725
Arab Univ.
J. Agric. Sci.,
Ain Shams Univ.,
Cairo, 26(2), 725 - 734, 2018

# SUSCEPTIBILITY OF DESERT LOCUST, *SCHISTOCERCA GREGARIA* (ORTHOPTERA: ACRIDIDAE) TO *BACILLUS CEREUS* ISOLATED FROM EGYPT

**[57]**

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**Keywords:** Schistocerca gregaria; biological control; entomopathogenic bacteria; 16S rRNA genes

## ABSTRACT

Examination was done at preliminary bracketing bioassay on one old 4th nymphal instar of desert locust. Results showed that two isolates, namely NDL1 and NDL2 were having highly potentiality as entomopathogenic bioagents. Thirty isolates were isolated from dead/ infected nymphs of desert locust occurred in raring cages at Department of Locust and Grasshoppers Research, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. Molecular identification of isolated bacteria was done using universal primers of 16s rRNA, followed by DNA sequencing. Nucleotides were blasted at (https://www.ncbi. nlm.nih.gov /genbank/) to recognize that NDL1 and NDL2 isolates were two different isolates of *Bacillus cereus* with a high similarity (100%). Susceptibility of 4th nymphal instar of Schistocerca gregaria (Forskal) to the isolated *B. cereus* was determined using two bioassay procedures, Leaf-dip and per os. The insecticidal activity of both isolates against locust nymph in leaf dipping showed that NDL2 was more efficient than NDL1. However, the opposite trend was observed in using per os. Both Isolates have the potential to be a successful biocidal agent to control desert locust.

## INTRODUCTION

Locust has been considered as one of the most destructive pest for economic crops/plants, all over the world due to the nature of damage that caused by locust swarm (**Lecoq, 2001**). Conventional control of locust is mainly relied on chemical insecticides which are effective in suppressing the locust swarm (**FAO, 2009**).Several research programs have brought up to develop biocontrol agents against locust as a part of an integrated locust management (**Prior & Greathead 1989 and Lomer et al 2001**). The major bacteria isolates tested as biocontrol agents are belonging to spore forming bacteria such as *Bacillus thuringiensis*, *B. sphaericus, B. cereus* and *Paenibacillus popilliae*, the entomopathogenic bacterium *Yersinia* sp. (MH96), isolated from grass grub larva (*Costelytra zealandica*) (white) from New Zealand soils, showed high virulence against *Locusta migratoria* (Linnaeus, 1758), (Orthoptera: Acrididae) (**Hurst and Glare, 2006**). This bacterium is causing death through secretion of toxin followed by rapid invasion of the host hemocoel overwhelming the insect within 72 h of infection (**Hurst et al 2007**). Bacterial insecticides have been primarily developed for control of lepidopteran and dipteran pest. A few products have been developed for control of beetle larvae (**Sarwar, 2015**).

The present study was aimed to isolation and identification of entomopathogenic bacteria from dead/infected nymphs of desert locust as well as the assessment of the entomocidal effects.

**(Received 20 August, 2017)**
**(Revised 10 September, 2017)**
**(Accepted 13 September, 2017)**

## MATERIALS AND METHODS

### 1. Insects

Nymphs and adults of Desert locus *S. gregaria* reared in locust laboratory; at Department of locust and grasshoppers Research, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt; were examined daily for separating from dead / infected ones. Separated insects were brought to Microbiological Resources Center (MIRCEN)) at Faculty of Agriculture, Ain shams university, Cairo, Egypt, in sterilized tubes for bacterial isolation. Infected insect showing symptoms that suggest bacterial infection; they become flaccid, lethargic, reddish or brownish and stop eating.

