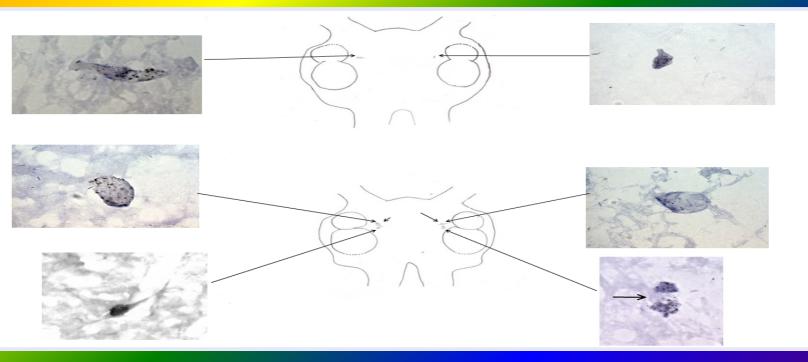


A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY and Covering Its Many Hues, Tints, Tones & Shades



Regular issue: mRNA PDH expression in the brain of crayfish Procambarus clarkii

Produced and hosted by *Centro de Investigación Científica de Yucatán, A.C.*, in collaboration with *Universidad Nacional Autónoma de México*, and the *International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society* Int. biotechnol. color j., ISSN 2226-0404

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### The INTERNATIONAL BIOTECHNOLOGY COLOR JOURNAL (IBCJ)

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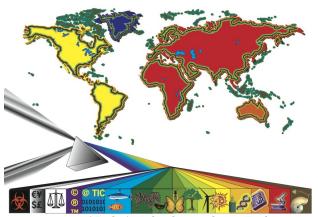
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Its main goal is to help bringing together efficient biotechnological solutions for many human problems, with the need for environmentally friendly and sustainable processes.

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Susana Lozano Muñiz President of the Foundation



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### **Editorial section**

International Biotechnology Color Journal (IBCJ) is an electronic Open Access journal, devoted to the publication of peer-reviewed articles covering all the fields of biotechnology.

With this number we celebrate our first year of publication with main focus on the dissemittion of scientific peer reviwed papers. In addition ICBJ intent is to provide 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. The present issue offers a sample of such diversity.

Instructions for every type of contribution are presented in the journal's Homepage and at the end of the present issue. The Editorial Board of IBCJ is fully committed to publish novel contributions in all areas of biotechnology. Submissions are reviewed from a rigorous optic of scientific criticism and all original contributions within the scope of the journal are welcomed.

If you are interested in participating as reviewer, please send us a letter, and a CV stressing your experience in the filed. Send this information by e-mail to "Dr. Jose Juan Zúñiga Aguilar" <zuniga@cicy.mx>

### Editorial comments to the contents of this issue

### By José Juan Zúñiga-Aguilar, Chief editor.

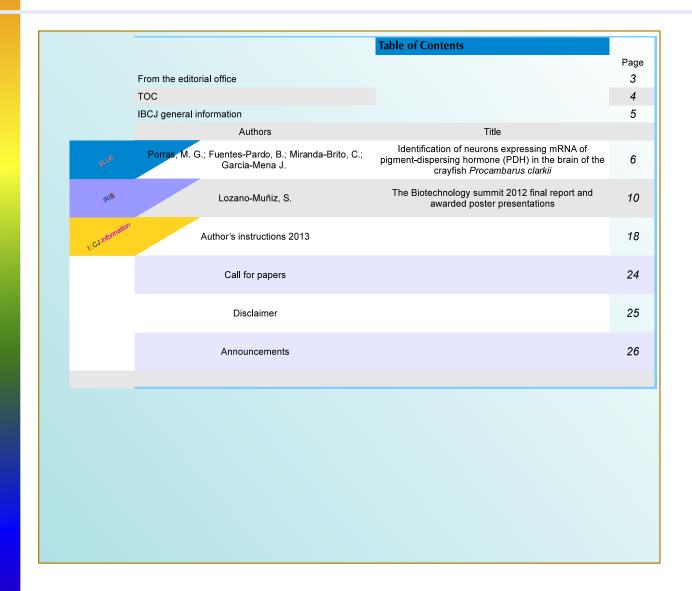
In this issue, Porras-Villalobos *et al.* cloned a cDNA segment of a pigment-dispersing hormone (PDH) beta isoform from the brain of the crayfish *Procambarus clarkii*. PDH peptides from many crustaceans have been isolated and characterized, but information about their gene expression is scarce. The authors presented here an interesting studio whose results about the spatial and temporal expression of the  $\beta$ -PDH isoform support its role as a key element of the circadian clock of crustaceans.

