UNITED NATIONS DEVELOPMENT PROGRAMME

Al servicio
de las personas
y las naciones

FINAL PROJECT REPORT

Project 79333: "Production and Application of Bio-products in Cultures of the Economical Importance"

March 2015

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de las personas
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FINAL PROJECT REPORT

a) BASIC PROJECT INFORMATION

Number and title of the project:
79333 "Production and Application of Bio-products in Cultures of the Economic Importance"

Coordinator: Cuban Research Institute for Sugar Cane By-Products (ICIDCA)

Other responsible parties (if any)
Four institutions recognized research Argentina, Mexico, Brazil and Cuba made packages proposed work. (UAC), México

- Autonomous University of Coahuila
- National University of Tucuman (NUT), Argentina
- Ribeirão Preto University, Brasil
- Cuban Resear Institut on Sucarcane Derivatives

Originally Preview: January 2011 Real: January 2012

Date of completion

Originally Preview: January 2013 Estimate: 2014

Reporting period: January 2012-November 2014

b) PROGRESS IN IMPLEMENTING THE PROJECT

a**) Project objectives:**

In the last times, natural compounds and bioproducts have been in the center of the attention of investigations towards the discovery of new products being ecologically safer. This way, the bio-products represent a big reservoir of safer chemical structures with biological activity.
This capacity of the bio-products is being used successfully as an effective alternative environmentally safer in the vegetal protection and development by the participant countries in this project: México, Argentina, Brazil and Cuba and other regional countries.
The Project is in the development program of ecological agriculture, starting from developing technologies with a commercial output of the bio-products obtained in a microbial way in economic interest cultures, for which it is necessary to improve current practices of bio-products production, to increase its scale and to establish a quality system allowing productions with commercial images, as a way to guarantee the extensive and general use in agriculture.

In this regard it is necessary to give an impulse to investigations towards applications and cooperation among countries, to allow the introduction in short term, of biological products in order to generalized way within producing sector, putting special emphasis in the prevention of the use of agrochemicals as main options to speed up the development of an environmentally friendly agriculture.
The research work aims at improving our knowledge of the bioactive products from microbial production using as substrate sugar by-products.

This objective will be reached completing the following stages:
1. Production of bio-products and microbial metabolites with bioactives properties on plants, increasing the mechanism of defense in plants, biocontrols and bioestimulaters characteristics in different cultures.
2. Dose, conditions and application definition of the microbial metabolites in the agriculture.
3. Improves of the agricultural yields and increasing the economical profits for introduction of the ecological agriculture.

Therefore, this project will provide of biotechnical tools in the production of bioproducts for agricultural, with the objective of:

1. Reinforce the viability of small farmers in rural areas by means of increasing incomes through the achievement of higher productivities.
2. To contribute to enlarge organic productions as well as the elimination or decrease of agro-chemicals in order to obtain environmentally friendly agriculture.

b) Project Outputs.

The outputs of the project were:

1. Isolation and selection of microorganism producers bioproducts and bioactive metabolites coming from the autochthonous regions of the integral countries of the project. This will allow having strains of diversity of soils and geographical regions, giving to the project a sense diverse in the projected outputs. A publication will be presented.

2. Optimization of the conditions of fermentation for the production of metabolites bioactives, using different substrates. Products with biocontrol and bioestimulaters characteristic will be defined and developed until the commercial presentation, and therefore they will impact directly in the agriculture. It will be presented in publications.

3. Design of a technological methodology for the application of bioactive products in the agriculture, defining dose and application conditions.

4. Economic report of the balances cost-benefit keeping in mind the cost of application of the biological products and their direct incidence on agriculture yields.

c) Project Activities

The activities of the project were distributed in 4 work packages, one of them being the coordination task.
Work packages.

Work Pack.	Work package title
	Project Management
1	Development of technologies for the production of agriculture bioproducts. Isolation and selection of microorganism producers of bio-products and bio-active metabolites coming from the autochthonous regions of the integral countries of the project.
2	Bioproducts and microbial metabolites production from microorganism. Optimization of fermentation.
3	Study of effect of the bioproducts and bioactive metabolites on different cultures.
4	Preliminary study of industrial validation. Economical evaluation. Prefeasibility study.

Evaluation of the Project objectives

All the objectives conceived for the project have been achieved.

In the following pages the main technical activities of the Project are described.

Developed activities

1. Development of technologies for the production of bioproducts for agriculture

a. Potential isolating microorganisms

b. Culture media design and production conditions

c. Scale of production volumes to provide products to apply.

d. Registration bioproducts

BIOTECHNOLOGY PRODUCTION OF BIOLOGICAL PRODUCT BIOJAS

It is presented in summary all stages culminating in the development of technology for the production of BIOJAS, whose active ingredient is jasmonic acid and jasmonates.

Microorganism *Botryodiplodia theobromae*, strains 715 and 1517, obtained from the collection of microorganisms of the National Institute for Basic Research in Tropical Agriculture (INIFAT), Havana, Cuba, which was preserved in inclined extract tubes malt-agar at 4 ° C. Culture medium: In the production of medium Miersch AJ (M-1, Table I) culture using sucrose as carbon source and potassium nitrate was used as nitrogen source.

Inoculum and fermentation conditions:

Petri plates were used with 25 mL of malt extract-agar, which was inoculated streak from mycelium of strains 715 and 1517 *Botryodiplodia theobromae* from the tubes grown for 3 days, the petri dishes were incubated for 5 days at 30 ° C. The culture medium was adjusted to pH 5.5 with NaOH (1N) and sterilized for 15 minutes at 120 ^{0}C, 10 mycelial fragments of 8mm obtained in Erlenmeyer flasks of 500 ml with 100 ml of medium Miersch culture used as seed culture, was incubated at 30 ^{0}C for 3 days were inoculated. Following the period growth of the microorganism, it is inoculated into Erlenmeyer flasks of 1 to 5 L total capacity at 30 ^{0}C, without shaking and aeration of 0.05 vvm for 15 days. At the end of fermentation Jasmonic Acid (JA) was quantified.

Detection and quantification of jasmonates. The fermentation medium was separated from the mycelium by vacuum filtration using Whatman filter paper No. 4. Aliquots of 5 ml of the cultivation filtrate were adjusted to pH 3.0 with HCl (4M) and subjected to extraction with ethyl acetate (1: 1). Fractions containing Jasmonic Acid (JA), were dehydrated with anhydrous sodium sulfate and taken to dryness by rotary evaporation at 50 ^{0} C. For the determination of AJ and related compounds, gas chromatography and HPLC was used.

<u>Kinetics of the Production of Jasmonic Acid</u>

Kinetic studies of the production of jasmonic acid were performed using strain 715.

Figure 1, shown the kinetics of the production of AJ and biomass, +pH and total reducer sugars consumption. It can be seen that for curve biomass production, first a latency phase close to

48 hours, wherein the microorganism is adapted to the new medium, followed by one logarithmic growth phase ends around the 144 hours in that maximum growth rate is on the order of 0,013 h-1 (average of 3 experiences). Start 144 hours begins the stationary phase, reaching a value of approximately 6.5 g / L of gravimetric dry matter.

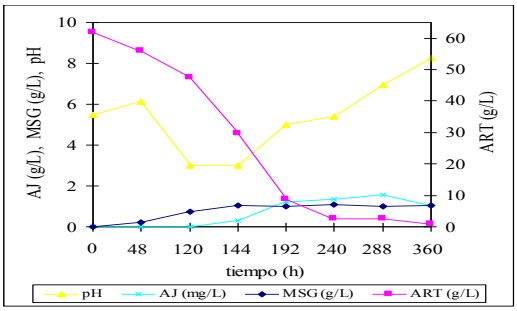


Figure 1 . Kinetics of jasmonic acid production, biomass, pH behavior and consumption of total reduced sugar in the cultivation of *Botryodiplodia theobromae* 715.

Conclusions

Microbiological production of Jasmonic Acid, is possible at concentrations in the order of 1000 mg / L, in static bioreactors up to 50 L of total volume with a superficial culture. This system is economic due to do not generate energy expenditure by the air supply, agitation and high costs for equipment.

Biojas registration

Biojas is a free biopreparation cell, obtained by fermentation in static culture, using *Botryodiplodia theobromae* strain 1517, whose active ingredient is jasmonic acid.

In the period, biological product BIOJAS was registered in the Cuban Central Register of Fertilizers, in November 28, 2013, the Certificate of Registration No 003/13 RCF enrolled in the first book, volume 1, page 031, being authorized its use in Cuba in all cultures valid until 28 November 2018.

STUDY OF THE PRESENCE AND QUANTIFICATION OF PHYTOHORMONES IN THE FERMENTATION BROTH OF *BOTRYODIPLODIA THEOBROMAE* BY LC-MS.

Chemical investigations of phytopathogenic fungi especially those associated with serious problems of agriculture, as the fungus *Botryodiplodia (Lasiodiplodia) theobromae*, has just begun. Achieve chemical characterization of novel metabolites present in both the broth and the biomass obtained during the microbial production of this fungus is of great importance, since it will expand the use and prospects of BIOJAS byproduct obtained in ICIDCA, favoring no only the production of a bio-stimulator, which allows the development of sustainable agriculture and is a benefit for feeding the population but also allowing evaluate new potential uses of this product, from the possible bioactive properties (antibacterial, antifungal and antitumor) of the metabolites found in the same.

For the determination of the phytohormone, indole acetic acid (IAA), indole propionic acid (AIP), Indole Butyric Acid (IBA) patterns (Cell Culture Reagents, SIGMA) and AJ (SIGMA) were used. Corresponding dilutions of a matrix solution, were made to prepare six working solutions for calibration curve in a range between 0.1 and 2.5 mg / L corresponding to each phytohormone. Fermented broths at the end of fermentation of the fungus *Botryodiplodia theobromae,* corresponding to 3 strains studied (3, 4, 83) were used.

Sample preparation:
pH of 5 mL fermentation broth, previously filtered and centrifuged, was adjusted between 2.5 and 3 with a solution of HCl (1 mol / L). Extraction was performed with ethyl acetate in a ratio of (1: 1), which was repeated for 3 consecutive times. The extract is subsequently rotoevaporated to dryness. For injection into chromatograph was rediluted in 1 mL of mobile phase and passed through a syringe filter of 0.45 µm.

Method HPLC-MS analysis
Analyses were performed on a HPLC-MS system with a HPLC equipment Agilent 1100 Series (Agilent Technologies, Santa Clara, CA, USA), which was equipped with a thermostatic autosampler and a quaternary pump connected to a Mass Spectrometer ion Trap with electrospray (ESI) interface. Samples and standards were injected onto a column of C18 reverse phase HPLC: Zorbax SB-C18, 3.5. µm, 2.1 X 150 mm, thermostatic to a 40 ^{0}C with a flow of 200 µl/min during the separation. Mobile phase A consisted of: 0.1% formic acid in MilliQ water (w/v) and mobile phase B 0.1% formic acid in methanol (w/v). Polarity gradient was used for separation. The mass spectrometer was operated in positive mode. Nebulizer gas pressure, drying gas flow and its temperature was 30 psi, 8 L/min, and 350 ^{0}C. Data were obtained in MS and MS / MS using MRM mode. The program used for the acquisition and processing of data was: Data Analysis Program for LC / MSD Trap Version 3.2 (Bruker Daltonik, GmbH, Germany).

Results and discussion
Were able to establish an analytical method for the simultaneous analysis of the AIA, AIP, AIB and AJ phytohormones, by LC-MS using the positive mode, although in the case of AJ no reports found in this way, when the optimization method is performed in this mode the best results for most phytohormones analyzed was found. When conditions were optimized for the analysis, masses transitions corresponding to each compound, and the retention time during the run were found. Linearity study was performed, for which the injection of working solutions was performed in triplicate and each correlation coefficient above 0.999, was obtained for the 4 compounds, in the range of concentrations studied. These data are summarized in Table 1. As can be seen the separation of compounds was achieved under the conditions employed.

Table 1 Transition of retention times and mass by LC-MS of analyzed phytohormones

Patrón	TR (min)	MS/MS	Recta calibrado	R^2
AIA	23,6	176/130	y = 1E+07x + 1E+06	0,9995
AIP	31,8	190/132	y = 8E+06x + 436955	0,9997
AIB	36,8	204/186	y = 5E+06x + 365342	0,9996
AJ	39,4	211/193	y = 1E+07x + 6E+06	0,9997

When broths extracts samples of the fungus 3 strains studied (C3, C4 and C83) were injected the results shown in Table below were obtained:

When samples corresponding to the extracts of the broths of 3 strains of the studied fungus (C3, C4 and C83) were injected, the results shown in Table below.

Fitohormona	Conc. Media (µg mL^{-1}) (R.S.D., %) AIA	AIP	AIB	AJ
Caldo fermentado C3	**0,047 (**0,002)	**0,0093 (**0,0004)	**0,025 (**0,001)	**99,84 (**0,15)
Caldo fermentado C4	**0,051 (**0,002)	**0.0023 (**0,0002)	**0,026 (**0,002)	**46,17 (**0,43)
Caldo fermentado C83	**0,017 (**0,002)	**0,0118 (**0,0008)	**0,0179 (**0,0006)	**65,50 (**0,02)

In the results, the presence of AIA is seen in the broths of the fungus even at very low concentrations between 0.02 and 0.05 $mg.L^{-1}$. In addition, the concentration of other auxinic phytohormones in broth such as AIP was below 0.05 and AIB in the order of 0.02 $mg.L^{-1}$. On the other hand, the concentrations of Jasmonic acid in the broths, was between 46 and 100 $mg.L^{-1}$.

CHARACTERIZATION OF A PIGMENT IN THE FUNGUS FERMENTATION BROTH

UV-Vis spectrum was carried out in the broth at the end of fermentation (10 days), due to the color intensity was diluted and a maximum at 500 nm is observed. IR spectrum was performed to fermented broth in his final hours (10 days) and the corresponding most significant bands were observed: a 3322.29 (ν OH inter- or intramolecular and N-H) 2945.17 and 2832.77 (C-H), 2361, 36 and 2337.45, 1644.96 (C = O), 1447.79, 1408.25, 1114.29, 1019.9 (νsim C-O ether forming ring or aryl ether.).

Fermentation broth of strain 83 was concentrated to 1/3 the initial volume in order to be characterized. When the characterization by HPLC-MS was performed, fermentation broth of strain 83 was concentrated to 1/3 the initial volume was used. This broth was subjected to the extraction process with solvents of increasing polarity: n-hexane, ethyl acetate and dichloromethane consecutively. Red pigment remains in the final aqueous phase after the extractions. This aqueous phase was rotoevaporated to near dryness in rotary evaporator at 40 ° C. This resulting aqueous sample was diluted in water and carried out a process of solid-liquid extraction using C18 Sep-Pak cartridges. From this process a fraction of ethyl acetate and methanol, was obtained and analyzed by HPLC with diode array detector.

ISOLATION OF MICROORGANISMS IN NATURAL ENVIRONMENTS (WORK IN THE AUTONOMOUS UNIVERSITY OF COAHUILA

Isolation of microorganisms was performed from 12 cocoa pods in the south of Mexico (Guerrero, Oaxaca, Chiapas and Veracruz).

Macroscopic and microscopic identification of isolates was done. The results are shown in Tables 2 and 3.

Tabla 2 Micro and macroscopic Identification of isolated strain

		COLOR DE LA COLONIA				CONIDIA
CEPA	CONIDIOFORO (µm)	TEXTURA	FONDO	MARGEN	REVERSO	ORNAM. Y TAMAÑO (µm)
4,1	18	Algodonoso	Blanco	Gris	Amarillo	2-3
4,2	12	Algodonoso	Blanco	Gris	Amarillo	3-4
4,3	16	Algodonoso	Blanco	Gris	Amarillo	2-3
4,4	14	Algodonoso	Blanco	Gris	Rosa (pigmento)	2-3
5,4	16	Algodonoso	Blanco	Gris	Amarillo	2-3
5,5	18	Algodonoso	Blanco	Gris	Amarillo	3-4
6,2	14	Algodonoso	Blanco	Gris	Amarillo	2-3
7,1	16	Algodonoso	Blanco	Gris	Rosa (pigmento)	2-3
7,2	16	Algodonoso	Blanco	Gris	Amarillo	2-3
7,4	17	Algodonoso	Blanco	Gris	Amarillo	2-3
7,5	18	Algodonoso	Blanco	Gris	Amarillo	3-4
10,1	19	Algodonoso	Blanco	Gris	Rosa (pigmento)	3-4
10,2	17	Algodonoso	Blanco	Gris	Rosa (pigmento)	2-3
10,3	16	Algodonoso	Blanco	Gris	Rosa (pigmento)	2-3
10,4	19	Algodonoso	Blanco	Gris	Rosa (pigmento)	3-4

Table 2a Micro and macroscopic Identification of isolated strain

CEPA PROBABLE	*LASIODIPLODIA SPP.*
ESPORANGIO	CONIDIÓFORO
ESPORA	BICELULAR
HIFAS	CEPTADAS, HIALINAS
COLONIAS	CAFÉ OSCURO DE TEXTURA ALGODONOSA

MACROSCÓPICA

The most likely species according to the comparison with Gilman j, will be the phytopatogenic fungus *Lasiodiplodia theobromae.*

Molecular identification was performed by displaying the extracted DNA by agarose gel electrophoresis, PCR and sequencing. 99% correlation with the fungus was found *Lasiodiplodia* spp.