### *2. Isolation of bacteria*

Dead and /or infected nymphs and adults were placed separately in different sterile tubes and subjected to microbiology lab for bacterial isolation. Isolation was carried out by three procedures body surface, internal swap and dead locust paste. Body surface of dead / infected locusts was swapped by sterilized swap into 10 ml of sterilized distilled water and then streaking was done from this solution on nutrient agar plates. Internal swap was taken from midgut and the cavity of dead / infect locusts into 10 ml of sterilized distilled water and then streaking was done on nutrient agar plates. Dead locust paste was swapped and directly streaked on nutrient agar plates. All the plates were incubated at 28°C for 72h. Isolates were distinguished based on colony color and morphology. Streaking was repeated periodically on nutrient agar plate every 72h using one separated colony in order to purify the isolated bacteria and have one isolate per each plate. Microscopic observation and parasporal inclusions and gram stain were preformed for each purified isolate.

### 3. Screening of the obtained isolates for bioactivity against locust

Fresh sticks and leaves of Egyptian clover; were used as a solo feed after dipping in each bacterial suspension individually for three min. Sticks and leaves were allowed to dry for 10 min under the ambient temperature. Each bacterial isolate was introduced to five one day old $4^{th}$ nymphal instar of desert locust using the treated sticks and leaves and placed in individual plastic boxes (30 cm in length and 18 cm in width). Daily examination was performed until 10 days and mortality % was recorded. Isolates showed potential activity against locust were subjected to bioassay and molecular identification.

### 4. Preparation of stock solution and dilutions of the selected bacterial isolates

Stock solutions of the selected bacterial isolates preparation of graduate dilutions were done according to **Mashtoly et al (2010 & 2011**). One hundred milliliters of nutrient broth media in 250 ml baffled flasks was inoculated with each isolate of bacteria and incubated overnight at 30°C with rotary agitation (150rpm). Exponentially growing cells (~1 $\times 10^6$ cells/ml) were harvested by centrifuging broth culture at 4000 rpm /15 min./4°C. The supernatantants were disposed off and pellets were washed by dissolving in 40 ml phosphate buffer saline (PBS) 1X diluted from 10X with double distilled sterilized water). The PBS wash was repeated a second time and after a third centrifugation the pellets were dissolved in 40 ml PBS and subjected to measurement the absorbance using a Eppendorf biophotometer at 600 nm. Seven dilutions ($10^{-1}$ to $10^{-7}$) of each bacterial isolate were made using PBS 1X. ODs of the last four dilutions ($10^{-4}$ to $10^{-7}$) were measured. Three replicates of nutrient agar media were inoculated with each of the last four dilutions (100ml/plate) of each isolate. Cell concentration (CFU) was determined after 48 hrs of incubation at 30°C. All measures were performed in triplicate and the mean values were used in growth equations. Each pure culture of the two bacterial isolates was also subjected to a Gram stain test. Parasporal and inclusion bodies were detected for each individual effective isolate. One hundred milliliters of nutrient broth media were inoculated with one loop of each isolate of bacteria individually and incubated at 30°C with rotary agitation 150 rpm for 4 days. Fifty microliters of each bacterial isolates were taken from its suspension on glass slid and covered and then examined by 100 x lenses using light microscope.

### 5. Bioassay

All prepared bacterial suspensions were subjected to bioassay on $4^{th}$ nymphal instar of desert locust using leaf dipping technique (**McGuire et al 1997**) and per os technique (**Mora et al 2007**).

### 5.1. Leaf dipping technique

Stock solution of selected bacterial isolates were prepared and CFU/ml of each were calculated and used four graduate dilutions of each stock solution for each isolate as showed in **Table (1)**. Adequate fresh sticks and leaves of clover were immersed in each bacterial concentration for 3 min and then allowed to dry at ambient temperature about 10 mins. treated sticks and leaves of the same isolate were introduced to 30 of 4th nymphal instar distributed in three replicates (10 nymph / each). Nymphs in each replicate were placed in individual small wooden framed (30cm in length, 18 cm in width and 20cm in height) with wire gauze in front and top to easily usage. Replicates were kept under the same rearing laboratory conditions. Control trial was done using the same procedures as treated ones except bacterial isolates. Sticks and leaves were dipped in autoclaved distilled water and then allowed to dry followed by introducing them to 30 nymphs. Daily examination of treated and control trails were done. The mortalities were recorded and subjected to statistical analysis.

