*British Microbiology Research Journal*
*11(5): 1-9, 2016, Article no.BMRJ.19130*
*ISSN: 2231-0886, NLM ID: 101608140*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Identification, Comparison, and Transfer of the pxo Gene between Members of Bacilli Species

**Shahcheraghi Seyed Hossein[1], Namazi Mohammad Javad[2*], Nouruzi Jamileh[3], Lotfi Marzieh[4], Hojatoallah Moradi[3] and R. Golmohammadi[2]**

*[1] Infectious and Tropical Diseases Research Center, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
*[2]Department of Microbiology and Immunology, Faculty of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.*
*[3]Basic Sciences College, Islamic Azad University, Tehran, Iran.*
*[4]Department of Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author SSH designed the study, performed tests and contributed to the final draft. Author NMJ contributed in design, supervising the project, writing the manuscript and corresponding responsibilities. Author NJ wrote the protocol, supervised the project, and critically monitored writing the manuscript. Author LM wrote the first draft of the manuscript and contributed in literature searches. Authors HM and RG revised the manuscript, performed statistical tests and managed the analyses of the study and literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BMRJ/2016/19130
Editor(s):
(1) Kenneth Lundstrom, Centre of Kenneth Lundstrcm Pan Therapeutics, Rue des Remparts, Switzerland.
(2) Preeti Bharaj, Dept. of Microbiology and Immunology, University of Texas Medical Branch (UTMB), Galveston USA.
Reviewers:
(1) Ilham Zahir, University Sidi Mohamed Ben Abdellah, Morocco.
(2) Manoel V. F. Lemos, Sao Paulo State University, Brazil.
(3) Mário Lettieri Teixeira, Institute Federal Catarinense, Brazil.
(4) Nazia Jamil, University of Punjab, Lahore, Pakistan.
Complete Peer review History: http://sciencedomain.org/review-history/12359

***Original Research Article***

***Received 26th May 2015***
***Accepted 13th October 2015***
***Published 20th November 2015***

## ABSTRACT

**Aim:** *Bacillus anthracis* causes anthrax in which the pxo gene and its associated plasmids, pXOP1and pXO2, encode toxin and capsule proteins, both of which are involved in the pathogenicity of anthrax. The possibility of transferring the pxo gene to other bacilli has recently been shown. The main aims of this study were to identify and compare the frequencies of the pxo

**Corresponding author: E-mail: mjnamazi@hotmail.com;*

gene in isolated bacilli members. The study examined possible pxo gene transfer from *B. anthracis* to other closely related members of the genus Bacillus. The findings presented here may be useful in the study of vaccination.
**Study Design:** The study design was cross-sectional and descriptive. Sixty-five soil samples were collected from different geographical regions in Iran.
**Place and Duration of Study:** The study was conducted in many provinces in Iran over several months. Samples were analyzed at the Mashhad University of Medical Sciences.
**Methodology:** Organisms were isolated from the soil, and the isolation of pXO plasmid was performed. Presence of the pXO1 plasmid was confirmed by agarose gel electrophoresis. Isolated proteins from each bacillus were examined by SDS-PAGE. The limits of proteins encoded by the pxo gene were specifically located and data were statistically analyzed using excel.
**Results:** Results showed that 13 samples out of 38 bacilli contained the plasmid of interest and protein bands related to proteins coded by the pxo gene.
**Conclusion:** We have determined that the pXO1 plasmid has been transferred from *B. anthracis* to 13 other isolates of *B. cereus* group members in different regions in Iran. No transfer of the pXO2 plasmid was observed. This was apart from the identification of the pxo gene and its plasmids in different members of bacilli.

*Keywords: Bacillus anthracis; SDS-PAGE; pXO1 plasmid.*

## 1. INTRODUCTION

Bacilli are obligatory aerobic, gram-positive bacteria that are present in water, soil, and air. Bacillus anthracis is the etiologic agent of anthrax. It is also used in the construction of biological weapons. This bacillus contains two plasmids, designated as pXO1 and pXO2 [1] which are responsible for producing the toxin and capsule, respectively. Copy numbers of these two plasmids have been studied [2]. The plasmids of pXO2 and pXO1 are 181 and 95 kb in size, respectively. The toxin of B. anthracis is an antigenic complex composed of several units, including Protective Antigen (PA), Lethal Factor (LF), and Edema Factor (EF). Molecular weights of these proteins are 82.7, 83, and 88.8 kDa, respectively [1].