Selecting a producer strain according to the production shows in Tables 3 and 3a.

TABLA 3 Selection of a producer strain of jasmonic acid

Muestra	Cepa	Ácido jasmónico
S	estándar	+
1	5,5	+
2	4,4	+
3	6,2	+
4	7,5	+
5	4,2	+
6	4,1	+
7	4,3	-
8	7,4	-
9	7,3	-

Table 3a Selection of jasmonic acid production strain

MUESTRA	CEPA	ÁCIDO JASMÓNICO
S	ESTÁNDAR	+
10	7,1	-
11	10,2	-
12	10,3	-
13	10,1	-
14	10,3	+
15	10,4	+

Jasmonic acid production was observed in only 9 of the 15 isolates. Table 4 shows the amount of jasmonic acid produced by the strains: : 4.4,5.5,6.2,7.5 y 10.4

Table 4 Quantification of jasmonic acid by selected strains

Cepa	Concentración mg/L
4,4	332
5,5	738.7*
6,2	48.2
7,5	443
10,4	208.4

Strain 5.5 is the higher jasmonic acid producer with a concentration of 738.7 mg / L, which shown biotechnological perspective and potential application in the field. This strain will be employed in fermentation studies.

BIOTECHNOLOGY PRODUCTION, CHARACTERIZATION AND USE OF PLANT PROTECTION BIOACTIVE METABOLITES OF *PSEUDOMONAS AERUGINOSA* PSS

The results obtained in the pilot plant production, characterization and evaluation of bio products Gluticid and HERBIO are presented. Both bioproducts are constituted by bioactive

metabolites of *Pseudomonas aeruginosa* PSS, for controlling phytopathogenic microorganisms and weeds. Isolation and purification of bioactive metabolites was performed from cell free supernatants by use different chromatography techniques, allowing to identify in Gluticid the siderophore pyoverdine type 11 as well as antimicrobial metabolite monoacetilphloroglucinol and in HERBIO, phytotoxins, which are small peptide with molecular weight around 1000 Da. Monoacetilphloroglucinol in Gluticid is 30-40 mg/g of commercial product and from 0.45 to 0.50 mg / g of Pyoverdine and pH between 5-6. The HERBIO, phytotoxin contains 10 mg of phytotoxins per gram of commercial product expressed as protein and pH 4.5.

Microorganism and culture conditions

Pseudomonas aeruginosa PSS from Culture Collection ICIDCA isolated from soil was used.

Pilot plant production of HERBIO and Gluticid, the most promising working conditions pilot plant stage were chosen, with appropriate adjustments relating to agitation and aeration at different stages: preparation of inoculum in the laboratory in orbital shaker; pre-fermentation using culture medium designed to each one, for Gluticid consists of glutamic acid as a carbon source, and salts, and the HERBIO glycerine as carbon source and salts in a Biolafite fermenter of 50 L with a working volume of 35 L fermentation stage was carried out in a 500L Marubishi fermenter with a working volume of 350 L, in the same medium in the pre-fermenter.

Down Stream

Centrifugation was performed in a Sharples centrifuge model AS26 IJV1 Laval eMBH, working at 12,000 rpm min^{-1}. Evaporation: The concentration of supernatant was performed in the evaporator batchwise. Drying: The drying was done in a type drier spray drying (spray-driers) Atomiser Niro, Copenhagen, Denmark, and ammonium sulfate was used as support.

Analytical determinations

Cell growth

Was estimated by measuring absorbance at a wavelength of χ 600 nm in a spectrophotometer PM.2^{a} OPTON.

Determination of Phytotoxins

Is performed to the cell free supernatants (CFS) by the method of microbiuret (Fankhauser, 2004). It is expressed as mg/mL protein.

Pioverdin determination

Is performed by measuring the absorbance at 400 nm and assuming the molar absorption coefficient for pyoverdines reported as 400 nm = 20,000 mol^{-1} x cm^{-1}-

HPLC determination. Gluticid pioverdin was extracted with methanol and was complexed with a solution 10 g/L Cl_3Fe. The mobile phase is: MeOH: H_2O: Acetic Acid 60: 40: 1

Phytotoxic activity *in vitro* was performed in petri dishes with moistened filter paper on *Bidens pilosa* L. freshly cut leaves. About 100 mL of sample were applied on the adaxial surface. 3 control leaves per treatment were employed, in which 100 mL of water was applied. The plates were sealed with parafilm and left at room temperature. The qualitative phytotoxic effect (Hartman et al, 1984) .was measured every 24 hours as a graduate scale of 1 to 4.

Thin layer e chromatography

Phytotoxin separation of the non-peptide pollutants in HERBIO, was performed on plates of silica gel 10 x 20cm, activated at 100 ° C, where it was applied 15 ml of ethanol extracts. The plates were developed in the following solvent systems: Ethyl acetate - isopropanol - water 15:30:20, methylene chloride - acetone 9: 2n-butanol - acetic acid - water 4: 1: 1.

HERBIO is a formulated as a wettable powder having the following characteristics: humidity: 4%, 9 mg of phytotoxin expressed as protein per g of commercial product, pH: 4.5.

Active ingredient of bioherbicide HERBIO, are phytotoxins produced by *Pseudomonas aeruginosa* PSS. The results obtained in cell growth, production of phytotoxins and glycerin consumption, shown in Figure 2. The kinetics of cell growth rapid growth was observed with a

minimal latency phase (2 hours), followed by a log phase ending around 16 hours, wherein the maximum specific growth rate (μ max), is on the order of 0.24 $h^{-1.}$. Biomass/substrate yield Y (x /s) 28%. and product/substrate yield Y (p/s) is 69.45%.

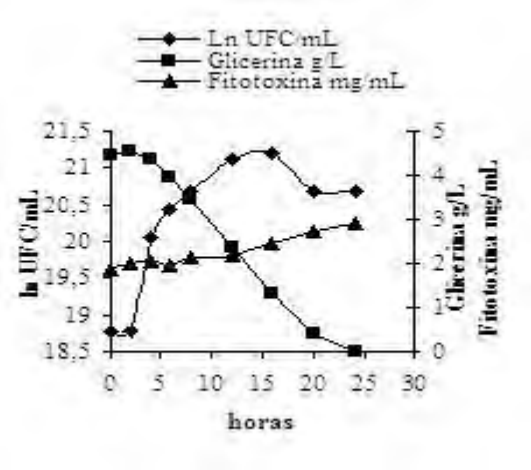


Figure 2 Cellular growth, phytotoxins production and glycerin consumption by Pseudomonas *aeruginosa* PSS

HERBIO Characterization

HERBIO characterization as a formulated wettable powder shown in Tables 5 and 6

Table 5 Characterization of HERBIO

Humedad %	2.13
pH	4.4
Fitotoxinas mg/g	9

Table 6 phytotoxic Activity *in vitro* of 10% HERBIO according scale on leaves of *Bidens pilosa* L

Días					
	mg de Fitotoxina aplicada	1	2	3	4
Fototoxicidad según escala					
Herbio 10 %	0.125	2	3	3	4

Phytotoxic activity measured every 24 hours according to the following scale graded from 1 to 4 where:

1 = no symptoms

2 = darkening of the edges and some dark spots

Dark spots 3 = 75% necrotic leaf

4 = total leaf necrosis

10 % HERBIO application, produced total necrosis of *Bidens pilosa* L leaves at 4 days. After 24 hours, the first signs of darkening of the edges of the leaves and some dark spots and at 48

hours, dark spots necrotic 75% of the sheet, and 4 days there is total necrosis demonstrating the effectiveness of the product under the studied conditions.

Conclusions

Gluticid and HERBIO bioproducts produced through biotechnology process and constituted by siderophores and monoacetilfloroglucinol metabolites for Gluticid and Phytotoxins for HERBIO represent a friendly alternative environment for controlling phytopathogenic microorganisms and weed control respectively with consequent reduction of chemical fungicides and herbicides.

PROSPECTION OF MICROBIAL METABOLITES WITH POTENTIAL APPLICATION IN AGRICULTURE.

Prospection of bioactive metabolites microorganisms isolated from the ultramafic deposits Cajalbana (Pinar del Río) and Cubanacan (Villa Clara) that could be promising for the development of new bio-agricultural purposes was performed. 84 were isolated microbial strains (34 and 50 Cajalbana Cubanacan). 47 bacterial cultures were placed taxonomically in a family of Enterobacteriaceae, genus *Azospirillum*, Azotobacter, *Beijerinckia Pseudomonas*. 100% of the strain fixed atmospheric nitrogen, inorganic phosphorus were solubilized by 58%, 57% had antagonistic activity against *Alternaria alternata*, *Fusarium solani*, *Fusarium moniliforme, Fusarium oxysporium esporotrichium* and *Fusarium*. 5 strains were selected as the most promising to be used in biotechnological processes, excrete metabolites that inhibit mycelial growth by over 30% compared with the control. Two as the most promising strains belonging to the genera *Azotobacte*r and *Azospirillum* sp, 2.8ay 3.8 (1) respectively are selected, both strains excreted protease enzymes to medium activity against a wide range of pH and temperature. The strain 2.8 SLC significantly increased the length of stem and root of radish plants, as well as the concentrations of nitrogen and phosphorus to the plant provided.

Results achieved:

84 microbial strains isolated from Cajalbana Pinar del Río and cuabal Cubanacan Villa Clara were characterized. 47 bacterial cultures were placed taxonomically as family Enterobacteriaceae (18), genus *Azospirillum* (12) *Azotobacter* (8) *Beijerinckia* (6) *Pseudomonas* (3) by biochemical and physiological tests.

Solubilization of inorganic phosphorus.

Of the 84 strains tested was found that 49 strains were able to grow on NBRIP medium supplemented with Ca_3 (PO_4) as a source of inorganic phosphorus, representing 58%. Phosphorus is one of the limiting nutrients for growth of plants and microorganisms in many ecosystems. This is often in an insoluble form. The major contribution of phosphorus to the soil due to the application of fertilizers, phosphates are rapidly immobilized in this and turn out this way unavailable to plants, so the ability to solubilize it would be of interest in the work of ecological restoration and phytoremediation because the hyper accumulator plants that are used in these processes are slow growing and solubilization phosphorus in the rhizosphere to be growth stimulatory.

Biological dinitrogen fixation

Taking into account that biological nitrogen fixation is another important factor to consider, bacterial cultures were inoculated into selective and differential semisolid medium Nbs and Ashby medium, allowing the qualitative study of dinitrogen fixation, and also allow the determination of nutritional requirements to fix atmospheric dinitrogen of different strains.

100% of strains grown in semisolid medium Nbs which means they all have the ability to fix atmospheric nitrogen although 45% required vitamins and micronutrients to do, the other 55% do not need it (growth amid Ashby). It should be noted that the fixing bacteria not symbiotic

dinitrogen are particularly at high concentrations in the rhizosphere of plants, region where the soil and roots make contact especially with grass, grass and turf, this aspect is well known to the rhizosphere of plant sugarcane, rice and other crops of commercial interest, not for the rhizosphere of Ni hyperaccumulators. These results are promising for further studies in order to obtain new biofertilizers.

Determination of antagonist activity in vitro

Was tested the antagonistic effect of the isolated against 5 strains of phytopathogenic fungi (*Alternaria alternata, Fusarium solani* (F-29), *Fusarium moniliforme* (F-22) *Fusarium esporotrichium* (F-13*) Fusarium oxysporium* (F-44). Around the 57% of strains showed antagonist effect against any of the fungal strains tested.

The most sensitive fungi were *Alternaria* and *Fusarium moniliforme*, with 32 and 35% respectively of all strains evaluated.

The strains VC 3.8 (1) VC 3.8 (2) VC 6.2 (a) VC 6.2 (b) and VC 2.8 (a), located in the genera *Azospirillum* and *Azotobacter r*espectively inhibited the mycelial growth of the five evaluated fungi which makes them promising to be employed in the development of products for biological control. Figure 3.

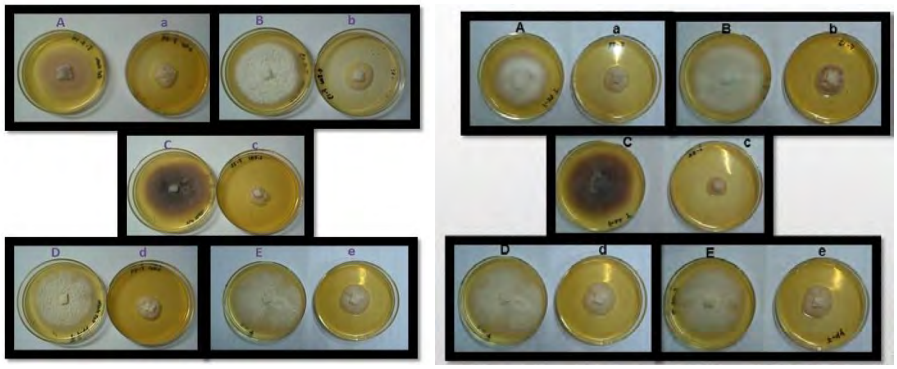


Figure 3 Effect of free cell supernatant of strains VC2 (left) and VC3 (right) on the growth of five strains of pathogenic fungi: A, to: *Fusarium solani* F-29; B, b: *Fusarium esporotrichium* F-13; C, c: *Fusarium moniliforme* F-22; D, c: *Fusarium oxysporium* F-44; E, e: *Alternaria alternata*. Control indicated in capital letters and Inhibition of growth of the strains indicated in lowercase. (B) Diameter of the fungal colony in the presence of SLC strains VC2 and VC3

Protease production

All strains showed proteolytic activity which was determined by the presence of halos on Agar translucent milk. This proteolytic activity could influence in antagonistic activity against phytopathogenic fungi.

Production of siderophores

The ability to produce siderophore in the two most promising strains was determined by measuring the absorbance of the supernatant at 400 nm. Siderophores concentration of 3.8 strain was 3 µM / L and strain 2.8ª of 6.35µM / L. These results are not significant if we compare the values with those produced by some species of the genus *Pseudomonas* are in the range of 140µM / L. However it is known that the siderophores absorbing at wavelengths that are of type Pioverdin. Spectrum of free cell supernatants absorption in presence of FeCl3 in both cases indicates a peak of absorbance at 300nm in the strain 3.8 (1) and in strain 2.8ª was 297nm which suggests some kind of iron chelation

Effect of metabolites excreted in the cell free supernatant of the two most promising strains of radish seeds under controlled laboratory conditions.

The effect of cell free supernatant (CFS) and strains over growth stimulation in stem and root of the plant (Figure 4) and increasing concentrations of nitrogen and phosphorus in plant, was determined. Strain 2.8 stimulated the growth of stem in 38 % and root in 41.3% and significantly increased supply of nitrogen and phosphorus to the plant.

Figure 4. Effect of the CFS and cell suspension of VC2 and VC3 strains on phenotype (A) and the lengths of the stem and the root (B) of radish (*Raphanus sativus)* plants variety Scarlet glove. *Azospirillum brasilense* strain 8I positive control and negative control water

BIOTECHNOLOGY DEVELOPMENT USING ORGANIC FERTILIZER MICROBIAL BIOPOLYMERS
Autonomous University of Tucumán

Synthesis of complexes formed by dextran, organic compounds and/or mineral salts.

For the synthesis of each complex was employed dextran obtained biotechnologically from *Leuconostoc. mesenteroides*, an inorganic iron salt and urea for agricultural use.

Determination of physical and chemical properties of the complexes obtained.

• Determination of dextran was conducted by phenol-sulfuric method.

• The iron determination was performed by indirect titration (iodometrically) using sodium thiosulfate as titrant and starch as indicator.

• Urea and nitrogen were measured by the Kjeldahl method

Stability studies of the complexes were determined following standard methods in time stability (15 ° C, 25 ° C, 35 ° C temperature for 12 months).

The ability to slow release nitrogen by the application of fertilizer on agricultural crops was studied. Plant growth was determined by planting in the greenhouse and sampling every 15 days from the application of fertilizers, using untreated control cultures treated with urea for agricultural use. At each sampling leaf area, fresh weight and dry weight was determined.

With the results obtained, the feasibility of agricultural use of the products studied as slow-release fertilizer nitrogen was evaluated.

