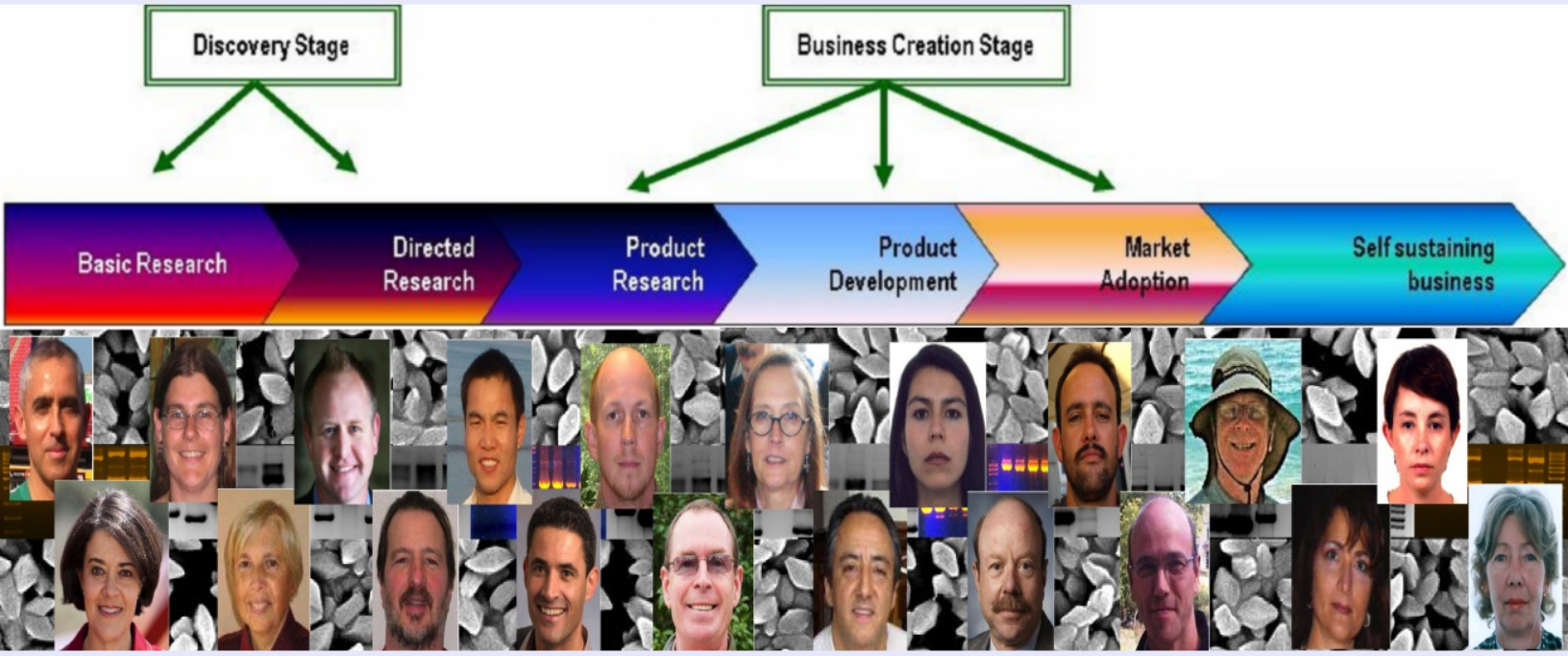
Number 2 Issue 1 February 2012

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY
and Covering Its Many Hues, Tints, Tones & Shades



Special issue:
Abstracts to the sessions of the Biotechnological Summit 2012

March, 12th to 21th of 2012.

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The International Biotechnology Color Journal appears every 4 months in the last week of February, June and October of each year, starting in October 2011. It is a non-profitable Electronic Publication. In its initial phase it does not requires neither publication nor access fees. Its goal is the publication of Original Scientific Information, previously unpublished, and provided by the contributing authors out of its own will. *Int. biotechnol. color j., ISSN 2226-0404.*

The INTERNATIONAL BIOTECHNOLOGY COLOR JOURNAL (IBCJ)

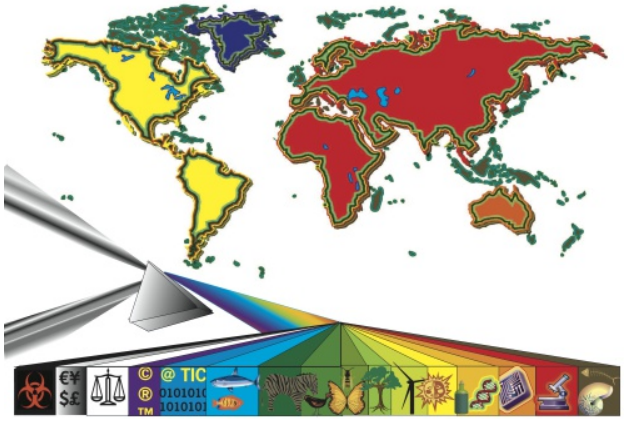
is the official quarterly publication of the **International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C.** (IFBR&ESCHNSAST&S).

The IFBR&ESCHNSAST&S is a civil association and nonprofit organization.

Its main goal is to help bringing together efficient biotechnological solutions for many human problems, with the need for environmentally friendly and sustainable processes.

As means to such goal, the Foundation counts on education to create social awareness to biotechnology's benefits and risks, and to promote the formation of highly qualified professionals and research scientists.

The constitutive act of the foundation was signed in the Heroic City of *Huajuapan de León, Oaxaca*, on September 14th, 2009, at the *NOTARÍA PÚBLICA No. 61 de los ESTADOS UNIDOS MEXICANOS*. As .



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Susana Lozano Muñiz
President of the Foundation

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Director: Rogelio Rodríguez Sotres, Faculty of Chemistry, Universidad Nacional Autónoma de México. Cd. Universitaria. Ave. Universidad 3000, C.P. 04510, Coyoacán, D.F. México. E-mail: sotres@servidor.unam.mx
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Editorial section

International Biotechnology Color Journal (IBCJ) is an electronic Open Access journal, devoted to rapidly publishing full peer-reviewed articles covering all the fields of biotechnology. Though the central focus of IBCJ is to publish scientific papers, it provides a forum for reviews of special interest, notes presenting relevant findings in a short format, essays with new technical advances or relevant updates of reported protocols, book reviews, scientific meetings, and letters to the editor. Instructions for every type of contribution are presented in the journal's Homepage and in PDF format in the last issue of each year.

The Editorial Board of IBCJ is fully committed to publish articles innovating in all areas of biotechnology. Contributions are reviewed from a rigorous optic of scientific criticism; thus, any original contribution that fits within the scope of the journal and promotes the advancement of biotechnology are particularly welcome.

Editorial comments to the contents of this issue

By José Juan Zúñiga-Aguilar, Chief editor.

In this special issue of the International Biotechnology Color Journal, we are particularly glad to present the abstracts to the sessions of the Biotechnology Summit 2012, held in the City of Merida, Yucatan, from March 12th to 20th, 2012.

Merida, a beautiful and peaceful City, the heir of the ancient scientific tradition of the Mayan people, hosted the first meeting organized by the International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology and Society. Biotechnology Summit 2012 was a forum for scientists working in diverse research areas, offering a comprehensive image of the different colors of biotechnology.

Biotechnology Summit 2012 also presented the Bt Symposium. Crops expressing Bt defense protein comprise one of the two largest groups of transgenic crops cultivated worldwide, the other group include herbicide-resistant plants. Modern societies are in urgent need of genetically modified crops with enhanced defense traits, but fulfilling international regulations, in terms of safety and ecological sustainability. The Bt symposium covered different aspects related to regulation of BT crops, the evolution of Bt resistance in the field, and the discussion of laboratory and field assays to understand molecular basis of Bt resistance. Particularly enlightening were the discussions on high throughput strategies and the use of molecular monitors of the Bt toxins' actions, as well as the presentation of novel molecular strategies to enhance BT toxicity in the field.

Research in biotechnology continuously changes and evolves, and scientific meetings like the Biotechnology Summit 2012 are crucial to the discussion of new findings and to the design of new strategies. Scientific meetings of high standards, such as this, endow with synergy the contributions of all fields of biotechnology. The International Biotechnology Color Journal is fully committed to disseminate the science shared during these activities.

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ABSTRACTS TO PRESENTATIONS[1]

Biotechnology Summit 2012

The International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C. organized the Biotechnology Summit 2012 in collaboration with International Centre for Genetic Engineering and Biotechnology (ICGEB), American Society for Microbiology (ASM), Sociedad Mexicana de Biotecnología y Bioingeniería (SMBB) Yucatán delegation, Centro de Investigaciones y Estudios Avanzados del IPN (CINVESTAV-IPN), Universidad Autónoma Metropolitana Unidad Cuajimalpa (UAM-C), Universidad Autónoma de Nuevo León, Facultad de ciencias Biológicas (UANL FCB), Secretaría de Educación Pública (SEP), Consejo Nacional de Ciencia y Tecnología (CONACYT), Universidad Nacional Autónoma de México (UNAM), among others.

The Biotechnology Summit was held in Merida, Yucatan, on March 12 to 21, 2012.