There are many characteristic differences between *B. anthracis* and other members of the *B. cereus* group regarding both pXO1 and pXO2 plasmids. The expression of the toxin is controlled by two genes, including *atxA* and *pagR*, whereas the acpB and acpA genes control the expression of the capsule [3,4].

Among many identified plasmids in *B. cereus*, there are small plasmids that are similar to many plasmids that belong to *B. thuringiensis*. A plasmid of 208 kb in size, called pBc10987, has been isolated from non-pathogenic *B. cereus* (ATCC10987). This plasmid bears extraordinary similarity to the pXO1 plasmid of *B. anthracis* [5].

Many members of the cereus group with the same genomes are economically important as they have been used in medicine or biological defense. These genetic similarities between *B. anthracis*, *B. cereus*, and *B. thuringiensis* suggest that they could be introduced as different members of one species [5-7].

Reports on activities of *B. thuringiensis* against vector mosquitoes have shown that they could be active as potential carriers of diseases, such as malaria and yellow fever. Products related to the spores of *B. thuringiensis* are considered very important biological insecticides [8].

*B. subtilis*, which is also known as *B. nato* and *B. globigi*, is a model species for investigations regarding growth and cell differentiation in *B. subtilis*, with large and flat colonies, including opaque, pink, yellow, or brown colors, and usually does not cause any disease in humans [9].

Andrup and colleagues [10] studied different plasmids and their similarities in *B. thuringiensis*, *B. cereus*, and *B. mycoides* separately. They found a large plasmid in *B. cereus*, called pBCXO1, which is similar to the pXO1 plasmid from *B. anthracis*. In addition, they showed that *B. thuringiensis* contains a plasmid similar to pXO2. They also found that two plasmids, pAW63 and pBT9727, in *B. thuringiensis* are similar to the pXO2 plasmid of *B. anthracis*. Comparative sequence analysis of these three plasmids showed that they have all been derived from a conjugative plasmid, such as pBT9727 and pAW63, both of which are involved in encoding capsule proteins in *B. thuringiensis*.

In another study, Xiaomin and colleagues [11] showed that a region of the pXO1plasmid that

resembled the same plasmid existed in *B. anthracis*, *B. cereus*, and *B. thuringiensis*. They included 24 isolates from *B. cereus* and *B. thuringiensis* that resembled the *B. cereus* isolate DBT248 which is used as a probiotic agent in some European countries. This finding suggested that this bacillus did not have the pXO1 plasmid. They also found that 6 isolates from *B. thuringiensis* and 12 from *B. cereus* contained a plasmid similar to pXO1.

In an isolate of *B. cereus* there is a circular plasmid called pBCXO1 with 99.6% similarity to the toxin-encoding pXO1 plasmid of *B. anthracis* [12]. There was a 272 kb plasmid that was smaller than many pXO1-like plasmids in *B. cereus* [13].

The above findings showed that the identification of different plasmids could be a valuable tool not only in systematic studies but also in vaccination studies. Finding plasmids that encode immunogenic antigens transferred between pathogenic and non-pathogenic members would provide safe induction of a protective immune response, considering the safety and efficiency in vaccine protocols. Therefore, the present study aimed to determine and compare the presence of common plasmids in different isolates derived from several members of bacilli. We examined the possibility of transfer the pXO1 plasmid from *B. anthracis* to *B. cereus*, *B. thuringiensis*, or *B. subtilis.* Our results may be considered in further development of vaccines against anthrax.

## 2. MATERIALS AND METHODS

### 2.1 Samples

Sixty-five samples were collected from deep and surface soils because these environments are protected from direct exposure to sunlight and they are a suitable source of the cereus group [14]. Samples were collected from nine different provinces of Iran, including Khuzestan, Kermanshah, Yazd, Khorasan Razavi, Tehran, Hamedan, Ilam, Fars, and Semnan, which are located in the southeast, north, and central areas. The collected soils were sieved. One gram of each filtered soil sample was poured into a sterile tube containing 9 ml distilled water. Soil samples were heated in a water bath at 80℃ for 20 min to destroy bacterial spores and were then incubated at 30 C to allow the soil and water to mix completely. After cooling, the suspension was cultured on nutrient agar maintained at 37C for 48 h.

Three identified cultures of *B. thuringiensis*, one of *B. cereus*, and one of *B. subtilis* were received from the division of Microbiology at the North Tehran branch of the Islamic Azad University. One culture of *B. cereus* and one of *B. subtilis* were received from the independent Microbiology unit at the Research Center at the Islamic Azad University.

