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BIOSAFETY PROGRAM

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NEWSLETTER

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SABP

The South Asia Biosafety Program (SABP) is an international developmental program initiated with support from the United States Agency for International Development (USAID). The program is implemented in India and Bangladesh and aims to work with the local governments to facilitate implementation of transparent, efficient and responsive regulatory frameworks that ensure the safety of new foods and feeds, and protect the environment.

SABP is working with its in-country partners to:

- Identify and respond to technical training needs for food, feed and environmental safety assessment.
- Develop a sustainable network of trained, authoritative local experts to communicate both the benefits and the concerns associated with new agricultural biotechnologies to farmers and other stakeholder groups.
- Raise the profile of biotechnology and biosafety on the policy agenda within India and address policy issues within the overall context of economic development, international trade, environmental safety and sustainability.

EVALUATION OF RABIES GLYCOPROTEIN EXPRESSED IN TRANSGENIC PLANTS FOR GLYCOSYLATION

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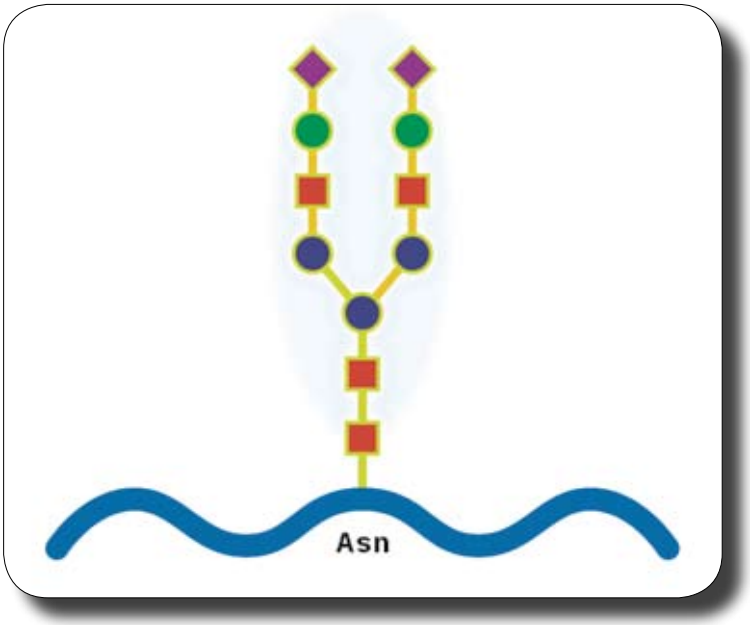
Under the Department of Science and Technology (India) and National Science Foundation (USA) (DST-NSF) programme, a research study was carried out to evaluate rabies glycoprotein expressed in transgenic plants for glycosylation. The study was carried out at the University of Agricultural Sciences in Bangalore, India and at Arizona Biodesign Institute in Tempe, Arizona,USA. The main objective of the study was to know whether glycosylation occurs in plant produced protein or not.

Transgenic plants have proven to be an efficient production system for the expression of therapeutic proteins. Differences in posttranslational modification such as glycoprotein have been one of the problems with plant derived proteins. In plants N-linked glycans and/or allergenic ß (1, 2) xylose (xyl) residues are attached to the proximal GlcNAc as for mammalian glycans. Plant glycans do not contain sialic acid residues and these residues may be important for successful topical passive immunization.

Protein glycosylation is a major posttranslational modification that generates a potentially large group of glycol forms from a single polypeptide chain. Since glycosylation can play an important role in the biological and pharmaceutical activity of the glycoprotein there is increasing interest in identifying and characterizing the oligosaccharide moieties of glycoprotein.