(1) The abstracts presented here were not peer reviewed

Bt-crops regulation in Mexico: An industrial point of view

J. Angel Saavedra, Ph. D.

Dow AgroSciences, Ave. Patria 2085 Piso 4. Frac. Andares, Zapopan, Jal., Mexico 45116. Tel 33 3678 2452, e-mail jsaavedra@dow.com

Regulation in Mexico for Bt-crops, has evolved from one initial Standard (NOM-056-FITO-1995) to the creation of the Biosafety Law (LBOGM March 18, 2005), the Bylaw (Reglamento de LBOGM, March 19, 2008), and some special documents, such as The Special Regime for Maize protection (REPM March 6, 2009), and some Standards (NOMs) dictated by the LBOGM still under discussion. Agencies that regulate Bt crops have also evolved, initially the Minister of Agriculture was the key authority regulating GM crops, whereas today three Ministers are involved in dictating approvals: Agriculture and Environment for cultivation; and Health for food and feed. The Intersecretariat Commission for Biosafety of GMO (CIBIOGEM) was officially formed in 1999 with the mandate to regulate all activities concerning GMO. Under this apparently good regulatory scenario, industry and academia have struggled to understand the principles underlying the process and their case by case application. Additionally, the number of studies required, timing and costs associated with registering a product has been hurdles in the process. The interpretation of regulations and data requirements need to be clear, consistent and predictable. Harmonization with requirements in other geographies also creates efficiencies. Fortunately, Government agencies are willing to discuss with stakeholders ways to reach Bt crop approvals faster. All these aspects are discussed in the presentation.

A program for monitoring the susceptibility of target lepidopteran pests to Bt proteins in three countries in Latin America

Miguel S. Serrano

TD&S Central America & Caribbean, Monsanto. E-mail: miguel.serrano@monsanto.com

A program to establish line base susceptibility prior to release, and to monitor susceptibility of target species after commercial release of Bt technologies has been carried out in Mexico, Colombia and Honduras. The scope of the program has varied among countries depending on regulatory approvals of technologies in cotton and corn. In Mexico the program was established in cooperation with scientists from INIFAP, COLPOS, ITSON and Universidad Autónoma Estado de Mexico, in Colombia, in cooperation with CIAT, and Honduras with Escuela Agrícola Panamericana Zamorano. In cotton, susceptibility to Cry1Ac and/or Cry2Ab has been monitored during each planting season for *Pectinophora gossypiella, Helicoverpa zea*, *Heliothis virescens*, *Spodoptera exigua* y *S. frugiperda* (Mexico) and *Alabama argillacea, Sacadodes pyralis, H. virescens*, *H. zea* and *S. frugiperda* (Colombia). Bt corn is commercially approved in Colombia and Honduras (Cry1Ab, Cry1A.105 and Cry2Ab) and a monitoring program was also established for *Diatraea saccharalis, H. zea*, and *S. frugiperda* in both countries and *H. virescens* in Colombia. With the experimental approval of Bt corn in Mexico, baseline susceptibility studies were initiated for target species, *S. frugiperda*, *D. saccharalis*, *D. grandiosella*, and *H. zea* at Colpos, Texcoco, Mexico. Data suggest that none of the target species in the three countries shows a decrease in susceptibility to any of the proteins evaluated. Evaluations will continue during the time the technologies are available to farmers in the approved countries.

US corn and cotton monitoring programs and current results

Ryan Kurtz

Cotton Incorporated, USA. E-mail: rkartz@cottoninc.com

For trait stewardship and as a condition of product registration, insecticidal trait registrants annually monitor for the presence of resistance alleles in populations of pest species through focus population sampling as well as follow up on field reports of unexpected damage. Registrants annually sample and bioassay populations of the key target pests *Diabrotica virgifera virgifera*, *Diatraea grandiosella*, *Ostrinia nubilalis*, *Heliothis virescens* and *Helicoverpa zea*. Sampling for the target pests is focused in areas identified as highest risk for resistance development. Bioassay methods are chosen for detecting field-relevant shifts in population response to the insecticidal protein and/or changes in resistance allele frequency in response to the use of traits expressing the protein. As much as possible, methods are kept consistent across years for comparisons with historical data. Additionally, registrants follow up each season on field reports of unexpected damage. Following up on field reports allow for early detection of resistance hot spots as well as information on secondary pests. Resistance monitoring serves as a means to measure the successfulness of an IRM plan as well as a trigger to implement appropriate remedial actions when necessary. An overview of US corn and cotton monitoring programs and current results will be discussed.

Simulation Modeling and Insect Resistance Management

J. Lindsey Flexner, Zaiqi Pan, Khai Tran, David Onstad and Bruce Stanley

USA

Computer simulation models have been used over the past three decades as a tool for comparing the importance of the assumptions thought to be critical in the development of insecticide resistance. These models have been used to assess the importance of many variables including: dose, resistant allele frequency, insect behavior, refuge strategy, and the durability of multiple toxins when used in mixtures, rotations or sequentially. Researchers build these simulations to enable the rapid assessment of the risk of pests developing resistance to insecticides or transgenic crops. In the United States, the use of models is a key component in the registration of transgenic plants expressing proteins from *Bacillus thuringensis*. This presentation will focus on simulation modeling used to predict the development of resistance by insect pests to transgenic maize under different agronomic scenarios. The authors will address the benefits and limitations of this approach, and will highlight the types of biological data needed to make these predictions more accurate and biologically relevant.

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Combination of field and laboratory trials to monitor Bt resistance, before and after, introduction of genetically engineered crops

Ingeborg Zenner de Polanía

U.D.C.A, Colombia. E-mail: ilenner@udca.edu.com

Based on the problems observed in Colombia after establishing both Bt cotton and Bt maize and in order to avoid future mistakes in approving the planting of Bt crops or other GM organisms, a comparative analysis of the conditions under which the Bt crops were obtained and the conditions under which they are now grown in the tropics are presented. Plant varieties, soil and climatic conditions, wind speed and directions that can influence pollen dispersal, distance and area of refugees and biosecurity aspects, as well as the respective mortality base lines are studies to be performed before releasing the genetically engineered crops.Emphasis is made on the failure of Bt cotton to control Spodopterafrugiperda, on the cross pollination by insects of Bt cotton and conventional grown cotton in refugees, which makes refugees unreliable for mitigating resistance, on the toxin concentration in plant parts of both Bt cotton and cornand its influence on the control of the target pests and, finally the adult fall army worm migration, which larvae fed on sub-lethal dosage of the Cry on cotton, to Bt maize, diminishing even more the control of this pest of economic importance in both crops, at least in Colombia.

Field-evolved resistance to Bt crops

Bruce E. Tabashnik

Department of Entomology, University of Arizona, Tucson, Arizona 85750, USA; Phone: (520) 621-1141; FAX: (520) 621-1150; email: brucet@cals.arizona.edu

Evolution of insect resistance threatens the continued success of transgenic crops that produce *Bacillus thuringiensis* (Bt) toxins to kill some key pests. Field-evolved resistance entails a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field. Although many pest populations remain susceptible, field-evolved resistance has been reported in some populations of at least six major pests. For Bt corn, resistance has been reported for *Spodoptera frugiperda* to Cry1F in Puerto Rico, *Busseola fusca* toCry1Ab in South Africa, and Diabrotica virgifera virgiferato Cry3Bb in the midwestern US. For Bt cotton, reported cases of resistance include Helicoverpa zea to Cry1Ac and Cry2Ab in the southeastern US, *Pectinophora gossypiella* to Cry1Ac in India, and *Helicoverpa armigera* to Cry1Ac in China. The cumulative number of pest species with field-evolved resistance increased from zero for the first 5 years (1996-2000), to one for the first 10 years (1996-2005), and to six for the first 15 years (1996-2010). Consistent with theory, field data indicate that abundant refuges of non-Bt host plants can delay resistance, particularly when resistance is inherited as a recessive trait.

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Pros and Cons of implementing an F2 screen for Bt-resistance monitoring

Carlos A. Blanco

USDA-APHIS Biotechnology Regulatory Services, Riverdale, MD, USA. E-mail: carlos.a.blanco@aphis.usda.gov

The planning and implementation of a sound scientifically-based approach to detect *Bacillus thuringiensis* resistance is of the upmost importance for an accurate monitoring program. Our ability to identify, isolate and quantify rare resistant alleles depends on accurate techniques, of which the F2 screen is the more effectively for detecting dominant and recessive alleles segregated in the second generation. Currently there are two major approaches for doing this, mass mating or single families of the parental generation. The first is an improper, less reliable method that involves possible fitness costs due to the resistance mutation producing results that do not properly isolate homozygous progeny and cannot accurately quantify the frequency of alleles in the sampled population. The former is a method that overcomes these objections but requires of more work. The question here would be if the F2 screen done with isofamilies is that cumbersome to jeopardize the quality of our work? A comparison of the two methods utilizing the same data set demonstrate that the F2 screen performed with mass mating would miss detecting resistant alleles, while the method using isofamilies would not.

Adaptive management of resistance to Bt-cotton in Australian *Helicoverpa spp*.