### 2.2 Gram and Ziehl–Neelsen Staining

Both Gram and Ziehl-Neelsen-like staining were performed. The process of destaining was performed using ethanol. Thick Fuchsin was poured onto the slides and heated for 5 min until steam rose, without allowing the sample to boil, and the slide was then washed with water. The color of each slide was cleared by adding ethanol until the red color remained on the slide. Each slide was then washed with water and covered with methylene blue for 1–2 min and dried. Under light microscopy, microorganisms were observed in blue and spores were observed in red [15]. Results of these biochemical tests are shown in Table 1.

### 2.3 Hemolysis Test

A hemolysis test was performed for the diagnosis of extracellular enzymes produced by bacteria. They were determined with an areola of hemolysis that appeared around bacterial colonies as described by Massie and colleagues [15].

### 2.4 Extraction of Mega Plasmid

The modified Kado and Liu [16] technique was used for large plasmid extraction. Bacteria were grown in 7 ml of Luria–Bertani broth. All tubes were then placed at 30℃ for 12–16 h with continuous shaking at 120 rpm. Cells (2 ml volume) were centrifuged at 13,000 rpm for 7 min, and the pellet was resuspended by repeated pipetting in 100 µl of elution buffer (15% wt/vol stoke, 40 mM Tris hydroxide, and 2 mM EDTA, pH 7.9). Cells were then lysed by addition of 200 µl of lysis solution (3% wt/vol) sodium dodecyl sulfate and 50 mM Tris, pH 12.6). The lysate was then heated at 60℃ for 30 min followed by addition of 20 µl (20 mg/ml) of proteinase K. The resulting solution was then inverted 20 times and incubated at 37℃ for 60 min. One milliliter of phenol:chloroform:isoamyl alcohol at a ratio of 25:24:1 (Sigma, USA) was added. The solution was again inverted 40 times. After centrifugation at 13,000 rpm for 7 min, the upper aqueous layer was collected for electrophoresis.

## 2.5 SDS-PAGE and Electrophoresis

A 0.5% agarose gel was used for electrophoresis with 1 kb standard marker to size DNA plasmids based on their base pair length. Gels were stained post electrophoresis using ethidium bromide (EtBr) and photographed under UV transillumination to observe plasmid bands. SDS-PAGE was then performed as previously described [17,18]. Briefly, the cultivated bacteria were centrifuged and overlaid onto a sucrose solution in order to remove all unnecessary components and soluble proteins. Lysozyme was then added to break down the peptidoglycan layer of the cells. Cells were then ruptured by osmotic shock and exposed to a rapid temperature change to cause cell collapse. Centrifugation was then performed twice to precipitate insoluble high molecular weight particles. In the second, higher speed centrifugation, membrane vesicles were precipitated. Thus, soluble proteins remained on the surface of the liquid, and a sediment was formed and used in both cases. Of the cultured overnight bacteria, 10 ml was then centrifuged at 5000 rpm for 5 min. The surface liquid was decanted and the remaining sediment was overlaid on 10 ml of 30 mM Tris HCl (pH 8.1) and vortexed. Cells were again centrifuged for 10 min at 5000 rpm and the surface liquid decanted. The pellet was overlaid on 200 µl of 20% sucrose with 30 mM Tris HCL (pH 8.1), and the mixture was then transferred to the reaction tube and placed on ice for 2 min. A lysozyme solution (1 mg/ml prepared in 20 µl of 1 M EDTA) was added to the tube and was then left on ice for 30 min. The tube containing cells was placed for 30 min on dry ice and 1.25 ml of 3 mM EDTA (pH 7.3) was added. The tube was vortexed and centrifuged, and the sediment was sonicated for 154 s. The sample was then transferred to a new reaction tube and centrifuged for 5 min at 5000 rpm until a precipitate formed. The surface liquid was transferred to another reaction tube and the resulting sediment removed. The latter surface liquid was centrifuged for 15 min at 15000 rpm, which finally resulted in a new sediment and the surface liquid. A 100 µl sample of the surface liquid was transferred into a new reaction tube with caution. To the tube containing 100 µl of the surface liquid, 50 µl of 3× LUG buffer was added, and the sample was kept on ice until required and the remaining surface liquid was poured. The deposit was overlaid on 100 µl of 1× LUG buffer and transferred to a reaction tube and placed on ice until required for SDS-PAGE.

