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SIGNIFICANCE OF PLASMIDS IN *BACILLUS CEREUS SENSU LATO* GROUP : A REVIEW

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Abstract

Bacillus cereus senso lato group includes three main species: *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis* in addition to the other species, but these three types play an important role in human life, for example *Bacillus anthracis* cause anthrax disease, which is spread by treatment with animals and livestock, which became resistant to vaccines in the last years. Furthermore, *Bacillus cereus*, which causes food poisoning with symptoms of nausea, vomiting and it is highly isolated from the products of children, milk and dairy products. The third *Bacillus thuringiensis* is an important factor in the control of the destructive insects of economic crops. The line between these the three species may be very thin so that is some research that these three species one single species on the other side there are those who give evidence of the absence of any genetic interactions among them. In this article, we discuss in this article a discussion about these species, their significance, their phenotype, their genetic structure and some detail about the causes of interference and its components if it exists between these species.

Key words: *Bacillus cereus senso lato*, plasmid, virulence, horizontal transfer gene.

Introduction

Bacillus genus and plasmid

Most of the described plasmids isolated from certain species of Bacillus bacteria are cryptic elements that lack genetic markers and are not suitable for selection as transformants colonies of genetic transformation. Therefore, plasmids those have clear properties so it can be developed and used as vectors in genetic engineering (Bernhard *et al.*, 1978). Mainly these plasmids are effective in replication and transcription as in INTA Fr7-4 Plasmid of the recently isolated *Bt* strain in Argentina. They contain a large plasmid that encodes for the killer Cry protein and it is multiplied by a bi-directional mechanism. In addition, conjugation genes were detected through sequencing also (Navas *et al.*, 2017). Others, such as the pBMB26 plasmid, isolated from the *Bt* strain YBT-020, a plasmid diffused in the *Bc* group, have a minireplicon and two open reading frames that support plasmid replication. This plasmid works on the aggregation of crystalline spores (Wang *et al.*, 2018). The plasmid may be small but integrated because of the presence of genetic elements necessary for cloning and replication as in strain *B. thuringiensis* var. *kurstaki* HD73, which contains a small hidden plasmid called pHT8_1 which contains a complete gene system that controls sporogenesis according to the feeding conditions of the insect (Fazion *et al.*, 2018). Plasmids have many applications in biological fields, such that modern research seeks to remove the genes of virulence from plasmids that are responsible of a particular pathogenicity. This method is safer than the used physical means such as gamma rays, through which a part of the resistant spores may remain a survivor during the treatment. Therefore, a recent study on the *B. anthracis* targeted the pathogenicity genes of pxo1plasmid by removing these genes, the bacteria have become completely non-pathogenic and thus have been confirmed by laboratory animal experiments (Plaut *et al.*, 2018). Genetic transformation of tetracycline-resistant plasmid pBC16 originally isolated from *Bc* to *Bs* and maintained constant in transformants (Bernhard *et al.*, 1978). Also between *Escherichia coli* and in thermophilic *Bacillus* has become possible. The resulting transformants have been shown the ability to grow in the high temperature incubator (Tominaga *et al.*, 2016). Genetic engineering

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experiment through designing plasmid that replicates transcript and multiply the genes responsible for the encoding of ethanol in large quantities in both *Bs* and *E. coli* (de Oliveira and Nicholson, 2016). The same process is also made via modification of special plasmid called Shuttle Plasmid can be genetically expressed in both positive *Bt* and negative gram bacteria *E. coli* (Trieu-Cuot *et al.*, 1987). The morphological and biochemical qualities alone don't be enough to return the isolates in their groups, so gene analyzes such as the whole-genome single nucleotide polymorphism analyzes and multilocus sequence typing are a guarantor to separate species from each other, although some genetic tests give high similarity, like the 16S rRNA gene sequences Moreover, the presence of responsible plasmids of pathogenicity in a certain species does not provide clear evidence that it is the same species itself as in ISS isolates whose physiological characteristics and biochemical tests indicate that they belong to the *Bc* group, but contain the like pxo1 pxo2 plasmids are toxins non-coded and genetic analyzes multilocus sequence typing and whole-genome single nucleotide polymorphisms analyzes implies their belonging to the species *Ba* (Venkateswaran *et al*., 2017).