Sharon J. Downes[1]; Rod Mahon[2]; Tracey Parker[1]; Bill James[2]

[1]CSIRO Ecosystem Sciences, Australian Cotton Research Institute, Locked Bag 59, Narrabri, NSW 2390 Australia, [2]CSIRO Ecosystem Sciences, Black Mountain Laboratories, PO Box 1700, Canberra, ACT 2601 Australia.

In Australia, monitoring *Helicoverpa* species for resistance to the Cry2Ab toxin in second generation *Bacillus thuringiensis* (Bt) cotton has fulfilled its intended function: to warn of increases in resistance frequencies that may lead to field failures. Prior to the widespread adoption of two-gene Bt cotton (Bollgard II), the frequency of Cry2Ab resistance alleles was at least 0.001 in *H. armigera* and *H. punctigera*. In the six years hence, there has been a statistically significant increase in the frequency of alleles conferring Cry2Ab resistance in field populations of *H. punctigera*. In this presentation we review the history of deploying and managing resistance to Bt cotton in Australia, outline the characteristics of the isolated resistance that likely impact on resistance evolution, and use a simple model to predict likely imminent resistance frequencies. We then discuss potential strategies to mitigate further increases in resistance frequencies, including the release of a third generation product that utilizes the novel vegetative insecticidal protein Vip3A. The robustness of the Vip3A inclusive variety will depend on resistance frequencies to Vip3A and to Cry2Ab when it is released (anticipated 2014) and the efficacy of Vip3A throughout the season. The area planted to Bt-crops is anticipated to continue to rise worldwide and many biotechnical companies intend to add Vip3A to existing products; therefore the strategies being considered in Australia are likely to relate to other situations.

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Current status of pink bollworm Bt resistance in Arizona

Jeff Fabrick

USDA-ARS, USA. E-mail: jeff.fabrickc@ars.usda.gov

Genetically engineered crops that produce insecticidal *Bacillus thuringiensis* (Bt) toxins kill some key insect pests of cotton and corn. Transgenic Bt cotton is a key component of a program designed to eradicate the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from the U.S. and adjacent areas of Mexico. Although this pest has evolved resistance to Bt cotton in India and similar resistance could undermine eradication efforts in North America, monitoring data show that pink bollworm field populations in the U.S. remain susceptible to the Bt toxins used in transgenic cotton. In all laboratory-selected strains of pink bollworm previously characterized, resistance to Cry1Ac involves three mutant alleles (r1, r2, and r3) of a cadherin gene that encodes a Cry1Ac toxin receptor. Examination of the r3 allele implicates the recent insertion of an active transposable element into the cadherin gene. Lab selection using Bt cotton bolls and Cry1Ac in diet produced a highly resistant strain of pink bollworm called Bt4R. An interstrain complementation test for allelism between Bt4R and a previously analyzed resistant strain (AZP-R) shows that mutations at a shared cadherin locus confer resistance to Cry1Ac in both strains. Molecular analysis identified a novel cadherin allele (r4) responsible for Cry1Ac resistance in Bt4R.

Characterization and estimation of Cry1F Resistance in Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith)

Ana Maria Velez[1,3], Terrence Spencer[1], Analiza Alves[2] and Blair Siegfried[1]

[1]Department of Entomology, University of Nebraska, Lincoln NE, [2]Pioneer Hi-Bred. [3]Email: anamaria.velez@gmail.com

Transgenic maize expressing Cry1F has been registered for *Spodoptera frugiperda* (J. E. Smith) control since 2003. Unexpected damage was reported in 2006 in Puerto Rico and Cry1F resistance in *S. frugiperda* was documented. The inheritance of resistance was determined in a resistant colony from Puerto Rico which displayed >300-fold resistance to purified Cry1F. Concentration-response bioassays of reciprocal crosses of resistant and susceptible parental populations indicated that the resistance is recessive and autosomal. Bioassays on the backcross of the F1 generation crossed with the resistant parental population suggest that a single locus is responsible for the resistance. Because the resistance is recessive and conferred by a single locus, we employed an F1 screening to estimate allele frequencies from populations in South Florida and Texas, field collected individuals of unknown genotype were crossed with the resistant colony. The offspring of these crosses were bioassayed using a discriminating Cry1F concentration, it was determined that resistance alleles could be detected from Florida and at lower frequencies in Texas. In addition, cross-resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ba and Cry2Aa was assessed. There was no significant cross-resistance to Cry1Aa, Cry1Ba and Cry2Aa. In contrast, low cross-resistance (< 20-fold) was observed for both Cry1Ab and Cry1Ac.

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Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Strains from Central Colombia to Two Insecticides and Cry endotoxins (Cry1Ac and Cry1Ab).

Juan Diego Ríos-Diez and Clara Inés Saldamando-Benjumea[1]

UNAL, Colombia. [1]E-mail: saldamando@yahoo.com

Spodoptera frugiperda (FAW) (J. E. Smith) is an important pest in western hemisphere and has genetically differentiated into two host–associated populations: the corn and the rice strains. In the US lines from corn, rice, bermudagrass and millet were used to evaluate the resistance of both strains to various insecticides and to the endotoxin Cry1Ac in transgenic cotton crops, and found that the corn strain is more resistant than the rice strain. However, all larvae tested were not genotyped. In Colombia genotyping of FAW is necessary because the rice strain can also be found in corn fields. In this work, collected larvae from corn and rice fields from Tolima (central Colombia) were genotyped and evaluated for the resistance to methomyl and lambda-cyhalothrin and for the Cry1Ac and Cry1Ab endotoxins. We found that the rice strain does not significantly differ in resistance to methomyl compared to the corn strain but it develops tolerance more rapidly to lambda-cyhalothrin. The eggs viability of treated females was also affected by methomyl on each generation. On the other hand, the corn strain developed resistance to both endotoxins more rapidly than the rice strain and concentrations of Cry1Ac needed for produce a mortality of 50% of *S. frugiperda* larvae were higher than for Cry1Ab.

Functional Genomics as a Tool to Study Insect Responses to Bt Intoxication

Brenda Oppert

USDA Agricultural Research Service, Center for Grain and Animal Health Research, Manhattan, KS. USA.

Bacillus thuringiensis (Bt) crystal (Cry) proteins are effective against some insect pests, but improvements are needed in both efficacy and lethal time of toxins that target coleopteran pests. Our laboratory has used functional genomics to study the effects of Bt Cry3Aa on *Tenebrio molitor* larvae. We compared the gut transcriptome of larvae that were fed a control (untreated) diet or a diet containing Cry3Aa at the median lethal dose for 24 hours. Using high throughput sequencing, we obtained sequence reads from control and Cry3Aa-treated larvae that were analyzed for functional groups. Enrichment analyses indicated that functions associated with mitochondrial respiration, maintenance of cell structure, membrane integrity, protein recycling/synthesis, and signaling were significantly increased and metabolic processes overall were reduced in Cry3Aa-treated larvae. We used these sequences for microarray analysis to evaluate temporal changes in gene expression after Cry3Aa exposure. Statistical analysis of transcript expression in control and Cry3Aa-treated larvae from all time-points indicated that an ortholog to Drosophila CG4367, induced in response to gram-negative bacterial infection, was most dramatically induced in Cry3Aa-intoxicated larvae. Overall, the data suggest that *T. molitor* larvae mount a complex response to Cry3Aa during the initial 24 hours of intoxication. This study demonstrates a novel approach to study Cry intoxication in insects lacking a sequenced genome while providing sequence data for future studies.

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Differential expression of peptidases and glycosidases in the midgut of *Bacillus thuringiensis* Cry3Aa-treated *Tenebrio molitor* larvae

Elena N. Elpidina

Moscow State University, Moscow 119991, Russia. E-mail: elp@belozersky.msu.ru

A stored product pest, *Tenebrio molitor,* is sensitive to Cry3Aa toxin from *Bacillus thuringiensis* (Bt). Larvae digest protein initially with cysteine peptidases in the anterior midgut and further with serine peptidases in the posterior midgut, and the latter activate Bt Cry protoxins and are a determining factor in toxicity and resistance. We evaluated the effect of Cry3Aa protoxin on the expression of cysteine and serine peptidase genes, as well as genes of glycosidases in the midgut of *T. molitor* larvae. High-throughput sequencing was used to obtain EST databases from midguts of one month old *T. molitor* larvae fed either a control diet or diet containing 0.1% Cry3Aa for 24 h. In addition, the temporal expression of peptidase genes was investigated by microarray analysis. Bt intoxication significantly reduced the expression of serine peptidases, potentially important in protoxin processing, while the insect maintained the production of critical digestive cysteine peptidases. We hypothesize that the insect may be attempting to decrease toxicity while maintaining efficient protein digestion. The data are the first application of high-throughput sequencing to the study of Bt intoxication and demonstrate that Cry3Aa intoxication in *T. molitor* induces widespread changes in peptidase gene expression.

Enhancement of insecticidal activity of Cry1 toxins against fall armyworm by site directed mutagenesis of toxin receptor binding epitopes.

