VACCINES

CONTENTS:

Introduction

A vaccine generally contains an agent that resembles a disease-causing microorganism and is usually made of the microbe, its toxins or one of its surface proteins. Scientists adopt many strategies to produce vaccines against a pathogenic microbe. These choices are directed by nature of pathogen and infection and as well as considering natural body immune response.Most current vaccines have their successdue to their ability to target pathogens that have low antigenic varaiability.This is the case for polio,tetnus,diphtheria.measles and hepatits B ,among others.As a result,vaccines that have capacity to neutralize antibodies against these pathogens were successful.

On the other hand, important cell mediated immunity against intracellular pathogens(which in most cases leads to chronic infection)is much more difficult using current vaccines strategies.In this regard,the recombinant vaccines are in use to produce immunity against pathogens.We can broadly classify the recombinant vaccines into Subunit recombinant vaccines,Attenuated recombinant vaccines and vector recombinant vaccines.

3. What is a vaccine?

In the most simplified terms, a vaccine is a biological preparation that provides active acquired immunity against a certain disease. Usually a vaccine consists of biological agent that represents the disease causing microorganism. It is generally made from a weakened or a killed form of the microorganism, its toxins or one of its surface protein antigens. The first successfully case of vaccination was performed by Edward Jenner in 1796.He noticed that individuals who had cowpox, did not touch smallpox even when coming in direct contact with disease.

4. What are the approaches for vaccines development?

Scientists take many approaches to make vaccines against a pathogenic microorganism. These choices are being selected by the nature of pathogen and infection as well as practical considerations about the use of the vaccines. Some of these options include live attenuated vaccines, inactivated vaccines, DNA vaccines and Recombinant subunit vaccines.

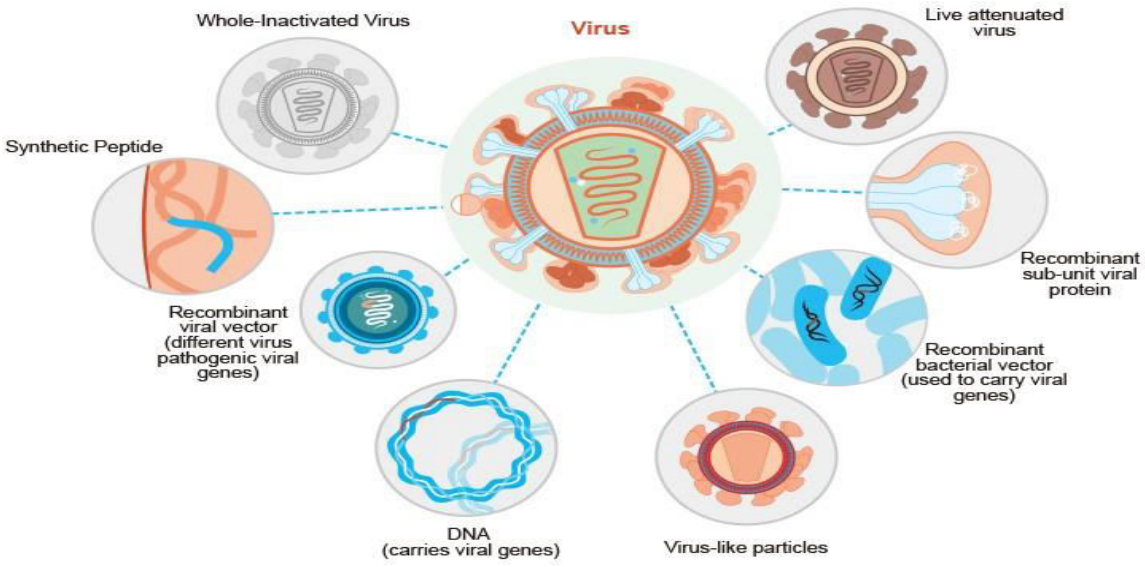

Figure 1: Various approaches for vaccine Development

5. What is a Recombinant vaccine?

Vaccine produced by using recombinant DNA technology (i.e. mixing of two DNA from different sources) is called recombinant vaccine. This involves inserting the DNA encoding antigen (such as bacterial surface protein) that stimulates an immune response into bacterial or mammalian cells, expressing the antigen in these cells and then purifying it from them. Recombinant vaccines are prepared with the help of expression system, such as bacteria, insect, yeast, and mammalian cells in which the DNA encoding the genetic determinant can be inserted and expressed. However many factors must be checked before choosing the system for antigen expression. The level of expression we get by using each expression vectors and promoter(Initiator) ,the selection marker of choice, the presence or absence of post- translational modifications by recombinant vector ,besides other are important characteristics

Bacterial expression system are most common in use because they are easy to handle and their ability for high level expression. On the other hand, for antigens in which post- translational modifications (e.g. glycosylation) are necessary, the use of mammalian or insect cells should be preferred.

6. Types of Recombinant vaccines:

The recombinant vaccines can be broadly classified into three groups:

6.1. Subunit recombinant vaccines:

These are the components of the pathogenic organisms. Subunit vaccines are proteins, peptides and DNA.

6.2. Attenuated recombinant vaccines:

In this method, genetically modified organisms (bacteria or viruses) that are made non-pathogenic are used as vaccines.

6.3. Vector recombinant vaccines:

These are the genetically modified viral vectors that can be used as vaccines to protect from several pathogens. Some of the advancements made in the preparation of recombinant vaccines against certain diseases are shortly described.

Type # 6.1.Subunit vaccines:

The subunit vaccines contain only a fraction of the pathogenic organism. Usually these are synthetic peptides that show protein component that induces immune response. The benefits of these vaccines include their purity in manufacturing, stability and safe use. Following are the some of the examples of diseases in which scientists achieved to prepare vaccines by using subunits of pathogens.

a) Hepatitis B:

Hepatitis B is a common viral disease in man. It basically affects liver causing chronic hepatitis and liver cancer. it contains a core having a viral genome(DNA) surrounded by phospholipids envelop carrying surface antigens. Scientists has identified the gene encoding for hepatitis B surface antigen (HBsAg).Recombinant hepatitis B vaccine as a subunit vaccine is produced by cloning(growing) HbsAg gene in yeast cells.*Saccharomyces cerevisiae,* a harmless baking and brewing yeast, is used in this purpose. The HBsAg assembles into virus like particles (VLPs),which are highly immunogenic, making the HBV vaccine, a very good vaccine. After expression in yeast system, it is purified.

Hepatitis B vaccine-the first synthetic vaccine:

In 1987, the recombinant vaccine for hepatitis B (i.e.HBsAg) was the first synthetic vaccine for public use. Hepatitis B vaccine is safe to use, very accurate and produces no allergic reactions.

