

PIPELINE OF CIGB'S AGBIOTECH PROJECTS

PROJECT	AREA	RESEARCH	DEVELOPMENT-PILOT SCALE	FIELD TESTS	REGISTER
GAVAC: IMMUNOGEN FOR BIOLOGICAL CONTROL OF BOVINE CATTLE TICKS.	VETERINARY				
HeberNem: ECOLOGICAL BIONEMATOCIDE AND GROWTH PROMOTER IN PLANT.	AGRICULTURE				
PorVac: SUBUNIT VACCINE AGAINST CLASSICAL SWINE FEVER VIRUS (PPC).	VETERINARY				
KestoZyme: BIOCATALIZER FOR THE PRODUCTION OF FRUCTOOLIGOSACÁRIDOS (FOS) FROM SACAROSE.	INDUSTRIAL ENZYME				
HIGHLY THERMOSTABLE BIOCATALYSTS FOR THE ENZYMATIC INVERSION OF SUCROSE AND MOLASSES.	INDUSTRIAL ENZYME				
ACUABIO V: NUTRITIONAL SUPPLEMENT AND GROWTH AND IMMUNE SYSTEM PROMOTER IN AQUATIC ORGANISM.	VETERINARY				
SOYBEAN TRANSGENIC PLANTS EXPRESSING <i>nmdef02</i> DEFENSIN TO PROTECT AGAINST FUNGI DISEASES.	AGRICULTURE				
CIGB -552vet: BIOLOGICAL CITOSTATIC FOR THE TREATMENT OF CANCER IN PET ANIMALS.	VETERINARY				
ACUABIO 4: NUTRITIONAL SUPPLEMENT FOR AQUATIC ORGANISMS	VETERINARY				
SalVac: VACCINE CANDIDATE AGAINST SEA LICE IN SALMONIDS	VETERINARY				
VACCINE P0: VACCINE CANDIDATE AGAINST GARRAPATAS BASED ON PROTEIN P0.	VETERINARY				
CunVac: RECOMBINANT VACCINE AGAINST HEMORRHAGIC DISEASE OF THE RABBIT	VETERINARY				
PLANTS AS BIOTEACTORS (PLANT MOLECULAR PHARMING)					
CIGB 42: FOR THE CONTROL OF INFECTIOUS DISEASES IN PLANTS.	AGRICULTURE				
CONTROLLING GEMINIVIRUS DISEASES	AGRICULTURE				
VACCINE FOR BIOLOGIC IMMUNOCASTRATION.	VETERINARY				
ANTIMICROBIAL PEPTIDES	VETERINARY				

Contacting point

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GAVAC: IMMUNOGEN FOR IMMUNOLOGICAL CONTROL OF BOVINE CATTLE TICKS

Area: Veterinary

OBJETIVE

The use of this immunogen for the purpose of incorporating it into integrated control strategies to reduce dependence on costly and environmentally erroneous chemical control of ticks affecting cattle.

DESCRIPTION

The bovine tick immunogen is based on the recombinant recombinant *Rhipicephalus boophilus* microplus tick Bm86 antigen formulated with an oily adjuvant. The application of this immunogen to the whole bovine mass of the farm, together with the appropriate integral strategies of control of ticks, has proven effective in field applications in Cuba and in several Latin American countries, drastically reducing parasite populations and consumption Of chemicals.

Active immunization of bovine cattle against the tick of the genus *Rhipicephalus (Boophilus) sp.* From a month of birth.

When GAVAC is administered to the bovine, the Bm86 antigen it contains is processed by the animal's immune system generating an immune response measured by antibodies. These antibodies pass to the tick when it suctions its blood and causes irreversible lesions that destroy its intestinal wall and cause damages that are manifested in the repletion, the oviposition and the viability of the larvae.

These damages have a cumulative effect on the generations of ticks, **causing the gradual reduction of the population in the grasses and in the animals.**

Progressively controls infestations and acts even against ticks resistant to chemical ticks, Does not interfere with the action of other vaccines or medications and has no withdrawal period or lack.

PATENT STATUS

The product in this moments not have patent, the technology is protected by the Know How of the whole productive process.

PROJECT STATUS

Product in commercial phase

TYPE OF COLLABORATION REQUESTED

Counterpart interested in efficacy trials with different tick species for the registration and commercialization of this product in different markets of interest.

COMPETITIVE ADVANTAGES AND MILESTONES

Only commercially available tick-borne vaccine. More than 4 million head of cattle have been immunized with GAVAC, its main advantages have been: Great reduction of the incidence of hemo-parasitic diseases, More than 80% reduction in chemical treatments for tick control, Drastic reduction Of tick control costs, completely ecological product.

HOST INSTITUTION

Centro de Ingeniería Genética y Biotecnología (CIGB). Ave 31, e/158 y 190 Cubanacán, Playa. PO Box 6162, La Habana, 10600, Cuba. Tel. (53-7)2504646 Fax. (53-7) 2736008; 2718070. E-mail: merardo.pujol@cigb.edu.cu Web site: <http://gndp.cigb.edu.cu>

Project code: AGBIO/CIGB/IAP-01

GAVAC: IMMUNOGEN FOR BIOLOGICAL CONTROL OF BOVINE CATTLE TICKS.

Area: Veterinary

DESCRIPTION

Tick-borne diseases constitute important constraints on the livestock industry, in particular for the production of cattle in tropical and sub-tropical regions of the world. Tick-borne protozoan diseases (babesiosis and theileriosis) and ehrlichial diseases (cowdriosis and anaplasmosis) are main health and management problems, which prevent upgrading of livestock in many developing countries. Integrated control strategies have been advocated based on host resistance to ticks and the diseases they transmit, strategic tick control taking into account the seasonal dynamics of tick infestation, availability of vaccines against tick-borne diseases, and cost/benefit analysis of chemical acaricide application.

The group at CIGB developed a vaccine against tick based on the recombinant expression of Bm86 antigen from *Rhipicephalus Boophilus microplus*. This vaccine, together with appropriate tick control integral strategies has shown to be effective in field applications in Cuba and in several Latin American countries, expressed in less amount of disease transmitted by ticks and reductions in the use of chemical product. (Figures 1A, B and C) Therefore the group has a vast experience in the cloning, expression, and testing of potential vaccine antigens against ticks.

More than 4 million cattle have been immunized with GAVAC under a Pest Management Program for controlling ticks showing:

Advantages

- Large reduction in the incidence of hemo-parasitic diseases.
- More than 80% reduction in Chemical treatments for tick control.
- A drastic reduction in expenses for tick control.
- Environmental friendly product.
- USDA has already tested GAVAC, showing more than 95% of efficacy against *R. B. annulatus*.

The Venezuela program report and effective results in the controlling the ticks and reduction of chemical treatments (Figure 2) with:

- Large scale field trial in Cuba wherein 509 758 cattle were vaccinated across the country.
- Reduction in frequency of acaricidal treatments: from 7-14 days to 115-1095 days (1095 days at certain locations).
- Reduction in chemical consumption: from 371 t annual average to 15 t.
- Reduction in incidence of death and sick by hemoparasites of 98%. Enzootic stability.
- Estimated reduction in about 15 Millions USD per year in tick controls.
- More than 2 million of cattle have received the vaccination with Gavac under the Integrated Management Program in Venezuela with an important reduction in the use of acaricides and in the expenses for the tick control.

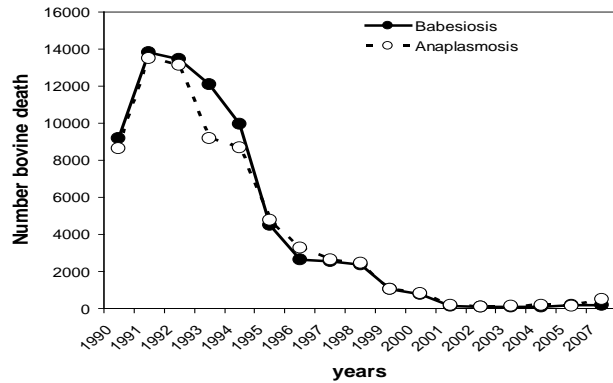
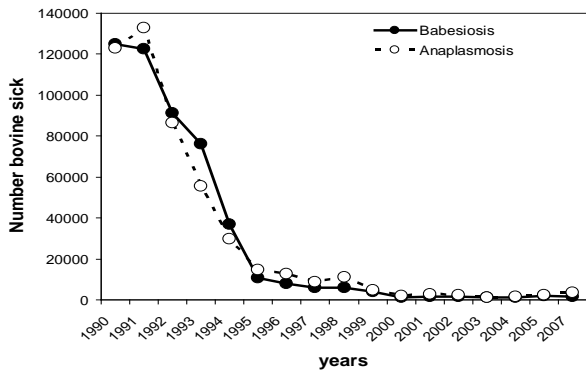
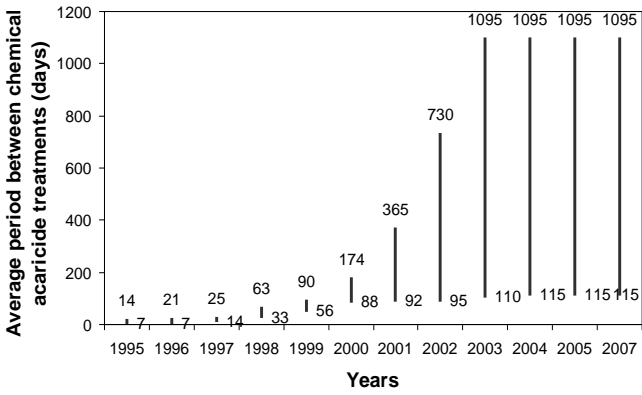
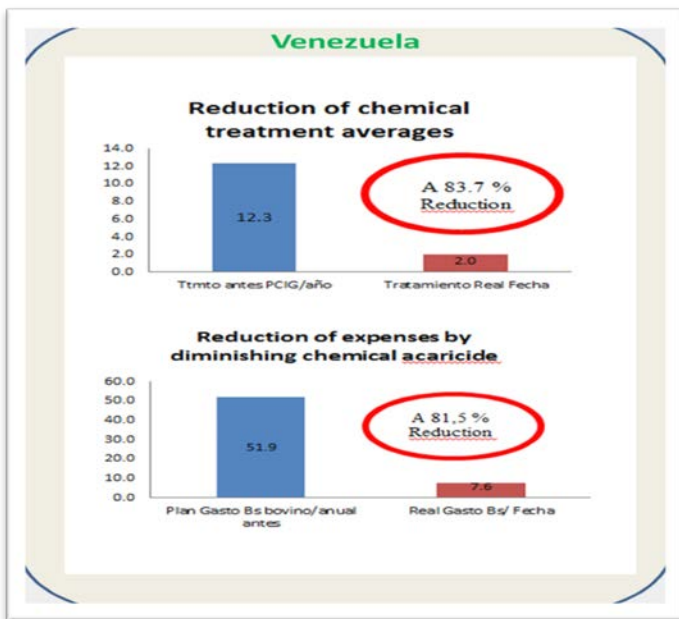


Figure 1. A: Average period between chemical treatments, B: Number of animals sick with babesiosis and Anaplasmosis, C: Number of death by Babesiosis or Anaplasmosis diseases.



The National program in the Republic Bolivariana of Venezuela included more than 2 million of bovines spread over 18 states.