RABIES ANTIGEN GLYCOSYLATION

The rabies virus antigen contains three potential glycosylation sites in the extra-cellular domain (Anilonis *et al*., 1981). This glycoprotein is the only protein on the surface of the virus and facilitates the virus uptake via the host cell receptors. It is also the target for host immune responses. Non- or under-glycosylated glycoprotein fails to express on the cell surface indicating that proper glycosylation of the glycoprotein is critical for its expression and function (Burger *et al*., 1991; Shakin- Eshleman *et al*., 1992). Therefore it is important that recombinant expressed glycoprotein carries proper glycosylation for its cell surface expression and to be functionally active. The overall significance and expected implications of this research project are multiple and of particular interest is the future role of plants as producers of biomedically important macromolecules, especially those that require complex posttranslational modifications. Both N-linked and GalNac α-1 ser/thr type of O-linked glycosylation are critical for the biological activity of many proteins.



Diagrammatic representation of glycosylation of an amino acid residue in a protein. The amino acid chain (blue) has certain residues which are glycosylated by the addition of different sugar molecules (coloured blocks). The type and pattern of the attached sugar molecules are thought to have an effect on the antigenicity of proteins.

METHODOLOGY

Analysis of glycosylation status of recombinant rabies antigen

Glycan analysis of plant produced rabies glycoprotein antigen was carried out. The semi quantitative biotin labeled lectin-binding assay was performed to determine the presence of glycosylation at the antigen. The lectin blot analysis was carried out to determine the presence of various sugars. The lectins ConA, WGA, RCA and UEA II were used for compari-

(continued on page 2 - see Evaluation)

NEWS & EVENTS

CALENDAR OF EVENTS

Event	Organization	Date	Place
INDIA			
Management of IPR in Biotechnology	Department of Biotechnology (DBT) and Biotech Consortium India Limited (BCIL)	March 19 - 20, 2009	Confederation of Indian Industry, Chandigarh
Consultations on Biosafety Capacity Building Project – Phase II	Ministry of Environment and Forests (MoEF) and BCIL	March 27 - 28, 2009	CSIR Science Centre, New Delhi
One-day seminar on Biodiversity and Agribiotechnology	DBT and Jaypee Institute of Information Technology University, Noida	April 25, 2009	Jaypee Institute of Information Technology University, Noida
Bangalore BIO 2009	Department of IT and Biotechnology, Government of Karnataka and the Vision Group on Biotechnology	June 18 - 20, 2009	Bangalore
INTERNATIONAL			
Theoretical and practical course 'Developments in Biosciences for Enhanced Food and Environmental Biosafety'	Department of Molecular Biology and Biotechnology, Faculty of Science, University of Dar es Salaam, Dar es Salaam, Tanzania	August 18 to 30, 2009	Department of Molecular Biology and Biotechnology, Faculty of Science, University of Dar es Salaam
ABIC 2009: Agricultural Biotechnology for Better Living and a Clean Environment	National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Ministry of Science and Technology (MOST) and ABIC Foundation	September 22 - 25, 2009	Queen Sirikit National Convention Center, Bangkok, Thailand
Biosafety workshop on 'Theoretical Approaches and Their Practical Application in the Risk Assessment for the Deliberate Release of Genetically Modified Plants'	Wendy Craig (Biosafety Unit, ICGEB, Trieste, Italy)	October 12 - 16, 2009	ICGEB Conference and Meetings, Padriciano 99, I-34012 Trieste, Italy

Evaluation - *continued from page 1*

son. The standard rabies commercial antigen, Rabipur, was used for the comparison.

Carbohydrate analysis

The following steps were carried out for carbohydrate analysis using the DIONEX. Separation and purification of the glycoprotein sample by SDS-PAGE. Electro transfer of the glycoprotein from the gel to PVDF membrane. Acid hydrolysis and monosaccharide analysis of the purified glycoprotein on the membrane.