Isabel Gómez[1], Guadalupe Peña[2], Josue Ocelotl[1], Alejandra Bravo[1] and Mario Soberón[1,3]

Instituto de Biotecnología Universidad Nacional Autónoma de México and Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos. [3]E-mail: mario@ibt.unam.mx

Bacillus thuringiensis Cry toxins are valuable tools for controlling insect pests when expressed in transgenic plants, as maize and cotton, resulting in lower use of chemical insecticides for crop production. Cry toxins are highly specific and harmless to other organisms as vertebrates and to most beneficial insects. However, some important insect pests as *Spodoptera frugiperda* show no or little susceptibility to Cry1A toxins produced in transgenic crops. *S. frugiperda* is an important maize and rice pest in North and South America. Cry1Ab Domain II exposed loops and Domain III b16-b22 regions have been shown to be important for binding to specific insect gut receptors mediating insect specificity. In this work we will present data on the site directed mutagenesis of certain Cry1Ab receptor binding regions that in some cases resulted in enhanced toxicity to *S. frugiperda* larvae without losing insecticidal activity against the susceptible *M. sexta* larvae. These mutants are likely to provide tools for crop protection against a broader number of lepidopteran insect species.

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Insertion of Cry toxin into the target membrane and role of CryMod toxins in suppressing resistance to different insect populations.

L. E. Zavala[1], L. Pardo-López[1], B. E. Tabashnik[2], F. Huang[3], B. D. Siegfried[4], Y. Wu[5], L. J. Gahan[6], D. G. Heckel[7], M. Soberón[1], and A. Bravo[1,8]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Mexico [2]University of Arizona, USA. [3]Louisiana State University, USA.4University of Nebraska, USA. [5]Nanjing Agricultural University, China. [6]Clemson University, South Carolina, USA. [7]Max Planck Institute for Chemical Ecology, Germany. [8]*Email:* bravo@ibt.unam.mx

Bacillus thuringiensis produces insecticidal Cry proteins that are used commercially for the control of insect pests. These are pore-forming toxins that interact with different receptors in the insect gut and form pores in the apical membrane causing cell burst and insect death. It is important to fully understand the mechanism of action of Cry toxins in order to improve them for the control of different insect pests. It was proposed that the hydrophobic hairpin formed by domain I helices α-4 and α-5 inserts into the phospholipid bilayer whereas the rest of helices of domain I are spread on the membrane surface in an umbrella-like conformation. However, a second hypothesis proposed that the three domains of the toxin insert into the bilayer. We constructed single Cys Cry1Ab mutants in the three domains of the toxin and labeled them with different fluorescent probes. Fluorescence and quenching analysis of Cry1Ab labeled proteins in solution and after membrane interaction revealed that the three domains of the toxin remain in the surface of the membrane and only a discrete region of domain I is inserted into the lipid bilayer supporting the umbrella model of toxin insertion. On the other side, the evolution of insect resistance is the mayor threat for the future use of Cry toxins. Resistance to Bt toxins in some insects is linked with mutations that disrupt cadherin receptor. Recently, it was shown that in other insects it is linked to mutation in ABC transporter or to aminopeptidase P genes. Cadherin receptor is involved in oligomerization Cry1A toxins after cleavage of helix α-1. The CryMod toxins lack the amino terminal region including helix α-1 and killed cadherin-silenced *M. sexta* and Bt-resistant *Pectinophora gossypiella* that had cadherin deletion mutations. The toxicity of CryMod toxins against five other Cry-resistant insect populations was analyzed. Our results show that CryMod toxins reduce resistant ratios of three different populations linked to mutations in the ABC transporter gene and one population linked to aminopeptidase P gene. In contrast, populations that show low resistance ratios due to cadherin mutations were not efficiently controlled by CryMod toxins, this may be due to the low potency of CryMod toxins against the corresponding susceptible populations. Overall our results indicate that CryMod toxins will be useful in the control of insects with different mechanisms of resistance. The role of ABC transporter and aminopeptidase P in the mechanism of action of Cry toxins remains to be analyzed.

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Characterization of resistance mechanisms to Dipel and transgenic Bt corn to improve resistance monitoring

Juan Luis Jurat-Fuentes

U. of Tennesee, USA. E-mail: jurat@utk.edu

Insecticidal products based on *Bacillus thuringiensis* (Bt) spores and toxins, and transgenic plants expressing Bt toxins (Bt crops) are used worldwide for pest control. One of the main issues related to the high adoption of these insect control products is the potential for evolution of resistance in target populations. Resistance monitoring is a crucial component of current insect resistance management programs for Bt crops. The development of sensitive DNA-based methods would greatly improve the efficacy and sensitivity of resistance monitoring efforts and greatly contribute to delay evolution of resistance. Towards this goal, we have characterized resistance mechanisms against the Bt product Dipel in laboratory-selected strains of the tobacco budworm (*Heliothis virescens*) and field-evolved resistance against transgenic Bt corn expressing Cry1Fa toxin in the fall armyworm (*Spodoptera frugiperda)*. Using proteomic techniques we identified proteins differentially expressed in resistant insects, and confirmed these identifications using transcription profiling and quantitative PCR studies. Data from our research identified proteins and genes involved in resistance to Bt toxins to target in the development of monitoring strategies for Bt resistance.

Enhancement of *Bacillus thuringiensis* Cry3Aa toxicity to vegetable Coleopteran larvae by a *Tenebrio molitor* cadherin fragment

Yu-Lin Gao and Zhong-Ren Lei[1]

IPP-CAAS, China. [1]*E-mail:* zrlei@ippcaas.cn

Bacillus thuringiensis (Bt) is a bacterium that produces toxins used in the control of insect pests. Bt toxins generally are not effective against many beetle pests, limiting the use of Bt toxins in integrated pest management. Based on the reported increase of Bt toxin oligomerization by a polypeptide from the Cry3Aa receptor cadherin in *Tenebrio molitor* (Coleoptera: Tenebrionidae), we hypothesized that this cadherin peptide, rTmCad1p, would enhance Cry3Aa toxicity towards coleopteran larvae. To test this hypothesis, we evaluated the relative toxicity of Cry3Aa with or without rTmCad1p against damaging chrysomelid vegetable pests of China. Cry3Aa toxicity was evaluated in the spotted asparagus beetle (*Crioceris quatuordecimpunctata*), cabbage leaf beetle (*Colaphellus bowringi*), and daikon leaf beetle (*Phaedon brassicae*). To assess effect of rTmCad1p on Cry3Aa toxicity, neonate larvae were fed Cry3Aa toxin alone or in combination with increasing amounts of rTmCad1p.The data demonstrated that Cry3Aa toxicity was significantly increased in all three vegetable pests, resulting in as much as a 15.3-fold increase in larval mortality. The application of rTmCad1p to enhance Cry3Aa insecticidal activity has potential for use in increasing range and activity levels against coleopteran pests displaying low susceptibility to Bt-based biopesticides.

Green: Wildlife, Ecology & Environment Biotechnology

International Biotechnology Color Journal

Benefits of pyramiding multiple Bt traits for resistance management

Nicholas P. Storer[1], Gary D. Thompson

Dow AgroSciences LLC, Indianapolis, IN, USA. [1]E-mail: nstorer@dow.com

The first insect-protected transgenic maize and cotton crops contained single Cry1A toxins from *Bacillus thuringiensis* Berliner (Bt). To slow the evolution of resistance in target insect populations, Bt crop growers have been required to plant separate portions of their fields to non-Bt varieties. These non-Bt patches serve as structured refuges for susceptible insects to develop and be available to mate with any resistant insects developing in the Bt fields, thus passing susceptibility on to their progeny. However, ensuring full grower adherence to structured refuge requirements has proven to be challenging. In recently introduced products, multiple Bt proteins with different modes of action against each key target pest species are combined in individual plants. These crops provide high levels of control of susceptible insects and those bearing resistance alleles. For target insects to have significantly increased fitness when feeding on these crops they are expected to need at least three resistance alleles (homozygous resistant against one protein, heterozygous against the second). Simulation models indicate that the evolution of resistance to these pyramided-trait products in target pest populations is expected to be dramatically delayed, permitting significant reductions in the refuge size. Furthermore, reduced refuge size and redundant killing of pests can allow the non-Bt refuge to be provided as a seed blend. This approach shifts the responsibility for refuge deployment from the grower to the trait provider, greatly simplifying farm management while ensuring the appropriate refuge is planted. Pyramiding multiple Bt traits and blending refuge seed reduce the uncertainties associated with resistance management for Bt crops and extend their expected durability.

Will conserving natural enemies with Bt plants affect resistance evolution?

Anthony M. Shelton

Department of Entomology, Cornell University/NYSAES, Geneva, New York USA 14456

There is also a large body of literature regarding Bt plants and how, compared to most conventional insecticides, they help conserve natural enemies. However, as is the case with conventional insecticides, the interaction of natural enemies and resistance evolution in their hosts is a topic of interest. The literature contains examples of natural enemies delaying resistance as well as accelerating the evolution of resistance, depending whether there is a differential impact on susceptible or resistant phenotypes. It is generally thought that those natural enemies that increase differential fitness between susceptible and resistant phenotypes on host plants will accelerate the evolution of resistance while those that decrease the differential will delay resistance. While this general concept is valuable, there are other factors that also influence resistance evolution. Using our unique system of diamondback moth and Bt plants and conventional insecticides, we have been exploring the question of whether conserving natural enemies will affect resistance evolution.