**Table1.** Preparation of different cell concentration (cfu/ml) of the two selected isolates for leaf-dip technique

| Isolates | Stock solution (CFU/ml) | Dilutions (CFU/ml) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| NDL1 | $1078X10^6$ | $539X10^6$ | $270X10^5$ | $135X10^4$ | $67X10^3$ |
| NDL2 | $467X10^6$ | $233X10^5$ | $117X10^4$ | $58X10^3$ | $29X10^2$ |

### 1.1. Per os technique

Stock solution of the selected bacterial isolates were prepared and cell concentration CFU/ml of each were calculated and serial different graduate dilutions of each were prepared and used per os as shown in **Table (2)**. All dilutions were expressed as (CFU/ml) and administered per os via 1cm Hamilton syringe equipped with 27-gauge needle to 4th instar nymph of *S. gregaria*. The treatment was done using different doses of CFU/ml in 0.2 ml (200µl) per individual insect and subsequently for other isolate. Five nymphs $4^{th}$ instar were treated and each of them were used as individual replicates. Daily examinations of the trails weredone. The mortalities were recorded and subjected to statistical analysis.

**Table2.** Cell concentration (cfu/ml) of the two se lected isolates for per os technique

| Isolates | Stock solution (CFU/ml) | Dilutions (CFU/ml) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| NDL1 | $539X10^6$ | $270X10^5$ | $135X10^4$ | $67X10^3$ | $34X10^2$ |
| NDL2 | $23X10^6$ | $11.5X10^5$ | $5.7X10^4$ | $2.8X10^3$ | $1.4X10^2$ |

### 2. Molecular Identification of the bacterial isolates using 16S rRNA sequencing

The selected bacterial isolates were identified using universal primers of 16S rRNA technique and then use its sequencing as molecular tools.

### 2.1. Extraction of bacterial DNA

Pure single colony from each bacterial isolates were picked up with a sterilized toothpick, and were suspended in 0.5 µl of sterilizes saline in a 1.5 µl centrifuge tube (**Mashtoly, et al 2010 & 2011**). Each tube was centrifuged at 10,000 rpm for 10 min. After removal of supernatant, the pellet was suspended in 0.5 µl of InstaGene Matrix (Bio-Rad, USA) as described by **Abolmaaty et al (2000).** The suspension was incubated at 56º C for 30 min and then heated at 100ºC for 10 min and then cooled to 5°C on ice.

### 2.2. Amplification of 16S rRNA

The 16S rRNA of each isolate was amplified by PCR using published Universal primer. Primer sequences are listed in **Table (3)**. The primer was synthesized and obtained from integrated DNA Technologies, Inc. The final standard PCR mixture consisted of 45 µl of Platinum PCR Super Mix 1.1X (Invitrogen Crop.) contains anti-Taq polymerase DNA antibody, Mg++ dNTPs, and recombinant Taq DNA polymerase at concentrations sufficient to allow amplification during PCR. One microliter of one forward and one reverse primer and 3µl of DNA sample was added in a final volume of 50 µl was processed in a PTC-100TM Programmable thermal controller (MJ Research, Inc.). the cycling conditions consisted of initial denaturation at 94˚C for 45 sec, annealing at 55˚C for 60 sec, and extension at 72˚C for 3 min. Final extension was performed at 72˚C for 10 min. Blanks were routinely used containing all components of the reaction

mixture except the DNA samples. PCR products were subjected to electrophoreses in 2% agarose gels and were stained with 5 µl of SYBR® safe DNA gel stain *10.000 X concentrate in DMSO* (Invitrogen Corp.) and were run in electrophoresis running buffer (0.2) M Tris base, 0.1 M Sodium acetate, 0.01 M NA2.EDTA, at PH 7.8). Digital images were obtained using a BIO-RAD Universal Hood Ⅱ ChemiDocTM CheXRS molecular imager and Mitsubishi P93 printer.

### 2.3. Purification of PCR product

PCR products were purified from unincorporated PCR primers and dNTPs using QIAquick PCR Purification Kit (Qiagen), following the manufacturer`s instructions for spin-column technology with the selective binding properties of a uniquely designed silica membrane. Special buffers (binding buffer (PB), washing buffer (PE) and elution buffer (EB) provided are optimized for efficient recovery of DNA. PCR products were purified from unincorporated PCR primers and dNTPs by using Montage PCR Clean up kit (Millipore).

