

Preface

Since its discovery about a century ago, *Bacillus thuringiensis* (Bt) has been used as a biopesticide in agriculture, forestry and mosquito control because of its advantages of specific toxicity against target insects, lack of polluting residues and safety to non-target organisms. Today Bt is the most successful commercial microbial insecticide, comprising about 90% of the biopesticide market. The insecticidal properties of this bacterium are due to the presence of insecticidal proteins, also called crystals, usually produced during sporulation. The new tools of biotechnology are changing the way scientists can address problems in the agriculture. Transgenic technology, involving a wide range of pesticidal genes from Bt, dominates the scenario of agricultural biotechnology. At the same time, Bt technology is also the most vehemently criticized area of agricultural biotechnology. Genetic improvement of Bt strains for the development of novel biopesticides entails increasing their potency against target insects, broadening the insecticidal spectra for specific crop applications, improving persistence on plants, and optimizing fermentation production.

On the other hand, Bt biotechnology comprises other aspects different from insecticidal proteins that has become an interesting topic of research such as secondary metabolites or enzymes with many applications in biotechnology which are deeply discussed in the last part of this book.

In this book, I have pretended to gather a great team of experts to bring together all recent studies regarding both fundamental and more applied research aspects related to biotechnology of Bt. The nineteen different chapters, written by the leading researchers in the field, give us a tour of the whole story of Bt biotechnology since its discovery and comprehensively update the entire subject.

- Part 1: *Bacillus thuringiensis*: an environmentally safety alternative

The first five chapters provide a general overview of *bacillus thuringiensis* as biopesticide describing what is Bt, how the mode of action of this bacterium is, how its use today in the market is, and giving an idea about how protein engineering is used to improve the efficacy of the toxins.

In the first chapter Sansinenea (pp. 3–18) presents a detailed history of the discovery of this bacterium and gives a brief description about Bt and its toxins.

In the second chapter Crickmore and George (pp. 19–39) continue nicely the description of the toxins of Bt and its mode of action and then explain the types of Bt products and their advantages over chemicals, disadvantages (narrow spectrum, low activity), the resistance as a threat to continue use of Bt, the use of synergists to improve activity, the use of genetic manipulation to improve activity and finally give a brief description of gene stacking.

In the third chapter Kaur (pp. 41–85) extensively and in depth describes the risk assessment of Bt transgenic crops from various aspects: the risk in non-target organisms, persistence of Bt in soil, risk analysis of Bt transgenic crops including studies in different countries to finish with conclusions and future prospect.

In the fourth chapter Abdullah (pp. 87–92) describes what are biopesticides and their market detailing the use and efficacy of *bacillus thuringiensis* as a biopesticide in integrated pest management.

To finish this first part of the book, in the fifth chapter Florez et al. (pp. 93–113) revise Cry proteins domains and analyze in an elegant way several protein modifications that have been successfully used for creating stable, functional proteins with minimal structural alterations. The understanding and proper use of protein engineering approaches may help in implementing appropriate pest management strategies by improving the efficacy of these toxins against insect pests.

- Part 2: Genetics of *Bacillus thuringiensis*

Due to the great importance that the genetic of Bt has had in biotechnological advances, this part is the longest and most extensive in the book and is composed of seven chapters. This part will provide a general view of the genetics of Bt from the beginning until today explaining all the genetic tools available for the improvement of the Bt strains.

To start this part, in the sixth chapter Økstad and Kolstø (pp. 117–129) describe with their great experience, the evolution of the *bacillus cereus* group, describing the main factors that differentiate *B. cereus*, *B. anthracis* and *B. thuringiensis*. This description provides a wide view of the characteristics of the *B. cereus* group including the sequencing of the whole genomes of Bt for the engineering of commercial *B. thuringiensis* strains with increased safety to humans.

In the seventh chapter, Sorokin (pp. 131–157) introduces in a nice way the genetics of the *B. cereus* group reviewing phage-mediated gene transduction and recombineering perspectives for the *B. cereus* group. In combination with new generation sequencing these approaches will constitute the gene identification methodologies in the post-genomics time.

In the eighth chapter, Vilas-Bóas and Santos (pp. 159–174) interestingly describe the studies focusing on conjugative transfer in *Bacillus thuringiensis*, involving the detection of *cry* genes in large conjugative plasmids, the genetic basis of the process, the main plasmids, and methodological variations of mating systems.

Continuing with a logical sequence of the book in the ninth chapter, Ochoa-Zarzosa and Lopez-Meza (pp. 175–184) describe the shuttle vectors of *B. thuringiensis* that have been constructed using essentially replicons from resident plasmids from this bacterium that replicate by the theta mechanism and also, the vectors that have been developed using plasmid replicons from other Gram-pos-

itive bacteria or RCR plasmids. The development of shuttle vectors with better characteristics and protocols with high transformation efficiency have greatly facilitated basic research and engineering of *B. thuringiensis*.

Following with the topic of the vectors, in the tenth chapter Xu et al. (pp. 185–199) take up the subject in great depth and provide a deep overview of current research and applications of *B. cereus* group plasmid vectors and prospects for further development, describing shuttle vectors, integration vectors both homologous recombination and transposons vectors, resolution vectors and expression vectors. In this way through two chapters the topic of vectors is explained at length to get started with recombination chapter.