- For the first time, the chemical complexes formed by microbial dextran polymer as controlled release vehicle, Fe as mineral micronutrient and urea as a source of N_2 was obtained.
- Chemical composition of the products obtained in Argentina regarding the complexes obtained in ICIDCA (Cuba) was studied. In the case of Argentine products, chemically-Fe-urea complex has 20% dextran, 2% iron and 60% urea. The fertilizer constituted by urea-dextran complex containing 16% dextran and 60% urea. Table IV.
- Both products were obtained in laboratory scale with yields of 82-87%.

- The physical composition, chemical and yield are slightly different from those obtained for the complex ICIDCA (Cuba), used as reference.
- Both contribute 27% of nitrogen to the soil as essential nutrient for plant growth that allow can be used as foliar fertilizers on agricultural crops.
- Early studies of application of fertilizers obtained in seasonal agricultural crops (pepper for paprika) shown that nitrogen is released from the complex and is absorbed by the plant stimulating growth.

Table 7 Chemical characterization of the complex Fe- Dextrana- Urea for use as controlled release fertilizer.

	Fertilizante Dextrana-Fe-Urea (FBQF-Arg)	Fertilizante Dextrana-Urea (FBQF-Arg)	Fertilizante Dextrana-Fe-Urea (ICIDCA-Cuba)
DEXTRANO %	20	16	8 -10
UREA %	60	60	47
HIERRO %	2	-	0,4-2,5
NITROGENO %	27	27	21
pH	5,5-8,5	6-8,5	3,5-9
Apariencia		Polvo marrón claro	Polvo marrón oscuro

Figure 5 fertilizer Dex-Fe-Urea FBQF- Argentina
Figure 6 Dex-Urea Fertilizer FBQF-Argentina
Figure 7 fertilizer Dex-Fe-Urea ICIDCA Cuba

Slow release fertilizer, first synthesized in Tucumán-Argentina, and were obtained with high production yield, possess similarity to those synthesized in Cuba, provide 270 g of nitrogen per kg of fertilizer applied and stimulate growth of agricultural crops foliar station.
Currently, he continues the study of technology in order to optimize the production process at pilot scale to achieve a more competitive and easily applicable in agriculture product parameters.

PRODUCTION, IDENTIFICATION AND CHARACTERIZATION OF METABOLITES WITH ANTIFUNGAL ACTIVITY PRODUCED BY BACTERIA EXTREMOPHILE PROMISING FOR BIOCONTROL

Determination of catechol-type siderophores by the *Bacillus* sp.VC3

The presence of siderophore catechol was determined by the colorimetric method Arnow (Arnow, 1937) using catechol as standard growth after growth of strain VC3in Fiss -Glucose medium with iron limiting conditions at 24 and 96 hours of incubation.

Table 8 Concentration of catechol siderophores produced by the *Bacillus* sp. strain VC3 to 24 and 96 hours

Cepas	Concentración de catecol mg/L	
VC3 24 horas	8,16 (0,31)	
VC3 96 horas	2,58 (0,16)	

Siderophores and specifically catecholates are other important metabolites with potentiality in biological control, due to they play a very important role in the chelation, mobilization and transport of ferric iron in soils with low concentrations of it. Table 1 shows the production of these metabolites by the *Bacillus S*p. VC3.

Table 1 shown that is obvious that as increasing the incubation time decreases drastically siderophore concentration, which can be attributed degradation in the environment.

In order to test different culture media cheaper and produced in Cuba, was tested molasses yeast hydrolyzate (medium BM2) and slop yeast hydrolyzate (medium BM3) as a source of organic nitrogen.

Figure 7 shown the proteolytic activities of cell free supernatant (CFS)

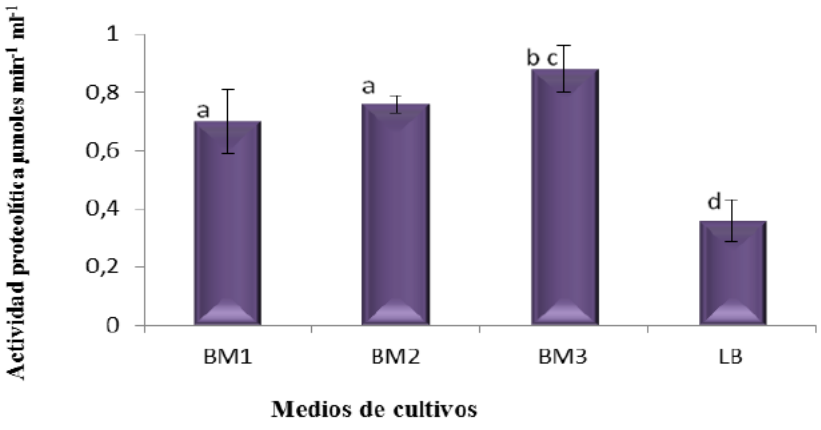


Figure 8 Proteolytic activity of the cell-free supernatants of *Bacillus* sp. strain VC3 in BM1, BM2 and BM3 medium, compared to the control LB.

The highest values of proteolytic activity were obtained with the CFS medium BM3 with significant differences compared to the rest of the medium tested. Meanwhile in SLC BM1 and BM2 medium proteolytic activity was higher to LB medium used as a positive control.
The results of the antimicrobial activity and proteolytic activity indicate that the BM3 medium constituted by cane molasses, salt and slop yeast hydrolyzate, is promising for producing a fungicide.
The most likely species according to the comparison with Gilman j. is the phytopathogenic fungus. *Lasiodiplodia theobromae*
Molecular identification was performed by displaying the extracted DNA by agarose gel electrophoresis, PCR and sequencing. 99% correlation with the fungus *Lasiodiplodia spp* was found.

EVALUATION OF COLLECTION STRAINS FOR THE PRODUCTION OF INOCULUM LB-1 (Efficient Microorganisms)

The concept and technology (EM Technology®, for its acronym in English) of Efficient Microorganisms was developed by Professor Teruo Higa, Faculty of Agriculture, University of Ryukyus- Okinawa, Japan.
Through years of experimentation he discovered the right mix of microorganisms and named "EM®" mixture, an acronym for Efficient Microorganisms, which are composed of highly efficient and beneficial organisms, no harmful or pathogenic, or genetically modified or chemically synthesized, that work synergistically as the sum of the three has more effect than each separately.
The EM ™ technology, originally developed as an alternative to chemical fertilizers and pesticides, has expanded in the last two decades of agriculture to water and effluent treatment, odor control, farms and animal health, human health and innumerable industrial treatments .
In Cuba are produced by hand, with very good results in its application mainly in agriculture, but the microorganisms containing are known, so it is of interest, producing an inoculum with collection microorganisms, allowing control quality of productions.
The aim of this work is the evaluation of microorganisms from Culture Collection ICIDCA in laboratory level for the production of inoculum LB-1 and product LEBAME consisting of effective microorganisms.

Table 9 Combination of evaluated microorganisms

No	*Lactobacillus*	Levadura	*Bacillus subtilis*
1	*Lactobacillus bulgaricum* B/103-4-1	Saccharomyces cerevisiae L/25-7-12	*Bacillus subtilis* B/23-45-10 Nato
2	*Lactobacillus plantarum* 1-5	Saccharomyces cerevisiae L/25-7-12	*Bacillus subtilis* B/23-45-10 Nato
3	▪ *Lactobacillus bulgaricum* B/103-4-1	Saccharomyces cerevisiae L/25-7-76	*Bacillus subtilis* B/23-45-10

			Nato
4	*Lactobacillus plantarum* 1-5	Saccharomyces cerevisiae L/25-7-76	*Bacillus subtilis* B/23-45-10 Nato
5	*Lactobacillus bulgaricum* B/103-4-1	Saccharomyces cerevisiae L/25-7-12	*Bacillus subtilis* B/23-45-3
6	*Lactobacillus plantarum 1-5*	Saccharomyces cerevisiae L/25-7-12	*Bacillus subtilis* B/23-45-3
7	▪ *Lactobacillus bulgaricum* B/103-4-1	Saccharomyces cerevisiae L/25-7-76	*Bacillus subtilis* B/23-45-3
8	*Lactobacillus plantarum* 1-5	Saccharomyces cerevisiae L/25-7-76	*Bacillus subtilis* B/23-45-3

Microorganisms were grown separately for 24 hours and subsequently in a mixed culture for 48 hours in a static way.

Inoculum activation

Was performed in 1 L Erlenmeyer flasks with plastic cover, containing cane molasses at a concentration of 30 g/L and 3% of inoculum.

Results

The results of cell growth after activated the inoculum, are shown in Table 12. All the strains combination studied, had cellular concentration between 10^6 and 10^7 CFU/mL, higher than those reported for commercial products which consist of effective microorganisms where the concentration of lactobacillus is 1×10^4 and yeasts 1×10^3.

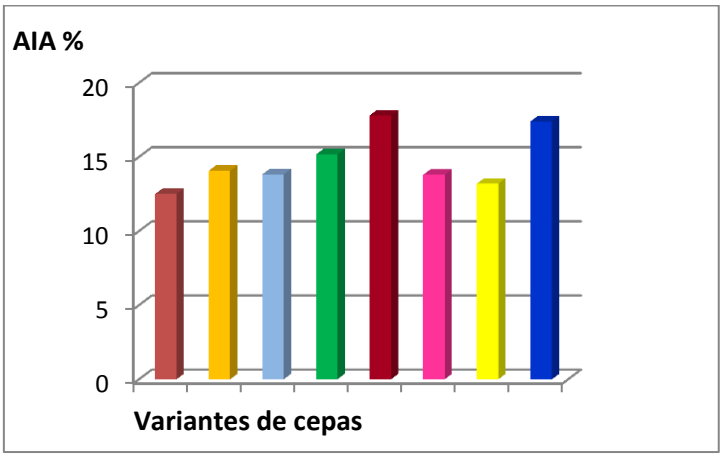
Table 10 Cellular concentration of bacteria and yeast tested

No	***Lactobacillus***	**Levadura**	***Bacillus subtilis***	
1	***L. bulgaricum* B/103-4-1**	***S. cerevisiae* (L/25-7-12)**	***Bacillus subtilis* B/23-**	

			45-10 Nato	
2	***L. plantarum* 1-5**	***S. cerevisiae* (L/25-7-12)**	***Bacillus subtilis* B/23-45-10 Nato**	
3	▪ ***L. bulgaricum* B/103-4-1**	***S. cerevisiae* L/25-7-76**	***Bacillus subtilis* -B/23-45-10 Nato**	
4	***L. plantarum* 1-5**	***S. cerevisiae* L/25-7-76**	***Bacillus subtilis* B/23-45-10 Nato**	
5	***L. bulgaricum* B/103-4-1**	***S. cerevisiae* (L/25-7-12)**	***Bacillus subtilis* B/23-45-3**	
6	***L. plantarum* 1-5**	***S. cerevisiae* (L/25-7-12)**	***Bacillus subtilis* B/23-45-3**	
7	▪ ***L. bulgaricum* B/103-4-1**	***S. cerevisiae* L/25-7-76**	***Bacillus subtilis* B/23-45-3**	
8	***L. plantarum* 1-5**	***S.cerevisiae* L/25-7-76**	***Bacillus subtilis* B/23-45-3**	

Indol Acetic Acid (AIA) is an auxin, which has a positive effect on the formation of the roots, and the initiation of lateral root hairs, which causes the reduction of wall pressure and induce the synthesis of specific enzyme and this leads to increase cell wall plasticity, promoting germination. Taking into account that LEBAME product (efficient microorganisms) has application in agriculture as biostimulant, is of interest to know which of the studied variants produced as much this auxin.

Figure 9 Production of indolaetic acid (IAA) for the different variants of microorganisms studied

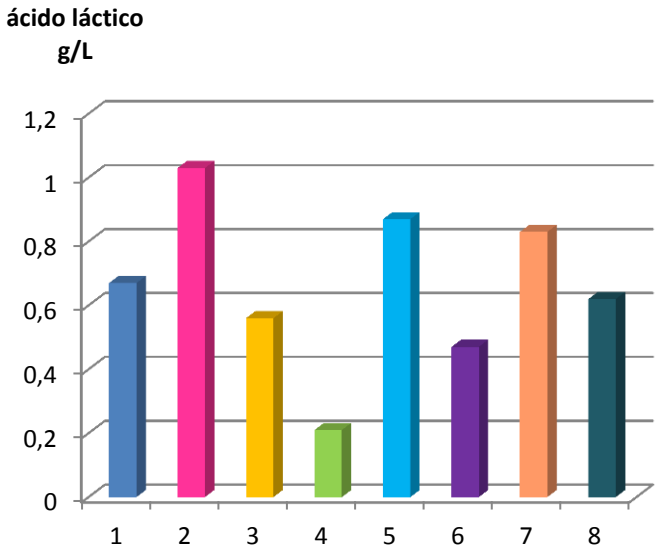


Among the applications of effective microorganisms, animal production is one with great importance, for which it is of interest the production of lactic acid produced by *Lactobacillus*, one of the effective microorganisms, because the potential benefits from the standpoint of nutritional and health (Sissons, 1989; Tannock, 1989,1992).

Currently, the use of lactobacillus is oriented triple sense
1)Growth stimulants and enhancers in food processing livestock products.
2) Control of intestinal imbalance in young animals, by developing the indigenous microflora and the resistance to colonization in the intestine
3) Predigestion of anti-nutritional factors (Havenaar and Huis, 1992)

In Figure 9 the production of lactic acid occurs. In it can be seen that the variant 2 having *Lactobacillus plantarum*, which is produced the largest amount of this compound.

Figure 9 Lactic acid production by the different variants of microorganisms studied



LEBAME is a biostimulating agricultural consisting of the following microorganisms ICIDCA culture collection:

- *Bacillus subtilis* B / 23-45-10 Nato (1 x 107 CFU / mL)
- *Lactobacillus bulgaricum* B / 103-4-1 (1 x 107 CFU / mL)
- *Saccharomyces cereviciae* L-25.07.12 (1.8 x106UFC / mL)

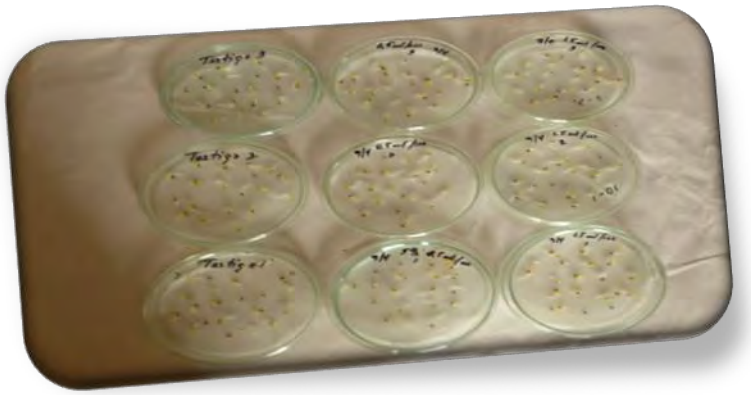
The inoculum is prepared in a first stage, growing each microorganism independently in their respective growth media at 32 ^{0}C for 48 hours, and in a second stage are grown together at 30 ^{0}C for 64 hours. Subsequently the same fermentation is performed statically using sugarcane molasses and ammonium sulfate until total consumption of reducing sugars and lower the pH. The final product has a pH of 3.8 and 1.5 g / L of ART.

IMPLEMENTATION OF A BIOASSAY IN VITRO AS AN INDICATOR OF QUALITY BIOPRODUCT LEBAME

Plant bioassays complement chemical analysis for the detection of phytotoxic metabolites in a product applied in agriculture, which can cause negative effects in plants.

Bioassays with seeds are used to determine the stimulatory effect of different biological products and for the detection and control of environmental toxic pollutants. The present study aimed to the design an in vitro bioassay with chard seeds (*Beta vulgaris*), to carry out the monitoring of the quality of the final output of LEBAME, byproduct consisting of *Bacillus subtilis* B / 23-45- 10 Nato, *Lactobacillus bulgaricum* B / 103-4-1 and *Saccharomyces cereviciae* L-25/07/12, by determining the rates of germination and growth of chard seeds (*Beta vulgaris*) as indicator plant. Significant effects LEBAME concentration was determined by Multiple Range Test for variables: Relative Percentage germination (RPG), Relative Growth Radicle (RGR), Relative Hypocotyl Growth (RGH) and germination index (GI). The following experimental procedure was followed:

- 20 chard seeds (*Beta vulgaris*) provided by National Institute of Agricultural Sciences per each Petri dish on filter paper moistened with 2.5 mL of distilled water in triplicate.
- The plates are placed in a plastic bag at a temperature of 22 ± 2 0C for 3 days to prevent moisture loss.
- At the end of exposure period (3 days), we proceeded to record the number of seeds that germinated normally, considering as a criterion for germination visible radicle.



- To facilitate measurement of the radicle and hypocotyl, we proceeded to freeze the Petri dishes after incubation at 4 0C completed.