Figure 2: Venezuela results in the integrate Gavac programs

PUBLICATIONS

1. Valle MR., Rubiera R., Penichet M., Montesinos R., Cremata J., Falacon V., Sánchez G., Bringas R., Cordovez C., Valdez M., Lleonard R., Herrera L. and De la Fuente J. **High level expression of a protective BM86 antigen from the cattle tick *Boophilus microplus* in yeast *Pichia pastoris***. Recombinant and Synthetic Vaccine, 1994, Narosa Publishing House, New Delhi, India.
2. Manuel Rodriguez, Roger Rubiera, Manuel Penichet, Raquel Montesinos, Jose Cremata, Viviana Falcon, Giselle Sanchez, Ricardo Bringas, Carlos Cordoves, Mario Valdes, Ricardo Lleonart, Luis Herrera, Jose de la Fuente II. **High level expression of the B. microplus Bm86 antigen in the yeast *Pichia pastoris* forming highly immunogenic particles for cattle**. Journal of Biotechnology 1994, 33: 135-146.
3. Machado H., Santizo C., **A rapid visual immunoassay for the quantification of bovine antibodies against recombinant *Boophilus microplus* BM86 antigen**. Recombinant Vaccine for the Control of Cattle Tick, 1995.
4. Mario Canales, Antonio Enriquez, Eduardo Ramos, Deborah Cabrera, Hubert Dandie, Alejandro Soto, Viviana Falcon, Manuel Rodriguez and Jose de la Fuente. **Large-scale production in *Pichia pastoris* of the recombinant vaccine Gavac® against cattle tick**. Vaccine, 1997, Vol. 15, No. 4, pp. 414-422.
5. Manuel Rodriguez, Jose de la Fuente, Carlos Montero, Miguel Redondo, Luis Mendez, Emerio Serrano, Mario Valdes, Antonio Enrique Mauriz, Eduardo Ramos, Mario Canales y Jorge Lodos. **ESTRATEGIA PARA EL CONTROL DE *Boophilus microplus* MEDIANTE LA INMUNIZACIÓN CON UNA VACUNA RECOMBINANTE QUE CONTIENE EL ANTIGENO Bm86**. Biotecnología Aplicada 1997, Vol. 14, No.2.
6. Labarta V., Rodriguez M., Penichet M., Lleonard R., Luaces L., De la Fuente J. **Simulation of control strategies for the cattle tick *Boophilus microplus* employing vaccination with a recombinant Bm86 antigen preparation**. Veterinary Parasitology 1996, 63: 131-160.
7. Jose de la Fuente, Manuel Rodriguez, Miguel Redondo, Carlos Montero, Jose Carlos Garcia-Garcia, Luis Mendez, Emerio Serrano, Mario Valdes, Antonio Enriquez, Mario Canales, Eduardo Ramos, Oscar Boue, Hector Machado, Ricardo Lleonart, C.A. de Armas, Sara Rey, J.L.Rodriguez, Mirna Artiles and Libertad Garcia. **Field studies and cost-effectiveness analysis of vaccination with Gavac™ against the cattle tick *Boophilus microplus***, Vaccine, 1998, Vol. 16, No.4, pp. 366-373.
8. Mario Canales, Jose A. Buxad, Lazaro Heynngnezz, and Antonio Enriquez. **Mechanical disruption of *Pichia pastoris* yeast to recover the recombinant glycoprotein BM86**. Enzyme and Microbial Technology 1998, 23:58-63.
9. Lodos J., Ochagavia ME., Rodriguez M., De la Fuente J. Simulations study of the effects of acaricide an vaccination on *Boophilus* cattle tick populations. Preventive Veterinary Medicine 1999, 38: 47-63.
10. Fragoso, P. Hoshman Radt, M. Ortiz, M. Rodriguez, M. Redondo, L. Herrera and J. de la Fuente. **Protection against *Boophilus annulatus* infestations in cattle vaccinated with the B. microplus Bm86-containing Vaccine Gavac**. Vaccine 1998, Volume 16 Number 20.
11. Oscar Bouea, Karina Sanchez, Grisel Tamayo, Laura Hernandez, Edel Reytor and Antonio Enriquez. **Single-step purification of recombinant Bm86 protein produces in *Pichia pastoris* by salting-out and by acid precipitation of contaminants**. Biotechnology Techniques, 1997, Vol11, No 8, pp. 561-565.
12. Boue, M. Redondo, C. Montero, M. Rodrigue and J. de la Fuente. **REPRODUCTIVE AND SAFETY ASSESSMENT OF VACCINATION WITH GAVAC™ AGAINST THE CATTLE TICK (*Boophilus microplus*)**. Theriogenology 1999, 51: 1547-1554.
13. O. BOUE, O. FARNOS, A. GONZALEZ, R. FERNANDEZ, J.A. ACOSTA, R. VALDES, L.J. GONZALEZ, Y. GUANCHE, G. IZQUIERDO, M. SUAREZ, I. DOMINGUEZ, H. MACHADO, M. RODRIGUEZ and R. LLEONART. **Production and biochemical characterization of the recombinant *Boophilus microplus* Bm95 antigen from *Pichia pastoris***. Experimental and Applied Acarology 2004, 32: 119-128.
14. Manuel Rodríguez Valle, Carlos Montero, Héctor Machado and Marisdania Joglar. **The evaluation of yeast derivatives as adjuvants for the immune response to the Bm86 antigen in cattle**. BMC Biotechnology 2001, 1:2.
15. Jose C. Garcia-Garcia, Carlos Montero, Manuel Rodriguez, Alejandro Sotot, Miguel Redondo, Mario Valdes, Luis Mkndez and José de la Fuente. **Effect of particulation on the immunogenic and protective properties of the recombinant Bm86 antigen expressed in *Pichia pastors***. Vaccine, 1998, Vol. 16, No. 4, pp. 374-380.

HEBERNEM: ECOLOGICAL BIONEMATOCIDE AND GROWTH PLAN ESTIMULATOR	
Agriculture Area: Agriculture	
GOAL	To implement a previous evaluation and business joint ventures for the commercial exploitation of the bionematicide HeberNem.
DESCRIPTION	At present, biological products for the control of pest and diseases are highly demanded worldwide. Their designs are oriented towards a friendly environmental nature compatible with organic agriculture and capable to strongly reduce or substitute the use of chemical pesticides. HeberNem is a biological nematocide, environmentally friendly, which has proven to be an efficient alternative, supplementing the action of chemical nematocides, under a system of integrated pest management. The product has been successfully tested in green houses dedicated to vegetable production in semi-protected crops and plantations of banana and guava. A nationwide mass marketing of bionematicide HeberNem is currently carried out by Permissions Nr. 001/2007 and 050/08 of National Pesticides Registry. A new registry of a more efficient solid formulation for greenhouses and semiprotected crops was recently obtained: Volume 6, Folio 0581, Permit 02616.
PATENT STATUS	<p>“Pesticides and antiparasitic compositions”. Cuba: CU2001-0004: PCT/CU01/00014 Granted in: Cuba, Europe, China, Vietnam, Israel, Mexico, Panama, United States, South Africa, Peru, Australia, Colombia, Russia, India, Canada, Brazil, Iran and Nigeria. Filed in Venezuela and Paraguay.</p> <p>“Biofertilizer Composition”. CU2007-0092: PCT/CU2008/000002 Granted in: Cuba, South Africa, Mexico, Panama, Canada and China. Requested in: Europe, USA, Canada, Brazil, Costa Rica, China, Argentina, Venezuela, Peru, Chile</p>
PROJECT STATUS	National Marketing and product effectiveness trials abroad.
TYPE OF COLLABORATION REQUESTED	Development and Evaluation Agreements to perform clinical trials to validate the effectiveness of Hebernem as biopesticide and biofertilizer. Identifying appropriate partners to extend its use and application. The results will lead to subsequent commercial agreements for specific territories and during a period of time.
COMPETITIVE ADVANTAGES MILESTONES	More than 18 years of toxicological and ecotoxicological studies (25 studies) have supported the product biosafety and bionematicide activity against several genera of plant parasitic nematodes. The product has proven to be environmentally friendly. Its mechanism of action has been elucidated. There are two formulations already registered: a liquid formulation (HeberNem-L) and solid (HeberNem-S).
HOST INSTITUTION	Centro de Ingeniería Genética y Biotecnología (CIGB). Ave 31, e/158 y 190 Cubanacán, Playa. PO Box 6162, La Habana, 10600, Cuba. Tel. (53-7)2504646 Fax. (53-7) 2736008; 2718070. E-mail: merardo.pujol@cigb.edu.cu Web site: http://gndp.cigb.edu.cu
Project code: AGBIO/CIGB/IAP-02	

HeberNem: ECOLOGICAL BIONEMATOCIDE AND GROWH PROMOTER IN PLANT.

Area: Agriculture

DESCRIPTION

At present, biological products for the control of pest and diseases are highly demanded worldwide. Their designs are oriented towards a friendly environmental nature compatible with organic agriculture and capable to strongly reduce or substitute the use of chemical pesticides. HeberNem is a biological nematocide, environmentally friendly, which has proven to be an efficient alternative, supplementing the action of chemical nematocides, under a system of integrated pest management. The product has been successfully tested in green houses dedicated to vegetable production in semi-protected crops and plantations of banana and guava. A nationwide mass marketing of bionematocide HeberNem is currently carried out by Permissions Nr. 001/2007 and 050/08 of National Pesticides Registry.

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Figure 1: Tomatoes plantation: Without HeberNem (left side) and with HeberNem (right side).



Figure 2: HeberNem powder formulation in 250 g bags.

Field experiment using HeberNem powder formulation in banana var. Cavendish: treatments with HeberNem through the irrigation system...

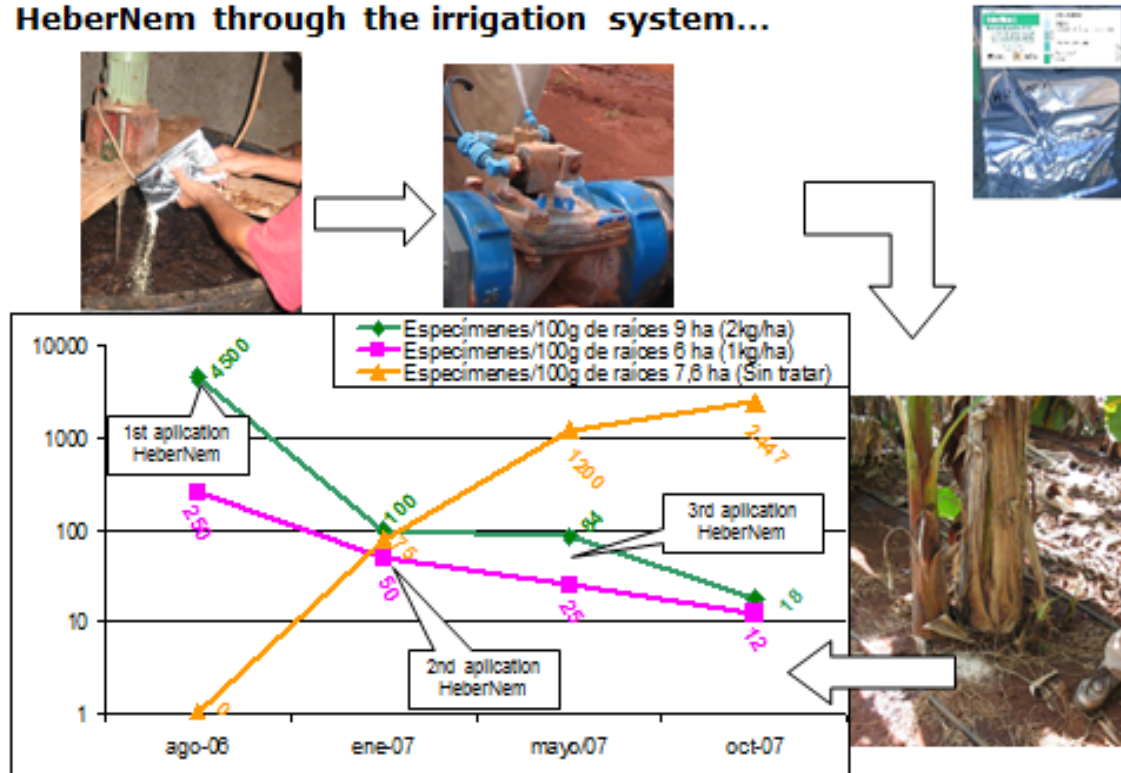


Figure 3: Example of application of HeberNem in banana plantations

PATENTS

“Pesticides and antiparasitic compositions”. Patent Number: US2004/0071663 A1. April 15, 2004. Granted in: Cuba, Europe, China, Vietnam, Israel, Mexico, Panama, United States, South Africa, Peru, Australia, Colombia, Russia, India, Canada, Brazil, Iran, Nigeria. Filed in Venezuela and Paraguay.