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Bt resistance, immunity and gut microbiota in *Plodia interpunctella*

Patricia Tamez-Guerra[1,3], María M. Iracheta[1], Brenda Oppert[2], Alberto Valadéz-Lira[1], Alonso A. Orozco-Flores[1], Ricardo A. Gómez-Flores[1] and Cristina Rodríguez-Padilla[1]

[1]Depto. de Microbiología e Inmunología, Fac. de C. Biológicas, UANL, A. P 46-F, San Nicolás de los Gza, N. L., MX. [2]USDA Agricultural Research Service, Center for Grain and Animal Health Research, Manhattan, KS. USA. [3]E-mail: patamez@hotmail.com

Plodia interpunctella (indianmeal moth), was the first insect in which resistance to *Bacillus thuringiensis* (Bt), was detected. This study was undertaken to evaluate midgut enzymatic activity, the expression of the antimicrobial peptide hemoline, and the Enterobacteriaceae community of *P. interpunctella* strains, one susceptible (PIS) and other resistant (PIR) to Bt, to determine their role in the Bt resistance mechanisms. Protease activity was evaluated by enzyme assays (zymograms and activity blots) on second, fourth, and five instar larvae. Hemolin expression was evaluated by both biological (antibacterial activity against several enterobacteria); and molecular (RT-PCR). Enterobacteriaceae community was also evaluated by biological assay (aerobic and anaerobic cultivable bacteria); and by molecular assay (Enterobacteriaceae general primers by RT-PCR. Activity blots and zymograms demonstrated that enzyme patterns in PIS and PIR larvae were different when chymotrypsin and casein were used as substrates, but no differences were found with trypsin. Hemolin expression was observed in PIR and PIS exposed to Bt, whereas Enterobacteriaceae molecular detection was observed in PIR and PIS exposed to Bt strains, but no with unexposed PIS. Overall, results indicate that proteases, hemolin and bacterial community change in Bt-resistant *P. interpunctella* larvae.

Next Generation Novel Traits

Isaac O. Oyediran[1], Von Kaster and David Laird

Syngenta Biotechnology, Inc, RTP, NC USA. SYNGENTA. [1]E-mail: isaacoyediran@syngenta.com

Syngenta is one of the world's leading companies with more than 26,000 employees in over 90 countries dedicated to our purpose: Bringing plant potential to life.

Our Crop Protection and Seeds products help growers increase crop yields and productivity. We contribute to meeting the growing global demand for food, feed and fuel and are committed to protecting the environment, promoting health and improving the quality of life. Syngenta has developed the next generation novel traits.

Green: Wildlife, Ecology & Environment Biotechnology

Innovation in biotechnology and the socioeconomic impact of biotechnology research.

Rodolfo Quintero-Ramírez

Department of Process and Technology, Universidad Autónoma Metropolitana, UAM-Cuajimalpa, Mexico City, e-amil: quintero@correo.cua.uam.mx

Abstract

In this presentation, I will discussed how biotechnology has shown to be a disruptive innovation, which has generated new products and services, has displaced products and producers already in the market, has created immense wealth and many new companies have emerged. To illustrate the disruptive nature of biotechnology three areas of application will be analyzed:

- Human healfth: bioterapeutics, biogenerics and biosimilars
- Agriculture: transgenic plants
- Energy: biofuels (ethanol and biodiesel)

Also it will be discussed how the socioeconomic impacts of its applications have been distributed unequally among industrialized countries and developing countries, and the trends that are emerging of food versus energy or food versus industry (bioplastics and new biomaterials) will be described.

The innovation process that biotechnology follows in industrialized countries (open innovation) will be compared with the process that is used in most developing countries (lineal model), and Mexico will be reviewed in more detail:

- Present situation of academia/research
- Negative impacts of biotechnology (loss of markets and producers; increase of bioproducts importation)
- Development of new bioproducts

A new way to approach innovation related to biotechnology for developing countries will be presented, based on the following model:

where: = innovation, = ideas, = resources, risk and rewards.

Some examples for Mexico and Latin America described; also some recommendations about future international collaboration will be done.

International Biotechnology Color Journal

Silver: BioEntrepreneurship & Economics of Biotechnology

Red biotechnology as an exciting career alternative for healthcare graduates

Dimitris Dogramatzis, R.Ph., Ph.D.

DOGRAMATZIS PHARMACY, Athens, Greece

The international healthcare (red) biotechnology arena is an exciting intersection of science and business, that has grown dramatically since its inception three decades ago. The present work summarizes the current state of bioeconomy and offers an exciting and rewarding career prospect for all healthcare graduates around the world.

Summary:

In 1953 Watson and Crick published a paper in Nature describing the double helix. In 1976 Swanson and Boyer founded Genentech, eventually succeeding in launching the first biosynthetic insulin in 1982. Today there exist several commercial sectors, each given its own corresponding coding color, e.g. red, green, blue, and white. Healthcare biotech is color-coded red (from the red blood cells) and includes the biosynthetic production of medicines and vaccines, stem-cell research, DNA sequencing and more.

In 2010, global red biotechnology had revenues of USD 84.6 billion, R&D expenses of 22.8, and net income of 4.7. It also employed 178,750 people, and had 622 public companies. Conclusion: Red biotechnology has evolved tremendously since its commercial inception in 1982. Today it represents an exciting and rewarding career prospect for all biological graduates around the world. Interested applicants should take dedicated commercial biotechnology courses, be hard-working, inquisitive, and meticulus, compare their prospects, network intensively and empower themselves. Finally, their professional career can not fully be predicted, will bring many sacrifices, changes and ups and downs, but rewards as well.

Current state of red biotechnology: Molecules, therapeutic areas, and manufacturers

Dimitris Dogramatzis, R.Ph., Ph.D.

DOGRAMATZIS PHARMACY, Athens, Greece

The international healthcare (red) biotechnology arena is an exciting intersection of science and business, that has grown dramatically since its inception three decades ago. The present review summarizes the state of bioeconomy, compares red biotechnology with pharmaceutical companies, and discusses the major bionations, manufacturers, therapy areas and molecules.

Summary:

In 1953 Watson and Crick published a paper in Nature describing the double helix. In 1976 Swanson and Boyer founded Genentech, eventually succeeding in launching the first biosynthetic insulin in 1982. Today there exist several commercial sectors, each given its own corresponding coding color, e.g. red, green, blue, and white.

Healthcare biotech is color-coded red (from the red blood cells) and includes the biosynthetic production of medicines and vaccines, stem-cell research, DNA sequencing and more.

The pharmaceutical lifecycle includes the pre-discovery, discovery, preclinical, clinical, regulatory approval, and post-marketing phases. In total, it usually scans through 10,000 promising molecules, tests thousands of human volunteers and patients, and requires 10-15 years and in excess of one billion USD to bring a new pharmaceutical to the marketplace.

Conclusion:

Red biotechnology has evolved tremendously since its commercial inception in 1982. To date, it has introduced more than 130 biotherapies, it has a positive global net income, it has helped save the lives of millions of patients around the world and it is on a pace to surpass the traditional pharmaceutical products at the top of the global sales list. The next decade will show whether the 21st century can finally be named the biotechnology century.

International Biotechnology Color Journal

Silver: BioEntrepreneurship & Economics of Biotechnology

Marine Biotechnology. Opportunities and challenges for Mexico´s XXI Century.

Fernando García Carreño., PhD.

CIBNOR. La Paz. BCS. México. e-mail: fgarcia@cibnor.mx

Continental México is ca 2 x 106 square kilometers, while Marine México is ca 3 x 106 sq km. Meaning that Marine México is two thirds of the total Country. Besides the size, Marine México is formed by a variety of disparate ecological niches. From estuarine waters, almost sweet water, to salt marshes with 6M NaCl; From two atmospheres to several hundreds; From subtropical or deep waters, to several hundred ºC in black hydrothermal vents at 3000 m deep in the Guaymas basin. All this causing specialized adaptations in organisms thriving in such environments. Leading to diverse metabolisms to be explored for biochemical mechanisms of production and transformation to become biotechnologies. Here we will define Biotechnology and how business based on biotechnologies is assessed and why basic research is needed to succeed and how success must be aimed; intellectual leadership. Also Biotechnology will be analyzed as a multidisciplinary science, what are the objectives of the science along with the tools of Biotechnology. Biotechnology is technology based on organisms, so organisms are quintessential in this paper.

Benefits of exploring marine organism are plentiful. Like generating and attracting novel, clean, leading-edge technology industries to the Country; Jobs for the wellbeing of Mexicans. Increasing opportunities to keep Mexican- and abroad-trained professionals of science at the region. Sustain and restore both land and marine habitats. Potential products are: food for humans and feed for land and aquatic animals; Food and feed ingredients and supplements; pharmaceuticals, cosmetics, fertilizers and all sort of biological reagents, from enzymes to pigments.

Of course there are potential benefits, as well as challenges. As long as we can keep a balance between them, México can achieve a better future based on science and biotechnologies.

The use of biotechnology crops in Argentina

Graciela del Valle Leguizamón.