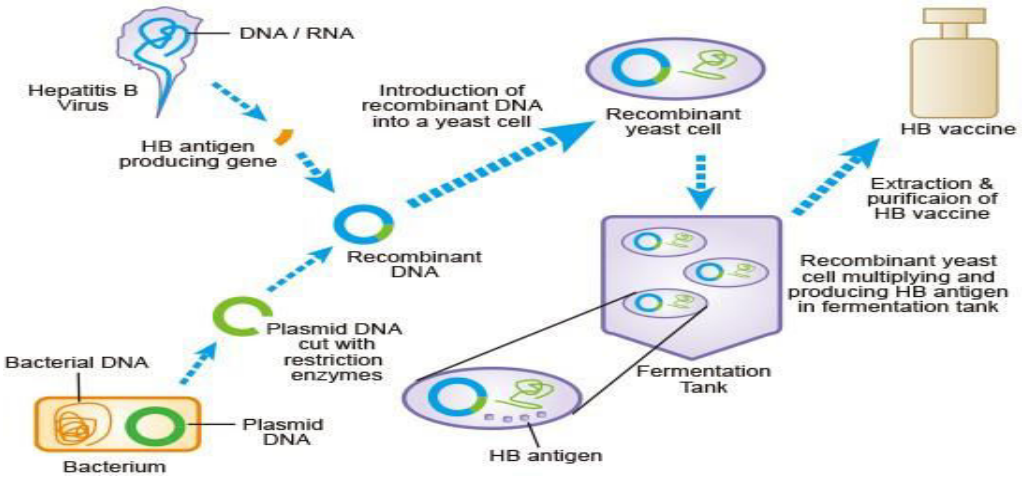

Figure 2: Hepatitis B vaccine production

Biotechnologists have been successful in adding hepatitis B gene into the cells of tomato plant. These genetically engineered plants produce hepatitis B antigens. The day is no longer to get immunized against hepatitis B by having a tomato in lunch!

b) t and Mouth Disease:

Foot and mouth disease (FMD) is a highly contagious disorder of cattle and pigs. A formalin killed foot and mouth virus (FMDV) was recently used to vaccinate against this disease. Four viral proteins(Vp1,Vp2,Vp3 and Vp4) surrounds the genome of FMDV.From these,Vp1 is immunogenic. The sequence of nucleotides for Vp1 was discovered from the genome of FMDV.A double stranded complementary DNA (cDNA) was made from single-stranded viral RNA(genome).Restriction enzymes used to cut this cDNA and the fragments were cloned by using plasmid pBR322 in E.coli.In this way ,the recombinant vaccine for FMDV in the shape ofviral protein 1 was used to vaccinate animals.

C) Human papillomaviruses viruses:

A recently developed example of recombinant vaccine is the vaccine against human papillomaviruses (HPVs) .HPV is a common sexually transmitted disease linked many kinds of muccotenus disorders in humans including cervical,valva and vagional cancer and genetial worts.Currently,two vaccines are in use against HPV.These both vaccines have been developed

based virus like particles (VLPs) obtained from HPV-6,-11,-16 and/or -18 subtypes.These vaccines use the L1 recombinant proteins of every subtype,produced either in yeast or an insect

cell system. The L1 is the major capsid protein that expresses in vitro causes the formation of VLPs.

DNA Vaccines (Genetic Immunization):

These vaccines usually consist of synthetic DNA containing the gene that encodes the disease agent protein.Normally,the plasmid DNA used as vaccine is cultivated in bacteria such as E.coli and they are separated and purified for injection. The concept behind a DNA vaccine is that the antigen can be expressed directly by host cells in a way that stimulates viral infection and starts an immune respons from the host.

DNA vaccines—plasmids can be administered to the animals by one of the following delivery method.

i. Nasal spray
ii. Intramuscular injection
iii. Intravenous injection
iv. Intradermal injection
v Gene gun or biolistic delivery (requires nanogram level of plasmids)

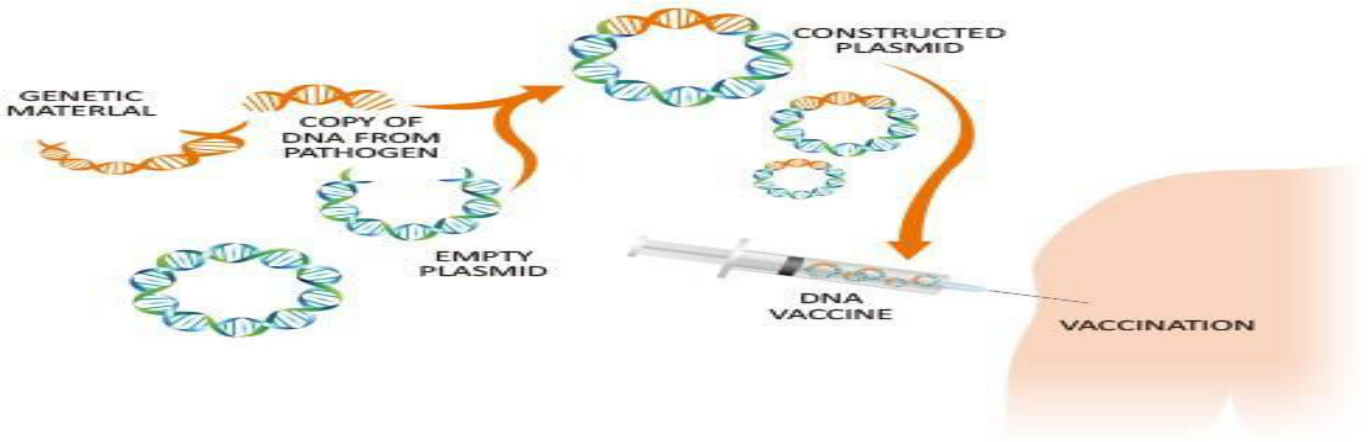

Figure 2: Principal of a DNA Vaccine

Present status of DNA Vaccines:

After 1990, many groups of workers World-wide have been trying to develop DNA vaccines against several diseases.Genetic immunization has been done against a number of pathogenic organisms.These include influenza A virus, rabies virus, hepatitis B virus,herpes virus,HIV and plasmodium speicies(malarial parasite).It must be noted that DNA vaccines have not been tried in humans for unknown risks of these foreign DNAs.

Plants as Edible Subunit Vaccines:

Plants serve as a cheap and safe production systems for subunit vaccines .The edible vaccines can be easily ingested by eating plants.This removes the processing and purification methods that are otherwise needed.Transgenic plants(tomato,potato) have been developed for expressing antigens derived from animal viruses(rabies virus,herpes virus).A selected list of recombinant against animal viruses produced in plants is given below in table.

vaccines

Table 1: A selected list of plant edible subunit vaccines

edible subunit vaccines

Antigen	*Host plant*
Rabies glycoprotein	Tomato
Foot and mouth virus (VPI)	*Arabidopsis*
Herpes virus B surface antigen	Tobacco
Cholera toxin B subunit	Potato
Human cytomegalovirus glycoprotein B	Tobacco

Type # 2 Attenuated Recombinant Vaccines

In the start of vaccine research, attenuated strains of some pathogenic organisms were prepared by long growth—weeks,months or even years.Although the reasons are not known ,the infectious organisms would lose its ability to cause disease but retains its capacity to act as an immunizing agent.This type of method is almost outdated now.