In the eleventh chapter Mesrati and Tounsi (pp. 201–214) present the genetic recombination in *Bacillus thuringiensis*. In the first part they describe the site specific recombination, including transposition by transposons and transduction by phage, in this bacterium and its exploitation in the construction of recombinant strains of *B. thuringiensis* improving their production as bioinsecticides and their insecticidal activities and *B. thuringiensis* mutagenesis. In the second part they describe the homologous recombination and its role in the construction of improved *B. thuringiensis* strains and in gene disruption.

To finish this second part of the book, in the twelfth chapter Sanchis (pp. 215–228) describes how recombinant DNA technology has been used to improve *Bacillus thuringiensis* (Bt) products and overcome a number of the problems associated with Bt-based insect control measures giving several examples describing how biotechnology has been used to increase the production of insecticidal proteins in Bt.

- Part 3: Bt as biopesticide: Applications in biotechnology

Once explained the second part of the book, begins the third part, which is composed of four chapters, and provides a detailed view of the applications of Bt in biotechnology until today including many details about Bt crops, such as food safety.

Continuing with the previous chapter and to start this part of the book, in the thirteenth chapter Li and Yu (pp. 231–258), firstly, in an elegant way, realize a very interesting and detailed summary of some chapters of the previous part that are important to this topic such as vectors, recipient strains and methods for constructing genetically modified Bt strains, to enter with great vision on the topic of genetically modified Bt strains viewed from different points such as broader insecticidal spectrum, high insecticidal activity, multifunctional activity and delayed pests resistance.

In the chapter fourteen Schnepf (pp. 259–281) retakes the recipient strains topic and describes in depth the insecticidal protein expression in gram-negative hosts, in Bt and in plants leading to biotechnological applications. Notable successes will be mentioned, however, more time will be spent on modifications that incrementally improve high production levels, unresolved issues with low-expressing proteins, or maintaining functionality of the expression host.

In the chapter fifteen Castagnola and Jurat-Fuentes (pp. 283–304) discuss key events in the history of Bt crop development and summarize current regulations

aimed at reducing the risks associated with increased adoption of this technology. By analyzing the history of Bt transgenic crops and the current marketplace trends and issues, they examine the outlook of current and impending Bt crops as well as potential issues that may emerge during their future use.

To finish this part an interesting sixteenth chapter is presented by Hammond (pp. 305–325) who relates a history of safe consumption of Cry proteins from use of Bt microbial pesticides on vegetable food crops, and summarizes the published literature addressing the safety of Cry insect control proteins found in both Bt microbial pesticides and those introduced into Bt agricultural crops. A discussion on the species-specific mode of action of Cry proteins to control target insect pests is presented. A human dietary exposure assessment for Cry proteins has also been provided. Lastly the food and feed safety benefits of Bt crops are briefly summarized including lower insecticide use and reduction in fumonisin mycotoxin contamination of grain.

- Part 4: Other *Bacillus* species in biotechnology

Finally this last part is composed of three chapters and provides a view about the all *bacillus* species that have biotechnological importance, and gives a new vision of the modern biotechnology that leads to discover new secondary metabolites of great importance in biotechnology.

To start this part of the book, in the seventeenth chapter Raddadi et al. (pp. 329–345) give an interesting survey on the most important biotechnological processes that have as effectors bacterial species in the genus *Bacillus*. Before highlighting the main biotechnological applications in which these species have been implicated, they briefly introduce the taxonomy, the ecology, the evolution and the natural variation that characterizes *Bacillus* genus.

In the eighteenth chapter Chaabouni et al. (pp. 347–366) summarize the most important secondary metabolites produced by members of genus *Bacillus*. Bacteriocins of *B. subtilis* and *B. thuringiensis* and other metabolites (as zwittermixin A, siderophore, surfactin and others) are described and the potential application of antimicrobial peptides in food, agriculture and pharmaceutical industries are discussed. This biotechnological potential will be highlighted and the safety evaluation of the metabolites and the species of the producer will be discussed.

To finish this last part of the book, in the nineteenth chapter Barboza-Corona et al. (pp. 367–384) review in depth the different kinds of chitinases that are synthesized by *B. thuringiensis*, their roles in nature, and their application in environment, agriculture and food industry. Additionally they, analyze bacteriocins of *B. thuringiensis* reported to date, how to enhance their production, and the methods for screening the bacteriocin activity. Finally, the future challenges and prospects of the antimicrobial peptides as biopreservatives, antibiotics, and nodulation factors are presented.

The biotechnological applications of Bt as biopesticide have increased in the last ten years and a new way of expanding the biotechnology of Bt has started. The challenge is great and there is still a need for research in different areas. I hope that this book will help to continue expanding the knowledge of Bt applied to biotechnology.

Finally the book is ready. From these lines, I would like to thank firstly Mr Max Haring from Springer SBM BV Publishing Editor who invited me to this interesting and exciting project and Mrs Marlies Vlot senior assistant for her very useful assistance in this project.

With a special mention, the editor gratefully acknowledges all the authors involved in this project, for their enthusiasm and their courage, for contributing chapters on the subjects of their expertise and giving so much of their time to make this book possible. I am deeply grateful for their generous and collegial spirit and their willingness to match their contributions to the view of the book and for all aspects of this adventure that, at the end, have crystallized in this book. For me, as Editor of this book, it has been really a privilege to interact with such a collection of scientists and present this useful work. I am very happy to have had the opportunity to contact and gather a great team of experts to bring together all recent studies related to biotechnology of Bt that I am completely sure will made this book an exit.



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