In the second contribution, the president of the "International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A. C.", Dra. Susana Lozano-Muñiz, presents the report of the Biotechnology Summit 2012, which was organized at the City of Merida, Yucatan. With special relevance, she informs that the Best Poster Presentation Awards was granted as recognition of the quality of the students' work currently developed in biotechnology. Winner groups are working in different but strategic topics exposing a broad area of interests either in medicine, biofuels or in vitro tissue culture, which represent a clear sample of the diversity of themes exposed in the congress.

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Regular Issue, October 2012 Editorial Board:

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Faculty of Chemistry, Universidad Nacional Autónoma de México

Tel (+52)5556225285; Fax (+52)5556225329; e-mail: sotres@unam.mx

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### Identification of neurons expressing mRNA of pigment-dispersing hormone (PDH) in the brain of the crayfish Procambarus clarkii

### Mercedes G. Porras<sup>a,\*</sup>, Beatriz Fuentes-Pardo<sup>a</sup>, Carolina Miranda-Brito<sup>b</sup>, and Jaime García-Mena<sup>c</sup>

- <sup>a</sup>Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, Mexico City, Mexico. E-mail: mg60@unam.mx
- <sup>b</sup>Departamento de Fisiología, Biofísica y Neurociencias. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Cinvestav), Unidad Zacatenco, Mexico City, Mexico.E-mail: cmiranda@cinvestav.mx
- <sup>c</sup>Departamento de Genética y Biología Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Cinvestav), Unidad Zacatenco, Mexico. E-mai l:jgmena@cinvestav.mx

\*To whom all correspondance should be sent

Chief Editor: José J. Zúñiga–Aguilar. Area Editor: Dr. Diego González Casati. Received: june 15, 2012. Revised version: september 30, 2012. Accepted: October 17, 2012.

### ABSTRACT

The expression of pigment-dispersing hormones (PDHs) have been reported in some crustaceans, however information on the nature of the genes and transcripts is limited. In this work we report the cloning of a portion of a messenger RNA encoding the isoform  $\beta$ -PDH, as well as the expression of this transcript in ten to eleven somata localized in the brain of the crayfish *Procambarus clarkii*, and the variations in 24 h of brain transcript expression. This  $\beta$ -PDH isoform is homologous to that found originally in the eyestalks of the crab *Uca pugilator*.

\*Contact address: Mercedes Graciela Porras Villalobos, Departamento de Fisiología, Facultad de Medicina, UNAM, Av. Universidad 3000 Universidad Nacional Autónoma de México, Delegación Coyoacán CP 04510, México City, México.

E-mail: mg60@unam.mx

Tel. 52 (55) 56 23 22 66, Fax 52 (55) 56 23 23 95

Abbreviations: PDH, Pigment dispersing hormone; PDFs, Pigment dispersing factors; ISH, In situ-hibridization; DIG, Digoxigenin; ERG, electroretinogram

Key words: crustacean, crayfish, mRNA, brain, eyestalk.

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### INTRODUCTION

The Pigment-dispersing hormone (PDH) is a key element in the circadian clock machinery of invertebrates. In crustaceans PDHs function as humoral promoters of tegument pigment migration (1,2); they are also proposed to be multifunctional peptides acting as local neurotransmitters/modulators (3,4). In insects, PDH peptides known as Pigment-dispersing factors (PDFs), having been identified in neurons exhibiting circadian variations; they have been proposed as central pacemakers (5,6).

The hormone PDH was initially isolated from eyestalks of shrimp *Pandalus borealis* (7), ( $\alpha$ -PDH, NSGMINSILGIPRVMTEAamide). In 1985, Rao and co-workers purified PDH from eyestalks of fiddler crab *Uca pugilator* ( $\beta$ -PDH, NSELINSILGLPKVMNDAamide); the classic crab isoform called  $\beta$ -Pigment dispersing hormone ( $\beta$ -PDH) (2). Since that time, all known crustacean PDH isoforms once purified or cloned from the eyestalk of crabs (2,7,8,9,10), crayfish (1,11), shrimp (12,13), or lobster (14).