“Biofertilizer Composition”. CU2007-0092: PCT/CU2008/000002 Granted in: Cuba, South Africa, Mexico, Panama, Canada and China. Requested in: Europe, USA, Canada, Brazil, Costa Rica, China, Argentina, Venezuela, Peru, Chile.

We are looking for partners interested in evaluating product effectiveness by conducting trials abroad to receive market rights for specific territories under exclusive status.

PUBLICATIONS

1. Mena J, de la Riva G, Vázquez RP, Fernández M, Coego A, García M, Pimentel E, López A, García R, Zaldúa Z, Mencho JD. **Nematicidic agent and method for the bio-control of nematodes**. International Application Published Under the Patent Cooperation Treaty (PCT 0397), 1996, WO 96/04749.
2. Mena J, Vázquez RP, León L, Ramírez Y, Hernández AT, Pimentel E. **Efectividad de *Corynebacterium paurometabolum* en el control de *Meloidogyne incognita***. Nematropica 2001, 31:144-145.
3. Mena J, Pimentel E, Hernández A T, Veloz L, Vázquez RP, León L, Mencho JD, Ramírez Y, Pujol M, Borroto, C. **Mechanism of action of *Corynebacterium paurometabolum* strain C-924 on nematodes**. Nematology 2002, 4: 287.
4. Jesús Mena Campos, Eulogio Pimentel Vázquez, Liuven Veloz González, Armando Tomás Hernández, Licette León, Yamilka Ramírez, Ileana Sánchez, Juan Diego Mencho, Alina López, Merardo Pujol, Carlos Borroto, Eralio Ramos, José Manuel Alvarez, Marieta Marín, Gilda Jiménez, Graciela García, Víctor M Pico, Manuel Expósito, Yaiza Coca, Miguel

Gómez, Amparo Olazabal, Abel Hernández, Viviana Falcón, María C De la Rosa, Ivón Menéndez, Manuel Raíces. **Aislamiento y determinación de cepas bacterianas con actividad nematocida. Mecanismo de acción de *C. paurometabolum* C-924 sobre nematodos.** Biotecnología Aplicada 2003, Vol.20, No.4.

5. Emilio Fernández, Jesús Mena, Julián González and María Elena Márquez. **Biological control of nematodes in banana.** Turner D.W. and F.E. Rosales (eds).. Banana Root System: towards a better understanding for its productive management: Proceedings of an international symposium / Sistema Radical del Banano: hacia un mejor conocimiento para su manejo productivo: Memorias de un simposio internacional. INIBAP, Montpellier, France. 2005, p 193-200.
6. Mena J, Pimentel E, Borroto C, Smith E, Mesa L, León L, Hernández M, Ramírez Y, Sosa A, García M, Raíces M, Hernández AT, Wong I, Basulto R, Oliva O, Herrera L. **Extensión Nacional del bionemática HeberNem, para el control de fitonematodos en los cultivos protegidos.** MINAG. Instituto de Investigaciones Hortícolas "Liliana Dimitrova". Investigaciones Científicas al servicio de la Agricultura tropical.-La Habana 2006. Liliana ISBN 959-7111-39-X.
7. Armando Hernández, Frédéric Weekers, Jesús Mena, Carlos Borroto, and Philippe Thonart. **Freeze-drying of the biocontrol agent *Tsukamurella paurometabola* C-924. Predicted stability of formulated powders.** GEN PUBLISHING INC., A MARY ANN LIEBERT INC. COMPANY • VOL. 2 NO. 3 • FALL 2006 INDUSTRIAL BIOTECHNOLOGY.
8. Mena J, Pimentel E, Hernández AT, León L, Ramírez Y, Wong I, Marín M, Mencho JD, Hernández M, del Castillo A, Sánchez I, Expósito M, Jiménez G, Fleitas M, García G, González N, Zamora J, Zalazar E, Olivera V, Rodríguez G, Álvarez B, Dandie H, Sánchez MC, Pimentel R, Pérez C, Compte O, Sardiñas, M, Martínez L, Salinas D, García C, Borroto C.. **Uso de bionemática HeberNem en los cultivos protegidos.** Resúmenes Taller Latinoamericano Biocontrol de fitopatógenos con *Trichoderma* y otros antagonistas. Fitosanidad 2006, 10: 168.
9. Mena J. **Manual de Aplicación del Bionemática HeberNem. 1er Seminario Técnico sobre Sanidad Vegetal en Cultivos Protegidos,** La Habana 2007: <http://revistas.mes.edu.cu/eduniv/evento/fitosanidad/Manual-Aplicacion-Bionemática-HeberNem.pdf>.
10. Mena J, Pimentel E, Marín M, Hernández AT, Sánchez I, Ramírez Y, González S, García M, Borroto C. **Composición biofertilizante.** Oficina Cubana de la Propiedad Intelectual, OCPI Nro. solicitud: 2007-0092.
11. Mena J, Pimentel E, Borroto C, Smith E, Mesa L, León L, Hernández M, Ramírez Y, Sosa A, García M, Raíces M, Hernández ET, Wong I, Basulto R, Oliva O, Herrera L. **Empleo del bionemática HeberNem como sustituto de productos químicos en los cultivos protegidos.** CITRIFRUT, 2007, ISBN 978-959-296-001-5.
12. Nemecio González, Idania Wong, Eulogio Pimentel, Jesús Zamora, Eladio Salazar, Jesús Mena. **EXPRESIÓN DE PROTEASAS EXTRACELULARES EN EL CULTIVO DE ALTA DENSIDAD DEL MICROORGANISMO CON ACTIVIDAD BIONEMÁTICA *Tsukamurella paurometabola*, C924.** TECNOLOGÍA QUÍMICA 2009, Vol. XXIX, No. 2.
13. Armando Hernández, Jesús Mena, Nemecio González, Jesús Zamora, Eulogio Pimentel, Eladio Salazar, María del C Sánchez, Carlos Borroto, Alain Moreira, Lourdes Mesa, Eduardo Smith, Viviana Falcón, Ivon Menéndez, Rafael Pimentel, Roger Ríos, Idania Wong. **Anhydrobiotic cells of the nematocidal agent *Tsukamurella paurometabola* C-924.** Biotecnología Aplicada 2009; Vol.26, No.4
14. Marieta Marín Bruzos, Jesús Mena Campos, Pavel Chaveli Chávez, Rolando Morán Valdivia y Eulogio Pimentel Vázquez. **Interaction among *Tsukamurella paurometabola* C-924 and *Rhizobium leguminosarum* biovar phaseoli CFH in bean plants.** Acta Agronómica. 2013, 62 (1), p 52-58.
15. Marieta Marín, Idania Wong, Graciela García, R. Morán, Rosa Basulto, E. Pimentel, J. Mena. **Actividad antagónica in vitro de *Tsukamurella paurometabola* C-924 frente a fitopatógenos.** Rev. Protección Veg. 2013, Vol. 28 No. 2: 132-137.
16. Marusia Stefanova, María Elena Díaz de Villegas, Jesús Mena Campos. Capítulo 8: **Control biológico de enfermedades de plantas en Cuba. Control Biológico de Enfermedades de Plantas en América Latina y el Caribe.** 2014, p 201-218.
17. Marieta Marín, Jesús Mena, Idania Wong, Rolando Morán, Ramón Franco, Marcia Rojas, Pavel Chavelis, Graciela García, Rosa Basulto, Armando Hernández, Eulogio Pimentel, Alain Moreira, Sonia González. **Characterization of the vegetal growth promoting capacity of *Tsukamurella paurometabola* C-924 and the main mechanisms involved.** Biotecnología Aplicada 2014, 31:168-171.

Porvac: SUBUNIT VACCINE AGAINST CLASSICAL SWINE FEVER	
Area: Veterinary	
OBJECTIVE	Development of a subunit vaccine against classical swine fever virus as part of a comprehensive approach to the control and eradication of this disease.
DESCRIPTION	<p>Classical swine fever is a highly contagious disease, classified in the list of notifiable diseases of the International Office of Epizootics (OIE).</p> <p>It is a viral infection that affects domestic and wild pigs. It is considered as one of the most devastating diseases for the swine industry worldwide, from the economic and health point of view.</p> <p>In the CIGB, a candidate vaccine against this virus has been developed, based on the expression in mammalian cells of a chimeric antigen comprising by E2 protein of classical swine fever virus. The antigen was fused to the porcine CD154 molecular adjuvant. This vaccine can stimulate cellular and humoral responses, very similar to those generated with the inactivated virus vaccine; we obtain early protection against the virus, only 7 days after the first dose applied. Porvac also cuts the horizontal and vertical transmission of the virus, and can considerate marker vaccine, that allows differentiating the vaccinated pigs from the infested ones. Our vaccine can be used in programs of control and eradication of this disease in zones of endemism.</p>
PATENT STATUS	CHIMERIC VACCINE ANTIGENS AGAINST CLASSIC SWINE FEVER VIRUS. Antígenos vacunales quiméricos contra el virus de la peste porcina clásica (PPC) Patente PCT/CU2007/000008, WO07/098717
PROJECT STATUS	For register in Cuba
TYPE OF COLLABORATION REQUESTED	Corporate association for licensing and marketing.
COMPETITIVE ADVANTAGES AND MILESTONES	It is a subunit vaccine based on the E2 protein of the virus to which the CD154 pig molecule was fused, this fusion protein is produced recombinantly in cells and mammals. It presents intellectual property and potential for use in government programs for the control and eradication of classical swine fever within a territory, prevents transmission from mother to offspring and allows differentiation between vaccinated and infested pigs.
HOST INSTITUTION	<p>Centro de Ingeniería Genética y Biotecnología (CIGB). Ave 31, e/158 y 190 Cubanacán, Playa. PO Box 6162, La Habana, CP 10600, Cuba. Teléf. (53-7) 2712397; 2716022. Fax. (53-7) 2736008; 2718070.</p> <p>Email: merardo.pujol@cigb.edu.cu Sitio web: http://gndp.cigb.edu.cu</p>
Código del Proyecto: AGBIO/CIGB/IAP-03	

Porvac: SUBUNIT VACCINE AGAINST CLASSICAL SWINE FEVER VIRUS (PPC).