For SDS-PAGE, the first resolving gel was poured using isobutanol until all bubbles were removed and the surface of the gel became smooth. Isobutanol was then poured away and the gel was washed and dried. The stacking gel was then poured. All wells were properly created and dried after hardening. The gel was installed on the electrophoresis system vertically and 1× tank buffer was added. Samples were boiled for 5 min using sample buffer before adding into the gel. Each sample (200 µl) was separately injected into the cavity with a Hamilton Ampoule. A protein molecular weight marker with a molecular weight of 170 kDa was injected into the middle well. The device was first connected to 85 V and after half an hour the voltage was increased to 105 V. The electrophoresis was stopped when the bromophenol blue dye front reached the end. The gel was then transferred to the rotator overnight and stained with Coomassie Brillant Blue R-250 (Sigma-Aldrich, Germany). The stain solution was slowly removed and de-staining solution comprising 8% acetic acid and 12% ethanol was used until the de-stain solution became clear and gel was removed for photography.

Statistics: Data were statistically analyzed using excel, including calculation of standard deviations and standard errors and application of the Student's t test. However, as the main objective of the study was to show gene transfer, there was no need to indicate any statistical results.

## 3. RESULTS

The distribution of identified bacteria that were obtained from 65 soil samples from different provinces and the total number of isolated bacilli and their details, including separate frequencies of 65 samples collected in each province across the country, are shown in Table 1. The highest isolation rates of *B. cereus* were found among the three provinces of Yazd, Kermanshah, and Tehran. The lowest isolation rates of this bacterium were in the provinces of Semnan and Hamadan. For *B. subtilis,* the highest rates were in the Kermanshah, Yazd, Khorasan Razavi, and Semnan provinces, whereas the lowest rates were in Ilam, Tehran, Khuzestan, and Fars. The most prevalent isolated bacilli from the soil were observed in Kermanshah and Yazd and the least often isolated bacilli were observed from Hamadan when compared with other provinces. Table 2 shows the different characteristics of the four species of the *B. cereus* group, separated by each province.

**Table 1. Total number of isolated bacilli and their details from 65 samples of soil collected from different provinces across the country. All collected bacilli were standard isolates form different units and departments of the Islamic Azad University**

| Province | Soil samples | Frequency of samples | *B. thuringiensis* | *B. subtilis* | *B. cereus* |
|---|---|---|---|---|---|
| Kermanshah | 6 | 5 | - | 2 | 3 |
| ILam | 10 | 3 | - | 1 | 2 |
| Hamadan | 9 | 1 | - | - | 1 |
| Tehran | 4 | 4 | - | 1 | 3 |
| Khorasan Razavi | 7 | 4 | - | 2 | 2 |
| Yazd | 9 | 5 | - | 2 | 3 |
| Semnan | 10 | 3 | - | 2 | 1 |
| Khusestan | 4 | 3 | - | 1 | 2 |
| Fars | 6 | 3 | - | 1 | 2 |
| Presented | - | 7 | 3 | 2 | 2 |
| Total | 65 | | 3 | 14 | 21 |

**Table 2. Different characteristics of four species of the *Bacillus cereus* group. The table shows the number and frequency of each isolated Bacillus from the soil in all provinces separately. The table shows different positive degrees in all tested samples. A (+) when 90%-100% of bacilli are positive. B (+): poor positive. C (+) 90% of bacilli are positive. D (−): less than 10% of the strains are positive. The lecithinase test for detection of B. cereus was performed. For more technical details, refer to the materials and methods section**

| Reaction | *B. anthracis* | *B. thuringiensis* | *B. cereus sub mycoides* | *B. cereus* |
|---|---|---|---|---|
| Egg yolk reaction1 | (+) B | + | + | + A |
| Catalase 2 | + | + | + | |
| Gram stain | + | + | + | + |
| Movement 3 | - | -+ | -D | + C |
| Hemolysis (sheep red blood cells) | - | + | + | + |
| Colony growth as root | - | - | + | - |
| Using anaerobic of glucose | + | + | + | + |
| Vp reaction 4 | + | + | + | + |

Fig. 1 shows the results of plasmid electrophoresis. We used a standard marker comprising DNA fragment sizes above and below 10 kbp. The pXO1 plasmid had a size of 189 kb and the pXO2 plasmid had a size of 95kb. According to the bands identified in Fig. 2, the appearance of large plasmids was confirmed to be above 10 kbp. The plasmid bands related to each *B. cereus* are clearly shown in Fig. 2, including bands representing the plasmid pXO1.