The aim of this study was to identify the cells in the brain of Procambarus clarkii in which the mRNA PDH is expressed. Therefore, we characterized the mRNA encoding PDH in the brain of P. clarkii and used a complementary RNA (cRNA) PDH probe for In-situ hybridization (ISH). By real-time RT-PCR, we quantified in one day the level of  $\beta$ -PDH mRNA expression in the brain of crayfish P. clarkii at different times during the day. The results presented here show 10 somata in clusters 9 and 11 of P. clarkii deutocerebrum expressing β-PDH mRNA. We found a maximum expression of β-PDH mRNA in the brain of P. clarkii at 6:00 am (circadian time 0, CT0), and the minimum at 12:00 am (circadian time 18, CT18). Based on the DNA sequence, the inferred amino acid sequence we found in P. clarkii's brain is the same originally (β-PDH found in  $U_{\cdot}$ pugilator eyestalks NSELINSILGLPKVMNDA-amide), and different from that isolated from P. clarkii eyestalk (1) (NSELINSILGLPLVMNEAamide).

Adult crayfish *P. clarkii* were collected from the Mexican state of Chihuahua. Animals were maintained in an aerated aquarium at a constant temperature (16°C) and under a photoperiodic regime of 12 h of Light and 12 h of Darkness (LD 12:12) To know the Circadian time (CT) of the population, we chose a random sample (five animals) from the crayfish pool and recorded the Electroretinogram (ERG) rhythm of each individual crayfish. For tissue collection, crayfish were anesthetized with packing ice for 10-15 min. For ISH, the brains were isolated by micro-dissection, immediately placed in liquid nitrogen, and 12- $\mu$ m slices of tissue were made.

PCR amplification of the cDNA encoding the PDH precursors was performed with cDNA as template, obtained by reverse transcription of total RNA. The degenerated forward PPF-primer (5'-CACAAGCGCAACTCNGAGC-3') and the degenerated reversed PPR-primer (5'-TTATCTCCBCCGGCVTCG-3') were used. After an initial denaturation step of 5 min at 95°C, the PCR reaction was carried out for 35 cycles with a denaturation step of 30 sec at 95°C, an annealing step for 30 sec at 58°C, and an extension step of 5 min at 72 °C. *In-situ* hybridization (ISH) was performed as described (15).

### RESULTS

A 75 pb predicted amino acid sequence for mRNA-PDH ( $\beta$ -PDH) is shown in the comparative alignment (first sequence, Figure 1) (Accession numbers FJ389457). Probing for PDH encoding mRNA containing cells in the brain of *P. clarkii* by the ISH method is depicted in Figure 2. The label of pdh-DIG probe was restricted to the pericarya and detected in dorsal and ventral regions in the deutocerebrum. According with Sandeman's nomenclature (16) somata were found in clusters 9 and 11. An extended PDH mRNA-DIG signal (~50 µm) corresponding to spliced kit three to four somata were found in bilateral–cluster–11 of the dorsal region (Fig 2A); a strong signal was found in a single cell ~20 µm localized in the opposite lobe of brain (Fig 2B). When exploring the ventral region of the deutocerebrum, two large PDH mRNA-DIG-labeled ~45 µm somata were identified in bilateral cluster 9, (Fig 2C and 2D); a small cell of ~15 µm was intensely labeled (Fig 2E). Two

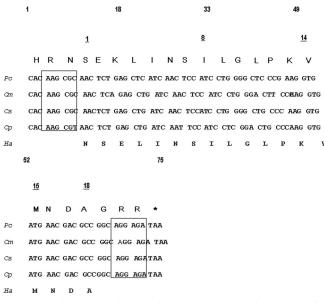
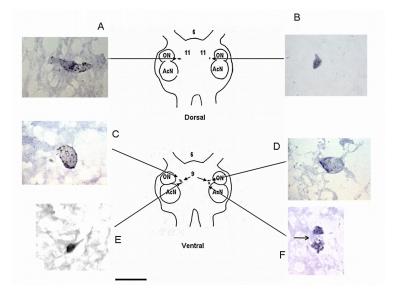


Figure 1. Alignment of selected DNA sequences of Pigment- dispersing hormones (PDH)s. Capital letters on top, indicate the predicted amino acids for the DNA sequence; capital letters underneath indicate the amino acids for the sequenced 18aa PDH peptide isolated by High performance liquid chromatography (HPLC) from the American lobster Ha, *Homarus americanus* (20). Pc, *Procambarus clarkii* Accession number FJ389457 (this work); Cm, *Carcinus maenas* (18); Cs, *Callinetes sapidus* (19); Cp, *Cancer productus* (16) Translation stop codons are indicated by an asterisk.