***Bacillus cereus sensu lato* Group**

It is a group of bacterial species belonging to the group *Bacillus* genus specifically, which are very closely related to each other and are linked together with developing relationships so that the close link between their members and differences are very limited among them .They're members of medical importance as they cause serious diseases to humans such as anthrax caused by *B. anthracis*, or they produce toxins that cause food poisoning, vomiting and gastrointestinal disorders such as *Bc* or it can be of environmental importance on the agricultural economic level of producing anti-insect toxins such as *Bt* (Maughan and Van der Auwera, 2011, Okinaka and Keim, 2016). However, the *Bc* group includes *B. mycoides*, *B. weihenstephanensis*, *B. pseudomycoides* and *B. cytotoxicus* (Jiménez *et al*., 2013). This is an overview of the three main species in this group.

Bacillus cereus: Bc is a common soil species and opportunistic pathogen for humans (Helgason *et al*., 2000). It produces a variety of toxins such as haemolysin, phospholipase, an emesis-inducing toxin and protease, gram positive aerobic, spore forming, motile, rod-shaped bacteria (Bottone, 2010, Granum and Lund, 1997). No capsule, beta-haemolysis , central spore site , 7.5% saline-tolerant, positive for starch degradation and gelatin analysis, TSI positive fermentative sugars, positive for methyl red, oxidase and catalase (Hadi, 2019). Regular colonies have regular boundaries, gray in color resistant to gamma phage and penicillin (van Tongeren *et al*., 2014), as well as the presence of Pilli that are spores associated (Venkateswaran *et al*., 2017). *Bc*, also it considers contamination factor in the industrial food such as dairy products (Okshevsky *et al*., 2017, Wong *et al*., 1988, Becker *et al*., 1994). It can also have therapeutic benefits and is used in the production of antimicrobial like antifungal agents, in particular soil isolates (Caballero *et al*., 2018).

The most important component of the plasmid found in *Bc* the pb16 (Bernhard *et al*., 1978), which is responsible for resistance to tetracycline, consist of open reading frames, as well as from the leader series, start and stop codon and it contains an independent unit for ending transcription (Palva *et al*., 1990). The plasmid was isolated in a closed circular manner of the *Bc* strain another plasmid is a bacteriocin called plasmid pBC7 (Bernhard *et al*., 1978).

Bacillus thuringiensis: *Bt* is a positive rod and has the same phenotypic characteristics as a *Bc* (Tongeren *et al*., 2014). It also has the inner space of the external spore, as well as the presence of the pilli or hairy appendages connected to the spore (Venkateswaran *et al*., 2017). These bacteria are capable of producing proteinaceous crystals, called Cry endotoxin, which is responsible for the toxicity of insects during the spawning phase (Agaisse and Lereclus, 1995). As a result of this property, researches and studies on this bacterial species have focused on their use in biological control as a pesticide in agriculture. As it represents about 90% of the pesticides used in the United States because there is no toxic effect on animal cells (Roh, 2007, EPA, 1998). It is usually sprinkled from airplanes as active spores on farms (Helgason *et al*., 2000). Although recent research has confirmed that insects has developed as a kind of resistance to these biological toxins, modern studies have sought to use phages in transmitting infection to insects in order to increase the infection activity (Badran *et al*., 2016).

It also has other applications in recent years because of the ability of protein crystals from different strains of *Bt* to kill preferentially of cancer cells (Saitoh *et al*., 2006, Mizuki *et al*., 1999). Five parental strains of *Bt* contained a plasmid complex through the studying of mutants, it was clear that the poisoning crystals producing–trait is connected to large single plasmid and these mutant strains (González *et al*., 1981) for this reason it was possible to transfer the killing insect trait to the plant by inserting the encoded genes into this internal toxin by bacteria *Agrobacterium tumefaciens* (Gatehouse, 2008, Barton *et al*., 1987).

Most of the serotypes of *B. thuringiensis* have plasmids that differ in their numbers and molecular weights

from one serotype pattern to another. The difference in the number and size of these plasmids gives a significant molecular weight variation ranging from (1.4-180 MD) (Stahly *et al.*, 1978). A study demonstrated that *B. thuringiensis* subsp. *israelensis* has a complex plasmid pattern consisting of eight molecular weight-different plasmids: 3.3 -4.2-4.9-10.6-68-75-105-135 MD (Gonzalez and Carlton, 1984) and can be more than 8 plasmids (Mizuki e*t al.*, 1999).