It is now possible to genetically engineer the organisms (bacteria or viruses) and use them as live vaccines,and such vaccines are also named as attenuated recombinant vaccines.The genetic manipulations for the production of these vaccines are widely of two types:

i. Deletion or modification of virulence genes (disaease causing) of pathogenic organisms.
ii. Genetic modification of non-pathogenic organisms to carry and express antigen determinants from pathogenic organisms.

Some of the important attenuated vaccines prepared by genetic modifications are briefly described.

a) Cholera:

Cholera is an intestinal disease. Its symptoms include diarrhea,dehydration,abdominal pain and fever. It is caused by the bacterium,*Vibro cholera*.On entering the small intestine,*V.cholera* grows and starts producing a toxic protein ,a hexameric entertoxin.This entertoxin causes the loss of ions from the cells and it leasds to diarrhea,dehydration and even death.

The currently used cholera vaccine is made up of phenol-killed V.cholera.The immune-protection lasts for 3-6 months .The DNA techniques have discovered the gene of enterotoxin(toxic protein).Entertoxic,a hexamer consists of one A subunit and five similar B subunits. The A subunit further has two functional domains-the A1 peptide which has the toxic activity and A2 peptide that links A subunits to B subunits.

By genetic engineering ,it was possible delete the DNA sequence encoding A1 peptide and produce a new strain of V.cholera.This is witout pathogen and it cannot produce entertoxin. The genetically enginerred V.cholera is a good candidate to use as an attenuated vaccine.

b) Salmonella Species:

Typhoid,enteric fever,food poisning and infant death are caused by different strains of Salmonella genus.Immunoprotection against Salmonella specie is really necessary.Some scientists have been successful in deleting aro genes and pur genes in Salmonella.

Type # 6.3 Vector recombinant vaccines:

Some of vectors (carriers) that may be bacteria or virus can used as vaccines after their genetically modification. Recombinant vector vaccines use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. Following is the some of the uses of this kind of approach:

Conclusions

Vaccines induce an immune response in the animal host that subsequently recognizes infectious agents and helps fight off the disease; vaccines must do this without causing the disease. Using recombinant DNA technologies, scientists have been able to develop live genetically modified organisms, recombinant killed vaccines, and genetic vaccines that no longer cause disease yet induce a strong immune response. Developing vaccines using rDNA technologies requires a thorough understanding of the disease agent, particularly the antigens critical for inducing protection and the factors involved in causing disease. In addition, it is important to understand the immune response of the host to ensure that the vaccine induces the appropriate immunological reaction.

Paralleling the development of new, more efficacious, stable, and safe recombinant vaccines is the study of vaccine delivery methods. In addition to using conventional delivery routes such as oral, intranasal, intradermal, transcutaneous, intramuscular, and IP, scientists are experimenting with needle-free systems and vaccine strategies that allow mass vaccination of

many individuals simultaneously.

Another active area of research is the study of compounds with the potential to enhance the immune response to vaccines. These approaches include incorporating immunomodulating compounds into vaccines that can affect the type of immune response directly and immunopotentiation compounds that strengthen the immune response. The antigenic pathway can thus be modulated to stimulate the appropriate arm of the immune response to provide solid protection. Also, new compounds that indirectly stimulate the immune response (such as microparticles and adjuvants) are being studied. These compounds are designed to present antigens to the immune system in such a way that optimal stimulation is achieved.

The promise of better vaccines to benefit animal agriculture and companion animals through rDNA technology is becoming a reality. A number of recombinant vaccines are available commercially, and many more are projected to be available soon, so the future of recombinant vaccines is bright. New efforts need to focus on delivery methodology as well as development of vaccines for economically important diseases for which no currently available vaccines exist or for diseases where poorly effective vaccines are currently in use.

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MICROBIAL POLYSACCARIDES

MICROBIAL POLYESTERS

Polyhydroxyalkanoates or PHAs are linear polyesters produced in nature by bacterial fermentation of sugar or lipids.

Key Points

- To produce PHA, a culture of a micro- organism such as Alcaligenes eutrophus is placed in a suitable medium and fed appropriate nutrients so that it multiplies rapidly.
- PHA synthases are the key enzymes of PHA biosynthesis.
- There are potential applications for PHA produced by micro-organisms within the medical and pharmaceutical industries, primarily due to their biodegradability.

Key Terms

- **Polyhydroxyalkanoates**: Polyhydroxyalkanoates or PHAs are linear polyesters produced in nature by bacterial fermentation of sugar or lipids.
- **fermentation**: Any of many anaerobic biochemical reactions in which an enzyme (or several enzymes produced by a microorganism) catalyses the conversion of one substance into another; especially the conversion (using yeast) of sugars to alcohol or acetic acid with the evolution of carbon dioxide.
- **biodegradability**: The capacity of a material to decompose over time as a result of biological activity, especially to be broken down by microorganisms

Polyhydroxyalkanoates, or PHAs, are linear polyesters produced in nature by bacterial fermentation of sugar or lipids. They are produced by the bacteria to store carbon and energy. More than 150 different monomers can be combined within this family to give materials extremely diverse properties. These plastics are biodegradeable and are used in the production of bioplastics. They can be either thermoplastic or elastomeric materials, with melting points ranging from 40 to 180°C.

The mechanical qualities and biocompatibility of PHA can also be changed by blending, modifying the surface or combining PHA with other polymers, enzymes and inorganic materials, making it possible for a wider range of applications.

PROCESS OF PHA PRODUCTION

To produce PHA, a culture of a micro-organism such as Alcaligenes eutrophus is placed in a suitable medium and fed appropriate nutrients so that it multiplies rapidly. The biosynthesis of PHA is usually caused by certain deficiency conditions (e.g. lack of macro elements such as phosphorus, nitrogen, trace elements, or lack of oxygen) and the excess supply of carbon sources. Recombinants Bacillus subtilis str. pBE2C1 and Bacillus subtilis str. pBE2C1AB were used in production of polyhydroxyalkanoates (PHA) and it was shown that they could use malt waste as carbon source for lower cost of PHA production. As raw material for the fermentation, carbohydrates such as glucose and sucrose can be used, but also vegetable oil or glycerine from biodiesel production. Researchers in the industry are working on methods with which transgenic crops will be developed that express PHA synthesis routes from bacteria and so produce PHA as energy storage in their tissues. Another group of researchers at Micromidas is working to develop methods of producing PHA from municipal waste water. Another even larger scale synthesis can be done with the help of soil organisms. For lack of nitrogen and phosphorus they produce a kilogram of PHA from three kilograms of sugar.