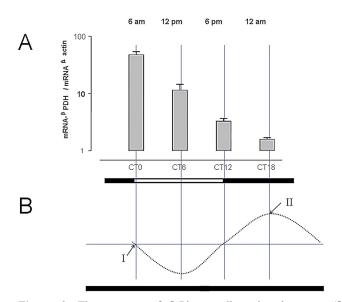
cells containing the PDH encoding mRNA were found in the opposite site close to the optical and accessory nuclei (Fig 2F). Figure 3 exhibits the variations of mRNA- $\beta$ -PDH expression in the brain measured by real-time RT-PCR in one day. The maximum expression level of the transcript 47.52 (±6.53) was obtained at CT0. Lower mRNA- $\beta$ -PDH levels 1.58 (±0.24) were obtained at CT18. In Figure 3B, the dotted line displays the circadian variation profile of the ERG determined as previously reported (17). It is observed that the minimum level of mRNA- $\beta$ -PDH expression detected matches with the maximum amplitude (arrow II) of the ERG, which is dependent on natural oscillations of groups of photoreceptors cells (18). The "middle point" of photoreceptor activity in the eyestalk is given when the ERG recording crosses the X axis (arrow I); this point is in agreement with the mRNA- $\beta$ -PDH maximum level expression in the brain.



**Figure 2.** *In-situ* hybridization of the dorsal region of the deutocerebrum of *P. clarkii*. The figure shows a sagittal section of *P. clarkii* brain probed with pigment-dispersing hormone (pdh)-Digoxigenin (DIG) which revealed sites of synthesis of PDH messenger RNA (mRNA). A) In dorsal region, cluster of at least four cells showing a strong signal localized proximal to the optical and accessory nuclei. B) In dorsal region a single soma with intense signal proximal to the same nuclei in the opposite anatomical area. In ventral region variable size of somata are located in between the vicinity of optical nucleus (ON) and accessory nucleus (AcN). Numbers 6 and 11 indicate anatomical areas according to Sandeman. ON, Optical nucleus; AcN, Accessory nucleus; Black bar scale is equivalent to 50 μm.

### DISCUSSION

PDH modulates spontaneous electrical activity of the brain and synchronizes EGR in *P. clarkii* (17). By other side, variation in the ERG amplitude in the visual system of *P. clarkii* has been reported (19). On measuring the electrical activity in the brain of this crayfish, some rhythm patterns in spontaneous multiunit activity and visual evoked potentials (VEPs) were found (20) These rhythms are 180° out-of-phase one to another. The rhythm of VEPs showed the main peak at midnight and is in closed phase relationship with the ERG amplitude rhythm (20); in addition, physiologically, this peak in the amplitude of the ERG signifies the highest natural oscillation activity of photoreceptor cells (9). In *P. clarkii* brain, the lowest level of mRNA-PDH synthesis coincides with the midnight peak of the VEPs.



**Figure 3.** Time course of β-Pigment-dispersing hormone (β-PDH) messenger RNA (mRNA) expression in 24-h. A) Graphic shows a determination of transcript concentration by quantitative Polymerase chain reaction (qPCR). The x-axis scale shows the day time (CT) in hours; the y-axis scale shows the ratio between β-PDH vs β-actin mRNA concentration. B) Graphic of variation profile of the Electroretinogram (ERG) in eyestalk photoreceptors of *P. clarkii*. The dotted line shows ERG minimum amplitude at CT6 and ERG maximum amplitude at time CT18 measured in synchronized animals to 12:12h light/dark condition cycle for 5 weeks prior to the experiments. The x-axis scale black bar shows time in hours and the dark light condition when the experiment was performed; the y-axis scale shows the ERG amplitude in μV. Arrow I indicates ERG amplitude at maximum β-PDH expression.

### CONCLUSION

In *P. clarkii*, the mRNA-PDH-expressing cells identified may be considered as molecular substrate of a circadian control system (oscillatory pacemakers) with PDH acting as a rhythm transmitter to distal brain areas and eyestalks in the crayfish. Electrophisiological study might provide evidence for neurons in *P. clarkii* deutocerebral area that produce an isoform of PDH, may constitute circadian oscillators of *P. clarkii* brain.