RESULT AND CONCLUSION

The recombinant rabies proteins from tobacco and muskmelon were analysed on SDS-PAGE gels and showed multiple bands, however column purified transgenic tobacco and muskmelon had a 68 KDa band similar to standard Rabipur vaccine. Two further SDS-PAGE gels were prepared, one for direct staining and another for western blotting, using PVDF membrane. The SDS-PAGE showed similar results upon staining with Coomassie blue stain and the other gel was used for western blotting onto PVDF membrane overnight using Biorad gel apparatus. The PVDF membrane was hybridised with a primary antibody (human rabies immunoglobulin, HRIg developed in mice) and a secondary antibody (goat anti human radish peroxidase conjugate). The western blot revealed binding to the commercial vaccine and also to the transgenic tobacco and transgenic muskmelon rabies glycoprotein. The commercial vaccine showed two bands instead of the single band in the transgenic. This may be because the commercial vaccine has the entire gene sequence of N and G whereas the transgenic plants were transformed with only the G protein gene.

The lectin blots were prepared using the lectins ConA / WGA/ RCA/ VVA and UEA II. The blot was cut in two after marking the protein on the membrane. One portion was used for acid hydrolysis to release the carbohydrates. The carbohydrate analysis showed that both the standard and transgenic contain monosaccharide sugars of galactose and glucose. The analysis of the rabies glycoprotein produced in tobacco and muskmelon showed glycosylation of the protein similar to the commercial vaccine. Hence we conclude that rabies vaccine can be produced in plants for commercial production.

Acknowledgement: I acknowledge Department of Science and Technology, New Delhi and National Science Foundation, USA for providing the grant. I also thank Dr. Lokesh Joshi and Dr. Vinay Nagaraj and Dr. Charles J Arntzen, Arizona Biodesign Institute, USA for their help.

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Algis Anilionis, Wunner, W.H. and Curtis, P.J. (1981). Structure of the glycoprotein gene in rabies virus. *Nature* **294(5838)**: 275-278.

Burger, S.R., Remaley, A.T., Danley, J.M., Moore, J., Muschel, R.J., Wunner, W.H., and Spitalnik, S.L. (1991). Stable expression of rabies virus glycoprotein in Chinese hamster ovary cells. *Journal of General Virology* **72**: 359–367.

(continued on page 4 - see references)

CREAM OF THE (WEB) CROP

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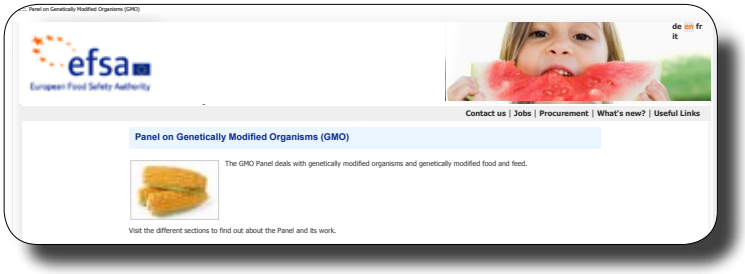
THIS MONTH'S PICK:

European Food Safety Authority (EFSA)

Panel on Genetically Modified Organisms (GMO)

http://www.efsa.europa.eu/EFSA/ScientificPanels/efsa_locale-1178620753812_GMO.htm



EFSA's role within the European regulatory framework for GMOs is to carry out scientific risk assessments or give scientific advice on GMOs. The GMO Panel provides independent scientific advice to the EFSA on the safety of:

- genetically modified organisms (GMOs) such as plants, animals and micro-organisms, on the basis of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms; and
- genetically modified food and feed, on the basis of Regulation (EC) No 1829/2003 on genetically modified food and feed

The Panel carries out risk assessments in order to produce scientific opinions and advice for risk managers. Its risk assessment work is based on reviewing scientific information and data in order to evaluate the safety of a given GMO. This helps to provide a sound foundation for European policies and legislation and supports risk managers in taking effective and timely decisions. The Panel carries out much of its work in the context of authorisation applications, since all GM food and feed products must be evaluated by EFSA before they can be authorised in the European Union (EU).

Subjects covered on the website include:

WHAT WE DO: An outline of how the Panel works and details on its four main areas of activity.