- The seedlings with a soft consistency are placed on a transparent glass plate and photographed at a height of 32 cm chamber.
- Length measurements radicle and hypocotyl, are performed digitally using Adobe Photoshop software program. Version 8.0.1.
-

The effect of different concentrations of LEBAME, on the RPG, RGR, RGH and GI shown in Figure 11.

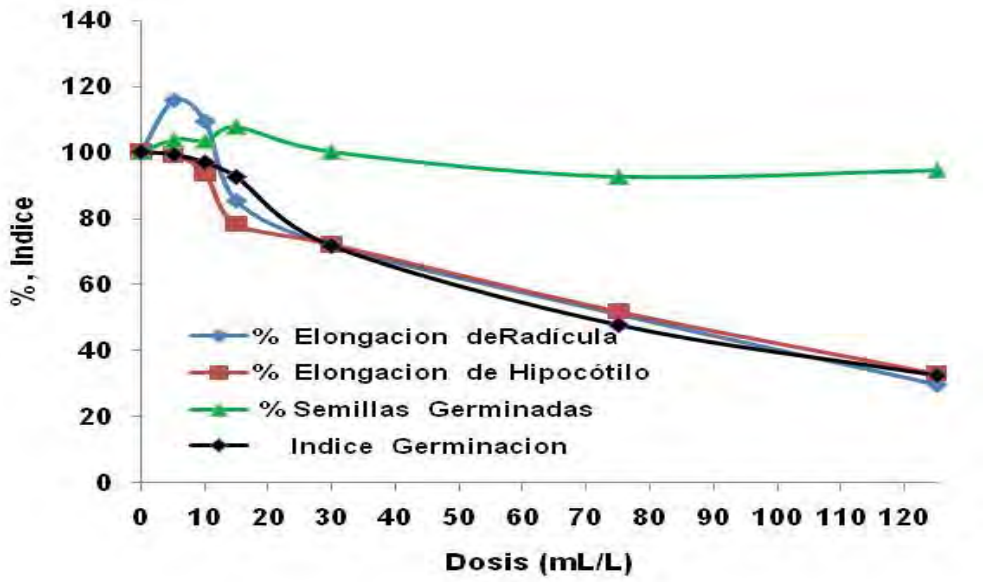


Figure 10 Effect of different concentrations of LEBAME, on the relative germination average (RGA), relative radicle growth (RRG), relative hypocotyl growth (RHG) and germination index (IG)

In all cases, the highest values of germination and growth are achieved at the concentration of 5 mL/L an decreasing at concentrations of 10, 15, 30, 75 and 125 mL/ L, with phytotoxic effect, which can be explained by the existence of hormesis phenomenon, characterized by low-dose stimulation and inhibition at high doses.

For a confidence level of $p < 0.05$, the results showed no significant differences between the concentrations of 5 and 10 mL / L.

The germination index (GI) is a more complete to describe the phytotoxic potential of organic material by ranges are indicator below:

- IG ≥ 80% No phytotoxic substances or who are at very low concentration
- IG between 50% and 80% indicates moderate presence of these substances.
- IG ≤ 50% Strong presence of phytotoxic substances

Conclusions

- The method allows monitoring and control LEBAME effects on the growth of chard taken as a model, and potential phytotoxic effects thereof.
- Concentration of 5 mL/L is chosen as it is for determining the stimulatory effect of the product by determining the relative growth of the radicle and CCR no phytotoxic effects.
- Length measurements radicle and hypocotyl, which are performed digitally using Adobe Photoshop CS software program, version 8.0.1, allows greater accuracy and speed of results.

STUDY OF STABILITY OF BIOPRODUCT LEBAME

The stability of a product is one of the parameters required for use and marketing. The study of the storage stability of a product, allows knowing the ability of this product to vary or degrade in the presence of different factors affecting the physical, chemical and biological characteristics.

The objective was to determine the stability of the product "LEBAME" in different storage conditions, for a period of six months.

Through a submerged fermentation process, batch "LEBAME" were obtained. They were packaged in plastic bottles of 1 liter of capacity, sealed.

Then each was stored at room temperature (25-30 ° C) and cooling (12 ° C) for a period of about 6 months.

Microbiological and analytical determinations:

Determinations were performed at different storage times and conditions in the two temperatures indicated.

- Count of microorganisms (bacteria and yeast cells) were performed as established in the literature
- Determination of pH
- Losses of microorganisms (bacteria and yeast) were determined by the value of the logarithm (base 10) counting cell respectively.

Statistical analysis of the results:

The results were processed using the Statgraphics Centurion XV program. 2007, by analyzing Multifactorial ANOVA, considering factors: temperature and time.

Multiple range tests were carried out in each case using the method: 95.0% Fisher LSD.

Resultados y Discusión

Los resultados del comportamiento celular, en diferentes lotes del producto “LEBAME”, almacenados cada uno en refrigeración (12 °C) y a temperatura ambiente (25-30° C), por un período de aproximadamente 6 meses, se muestran en las Tablas 12 y 13.

Table 11 Counting Microorganisms (CFU ml^{-1}) In a lot of the product "LEBAME stored 6 months in cooling (12 ^{0}C)

Número del Lote	Tiempo (días)	Conteo de bacterias (CFU. mL^{-1})*	Conteo de levaduras (CFU. mL^{-1})*
1	0	1.00 x10^8	5.00 x 10^6
	32	7.00 x10^6	4.00 x 10^5
	170	1.60 x 10^6	7.00 x 10^5
	200	9.50 x 10^6	4.50 x 10^5

2	0	1.00×10^{8}	5.00×10^{6}
	32	7.20×10^{6}	2.50×10^{5}
	170	3.25×10^{6}	2.00×10^{5}
	200	3.85×10^{5}	4.50×10^{4}
3	0	4.00×10^{5}	8.00×10^{6}
	32	3.50×10^{5}	1.55×10^{6}
	170	1.60×10^{6}	8.00×10^{5}
	200	3.70×10^{7}	1.80×10^{7}

***Average values**

Table 12 Counting Microorganisms (CFU mL^{-1}.) In a lot of the product "LEBAME" stored 6 months at room temperature (25-30 ° C), average values

Número del lote	Tiempo (días)	Conteo de bacterias (CFU. mL^{-1})	Conteo de levaduras (CFU. mL^{-1})
1	0	1.00×10^{8}	5.00×10^{6}
	32	1.10×10^{7}	4.00×10^{6}
	170	1.75×10^{6}	1.50×10^{5}
	200	3.40×10^{5}	5.00×10^{4}
2	0	1.00×10^{8}	5.00×10^{6}
	32	1.20×10^{6}	2.50×10^{5}

	170	1.50×10^{6}	6.00×10^{5}
	200	7.45×10^{5}	3.85×10^{5}
3	0	5.35×10^{6}	8.00×10^{6}
	32	1.40×10^{6}	4.00×10^{5}
	170	1.10×10^{5}	2.00×10^{5}
	200	2.70×10^{7}	1.00×10^{3}

LEBAME product obtained has a bacterial population between 10^{5}-10^{8} UFC. mL^{-1} and a population of yeast 10^{5}-10^{6} UFC. mL^{-1}.

ENRO, 2013 reported for product Efficient Microorganisms EM-1 at least the following microorganisms in thousands of CFU / mL in aqueous solution: Bacteria Lactic Acid 1×10^{4} and 10^{6} photosynthetic bacteria, yeasts 10^{3} sheet of Effective Microorganisms in Dominican Republic a single cell concentration for the same microorganisms $1x\ 10^{4}$ CFU / ml is reported.

The time in which a given original population of a product ratio remains viable after storage at conditions chosen, known as pot life or shelf life, so to analyze the cellular behavior in the storage time, from the above results microorganisms losses in both temperature conditions (cooling to 12 ° C and at room temperature 25-30 ° C) was calculated; analysis of variance was performed for variables: loss of bacteria and yeasts.
In Table 14 the results of the analysis of variance for the variable loss of bacteria.

Table 13 Analysis of Variance for the variable loss of Bacteria (Type III Sum of Squares)

Fuente	Suma de cuadrados	Gl	Cuadrado Medio	Razón-F	*Valor-P*
Efectos principales					
Temperatura	0.605252	1	0.605252	0.97	0.3309
Tiempo	8.24276	3	2.74759	4.39	**0.0090**
Residuos	25.6326	41	0.625184		
Total (corregido)	69.0784	47			

P-values prove the statistical significance of each factor.

P- value of the time factor is less than 0.05, that why has a statistically significant effect on the loss of bacteria, with 95.0% confidence level. The temperature factor shows no significant effect.
The results of the multiple range test for the variable loss of bacteria, with the factors: time and temperature, according to the method: 95.0% Fisher LSD, are shown in Figures 11 and 12

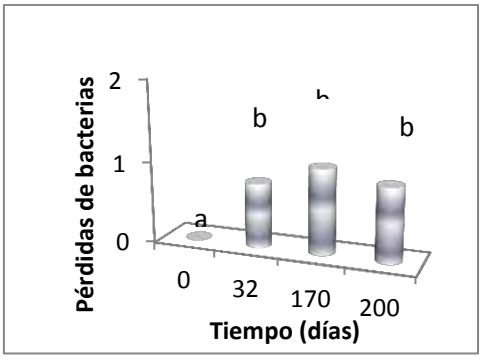


Figure 11 Behavior losses bacteria with time factor. Multiple range tests. (Different letters show significant differences at a level of 95.0% confidence)

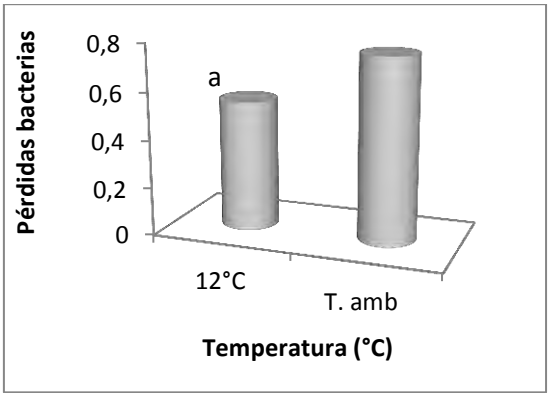


Figure 12: Behavior losses bacteria with the temperature factor. Multiple range test. Different letters show significant differences at a level of 95.0% confidence.

Bacteria losses increase significantly until 32 days. From now until 200 days no significant differences were observed. Temperature does not significantly influence these losses.
The analysis of variance for the variable loss of yeast shown in Table 14

Table 14: Analysis of Variance for the variable loss yeast

Fuente	Suma de cuadrados	Gl	Cuadrado Medio	Razón-F	Valor-P
Efectos principales					
Temperatura	1.97641	1	1.97641	3.78	0.0589
Tiempo	17.5762	3	5.85875	11.19	**0.0000**

Residuos	21.4624	41	0.523474		
Total (corregido)	41.4243	47			

P value for the time factor proved to be less than 0.05, so only time will have a statistically significant effect on yeast losses with a 95.0% confidence level.
The results of the multiple range test for analyzing the loss of yeast time and temperature factors, using the method: 95.0% Fisher LSD shown in Figures 13 and 14.

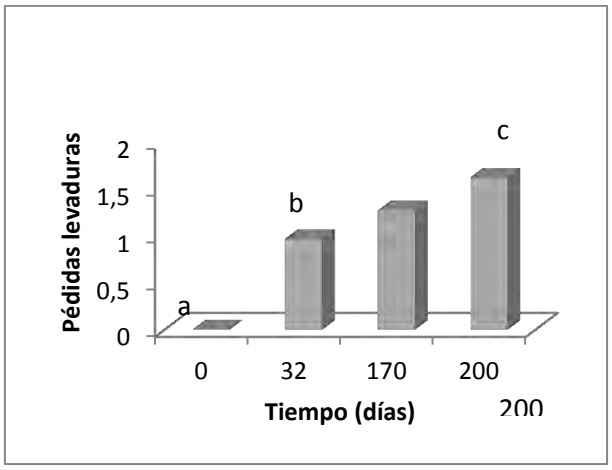


Figure 13: Behavior of losses yeast with the time factor. Multiple range tests. Different letters show significant differences at a level of 95.0% confidence.

Yeast losses increase significantly until 32 days.
Between 32-170 days and between 170 -200 days no significant differences were detected

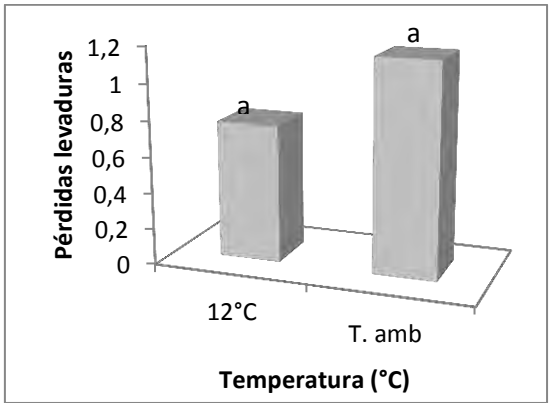


Figure 14: Behavior of losses yeast with the temperature factor. Multiple range test. Different letters show significant differences at a level of 95.0% confidence

When analyzing the losses of microorganisms, it can be noted that the product is stable until about 6 months storage conditions studied.

PH behavior during storage at different temperatures are shown in Tables 15 and 16.

Table 15 Results pH in lots of the product "LEBAME" stored under refrigeration (12) ° C (average values).

Número del Lote	Tiempo (días)	pH
1	0	3.86
	32	4.02
	170	4.05
	200	3.97
2	0	3.78
	32	3.98
	170	3.81
	200	4.02
3	0	4.08
	32	4.14
	170	4.07
	200	4.02

Table 16 Results of pH product "LEBAME" lots stored at room temperature (25-30 ° C), average values.

Número del Lote	Tiempo (días)	pH
1	0	3.86
	32	4.02
	170	3.98
	200	3.93
	0	3.78
	32	3.97

2	170	4.04
	200	4.10
3	0	4.08
	32	4.03
	170	4.03
	200	4.03

It can also be noted that the LEBAME product has a pH value between 3.78 and 4.14, a value very close to that reported by ENRO, (2008a), who earned a pH between 3.3 and 3.6. Future Technoday, 2013, states that a measurable indicator is whether the product has a pH greater than 4.0.
The analysis of variance for pH variable appears in Table 18.

Table 17 Variance analysis for pH variable

Fuente	Suma de cuadrados	Gl	Cuadrado Medio	Razón-F	Valor-P
Efectos principales					
Temperatura	0.000133333	1	0.000133333	0.02	0.9001
Tiempo	0.102767	3	0.0342556	4.10	0.0121
Residuos	0.359467	43	0.00835969		
Total (corregido)	0.462367	47			

Time factor was valued at P less than 0.05, so it has a statistically significant effect on the pH of the product "LEBAME" with a 95.0% confidence level.
Using multiple range test, using the method: 95.0% Fisher LSD could analyze the behavior of pH, time and temperature analyzing factors; these results are shown in Figures 14 and 15

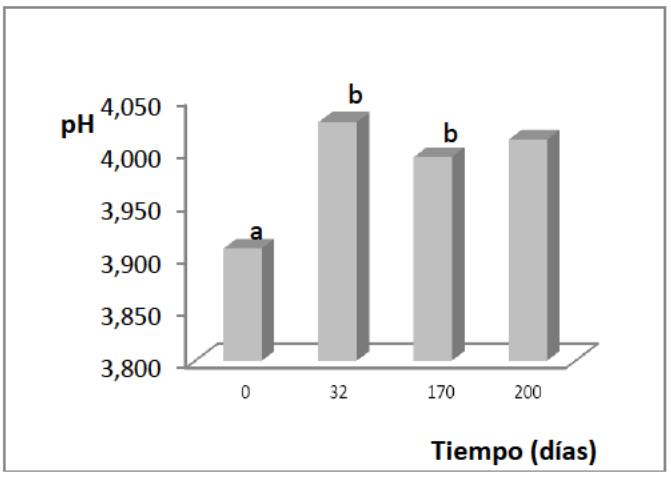


Figure 15: Behavior of pH with time factor, multiple range test. Different letters show significant differences at a level of 95.0% confidence.

Significant differences are detected on pH to analyze the corresponding times of 32, 170 and 200 days of storage from the initial day. From 32 days to 200 days no significant differences were detected.
Next shown in Figure 1 6, the behavior of the temperature by analyzing the pH factor.