### 2.4. Elution of DNA in low salt solution

Elution efficiency was strongly dependent on salt concentration and pH of the Elution buffer. Contrary to adsorption, elution was the most efficient under basic conditions and low salt concentration. DNA was eluted with 30µl, applied to the column center, of buffer EB (10mM Tris. C1, Ph 8.5) or Ultra-pure DNAse/RNAse free water and then the column was incubated for 1 min. at room temperature, the maximum Elution efficiency was achieved between PH 7.0 and 8.5.

**Table 3**. Primers of amplification and sequencing

| Primers | Code | Direction | Primer Pairs | Sequence | Reference |
|---|---|---|---|---|---|
| Primers for amplification | A | F | 27 | AGAGTTTGATCMTGG CTCAG | Nucleic Acids Research, Vol. 18, Supplement |
| | B | R | 1492 | TAC GGYTACCTTGTT AC-GACTT | |
| Primers for sequencing | A | F | 518 | CCAGCAGCCGCGGTA ATA CG | |
| | B | R | 800 | TACCAGGGTATCTAATCC | |

### 5.2. Sequencing of the purified bacterial 16S rRNA

The sequencing of the purified PCR products of approximately 1,400 bp was carried out in the Macrogen sequencing service by using 2 primers as described **(Table 3)**. Sequencing were performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA).The quality of the sequences was checked by the soft ware named Sequence Scanner Version: 1.0(c) copyright Applied Biosystem. 2005.

### 5.3. Sequencing of 16S rRNA region and data analysis

Nucleotide sequences were analyzed by BLAST (blastn) search and were compared against bacterial 16S rRNA sequences data available in the Gene bank data base **(Altschul et al 1990)**. The sequences were align by using Clustal W 1.74 **(Thompson et al 1994)**, and were followed by construction of neighbor joining phylogenetic tree using MEGA4. (http://www.megasoftware.net). The nucleotide sequences were submitted in the GenBank database (https://www.ncbi. nlm.nih.gov/genbank/).

## RESULTS AND DISCUSSION

### 1. Susceptibility of 4th nymphl instar of desert locust to isolated bacteria

The promising bacterial isolates were assessed against one day old 4th nymphal instar of desert locust S. gregaria. Two methods of bioassay; Leaf dipping and per os techniques were followed resulting graduate mortality percentage which werethe toxicity values were obtained using the software Ldp Line (http://embakr.tripod.com/ldpline). according to **Finney (1971)**.

### 1.1. Leaf dipping technique

NDL1 isolate showed mortality after 24 h with the tested concentrations, i.e.539X10$^6$, 270X10$^6$, 135X10$^6$, 67X10$^6$ and 34X10$^6$ CFU/ml. The corresponded % mortality were: 50, 35, 30, 20 and 5% respectively. Such mortality reached to 90,75, 35, and 25% ,respectively by 48 h post treatment while mortality in control treatment was 5%. **(Table 4)**.

**Table 4.** Efficacy of NDL1 isolate on 4th nymphal instar of *S. gregaria* at 24 and 48 hours after administration by leaf dipping technique

| Cell concentration (CFU/ml) | 24 h | 48 h | | |
|---|---|---|---|---|
| | %Observed Mortality | %Observed Mortality | %Corrected Mortality | %Computed Mortality |
| 539X10$^6$ | 50 | 100 | 98.94 | 98.40 |
| 270X10$^5$ | 35 | 90 | 89.47 | 90.33 |
| 135X10$^4$ | 30 | 75 | 73.68 | 70.24 |
| 67X10$^3$ | 20 | 35 | 31.57 | 40.96 |
| 34X10$^2$ | 5 | 25 | 21.05 | 16.14 |
| **control** | **0** | **5** | **---** | **---** |

Data in **Table (5)** show the Mortality percentages of NDL2 isolate at the concentrations, i.e. 23X10$^6$, 11.5X10$^6$, 5.7X10$^6$, 2.8X10$^6$, 1.4X10$^6$ CFU/ml. The observed mortality after 24 h were: 90, 60, 55, 30 and 16% respectively. While after 48h such mortality reached to 100, 85, 75, 60, and 30 %, respectively. However, the mortality in control treatment was 10% after 24 or 48 h.