Fig. 2 shows the results of protein extraction and SDS-PAGE. Of note was that the protein bands in the range of 72–95 kDa were observed in 13 samples. All of these are related to the bacterial toxin and hence, to the locus of the pXO1plasmid gene. Therefore, the existence of protein bands related to the pXO1 plasmid was proved in all 13 isolates of *B. cereus*. However, no bands related to the protein encoded by the pXO2 plasmid were observed in any of the bacilli isolates we collected. The distribution of the above 13 protein bands was shown in Fig. 2. Two samples were collected from each provinces of Tehran, Khorasan Razavi, Khuzestan, Fars, and Kermanshah. One sample was obtained from each provinces of Semnan, Yazd, and Hamadan.

## 4. DISCUSSION

Among members of the *B. cereus* group, *B. anthracis* is the causative agent of anthrax, a lethal infectious disease; *B. thuringiens* is used as a biological insecticide; *B. subtilis* is used for producing antibiotics at the industrial level; and *B. cereus* is a poison producer. The above-mentioned species have two plasmids responsible for their pathological aspects, the

pXO1 plasmid for producing toxin and the pXO2 plasmid, which contains the capsule synthetic genes. Studies have shown that gene transfer between bacilli is possible. With this regard, some researchers suggested that the pxo gene has been transferred from *B. anthracis* to other members of the group, which may be used to develop a safe and protective method for producing vaccine. In the present study, the frequency of the pxo gene and related plasmids in *B. cereus*, *B. thuringiensis*, and *B. subtilis* were observed. Our results confirmed that the pxo1 gene plasmids have been transferred to other bacilli members. We demonstrated that 13 positive samples from different isolates of bacilli carried the pxo1 gene. In contrast, the same method did not show any protein band related to the transfer of the pXO2 plasmid. Andrup and colleagues [10] found a large plasmid in *B. cereus*, which was designated as pBCXO1. This was similar to the pXO1 plasmid of *B. anthracis*. They also found that there is an isolate from *B. thuringiensis* which contains a plasmid similar to pXO2. They examined isolates from *B. thuringiensis*, *B. cereus*, and *B. micoides*. They demonstrated by electrophoresis that the isolated plasmid from *B. thuringiensis* had a molecular size of approximately 128 kb. They also found different types of large plasmids with molecular sizes of approximately 350 kb. They concluded that *B. cereus* and *B. thuringiensis* contained large plasmids as do other members of the *cereus* group. In agreement with our results, Lopez-Valdez and colleagues [9] showed that transferring the pXO1 plasmid from *B. anthracis* to *B. cereus* was possible. Similar to our study, Van der Auwera and colleagues [19] proved that the two plasmids pBT9727 and pAW63 in *B. thuringiensis* existed using their similarity to the pXO2 plasmid of *B. anthracis*. They also found that the plasmids pBT9727 and pAW63 were involved in encoding capsule proteins of *B. thuringiensis*. They checked 19 isolates of *B. thuringiensis*, of which five isolates had both of the mentioned plasmids and one had the pAW63 plasmid. They also demonstrated that there was more similarity between the pAW63 plasmid of *B. thuringiensis* and the pXO2 plasmid of *B. anthracis* than that between the BT9727 and pXO2 plasmids. Moreover, their study showed that plasmids, such as pAW63 and pBT9727, in some bacilli, particularly of the cereus group, were similar to those responsible for encoding toxin and capsule. Our study revealed a plasmid that was similar to that which encodes the capsule in *B. anthracis*; however, no toxin-like plasmid in *B. thuringiensis* was determined.