Polyesters are deposited in the form of highly refractive granules in the cells. Depending upon the microorganism and the cultivation conditions, homo- or copolyesters with different hydroxyalkanic acids are generated. PHAs granules are then recovered by disrupting the cells. In the industrial production of PHA, the polyester is extracted and purified from the bacteria by optimizing the conditions of microbial fermentation of sugar or glucose. Once the population has reached a substantial level, the nutrient composition is changed to force the micro-organism to synthesize PHA. The yield of PHA obtained from the intracellular inclusions can be as high as 80% of the organism’s dry weight.

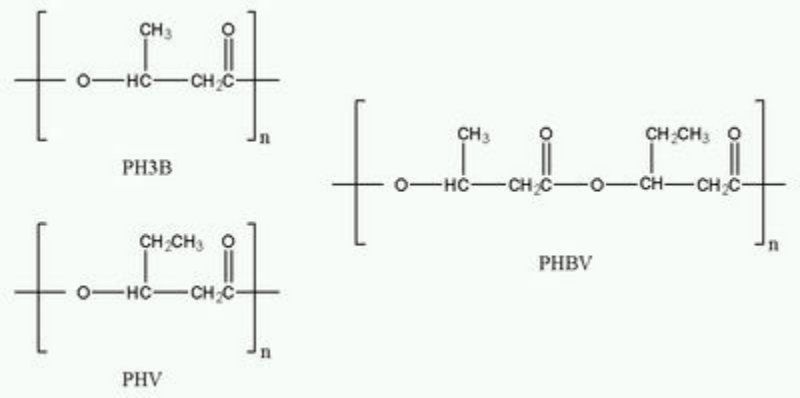

Figure: **Chemical structures of P3HB, PHV and their copolymer PHBV**: Chemical structures of P3HB, PHV and their copolymer PHBV

PHA SYNTHASES

PHA synthases are the key enzymes of PHA biosynthesis. They use the coenzyme A – thioester of (r)-hydroxy fatty acids as substrates. The two classes of PHA synthases differ in the specific use of hydroxyfattyacids of short or medium chain length. The resulting PHA is of the two types: Poly (HA SCL) from hydroxy fatty acids with short chain lengths including three to five carbon atoms are synthesized by numerous bacteria, including Ralstonia eutropha and Alcaligenes latus (PHB). Poly (HA MCL) from hydroxy fatty acids with middle chain lengths including six to 14 carbon atoms, can be made for example, by Pseudomonas putida. A few bacteria, including Aeromonas hydrophila and Thiococcus pfennigii, synthesize copolyester from the above two types of hydroxy fatty acids. The simplest and most commonly occurring form of PHA is the fermentative production of poly-beta-hydroxybutyrate) (poly-3-hydroxybutyrate, P3HB), which consists of 1000 to 30000 hydroxy fatty acid monomers.

PHA APPLICATIONS

PHAs are processed mainly via injection molding, extrusion and extrusion bubbles into films and hollow bodies. A PHA copolymer called PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) is less stiff and tougher, and it may be used as packaging material. There are also applications for PHA produced by micro-organisms within the medical and pharmaceutical industries, primarily

due to their biodegradability. Some of the fixation and orthopaedic applications that have been devised for these polymers include:

- sutures and suture fasteners
- meniscus repair and regeneration devices
- rivets, tacks, staples, and screws
- bone plates and bone plating systems
- surgical mesh, repair patches, and cardiovascular patches
- vein valves, bone marrow scaffolds
- ligament and tendon grafts
- ocular cell implants
- skin substitutes, bone graft substitutes, and wound dressings

MICROBES AS BIOCONTROL AGENTS

They come from naturally occurring or genetically altered bacteria, fungi,algae, viruses or protozoans. Microbial control agents can be effective and used as alternatives to chemical insecticides. A microbial toxin can be defined as a biological toxin material derived from a microorganism, such as a bacterium or fungus. Pathogenic effect of those microorganisms on the target pests are so species specific. The effect by microbial entomopathogens occurs by invasion through the integument or gut of the insect, followed by multiplication of the pathogen resulting in the death of the host, e.g., insects. Studies have demonstrated that the pathogens produce insecticidal toxin important in pathogenesis. Most of the toxins produced by microbial pathogens which have been identified are peptides, but they vary greatly in terms of structure, toxicity and specificity.

These microbial pesticides offer an alternative to chemical insecticides with increased target specificity and ecological safety so that they are used either uniqly or in combination with other pest management programmes. One definition for integrated pest management (IPM) which is most relevant to this practice comes from Flint and van den Bosch [1981]: "It is a ecologically based pest control strategy that relies heavily on natural mortality factors and seeks out control tactics that disrupt these factors as little as possible. Ideally, an integrated pest management program considers all available pest control actions, including no action, and evaluates the potential interaction among various control tactics, cultural practices, weather, other pests, and the crop to be protected".

These microbials as biocontrol agents present u beneficiancy. They have efficiency and safety for humans and other nontarget organisms. They leave less or no residue in food. They are ecologically safe, so that other natural enemies are free of their threatening, leading to preservation of other natural enemies, and increased biodiversity in managed ecosystem. So, microbial agents are highly specific against target pests so they facilitate the survival of beneficial insects in treated crops. This may be the main reason that microbial insecticides are being developed as biological control agents during the last three decades.

Microorganism e.g., a bacterium, fungus, virus or protozoan as the active ingredient can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest. For example, there are fungi that control certain weeds, and other fungi that kill specific

insects. One bacterial species like *Bacillus thuringiensis* may be more effectiv on *Aedes aegypti* while one another *B. sphaericus* strain can be effective on a different types of mosquito like *Culex quinquefasciatus*.

Advantages of microbial insecticides

Individual products differ in important ways, but the following list of beneficial characteristics applies to microbial insecticides in general.

- The organisms used in microbial insecticides are essentially nontoxic and nonpathogenic to wildlife, humans, and other organisms not closely related to the target pest. The safety offered by microbial insecticides is their greatest strength.
- The toxic action of microbial insecticides is often specific to a single group or species of insects, and this specificity means that most microbial insecticides do not directly affect beneficial insects (including predators or parasites of pests) in treated areas.
- If necessary, most microbial insecticides can be used in conjunction with synthetic chemical insecticides because in most cases the microbial product is not deactivated or damaged by residues of conventional insecticides. (Follow label directions concerning any limitations.)
- Because their residues present no hazards to humans or other animals, microbial insecticides can be applied even when a crop is almost ready for harvest.
- In some cases, the pathogenic microorganisms can become established in a pest population or its habitat and provide control during subsequent pest generations or seasons.
- They also enhance the root and plant growth by way of encouraging the beneficial soil microflora. By this way they take a part in the increase of the crop yield.

