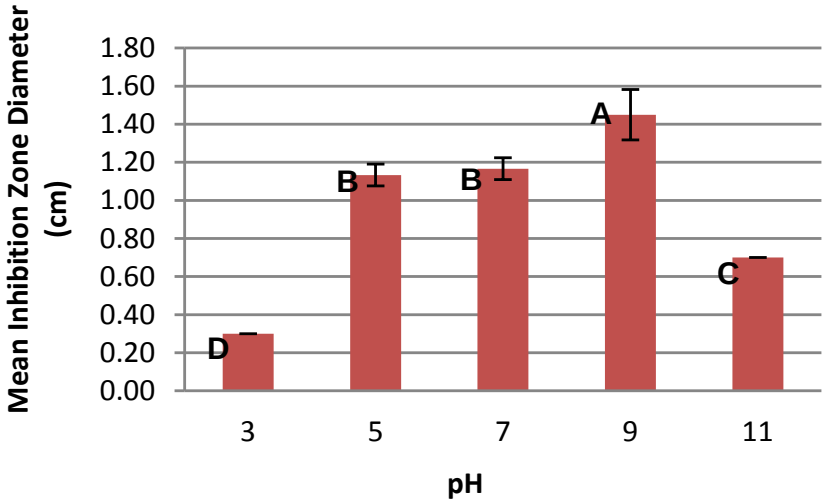

Figure 6. The effect of pH on production of 2B-1B antimicrobial agents using Luria Bertani broth after 20 hours of incubation at 35°C and 200 rpm. The error bars refer to standard deviations.

identify Bacillus strains from coastal soils that exhibit antimicrobial activity which are mostly antagonistic against Gram-positive bacteria (88% of the total isolates) compared to Gram-negative bacteria (20% of the total isolates). Agar well diffusion method was a good method to explore such activities which yielded inhibition zones ranging from 0.1 to 0.83 cm. Strain 2B-1B isolated from site B3-3, which is closer to the coast, was found to have wide range of antimicrobial activities on both Gram-positive and Gram-negative bacteria (highest mean inhibition zone against *S. epidermis*). 2B-1B optimization tests on its antimicrobial activity at different conditions confirmed that it is a promising antibiotic producing agent as it managed to perform best (yielding 1.45 cm mean inhibition zone) using Luria Bertani broth, at 35°C, and at pH 9.0, under effective conditions. Therefore, strain 2B-1B antimicrobial agents as a potent antibiotic can be a good candidate for further studies leading toward comprehensive investigation for its unexplored mechanism of action.

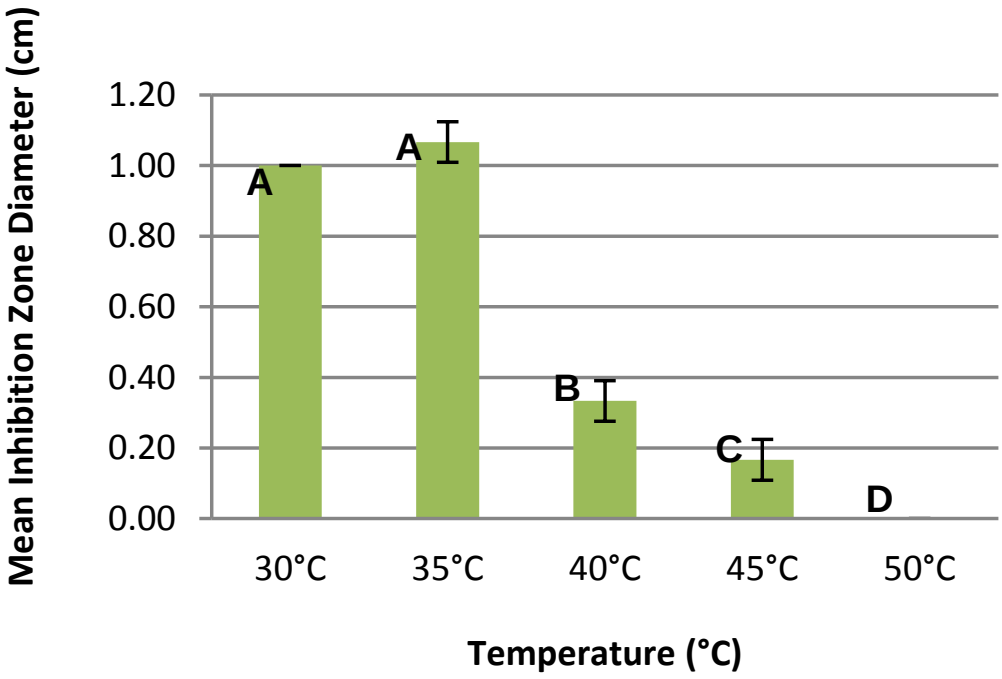

Figure 7. The effect of different temperatures on production of 2B-1B antimicrobial agents using Luria Bertani broth at pH 7, after 20 h of incubation at 200 rpm. The error bars refer to standard deviations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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