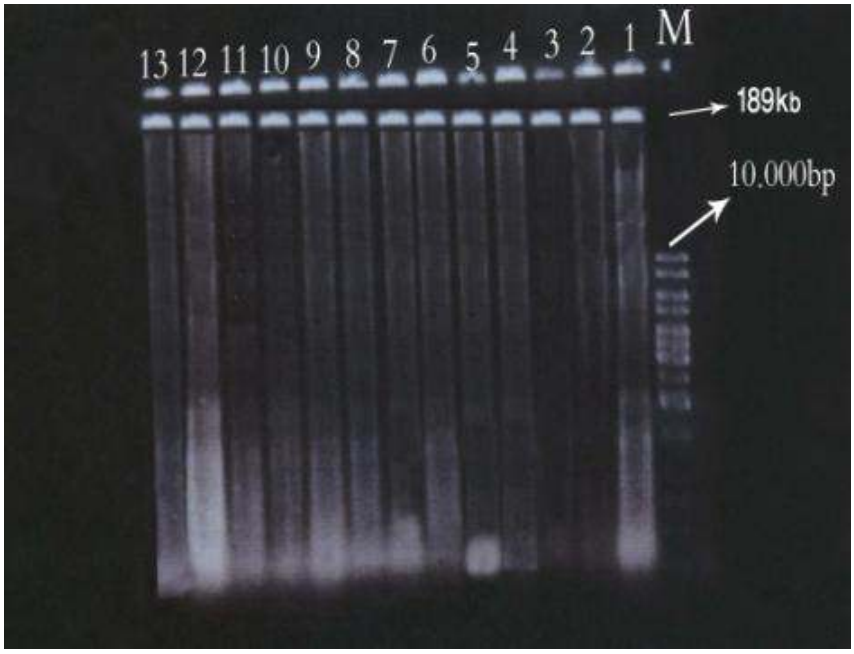


**Fig. 1. The figure shows plasmid bands related to each isolate of *B. cereus*. They are clearly shown from numbers 1 to 13 in comparison with the 1 kbp marker line, M (Fermentas, UK). The bands of the marker include 1110, 2230, 3370, 4590, 5039, 6470, 7700, 8940, 9500 and 10000 bp**

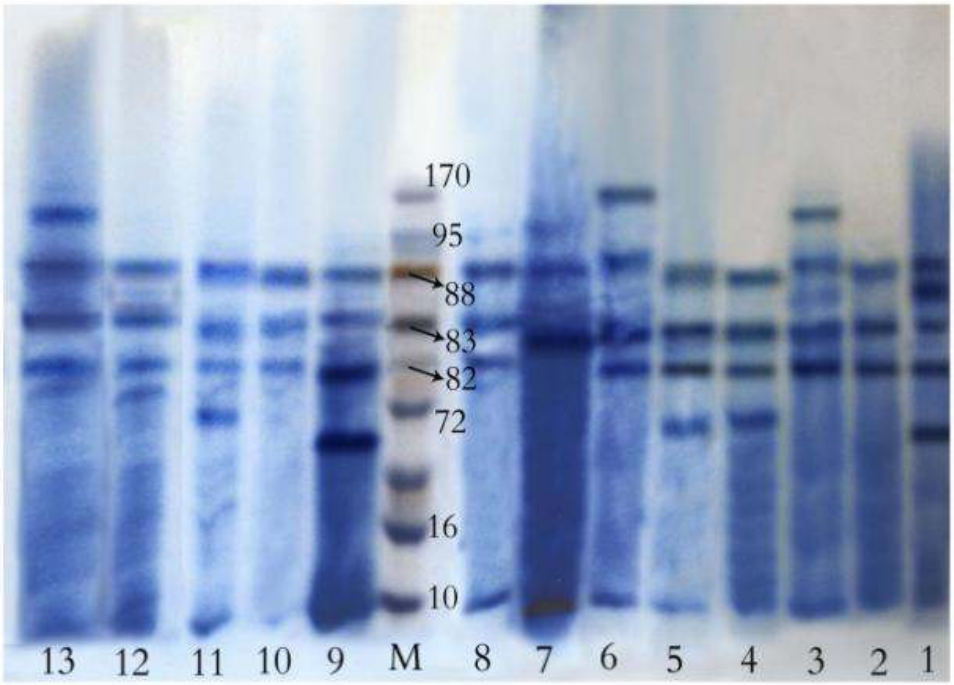


**Fig. 2. Shows that all 13 bands of positive samples can be observed. These bacteria were all *Bacillus cereus* isolates. The protein bands are determined between 72 and 95 kDa in which the range of toxin-related proteins bands are present**

*M: The marker bands are shown as: 10, 16, 34, 72, 82, 83, 88, 95, and 170 kDa, respectively. The 16 kDa band that was related to the protein encoded by the pXO2 plasmid was not observed in any of above bacilli isolates*

Xiaomin and colleagues [11] demonstrated that within the pXO1 plasmid there was a sequence with partial similarity to the plasmid present in *B. anthracis*, *B. cereus*, and *B. thuringiensis*. They determined that 6 *B. thuringiensis* and 12 *B. cereus* isolates shared 90% similarity with this sequence within the pXO1 plasmid. They expressed the proteins encoded by these sequences and investigated their toxic effects on hamster ovary cells, showing no toxic effects. It is, therefore, worthy to note that the above study and our results confirmed the transfer of the pXO1 plasmid and its consequent effect. However, we showed that although the pXO1 plasmid has been transferred to *B. cereus* and *B. thuringiensis*, the pXO2 plasmid was not transferred; therefore, the toxic effect could not be elicited.