Area: Veterinary

DESCRIPTION:

Classical swine fever virus (CSFV) elicits high mortality in infected herds during recurrent outbreaks. E2-viral envelope glycoprotein is responsible for the induction of neutralizing antibodies, which become this molecule in a prospective candidate to develop vaccines.

In order to obtain a high immunogenic vaccine, viral E2 glycoprotein was fused to the swine CD154 as a molecular adjuvant. This candidate vaccine has been produced by genetic engineering in CHO-DG44, CHO-K1 and HEK 293 cells. The protective capacity of the chimeric E2-CD154 glycoprotein against a virulent strain of CSFV was demonstrated in a challenge experiment using a biphasic immunization schedule with doses of 50 and 25 µg/pigs. The immunized animals developed neutralizing antibodies that were protective when the animals were faced to a challenge with 10^5 LD₅₀ of the homologous CSFV ‘‘Margarita’’ strain administered by intramuscular injection. Consequently, no clinical signs of the disease were detected in the vaccinated pigs (Figure 1). The E2-CD154 immunized animals were also able to overcome the challenge with a high virulent CSF virus strain performed 7 days after a unique dose of the vaccine without clinical manifestations of the disease. Specific anti-CSFV neutralizing antibodies and IFN-γ were induced 8 days after challenge equivalent to 14 days post-vaccination. This is the first subunit vaccine able to confer complete protection by the end of the first week after a single vaccination. In addition, this vaccine is also able to inhibit horizontal and vertical transmission of the virus. These results demonstrate that the E2-CD154 antigen could be potentially used under outbreak conditions to stop CSFV spread and for eradication programs in CSF enzootic areas.

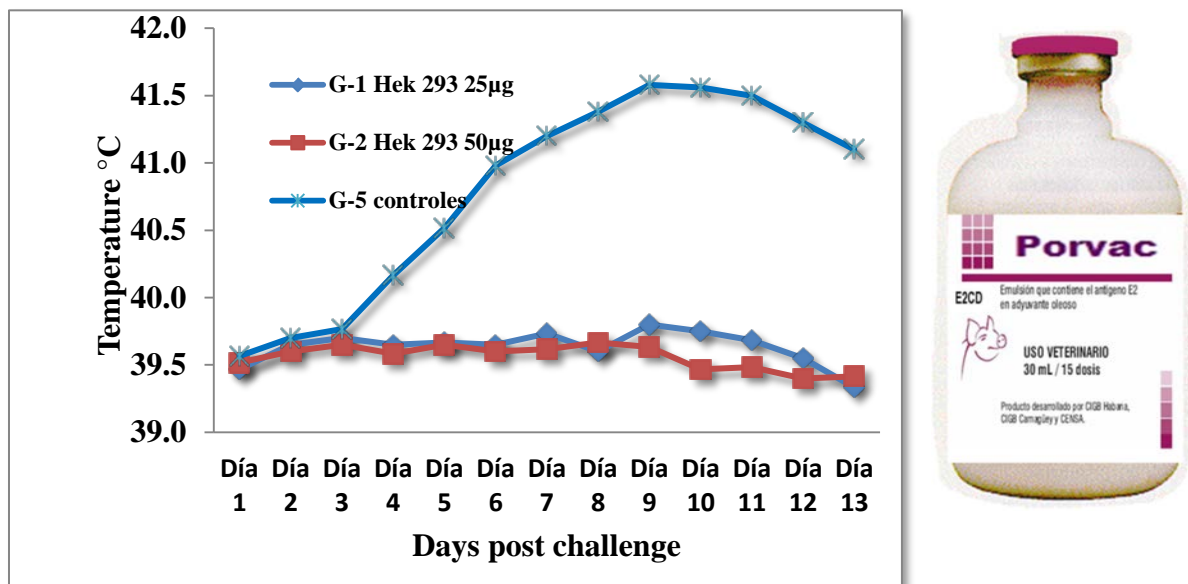


Figure 1: Vaccine Protect in challenge pigs.

In parallel, a DIVA diagnostic system has been developed (Figure 2) which allows distinguishing vaccinated animals from infected animals. Both tools will allow establishing a program to assist in the eradication of Classical Swine Fever in countries where this disease is endemic.

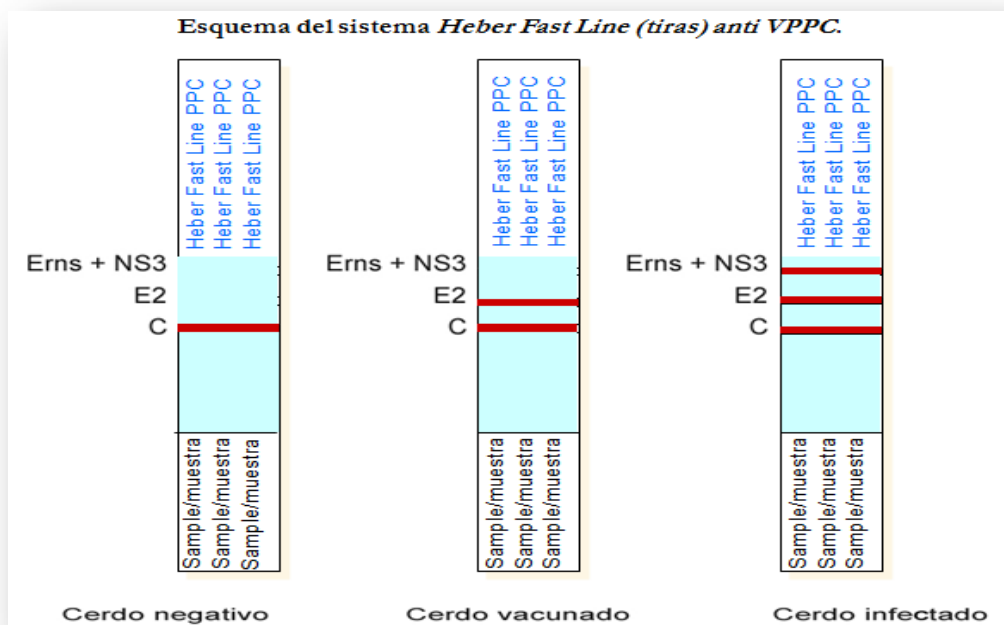


Figure 2: DIVA diagnostic system.

The main achievements of this project are:

- ✓ Cloning and characterization of CSFV E2 glycoprotein fused to swine CD154 molecule.
- ✓ Expression of chimeric E2-CD154 protein in mammalian cell cultures (CHO-DG44, CHO-K1 and HEK 293).
- ✓ Demonstration of protective capacity of the E2-CD154 glycoprotein in an experiment using a biphasic immunization schedule and a challenge with 10^5 LD₅₀ of the high virulent CSFV “Margarita”.
- ✓ Demonstration of protective capacity against 10^5 LD₅₀ of the high virulent homologous CSFV “Margarita” after 7 days post vaccination with E2-CD154 glycoprotein using a single immunization.
- ✓ Demonstration of trans placental transmission inhibition of CSFV.
- ✓ Vaccination with this subunit marker vaccine E2-CD154 allows distinguishing vaccinated animals from infected animals.

PATENTS

CHIMERIC VACCINE ANTIGENS AGAINST CLASSIC SWINE FEVER VIRUS. Antígenos vacunales quiméricos contra el virus de la peste porcina clásica (PPC) Patente PCT/CU2007/000008, WO07/098717

PUBLICATIONS

1. Marisela Suárez, Yusmel Sordo, Yanet Prieto, María P. Rodríguez, Lídice Méndez , Elsa M. Rodríguez, Alina Rodríguez-Mallon, Elianet Lorenzo, Elaine Santana, Nemesio González, Paula Naranjo, María Teresa Frías, Yamila Carpio, Mario Pablo Estrada. **A single dose of the novel chimeric subunit vaccine E2-CD154 confers early full protection against classical swine fever virus.** Vaccine 2017.
2. Prieto Y, Mendez L, Rodríguez E , Carpio Y, Rodríguez A, Venereo A, Lorenzo E, Rodríguez MP, Suárez M, Sordo Y, Joglar M, Santana E, Oramas N, González E, Pons T and Estrada MP. **Generation and efficacy assessment of a chimeric antigen E2-CD154 as a marker Classical Swine Fever Virus subunit vaccine produced in HEK 293 mammalian cells.** (artículo en escritura)
3. Lilianne Ganges, Sara Muñoz-González, Yusmel Sordo, Marta Pérez-Simó, Marisela Suarez, Albert Canturri, Maria Pilar Rodríguez Molto, Maria Teresa Frías-Lepoureau, Mariano Domingo, Mario Pablo Estrada. **Efficacy of E2 glycoprotein fused to porcine CD154 as a novel chimeric subunit vaccine to prevent classical swine fever virus vertical transmission in pregnant sows.** Veterinary Microbiology 2017.

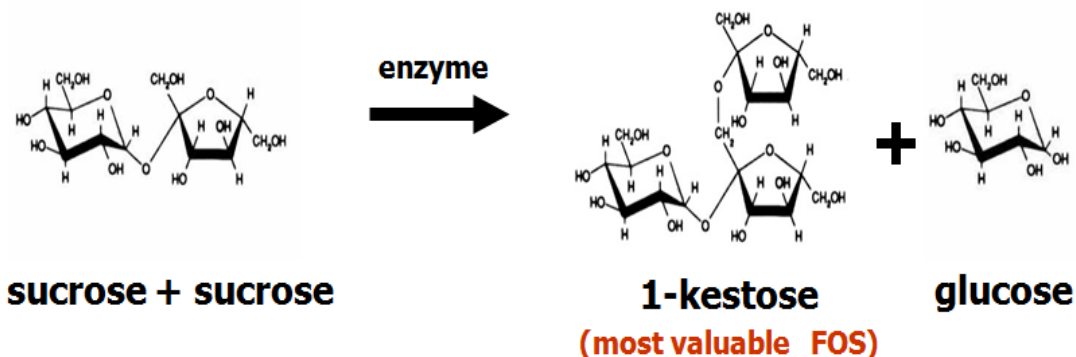
KestoZyme: BIOCATALIZER FOR THE PRODUCTION OF FRUCTOOLIGOSACÁRIDOS (FOS) FROM SACAROSE	
Area: Industrial Enzymes	
GOAL	Commercial Exploitation of the enzyme Fructosyltransferase (KestoZyme) for the production of high quality FOS from sucrose.
DESCRIPTION	Fructooligosaccharides (FOS) are natural nondigestible sugars, consisting of 2-4 units of fructose attached to a terminal glucose. Given its activity as soluble fiber and its demonstrated prebiotic effect in humans and animals, FOS maintains an increasing demand in the functional foods market. At present, commercial production of FOS from sucrose is carried out using biocatalysts based on fungal cells immobilized on calcium alginate spheres. CIGB has developed a new biocatalyst for the production of FOS, based on a fructosyltransferase of vegetable origin produced at high levels in the yeast <i>Pichia pastoris</i> . The recombinant enzyme (1-SSTrec) is formulated as a lyophilized powder of specific high activity (> 10,000 U / g), is fully soluble in water and can be operated at temperatures of 40 ° C. It is stable at room temperature (25-30 ° C) for one year. The composition (w / w) of the reaction products are: F (1%), G (21%), GF (20%), GF2 (55%), GF3 (3%). The enzyme can be operated without reuse in a batch reactor or with continuous reuse in a membrane bioreactor.
PATENT STATUS	The technology for the production and operation of the Fructosyltransferase enzyme (1-SSTrec) is backed by the patent "Method of obtaining 1-kestose". No. of application in Cuba: CU2012-0138. International Application No.PCT / CU2013 / 000005. International Publication No. WO 2014/044230.
PROJECT STATUS	In Commercial step.
TYPE OF COLLABORATION REQUESTED	License of our technology for the production of FOS by the use of the enzyme Fructosyltransferase (KestoZyme).
COMPETITIVE ADVANTAGES MILESTONES	<ul style="list-style-type: none"> • No recombinant fructosyltransferases are available for the commercial production of FOS. • The enzyme 1-SSTrec obtained in the CIGB has a high specific activity (> 10,000 U / g) has competitive advantages over the current biocatalysts based on cells / fungi enzymes, in terms of: <ul style="list-style-type: none"> • High performance of total FOS (55-60%, w / w) in reaction products. • High quality in the final composition of FOS (90% 1-kestose and 10% nistosa). • No hydrolysis activity of sucrose substrate. • Simple and flexible operation to produce small and large scale FOS.
HOST INSTITUTION	<p>Centro de Ingeniería Genética y Biotecnología (CIGB). Ave 31, e/158 y 190 Cubanacán, Playa. PO Box 6162, La Habana, 10600, Cuba. Tel. (53-7)2504646 Fax. (53-7) 2736008; 2718070.</p> <p>Email: merardo.pujol@cigb.edu.cu Web site: http://gndp.cigb.edu.cu</p>
Project code: AGBIO/CIGB/IAP-04	