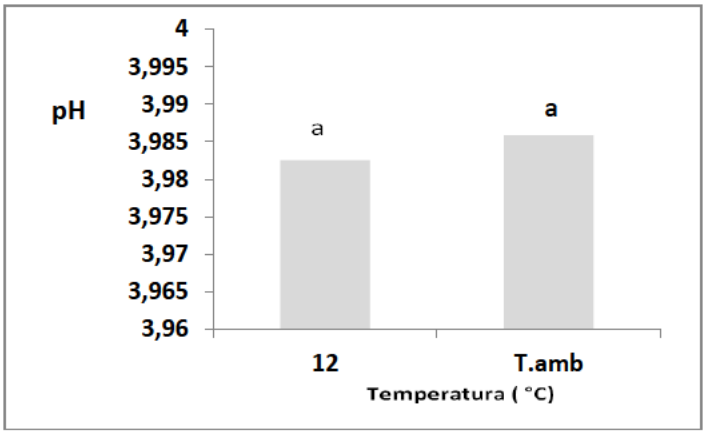


Figure 16 Behavior of pH with temperature factor, multiple range tests. Different letters show significant expats at a 95.0% level of confidence

The results show that the temperature does not vary significantly on pH. Product "LEBAME" is stable under working conditions studied, until 6 months of storage. It is inferred that the product can be stored at room temperature (25-30 ° C), unaffected, which would lead to savings from the economic point of view, not having the need for refrigerated storage environments.

CHEMICAL AND ENZYMATIC ESTER SYNTHESIS
EXPERIENCES IN OBTAINING AND APPLICATIONS IN COVERING POST-HARVEST OF FRUITS AND VEGETABLES

In recent years, the industry has begun to replace surfactants derived raw materials in the petrochemical industry for compounds friendlier to the environment.
With this premise, have been developed a group of surfactants fatty acid esters of high molecular weight molecules are dual hydrophobic and hydrophilic behavior. These molecules

have numerous industrial applications such as non-ionic surfactants and emulsifiers. The synthesis of these esters is performed chemically and enzymatically. In most often by trans esterification processes with fatty acids from vegetable oils. They are environmentally non-toxic non-ionic compounds, and so can be used as cover postharvest fruit and vegetables, allowing minimize the loss of quality of these fruits during storage and marketing.

Obtaining sucrose esters was performed in a process of interesterification in the absence of solvents, including sucrose, natural esters (corn oil), glycerin and methyl oleate as catalyst in a stirred reactor at 120 ªC. The reaction products are a mixture of sucrose mono- and diesters of stearic, palmitic and oleic acids, and mono and di glycerides potassium soaps.

acylglycerides were obtained in an enzymatic esterification way. Enzymatic synthesis was performed with glycerol and as a source of fatty acids, sunflower, soybean and olive oil in molar ratio 2: 1. The enzyme used was a commercial lipase: Type XIII Lipase from *Pseudomonas* specie, Sigma, lyophilized . the most favorable reaction conditions to obtain greater amount of mono- glycerides (MG) and diglycerides (DG), have been studied To determine the products of the reaction and their concentrations, the samples were subjected to qualitative analysis by TLC (eluent n-hexane / ethyl ether / acetic acid 75: 25: 1) and quantitate analysis by scanning in a Shimadzu scanning densitometer CS-9000.

Once fractions were identified, as comparative effect between acylglycerides and sucrose ester, was studied as coverage postharvest strawberry (Fragaria x ananassa Duch, variety Camarosa). The fruits were selected by degree of commercial maturity, on the basis of a uniform color and size, absence of mechanical and / or visible signs of fungal attack. Washed with chlorinated water (250 ppm chlorine) for 30 seconds and air dried. The fruit is separated into 100 gram lots.

Lots of fruits were subjected to the following treatments:

-Control: Strawberries, washed and air dried.

Treatment1: fruit dipped in 2% aqueous solution of sucrose esters and natural convection dried at room temperature for one hour.

-Tratamiento2: Fruit dipped in 2% aqueous solution of acylglycerides and convection dried at room temperature for one hour.

Simulating the conditions of the chain of commercial refrigeration, samples placed in propylene without plastic tray covered and refrigerated at 4 ° C for 15 days. Weight loss, Total Soluble Solids (TSS), titratable acidity, maturity index, number of missed fruits and general appearance (photo up): The following variables were analyzed.

For applications sucrose esters coverage in fruits and vegetables, it was observed that the weight losses of treated fruits were lower in all concentrations tested.

Soluble solids increased during days but showed lower values in treated samples than in controls. Controls deteriorated faster than the samples treated in the number of lost by decay or disrepair sensory units.

Postharvest suitable for coverage concentrations were 0.5% to 5% because at higher concentrations observed alterations in the metabolism of sugars in the fruits evidenced / acid ratio and a higher degree of maturation.

From a sensory standpoint appropriate concentrations were 0.5% to 2%

In the subsequent application with fractions of MG and DG olive oil and sucrose esters in postharvest coverage was found to ninth day there was no difference between the two treatments. From then until day 15 of the trial reported improved behavior sucrose esters as postharvest retail coating weight loss, least amount of soluble solids, lower acidity and consequently lower rate of maturity.

We also found that both treatments achieved prolong the life and fresh fruit strawberries compared to the control without coverage.

For applications sucrose esters coverage in fruits and vegetables was observed that the weight losses of the treated fruits were lower in all concentrations tested

In olive oil acylglycerides proved suitable substrate for the production of mono- and diglycerides in the enzymatic esterification of glycerol in non-aqueous medium

With the esters obtained is achieved enzymatically delay changes related to aging of strawberries and therefore prolong its life as fresh fruit (Figure 16, 17, and 18)

Comparing the behavior of the esters obtained chemically and enzymatically as strawberry protective cover was favorable to postharvest sucrose esters used in concentrations of 2% aqueous solutions.

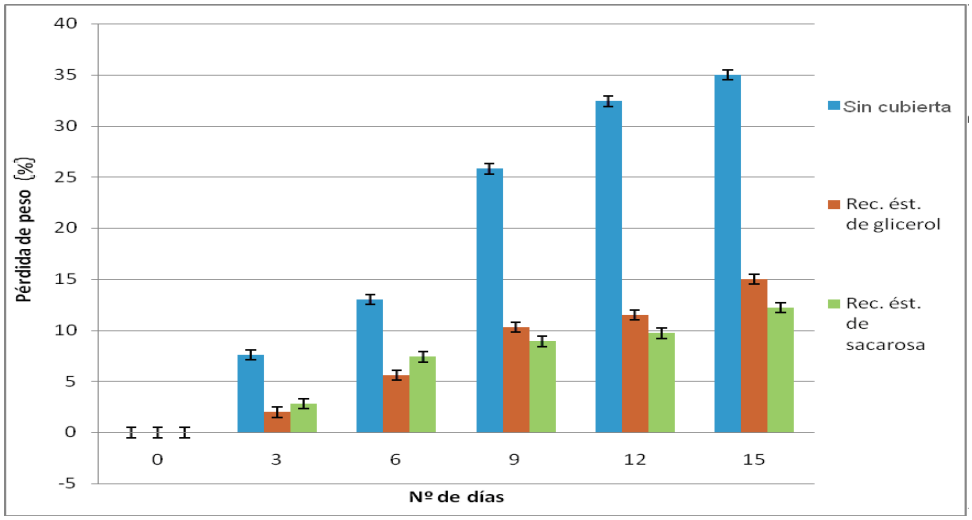


Figure 17 Loss weight percent during storage of fruits without cover and coated

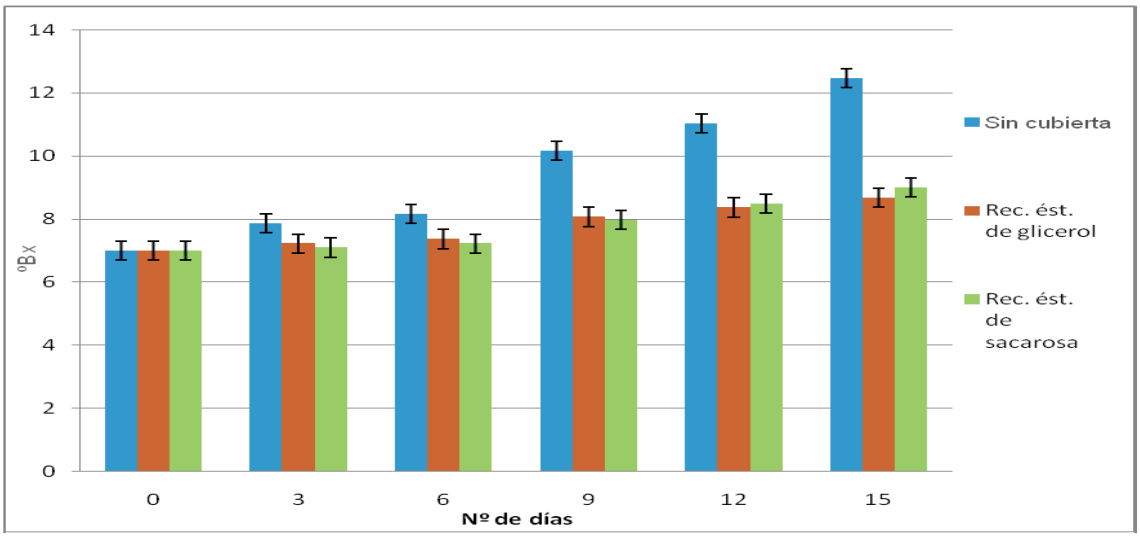


Figure 18 Values of total soluble solids (TSS) of the fruits on the day of harvest and during storage in fruits without cover, treated with glycerol and sucrose esters.

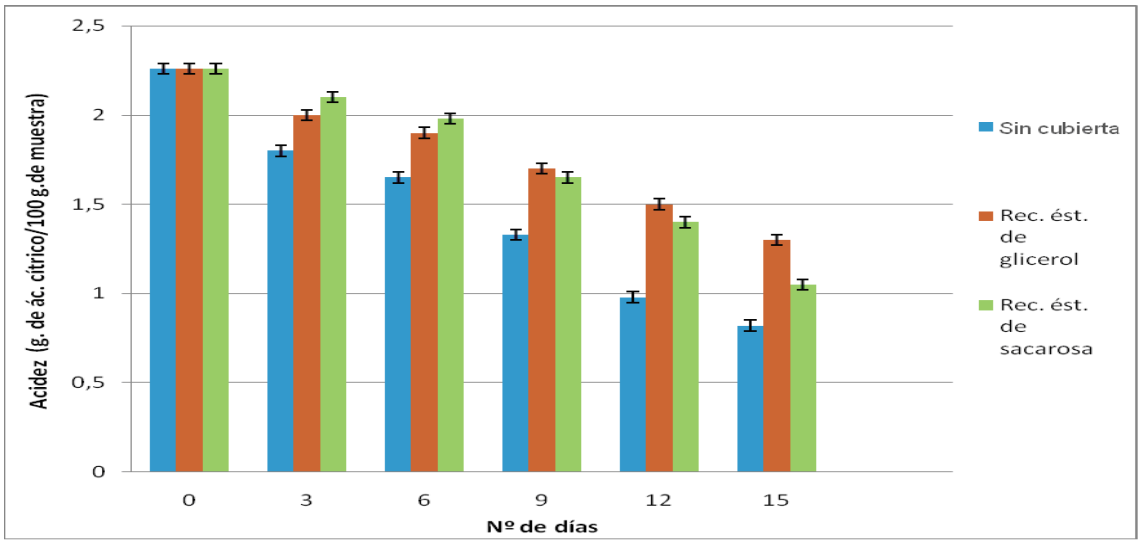


Figure 19 Values of total soluble solids (TSS) of the fruits on the day of harvest and during storage in fruits with cover, treated with glycerol and sucrose esters.

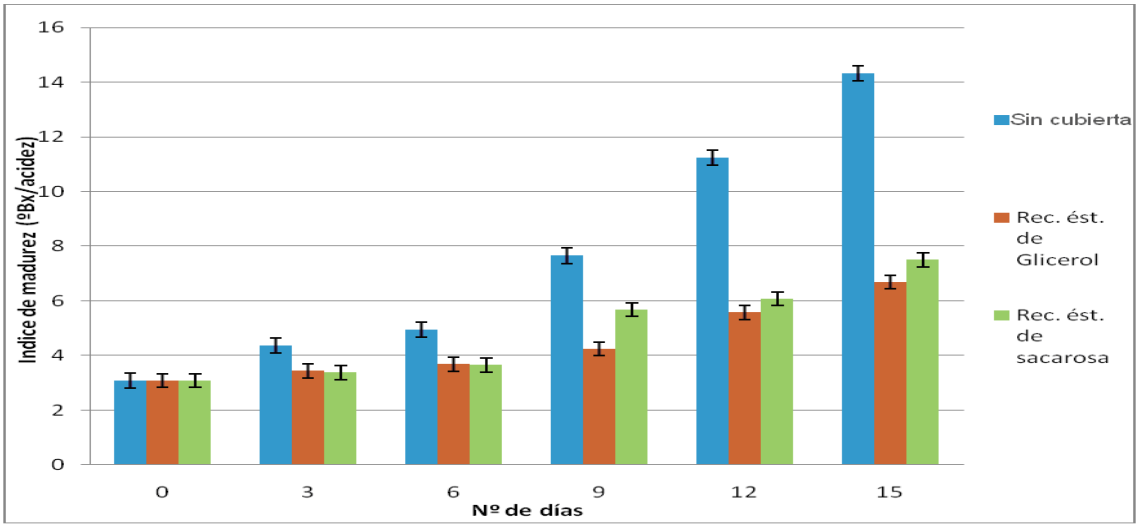


Figure 20 Index of fruit maturity on the day of harvest and during storage in control fruits, coated with glycerol esters of sucrose.

3. ASSESSMENT OF APPLICATION OF AGRICULTURAL BIOPRODUCTS

BIOJAS

BIOJAS has been evaluated as biocontrol of pineapple plants cv "Cayenne lisa" under plantation in the province of Ciego de Avila where he was effective in attacking the mealy bug representing the most damaging pest cultivation to constitute the agent will propagator of the virus. This seems due to an insecticidal effect but the induction of metabolic mechanisms of the plant resistance. In string bean Escambray crop, to control aphids (aphids), killing 100% of aphids and prevented further outbreaks of aphids, white flies. At doses of 60 mg / L. In vitro test produced inhibition of almost 50% growth of *Sclerotium* Sp.. Always reduced the presence of the fungus, demonstrating protective action, increased seedling, and survival pineapple ex vitro conditions against *Phytophthora nicotianae* var. parasitica. In zucchini with *Nesidiocoris tenuis*, biological predator introduced to control whiteflies, small aphids, aphids and thrips, which were affecting plants and fruits, was controlled mixture containing 25 mg / L of jasmonic acid,. had effectiveness of eggplants and cucumbers with presence of powdery mildew.

Indications of proposed use

From the mode of action for jasmonic acid and developed bioagricultural ongoing studies in the country, indications proposed use are:

Doses of 60 mg / L in Smooth Cayenne pineapple: significant reduction of mealy bug attacks.

Doses of 60 mg */. Sclerotium* sp. In vitro inhibition of almost 50% growth.

Doses of 10 mg / L in growing string bean Escambray to control aphids, killing 100% of aphids and prevents further outbreaks of aphids, whiteflies and thrips .

In the interaction banana-*Fusarium oxysporum F. cubense* race 2, jasmonic acid (1.0 mg / L) always reduced the presence of the fungus, demonstrating protective action.

Doses of 1 mg / L in vitro increases survival of seedlings pineapple ex vitro conditions against *Phytophthora nicotianae* var. parasitica.

Application of BIOFERTILIZER BIOJAS IN SOYBEAN IN THE ALIAR COMPANY IN COLOMBIA

Was observed greater plant height, increased nodulation and capsule counting 113 in lot control, against 144 in lot with BIOJAS, with a number of 6 plants in the control and 6 in lot with Biojas.

EVALUATION OF THE EFFECTIVENESS OF HERBIO IN DICOTYLEDONOUS WEED CONTROL AND TOLERANCE IN SUGARCANE

In sugarcane plant variety CP52-43 in the Production Unit Manuel Fajardo, Quivicán, Havana, on red ferrallitic soil, the application was performed 35 days after planting field, with height cultivation of 30-40 cm in a randomized block design with 6 replications and plot area of 12 m^2. Treatments show in Tables 19 and 20.