- Risk assessment of GM food and feed applications.
- Development of guidance documents.
- Scientific advice in response to ad-hoc requests from risk managers.
- Self-tasking activities under which, on its own initiative, the Panel identifies scientific issues related to GMO risk assessment that require further attention. For instance, the Panel has produced a scientific report on the use of animal feeding trials in GMO risk assessment.

TOPICS A-Z: Subjects covered are (1) Antimicrobial Resistance, (2) Feed and (3) Genetically Modified Organisms. Information includes a definition of the subject and a summary of how it is dealt with under the mandate of the Panel and its place in the European Union regulatory framework.

PANEL MEMBERS: A summary of the appointment process and a list of Panel members.

WORKING GROUPS: Lists of members of the 11 working groups and minutes of meetings.

OPINIONS: Links to the latest Panel risk assessment opinions and a searchable archive of past opinions.

STATEMENTS: Links to the latest Panel official statements and a searchable archive of past statements.

GUIDANCE: Links to the latest Panel guidance documents and a searchable archive of past guidance documents.

GMO APPLICATIONS: A summary of the application process for EU approval of a GMO. Includes a list of the most recent scientific risk assessments submitted by the EU to the Panel, a searchable database, known as the "Register of Questions", which provides information on the progress, deadlines and status of the EU requests for risk assessment.

OTHER SCIENTIFIC DOCUMENTS: Links to official documents other than scientific opinions such as statements, reports and advice that have been adopted by the Panel.

REQUESTS AND MANDATES: Requests for opinions are received by EFSA mainly from the European Commission and occasionally from the European Parliament. Member States can also request opinions. A request outlines what is being asked of EFSA: the issue, the terms of reference, the timeframe, *etc*. Information about each request, including supporting documents and status, is available in the Register of Questions database, which is accessible through this section.

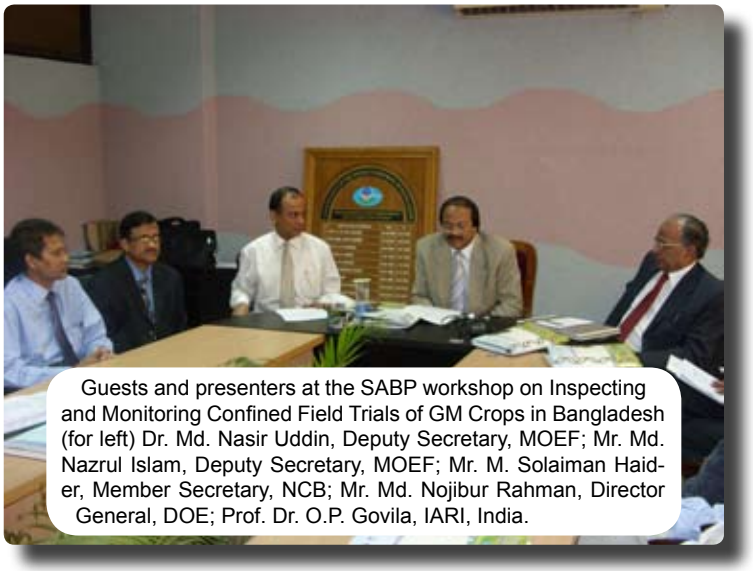
references - *continued from page 2*

Shakin-Eshleman, S.H., Remaley, A.T., Eshleman, J.R., Wunner, W.H. and Spitalnik, S.L. (1992). N-linked glycosylation of rabies virus glycoprotein. Individual sequons differ in their glycosylation efficiencies and influence on cell surface expression. *Journal of Biological Chemistry* **267**: 10690-10698.