### ACKNOWLEDGMENTS

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## **BIOTHECHNOLOGY SUMMIT, 2012.**

### **REPORT OF THE EVENT**

Susana Lozano-Muñiz\*

Instituto de Biotecnología, Universidad del Papaloapan, campus Tuxtepec, Tuxtepec, Oax. México

\*To whom all correspondence should be sent.

### Notes from the President of the Foundation

The Biotechnology summit 2012 (BS12) was the first General Meeting of the International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C. a Nonprofit Organization, was sheeduled this year for March 12-21 in Mérida Yucatán México. The organizing committee developed an extensive program highlighting not only recent progress in biotechnology, but also the trends in biotechnology for future commercial application. The Biotechnology summit is an event inclusive of all biotechnology-related topics, from issues of bioethics, biotechnology applications in basic and applied research. The areas were color-coded, following the color codes used by the IBCJ.

\*Contact address: Instituto de Biotecnología, Universidad del Papaloapan, Campus Tuxtepec, C. Circuito central No. 200, Col. Parque Industrial.C.P. 38301, Tuxtepec, Oax.

Tel. 01(287)8759240 ext 220. E-mail: susana lozano@hotmail.com

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# Remarks by the President of the Foundation

We hope that the biotechnology summit 2012 has contributed to the updating, distribution, intercom and improvement of biotechnology and research activities in Mexico and other countries, so as to boost the development and management of communication, collaboration academic and productive. In biotechnology summit 2012 discussion on the grounds and actions aimed at developing new processes, products and benefits for different needs and regions of our country were undertaken. We choose to amalgamate into one place the various areas of application of biotechnology, in order to provide all participants, but especially young biotechnologists, with a forum for the interaction between academic researchers and industry in the field of Biotechnology. The diversity of all areas of biotechnology and sustainable development, viewed from a scientific, technological and productive, made the event a chalenge, but also an opportunity.

As highlights of this year' program, the 2012 summit's Biotechhnology Scientific Sessions comprised 15 areas/color of biotechnology and its applications, and one core symposium: "Strategies to Monitor and Reduce Resistance to Bacillus thuringiensis among Targeted Insects".

### **Other Symposia**

### Symposia with Participation of Students

1. Effect of temperature and protease inhibitors on the proteases of sea cucumber (*Isotichopus fuscus*) (Hernández *et al.*).

2. Pathogenicity of *Isaria fumosorosea* on immature whitefly *Bemisia tabaci* (Hemiptera:Aleyrodidae) (Ruiz *et al.*).

3. Study of the fermentative capacity and ethanol production of two microorgnisms isolated from bovine rumen (Estrada-Martínez *et al.*).

4. Morphological, biochemistry and molecular characterization and selection of genotypes with flesh color red orange in *Carica papaya* L. (Vázquez *et al.*).

5. Differential induction and repression patterns of  $\beta$ -fructofuranosidases of *Aspergillus niger* in submerged and immobilized culture (Trenado-Uribe *et al.*).

6. Study of BMP-15 and BMPR-1B Gene Polymorphism in West African Sheep of Yucatán (Couoh-Puga *et al.*).

### Workshops Within the Conference

1. Somatic embryogenesis and embryo rescue - Preparation and in vitro propagation of disease-free plants.

In charge of Dr. Hector Gonzalez-Rosas, COLPOS.

2. Every Day We Learn Biotechnology

In charge of the National Research and Technology Transfer for Sustainable Rural Development (SNITT) of SAGARPA (Mexican government).

### **Organizers and Participants**

In the organization of the event several committees were involved (Fig. 1). The Organizing Committee, responsible for all the planning, logistics, and communication with the attendees. The Scientific Committee, responsible for the quality of scientific presentations and symposia within the event. The organizers of the individual symposia and workshops and the Meeting staff, in charge of the technical details, and providing help to the attendees.

Ma del Carmen Montes-Horcasitas

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Workshops Organizer:

### Meeting staff

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### Figure 1. List of participants in the organization of the event.

The people involved in every one of the above committees is listed in figure 1.

Figure 2 shows a picture of the presidium at the Summit's opening ceremony and figure 3 is a picture of some of the participants. The participation of authorities form the Mexican Government reflects the recognition acknowledged to the event by the local authorities.