In another study, Hoffmaster and colleagues [12] examined the toxin-producing genes of *B. anthracis* and related genes in *B. cereus*, which elicit an anthrax-like illness. They found a plasmid which was nearly 99.6% similar to the pXO1 plasmid of *B. anthracis*. This plasmid was a circular plasmid called pBCXO1 and bore very high sequence similarity with the plasmid responsible for encoding the toxin of *B. anthracis*. Additionally, this plasmid in *B. cereus* G9241 has been shown to be responsible for encoding the toxin. The virulence of *B. cereus*, which was used in the above study, was confirmed during experiments in mice.

However, the study did not confirm the same homology to the pXO2 plasmid which encodes the *B. anthracis* capsule. They also showed, through phenotypic examinations and analysis of 16s rRNA in mice using *B. cereus*, that *B. cereus* G9241was able to create an anthrax-like disease. A result very similar to ours has been reported by Hoffmaster and colleagues, where the presence of a similar pXO1 plasmid and simultaneous absence of the pXO2 plasmid in *B. cereus* G9241 was observed.

Many plasmids from *B. cereus* were separated by Rasko and co-workers [13]. Their experiments showed that the pXO1-like plasmids generally have molecular sizes of approximately 272 kb, which is larger than that of the pXO1 plasmids. The largest pXO1-like plasmids include

pPER272 and pCER270 with a size of 270 kb which may act as carriers. It has been shown that the number of copies of the pXO1 plasmid in *B. anthracis* is between 13 and 40 per chromosome [13]. It has been shown that chromosomes of the cereus group members are similar to each other and that plasmids of these members have important roles in the virulence and ecology of those organisms. Analysis of pXO1-like plasmids has led to the identification of areas related to the virulence of other plasmids, particularly the pCER270 plasmid. According to these investigations, the pCER270 plasmid has been shown to be related to the pXO1 plasmid. Researchers have demonstrated that the pPER272 and pCER270 plasmids and their related genes are involved in the genetic variation between chromosomes and plasmids among different members of the cereus group. According to chromosomal sequencing in the above species with pXO1 and pXO2 plasmids, future studies should continue to identify the similarities between their genes. To date, studies that have focused on these groups have revealed that the pPER272 plasmid has many similarities with the pBC10987 plasmid. Although there is a 90 kb region in the pPER272 plasmid, there is no such region in the pXO1 plasmid [13]. Considering the above studies, in future studies that aim to clarify the frequencies of such gene copies, modifying various factors, such as time and growth conditions in the suitable media, would be critical to obtain more accurate results. The existence of pXO1-like plasmids in *B. cereus* was demonstrated in the studies mentioned. However, the lack of virulence in these bacilli may be due to a lack of the complete pXO1 plasmid structure.

Moreover, Raymond and Bonsall [20] studied the importance of the maintenance of virulence factors in the *B. cereus* group in the face of social interactions in a population. They suggested that this group would be a novel study system for better understanding of the evolutionary and virulence factor distribution in a community. All of these studies encourage us to continue working to identify more accurate information regarding factors that can be used to control these bacilli-borne diseases.