**Table 5.** Efficacy of NDL2 isolate on 4th nymphal instar of *S. gregaria* at 24 and 48 hoursafter administration by leaf dipping technique

| Cell concentration (CFU/ml) | 24 h | 48 h | | |
|---|---|---|---|---|
| | %Observed Mortality | %Observed Mortality | %Corrected Mortality | %Computed Mortality |
| 23X106 | 90 | 100 | 94.44 | 95.22 |
| 11.5X105 | 60 | 85 | 83.33 | 85.33 |
| 5.7X104 | 55 | 75 | 72.22 | 66.7 |
| 2.8X103 | 30 | 60 | 55.55 | 42.47 |
| 1.4X102 | 16 | 30 | 22.22 | 36.97 |
| control | 10 | 10 | --- | --- |

**Table 6.** Toxicity values of 2 bacterial isolates to 4th nymphal instar using Leaf dipping techniqueat 48hoursafter treatment

| Isolates | $LC_{50}$ (CFU/ml) | $LC_{25}$ (CFU/ml) | $LC_{90}$ (CFU/ml) | slope | Toxicity index at $LC_{50}$ |
|---|---|---|---|---|---|
| NDL1 | 1.6X10$^5$ | 1.1X10$^4$ | 2.5X10$^7$ | 0.59 | 4.28 |
| NDL2 | 7.0X10$^3$ | 2.6X10$^2$ | 3.5X10$^6$ | 0.48 | 100 |

Toxicity index and Relative Efficiency compared with LC50 of NDL2

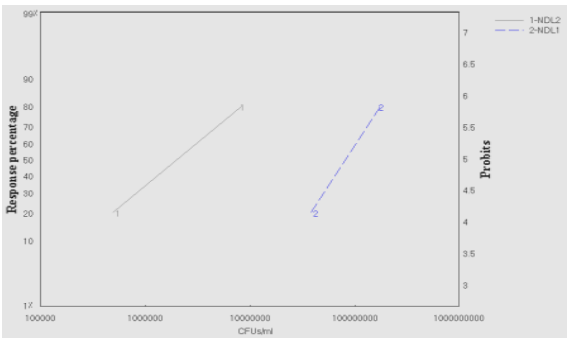


**Fig. 1.** Toxicity Regression lines of 2 bacterial isolates to 4thnymphal instar of *S. gregaria* using Leaf dipping techniqueat 48 hourspost treatment.

### 1.1. Per os

Results in **Table (7)** show the mortality percentages of NDL1 at doses of: 1078X10$^6$, 539X10$^6$, 270X10$^6$, 135X10$^6$ and 67X10$^6$CFU/ml/nymph. The corresponded % mortality were: 100, 70, 60, 40 and 20%, respectively. The mortality in control treatment was 0 %.

**Table 7.** Efficiency of NDL1isolate to 4th nymphal instar of *S.gregaria* at 6 h using per os technique

| Cell concentration (CFU/ml) | %Observed Mortality | %Corrected Mortality | %Computed Mortality |
|---|---|---|---|
| $1078X10^6$ | 100 | 100 | 86.11 |
| $539X10^6$ | 70 | 70 | 82.74 |
| $270X10^5$ | 60 | 60 | 63.04 |
| $135X10^4$ | 40 | 40 | 39.02 |
| $67X10^3$ | 20 | 20 | 18.63 |
| Control | 0 | --- | --- |

Data in **Table (8)** show the observed mortality percentages of NDL2isolate. Which were: 100, 85, 80, 70 and, 60% 6 hr post treatment at the doses $467X10^6$, $233X10^6$, $117X10^6$, $58X10^6$ and $29X10^6$ CFU/ml/nymph, respectively. The mortality in control treatment was 20%.