There were 303 assistants and 58 poster contributions from six areas/color plus 16 oral presentations (74 contributions). The Abstracts to these oral presentations were published in the February issue (vol. 2, issue 1, 2012) of IBCJ. For the areas associated to each color please refer to the last section of this issue at pg. 19.

José Martín González Llanes (SMBBY)

A statistical summary of the number of contributions by color/area is given in figure 4.

### Funding

In addition to the registration fees and the contribution by all the attendees, the event was possible thanks to the financial aid provided by several organizations and companies. Those companies and institutions providing financial support to the event are included in figure 5. The organizers are indebted to all of them.

	MaryAnn Principato	DT FDA
COL-POS Tab	Maximiliano Wilda	CONICET
West Visayas State University	Miriam de la Garza- Ramos	FO CDICS UANL
UAM-Iztapalapa	Monica Rosales-Pérez	Asoc Poblana de Cienc Micro AC
CIBA IPN	Muhammad Azhar	Microbiologist Islamabad, Pakistan
National University of San		Univ. of

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Addy Leticia Zara-García UAC

Garcia

FCQ-UNACH

Centro de Inv

BUAP

UACH

Baylor College of Medicine

Micro del ICUAP

Alejandro Ruíz-Sánchez

Antonio Rivera

Blanca E Sanchez-

Bautista-Muñoz

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Figure 2. OPENING CEREMONY (Monday March 19) - Welcome and Introduction of the Presidium. From left to right Dra. Leticia Olivera Castillo (Master of Ceremony, CINVESTAV IPN Mérida), Dr. Jaime Padilla Acero (Scientific Director of AgroBio México), Dra. Marcela Zamudio Maya (Director FIQ-UADY), Dr. Inocencio Higuera Ciápara (director of CICY representative from CONACYT), Dra. Susana Lozano Muñiz (president of the Foundation), Dr. Tomás González Estrada (Director General of CONCYTEY representative of the Governor), M.C. Jaime Piña Razo (Director of INIFAP Southeast) y M.C. Abel Zapata Dittrich (Director of Instituto Tecnologico de Merida). As special guest: Dr. Romeo de Coss Gómez (director CINVESTAV-Mérida), Dra. Ingrid M. Rodríguez Buenfil (CIATEJ Unit Southeast Director, M.C. Roger F. Vázquez Aguilar (IT Conkal Director), Dr. Jorge Zavala (Director CIR-Hideyo Noguchi)..



Figure 3. Some Participants. From left to right Carlos Alberto Blanco (USA), Zhong-Ren Lei (China), Ryan W. Kurtz (USA), Susana Lozano-Muñiz (Mexico), Elena Elpidina (Rusia), Patricia Tamez-Guerra (Mexico), Alejandra Bravo (Mexico), Clara Inés Saldamando-Benjumea (Colombia), Ana María Vélez (Colombia), Ingeborg Zenner de Polonía (Colombia), Nicholas Storer (USA), Robert W. Behle (USA), Jeffrey A. Fabrick (USA), Miguel Serrano (Honduras), Isaac Oyediran (USA), Juan Luis Jurat-Fuentes (España), Jaime Padilla Acero (Mexico), Mario Soberón (Mexico), Bruce E. Tabashnik (USA), Nicholas P. Storer (USA), J. Angel Saavedra (Mexico), J. Lindsey Flexner (USA), Michael Caprio (USA), Anthony M. Shelton (USA), Yulin Gao (China).

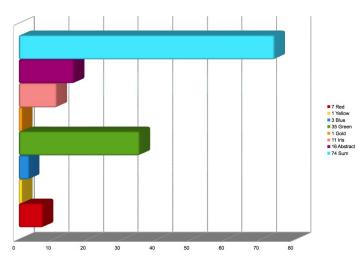


Figure 4. Number of presentations by color (area).

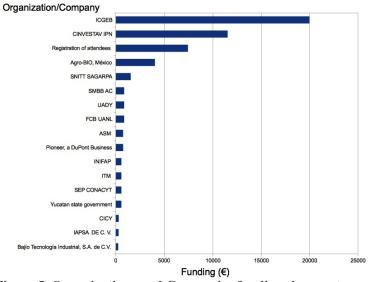


Figure 5. Organizations and Companies funding the event.