Belgrano(S) Avenue, 1912. Santiago del Estero. Argentina ZIP CODE 4200 54-3854599528, e-mail: graciela@unse.edu.ar

Argentina is mainly an agricultural country. In recent times, lands considered marginal were incorporated to the production, impulsed by technological changes and introduction, in 1996, of genetically modified crops. Currently 21.3 million ha. are cultivated with transgenic soybean, corn and cotton. The transgenic maize use contributes to control of mainly Lepidoptera pests, *Spodoptera frugiperda* and *Diatraea saccharalis*, but also others such as *Heliothis zea* and *Agrotis ipsilon*, especially in the northwest, where tropical corn is cultivated. Other traits give herbicide tolerance (gliphosate and ammonium gluphosinate). The last transgenic events approved in Argentina were stacked. This type of events has been incorporated into cultivation ,similarly to the simple events and this arises from the accumulation of events developed and approved previously. These belong to the same company or rival companies. In these cases, plants are modified with many traits which give an integral protection to the crop (Lepidoptera and root worms, herbicides). In relation to soybean, around 90% of the total cultivated are gliphosate resistant, what caused a true revolution in this crop. Transgenic cotton, BT and gliphosate resistant, is less cultivated, compared to soybean and corn. Currently, companies are working in order to get new traits with wide spectrum herbicides sensibility; more efficient in the water and critical nutrients use; salt, flooding and high temperature tolerance. In conclusion, in Argentina, the adoption rate of transgenic crops is high because growers are satisfied, due to low costs, decrease in insecticide use, and high crop yields with high quality. However challenges are faced, such as responsible management of these crops to avoid pests resistant and damages to native biodiversity.

Cloning embryos: Application to wildlife species as endangered bighorn sheep

María del Carmen Navarro-Maldonado

Doctor in Veterinary Sciences, Animal Production Coordination, Biology of Reproduction Department Biological and Health Sciences Division, Universidad Autónoma Metropolitana UAM Iztapalapa, Mexico City,

Embryo cloning is a biotechnology that has been applied since the 30s, to reproduce different animal species. There, we have some works from Hans Spemann, who divided a fertilized salamander oocyte using a baby hair, obtaining more than two viable embryos; to Wilmut and Campbell who, working with domestic sheep, transferred a mammary gland cell into an enucleated oocyte, giving birth to the very first cloned sheep from adult cells "Dolly". What to say about the uses that cloning biotechnologies have, for the reproduction of selected productive animals, for the economical profits that this represents and for the ecological interest in attempt to preserve or avoid the species extinction, as it happened for Dr. Lanza, who cloned the first wild gaur "Noah". It had occurred several works in which this biotechnology has won supporters and enemies, rumors and scientific evidences, everything around the need and pertinence of its application in humans. In this conference it will be explained the different ways for embryo cloning, its applications and will see some examples, giving emphasis to its application to wild animal still in existence and that already extinct.

International Biotechnology Color Journal

Green: Wildlife, Ecology & Environment Biotechnology

International Biotechnology Color Journal

The "omics" Approach for Solving the Pre-harvest Aflatoxin Contamination problem: Understanding the genomics and metabolomics of the fungus and proteomics of the affected corn crop

Dr. Deepak Bhatnagar, Research Leader

Food and Feed Safety Unit USDA/ARS/SRRC USA

Aflatoxins are highly carcinogenic secondary metabolites produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin contamination of food and feed has been of particular concern over the last four decades because of the toxicity of these compounds. Regulations exist in over 100 countries of the world against sale of contaminated commodities, causing severe economic burden on the farmers. Therefore, this problem is both a food safety and an economic issue. Now, for the first time control measures for this problem appear within reach. For practical and sustainable control of pre-harvest aflatoxin contamination to be realized, however, additional information is needed about the fungus, the affected crops and the specific molecular factors (both in the plant and the fungus) involved during host plant-fungus interaction. The information derived from the use of novel tools such as genomics, proteomics and metabolomics provides us with the best and the quickest opportunity to achieve a clear understanding of the survival of toxigenic fungi in the field, the ability of the fungus to invade crops, and the process of toxin contamination under various environmental conditions. Significant progress has been made recently in understanding the genomic makeup of the most significant aflatoxin producing field fungus, namely *Aspergillus flavus*. Progress also has been made in the study of host crop resistance to fungal invasion through the use of proteomics. The information available on production of aflatoxin and other metabolites by *Aspergillus flavus* is reasonably extensive, although the application of metabolomics as a tool in this study is relatively new. In this presentation, the use of genomics, proteomics and metabolomics in deriving the requisite information for developing effective strategies to interrupt the machinery in the fungus for production of these toxins, as well as to enhance host-resistance against fungal invasion and aflatoxin contamination of crops will be discussed.

Green: Wildlife, Ecology & Environment Biotechnology

Biotechnology-derived products for insect pest control

Patricia Tamez-Guerra[1] and Robert W. Behle[2]

[1]UANL, NL,Mexico. [2]USDA-ARS, USA. [2]Email: robert.behle@ars.usda.gov

Efforts to produce microbial-based insecticides have resulted in development of new and improved methods in biotechnology. Microorganisms, metabolites from plants and microorganisms, and transgenic crops have been used to make biotechnologically-derived products for control of insects. New biotechnology has confronted two biggest challenges for biological pest control agents: to reduce the production cost, and demonstrate efficacy comparable with current chemical pesticides. Among entomopathogens, bacteria, viruses, fungi and nematodes are widely used as bioinsecticides. Bacteria and fungi can be produced in vitro using liquid or solid fermentation techniques, however, viruses and nematodes must be produced in vivo. Production biotechnology involves the following aspects: liquid fermentation/spray-drying for bacteria, in vivo (insects) or in vitro (insect cells by liquid fermentation)/emulsion for viruses, mixed (liquid and/or solid) fermentation/oil emulsion by fungi, and in vivo production along with dry or liquid as matrix stabilizers by nematodes. In order to improve the potential for large scale biopesticides commercialization, state-of-the-art biotechnology has been focused to transgenic microbial and seed products, along with formulation and application techniques. Continuous scientific discoveries in basic biology combined with improved and novel methods in biotechnology will contribute to future safe and effective pest control.

Sustainable agro-industrial production in Chiapas México as an alternative technology for food security.

Peggy E. Alvarez-Gutiérrez.

Universidad Politécnica de Chiapas, México. E-mail: peggy.alvarez@hotmail.com

The State of Chiapas is located in the south region of Mexico. Despite of having vast natural resources and an immense biodiversity, this state is one of the world's poorest regions.

The main purpose of this study was to develop a sustainable technology for mushroom production (*Pleurotus spp*) for low-income women living in the Lacandona Rainforest Area and also Los Altos Region area in Chiapas Mexico.

The results of this study showed the comparison of three different systems for mushroom production in two different regions of the state of Chiapas, and the mechanism of technology transfer to three groups of low income population, with a special focus on the female population.

This study is meant to support the United Nation's Development Goals: end poverty and hunger, gender equality. Additionally, this study elucidates how a sustainable environment can be possible by producing a high protein food using agro-industrial residues as raw material.

Genomics of wheat under salt stress

José Luis Díaz De León Álvarez,

Universidad Autónoma de Baja California Sur (UABCS), e-mail: jldeleon@uabcs.mx

Mapping of quantitative trait loci (QTL) was carried out in a set of 114 RILs of the International Triticeae Mapping Initiative (ITMI) mapping population under salt stress. Seedling population was grown during 8 days under salt treatment (Hoagland's ½ strength + 110 mM NaCl, EC 12.4 mS/cm) and normal treatment (Hoagland's ½ strength, EC 0.9 mS/cm). We calculated starch degradation, measuring the dry weight of the grains on the 4th, 6th, and 8th days of culturing. Formation of biomass was calculated measuring leaf and root length on the 4th, 6th, and 8th days of culture. Interval mapping resulted in 13 QTLs, 2 major QTLs (LOD >3) and 11 minors QTLs (LOD >2). A total of 10 QTLs were associated with saline treatment and 3 QTLs at normal treatment. The data show that a high percentage of QTLs were in chromosomes 2B (3, 23.0%) and 1A (3, 23.0%), followed by 4D (2, 13.6%).

The differential response to field salinity of the parents of the ITMI wheat mapping population (cv. Opata 85 and the synthetic hexaploid W7984) was exploited to perform a QTL analysis of the response to salinity stress of a set of agronomic traits over two seasons. The material was irrigated either with potable water (EC of 1.0 dS m–1) or with diluted seawater (12.0 dS m–1). Grain yield was positively correlated with tiller number, plant height, percentage survival, ear weight, ear length, grain number per ear, grain weight and thousand grain weight, and negatively with time to booting, anthesis and physiological maturity, under both the control and salinity stress treatments. In all, 22 QTL were detected under control conditions, and 36 under salinity stress. Of the latter, 13 were major loci (LOD >3.0) and eight were reproducible across both seasons. Chromosome 2D harboured 15 salinity stress associated QTL and chromosome 4A six such QTL. The remaining loci were located on chromosomes 2A, 5A, 6A, 7A, 1B, 4B, 3B, 6B, 7B and 6D.