## 5. CONCLUSION

To the best of our knowledge, this is the first report to indicate the transfer of the pXO1 plasmid from *B. anthracis* to *B. cereus* in Iran. We have shown that the transfer of the pXO1 and pXO2 plasmids was not observed from *B. anthracis* to *B. subtilis* and *B. thuringiensis*. Our results provide beneficial information to vaccination studies attempting to produce a safe, protective vaccine against anthrax as a fatal disease.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Rasko DA, Altherr MR, Han CS, Ravel J. Genomics of the *Bacillus cereus* group of organisms. FEMS Microbiology Reviews. 2005;29:303-329.
2. Rui J, Fang Y, Rongqiang Y, Lingbin M, Xiaodong M. The preparation and evaluation of DNA microarray probes for detecting *Bacillus anthracis*. J Mıcrobıol and Mıcrobıal Research. 2014;2:8-16.
3. Koehler TM. *Bacillus anthracis* genetics and virulence gene regulation. Curr Top Microbiol Immunol. 2002;271:143-164.
4. Delvecchio V, Connolly J, Alefantis T, Walz A, Quan M, Patra G, Ashton JM, Whittington JT, Chafin RD, Liang X, Grewal P, Khan A, Mujer S, Cesar V. Proteomic profiling and identification of immunodominant spore antigens of *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*. App Environ Microbio. 2006; 72:6355-6363.
5. Han CS, Xie G, Challacombe JF, Altherr MR, Bhotika SS, Brown N, Bruce D, Campbell CS, Campbell ML, Chen J, Chertkov O, Cleland Cathy, Dimitrijevic M, Doggett NA, Fawcett JJ, Glavina T. Pathogenomic sequence analysis of *Bacillus cereus* and *Bacillus thuringiensis* isolates closely related to *Bacillus anthracis*. J Bacteriol. 2006;188:3382-3390.
6. Pannucci J, Okinaka RT, Sabin R, Kuske CR. *Bacillus anthracis* pXO1 plasmid sequence conservation among closely related bacterial species. J Bacteriol. 2001;184:134-141.
7. Reddy DM, Paul D, Reddy HK, Reddy GM. Characterization and identification of *Bacillus cereus* GMHS: An efficient 2-picoline degrading bacterium. Int J Integ Biol. 2009;5:187-191.
8. Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P. *Bacillus thuringiensis*:

A century of research development and commercial applications. Plant Biotechnol J. 2011;9:283-300.

9. Lopez-Valdez F, Fernandez-Luqueno F, Ceballos-Ramirez JM, Marsch R, Olalde-Portugal V, Dendooven L. A strain of *Bacillus subtilis* stimulates sunflower (*Helianthus annuus* L.) growth temporarily. Scientia Horticulturae. 2011;128:499-505.
10. Andrup L, BarfodK K, Jensen GB, Smidt L. Detection of large plasmids from the *Bacillus cereus* group. Plasmid. 2008;59: 139-143.
11. Xiaomin H, Munk HB, Bohse HN, Zhiming. Detection and phylogenic analysis of one anthrax virulence plasmid pXO1 conservative ORF ubiquitous presented within *Bacillus cereus* group. Biochem and Biophysics Res commun. 2006;349:1214-1219.
12. Hoffmaster AR, Ravel J, Rasko DA, Chapman GD, Chute MD, Marston CK, De BK, Sacchi CT, Fitzgerald C, Mayer LW. Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax. Proc Natl Acad Sci USA. 2004;101:8449-8454.
13. Rasko DA, RosovitzMJ, Økstad OA, Fouts DE, Jiang L, Cer RZ, Kolstø AB, GillSR, Ravel J. Complete sequence analysis of novel plasmids from emetic and periodontal *Bacillus cereus* isolates reveals a common evolutionary history among the *Bacillus cereus* group plasmids, including *Bacillus anthracis* pXO1. J Bacteriol. 2007;189:52-64.
14. Chikerema SM, Pfukenyi DM, Hang'ombe BM, L'Abee-Lund TM, Matope G. Isolation of *Bacillus anthracis* from soil in selected high-risk areas of Zimbabwe. J Appl Microbiol. 2012;113:1389-1395.
15. Massie J, Roberst G, White PJ. Selective isolation of *Bacillus sphaericus* from soil by use of acetate as the only major source of carbon. Appl Environ Microbiol. 1985;6: 1478-1481.
16. Kado CI, Liu ST. Rapid Procedure for detection and isolation of large and small plasmids. J bacterial. 1981;145:1365-1373.
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227: 680-685.
18. Ismet B. Characterization of Bacillus species by numerical analysis of their SDS-PAGE protein profiles. Journal of Cell and Molecular Biology. 2004;3:33-37.
19. Van der Auwera GA, Andrup L, Mahillon J. Conjugative plasmid pAW63 brings new insights into the genesis of the Bacillus anthracis virulence plasmid pXO2 and of the Bacillus thuringiensis plasmid pBT9727. BMC Genomics. 2005;26:103.
20. Raymond B, Bonsall MB. Cooperation and the evolutionary ecology of bacterial19- 20- virulence: The *Bacillus cereus* group as a novel study system. Bioessays. 2013;35: 706-16.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://sciencedomain.org/review-history/12359*