Disadvantages of microbial insecticides

Naturally there are also the limitations which are listed below, but do not prevent the successful use of microbial insecticides. These factors just provide users to choose effective microbial products and take necessary steps to achieve successful results.

- Because a single microbial insecticide is toxic to only a specific species or group of insects, each application may control only a portion of the pests present in a field and garden. If other types of pests are present in the treated area, they will survive and may continue to

cause damage. Conventional insecticides are subject to similar limitations because they too are not equally effective against all pests. This is because of selectivity indeed and this negative aspect is often more noticeable for both general predators, chemicals and microbials. On the other hand predators and chemicals may be danger for other beneficial insects in threatened area.

- Heat, desiccation (drying out), or exposure to ultraviolet radiation reduces the effectiveness of several types of microbial insecticides. Consequently, proper timing and application procedures are especially important for some products.
- Special formulation and storage procedures are necessary for some microbial pesticides. Although these procedures may complicate the production and distribution of certain products, storage requirements do not seriously limit the handling of microbial insecticides that are widely available. (Store all pesticides, including microbial insecticides, according to label directions.)
- Because several microbial insecticides are pest-specific, the potential market for these products may be limited. Their development, registration, and production costs cannot be spread over a wide range of pest control sales. Consequently, some products are not widely available or are relatively expensive (several insect viruses, for example).

PATHOGEN	**PRODUCT NAME**	**HOST RANGE**	**USES AND COMMENTS**
BACTERIA			
***Bacillus thuringiensis* var. *kurstaki* (*Bt*)**	Bactur®, Bactospeine®, Bioworm®, Caterpillar Killer®,	caterpillars (larvae of moths and butterflies)	Effective for foliage-feeding caterpillars (and Indian meal moth in stored grain). Deactivated rapidly in sunlight; apply in the evening or on overcast days and direct some spray to lower surfaces

	Dipel®, Futura®, Javelin®, SOK-Bt®, Thuricide®, Topside®, Tribactur®, Worthy Attack®		or leaves. Does not cycle extensively in the environment.
***Bacillus thuringiensis* var. israelensis (*Bt*)**	Aquabee®, Bactimos®, Gnatrol®, LarvX®, Mosquito Attack®, Skeetal®, Teknar®, Vectobac®	larvae of *Aedes* and *Psorophora* mosquitoes, black flies, and fungus gnats	Effective against larvae only. Active only if ingested. *Culex* and *Anopheles* mosquitoes are not controlled at normal application rates.. Does not cycle extensively in the environment.
Bacillus thuringiensis* var. *tenebrinos	Foil® M-One® M-Track®, Novardo® Trident®	larvae of Colorado potato beetle, elm leaf beetle adults	Effective against Colorado potato beetle larvae and the elm leaf beetle. Like other *Bt*s, it must be ingested. It is subject to breakdown in ultraviolet light and does not cycle extensively in the

			environment.
Bacillus thuringiensis* var. *aizawai	Certan®	wax moth caterpillars	Used only for control of was moth infestations in honeybee hives.
Bacillus popilliae* and *Bacillus lentimorbus	Doom¨, Japidemic¨,® Milky Spore Disease, Grub Attack®	larvae (grubs) of Japanese beetle	The main Illinois lawn grub (the annual white grub, *Cyclocephala* sp.) Is NOT susceptible to milky spore disease.
Bacillus sphaericus	Vectolex CG®, Vectolex WDG®	larvae of *Culex, Psorophora,* and *Culiseta* mosquitos, larvae of some *Aedes* spp.	Active only if ingested, for use against *Culex, Psorophora,* and *Culiseta* species; also effective against *Aedes vexans.* Remains effective in stagnant or turbid water
FUNGI			
Beauveria bassiana	Botanigard®, Mycotrol®, Naturalis®	aphids, fungus gnats, mealy bugs, mites, thrips, whiteflies	Effective against several pests. High moisture requirements, lack of storage longevity, and competition with other soil microorganisms are problems that remain to be solved.
Lagenidium giganteum	Laginex®	larvae of most pest mosquito species	Effective against larvae of most pest mosquito species; remains infective in the environment through dry periods. A main drawback is its inability to survive high

			summertime temperatures.
PROTOZOA			
Nosema locustae	NOLO Bait®, Grasshopper Attack®	European cornborer caterpillars, grasshoppers and mormon crickets	Useful for rangeland grasshopper control. Active only if ingested. Not recommended for use on a small scale, such as backyard gardens, because the disease is slow acting and grasshoppers are very mobile. Also effective against caterpillars.
VIRUSES			
Gypsy moth nuclear plyhedrosis (NPV)	Gypchek ® virus	gypsy moth caterpillars	All of the viral insecticides used for control of forest pests are produced and used exclusively by the U.S. Forest Service.
Tussock moth NPV	TM Biocontrol-1®	tussock moth caterpillars	
Pine sawfly NPV	Neochek-S®	pine sawfly larvae	
Codling moth granulosis virus (GV)	(see comments)	codling moth caterpillars	Commercially produced and marketed briefly, but no longer registered or available. Future re-registration is possible. Subject to rapid breakdown in ultraviolet light.
ENTOMOGENOUS NEMATODES			
Steinernema	Biosafe®,	larvae of a wide variety of	*Steinernema riobravis* is the

***feltiae* (=*Neoaplectana carpocapsae*) *S. riobravis*, *S. carpocapsae* and other *Steinernema* species**	Ecomask ®, Scanmask ®, also sold generically (wholesale and retail), Vector®	soil-dwelling and boring insects	main nematode species marketed retail in the U.S. Because of moisture requirements, it is effective primarily against insects in moist soils or inside plant tissues. Prolonged storage or extreme temperatures before use may kill or debilitate the nematodes.
Heterorhabditis heliothidis	currently available on a wholesale basis for large scale operations	larvae of a wide variety of soil-dwelling and boring insects	Not commonly available by retail in the U.S.; this species is used more extensively in Europe. Available by wholesale or special order for research or large-scale commercial uses.
PATHOGEN			
Steinernema scapterisci	Nematac ®S	late nymph and adult stages of mole crickets	*S. scapterisci* is the main nematode species marketed to target the tawny and southern mole cricket. Best applied where irrigation is available. Irrigate after application.

Table 1.

Microbial Insecticides: A summary of products and their uses.

(Agricultural Entomology, University of Illinois at Urbana-Champaign. ENY-275 IN081)

Entomopathogenic fungi

Entomopathogenic fungi are important natural regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture. These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins, and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses. Entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application. The use of fungal entomopathogens as alternative to insecticide or combined application of insecticide with fungal entomopathogens could be very useful for insecticide resistant management.