KestoZyme: BIOCATALYZER FOR THE PRODUCTION OF FRUCTOOLIGOSACÁRIDOS (FOS) FROM SACAROSE.

Area: Industrial Enzyme

DESCRIPTION

The Fructooligosaccharides (FOS) are sucrose-derived undigestible sugars, consisting of 2-4 fructose units and a terminal glucose. Due to its activity as soluble fibers and its demonstrated prebiotic effect in human and animals FOS maintain an increasing demand in the nutraceuticals and functional food markets. FOS intake has been proven to promote the selective proliferation of intestinal Bifidobacteria and Lactobacilli with important benefits to health, including: competitive exclusion of intestinal pathogens, reduction of serum cholesterol, increase of calcium and magnesium absorption, prevention of colon cancer, and production of B-vitamins. The trisaccharide 1-kestose (GF₂) is commercially more attractive FOS due to its superiority in terms of bifidogenic activity and more sweetening power, in comparison with nystose (GF₂) and fructosylnystose GF₃, so it is more appropriate for use as a low-caloric sweetener for diabetics. Current systems for FOS production from sucrose are based on fungal cells entrapped in calcium alginate beads. CIGB has developed a novel biocatalyst for the production of FOS, based on a plant Fructosyltransferase produced at high levels in the yeast *Pichia pastoris* (*KestoZyme*). The recombinant enzyme (1-SSTrec) is formulated as a freeze-dried powder with high specific activity (>10,000 U/g), is totally soluble in water and can be operated at temperature of 40°C. This enzyme is stable at room temperature (25-30°C) during one year. The average product composition (w/w) is as follows: F(1%), G(21%), GF(20%), GF₂(55%), GF₃(3%). The soluble enzyme is operated, without reuse, in a batch stirred tank reactor or, with continuous reuse, in a membrane bioreactor.



There are no Free Fructosyltransferase enzymes available for commercial FOS production.

The enzyme 1-SSTrec developed at CIGB is recovered from the culture supernatant with purity above 90% and has competitive advantages over current production systems based on fugal cells/enzymes, in terms of:

- High specific activity (>10000 U/g)
 - High yield of total FOS (55-60%, w/w) in the reaction products.
 - High quality of final FOS composition (90% 1-kestose and 10% nystose)
 - Sucrose-hydrolyzing activity is negligible.
- Simple and flexible operation processes for FOS production at different scales.

Table 1: Comparison between Immobilized fungus enzyme and KestoZyme.

Biocatalyst	Company	FOS content (dry matter)	Composition at highest GF2	Composition at highest FOS
<i>I-SSTrec</i> <i>KestoZyme</i>	HeberBiotec	55-60%	GF2 ~ 91% GF3 ~ 9% GF4 ~ 0%	<u>GF2 ~ 86%</u> GF3 ~ 14% GF4 ~ 0%
<i>Aspergillus niger</i> <i>Immobilized</i>	Beghin Meiji, Meiji Seika	55-60%	GF2 ~ 76% GF3 ~ 22% GF4 ~ 2%	GF2 ~ 37% <u>GF3 ~ 53%</u> GF4 ~ 10%

Technologies have been developed for:

- The large-scale production of the Fructosyltransferase enzyme KestoZyme and the formulation as a stable enzyme powder with high specific activity.

The enzyme operation for FOS production in batch stirred tank reactors.

Corporate partnership for licensing our FOS production technology using KestoZyme biocatalizer.

PATENTS

The technology for the production and operation of the Fructosyltransferase enzyme (KestoZyme) is backed by the patent "Method of obtaining 1-kestose". No. of application in Cuba: CU2012-0138. International Application No.PCT / CU2013 / 000005. International Publication No. WO 2014/044230.

PUBLICATIONS

1. E.R. Pérez, L.E. Trujillo, J.G. Arrieta, H. Pérez, M.A. Brizuela, G. Trujillo, L. Hernández. **A pH shift-based procedure to screen fructooligosaccharides fermenting yeast and bacterial strains.** *Biotechnologia Aplicada* 2010, 27:216-220.
2. C. Menéndez, D. Martínez, L.E. Trujillo, Y. Mazola, E. González, E. Pérez, L. Hernández. **Constitutive high level expression of a codon-optimized β -fructosidase gene from the hyperthermophile *Thermotoga maritima* in *Pichia pastoris*.** *Applied Microbiology and Biotechnology* 2012, 97:1201-1212.
3. D. Martínez, B. Cutiño-Avila, E.R. Pérez, C. Menéndez, L. Hernández, A. del Monte-Martínez. **A thermostable exo- β -fructosidase immobilized through rational design.** *Food Chemistry* 2014, 145:826-831.
4. D. Martínez, C. Menéndez, F.M. Echemendia, E.R. Pérez, L.E. Trujillo, A. Sobrino, R. Ramírez, Y. Quintero, L. Hernández. **Complete sucrose hydrolysis by heat-killed recombinant *Pichia pastoris* cells entrapped in calcium alginate.** *Microbial Cell* 2014, Factories doi: 10.1186/1475-2859-13-87.
5. C Menéndez, D Martínez, LE Trujillo, R Ramírez, A Sobrino, BV Cutiño-Ávila, L Basabe, A del Monte-Martínez, ER Pérez, L Hernández. **Development of soluble and immobilized biocatalysts based on a recombinant thermostable β -fructosidase enabling complete sucrose inversion at pasteurization temperatures.** *Biotechnologia Aplicada* 2014, 31:249-253.
6. D Martínez, C Menéndez, FM Echemendia, L Hernández, A Sobrino, LE Trujillo, I Rodríguez, ER Pérez. **Kinetics of sucrose hydrolysis by immobilized recombinant *Pichia pastoris* cells in a batch reactor.** *Journal of Microbial & Biochemical Technology*, 2015, 7(5):294-298 doi:10.4172/1948-5948.1000228.

HIGHLY THERMOESTABLE BIOCATALIZADORES FOR THE PRODUCTION OF SIROPE INVESTED FROM SACAROSE	
Area: Industrial Enzymes	
OBJECTIVE	Commercial exploitation of thermostable biocatalysts for the enzymatic inversion of sugar and honey.
DESCRIPTION	<p>The hydrolysis of sucrose produces a mixture of glucose and fructose in equal proportion (known commercially as Inverted Sugar or Inverted Syrup). The use of Inverted Syrup presents advantages over its precursor, sucrose, in terms of higher total dry matter, higher solubility, higher retention of moisture, greater sweetening power and faster action as an energy food. The enzymatic hydrolysis of sucrose, unlike the conventional inversion with acid, does not use chemical substances nor generate toxic byproducts, and is therefore the most recommended process for the production of Inverted Sugar, with a destination in the food industry.</p> <p>CIGB has developed two biocatalysts for total or partial inversion of sucrose:</p> <ol style="list-style-type: none"> 1. The first biocatalyst is based on the immobilization of <i>Pichia pastoris</i> cells containing high levels of a thermostable invertase (TmINVrec) of bacterial origin in periplasm. A heat treatment eliminates the viability of the cells prior to immobilization in calcium alginate. This biocatalyst can be stably operated at 60-70 ° C to reverse concentrated solutions (55-60 ° Bx) of sucrose or honey, either in repeated batch cycles or in continuous flow columns. 2. The second biocatalyst consists of the recombinant enzyme (TmINVrec) recovered from the culture supernatant and formulated as a soluble high specific activity powder (> 15,000 U / g). The enzymatic activity is stable after one year of storage at room temperature (25-30 ° C). In both cases, the hydrolysis reaction is completed in a short period of time and results in syrup directly fit for human consumption.
PATENT	Specific Know-how
PROJECT STATUS	<p>The following technologies have been developed:</p> <ul style="list-style-type: none"> - Pilot scale production and operation of the immobilized cell biocatalyst. - Production and pilot scale operation of the free enzyme biocatalyst.
TYPE OF COLLABORATION REQUESTED	Strategic alliance and association for the production and / or commercialization of thermostable biocatalysts for the enzymatic inversion of sucrose.
COMPETITIVE ADVANTAGES AND MILESTONES	<p>The biocatalysts developed in the CIGB are more competitive than the current enzymatic systems of sugar inversion, in that:</p> <ul style="list-style-type: none"> • Its high thermal stability allows to operate in continuous reuse cycles. • Total sucrose inversion is achieved in short reactions. • Operating costs are low.
HOST INSTITUTION	<p>Centro de Ingeniería Genética y Biotecnología (CIGB). Ave 31, e/158 y 190 Cubanacán, Playa. PO Box 6162, La Habana, 10600, Cuba. Tel. (53-7)2504646 Fax. (53-7) 2736008; 2718070.</p> <p>Email: merardo.pujol@cigb.edu.cu Web site: http://gndp.cigb.edu.cu</p>
Código del Proyecto: AGBIO/CIGB/IAP-05	

BIOCATALIZADORES ALTAMENTE TERMOESTABLE PARA LA PRODUCCIÓN DE SIROPE INVERTIDO A PARTIR DE SACAROSA

Area: Technology of enzymes.

DESCRIPTION

The Center for Genetic Engineering and Biotechnology has developed two biocatalysts for total or partial inversion of sucrose:

The first biocatalyst is based on the immobilization of *Pichia pastoris* cells containing in periplasm high levels of a thermostable invertase (TmINVrec) of bacterial origin. A heat treatment eliminates the viability of the cells prior to immobilization in calcium alginate. This biocatalyst can be stably operated at 60-70°C to reverse concentrated solutions (55-60°Bx) of sucrose or honey, either in repeated batch cycles or in continuous flow columns. The second biocatalyst consists of the recombinant enzyme (TmINVrec) recovered from the culture supernatant and formulated as a soluble high specific activity powder (> 15,000 U/g). The enzymatic activity is stable after one year of storage at room temperature (25-30°C). In both cases, the hydrolysis reaction is completed in a short period of time and results in a syrup directly suitable for human consumption.

