International Journal of Innovation in Agriculture Sciences and Rural Development
Vol.3, NO.3 , P: 53 - 58
Received : 29 June 2021
Accepted : 23 July 2021

Biological application of Bacillus thuringiensis of agricultural

Reyhaneh Ghorbani Zavareh
Master student of industrial Microbiology, Shahid Ashrafi Esfahani University, Esfahan, Iran
re73ghorbani73@gmail.com
Masoume Dehghani
Master student of industrial Microbiology, Shahid Ashrafi Esfahani University, Esfahan, Iran
masoume.dehghani4033@gmail.com
Maryam Sadat Mirbagheri Firozabad
Department of Biology, Faculty of Sciences, Yazd University, Yazd, Iran
m.mirbagheri@yazd.ac.ir
Maryam Sadat Jalili Tabaii *
Department of Biotechnology, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani University, Esfahan, Iran
maryjtabaii@gmail.com

Abstract:
Bacillus thuringiensis (Bt):
Gram-positive bacteria are sporulation that its main habitat is soil. The crystalline toxins produced during the sporulation phase are toxic to insects but non-toxic to humans, and are the main microorganisms used in the biological control of agricultural pests. With increasing demand for foodstuffs free of chemical toxins and with the implementation of agricultural methods that are less harmful to the environment, the use of toxins has expanded. Bacteria in the sporulation phase secrete protein crystals consisting of different groups cry and cyt. Cry proteins are toxic to insects including Lepidoptera, Coleoptera, Hymenoptera, Diptera, as well as nematodes. Severe toxicity of cry proteins has also been reported in the Diptera order. Since each type of cry toxin has a specific mechanism as an insecticide, it usually affects only a few species of a particular order. Recently, researchers have produced new pesticide proteins (VIPs) in the vegetative growth phase of bacteria. And have a broader functional mechanism, they reported. These toxins are lethal to a wide range of the order Lepidoptera. Bt also produces pathogens, including insecticide-secreting protein toxins (exotoxins). However, the mechanism of action of these products is not fully understood. There are two proposed models: first, that Bt causes osmotic imbalance in response to the formation of pores in the cell membrane, and second, that it opens ion channels that activate the process of cell death. There are several methods that resist Bt is induced in them: changes in receptors that do not detect cry toxin, the synthesis of membrane transporters that kill peptides from the cytosol, and the development of regulatory mechanisms that disrupt the production of toxin receptors.

Keywords: *Bacillus thuringiensis*, Agricultural pests, Toxins, Secretory proteins

Introduction:

Ever since man began farming, he has been one of the human competitors in the production of better and more insect products, first using mechanical and agricultural methods and then turning to chemical methods, and the primary chemical pesticides that are composed of minerals and Organic arsenic was used. These compounds included organochlorines, organophosphates, carbamates, formamidines, and proteroids (Namdar Khojasteh et al). These pesticides had disadvantages, including the selective superiority of some insects over pesticides, such as houseflies. Their durability and toxicity (resistance) have been established for a long time. It harms beneficial soil insects. These pesticides also had a number of disadvantages and a number of advantages, One of the benefits of these pesticides is that they prevent pests from becoming resistant over time. Specific effect: They are not harmful to humans and other organisms and do not harm beneficial and pollinating insects. No damage to soil texture and soil conditions and soil fertility. Biodegradability and finally low secondary costs and the possibility of use in a short time until harvest (Iranmanesh et al.; 2010). But the disadvantages of these pesticides: high price, high production and distribution costs, less stability, the need for knowledge and awareness to use these pesticides, relatively slow performance, no effect on adult insects, which usually cause the most damage. (Dunbar and Beard, 1975). Many pests are controlled by biological pesticides. (Marzban et al., 2014).