The commercial mycoinsecticide 'Boverin' based on *B. bassiana* with reduced doses of trichlorophon have been used to suppress the second-generation outbreaks of *Cydia pomonella* L. Anderson *et al.* (1989) detected higher insect mortality when *B. bassiana* and sublethal concentrations of insecticides were applied to control Colorado potato beetle (*Leptinotarsa decemlineata*), attributing higher rates of synergism between two agents.

The use of the insect-pathogenic fungus *Metarhizium anisopliae* against adult *Aedes aegypti* and *Aedes albopictus* mosquitoes has also been reportedThe life span of fungus-contaminated mosquitoes of both species was significantly reduced compared to uninfected mosquitoes. The results indicated that both mosquito species are highly susceptible to infection with this entomopathogen.

Viral pesticides

There are more than 1600 different viruses which infect 1100 species of insects and mites. A special group of viruses, called baculovirus, to which about 100 insect species are susceptible, accounts for more than 10 percent of all insect pathogenic viruses. Baculoviruses are rod-shaped particles which contain DNA. Most viruses are enclosed in a protein coat to make up a virus inclusion body. Alkaline condition of insect's midgut dissolves the protein covering and the viral particles are released from the inclusion body. These particles fuse with the midgut epithelial cells, multiply rapidly and eventually kill the host. But, viral pesticides are more expensive than chemical agents. Furthermore, many baculoviruses are host specific. Therefore they cannot be used to control several different pests. The action of baculoviruses on insect larvae is too slow to satisfy farmers. These viral preparations are not stable under the ultraviolet rays of the sun. Efforts are being made to encapsulate baculoviruses with UV protectants to ensure a longer field-life.

NPVs and GVs are used as pesticides but the group based on nucleopolyhedrosis viruses is much larger. The first viral insecticide Elcar™ was introduced by Sandoz Inc. in 1975Elcar™ was a preparation of *Heliothis zea* NPV which is relatively broad range baculovirus and infects many species belonging to genera *Helicoverpa* and *Heliothis*. HzSNPV provided control of not only cotton bollworm, but also of pests belonging to these genera attacking soybean, sorghum, maize, tomato and beans. In 1982 Sandoz decided to discontinue the production. The resistance to many chemical insecticides including pyrethroids revived the interest in HzSNPV and the same virus was registered under the name GemStar™. HzSNPV is a product of choice for biocontrol of *Helicoverpa armigera*]. Countries with large areas of such crops like cotton, pigeonpea, tomato, pepper and maize, e.g. India and China, introduced special programs for the reduction of this pest by biological means. In Central India, *H.armigera* in the past was usually removed by shaking pigeonpea.

The well-known success of employing baculovirus as a biopesticide is the case of *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) used to control the velvetbeen caterpillar in soybean. In the early eighties this program was performed in Brazil. Since then, over 2,000,000 ha of soybean have been treated with the virus annually. Recently, after many new emerging pests in the soybean, this number dropped down. Although the use of this virus in Brazil is the most impressive example of viral bioregulation worldwide, the virus is still obtained by *in vivo* production mainly by infection of larvae in soybean farms. The demand for virus production has increased tremendously for protection of four million hectares of soybean annually. Because large scale *in vivo* production of baculoviruses encounters many difficulties the high demand for AgMNPV require studies dealing with inexpensive *in vitro* production of the virus. The use of AgMNPV in Brazil brought about many economical, ecological and social benefits. On the basis of this spectacular success of a baculovirus pesticide, it is needless to say that the advantages of biopesticides over chemical pesticides are numerous.

Protozoa

Protozoan pathogens naturally infect a wide range of insect hosts. Although these pathogens can kill their insect hosts, many are more important for their chronic, debilitating effects. One important and common consequence of protozoan infection is a reduction in the number of offspring produced by infected insects. Although protozoan pathogens play a significant role in the natural

limitation of insect populations, few appear to be suited for development as insecticides.

As an other example, the Microsporidia include species promising for biological control. Microsporidian infections in insects are thought to be common and responsible for naturally occurring low to moderate insect mortality. But these are indeed slow acting organisms, taking days or weeks to make harm their host. Frequently they reduce host reproduction or feeding rather than killing the pest outright. Microsporidia often infect a wide range of insects. Some microsporidia are being investigated as microbial insecticides, and at least one is available commercially, but the technology is new and work is needed to perfect the use of these organisms.

Microscopic nematods

To be accurate, nematodes are not microbial agents. Instead, they are multicellular roundworms. Nematodes used in insecticidal products are, however, nearly microscopic in size, and they are used much like the truly microbial products discussed previously. Nematodes are simple roundworms. Colorless, unsegmented, and lacking appendages, nematodes may be free-living, predaceous, or parasitic. Many of the parasitic species cause important diseases of plants, animals, and humans. Other species are beneficial in attacking insect pests, mostly sterilizing or otherwise debilitating their hosts. A very few cause insect death but these species tend to be difficult (e.g., tetradomatids) or expensive (e.g. mermithids) to mass produce, have narrow host specificity against pests of minor economic importance, possess modest virulence (e.g., sphaeruliids) or are otherwise poorly suited to exploit for pest control purposes. The only insect-parasitic nematodes possessing an optimal balance of biological control attributes are entomopathogenic or insecticidal nematodes in the genera *Steinernema* and *Heterorhabditis*Nematodes used for insect control infect only insects or related arthropods; they are called entomogenous nematodes.

The entomogenous nematodes *Steinernema feltiae* (sometimes identified as *Neoaplectana carpocapsae*), *S. scapteriscae*, *S. riobravis*, *S. carpocapsae* and *Heterorhabditis heliothidis* are the species most commonly used in insecticidal preparations. Within each of these species, different strains exhibit differences in their abilities to infect and kill specific insects. In general, however, these nematodes infect a wide range of insects. On a worldwide basis, laboratory or field applications have been effective against over 400 pest species, including numerous beetles, fly larvae, and caterpillars.

The infectious stage of these nematodes is the third juvenile stage often referred to as the J3 stage or the "dauer" larvae. Nematodes in this stage survive without feeding in moist soil and similar habitats, sometimes for extended periods. *Steinernema* species infect host insects by entering through body openings--the mouth, anus, and spiracles (breathing pores). *Heterorhabditis* juveniles also enter host insects through body openings, and in some instances are also able to penetrate an insect's cuticle. If the environment is warm and moist, these nematodes complete their life cycle within the infected insect. Infective juveniles molt to form adults, and these adults produce a new generation withing the same host. As the offspring mature to the J3 stage, they are able to leave the dead insect and seek a new host.