The project status is: The following technologies have been developed: Pilot scale production and operation of the immobilized cell biocatalyst and the free enzyme biocatalyst.

The table summarizes the productivity in each of the concentrations tested with two biocatalyst charges given by the biocatalyst (g) mass ratios: reaction volume (mL) or U/g sucrose, according to the amount of biocatalyst obtained from 1 L of culture. Under all tested conditions more than 50% hydrolysis of the sucrose was achieved.

Productivity of biocatalysts at different initial concentrations of sucrose

Biocatalysts	Biocatalyst /L*	Biocatalyst charges	500 g/L	600 g/L	700 g/L
PpABfrA	725 g	1:10	445,6	348,3	316,5
		1:5	226,3	226,8	215,4
BfrA libre	97379,8 U	4 U/g	2233,5	2160,0	2178,8
		8 U/g	2066,0	2000,0	1531,3

The productivity (P) represents the amount of hydrolyzed sucrose (500 g/L, 600 g/L, 700 g/L) per amount of biocatalyst obtained from 1 L of culture in the reaction time (g/L/h). Calculations were performed for more than 98% hydrolysis or at 10 h reaction. * Quantity of biocatalyst obtained by L of culture.

The stability of the biocatalysts under reactive conditions showed average life times of 360 and 168 h for PpABfrA and free BfrA, respectively. The biocatalysts developed in the CIGB are more competitive than the current enzymatic systems of sugar inversion (invertase from *Saccharomyces cerevisiae*), in that: Its high thermal stability allows to operate in continuous reuse cycles, total sucrose inversion is achieved in short reactions and operating costs are low.

PATENT: Specific Know-how

PUBLICATIONS

1. C. Menéndez, D. Martínez, L.E. Trujillo, Y. Mazola, E. González, E. Pérez, L. Hernández. **Constitutive high level expression of a codon-optimized β -fructosidase gene from the hyperthermophile *Thermotoga maritima* in *Pichia pastoris*.** Applied Microbiology and Biotechnology 2013, 97:1201-1212.
2. D. Martínez, C. Menéndez, F.M. Echemendia, E.R. Pérez, L.E. Trujillo, A. Sobrino, R. Ramírez, Y. Quintero, L. Hernández. **Complete sucrose hydrolysis by heat-killed recombinant *Pichia pastoris* cells entrapped in calcium alginate.** Microbial Cell Factories 2014, doi: 10.1186/1475-2859-13-87.
3. C Menéndez, D Martínez, LE Trujillo, R Ramírez, A Sobrino, BV Cutiño-Ávila, L Basabe, A del Monte-Martínez, ER Pérez, L Hernández. **Development of soluble and immobilized biocatalysts based on a recombinant thermostable β -fructosidase enabling complete sucrose inversion at pasteurization temperatures.** Biotecnología Aplicada 2014, 31:249-253.
4. D Martínez, C Menéndez, FM Echemendia, L Hernández, A Sobrino, LE Trujillo, I Rodríguez, ER Pérez. **Kinetics of sucrose hydrolysis by immobilized recombinant *Pichia pastoris* cells in a batch reactor.** Journal of Microbial & Biochemical Technology, 2015, 7(5):294-298 doi:10.4172/1948-5948.1000228.
5. D Martínez, C Menéndez, L Hernández, A Sobrino, LE Trujillo, I Rodríguez, ER Pérez. **Scaling-up batch conditions for efficient sucrose hydrolysis catalysed by immobilized recombinant *Pichia pastoris* cells in a stirrer tank reactor.** Electronic Journal of Biotechnology 2017, 25:39-42) doi:10.1016/j.ejbt.2016.11.003.

ACUABIO V: NUTRITIONAL SUPPLEMENT, IN AQUATIC SPECIES FOR AQUACULTURE IMPROVEMENTS OF GROWTH AND IMMUNE SYSTEM	
Area: Aquaculture	
GOAL	To assess in important species of aquatic organisms o (eg, shrimp, salmon, tilapia, carp, bivalves and other) a potent stimulator of growth in the early life stages.
DESCRIPTION	<p>A formulation able to improve the survival, growth and homogeneity in sizes of aquatic species grown in aquaculture. The CIGB 777 has been evaluated in Cuba at laboratory and pilot scales. The product is based on Releasing Peptide Growth Hormone (GRHP6), a secretagogue of growth hormone.</p> <p>During its assessments on fish and shrimp CIGB 777 showed:</p> <ul style="list-style-type: none"> • Increase in fish and shrimp's larvae surviving during farming (10-20% in larvae stage and ponds culture). • Increase in standard size and weight of whole fish and shrimps population during fish/shrimp farming.
PATENT STATUS	<p>"Analogue compound to peptide secretagogues of the growth hormone and preparations containing them": PCT/CU2007/000007. Date of International Application: February 28th, 2007. Granted in Australia, Europe, USA, Singapore, Japan, China, India, Mexico, South Korea, Canada, Cuba, Russia and South Africa.</p> <p>Filed in: Brazil, Chile and Malaysia</p>
PROJECT STATUS	<p>In assessment a formulation to be applied by immersion baths.</p> <p>In Development oil formulation to be added to the aquaculture feed.</p>
TYPE OF COLLABORATION REQUESTED	<p>Corporate partnership for out-licensing, co-development and commercialization.</p> <p>Identification of appropriate partners to:</p> <ul style="list-style-type: none"> • Register CIGB 777 formulation to be applied by immersion baths in regional aquaculture sectors linked to production. • Jointly develop the oily formulation of CIGB 777 to be added to the aquaculture feed.
COMPETITIVE ADVANTAGES MILESTONES	It promotes growth, survival, innate immune responses and antioxidant defense of economic importance's aquaculture species.
HOST INSTITUTION	<p>Centro de Ingeniería Genética y Biotecnología (CIGB). Ave 31, e/158 y 190 Cubanacán, Playa. PO Box 6162, La Habana, 10600, Cuba. Tel. (53-7)2504646 Fax. (53-7) 2736008; 2718070.</p> <p>Email: merardo.pujol@cigb.edu.cu Web site: http://gndp.cigb.edu.cu</p>
Project code: AGBIO/CIGB/IAP-06	

ACUABIO V: NUTRITIONAL SUPPLEMENT IN AQUATIC SPECIES FOR IMPROVEMENTS OF GROWTH AND IMMUNE SYSTEM.

Area: Aquaculture

DESCRIPTION

The Center for Genetic Engineering and Biotechnology has developed a formulation able to improve the survival, growth and homogeneity in sizes of aquatic species grown in aquaculture. It has been evaluated in Cuba at laboratory and pilot scales.

The product is based on Releasing Peptide Growth Hormone (GRHP-6), a secretagogue of growth hormone.

During its assessments on fish and shrimp Acuabio V showed:

- Increase in fish and shrimp's larvae surviving during farming (10-20% in larvae stage and ponds culture).
- Increase in standard size and weight of whole fish and shrimps population during fish/shrimp farming.
- Increase immune innate response and antioxidant defense in the species evaluated.
- Increase pathogens resistance in animals treated compared with controls one.

The project status is: in assessment a formulation to be applied by immersion baths and

In Development, formulation to be added to the aquaculture feed.



33 days of culture in ponds shrimp samples after four treatments with the Acuabio V. Control group represents larvae treated with BSA.

Innate immune Parameters and weight in tilapia larvae treated with Acuabio V after 30 days of culture.

	Control	Acuabio V
Weight (g)	0.188 ± 0.06*	0.231 ± 0.09*
Protein concentration (mg/g of tissue)	14.47 ± 5.92	9.53 ± 1.75
Antiprotease activity (anti-trypsin)	13.19 ± 2.31	23.11 ± 3.98*
Lectins (titre)	6.00 ± 2.19	14.67 ± 9.35*
SOD activity (U/mL·min)**	1.06 ± 0.09*	7.54 ± 1.36*

* Statistical differences t student $p < 0.05$

PATENT

Method of stimulating growth and resistance to disease of aquatic organisms. No. International Application: WO 03/080102 A1. Granted in Australia, Europe, USA, Singapore, Japan, China, India, Mexico, South Korea, Canada, Cuba, Russia, South Africa, Chile, Malaysia.

PUBLICATIONS

1. Rebeca Martinez, Yamila Carpio, Antonio Morales, Juana María Lugo, Fidel Herrera, Claudina Zaldívar, Olimpia Carrillo, Amílcar Arenal, Eulogio Pimentel, Mario Pablo Estrada. **Oral administration of the growth hormone secretagogue-6 (GHRP-6) enhances growth and non-specific immune responses in tilapia (*Oreochromis sp.*).** *Aquaculture* 2016, 452: 304–310 <http://dx.doi.org/10.1016/j.aquaculture.2015.11.014>.
2. Martinez R., Ubieta K, Herrera , F, Forellat, A , Morales R , de la Nuez A, Rodriguez, R, Reyes,O, Oliva , A, Estrada , MP. **A novel GH secretagogue, A233, exhibits enhanced growth activity and innate immune system stimulation in teleosts fish.** *Journal of Endocrinology* 2012, 214 : 409- 419, 2012. DOI: 10.1530/JOE-11-0373.

OBTAINING SOYBEAN PLANTS BEARING THE DEFENSIN GENE nmdef02 AND ITS EVALUATION AGAINST THE HONGO *Phakopsora pachyrhizi*

Agriculture Area: Agriculture

GOAL	To evaluate resistance to the fungus <i>Phakopsora pachyrhizi</i> in soybean plants transformed with the defensin gene nmdef02.
DESCRIPTION	<p>Plant diseases caused by fungi and oomycetes have an important impact for crop production in Cuba and worldwide. Every year huge amounts of fungicides for control are applied, with the effect thereof on the environment and human health. Find alternatives with biotechnological approaches for disease control is the biggest challenge for researchers related with this topic. Plant defensins are small, cysteine-rich peptides, which inhibit the growth of a wide range of microorganisms and are part of the defense mechanisms of plants against diseases.</p> <p>The application of the plant defensin as alternative to reduce crop losses due to the attack of pathogen constitutes an advantage with regard to the application of chemical fungicides. First: plant defensin are derived from seeds, roots and tubers, for what they constitute nature substances that are not toxic to the host plant and neither to people that consume the products from these plants. Second: as other protein, the defensin quickly degrades like native substances not leaving any residual after their effectiveness expires. This defensin was isolated from a plant and develops a strong anti- microbe activity in vitro and ex vitro against important bacteria, fungus and oomycetes pathogens. Herewith, the constitutive expression of this defensin in transgenic plants increased the resistance to many plant diseases with high economic impact.</p>
PATENT STATUS	<p>"Nucleic and amino acid sequences for the control of pathogen agents". CU2009-000003/WO 2009/117975 A1. Granted in: Cuba, United States, Europe, China, Japan, Vietnam, Israel, Mexico, South Africa, Australia, Colombia, Russia, India, Canada, Brazil, Iran, Nigeria.</p> <p>Publication: <i>NmDef02</i>, a novel antimicrobial gene isolated from <i>Nicotiana megalosiphon</i> confers high-level pathogen resistance under greenhouse and field conditions. <i>Plant Biotechnology Journal</i>, 8: 678-690, 2010.</p>
PROJECT STATUS	In field test
TYPE OF COLLABORATION REQUESTED	Corporate partnership for out-licensing , co-development and commercialization
COMPETITIVE ADVANTAGES MILESTONES	More than 10 years of efficacy studies in experimental areas of several crops against different important from the economic point of view plant pathogens.
HOST INSTITUTION	<p>Centro de Ingeniería Genética y Biotecnología (CIGB). Ave 31, e/158 y 190 Cubanacán, Playa. PO Box 6162, La Habana, 10600, Cuba. Tel. (53-7)2504646 Fax. (53-7) 2736008; 2718070.</p> <p>Email: merardo.pujol@ciqb.edu.cu Web site: http://gndp.ciqb.edu.cu</p>
Project code: AGBIO/CIGB/IAP-07	

SOYBEAN TRANSGENIC PLANTS EXPRESSING *nmdfe02* DEFENSIN TO PROTECT AGAINST FUNGI DISEASES.