### **Best Poster Presentation Awards**

Presentations at poster sessions were evaluated by the organizers, and the best porter presentations were awarded:

First place

### CUCURBITA FICIFOLIA FRUIT AS INSULIN SECRETAGOGUE IN RINM5F CELLS

- María Elizabeth Miranda Pérez<sup>1</sup>, María Del Carmen Escobar Villanueva1, Jesús Vladimir Hernández Rosado<sup>1</sup>, Fausto Sánchez Muñoz<sup>1</sup>, Julio César Almanza Pérez<sup>1</sup>, Francisco Javier Alarcón Aguilar<sup>1</sup>, Clara Ortega Camarillo<sup>2</sup>
- <sup>1</sup>Laboratorio de Farmacología Universidad Autónoma Metropolitana unidad Iztapalapa (UAM-I) Avenida San Rafael Atlixco 186 Vicentina, 09340 Ciudad de México, Distrito Federal. <sup>2</sup>UIM Bioquímica, Hospital de Especialidades, CMN SXXI, IMSS. Corresponding author: *mirandapme@hotmail.com*

Abstract: Taking into account that the diabetes mellitus (DM) is an important public health problem globally, like part of a work to development a product added with an aqueous extract of Cucurbita ficifolia fruit with a possible use to the treatment of DM, a aqueous extract of this fruit was obtained and chemically characterized by it's content of D-quiroinositol, the principal hypoglycemic compound of the fruit. To study the mechanism of hypoglycemic activity of this extract and of the D-quiroinositol alone, RINmF5 cells were exposed to different concentrations of both, and production insulin and Kir 6.2 channels were measured. Cells treated with D-quiroinositol and C. ficifolia increased mRNA expression of insulin and Kir 6.2 compared with control group, suggesting a mechanism of action throughout of increment in the expression of the gen of insulin. This research supports the idea of develop a new nutraceutic product from C. ficifolia fruits like a co-adjutant in the treatment of DM. Keywords: DM /Insulin /Kir 6.2 /C. ficifolia

Second place:

# AXENICESTABLISHMENTANDINVITROFORMATIONOFADVENTITIOUSSHOOTSINNARDO(POLIANTHES TUBEROSE L.)

Addy Yolanda Tejero Peña<sup>1</sup>, Luis Leonardo Pinzón López<sup>1</sup>, Eduardo Villanueva Couoh<sup>1</sup>, Arturo Reyes Ramírez1, Delfino Reyes López<sup>2</sup>.

<sup>1</sup>Instituto Tecniologico de Conkal, Yucatán, México. <sup>2</sup>Benemérita Universidad Autónoma de Puebla, México. Corresponding author: *addy\_210505@hotmail.com* 

Abstract: The nard (Polianthes tuberosa L.) is a plant endemic to Mexico that is used in the pharmacological and fragrances industries, as well as ornamental plant (Herrera, 1990). In ornamental exploitation, the species offers little or no genetic variability, which reduces the opening of new markets, limiting their profitability. In this context. biotechnology through genetic transformation or induced mutagenesis, offer possibilities for breeding of new varieties. However, techniques depend for both their success of micropropagation protocols that enable there generation and mass propagation of new varieties. Therefore in the present study wee valuated three disinfectant agents (H2O2, Bioaxénico® and NaClO) for establishing aseptic tissue bulbs and 11 combinations ANA/BA to form adventitious shoots, in a basal medium. It was determined that using NaOCl (3% a.i) the axenic tissue percentage was 65% and the formation adventitious shoots via direct organogenesis was achieved with a frequency of up to 4 shoots per explants in the treatment of 7mM BA in the absence of ANA. Keywords: Axenic /Benzyladenine /Naphthalenaceticacid /Organogenesis.

Third place:

### ISOLATION OF CELLULOSE-HYDROLYTIC BACTERIA CAPABLE OF HYDROLYZING CITRUS PEEL WASTE

López-Domínguez Cyndi, Rodríguez-Buenfil Ingrid, Ucan-Hernandez Xemón, Evangelista-Martínez Zahaed, and Sánchez-Contreras Angeles

Unidad Sureste del Centro De Investigación y Asistencia en Tecnología y Diseño del Estado De Jalisco A.C. Corresponding author: *msanchez@ciatej.net.mx* 

**Abstract:** A cellulose-hydrolytic bacterium isolated from the rumen of Bos indicus was examined for their ability to hydrolyze citrus peel waste. Cellulosehydrolytic ability was screened using microcrystalline cellulose as a carbon source and Congo Red Assay. The cellulose-hydrolytic bacterium was identified by 16RNAlike *Klebsiella sp.* This strain is a cellulolytic microorganism that produces large extracellular multienzyme complexes called cellulosomes in culture broth of citrus peel. **Keywords:** *Klebsiella sp.* /Citrus peel /Hydrolysis

### **Related References**

1. Fernández-Luqueño F, López-Valdez F, Lozano-Muñiz S. (Eds.). Biotechnology Summit 2012, Yucatán México. pp. 1-5. 12-21 March 2012.