History:

In 1901, Ishivata, a Japanese scientist, isolated a parallel spore-bearing bacterium from a diseased silkworm and named it Sotobacillus (Fernandez-chapa et al, 2019). In 1911, Berliner isolated a similar bacterium from Mediterranean flour flies in the German region of Thuringia and named it Bacillus thuringiensis (Samir and Abbas, 2018). The first commercial use of the toxin by Hose was in 1928 in France against corn pests (Milner, 1994). But in 1983, they were able to isolate the strains that cause disease on coleoptera. From now on, new research on bacteria, especially on molecular biological aspects, began. This year, the commercial products of this bacterium called Sporin was produced commercially in France. Commercial production took place in the United States in 1985, and Bt is now produced worldwide under a different brand (Ebrahim et al, 2010).

Microscopic and macroscopic characteristics of Bacillus thuringiensis:

This bacterium is one of the prokaryotes and does not need light to grow (Qalichi et al., 2013). This bacterium grows easily in many laboratory environments, such as a nutritious broth (NB: Nutrient broth) under aerobic conditions and at temperatures between 15-40 degrees. Gram-positive,Optional anaerobes produce spores (Moazamian et al, 2010). Most are isolated in soil environments, insect carcasses, water, fresh or rotten leaves, storage products (Mane et al, 2015) and rainwater (Hamedo, 2015).

Types of toxins produced by Bt:

Alphaexotoxin(α-exotoxin) in addition to insects, this toxin has a toxic effect on mice and other vertebrates. Beta-exotoxin(β-exotoxin): Produced in some strains of this bacterium and has major effects on mammals. It is toxic and dangerous to blood cells and its use is banned in many countries. Gamma exotoxin: This toxin is toxic to insects. Deltaendotoxin (δ-endotoxin): Formed at the stage of bacterial death and spore formation. Today, commercial products use strains of B. thuringiensis, which produces deltaendotoxin (δ- endotoxin), and deltaendotoxins are the same as crystalline proteins (Carpets et al., 2015). Vip toxins are produced in the growth cells of some bacteria. For example, Bacillus cereus: Vip1 and Vip2 are lethal to insects. Bacillus thuringiensis Vip3 is lethal to Agrotis and Sprodoptera (Estruch et al, 1995).

Crystalline proteins:

They are detected by the production of one or more spore crystals during the sporulation period. These are protein crystals called deltaendotoxins(δ-endotoxin) (Swiecicka et al, 2008) and have a specific toxic effect on many different insects. There are cry (specific) proteins and cyt (nonspecific) cytolytic proteins. In 1989, four classes of cry genes and two classes of cyt genes were identified. Cry1 genes encode proteins that are toxic to the order Lepidoptera. Cray 2 genes on the order Lepidoptera and Diptera and Cray 3 genes on the order Coleptera and cry4 genes specifically on the Diptera toxicity (Gray Moradi et al., 2014).

The mechanism of action:

The bacterium is sprayed on agricultural foliage so that it can be eaten by small pest larvae. This bacterium only poisons insect larvae. It does not affect eggs, pupae, or whole insects. During the sporulation phase, the Bt strain produces one spore and one or more protein crystalline bodies. These cry proteins are linked together by hydrophobic bonds and disulfide bridges. The most obvious forms of toxin are two-dimensional structures. The entry of protein crystals into the gastrointestinal tract of susceptible insects causes their death.The crystals are dissolved by the alkaline sap of the middle intestine of the cornea, then the toxin is broken down by intestinal proteases to form the active protein toxin. The active toxins bind to specific receptors on the plasma membrane of the epithelial cells of the middle intestine of the cornea and then enter the cytoplasmic membrane, forming cation-conducting pores 10 to 20 angstroms in diameter, thereby inhibiting the relative ion permeability And protons. The flow of water with the ponies goes into the cells of the middle intestine of the cornea and causes swelling and disintegration of the cell. Falls (Villalon et al, 1998).