Table 18Tratament with bioherbicide HERBIO evaluated

No	Productos	% P/V	Kg/ha	g or ml for 6 lots (72 m^2)
1	Testigo absoluto	-	-	-
2	2.4-D Ester.	0.75	1.88	14
3	HERBIO	2.5	6.25	47
4	HERBIO	5	12.5	94
5	HERBIO	10	25	188
6	HERBIO	20	50	376
7	HERBIO + AG-5 (PH-5)	2.5	6.25	47
8	HERBIO + AG-5 (PH-5)	5	12.5	94
9	HERBIO + AG-5 (PH-5)	10	25	188
10	HERBIO + AG-5 (PH-5)	20	50	376

Table 19 Evaluation of HERBIO herbicide efficacy and crop tolerance EWRS (European Weed Research Society)

Índice de evaluación	Estimación visual de eficacia o de fitotoxicidad	
	Sobre malezas	Sobre el cultivo
1	Total 100	Ningún efecto como el testigo
2	Muy buena	Muy ligeros síntomas
3	Buena	Ligeros síntomas
4	Suficiente en la práctica	Daños sin influencias cosechas
5	Dudosa	Dudosa
6	Mediocre	Daños bastantes fuertes
7	Mala	Daños fuertes
8	Muy mala	Daños muy fuertes
9	Nula como testigo	Destrucción total 100 %

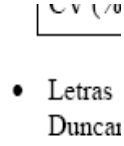
Treatments with HERBIO only, at doses of 25 and 50 kg / ha, at 15 and 30 days after application, were acceptably effective (though worse than the standard 2,4-D ester at dose of 1.88 L / ha), in the control of dicotyledonous weeds Ipomoea trifida, Croton lobatus, Chamaecyse hyssopifolia and Euphorbia heterophylla. Mixtures of HERBIO plus surfactant - acidifying AG-5 (AG-5 adjusted to pH 5 or violet coloration, at 0.2-0.3 L / ha) result in a satisfactory control of dicotyledonous weeds *as Ipomoea trifida*, *Croton lobatus*, *Chamaecyse hyssopifolia*, *Euphorbia heterophylla* and *Vigna vexillata* at doses of 6.25, 12.5, 25 and 50 kg /ha, being comparable to the standard of 2,4-D ester at the maximum dose of HERBIO (50 kg / ha). The species *Cyperus rotundus* also resulted acceptably controlled and better than the standard at higher doses (25 and 50 kg/ha). No phytotoxic damage were seen in the variety of CP52-43 cane.

Evaluation of Gluticid in the control of phytopathogenic fungi

In Homes Protected Cultivation:
The product was evaluated in Cienfuegos Several Crops Company in growing houses intended for tomato (*Lycopersicon esculentum* Mill) on the varieties, Aro 84-84, against *Alternaria solani*

pathogen with the highest incidence in the province under these conditions, which produces the early blight disease.
Gluticid at a concentration of 0.08 kg ai / ha, compared with chemical fungicides: Mancozeb 80 PH (2.4 kg ai / ha), and Tebuconazol (Orius 25 EC) (0.25 kg ai / ha).
A completely randomized design with plots of two rows of 30 m long was used in both cases. Treatments were made with a backpack GN-16 and were initiated when the culture was 7 days after planting with a frequency of 4-5 days for protective fungicides and 9 - 10 days for systemic. The intensity of attack was evaluated in 20 plants per plot based on the scale of 6 degrees (IISV, 1978). The average degree of intensity is determined by the formula of Townsend and Heuberger, cited by CIBA GEYGI (1981). The data obtained from intensity distribution and effectiveness was processed by the statistical package, ESTATISTICA for Windows version 4. The averages were compared according to the multiple range test of Duncan.

Table 20 Results of the Gluticid evaluations in controlling *Alternaria solani* on tomato (*Lycopersicon esculentum* Mill) variety Aro 84-84 in crop houses



The control of Gluticid against *Alternaria solani* in tomato (Lycopersicon esculentum Mill) in crop house was 58.25% with no significant difference in treated plots with Mancozeb whish was 60.25%, these were maintained until the last evaluation at 120 days culture results in controlling the disease. Plots treated with tebuconazole against *Alternaria solani* , showed lower values, significantly different from those obtained with Gluticid and Mancozeb.
Different types of fungicides, both contact and systemic, among the latter, the triazoles are used in the country, however, can cause resistance of the fungus under control; so the effectiveness of Gluticid against this disease, constitute a promissory bioproduct.

Replacing the Fundazol by Gluticid in the cultivation of edible mushrooms

A hybrid strain of *Pleurotus osteatus* was used. As substrate sugarcane crop residues from a storage facility, sliced into fragments used 5 - 10 cm in length and subjected to heat treatment at 95 ^{O}C for 2 hours. A solution of a chemical fungicide Fundazol 0.02% was added to a portion of substrate at a concentration of 200 ppm and the final wet was adjusted to 75%. The other part of substrate was treated with a solution of Gluticid at concentrations of 0.02-0.08% and the control doesn't have any fungicide. They were packed in bags of 4 kg and 10 kg in a Pilot Plant and in Semi-Industrial Plant and subsequently inoculated. Fungi were cultured for two harvests approximately 40 days.

In Figure 20 the results of efficiency (expressed as %) achieved in mushroom cultivation trials with and without fungicide application are discussed. It is observed that higher values yield was chieved in the bags with Gluticid (9.5%) followed with the bags with Fundazol (7.4%), while where in the control without fungicide, and was reached only 2%, demonstrating the need to use in the culture of the mushrooms, fungicides eliminate in order to eliminate the lower fungi contamination.

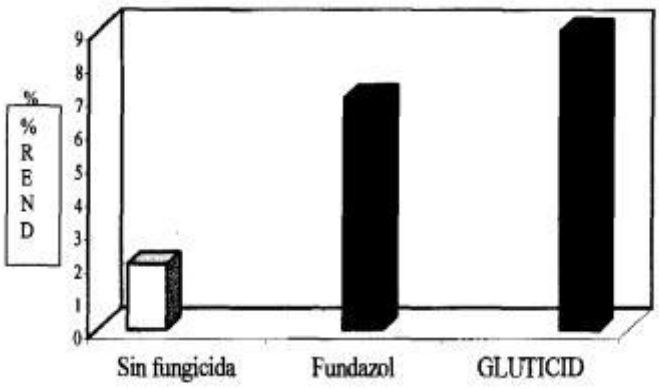


Figure 21 Yield achieved in *Pleurotas ostreatus* (mushroom) cultivation with application of chemical fungicide (Fundazol) and biological fungicide (Gluticid)

Lower fungi contamination was observed in 83.0% of the bags where there is fungicide applied while not contaminated was presented in bags with Gluticid and Fundazol .This results indicating the feasibility of used a biological product in mushroom cultivation technology resulting in a clean technology with reduced environmental pollution.

Evaluation of the Cuban biofungicide Gluticid for the control of black sigatoka (*Mycosphaerella fijiensis*) disease in bananas, Ecuador

Taking into account that black sigatoka disease is considered of the first order for the Republic of Ecuador due to the severity of the attack and the economic losses in banana plantations, preliminary test was conducted to evaluate the effectiveness of Gluticid in control of this disease.

Damage symptoms of black sigatoka:

Initially the disease occurs with yellowish spots watery and translucent appearance, that are located anywhere in the pseudostem. Then these colors become reddish brown and extend in all directions, covering sheaths partially or completely leaves. Finally affected areas take a dark and surrounding tissues, when pressed, a fetid liquid is clear. These rots move progressively to the pseudostem base, while the bacterium penetrates the tissues of the internal shuck by contact with external affected. This results in a weakening of the pseudostem, causing bending of the plant by the most affected part. If the attack occurs when plants are adult, cluster weight easily contributes to the collapse of the plant before the total fruit development, reducing the commercial value of these.

Foliar damages by black sigatoka

Gluticid is a biochemical biofungicide, for plant protection use, obtained by biotechnological procedure from *Pseudomonas aeruginosa* PSS. It is a wettable powder formulated, constituted by antimicrobial metabolites monoacetilfloroglucinol, siderophore pyoverdine and salicylic acid. Does not contain cells.

Experimental protocol in organic banana production areas in Ecuador

The tests were conducted in productive areas cultivated with organic bananas from Novoa Corporation to evaluate its effectiveness in the control of black Sigatoka disease of banana.
Applications were made in 3 ha of crop covering lots 6, 7 and 8 of this company, on a weekly basis for 21 days. The product was applied with hand sprinkler in the early hours of the morning at a concentration of 1.5 kg / ha.
At the end of 21 days, the effectiveness was assessed in 80 plants per treatment from the comparison with biofungicide Serenade (biofungicide constitute by *Bacillus subtilis*)

The results showed that the effectiveness of Gluticid technique was good due to the development of the disease is stopped. Actual development index was reduced to about 60 percent in the treated plants with this product compared to the treated with SERENADE. The new leaves that emerged after product application showed no visible symptoms of disease observed in general a good look at the banana plantations treated with this byproduct.

AVERAGE OF ACTUAL DEVELOPMENT INDEX (ADI) OF THE DISEASE BLACK SIGATOKA IN EVALUATES PLANTS

ADI Plants with Serenade	ADI Plants with Gluticid
242	143

Gluticid demonstrated the control of black Sigatoka of banana, superior to conventional products, that why is interest in applying the product on larger areas.

Application of bioproducto Bioenraiz

Crops proposed

From the general mode of action for the indole-3-acetic acid and developed bioagricultural ongoing studies in the country, the indications for use proposals are:

- Red dwarf guava cuttings 18-40
- In vitro plants of potato and sexual seed
- In vitro plants of banana
- Sugarcane (Ja 60-5), agamic seed
- ornamental flowers (red carnations and dahlias)

Main benefits of the product

- Increase the length of the root and bud formation (sugarcane Ja-60-5 agamic seed).
- Promote emission and increase root hairs, and in nursery garden, increase the percentage of survival (guava cuttings Red Dwarf 18-40)
- Increasing height and number of internodes in potato plantlets (cuttings) and potato seed. Increase rooting cuttings and potato apices (Var LT-7 and Var.DTO-2).
- Increase the rates or multiplication and rooting of ornamental flowers (red carnations and dahlias)

During these years were developed the market tests BIOENRAIZ product produced in ICIDCA Sales management was directed towards the most important companies of agriculture. Consequently a marketing strategy sales were made to companies of the Ministry of Agriculture and other companies. Demand greater than the volume traded that why considered the BIOENRAIZ product has a broad market and its use is recognized as rooting and plant growth stimulator was observed. The criteria received technical staff who is making the application are satisfactory as expressed guarantees customer.

Listed below are some of the companies that was provided product BIOENRAIZ

	Quantity (L)
MICONS “Matanzas”, Matanzas	3
Emp. Cultivos Varios “Horquita”, Cienfuegos	5
EMA “Victoria de Girón”, Playa Larga, Mtzas	5
TCA Cotorro, La Habana	1
Empresa Cítricos de Ceiba, Caimito	7
Empresa Cítricos de Ceiba, Caimito	8
Empresa Cítricos de Ceiba, Caimito	6
Empresa Cítricos de Ceiba, Caimito	9
UBPC “Vivero Alamar”, La Habana	2
CPA Camilo Cienfuegos, Pinar del Río	0,5

CCS Ovidio Estévez, La Habana	2
Emp. Cítricos "Vict. de Girón", Jagüey Grande	10
CCS Daniel Hernández, La Habana	10
Cultivos Varios Villa Clara	10
Empresa Agropecuaria MININT, Villa Clara	2
Finca CTC, La Habana	16
CPA Omar Rivero, Granma	15
CCS Fructuoso Rodríguez, La Habana	5
Vivero Alamar, La Habana	3
CCS Ana Betancourt, Mayabeque	1
CCS Humberto Hernández, Mayabeque	10

The productive capacity of the facilities of ICIDCA was demonstrated and a growing market was identified.

LEBAME

Effect of dose, and application time of LEBAME in tomato crop.
Planting date: November / 2013
Variety: Mara.
Treatments: 21
Repetitions: 3
Plot: 1m²
Experimental Design: Completely randomized.
Statistical processing: Simple Variance Analysis

Applications
Imbibition of seeds: November 5, 2013
First application (foliar spray): November 15, 2013 (10) days after sowing (DAS)
Second application (foliar spray): November 28, 2013 (20 DAS)

Evaluations
(5 plants / treatment)
First evaluation 10 days after germination (DAG), 15 November 2013
Second evaluation: 15 DAG, 28 November 2013
Third Evaluation: 28 (DAS) December 3, 2013

Assessments growth

Number of leaves per plant
Plant height (cm)
Root length (cm)
Stem diameter (cm)
Foliar and root dry mass

Table 22. Effect of LEBAME on germination and growth of tomato seedlings at 10 days after germination (DAG)

First evaluation November 15 2013							
Tratamientos	**Descripción**	**No Hojas**	**Altura (cm)**	**L.Raíz (cm)**	**Diámetro (cm)**	**M. S. Foliar (g)**	**M.S. Radical(g)**
T1-T2-T3	Imbibición 2.5mL.L^{-1}	2	4.96 c	4.96 c	2.31	0.03 b	0.0031 c

T6-T7-T8	Imbibición 5 mL.L^{-1}	2	7.45 b	7.45 b	2.1	0.07 b	0.0048 bc
T11-T12-T13	Imbibición 10 mL.L^{-1}	2	7.23 b	7.23 b	2.31	0.08 b	0.0069 a
T16-T17-T18	Imbibición 15 mL.L^{-1}	2	8.8 a	8.8 a	2.55	0.19 a	0.0064 ab
T21	Control	2	6.74 b	6.74 b	2.22	0.09 b	0.007 a
Esx		0.04NS	0.37*	0.37*	0.25 NS	0.032*	0.0007*

Average with common letters are not statistically different for Duncan p≤0,001

In the second evaluation to the 15 days old plants, highly significant differences for each of the variables evaluated were obtained. The number of leaves was stimulated with 2.5 mL/L , 10 days after germination (DAG) and 5mL/L at 15 DAG. The plant height was higher in the treatment of 5 ml/L at 15 DAG; The root length was higher with treatment application imbibition of seeds in 15 ml/L; stem diameter greater thickness reached in the treatment of 5 mL/L at 10 DAG. The foliar and root dry mass were higher in the treatment of 15 ml/L at 10 DAG. As denoted for each variable there was a different response to treatments except the last two.

Table 23. Effect of LEBAME on growth of tomato seedlings at 15 days after germination Second evaluation 28 nov 2013

Trat	Descripción	No Hojas	Altura (cm)	L.Raíz (cm)	Diámetro (cm)	M. S. Foliar(g)	M.S. Radical(g)
T1	Imbibición 2.5mL.L^{-1}	2,4 cde	13,02 i	8,0 bcde	0,45 cdefg	0,33 f	0,04 d
T2	I/AF 2.5mL.L^{-1} 10 ddg	2,6 bcd	21,7 cdef	7,8 bcde	0,40 g	0,41 cdef	0,056 bcd
T3	I/AF 2.5mL.L^{-1} 15 ddg	2,0 e	21,72 cdef	9,7 abc	0,41 g	0,29 f	0,03 d
T4	AF 2.5mL.L^{-1} 10 ddg	3,2 a	20,56 defg	8,74 abcde	0,41 fg	0,31 f	0,05 cd
T5	AF 2.5mL.L^{-1} 15 ddg	2,4 cde	19,6 fg	8,88 abcde	0,48 abcdef	0,39 def	0,04 d
T6	Imbibición 5mL.L^{-1}	2,6 bcd	21,08 cdefg	7,68 cde	0,45 bcdefg	0,34 ef	0,05 cd
T7	I/AF 5mL.L^{-1} 10 ddg	3 ab	25,04 b	8,72 abcde	0,42 efg	0,62 ab	0,058 bcd
T8	I/AF 5mL.L^{-1} 15 ddg	3,2 a	23,4 bcd	8,70 abcde	0,50 abcd	0,59 abc	0,08 ab
T9	AF 5mL.L^{-1} 10 ddg	2,8 abc	23,98 bc	8,62 abcde	0,53 a	0,46 abcdef	0,054 bcd
T10	AF 5mL.L^{-1} 15 ddg	2,2 de	20,94 cdefg	7,36 de	0,42 efg	0,37 def	0,05 cd
T11	Imbibición 10 mL.L^{-1}	2,6 bcd	22,2 bcdef	8,58 abcde	0,42 efg	0,41cdef	0,052 bcd
T12	I/AF 10 mL.L^{-1} 10 ddg	2,6 bcd	23,2 bcd	8,72 abcde	0,44 cdefg	0,35 ef	0,062 bcd
T13	I/AF 10 mL.L^{-1} 15 ddg	3 ab	23,08 bcde	9,74 ab	0,458 bcdefg	0.54 abcde	0,056 bcd
T14	AF 10 mL.L^{-1} 10 ddg	2,2 de	23,28 bcd	8,38 bcde	0,44cdefg	0.38 def	0,042 cd

T15	AF 10 mL.L-1 15 ddg	2,4 cde	16,26 hi	8,00 bcde	0,458 bcdefg	0.28 f	0,04 d
T16	Imbibición 15 mL.L-1	3 ab	21,7 cdef	10,60 a	0,49 abcde	0.57 abcd	0,07 abc
T17	I/AF 15 mL.L-1 10 ddg	3 ab	25,5 ab	9,66 abc	0,52 ab	0.64 a	0,09 a
T18	I/AF 15 mL.L-1 15 ddg	3 ab	28,6 a	9,16 abcd	0,51 abc	0.60 abc	0,08 ab
T19	AF 15 mL.L-1 10 ddg	2,6 bcd	21,46 cdef	7,11 e	0,45 bcdefg	0.43 bcdef	0,03 d
T20	AF 15 mL.L-1 15 ddg	2,e	18,2 gh	8,20 bcde	0,39 g	0.46 abcdef	0,054 bcd
T21	Control	2,8 abc	19,86 efg	6,96 e	0,43 defg	0.32 f	0,03d
	ESx	0.02*	1.175*	0.73*	0.026	0.073*	0.01*