The high production of insecticides depends largely on the production of spores, which in turn depends on the promoters (Park *et al*., 2016). There are many promoters that control the production of anti-insect poison at the stage of forming spores in *Bt* such as Cry4Ab, Cry11Aa, Cyt1Aa and Cry4Aa (Federici et park, 2006, Federici *et al*., 2010). The Cry promoter is the highest in the production of anti-insect toxicity in the *Bt* subsp. *israelensis* than Cyt. promoter Therefore, researchers seek to increase its toxicity by increasing promoter number which increasing the plasmid number copies then increasing anti-insect protein crystals production (Park *et al*., 2016).

The majority of the encoded plasmids for protein crystals are conjugative plasmids (Gonzalez *et al.* , 1982) can be transferred among bacterial species *Bt*, *Ba*, *Bc* and *B. megaterium* (1994 Battisti *et al*., 1994, Wiwat *et al*., 1985, Bora *et al*., 1990). Aronson demonstrated that the conjugation frequency in genes that are from plasmid is higher than that of chromosomal origin (Aronson and Beckman,1987).

As well as toxins such as Cry and two known Cyt toxins which previously described in *Bt* subsp. *israelensis*, third type has been described and through the plasmid series pBtox is found in the strain mentioned, it was found that it is responsible for several functions in addition to genes encodes for toxin such as spore producing, germination and antibiotics production. (Berry *et al*., 2002). Electron microscope studies on this bacterium showed the formation of nanotubes, which are double strips of actin, different from the actin filaments in the eukaryotic cells which is called f-actin. They have a similar function to filamentous system isolation the eukaryotic cell, this filamentous system has a role in the isolation of plasmids and their distribution to the daughter cells (Jiang *et al.*, 2016).

Bacillus anthracis: Gram positive rod shaped bacteria from *Bc* group plcR gene, is a genetic mutation Nonsense found in 89 isolate from *Ba*, but is not present in members of the *Bc* group that most closely related to *Ba* (Easter *et al.*, 2005, Mignot *et al*., 2001). It is also spores forming bacteria, its spores are the mean of transmission and spread of anthrax disease which remain in the soil for hundreds of years until vegetative cells germination. They have a global spread and their diseases are dangerous and resistant to most of the vaccines. They are transmitted to human, either through Inhalation Contaminated soil only, or dealing with infected animals, mostly herbivorous animals and despite the availability of anti-vaccines, they prevent only a small part of this disease, (Krakalik *et al*., 2017, Hugh-Jones, 1999, Hugh-Jones and Blackburn, 2009). *Ba* is a capsule forming, 16S RNA type, sensitive to penicillin and gamma phage, also it possesses other cellular walls (Hoffmaster *et al*., 2004).

There are two types of plasmids in *Ba* pxo1 and pxo2, which carry major factors for fermentation. The number of copies of these plasmids varies and also their relations with other plasmids that are similar to them like pxo1 pxo2 plasmids (Pena-Gonzalez *et al*., 2018). Pxo1 plasmid is responsible for Anthrax toxin encoding, which includes two types of anthrax toxin are lethal in the form of edema toxin (PA and EF), which in turn are made up of three proteins are the protective antigen PA, lethal factor (LF) and the edema factor (EF). Plasmid pxo2 is the one that encodes the capsule for polyglutamate which produce capsule and that leads to penetrate the immune system of the host, by protecting the bacteria from phagocytic cells attacking (WHO, 1998, Uchida *et al*., 1986, Hugh-Jones and Blackburn, 2009).

As a result of the seriousness of the disease caused by this serious pathogen and the catastrophic effects that the biologists expect, especially when used as a destructive weapon in the terrorist wars, it is therefore important to focus fully on the genes needed for virulence and the occurrence of the disease, especially that it is encoded by the plasmids mentioned earlier, Therefore plasmids are continuously exposed by scientists to study, analyze, characterize and through specific markers and the use of PCR techniques (Riojas *et al.*, 2015, Irenge and Gala, 2012).

Number of plasmids were significantly differentiated from one study to another, as in (Coker *et al*., 2003), 40.5 of plasmids pxo1 and 5.4 of plasmids pxo2, whereas in another study 10.89 of pxo1 and 1.59 of pxo2 were recorded as averages of the plasmids number of each genome (Pilo *et al*., 2011), while in another study it was found that the number of plasmid copies of each chromosome was respectively 3-4 and 1-2 (Straub *et al*., 2013). Furthermore, studies based on the gene series suggested that the number of pxo1 plasmids is 2.3, while a recent study showed that the number of pxo1 plasmid copies was 3.86 and the number of pxo2 plasmid copies

was 2.29 (Pena-Gonzalez *et al.*, 2018).

Is there a genetic overlap between species of *Bacilus cereus senso lato* group?