**Table 8**. Efficiency of NDL2isolate to 4th nymphal instar of S. gregaria at 6 h using per os technique

| Cell concentration (CFU/ml) | %Observed Mortality | %Corrected Mortality | %Computed Mortality |
|---|---|---|---|
| $467X10^6$ | 100 | 98.75 | 92.95 |
| $233X10^5$ | 85 | 81.25 | 87.62 |
| $117X10^4$ | 80 | 75 | 77.21 |
| $58X10^3$ | 70 | 62.50 | 63.05 |
| $29X10^2$ | 60 | 50 | 46.89 |
| Control | 20 | --- | --- |

**Table 9**. Toxicity values of 2 bacterial isolates to 4th nymphal instar of *S. gregaria* using per os treatment at 6 hours after treatment

| Isolates | $LD_{50}$ (CFU/ml) | $LD_{25}$ (CFU/ml) | $LD_{90}$ (CFU/ml) | slope | Toxicity index at $LD_{50}$ |
|---|---|---|---|---|---|
| NDL1 | $5.1X10^3$ | $0.7X10^2$ | $5.7X10^7$ | 0.32 | 100 |
| NDL2 | $5.2X10^6$ | $1.9X10^5$ | $2.8X10^9$ | 0.47 | 0.097 |

Toxicity index and Relative Efficiency compared with $LD_{50}$ of NDL1

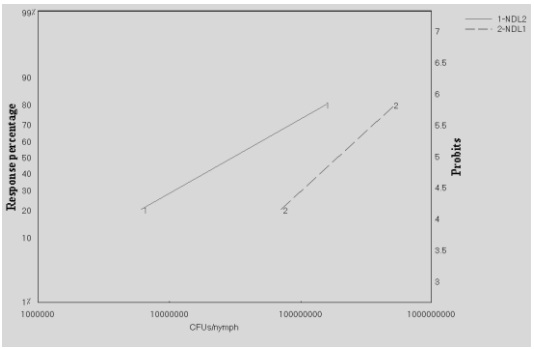


**Fig. 2.** Toxicity Regression lines of 2 bacterial isolates to 4thnymphal instar of *S. gregaria* using per os treatment at 6 Hours After treatment.

Reviewing the obtained data, it is clearly observed that the efficacy of each bacterial isolates varied between the two tested methods of administration. The descending order of tested isolates according to their toxicity in leaf dipping technique was, NDL2 then NDL1. The opposite order was observed in per os technique were NDL1 then NDL2. The contradiction trends between the two different bioassay techniques suggest that NDL2 isolate might produce enteric toxin or antibiotic which may be very sensitive to degradation when introduced to nymphs by leaf-dip method. However, these toxins are highly activated if it goes through vocal cavity. The non-homogenous distribution of colony forming units of NDL2 may ledto sub lethal concentration which decreases the mortality level. But in per os techniques the dosage goes through the vocal cavity directly to insect gut at lethal concentration. Also, may be due to the amount of spores consumed in the first method which varied from one nymph to another, while in the second method each nymph received the same amount of spores.

**Goettel and Johnson (1997)** provide an overview of the pathogens that affect acridids, including bacteria, viruses, nematodes, microsporidia, and fungi. The first microbial control agent developed for acrididae control was *Nosema locustae* Canning but a demand for more rapid speed of kill led to the development of fungi capable of penetrating insect cuticle. (**Johnson 1997**) the majority of virulent isolates found so far were *Metarhizium acridum*. (**Hunter et al 2001**) formerly *M. anisopliae* var. acridum Metsch. Sorr., but In field use, *Metarhizium* has a relatively slow and variable speed of kill, which may create practical problems for its use **(Lomer et al 2001** and **Abdelatef 2015)**, therefore the tested isolates in the present study may became a promising entomopathogenic for desert locust control for their rapid speed of kill.