The parents (the landrace Chinese spring (CS) and a synthetic hexaploids (S6x)) and 17 derived single chromosome substitution lines (SL) were grown in parallel in the field under non-saline (1.0 dSm-1) and saline (12.0 dSm-1) conditions, and evaluated for a set of phenotypic traits. The performance of CS indicated it to have borderline salinity tolerance with respect to all of the traits except for leaf area (for which it behaved in as a salinity sensitive type). The SL 4D was early in booting, ear emergence, flowering and maturity, while 5D and 2B SLs were both late. The 2B SL produce 33% more ears than CS. The 5D SL under-performed with respect to ear weight, grain number per ear, grain weight per ear and 1000 grain weight both under non-saline and saline conditions. Under saline conditions, four SLs (1A>5A>1D>2B) outperformed Cs for ear length, and six SLs (1D>6A>4B>3A>3B>3D) showed an improved grain weight. The grains produce by the 2B SL were smaller than those of CS. Leaf area developed better in four SLs (4D>2B>1A>7D) than in CS.

Bioprocess intensification through high cell-density cultivation in batch mode of metabolically engineered *E. coli* strains

Dr. Alvaro R. Lara

Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana, UAM-Cuajimalpa, Mexico City.

High cell-density cultivation (HCDC) is the preferred option for industrial bioprocesses using *Escherichia coli*. Despite its wide use, HCDC present several difficulties. Attaining high cell-densities require the use of elevated amount of carbon source (usually glucose). Due to the production of acetate by overflow metabolism in E. coli, glucose has to be fed to the bioreactor in order to keep the specific uptake rate below critical levels for overflow metabolism triggering. This leads to extended cultivation times and substrate gradients in the large-scale. To avoid overflow metabolism, we developed a strain with an alternative glucose transport system and decreased acetate production. The modified strain has been cultivated using up to 130 g/L of glucose in batch mode with a very low acetate accumulation. Compared to the parent strain, superior plasmid DNA and recombinant protein production have been achieved. Another challenge of HCDC is the large oxygen demand, which can not be easily satisfied in conventional bioreactors. In order to improve oxygen transfer, we evaluated the use of high pressure cultivations. A total pressure of up to 8 atm in the bioreactor allowed maintaining aerobic conditions whereas the kinetic behavior of the strains was not affected. A molecular alternative to contend with oxygen-limiting conditions is the expression of a recombinant hemoglobin in the engineered strain, which has improved its performance under microaerobic conditions in HCDC in batch mode.

International Biotechnology Color Journal

Gray: Classic Biotechnology

Chloroplast transformation: a tool to minimize horizontal gen transfer

Quintín Rascón-Cruz

Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua, e-mail: qrascon@uach.mx

Due to the growing concern that genetically (GM) modified crops can transfer genes through pollen to related plants (wild), it is necessary to propose strategies for containment of these. The use of genetic engineering of the chloroplasts to promote maternal inheritance of the transgenes is highly desirable for those instances that involve a potential risk of flow of genes between GM crops or plants wild type and GM crops. Genetic transformation of chloroplast genome can reduce or avoid unwanted gene transfer because the chloroplast genes are through maternal inheritance in most plants. This project used three delivery plasmids for genetic transformation of the chloroplasts of maize (*Zea mays*). Plastid transformation offers advantages over conventional nuclear genome transformation which includes high levels of protein expression, introduction of the transgene into the genome by homologous recombination cloroplastídico (site specific), absence of position effects and gene silencing expression of various transgenes in tandem (polycistronic) and dramatically decreased gene flow through pollen since chloroplasts are maternally inherited. Currently, there is a real concern that genetically modified crops can transfer their transgenes to other plants, therefore, in our laboratory have constructed vectors for chloroplast transformation by homologous recombination of *B. gracilis* and *Zea mays*. Transplantomic plant could contribute to maximize the benefits of transgenic plants to significantly reducing the potential risk of horizontal gene transfer to other crops.

Non-conventional methods for protein crystallization: using physical parameters to control de crystal quality for x-ray crystallography

Dr. Abel Moreno Cárcamo

Instituto de Química, UNAM, Mexico City, Mexico.

Medium-sized single crystals with near-to-perfect habits made of molecular arrangements with no defects that produce well resolved and intense diffraction patterns are the dream of every protein crystallographer. Four basic crystallization methods are presently at the disposal of crystal growers to grow such crystals. As half a century ago in chemistry labs, crystallization assays can be set up using either (i) in batch, (ii) by the diffusion of vapor between hanging (or sitting) droplets and a reservoir of precipitant, (iii) by dialysis across a semi-permeable membrane or (iv) free interface diffusion at the interface of two liquids.

In this plenary talk an overview of crystal growth methods from Nature grown crystals until conventional and non-conventional (using electric and magnetic fields) experimental methods of crystal growth of proteins will be presented. In order to control the kinetics of the crystallization process, it is also presented what physical and chemical parameters allow us to control the nucleation, and crystal growth of biological macromolecules. Finally, a short overview of the main counter-diffusion methods, and new strategies for enhancing the crystal quality for high resolution X-ray crystallography will be also revised.

Innovative Inventions and Patents

Ricardo Gómez.

Centro de Incubación de Empresas y Transferencia de Tecnología, UANL, NL., Mexico.

Innovations deeply impact not only areas related to medicine, engineering, biotechnology, physical sciences, and information technology, but also, education, business and social sciences, some of which stimulate research and development, commercialization, and technology transfer derived from products improvement, creation of novel products or disruptive technologies. It is well known that investing in research and development are more likely to compete or take leadership in areas of opportunity. The parties involved include universities, companies and research centers. Innovations provide the advantage of creating new products or services that give vitality to those involved; this encourages more competitive advantage. However, innovation is considered not a general feature of all individuals, but rather an elitist aspect among individuals with a talent for seeing beyond the picture presented to them daily, and who have the vision of prosperity through the implementation of changes in their environment and transformation of matter or energy; nevertheless, there exists systematic innovation through TRIZ which can be thought and used for those who want or are required to innovate and that does not require a special talent or mental gift. If innovation is not protected, it is at risk of being duplicated or plagiarized and this would lead to losing any right to it. The intellectual property protection in the form of patents, utility models and copyrights is a legal tool for the inventor that entitles him (her) to define the way of exploiting it.

International Biotechnology Color Journal

PURPLE: Intellectual property protection & Patenting

International Biotechnology Color Journal

Enzymes as biotechnological tools for the synthesis of natural food additives

Dolores Reyes-Duarte,

Artificios 40, 6o. piso. Col. Miguel Hidalgo. Deleg. Álvaro Obregón, México, D.F. C.P. 01120. (55)26363800 ext. 3854. E-mail: dreyes@correo.cua.uam.mx

The modification of natural antioxidants to improve their chemical, oxidative and/or thermal stability, or to alter their hydrophile–lipophile balance (HLB), yields a series of "semisynthetic" antioxidants with a great impact in industry. On the other hand, the synthesis of carbohydrate fatty acid esters has become of interest due to the wide range of applications in the food, cosmetics, oral-care, detergent and pharmaceutical industries.

The chemical synthesis of these derivatives generally uses harsh conditions with strongly corrosive acids or also bases at high temperatures, resulting in poor selectivity and the formation of undesirable by-products.

To overcome these shortcomings, new approaches based on the use of biological catalysts are being evaluated.

Biocatalysts are biodegradable, use mild reaction conditions, low energy requirements and display chemo-, regio- and/or stereospecificity resulting in decreased by-product formation thus avoiding the need for functional group protection and activation usually required in the chemical synthesis.

In this occasion, I will review the applications of enzymes such as lipases and esterases in the synthesis and modification of food additives as potential nutraceuticals like fatty acid esters of antioxidants, sugar esters and other natural products.

YELLOW: Food & Nutrition Biotechnology

International Biotechnology Color Journal

Metabolic engineering to increase the production of plasmid DNA vaccines by *Escherichia coli*

Dr. Alvaro R. Lara

Departamento de Procesos y Tecnología. Universidad Autónoma Metropolitana-Cuajimalpa, Artificios 40, Col. Miguel Hidalgo, Del. Alvaro Obregón CP 01120. México. Tel 26363804; fax 26363800, ext. 3832.

Plasmid DNA (pDNA) is the base for upcoming vaccines and gene therapy drugs. As the demand of pDNA is expected to grow, better *Escherichia coli* (the usual host for pDNA synthesis) strains for industrial production need to be developed. We have tested *E. coli* strains with a very low overflow metabolism for the production of pDNA in high cell-density cultivations in batch mode. The engineered strain has shown a superior performance compared to its parent. Further modifications in the activity of the pyruvate kinase resulted in improved pDNA production and reduced oxygen consumption. A second generation engineered strain was developed by mutating genes related to plasmid regulation and nucleotides synthesis and transport. Three mutations additional to the modification of the glucose transport system allowed increasing the pDNA yield nine times compared to the wild type strain. The second generation mutant produced more than twice as much pDNA than the commonly used DH5alpha strain. The mentioned results show the potential of rational modifications for increasing pDNA production by *E. coli*.