Area: Agriculture

DESCRIPTION

This is an applied research project aimed to develop molecular strategies to inhibit fungi diseases like asian rust in the commercial soybean genotypes come together with it resistance to herbicide glyphosate.

The widespread and sustainable exploitation of crops around the world is continuously challenged to pest control (Delgado, 2015) and particularly those come from fungi. The scale-up of crops is a factor that triggers the emergence of diseases that affect its yielding; such is the damage of Asian rust *Phakopsora pachyrhizi* associated with the extending of soybean around the world. Soybean rust is a severe foliar disease of soybean that occurs throughout most soybean producing regions of the world. Soybean rust causes significant yield loss to soybean crops in Asia, Africa, Australia, and nearly all tropical countries in the Eastern where soybeans are grown have reported its occurrence. Rust is considered to be a major threat to soybean production in the Western hemisphere. In Brazil, this disease was estimated to cost growers approximately \$1.2 billion (USD) in 2003 alone. If *P. pachyrhizi* becomes established in the continental US, serious yield losses are likely to occur (Aragao, 2011; Enriquez et al., 2011). There are other fungal diseases associated with the cultivation of soybean that are of great interest because of their high frequency of incidence on the crop.

To solve the problem of diseases in cultivable plants, several interesting strategies have been approached (Borras et al., 2009; Portieles et al., 2010; Enriquez et al., 2011).

Thus, one of the researches carried out in the laboratory was to express the defensin NmDef02 in the Cuban soybean genotype in order to protect it against Asian rust produced by *P. pachyrhizi*, we followed for plant regeneration the procedure applicate by Cuban soybean cv Incasoy36, Figure 1.

Figure 1: Procedure for plant regeneration in Cuban soybean cv Incasoy36



Several Incasoy36 and DT84 transgenic lines *nmdfe02*- and *cp4epsps*- expressing genes were obtained and challenged in two independent field trials. It demonstrated that some of these lines were able to protect against not only for asian rust but also to the most important fungi of soybean under field condition. Figure 2.

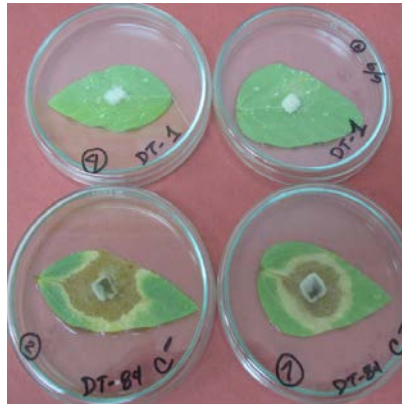


Figure 2: Transgenic defensin-expressing soybean cv DT84 were tested for fungus in an in vitro growth assay.

In experiments with this transgenic events in the field trial comparing with the not transgenic soybean plant was demonstrated the protections of the transformed plants against challenged with *P. pachyrhizi*, Figure 3.

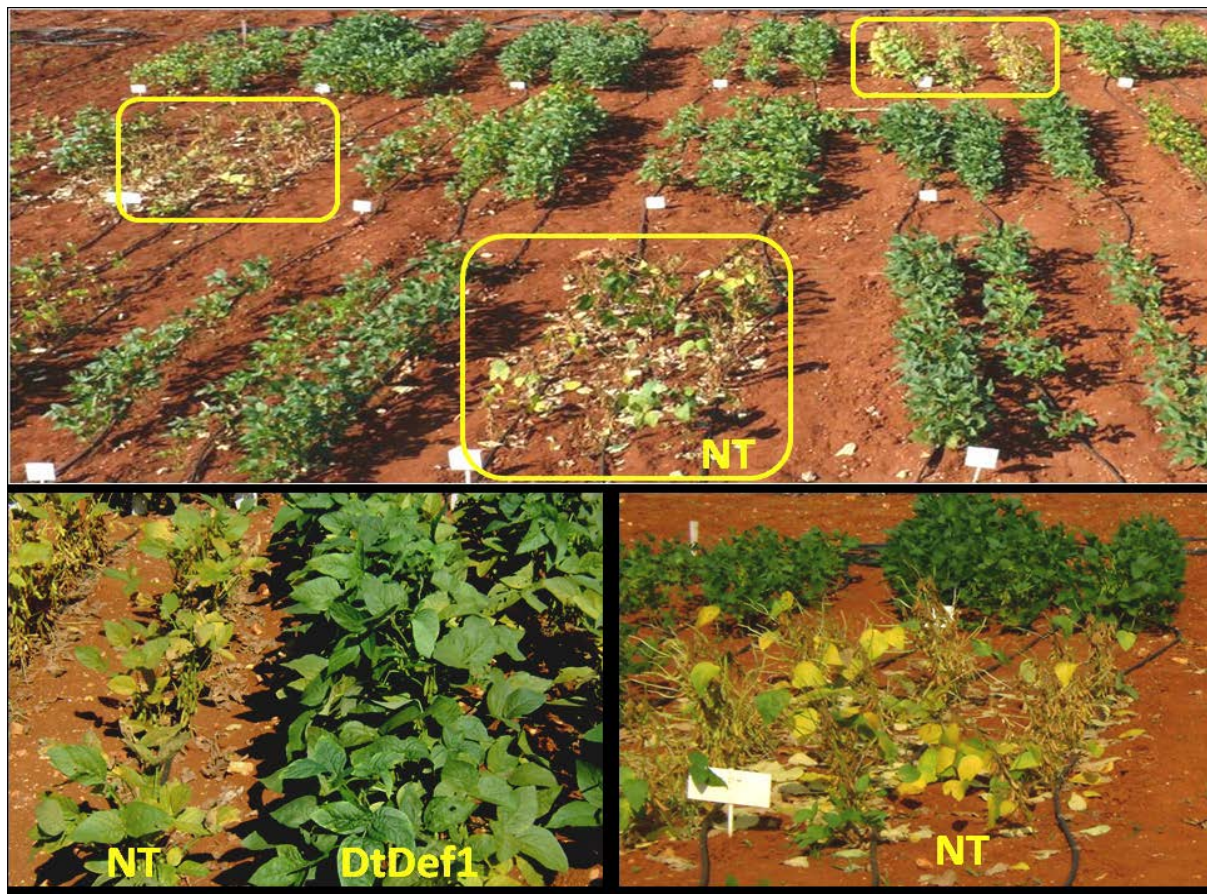


Figure 3: Field trial with soybean expressing defensin nmdef02, challenged with *P. pachyrhizi*. NT, conventional plants that were used as control.

PATENT

Aminoacids and nucleic acid sequences for controlling pathogens. International. Application Number PCT/CU2009/000003. International Publication Number: WO 2009/117975A1 Borrás, R Portieles, M Pujol, G Enriquez, E Gonzalez, C Ayra-Pardo, C Borroto (2009).

PUBLICATIONS

1. R Portieles, C Ayra, E González, A Gallo, R Rodríguez, O Chacón, Y López, M Rodríguez, J Castillo, M Pujol, G Enriquez, C Borroto, L Trujillo, B.P.H.J. Thomma, O Borrás-Hidalgo. **NmDef02, a novel antimicrobial protein isolated from *Nicotiana megalosiphon* confers high-level pathogen resistance under greenhouse and field conditions.** Plant Biotechnol. J. 2010, 8: 678-690.
2. G Enriquez et al, **Biotechnology for the genetic improvements of Cuban soybean varieties.** In book of Abstracts of Biotechnology Havana'2011 "Ag-Biotech: contributing to face global challenges", 2011, Havana, Cuba.
3. F Aragao, **Development of the imidazolinone tolerant soybean by Embrapa/BASF and future perspectives.** In book of Abstracts of Biotechnology Havana'2011 "Ag-Biotech: contributing to face global challenges", 2011, Havana, Cuba, pp. 157.
4. C Delgado, G Enriquez, R Ortiz, O Cespedes, N Soto, Y Hernandez, M Pujol. **Glyphosate resistance trait into soybean Cuban varieties: agronomical assessment of transgenic lines until F6 generation.** International Journal of Agronomy and Agricultural Research. 2015, 7, (4): 75-85.
5. Soto, N., Delgado, C., Hernández, Y. et al. **Efficient particle bombardment-mediated transformation of Cuban soybean (INCASOY-36) using glyphosate as a selective agent.** Plant Cell Tissue and Organ Culture 2017, 128:187–196.

CIGB -552vet: BIOLOGICAL CITOSTATIC FOR THE TREATMENT OF CANCER IN COMPANY ANIMALS.

Área terapéutica: Veterinaria

OBJECTIVE	To conclude the development, license the patent and assume registration and commercialization of the product in diverse markets of interest, this is a new drug directed to molecular targets useful in the treatment of cancer and its metastases.
DESCRIPTION	CIGB-552 is a second-generation peptide that increases the levels of COMMD1 and causes selective degradation of the RelA subunit (p65) of the NFkB transcription factor. CIGB-552 induces apoptosis and blocks cell cycle progression in several tumor cell lines without causing negative effects on normal cells. It has been shown to elicit a potent antitumor effect in various tumor models in immunocompetent animals and a broad therapeutic window in xenograft models of human colon cancer lines in nude mice. In addition, its antimetastatic effect has been demonstrated in the model of lung metastasis of Lewis carcinoma. Its subcutaneous administration is effective, and its biodistribution guarantees a direct apoptotic effect on the tumor. Its mechanism of action presupposes a new therapy directed to the regulation of the antiapoptotic activity of the factor NFkB in cancer cells that would provide greater selectivity and specificity. This new peptide represents a potential therapy for various types of cancer that affect companion animals as has been demonstrated in the tests performed on dogs such as sarcomas, lymphomas and others.
PATENT	<ol style="list-style-type: none"> 1. Immunomodulatory and antitumor peptides, PCT/CU2007/000006 Otorgada en Europa, China, México, India, Australia, Rusia y EUA. 2. Cell penetrating peptides and their use fused to biomolecules with therapeutic action, PCT/CU2008/000006. Otorgada en EUA, Canadá, México, China, Corea y Sudáfrica. 3. CIGB-552 second-generation peptide and COMMD1 protein as a new molecular target for cancer treatment. (PCT/CU2011/000003). Otorgada en EUA, México, China, Sudáfrica, Australia, Japón, y Ucrania.
PROYECTO STATUS	Extensive laboratory data, conducted two clinical trials in dogs with spontaneous tumors.
TYPE OF COLLABORATION REQUESTED	Corporate association for licensing, co-development, registration and marketing.
COMPETITIVE ADVANTAGES AND MILESTONES	<p>Product "First in its class". There are no drugs in the market whose mechanism is based on the inhibition of the transcription factor NF-kB by levels recovered from the COMMD1 protein in cancer cells. Biological properties of as cell penetrator, cytotoxic and immunomodulatory. It does not produce immunosuppression and produces few adverse effects.</p> <p>It can be combined with existing therapies. The mechanism of action demonstrates that the peptide has a great potential for application in various solid tumors, including colorectal, breast, lung and pancreatic cancer, in addition to those inflammatory diseases at risk of developing cancer.</p>
HOST INSTITUTION	Centro de Ingeniería Genética y Biotecnología (CIGB). Ave 31, e/158 y 190 Cubanacán, Playa. PO Box 6162, La Habana, CP 10600, Cuba. Teléf. (53-7) 2712397; 2716022. Fax. (53-7) 2736008; 2718070. Email: merardo.pujol@cigb.edu.cu Sitio web: http://gndp.cigb.edu.cu