Most of the analyzes, techniques and genetic tests applied on the three species of this group to determine the extent of genetic affinity or divergence or the extent of the interplay of evolutionary relationships between them depend largely on the genes carried by plasmids (Maughan and Van der Auwera, 2011).

However, most of the scientists justify the theory of one species and that is because of the large number of interrelated relationships and genetic relations very closely related to each other in this group. It is through that *Ba* and *Bt* are of one type or species which is *Bc*. The phenotypic traits that give the distinct differences are carried in plasmid genes In each species, while the other characteristics, which are similar in all species, such as resistance or sensitivity to beta-lactam antibiotic or motility or non-and haemolysis pattern, are all carried on the chromosome and that the differences in these characteristics occur because of certain mutations in individual genes in those species (Helg ason *et al*., 2000). This has already been demonstrated in the nitrous using as a chemical mutagen in order to determine the location of the mutation in the chromium resistance gene weather it is in the plasmid or chromosome and it has already been shown that the mutation occurred on the plasmid that is responsible for chromium resistance, which resulted in high sensitivity to chromium in mutant isolates treated with nitrous acid that was in *Bc* which isolated from contaminated soils in the Mosul city (Hadi, 2019).

While some researchers suggest that the differences between the species of this group is the presence of plasmid or not, like what happens in the case of *Bt* and *Bc*, since the only difference between them is the presence of encoding genes for anti-insect toxin which are present within plasmid and if plasmid loss it then they cannot distinguish between these two species (Thorne, 1993). So that the horizontal transmission of plasmids in fact led to a large similarity between them, which caused a problem in classification, pathologenicity and virulence (Patra *et al*., 1996, Gonzales *et al*., 1982, Sabelnikov and Ulyashova, 1990, Wilcks *et al*., 1998).

Bacterial clustering or aggregations may also be a cause of horizontal genes transport, which has been demonstrated in the *Bt* species by causing a mutation in the aggregation gene. A small plasmids transfer from Agr + to Agr- that is carried on pXO16 plasmid occurred. The aggregation phenomenon was recognized by the pheromone elimination through inducing it and using protease enzyme to combine donor and recipient cells (Andrup *et al*., 1993, Jensen *et al*., 1995).

Aggregation substrates are plasmid-encoded flat proteins that help contacting one cell to another to facilitate the plasmids exchange between donor and recipient strains because it is an important component of the genetic exchange system in this genus. This helpes plasmid spreading that encode for virulence factors such as the cytolycin production determinants and antibiotic resistance within species. Aggregation substances contribute to bacterial pathogenicity within the organism body through a variety of mechanisms, including bacterial adhesion of to the epithelial cells of the intestines and kidneys, colonization of tissues and protection against Macrophage cells or PMN cells by enhancing the phagocytic process in a manner that stimulates macrophages and PMN cells but does not kill bacterial cells. The aggregation substances act synergistically with cytolycin to increase the bacteria virulence the to stimulate the QS phenomenon, which results in deeper breakdown and invasion of tissue (Mundy *et al*., 2000, Upadhyaya *et al*., 2009). The aggregation materials have also been found in the *Bc* group and it performs variable functions in resistance to different environmental conditions and formation of cellular biofilms (Majed *et al*., 2016).

The pheromones produced by the recipient strain that specialized to plasmid or certain plasmids group which stimulate the donor strains to produce a specialized plasmid encodes for surface proteins that facilitate the beginning of the mating pair formation to initiate the bacterial conjugation process which is a system was proved in *B. genus* (Tortosa *et al*., 2001, Donelli *et al*., 2004). As well as that the pheromone system has been shown in 29 strains of the *Bc* group, which encodes for cellular signals through pentapeptide, which is also related to the evolution of species in this important group (Magnuson *et al*., 1994).

It is a system that is different from the conjugation systems found in the gram negative bacteria that it does not require sexual pilli and it contains a pheromone induced system in which the donor cell contains conjugation plasmids responsive to the pheromones for genetic exchange and this cell has a specific range of recipient hosts for conjugation including closely related species as a result This extraordinary transmission leads to the rapid spread of antimicrobial resistance (Fisher and Phillips, 2009).