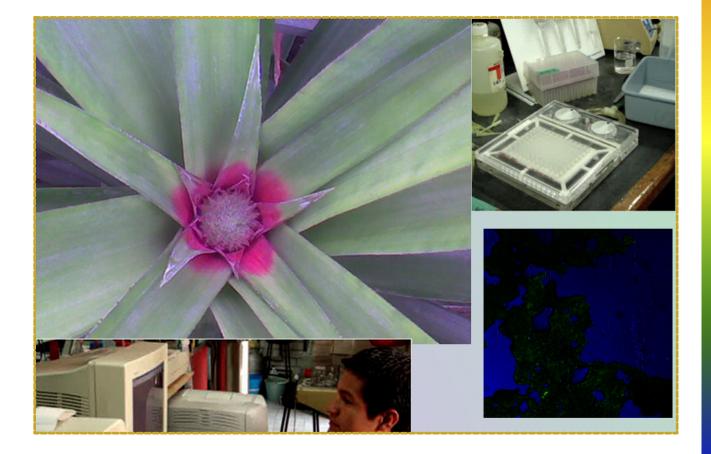
2. Int. Biotech. Color J. 2(1) 7 - 36. Special issue: Abstracts to the sessions of the Biotechnological Summit 2012, March, 12th to 21th of 2012.

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PLATINUM	Synthetic Biology	Design and obtention of new biological components, devices, and systems. Applications of re-designed natural biological systems.
SILVER	Biobusiness, Bioentrepreneuship & Marketing:	Development economics, Biobusiness and marketing. Strategy for innovative economical development. Improvement of the system of the S&T and innovation activities management
TRANSPARENT	Biotechnology, Bioethics & Society:	Assesment of the public support to the scientific activity. Biotechnological potential and human resources
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Theses:

Smith I J K (1991) Title of thesis. PhD thesis. University, City

#### Online document:

Smith I J K (1991) Title. Source Title, http://www.domain.class.ntw/pub/info/docs/essay.html, accessed january 1, 1991. Patent:

# Smith I J K, Brown I K, , Lee J L, Wilson I M, Martin L M, Patel H G, Taylor F G, Wong T R, Campbell F R, Williams H R et al., inventors. January 1, 1991. Patent's full title. Patent Application Office No. 12345

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Smith I J K, Brown I K, , Lee J L, Wilson I M, Martin L M, Patel H G, Taylor F G, Wong T R, Campbell F R, Williams H R et al. (year of publication) Article's full title. DOI: xxxx.yy-zz.yy

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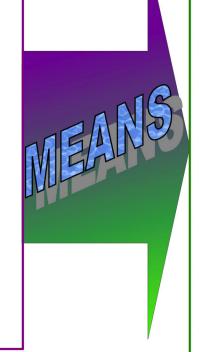
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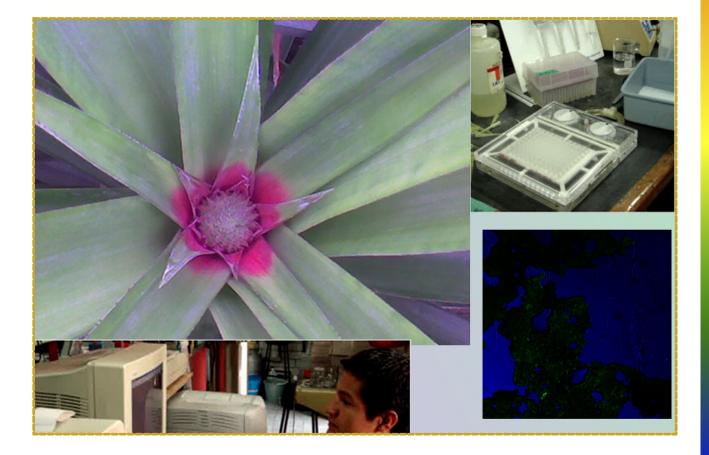
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