Average with common letters are not statistically different for Duncan p≤0,001

Table 24. Effect of LEBAME on growth of tomato seedlings at 25 days after germination (DAG)

Tercera evaluación 3 Diciembre 2013							
Trat	**Descripción**	**No Hojas**	**Altura (cm)**	**L.Raíz (cm)**	**Diámetro (cm)**	**M. F. Foliar(g)**	**M.F. Radical(g)**
T1	Imbibición 2.5mL.L-1	4,4 ab	31,2 ij	9,8 bcd	0,53 bcd	1,04 abcd	0,14 bc
T2	I/AF 2.5mL.L-1 10 ddg	4 abcd	37,8 def	7,9 cd	0,46 d	1,18 abc	0,21 bc
T3	I/AF 2.5mL.L-1 15 ddg	3,4 de	36,2 efg	10,5 abc	0,55 abcd	0,93 abcd	0,21 bc
T4	AF 2.5mL.L-1 10 ddg	3,4 de	29,4 j	9,8 bcd	0,53 bcd	0,86 bcd	0,10 bc
T5	AF 2.5mL.L-1 15 ddg	4,0 abcd	34,0 ghi	9,8 bcd	0,50 bcd	0,74 cd	0,09 bc
T6	Imbibición 5mL.L-1	4,0 abcd	36,2 efg	10,2 bcd	0,48 cd	0,96 abcd	0,18 bc
T7	I/AF 5mL.L-1 10 ddg	4,0 abcd	42,8 ab	8,0 cd	0,51 bcd	0,85 bcd	0,17 bc
T8	I/AF 5mL.L-1 15 ddg	3,6 cde	31,8 hij	9,6 bcd	0,45 d	0,78 bcd	0,09 bc
T9	AF 5mL.L-1 10 ddg	3,8 bcde	35,4 fgh	10,5 abc	0,58 ab	1,09 abcd	0,16 bc
T10	AF 5mL.L-1 15 ddg	4,6 a	37,6 defg	9,6 bcd	0,58 ab	1,20 ab	0,23 b
T11	Imbibición 10 mL.L-1	4,6 a	45,6 a	9,6 bcd	0,48 bcd	1,18 abc	0,43 a
T12	I/AF 10 mL.L-1 10 ddg	4 abcd	42,2 abc	8,8 bcde	0,47 d	1,11 abcd	0,23 b
T13	I/AF 10 mL.L-1 15 ddg	3,2 e	35,8 efg	9,0 bcde	0,45 d	0,76 bcd	0,07 c
T14	AF 10 mL.L-1 10 ddg	3,4 de	31,8 hij	9,4 bcde	0,53 bcd	0,91 bcd	0,13 bc
T15	AF 10 mL.L-1 15 ddg	3,8 bcde	32,0 hij	9,6 bcd	0,48 cd	0,95 abcd	0,18 bc
T16	Imbibición 15 $mL.L^{-1}$	3,8 bcde	35,4 fgh	9,2 bcde	0,57 abc	1,02 abcd	0,16 bc
T17	I/AF 15 $mL.L^{-1}$ 10 ddg	3,8 bcde	39,4 bcde	10,7 ab	0,52 bcd	1,09 abcd	0,18 bc
T18	I/AF 15 $mL.L^{-1}$ 15 ddg	4,2 abc	39,2 bcde	12,7 a	0,56 a	1,37 a	0,21 bc
T19	AF 15 $mL.L^{-1}$ 10 ddg	3,2 e	38,6 cdef	7,8 cd	0,49 bcd	1,04 abcd	0,10 bc
T20	AF 15 $mL.L^{-1}$ 15 ddg	3,4 de	39,4 bcde	7,3 b	0,484 bcd	0,72 d	0,09 bc

T21	Control	4 abcd	40 ,0 bcd	8,5 cde	0,53 bcd	1,12 abcd	0,14 bc
ESx		0.26*	1.33*	0.79*	0.03*	0.15*	0.05*

Average with common letters are not statistically different for Duncan p≤0,001

The evaluation carried on at the end of the seedlings cycle, also denoted differences between treatments for each of the variables evaluated. For the number of leaves per plant, there were no differences between treatments of 5m/L and 10 ml/L at 15 DAG; for height and root dry mass the best treatment was of the seed imbibition on 10 ml/L, and for variables root length, stem diameter and leaf fresh mass, the best treatment was combined the imbibition with foliar spray at 15 DAG at dose of 15 ml.l-1

Continued use of Efficient Microorganisms (EM) is a sustainable form of agricultural production. For the farmer resulting in increased harvests and less use of pesticides and mineral fertilizers, however his biggest profit, will be the recovery of their main richness , the soil of his farm (Red SICTA Project, 2013).
Several studies have demonstrated the effectiveness of effective microorganisms (EM) in agricultural crops, as demonstrated by Gutiérrez et al, (2012) to prepare two organic fertilizers with ME and check that these are efficient for tomato and corn crops.

Lara and Marco (2014) concluded that the application of effective microorganisms favorably influenced the growth and development of plants of broccoli, reporting the best results, with greater growth in length of the leaf blade (52.49 cm) in pellet diameter (19.48 cm), as in the weight of the pellet (0.40 kg), thereby obtaining the best yields (16.04 t / ha).
Acosta et al, (2013) in maize also found that the best treatment was combined into the mix where the substrate to the MS, which exceeded significantly (P <0.05) the witness with chemical fertilization growth variables evaluated.

Conclusion
There is a positive effect in stimulating the growth of tomato plants from the use of bioproduct LEBAME. The combination of imbibition of seed and foliar spray after 15 days of germination at dose of 5mL/L, results in the greatest stimulation of the variables plant height, stem diameter, root length and leaf fresh mass, important aspects to consider in the quality of the plants for transplantation.

Application of LEBAME on lettuce
Date: February 2014
Variety: Black Sympson
Location: INCA
Foliar sprays: July 15 DDS and DDS

Results
There are significant difference for each variables evaluated. For the height and fresh mass, had a larger size and higher weight plants receiving 5mL/L dose, however, also the dose of 10 mL/L was superior to the control treatment; in the case of the number of leaves per plant, there were no differences between doses, and the dry weight was also similar for both treatments. Therefore, to achieve a stimulating plant growth is sufficient dose of 5 mL/L.

The analysis realized in the time of harvest, shows that in correspondence with the growth, plants that received the dose of 5 mL/L have a greater leaf area, followed by a dose of 10 mL/L and both were higher than the control. The content of chlorophyll pigments is also higher at the dose of 5 mL/L and both exceed the control.

Knowledge management in terms of training and preparation of 45 small farmers and at least 10 specialists, involved in the industry responsible for the of bioproducts production.

Developed activities

The study of patents and reports related to the subject to was contracted to the Patent National Office. The information obtained was mainly obtained through QPAT online service, from de company Questel Orbit, possessing high level of renovation and provides funding and publications of patent families of 78 authorities as well as full texts of PCT, EPO, FR, GB, US and DE. Another literature were also consulted through sites www.scirus.com and www.scholar.google.com

a) Evolution of patents. leaders in research and production. Current Status

According to international patent studies, the development of biofertilizers began in the 80s of XX century, with accelerated from 2000, a process that continues today, as an alternative for sustainable agriculture increased. China was the largest producer of patents on this subject, followed by the US, Russia and Romania. United States is the most widely distributed and registration of their patents on several continents. Most companies that sell biofertilizers are Indian. Among the headlines with an enrollment over their inventions are The National Autonomous University of Mexico and several US firms.

Biofertilizers contribute to improving the quality and productivity of crops by partial or total elimination of the addition of chemical fertilizers. The beneficial effects are associated not only with the ability to fix nitrogen but also with the ability to produce antibacterial and antifungal compounds and growth regulators. Saving nitrogen fertilizer is a parallel saving fossil fuels used in the ammonia synthesis. Despite its advantages, the traditional fertilization with chemical fertilizers remains the most widely used technology in general be less expensive and have a technology, agricultural machinery and established cultures. Today, the increased use of biofertilizers and bioproducts is in organic agriculture.

-Stays mexican students of the Autonomous University of Coahuila:. Cecilia Balvantín, Fabiola Veana, Erika Nava, José Humberto Sánchez and Elan Iñaki Laredo in the laboratories of the Department of Biotechnology, ICIDCA. The work developed as part of their master's thesis (2 of which concluded in the period) and the Doctoral Program.

-Dr. Graciela Cerutti and Ing. Patricia Albarracínfrom National University of Tucumán, made a work visit for 15 days in ICIDCA. They dictated the course ¨Development of microbial bioproducts with sustained action.

-Dra. Georgina Michelena and MSc PhD student Yaniris Lorenzo carried out a mission in Colombia in order to check in the application test of a bioproduct BIOJAS (jasmonic acid) in soybeans crop.

In this mission they visited a company BIOFARM Colombia SA, that produces solutions for plant nutrition, based on biotechnology products. At the meeting in this company Dr. Georgina Michelena, made a presentation about potentiality of the project and specifically the relation of ICIDCA agriculture bioproducts.
Then they were exposed by the Ing. Cesar Augusto Colorado the results obtained in the application on soy and rice of Biojas in Colombia, and the interest in collaborate in the development of the introduction of biotechnology processes and products in Colombia.

-Dr Georgina Michelena from ICIDCA, imparted a curse: Agricultural Biotechnology applied to bioproducts production with attendance of 23 participants.

Objectives: The main objective of this course was to provide participants with the basic principles and concepts of fermentation processes as well as those related to production by fermentation pathway of the main microorganisms used in the production of biopesticides and biofertilizers made both by microorganisms intact as they produce metabolites.

Lecture 1 Introduction to Industrial Microbiology: Basic concepts, general characteristics of microorganisms and metabolites.

Lecture 2. Substrates employed in bioprocesses: preparation and characteristics, growth of microorganisms, batch culture, fed batch, continuous, self-continuous culture, transfer and oxygen requirements, yeast physiology

Lecture 3 Importance of microorganisms used in biotechnological processes for use in biofertilization and biological control of pests and plant diseases. Alternative use of biomass or active metabolites. Bioplaglicides to control insects, diseases and weeds. General concept. Main microorganisms employed: fungi (*Trichoderma* and *Metharizium*), bacteria (*Bacillus thuringiensis*), yeasts for postharvest control.

Lecture 4 active Main active metabolites produced by *Pseudomonas*: antibiotics, siderophores and Phytotoxins.

Lecture 5 Biofertilizers. General concept. Main microorganisms employed: mycorrhizae fungi, *Azospirillum, Azotobacter, Rhizobium*, plant growth promoting bacteria

Lecture 6. Biostimulyators General concept. Main metabolites and microorganisms:. indole acetic acid (IAA) jasmonic acid (JA), gibberellic acid (GA).

Lecture 7 Steps involved in development of bioproducts. Biosecurity: regulatory measures for implementation. Strategic decisions in Agricultural Biotechnology, development and implementation of research programs, socio-economic considerations.

FIELD DAY : BIOPRODUCTS MANAGEMENT

On March 6, 2014, Field Day bioproducts management was held in the garden of producer Orlando Padrón in San José de las Lajas, with the primary aim of publicizing new bioproducts to stimulate growth and productivity crops.
The products presented were: LEBAME (ICIDCA) Fitomas-E (ICIDCA), Biogen (CENSA) and Quitomax (INCA).
The meeting was attended by 24 participants, among who were: producers, researchers, students and teachers of the Polytechnic Institute of Agronomy, of which 9 are women for a 39% of participation.

Producers presented the results obtained with the bioproducts. In the case of LEBAME producer Orlando Padrón applied it in lettuce in his garden at a concentration of 2.5 mL / L, and obtained a faster development of this crop, which allowed him to advance the harvest in 10 days. He is very satisfied with the results.

The producer Benigno Pérez Molina, applied LEBAME in radish also at the concentration of 2.5 mL / L, and obtained a vigorous and healthy product as seen in the pictures below.

Día de campo para el intercambio entre productores e ntegrantes del proyecto Producc ón y Aplicac ón de Bioproductos de Importanc a Económica, celebrado el 6 de marzo 2014 en el huerto del productor Orlando Padrón en San José de las Lajas, La Habana, Cuba

The producers expressed their deposition to continue evaluating the bioproducts and issue the results obtained thereby contributing to the development of the project. They also expressed the need for provide the bioproducts once marketed to guarantee friendly harvests to the environment.

OTHER ACTIVITIES

Elan Iñaky Laredo, student of Doctoral Program at the Autonomous University of Coahuila, carried out a stay of three months in the Department of Bioproducts ICIDCA, during which, he conducted an experimental study on the evaluation of two systems of fermentation for the production of jasmonic acid from *Lasiodiplodia* spp.

Dra Graciella Cerutti, Ing. Patricia Albarracin and Ing. Mónica Coronel carried out a stay of 15 days in the Department of Bioproducts ICIDCA and they dictate a lecture related to the use of sucrose in the conservation of post-harvest fruits and reviewed the development of bioproducts with sustained action supported in microbial biopolymers.

A working meeting was held in Havana between Fidel Domenech and María Elena Díaz de Villegas from ICIDCA, with Dr. José Luis Martínez from the Autonomous University of Coahuila, where the project results and future prospects were analyzed.

A technical mission of the MSc María Elena Díaz de Villegas and Technologist Emilia Carrera was carried on in Tucumán, Argentina for academic exchange on the production and application of bioproducts for agriculture. In the Faculty of Biochemistry, Chemistry and Pharmacy, two conferences were held during the Day Update: Development and Application of Bioproducts in Pharmacy and Agriculture (MSc Elena Diaz de Villegas), and Main Analytical Technics (Emilia Carrera).

In the Regional Tucumán Faculty - National Technological University, the conference ¨Production and Potential of Effective Microorganisms (EM) in Agriculture, Animal Production, Postharvest, Hygiene and effluent treatment¨ was given.

An exchange with students from the Faculty of Exact Science and Technology and Ma Elena Díaz de Villegas was performed, made a presentation on Production and potential of effective microorganisms (EM) in agriculture, animal production, postharvest, hygiene and effluent treatment and Overview of Biotechnology applied to the development of bioproducts for agriculture in Cuba.

Available in http://www.frt.utn.edu.ar , university knews: A Cuban researcher gave two lectures of the Cuban Research Institute on Sugarcane Derivatives.

The MSc María Elena Díaz de Villegas of the Cuban Research Institute of Sugar Cane Derivatives (ICIDCA) gave two lectures on bioproducts for agriculture and the use of effective microorganisms in agriculture.

The Cuban specialist spoke on Wednesday 14 to a a full room: "The Overview of Biotechnology Applied to Developing Agricultural Bioproducts" and "Production and Potential of Efficient Microorganisms (EM) in Agriculture, Animal Production, Postharvest and Hygiene Effluent Treatment ". At the same, the MSc María Elena Díaz presented the work being done in the Cuban Institute on the development of bioproducts for agriculture, "there is the need to replace agrochemicals with the problems of environmental pollution," the specialist said

The lecture deepens into what biotechnology is, what can we use it, and what are the main benefits. In another dissertation was specified on the use of effective microorganisms that are used for different purposes, pointing to how to produce and what are the main benefits.

Cuban researcher referred to the interest for technology students: "I hope you find interesting to know what is being done in another country like Cuba, in which we find ourselves at the moment and how to deepen about biotechnology."

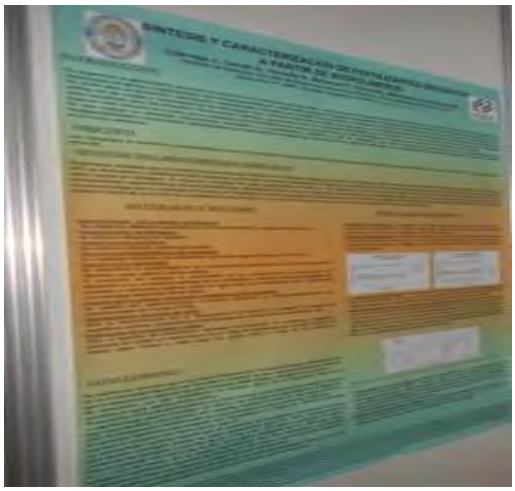
Meanwhile, the organizer, technology teacher Patricia Albarracín, (Assistant Professor of Chemical Engineering in Information Systems) explained: "The Faculty has an authorized Perez Guerrero project of the United Nations, which has great prominence the area of biotechnology The idea with these conferences was to be known, is disseminated and research group believes that area.