Ba is considered a developmental linear extension of *Bc* depending on MLST technique and through analyzing nine chromosomal genes of *Bc* that produce anthrax toxin is also considered *B. anthracis* through its ability to have

virulence and the disease occurrence and based on genetic similarities and complex evolutionary principles and horizontal transfer gene capacity (Helgason *et al*., 2000). Other studies have also shown that the isolates which were taken from anthrax-endemic areas of *Bc* appear to be genetically similar to *Ba* except that they contain the infection plasmid required for anthrax virulence (Patra *et al*., 1998). Furthermore, it is considered *Bc* produced anthrax toxin is a *Ba* through its virulence, the disease occurrence, genetic similarities, complex evolutionary and horizontal transfer gene capacity (Helgason *et al*., 2000).

Additionally, *Bt* is closely related to Bc (Carlson *et al*., 1994), which is also associated with *Ba* (Helgason *et al*., 2000). There are a high genetic similarities proportion of f pXO1 plasmid genes of the anthrax toxin regulation and production of in the *Bc* LA2007 strain which was isolated from a fatal pneumonia case, this resemblance indicate a lateral transmission of genes between the two species of *B.* or an evolutionary relationship between them (Pena-Gonzalez *et al*., 2017).

Some researchers consider the phenotype or morphological characteristics differences are not reasons to considered species are different, but the plasmid genes that encodes virulence factors and toxins are the main principles for classifying the species of one group. Despite of the miscellaneous typical phenotypic properties of the three species that belong to the known *Bc sinso lato* group, MEE and the sequencing processes of nine chromosomal genes of *Ba* and comparing it with *Bc* and *Bt*, it showed that the high correlation in these comparisons (Helgason *et al*., 2000).

For example, some tests that is sensitive to *Ba* interfering with other *B.* species like *Bt* and *Bc*. These tests include DFA such as CW-DFA a test which depend on galactose / N-acetylglucosamine antigens of *Ba* cellular wall. However, this test gave positive results with *Bt* isolates of and *Bc*, while the unique capsule proteins produced by *Ba* are investigated by the (CAP-DFA) assay. The capsule internally of the poly-g-D-glutamic acid also gives a positive result with *Bt* strains (De *et al*., 2002). Half of the *Ba* and *Bc* G9241 isolates showed a difference in the colonies phenotype, in the *Bc* G9241 species that gave dry colonies on the bicarbonate agar medium whereas *Ba* isolates showed mucous colonies consequently the colony's variation in this medium is not conclusive evidence to distinguish between this group members(Beesley *et al*., 2010).

Other suggest that there is no any definitive genes exchange through the plasmids between the *Ba* and *Bc s.l.* group as a result of the conservation or stabilization of these genes of the same origin ancestors or predecessors of both species (Pena-Gonzalez *et al*., 2018). On the other hand, there is reverse proof of this theory that despite the *Bc s.l.* group isolates from environmental original, previously recorded in (Van der Auwera *et al*., 2013), which contains the pxo1 and pxo2 like plasmids, but does not encode for the anthrax toxins. However, they contain genetic markers for the capsule production, which are a major cause of pathogenicity (Pena-Gonzalez *et al*., 2018).

The convergence or overlap between the members of this group can be due to the high conjugation potentials of their plasmids. Through the conjogation experiments in the *Bt* serovar *israelensis* strain, it was found that the large pXO16 plasmid has high conjugation capabilities enhanced by high mobility and movement capabilities by single strand DNA (ssdNA) from one bacteria to another with the presence of genes assigned to ATPase activity (Makart *et al*., 2018).

Plasmids transferred to the recipients' cell by conjugation are called conjugative plasmids and these plasmids contain genes responsible for the transmission (Madigan *et al*., 2000). Non-Conjugative Plasmids are plasmids that are unable to move to the recipients' cell because they do not contain the genetic information needed to perform the conjugation process (Brown, 1986). It is believed that the genetic interference between species and the genes transfer between the chromosome and plasmids occurs because of the Mobile Elements and thus leads to genetic diversity and the pathogenic strains production through the horizontal transfer genes from one strain to another. For example, Cry genes that spread among several strains From *Bt*. In order to investigate the mobile elements between the chromosome and the plasmid, the chromosome and plasmid genes had to be fully examined or sequenced (Méric *et al*., 2017).

Transposons are pieces of DNA that move easily from one location to another between the plasmid and the chromosome. They are transferred between plasmids and have the ability to change their genetic position. They jump between different sites on the same plasmid. They are main factors for reorganizing the genetic information. They can combine several genes into one plasmid due to their ability to Jump and input, also it can encode for antibiotic-resistant enzymes, toxins, enzymes and various metabolic enzymes and can cause various gene mutations in which the transposons inserted in it or change in gene expression (Levinson, 2012). These elements are also widely available in Bc group species and they are also responsible for the transfer of certain traits from one species to another and hence increasing genetic diversity

within this group (Agersø *et al*., 2002).