International Biotechnology Color Journal

Protein research in the XXI century

Eduardo Armienta-Aldana, Profesor - Investigador Titular "C"

Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, e-mail: eduardoarmienta@gmail.com

Protein research has shown a great diversification in the late twentieth century. It is no longer enough to know and understand the features and functions of proteins; today want to know how they work *in vivo*, understand their interactions and structural conformations in an active organism. This will allow us to have a better understanding of what they really are, and design proteins, both with structural and enzymatic functions. Progress has been made about the study and research of proteins, not only just the traditional electrophoretic and chromatographic analysis routinely performed by laboratories dedicated to the study of proteins. We have now covered more than 10 years of the new millennium and expectations in the study of proteins has grown exponentially, key examples, the Proteomics Unit of CINVESTAV-IPN, Campus Guanajuato (Mexico), which is one of the most modern in the country and also the Biostructural Nuclear Magnetic Resonance Unit of the Leloir Institute (Argentina), to name a few. What else can we expect in the coming years?, Perhaps only our imagination can give us that answer.

Genomics of airborne bacteria in megacities

Jaime García Mena.

Laboratorio de Genómica Ambiental, Departamento de Genética y Biología Molecular. Cinvestav-IPN Unidad Zacatenco.

Megacities all over the world congregate huge amounts of human beings in high density populated areas with activities imposing deleterious consequences for the environment and all aspects of their life. One of such problems are the outdoor bioaerosols which disperses high amounts of microorganisms during certain periods of the year. Megacities such as Mexico City, with a population of ca. 20 million inhabitants in its metropolitan area, reports data for several respiratory and gastrointestinal diseases, in which the infectious agent could be an airborne bacteria. We performed a continuous systematic analysis of the bacteriological content of the low outdoor atmosphere in Mexico City, following the airborne bacteria by microbiological and molecular biology methods, during the spring, summer, fall, and winter seasons. Bacterial diversity has been determined by metagenomic analysis of massively isolated DNA from the samples, and also by identification of single isolated bacteria. Our data recollection shows a reasonable expected diversity of the bacterial community in the analyzed bioaerosols but an unexpected variation in the type and abundance of the identified bacteria which suggest a correlation with the prevalence of certain diseases in this megacity during the year. This work has been financed by Cinvestav and ICyTDF.

IRIS: Multidisciplinary Biotechnology

Molecular structural bioinformatics of integral membrane proteins: the H3 receptor, a target for cognitive diseases

Charbel Maroun

CIRB, Collège de France. 11 pl. Marcelin Berthelot, 75014, Paris. FRANCE

G protein-coupled receptors (GPCRs) are a subclass of the seven-transmembrane helix proteins (7TM) that transmit signals to cells in response to stimuli, thus mediating physiological functions through interaction with hetero-trimeric G proteins. Many diseases involve the malfunction of these receptors, making them important drug targets. The histaminergic system plays a major role in cognition and the H3 GPCR plays a regulatory role in the pre-synaptic release of histamine by inhibiting its release in brain and making it an attractive target for CNS indications including cognitive disorders, narcolepsy, ADHD and pain. H3R inverse agonists, by suppressing this brake, enhance histamine neuron activity.

In spite of serious difficulties, the 3D structures of several GPCRs have recently been determined experimentally. Nevertheless, hundreds of sequences of interesting “druggable” GPCRs are still awaiting structure determination. Molecular modeling and simulation methods attempt to fill this gap by generating reliable 3D models of GPCRs. In order to study its behavior, once a receptor model is obtained, it is embedded in a phospho-lipid bilayer and surrounded by aqueous solvent and counter-ions, so as to constitute a realistic system comprising hundreds of thousands of atoms.

Here, we will describe and illustrate the modeling and simulation of the H3 receptor.

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GOLD: Bioinformatics e³ Nanobiotechnology

Germination of soybeans and its effect on chronic diseases

Robles-Ramírez María del Carmen, González-Espinosa Laura Aideé, Mora-Escobedo Rosalva

Departamento de Graduados en Alimentos, Escuela Nacional de Ciencias Biológicas, I.P.N. Prol. Carpio y Plan de Ayala S/N. Col. Sto. Tomás, México, D.F. C.P. 11340. Tel. (55)57296000 Ext. 62462. Corresponding author: rosalmora@hotmail.com

Soybean is an abundant source of proteins with high nutritional value and excellent physicochemical properties in foods, and also because it is a rich source of non-nutritive components with potential health benefits. Soy consumption has beneficial effects in the treatment of obesity due to its proven ability to decrease several markers, such as the amount of lipids stored inside adipocytes. On the other hand, extensive epidemiological, in vitro, and animal data suggest that soybean consumption reduces the risk of developing several types of cancer. However, little is known about the effect of germination on the antiobesity and anticancer properties. The aim of this work is to study the influence of soybean germination time (0, 2, 4, 6 days) on some obesity markers and on tumor growth both studies in mice. The results found indicate that a process as germination may help to increase antiobesity and anticancer properties. This effect can be correlated with the change in the protein pattern, and the increase of phytochemicals along the germination process.

Nutrigenetics and Nutrigenomics: Implications in Type 2 Diabetes

Laura del Bosque-Plata, PhD

National Institute of Genomic Medicine, Mexico

Nutritional genomics provides powerful approaches to decipher the complex relationship between bioactive molecules, genetic variation and biological system, and can help to develop personalized nutrition and dietary recommendations. The data and high-throughput molecular technologies generated through international genomic projects are generating an unprecedented opportunity to facilitate the understanding of diet-related diseases.The impact of nutrients can be evaluated through a multitude of 'omic' technologies and biomarkers. There is a significative familiar aggregation in type 2 diabetes (T2DM) and related phenotypes, among them are obesity, hypertension and dislypidemia; the aggregation of metabolic characteristics is probably due to genetic effects that interact with unique life style/environmental shared factors. It has been reported several interactions between genotypes and metabolic diseases. Dietary fat is a fundamental environmental factor that may interact with genetic determinants and several genes previously associated to T2DM have been reported to interact with dietary fat, PPARγ, TCF7L2, CAPN10. The discovery of genetic factors in T2DM will contribute to the knowledge of its pathophysiology and may help the understanding of gene-nutrients interactions in this disease. Personalized interventions according to genotype may be considered. Furthermore, the future progression of nutritional genomics in T2DM needs diverse "omics" studies in a systems-biology-orientated approach, and also studies in diverse animal and in vitro models.

Biotechnological approach to global warming effects on Agriculture.

Christian Alcocer, Francisco Espadas, Fabio Idrovo, Eduardo Blumwald[1], Gabriela Fuentes y Jorge M. Santamaría

Yucatan Scientific Research Center (CICY). [1]University of California, Davis

Global warming is a serious threat to food production worldwide. Most plant species die after a short exposure to temperatures close to 50 ºC . Summer temperatures at sites around the world are reaching 45 ºC lately. If the experimental data proves right at the level of whole crop, a scenario of desertification might occur at various regions of the world. Plants have evolved mechanisms to deal with excessive temperatures. Among those mechanisms at the cell level, the transcription factors called Heat Shock Factors (HSF) might play a key role in preventing cell death. From the Biotechnological view point, it is possible that the over-expression of this HSF might result in increased heat resistance in crops. In our preliminary experiments, papaya plants did not die even after 1 hour exposure to 50 ºC. This appears to be related with an increased expression in most of the HSF evaluated. Experiments are in progress now, to evaluate if plants that over-express this HSF, prove to show increased heat tolerance.

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BROWN: Geomicrobiology & Arid zones Biotechnology

Early stimulation on science and technology in basic school

Yozabed Marina Reyes Vidal, Ismael Ortiz Miguel, Tito Adabel Perez Felipe, Flor Garza Vargas, Gerardo Nuñez Medina, Maria del Carmen Urzua Hernandez, Susana Lozano Muñiz

[1]Universidad del Papaloapan. [2]Universidad Nacional Autónoma de Mexico, E-mail: susana_lozano@hotmail.com

In recent decades, Mexico has made significant progress with respect to the coverage of basic education, which has been achieved tanks to important advances in data recollection from the education system, right through the annual implementation of the test called The National Assessment of Academic Achievement in Schools (ENLACE, for its acronym in Spanish). This test was held in the different states of the Mexican Republic. This test focuses on the work strategies that allow the children in primary education to develop better intellectual, social and physical abilities. For example, with tools, very basic these days as it is a computer, from an early age, children begin to stimulate their mind as their activate their imagination and attention span. For that reason it is important to basically outline the material resources available to the School. Activities that can be made through the Information and Communication Technology (TIC´s) in education are, in general, motivating to the students, an encourage their playful character, by the use of visuals, colors and three-dimensional figures and hearing, among other advantages. We used National Institute of Statistics, Geography and Informatics (INEGI, for its acronym in Spanish) data to analyze the social situation, education and technology availability of the Mexican population. Early stimulation is the set of actions to promote the physical, mental and social aspects of child psychomotor retardation prevent, cure and rehabilitation of motor impairment, sensory deficits, intellectual disabilities, language disorders. However, to introduce these children in their midst, replacing the burden of a useless life for the joy of becoming a useful life and feelings of aggression, indifference or rejection in solidarity, cooperation and hope. Educational backwardness and poverty are the main factors that exist in the rural states, which affects the child population, and lead to a high index of infant mortality.

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Rogelio Rodríguez-Sotres

Faculty of Chemistry

Universidad Nacional Autónoma de México

Tel (+52)5556225285

Fax (+52)5556225329

e-mail: sotres@unam.mx

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