When these crystalline proteins are placed in the alkaline environment of the insect intestine, they are dissolved and proteolytic action is performed in the N-terminal or C-terminal by alkaline proteases of the midgut. The structure of the toxin is such that domaim1 contains seven alpha-helixes. The surrounding spirals are hydrophobic and the central spirals are hydrophilic. Domain1 role plays a role in the permeability of the membrane and the formation of pores in the epithelial cells of the midgut of target insects due to the long presence of long hydrophobic and hydrophilic spirals. Domaim2 of a triangular column consists of three beta plates formed to bind the toxin to the cells lining the middle intestine of the cornea and the Domine 3 includes the anti-parallel beta species, which, like region 2, plays a role in binding the toxin to the receptor and regulating ion channels. After passing through the matrix, the toxin binds to the receptor in the intestinal cell membrane and, by binding to the receptor, acts as an important determinant of toxin specificity. In the cell membrane, which causes ionic disorder between the blood and the intestine, which causes cell lysis and damage to the epithelial intestinal tissue and the death of larvae (Froutni Bella Biglou, 2016).

Molecular methods:

Includes transgenic plants and cloning of the deltaendotoxin (δ-endotoxin) gene in maize.

Creating transgenic plants:

Gene transfer from one organism to another is an example: Transfer of Bacillus thuringiensis toxin to control corn pests. The toxin is released from the bacterium Bacillus thuringiensis into the plant, which is corn, and causes poisoning and death when the insect uses the corn (Swamy and Asokan, 2013).

Other methods:

Selective plasmid deletion:

In many strains of Bacillus thuringiensis, each bacterium has multiple plasmids, some of which may c one or more cry genes.The expression of each of the cry genes is encoded by the amount of cry protein encoded by those pretoxin cry genes with little activity against that pest. In such cases, providing highly active toxins in each cell This can be done by deleting genes encoding low-activity pro-toxins (Malek zadeh et al., 2006).

Making conjugation strains:

Some plasmids in the strains of Bacillus thuringiensis are self-transmitting. Such plasmids can be used to construct new strains by conjugation. None of the strains of Bacillus thuringiensis, which is toxic to all of these insects, have been isolated in the wild. However, an effective strain against all three of these pests is as follows: The active strain was isolated from Bacillus thuringiensis against the Colorado potato beetle, and its cry genes were found to be located in a transmissible plasmid. This strain is mixed with a strain of the acceptor Bacillus thuringiensis, which is active against European corn worms. (Swokan and Asokan, 2013).

Gene transfer to other microorganisms:

With genetic engineering, cry genes and their modified versions can be inserted and expressed in any microorganism. In fact, cry genes are already transmitted to various bacteria and plants. Insect-resistant tragic agricultural products express the cry proteins of Bacillus thuringiensis. Transmission of cry genes to cyanobacteria , which growen masse in aquatic environments, is a way to maintain high levels of toxins in mosquito-infected habitats. Modern protection of agricultural products and repression of insects opens the pest (Malekzadeh et al., 2006).

Formulation:

Available as a combination of endotoxin crystals and live bacterial spores. Some products are prepared and supplied as capsules in bacterial cells (Marzban et al., 2014).

Bt is used today in two ways:

1- Production of insecticidal microbial toxins: Bt commercial products are presented as microbial insecticides in the form of powder or powders suspended in water and today it is one of the most widely used agents of microbial control of insects. 2- Production of Bt plants: These plants contain toxin-producing genes derived from the bacterium Bacillus thuringiensis. These plants are able to produce toxins and kill the insect pests that feed on these plants (Sarraf zadeh, 2012).

Some insecticides, such as toxins from a particular species or subspecies of Bacillus, can be poisonous to whole orders of insects, while others may be effective against only a few species or even just one species. Bacteria are one of the most widely used microbial insecticides in the 1960s. The amount of these pesticides is 4-6 grams per hectare, which are used to control more than 40 different insects that cause damage to agriculture and forests, and some are the cause of human disease.The endotoxins produced by B. thuringiensis act specifically for a number of insects and when converted to ultraviolet light or other environmental factors, they are rapidly converted to non-toxic compounds. Known as environmentally friendly products (Waites et al, 2001)

Conclusion: The best way to control pests is biological control to prevent many dangers including poisoning, abortion, skin and neurological complications, behavioral disorders, cancer. Increasing the production of biological pesticides also reduces production and distribution costs.

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