Nosema locustae has been used to reduce grasshopper populations in rangeland areas, and adequate control has been achieved when treatments were applied to large areas while hoppers were still young. Although not all grasshoppers in the treated area are killed by *Nosema locustae,* infected hoppers consume less forage and infected females produce fewer viable eggs than do uninfected females. *Nosema locustae* persists on egg pods to provide varying degrees of infection the following season. The effectiveness and utilization of *Nosema locustae* for rangeland grasshopper control are likely to increase as research continues. This single-celled protozoan infects and kills over 90 species of grasshoppers, locusts, and some species of crickets. Nosema Iocustae is non-toxic to humans, livestock, wild animals, birds, fish, and pets. Should be applied early in the season as over-wintering hoppers emerge. Unfortunately, small, one-pound packages of *Nosema locustae* preparations developed for sale to gardeners and homeowners offer much less utility or none. The mobility of grasshoppers, coupled with the fact that infected hoppers are not killed until a few weeks after they ingest the pathogen, means that application of baits containing *Nosema locustae* to individual lawns or gardens is unlikely to reduce grasshopper densities or damage substantially.

Bacterial biopesticides

Bacterial biopesticides are the most common and cheaper form of microbial pesticides. As an insecticide they are generally specific to individual species of moths and butterflies, as well as species of beetles, flies and mosquitoes. To be effective they must come into contact with the target pest, and may require ingestion to be effective. Bacteria in biological pesticides survive longer in the open than previously believed. Bacterial pathogens used for insect control are spore-forming,

rod-shaped bacteria in the genus *Bacillus*. They occur commonly in soils, and most insecticidal strains have been isolated from soil samples. The *Bacillus* genus encompasses a large genetic biodiversity. *Bacilli* are present in an extremely large area of environments ranging from sea water to soil, and are even found in extreme environments like hot springs. This bacterium could be one of the major sources of potential microbial biopesticides because it retains several valuable traits.

First of all, *Bacilli*, like *B. subtilis*, are well-studied organisms. Secondly, the US Food and Drug Administration (USFDA) has granted the "generally regarded as safe" (GRAS) status to *Bacillus subtilis* which is thus recognized non-pathogenic. This is of course essential with respect to its application as a biopesticide. Thirdly, *Bacilli* have the capacity to produce spores which are extremely resistant dormancy forms capable to withstand high temperatures, unfavorable pH, lack of nutrients or water, etc. They are produced by the bacteria when environmental conditions are unfavorable which probably helps these microorganisms to survive in the phytosphere. The phenomenon can also be exploited in industrial production as sporulation can be induced at the end of cultures. course essential regarding its application as a biopesticide.

Bacterial insecticides must be eaten to be effective; they are not contact poisons. Insecticidal products comprised of a single *Bacillus* species may be active against an entire order of insects, or they may be effective against only one or a few species.

***Bacillus thuringiensis*, BT**

Bacillus thuringiensis (Bt) is an aerobic, gram positive, spore forming soil bacterium that shows unusual ability to produce endogenous different kinds of crystals protein inclusions during its sporulation. *B. thuringiensis* (commonly known as 'Bt') is an insecticidal bacterium, marketed worldwide for control of many important plant pests - mainly caterpillars of the Lepidoptera (butterflies and moths) but also mosquito larvae, and simuliid blackflies that vector river blindness in Africa. The commercial Bt products are powders containing a mixture of dried spores and toxin crystals. They are applied to leaves or other environments where the insect larvae feed. The toxin genes have also been genetically engineered into several crop plants. The method of use, mode of action, and host range of this biocontrol agent may differ within other Bacillus insecticidal species.

The *Bacillus* species, *Bacillus thuringiensis,* has developed many molecular mechanisms to produce pesticidal toxins; most of toxins are coded for by several *cry* genes. Since its discovery in

1901 as a microbial insecticide, *Bacillus thuringiensis* has been widely used to control insect pests important in agriculture, forestry and medicine. Its principal characteristic is the synthesis of a crystalline inclusion during sporulation, containing proteins known as endotoxins or Cry proteins, which have insecticidal properties. The crystal protein inclusions are composed of one or more crystal (Cry) and cytolytic (Cyt) toxins which are also called δ-endotoxins or insecticidal crystal proteins. Some of these proteins are highly toxic to certain insects but they are harmless to most other organisms including vertebrates and beneficial insect. Since their insecticidal potential has been discovered, it has been produced commercially and accepted as a source of environment friendly biopesticide all over the world.

There are different strains of *B. thuringiensis*. Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. While some Bt's control moth larvae found on plants, other Bt's are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bt produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.The most widely used strains of *B. thuringiensis* have started against three genera of mosquitos; *Culex*, *Culiseta* and *Aedes*[

Their study has shown that Bt spores can survive both on the ground and in animals. What's more, wind, rain and animals can carry them to neighbouring areas. In the splashing rain drops they can even "hop" from the ground up onto leaves - another means of transport. Bt bacteria are also known to be able to easily transfer their toxicity genes to other bacteria in the application area.

When the bacteria were sprayed on cabbage plants, and they were found to have killed all the cabbage white butterfly larvae. In addition, though, the field study revealed that the bacteria are able to survive for a considerable time. After spraying, by far the majority of the spores were found to be present in the upper two centimetres of the soil, *National Environmental Research Institute of Denmark*. "Their toxic effects disappeared after a few days, but half of the bacteria were still surviving as spores 120 days later, and one fifth were still alive after a year. They existed in a dormant state, however, and did not produce toxins, although the spores are able to germinate later and produce insecticide again," explain microbiologists Bjarne Munk Hansen and Jens Chr. Pedersen of the National Environmental Research Institute. Until now it has generally been believed that the majority of Bt bacteria disappear rapidly after they have been sprayed. "It was thought that when the toxic effect disappeared, the bacteria had also disappeared. What in fact

happens, though, is that the bacteria convert to a dormant stage and become spores," continue the two scientists.

In the present era of transgenic technology, insecticidal toxins of *Bacillus thuringiensis* (Bt) assume considerable significance in the production of insect resistant crops such as cotton, maize, potato, rice etc. This review also describes about biology of Bt toxin, recent progress in the development of Bt technology, evolution of resistant insect populations against Bt and management strategy.

Different domains involved in the toxicity of B.thuringiensis toxin in the mid-gut of targeted insect. Source:Sharma et al., 2000. Bt bacteria are used by farmers, foresters and gardeners to destroy butterfly larvae, mosquito larvae and beetles. The Danish field study, which was undertaken in 1993 and 1994, is one of the first in the world where plants have been systematically sprayed with Bacillus thuringiensis bacteria, and where the research has been ecologically oriented. In contrast, the numerous field studies undertaken by the producers of biological pesticides have been oriented to commercial considerations.

Scientists Per Damgaard and Jørgen Eilenberg at the Royal Agricultural University in Denmark, have also observed examples of spores germinating in living but weakened flies. The flies were already suffering from a severe fungal infection of the lower abdomen, and it was exactly there that the spores germinated. They showed that, the bacterial spores germinate well in dead insects, as the two scientists confirmed by feeding spore and toxin-treated food to larvae of the large cabbage white butterfly.