Transposons surround by two entrance strands called insertion sequences. These elements are present in one or several copies of the chromosome or plasmid or both. In contrast to plasmids, the transposons or jumping elements have no self-replication ability, they multiply as part of the received DNA (Dxepardieu *et al*., 2007). Conjugative Transposons are mobile DNA molecules that encode for all the functions required for cell substitution and also bacterial cell conjugation. They include a series of synthetic genes linked by IS from the sides that are repeated in the inverse order of the transposons are found in a wide range of gram positive bacteria (Tn961 in *Enterococcus faecalis*) and gram negative bacteria such as (Tn455 with Tetracycline resistance gene in *Bacteroides*), which is important for the diffusion of antibiotic resistance genes and the transport frequency of the conjugate transposons ranges between10^4-10^9 (Mihaeseu *et al*., 2008). Within the *Bc* species, transposons play a significant role in the horizontal transfer of genes. They play an important role in the transfer of cellular functions, particularly antibiotic resistance (Mahillon and Lecrelus, 1988, Nicolas *et al*., 2015) Virulence plasmids transport and their genes as happened in the cryptic transposon Tn4430 that was present in *Bt* pxo12 plasmid which transfer genes that encode for the insecticide pxo12, as well as the transfer of pXO1 and pXO2 plasmids genes from *Ba* to the rest of the group species like *Bc* (Green *et al*., 1989). It was also diagnosed 16 type of IS and transposons from the Tn3 family in group of *Bc senso lato* (Fayad *et al*., 2019). It was also diagnosed 16 type of Insertion Sequence and transposons from the Tn3 family in group of *Bc. s.l.* (Fayad *et al*., 2019). Furthermore, Connections between the chromosome and plasmid lead to spread of pathogenicity and virulence among bacterial strains and plasmids alter their genetic content through rapid correlation with the chromosomes (Kiem and Wagner, 2009).

The integrons are sequences of DNA often formed as transposons part, which contains genetic determinants of the recombination system in specific sites. Integrons are characterized by merging and thus mediating the movement of a short DNA sequence called Gene Cassettel, most of gene cassette contain one antimicrobial gene. Integrons groups combine many gene cassettes and it plays an important role in the propagation and development of antibiotic resistance genes and the distribution of resistant bacteria to multiple treatments (Rajaei *et al*., 2014). Whereas, some researchers suggest that suitable environmental conditions should be available for horizontal transfer gene among plasmids because this genetic transport was not observed among *Bt*., *Bc* and *Ba*. It also refers to possible genetic causes like restricted plasmids within one species (Méric *et al*., 2017).

On the other hand, in many countries in the world, the whole gene sequencing and SNPs tests were done in the hundreds isolated strains of *Ba* and in an attempt to make a molecular fingerprint of these isolates, which showed that the genes had a high relativity stability and did not have variations or transitions between the species in the studied isolates (Derzelle *et al*., 2015, Girault *et al*., 2014). There are also other factors that affect the identification of species genes depending on the plasmids, like the used strains type, either environmental or clinical, as well as the number of isolates, especially as it is preferred to have a large number in order to be environmental similarity, also the technology that used. All these factors have a significant role in determining the plasmids number or genetic transitions that occur between species as the results are affected by the impact or variation of these conditions (Pena-Gonzalez *et al*., 2017).

Conclusion

Despite of the views divergence on the extent of genetic overlap between the species of *Bacillus ceres senso lato* group, However, this does not negate the clear role of plasmid in the horizontal or vertical transfer of genes, except the contribution of other genetic elements to help it in this matter, such as transposons, integrons, conjugated elements and IS where these factors help with the plasmid presence to spread genetic diversity among these members of a group.

Abbreviations

Bacillus: *B, Bacillus thuringiensis: Bt, Bacillus anthracis: Ba, Bacillus cereus: Bc, Bacillus subtilis: Bs,* sensu lato*: s.l.,* Crystal or delta-endotoxin gene: Cry, Cytotoxin gene*:* Cyt, Open reading frames: ORF, Multilocus enzyme electrophoresis: MEE, Polymorphonucleur leukocyte: PMN, Phenomenon Quorum Sensing: QS, Direct fluorescent antibody assay: DFA, Cell Wall Direct fluorescent antibody assay: CW-DFA, Single-nucleotide polymorphism: SNP, Transposons: Tn.

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