SABP BANGLADESH GM FIELD TRIAL INSPECTION WORKSHOP

A day-long South Asia Biosafety Program (SABP) workshop on the Inspection and Monitoring of Confined Field Trials of Genetically Modified Crops in Bangladesh was held at the Bangladesh Department of Environment (DoE) on February 19, 2009. Among the participants were eight of the nine members of the Field-Level Biosafety Committee (FBC) assigned to inspect field trials of transgenic potato currently in progress in Bangladesh. Also present was Mr Solaiman Haider, Secretary to the National Committee on Biosafety and Member Secretary of FBC, and staff from Bangladesh Agricultural Research Institute who may be FBC Monitoring Officers for future trials.

Mr. Md. Nojibur Rahman, the Director General of DoE was the Chief Guest and assured the participants of his full support and cooperation in strengthening the capacity of DoE so that the field trial experiments with GM crops can be conducted in safe and sound manner. Mr. Haider welcomed the participants and guests and gave an overview of the FBC and its function as well as a summary of SABP's role in the biosafety programs being implemented by DoE. Prof. M. Imdadul Hoque, SABP Bangladesh country coordinator, outlined the workshop programme and objectives and provided support to the main workshop presenter, Dr. O.P. Govila, former Professor of Genetics, Indian Agricultural Research Institute and a member of numerous review and inspection teams for confined field trials in India.



Guests and presenters at the SABP workshop on Inspecting and Monitoring Confined Field Trials of GM Crops in Bangladesh (for left) Dr. Md. Nasir Uddin, Deputy Secretary, MOEF; Mr. Md. Nazrul Islam, Deputy Secretary, MOEF; Mr. M. Solaiman Haider, Member Secretary, NCB; Mr. Md. Nojibur Rahman, Director General, DOE; Prof. Dr. O.P. Govila, IARI, India.

The purpose of the workshop was to train Monitoring Officers from the FBC to inspect confined field trials. The training, which is based on the Manual for Inspectors developed by SABP and currently before the National Committee on Biosafety for approval, included a review of the roles and responsibilities of Monitoring Officers and the standard operating procedures by which confined field trials should be conducted in Bangladesh. At its conclusion the participants, most newly appointed members of the FBC, expressed satisfaction with what they had learned and the new-found comfort it had provided them regarding the role of the FBC in monitoring confined field trials of transgenic crops.

200,000 RICE MUTANTS AVAILABLE WORLDWIDE FOR SCIENTIFIC INVESTIGATION

Science Daily - March 10, 2009

Scientists across the world are building an extensive repository of genetically modified rice plants in the hope of understanding the function of the approximately 57,000 genes that make up the genome of *Oryza sativa*. The International Rice Functional Genomics Consortium recently announced the public availability of more than 200,000 rice mutant lines, which represent mutations in about half of the known functional genes mapped for rice to date. [* Krishnan A, Guiderdoni E, An G, Hsing YI, Han CD, Lee MC, Yu SM, Upadhyaya N, Ramachandran S, Zhang Q, Sundaresan V, Hirochika H, Leung H, Pereira A (2009) *Plant Physiology* **149(1)**: 165-170.]

Researchers have estimated the number of different rice mutants needed to have a mutant for every gene as somewhere between 180,698 and 460,000. Two hundred thousand rice mutants are now available and have been mapped by the insertion of what are known as flanking sequence tags – small pieces of DNA or molecular tags that integrate into the rice genome. This approach is useful because it allows scientists to link a physical location on the genome to a specific gene and its visible feature or phenotype.

Arjun Krishnan, first author on the paper and a graduate student in Andy Pereira's laboratory at the Virginia Bioinformatics Institute, stated: "Bioinformatics is making it possible to visualize the vast amounts of sequence information available to researchers. The resources described in this paper, which are the combined output of many leading international rice research laboratories, mean that researchers can see and explore on their computers the precise positions of mutations in the rice genome sequence, for each rice mutant plant. About 50 percent of the protein-coding genes have knockout mutations, which probably abolish their expression and can provide valuable information on the genes by virtue of their loss of function. This is a significant milestone for the project and the availability of these rice plants represents a powerful resource for the rice genomics community."

See the full article at
http://www.agbios.com/static/news/NEWSID_10508.php

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