Under good growth conditions a spore can produce up to a thousand million new spores in a single insect larva.

"There are no previous examples of the spores reproducing in living organisms, although they appear to be able to do so in dead flies. The advantage for the bacterium is that the spores can be spread when the fly moves around," continue Bjarne Munk Hansen and Jens Chr. Pedersen.

Gene	**Target pest**	**References**
Cry 1A(b)	Striped stem borer and leaf folder	Fujimoto et al. (1993)

Cry 1A(b)	Yellow stem borer and striped stem borer	Wunn et al. (1996)
Cry 1A(b)	Yellow stem borer and striped stem borer	Ghareyazie et al. (1997)
Cry 1A(b)	Yellow stem borer	Datta et al. (2002)
Cry 1A(b)	Yellow stem borer	Alam et al. (1999)
Cry 1A(b)/ Cry 1A(c)	Leaffolder and yellow stem borer	Tu et al. (2000)
Cry 1A(b)/ Cry 1A(c)	Yellow stem borer	Ramesh et al. (2004)
Cry 1A(c)	Yellow stem borer	Nayak et al. (1997)
Cry 1A(c)	Yellow stem borer	Khanna and Raina (2002)
Cry 1A(c)	Striped stem borer	Liu et al. (2002)
Cry 2A	Leaffolder and yellow stem borer	Maqbool et al. (1998)
Cry 2A/ Cry 1A(c)	Leaffolder and yellow stem borer	Maqbool et al. (2001)
Cry 1Ie	Corn borer	Liu et al., 2004

Table 2.

Successful examples to show *B. thuringiensis* genes (originated from *Bacillus thuringiensis*) integration for insect resistance in rice.

Insects can be infected with many species of bacteria but those belonging to the genus Bacillus, as already mentioned, are most widely used as pesticides. *Bacillus thuringiensis* has developed many molecular mechanisms to produce called cry genes. Since its discovery in 1901 over one hundred *B. thuringiensis*-based bioinsecticides have been developed, which are mostly used against lepidopteran, dipteran and coleopteran larvae. In addition, the genes that code for the insecticidal crystal proteins have been successfully transferred into different crops plants by means of transgenic technology which has led to significant economic benefits. Because of their high specificity and their safety in the environment, *B. thuringiensis* and Cry protein toxins are efficient, safe and sustainable alternatives to chemical pesticides for the control of insect pests. The toxicity of the Cry proteins have traditionally been explained by the formation of transmembrane pores or ion channels that lead to osmotic cell lysis. In addition to this, Cry toxin monomers also seem to promote cell death in insect cells through a mechanism involving an adenylyl cyclase/PKA signalling pathway. However, despite this entomopathogenic potential, controversy has arisen regarding the pathogenic lifestyle of *B. thuringiensis*. Recent reports claim that *B. thuringiensis* requires the co-operation of commensal bacteria within the insect gut to be fully pathogenic.

The first developed *Bacillus thuringiensis* insecticidal agent is a mixture of *Bacillus thuringiensis* spores and its toxin. As a pesticide, (BT*)* accounts for over 90 percent of total share of today's bioinsecticide market and has been used as biopesticide for several decades. The discovery of the strain *B. thuringiensis* serovar *israelensis* made possible efficient microbiological control of Diptera Nematocera vectors of diseases, such as mosquitoes (Culicidae) and black flies.

In most countries of the world, products are available for control of caterpillars (var. *kurstaki*, *entomocidus*, *galleriae* and *aizawai*), mosquito and blackfly larvae (var. *israeliensis*) and beetle larvae (var. *tenebrionis*). Actively growing cells lack the crystalline inclusions and thus are not toxic to insects. The BT preparations remain stable without any disentegration over years even in the presence of UV sun rays. As the insect feeds on the foliage, the crystals too are eaten up. These are hydrolysed in the insect's midgut to produce an active endotoxin. The active toxin binds to receptor sites on gut epithelial cells and creates imbalance in the ionic make-up of the cell. This is seen by swelling and bursting of the cells due to osmotic shock. Subsequent symptoms are paralysis of the insect's mouthparts and gut. So obviously the feeding process is inhibited.

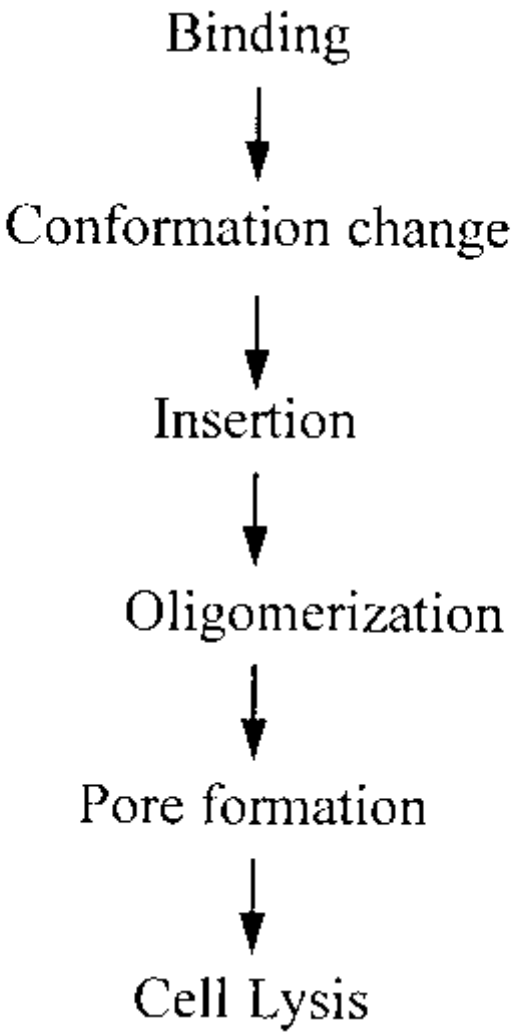

Figure 3: Characterization of the steps require for formation of pores in cell membranes

Also, a relatively new mechanism of action of Cry toxins have been proposed which involves the activation of Mg2+-dependent signal cascade pathway that is triggered by the interaction of the monomeric 3-domain Cry toxin with the primary receptor, the cadherin protein BT-R1. The triggering of the Mg2+-dependent pathway has a knock-on effect and initiates a series of cytological events that include membrane blebbing, appearance of nuclear ghosts, and cell swelling followed by cell lysis. The Mg2+-dependent signal cascade pathway activation by Cry toxins have been shown to be analogous to similar effect imposed by other pore forming toxins on their host cells when they are applied at subnanomolar concentration.

Though the two mechanisms of action seem to differ, with series of downstream events following on from toxin binding to receptors on target cell membranes, there is a degree of commonality in that initially the crystals have to be solubilised in vivo or in vitro, and activated by proteases before

and/or after binding to receptors such as cadherin.