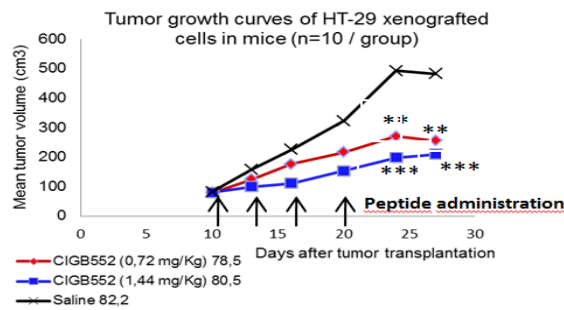
Código del Proyecto: BIO/CIGB/IAP-08

CIGB-552vet: BIOLOGICAL CITOSTATIC FOR THE TREATMENT OF CANCER IN PET ANIMALS.

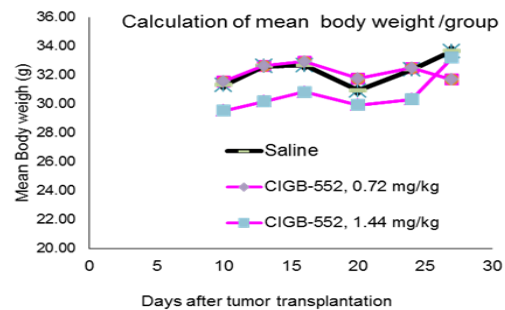
Therapeutic area: Veterinary

DESCRIPTION

CIGB-552 is a novel synthetic peptide derived from the antimicrobial peptide LALF 32-51 (*Limulus* sp) which has been shown to be a potential candidate for the anticancer therapy and one of its useful property is the cell-penetrating capacity. COMMD1 is a newly recognized pleotropic protein that plays an important role in inflammation, hypoxic response and cell survival. The recovery of COMMD1 protein by CIGB-552 inhibits NF- κ B, a key factor involved in the survival, proliferation and dissemination of tumor cells⁽²⁾. In addition, CIGB-552 inhibits the transcriptional activity of NF κ B by TNF α and IL-1 β in human colon cancer cells. The peptide CIGB-552 demonstrates potent *in vivo* efficacy against human colon and lung cancer with a considerable therapeutic window in murine xenograft models⁽³⁾ (Figure 1). Besides, safety and tolerability of the therapy with the peptide CIGB-552 in a model of spontaneous tumor in dog was demonstrated.



HT-29 (10^7 cells) day 0. At day 10 mice were randomised to 3 groups and treated s.c with CIGB-552 (0,72-1,44 mg/Kg) for two weeks (two dosis/week). One week after the last administration, tumors were taken and the respective sections were prepared for CD31 analysis.



The body weight of the animals was measured three times/week as an indication of gastrointestinal toxicity.

Figure 1 *In vivo* efficacy on a xenograft mouse model: colorectal (HT-29 human xenograft). Development by Experimental Pharmacology Oncology-Berlin

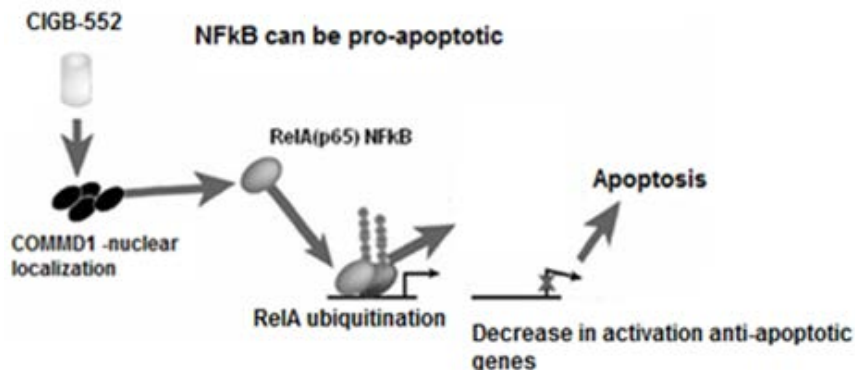


Figure 2: Propose Mechanism: We have demonstrated that CIGB-552 mediated-estabilization of COMMD1 promotes ReIA (p65) ubiquitination and subsequent repression of the NF κ B activity, an event linked to the apoptosis of cancer cells.

The mechanism of action of CIGB-552 assumes a new targeted anticancer therapy to regulate oncogenic and inflammatory activity of NF κ B in cancer cells, providing greater selectivity and specificity. This novel peptide presupposes a potential

applicability in solid tumors and inflammation associated- cancer including colorectal, breast, lung, melanoma, lymphomas and others.

Phase I clinical trial in Dogs.

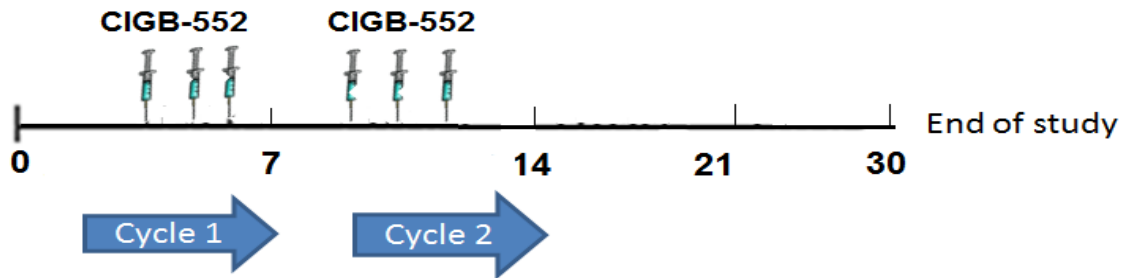


Figure 3. Design of study. CIGB-552 was evaluated at three dosage levels: 0.075, 0.15 and 0.3 mg/kg, s.c. Schedule: Three injections per week every other day for 2 weeks. Clinical assessments were done on days 0 , 7, 15 and 30 of the study.

Table 1: Responding population of dogs treated with CIGB-552vet

Breed	Dose of CIGB-552 (mg/Kg)	Tumor histology	TNM stage ¹	Response	Response duration (days)
1. Boxer	0.075	Cutaneous lymphoma	Stage IV	PD	-
2. Pekinese	0.075	Soft tissue sarcoma	T2bN0M0 Stage III	PD	-
3. Mexican hairless terrier	0.075	Cutaneous lymphoma	T1N0M1	CR	250
4. Teckel	0.15	Orbital melanoma	Stage III	SD	60
5. Cross- breed	0.15	Cutaneous lymphoma	Stage III	SD	60
6. Bichon habanero	0.15	Oral carcinoma (SCC)	T3bN0M0 Stage III	PD	-
7.Labradors	0.3	Spleen lymphoma	Stage I	PR	120
8. Cross- breed	0.3	Cutaneous mastocytoma	Stage III	PR	□60

Abbreviations: SCC, squamous cell carcinoma; TNM, tumor-node-metastasis; CR, comple esponse; PD, progressive disease; PR, partial response; SD, stable disease.

PATENTS

1. A patent was filed claiming the anti-tumor activity of the novel peptide and their mimetics (PCT/CU2007/000006) Granted: Europe, China, India, Mexico, Australia, Rusia, USA. Expire: 2027
2. Cell penetrating peptides and its use fused to biomolecules with therapeutic action (PCT/CU2008/000006). Granted in USA, Canada, Mexico, China, Korea, and Sudafrica. Expire: 2028
3. Cancer Therapy Method (PCT/CU2011/000003). The patent is claiming the sequence of the second generation peptide CIGB-552 and COMMD1 as a target for cancer therapy. Granted in USA, Mexico, China, Sudafrica, Australia, Japon, Ucrania. Expire: 2031

PUBLICATIONS

1. Maribel G. Vallespí, Julio R. Fernandez, Isis Torrens, Isbel Garcia, Hilda Garay, Osmani Mendoza, Milaid Granadillo, Viviana Falcon, Boris Acevedo, Raimundo Ubieta, Gerardo E. Guillen and Osvaldo Reyes. **Identification of a novel antitumor peptide based on the screening of an Ala library derived from the LALF₃₂₋₅₁ region.** J Pept Sci. 2010, Jan;16(1):40-7.
2. Julio R Fernández Massó, Brizaida Oliva Arguellez, Yelaine Tejeda, Soledad Astrada, Hilda Garay, Osvaldo Reyes, Livan Delgado-Roche, Mariela Bollati-Fogolín and Maribel G Vallespí. **The Antitumor Peptide CIGB-552 increases COMMD1 and Inhibits Human Lung Cancer Cells Growth.** Journal of Amino Acids, 2013, Article ID 251398.
3. Maribel G. Vallespí, Gilmar Pimentel, Ania Cabrales-Rico, Julio Garza, Brizaida Oliva Arguellez, Osmani Mendoza, Yolanda Gomez, Tais Basaco, Iraida Sánchez, Carlos Calderón, Juan C. Rodriguez, Maria Rivera Markelova, Iduna Fichtner, Soledad Astrada, Mariela Bollati-Fogolín, Hilda E. Garay and Osvaldo Reyes Acosta. **Antitumor efficacy, pharmacokinetic and biodistribution studies of the anticancer peptide CIGB-552 in mouse models.** Journal of Peptide Science. 2014, Volume 20, Issue 11, pages 850–859.
4. Teresa Núñez de Villavicencio-Díaz, Yassel Ramos Gómez, Brizaida Oliva Argüelles, Julio R. Fernández Mass, Arielis Rodríguez-Ulloa, Yiliam Cruz García, Luis Javier González, Vladimir Besada Pérez, Inés Tiscornia, Victoria, S., Mariela Bollati-Fogolín and Maribel G Vallespí. **Comparative proteomics analysis of the antitumor effect of CIGB-552 peptide in HT-29 colon adenocarcinoma cells.** Journal of Proteomics 2015, 126: 163–171.
5. Arielis Rodríguez-Ulloa, Jeovanis Gil, Yassel Ramos, Lilian Hernández-Álvarez, Lisandra Flores, Brizaida Oliva, Dayana García, Aniel Sánchez-Puente, Alexis Musacchio-Lasa, Jorge Fernández-de-Cossio, Gabriel Padrón, Luis J. González López, Vladimir Besada, and Maribel G Vallespí. **Proteomic Study to Survey the CIGB-552 Antitumor Effect.** BioMed Research International 2015, Article ID 124082, 18 pages.