**2. Molecular Identification of the bacterial isolates using 16S rRNA sequencing**

PCR product of each purified DNA for each Isolates were tested on 2% agarose gel to confirm band clearance with no background or overlap. Sequence scanner software v 1.0 obtained from Applied Bio-system. was used to read the sequencing data generated by 3130xl Genetic Analyzer (Applied Biosystems, Foster City,CA) of the PCR amplicons of unknown isolated bacteria. The primer was successfully used for sequencing amplicons obtained from DNA template isolated from NDL1 and NDL2. Analysis of the 16S rRNA of isolates demonstrated that they shared high homologies with other *Bacillus* sp. All these sequences were submitted to NCBI gene databank. The partial 572 bp of 16S rRNA sequence of NDL1 showed 100 % identity to the isolate of *B. cereus*. The partial 463 bp of 16S rRNA sequence of NDL2 showed 100 % identity to another isolate of *B. cereus* according to the submitted data on genbank. It is of interest to notice that 572 bp and 463 bp of the NDL1 & NDL2 sequences were highly aligned (100%) when they were tested according to BLASTn, nucleotide alignment (bl2seq).This 100% identity shows that PCR product was almost fully sequenced. Based on analysis of the 16S rRNA gene sequence, the bacteria isolated from dead/infected Desert locust were identified as *Bacillus cereus* isolate NDL1 and *B. cereus* isolate NDL2.

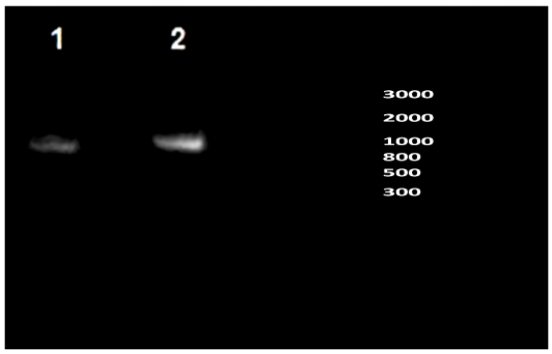


**Fig. 3.** Gel electrophoresis of PCR amplification products with unknown 2 isolates (NDL1 and NDL2)

The *B. cereus* sensu lato group comprises six related species: *B. cereus*, *B. anthracis, B. thuringiensis, B. mycoides, B. pseudomycoides* and *B. weihenstephanensis*. (**Soufiane and Cote 2010**). *B. cereus* has been used successfully as a microbial control agent for the grubs of *Amphimallon solstitiale*, *Melolontha melolontha,* Anomaladimidiata and Holotrichia seticollis (**Sezen et al 2005; Selvakumar et al 2007 and Sushil et al 2008**). Though some isolates of *B. cereus* have been known as opportunistic human pathogens with their ability to produce enteric toxins, some isolates of *B. cereus* have many agronomically useful traits, such as antibiotic production for plant disease suppression (**Handelsman et al 1996**). The insecticidal property of *B. cereus* has been attributed to the production of the lipase toxin phospholipase C, (**Lysenko, 1972a and b**) and the paralytic toxin sphingomyelinase C (**Nishiwaki et al 2004**). A synergistic action between the antibiotic zwittermicin produced by *B. cereus* and the crystal toxins of *B. thuringeinesis* var. kurstaki has been reported in controlling the gypsy moth **(Broderick et al 2000**). PCR primers were designed to detect genes for phosphatidylcholine specific phospholipase C, phosphatidylinositol specific phospholipase C, immune inhibitor A, vegetative insecticidal protein 3A, a protein proposed to be involved in capsule synthesis, a newly identified Ser/Thr kinase homologue and enterotoxin entS. Motility, the presence of flagella, haemolysis, chitinase and lecithinase production were also evaluated. The widely varying profiles of the 23 isolates from the complex provide a pool of different genotypes that can help to identify factors involved in pathogenicity **(Guttmann and Ellar, 2000).** Recently, **(Abi Khattar et al 2009**) reported that dlt operon of *B. cereus* is required for resistance to cationic antimicrobial peptides and virulence in insects. Pathogenicity of *B. cereus* against other scarab grub species has also been reported previously. With the exception of IB825, all the *B. thuringiensis* and *B. cereus* isolates were able to utilize chitin as their sole carbon and nitrogen source. If *B. cereus* was able to infect insects, chitinase production could be the suggested mechanism involved in the infection.

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