MSC María Elena Díaz de Villegas imparting a lectures in the Regional Tucumán Faculty – Tecnological National University

b) Celebration of a Workshop **Bioproducts for Agriculture** during the International Congress on Sugar and Sugarcane Derivatives ¨Diversification 2013¨, Havana, Cuba, October 14-18, 2014

14 papers were presented, including 5 developed by specialists of Argentina, Mexico and Cuba participants in the project.

Presentation of Ing Patricia Albarracin from the National University of Tucumán, Argentina in a Workshop Bioproducts for Agriculture



Dra Graciela Cerutti Poster fom Autonomous University of Tucuman, Argentina presented in a Congress Divesification 2013
Synthesis and obtaining of complex constituted by dextrans

Patricia Albarracin (Tucumán Argentina, Antonio Bell y Emilia Carrera (Cuba) during the Congress Diversification 2013

Presentation of Dr José Luis Martínez from Autonomous University from Coahuila, Mexico in a Workshop Bioproducts for Agriculture

c) Celebration of Seminar ICIDCA 2013, where in Session Bioproducts and Bioprocesses, the work developed in the project were presented.

Presentation of the head of the project María Elena Díaz de Villegas during the Session Bioproducts and Bioprocesses

during the Congress LABIOFAM 2014 in the session Bioplaguicides, Biofertilized and Bioestimulant for Agriculture , were presented 5 papers.

1. Evaluación de cepas de colección para la producción del inoculo LB-1 (microorganismos eficientes)

María Elena Díaz de Villegas, Grizel Delgado. Aidin Martínez, Gisela González, Nnoylin Sánchez, Emilia Carrera, Silvano Legrá, Silvia Armenteros.

2. Potencialidades del bioproducto IC-GIB como bioestimulante para la agricultura

Grizel Delgado, Grolamys Castillo, Grisel Ortega

3. Tecnología de secado y aplicaciones del bionutrinte FitoMas-e

José Villar Delgado, Felipe Campo Rodríguez, Bárbara Hernández Cruz

4. Biofertilizante Nitrofix. caracterización de los metabolitos obtenidos en su producción y la evaluación en diferentes cultivos.

Gómez, E., Guevara, Y., San Juan, A. N., Borges, D., Lemes, T., Serrano, J., Pérez, M1.,Lami, L., Fraga, R., Readigos, R., Hernández, A., Morales, F.

5. Estudio de estabilidad del bioproducto LEBAME

Grisel M. Ortega Arias-Carbajal, María Elena Díaz de Villegas, Grizel Delgado Arrieta,Gisela González Pardo, Silvia Armenteros Galarraga, Emilia Carreras Bocourt.

d) A technical mission of the MSc María Elena Díaz de Villegas and MSc Grisel Ortega, about the production and application of bioproducts for agriculture.

They gave the following lectures:

- ICIDCA: Líneas de investigación, producciones, servicios y gestión de la calidad.

MSc Grisel Ortega

- BIOJAS: Bioproducto agrícola de ácido jasmónico; surgimiento y desarrollo en el ICIDCA

MSc Grisel Ortega

- Evaluación de cepas de colección para la producción del inoculo LB-1 (microorganismos eficientes).

MSc María Elena Díaz de Villegas.

- Producción de bioplaguicidas por fermentación sumergida para el control de hongos fitopatógenos y malezas.

MSc María Elena Díaz de Villegas.

MSc María Elena Díaz de Villegas during the lecture in the Technological Institute of Celaya

MSc Grisel Ortega during the lecture in the Technological Institute of Celaya

MSc Grisel Ortega and MSc María Elena Díaz de Villegas with Prof. Dr Eleazar Escamilla in the Technological Institute of Celaya

4. FINANCIAL INFORMATION

Source of funds	TFPG
Total budget	34 000,00
Executed budget	28 154.74

5. DIVULGATION RESULTS ACTIVITIES

-Project website within the WEB page ICIDCA, showing Bioproducts Project activities
www.icidca.cu/

Publications

-Development, characteristics and use of biopesticides to control pathogens in Cuba. Chapter of Control of plant diseases in Latin America and Caribbean.
http://portal.fagro.edu.uy/index.php/intensific-agr/file/367-control-biologico-de-enfermedades-de-plantas-en-america-latina-y-el-caribe.html

- Rhizobium for Non-legume. Journal Plos One. Improvement of phosphorus solubilizing bacteria. Editorial of the Spanish Academy

Phytoremediation, a technology that involves plants and microorganisms in environmental sanitation. ICIDCA Journal, 2012

Simultaneous Quantification of phytohormones in Fermentation Extracts of *Botryodiplodia theobromae* by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. World Journal of Microbiology and Biotechnology

The Bioproducts an alternative for agriculture: Oscar Almazan, Eulalia J. Gómez, Rosa María Álvarez, Grisel Ortega, Grizel Arrieta Delgado, José Villar Delgado, Ma Elena Diaz de Villegas ISBN- 978-959-7165-42 (CD).

INDEX

- Compost
- Promoting growth bacteria and phosphorus soil solubilization

Bioestimulats
- BIOJAS (Jasmonic acid)
- BIOENRAIZ (Indolacetic acid)
- Gibberellins
- FitoMas

Bioplaguicides
- *Metarhizium anisopliae*. (entomopathogenic)
- *Trichoderma sp.*
- *Beauveria bassiana*
- Verticid
- Nemacid
- *Paecilomyces lilacinus*
- Gluticid
- HERBIO

PROJECTIONS FOR BIOPRODUCTS FOR AGRICULTURAL USE

-International course Sugar Agroindustry and its derivatives to students from University of Haute École Provinciale de Hainaut - Condorcet (Bélgica)

-Diploma Thesis Biology Faculty : Isolation and characterization of phosphorus solubilizing
Tutor: Reynaldo Fraga

-Doctoral Thesis Biology Faculty : Production and characterization of jasmonic acid from *Botryodiplodia* sp., 2013. Felipe Eng

-Master Thesis Biology Faculty:. Characterization of metabolites excreted by two rhizobacteria isolated nickel accumulator plants, 2013. Isis Amores

-Diploma Thesis, Biology Faculty Influence the culture medium on the antifungal effect of the metabolites excreted by Bacillus sp. VC3 strain isolated from ultramafic soils
Tutor: Nayra Ochoa

6. LESSONS LEARNED
The transformation of knowledge in the production of bioproducts, is a cooperative action SS. The working methods and procedures for the isolation of strains and production of jasmonic acid and Fe-urea dextran from the provider country: Cuba to host countries: Mexico and Argentina allowed short time, save resources and promote technologies in biotechnology development themes.

Briefly describe the lessons learned during the project:

1. Combine several objectives to reduce the costs of achieving results. This recommendation applies the same to achieve the objectives abroad as at home industry where you have to make visits to the countryside.
2. The dissemination of the project results in other countries of the region allows the future incorporation of other working groups and the formation of other alliances to develop it.

3. The establishment of multidisciplinary teams in companies helps ensure the sustainability of the activity

7. WORK AREAS FOR PERFORMANCE IMPROVEMENT

1. Joining forces with participating institutions and AZCUBA for meeting objectives

2. Involve factors involved in executive decisions for the introduction of results and service in national industry with AZCUBA project commitments, to join forces and achievement the objectives.

Prepared by:

María Elena Díaz de Villegas _____

Name **Signature**

Project Director

Date: February 2015

Organization: Cuban Research Institute on Sugar Cane Derivatives

Universidad de La Habana

Facultad de Biología

Tesis presentada en opción al grado científico de Licenciado en Microbiología

Influencia del medio de cultivo sobre el efecto antifúngico de metabolitos excretados por *Bacillus* sp. cepa VC3, aislada de suelos ultramáficos.

Aut: Jéssica Mendoza Rodríguez

Tutores: Lic. Nayra Ochoa Viñals

MCs. Isis Amores Sánchez

Asesor: DraCs. Orquídea Coto Pérez

Instituto Cubano de Investigaciones de Derivados de la Caña de Azúcar

La Habana, 2014

UNIVERSIDAD DE LA HABANA

FACULTAD DE BIOLOGÍA

Caracterización de dos rizobacterias aisladas de plantas acumuladoras de níquel.

Tesis presentada para optar por el título de Máster en Microbiología
Mención Microbiología General

Autor: Lic. Isis Amores Sánchez

Tutores: Dra. Jeannette Marrero Coto
Dra. Orquídea Coto Pérez
Asesor: Msc. Maria Elena Díaz de Villegas

Instituto Cubano de Investigaciones de los Derivados de la Caña de azúcar

ICIDCA
2013

Universidad de la Habana

Facultad de Química

Departamento de Química Orgánica

ESTUDIO DE METABOLITOS PRODUCIDOS EN EL PROCESO FERMENTATIVO DEL HONGO BOTRYODIPLODIA THEOBROMAE POR MÉTODOS CROMATOGRÁFICOS Y ESPECTROSCÓPICOS

Tesis presentada en opción al grado científico de Doctor en Ciencias Químicas

Autora: MSc. Grolamys Castillo Portela

Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar

Tesis presentada en opción al grado científico de Doctor en Ciencias Químicas

Autora: MSc. Grolamys Castillo Portela
Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar
La Habana, 2014

CONGRESO INTERNACIONAL LABIOFAM 2014 CON LOS ★

CERTIFICADO DE AUTOR

SE OTORGA A: Grolamys Castillo

TÍTULO DEL TRABAJO: Determinación por HPLC-ESI-MS del perfil de fitohormonas presentes en el BIOSAS, bioestimulante y bioplaguicida de origen microbiano

EN LA MODALIDAD DE:

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- ■ CONFERENCIA
- ■ SIMPOSIO
- ■ TEMA LIBRE
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- ■ MODERADOR

ING. ISBEL GONZÁLEZ MARRERO
Presidente del Comité Científico

DR. JOSÉ A. FRAGA CASTRO
Presidente del Comité Organizador

CONGRESO INTERNACIONAL LABIOFAM 2014 CON LOS 5

CERTIFICADO DE AUTOR

SE OTORGA A: Gnsel M. Ortega Arias Carbajal

TÍTULO DEL TRABAJO: Estudio de estabilidad del biopreducto LEBAME

EN LA MODALIDAD DE:

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- ■ CONFERENCIA
- ■ SIMPOSIO
- ■ TEMA LIBRE
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- ■ SECRETARIO DE SESIÓN
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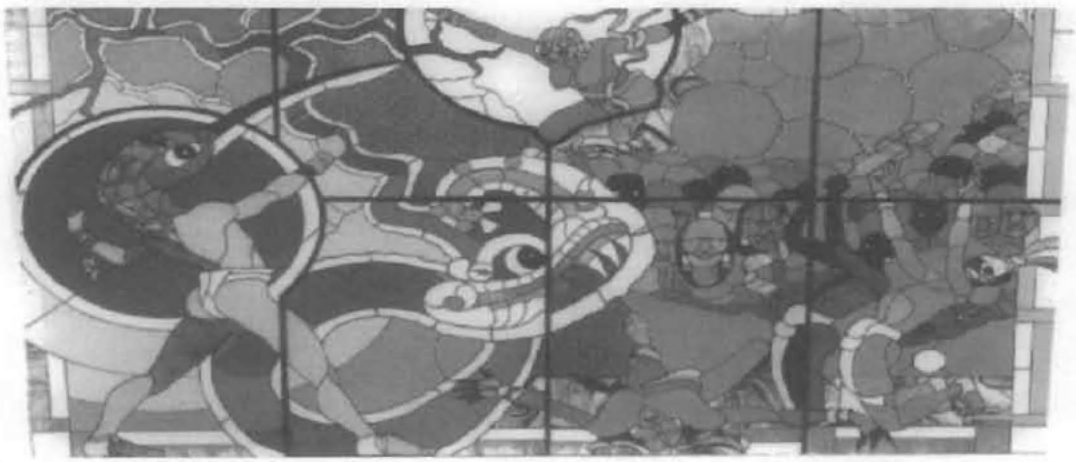
Presidente del Comité Cientifico

DR. JOSÉ A. FRAGA CASTRO
Presidente del Comité Organizador

SEPTIEMBRE, DEL 22 AL 25 PALACIO DE CONVENCIONES, LA HABANA



Departamento de Ingeniería Química
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RECONOCIMIENTO

al

DRA. GRISEL M. ORTEGA ARIAS-CARBAJAL

Por su valiosa exposición con la conferencia

"EVOLUCIÓN Y TENDENCIAS ACTUALES DE LA INGENIERÍA DE PROCESOS"

En el SEMINARIO DEPARTAMENTAL

Celaya, Gto., 10 de noviembre de 2014

Ramiro Rico M.

Dr. Ramiro Rico Martínez
Jefe de Departamento

LABIOFAM Grupo Empresarial · Organización Panamericana de la Salud · Ministerio de la Agricultura · CITMA · IPK Instituto Pedro Kourí · Palacio de Convenciones de La Habana

LABIOFAM 2012

Congreso Internacional LABIOFAM 2012

Simposio de Productos Naturales en la Terapia contra el Cáncer

Del 24 al 28 de Septiembre del 2012
Palacio de Convenciones de La Habana, Cuba.

CERTIFICADO de AUTOR

Se otorga a: María Elena Díaz de Villega

Título del Trabajo: Sesión Bioplaguicidas y Biofertilizantes

En la modalidad de:

- ☐ Conferencia Magistral
- ☐ Conferencia
- ☐ Simposio
- ☐ Tema libre
  - — Oral — Póster
- ☐ Mesa redonda
- ☑ Presidente de Sesión
- ☐ Secretario de Sesión
- ☐ Coordinador
- ☐ Moderador

LABIOFAM Grupo Empresarial
Dr. José Antonio Fraga Castro
DIRECTOR GENERAL

Dr. José A. Fraga Castro
Presidente del Comité Organizador

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DIRECTORA
INVESTIGACIÓN Y DESARROLLO

Ing. Isbel González Marrero
Presidente del Comité Científico

ICIDCA

Seminario Científico ICIDCA

6-8 de noviembre 2012

Diploma de Participación

Ponente: María Elena Díaz de Villegas

Autores: Ma. Elena Díaz de Villegas, Georgina Michelena, Griegel Delgado

Trabajo presentado: Participación del ICIDCA en el Programa para la recuperación y desarrollo de los biofertilizantes, biofitomejoradores y biopla.

Sesión: Taller Bioproductos Fecha: 8 de Noviembre 2012

Luis O.Gálvez Taupier
Director General
ICIDCA

ICIDCA
INSTITUTO CUBANO DE INVESTIGACIONES DE LOS DERIVADOS DE LA CAÑA DE AZUCAR
DIRECCIÓN GENERAL

LABIOFAM Grupo Empresarial · Organización Panamericana de la Salud · Ministerio de la Agricultura · CITMA · IPK Instituto Pedro Kouri · Palacio de Convenciones de La Habana

LABIOFAM 2012

Congreso Internacional LABIOFAM 2012

Simposio de Productos Naturales en la Terapia contra el Cáncer

Del 24 al 28 de Septiembre del 2012
Palacio de Convenciones de La Habana, Cuba.

CERTIFICADO de AUTOR

Se otorga a: María Elena Díaz de Villega

Título del Trabajo: Producción biotecnológica, caracterización y uso fitosanitario de metabolitos de Pseudomonas aeruginosas PSS.

En la modalidad de:

- ☐ Conferencia Magistral
- ☐ Conferencia
- ☐ Simposio
- ☑ Tema libre
  - — Oral — Póster
- ☐ Mesa redonda
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ICIDCA 50 Aniversario 1963-2013

II TALLER PROVINCIAL DE BIOPRODUCTOS Y ALIMENTO ANIMAL

UEB BIOPROCESOS CUBA 10. ICIDCA

Diploma de Participación

Título: Implementación de un bioensayo in vitro como indicador de la calidad del Bioproducto LEBACID

Autores: Yaelena Díaz de Villegas, Griezel Delgado Antonio Bell, Yayna Ochoa, Neglen Sánchez

La imaginación es la vanguardia y como el profeta de la ciencia.

José Martí

"Año 55 de la Revolución"
mayo 21-22 de 2013

INSTITUTO CUBANO DE INVESTIGACIONES DE LOS DERIVADOS DE LA CAÑA DE AZÚCAR
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Luis O.Gálvez Taupier
Director General
ICIDCA

UNIVERSIDAD NACIONAL DE TUCUMÁN
FACULTAD DE BIOQUÍMICA, QUÍMICA Y FARMACIA
CÁTEDRA DE GARANTÍA DE CALIDAD
DE DROGAS Y MEDICAMENTOS
DEL INSTITUTO DE FARMACIA "Dr. A. F. ROVELLI"

Certificamos que la **LIC. EMILIA CARRERA**

ha participado como **Disertante** en la **Jornada de Actualización "Desarrollo de Bioproductos de Aplicación en Farmacia y Agricultura".**

Organizado por la Cátedra de Garantía de Calidad de Drogas y Medicamentos del Instituto de Farmacia "Dr. A. F. Rovelli" de esta Facultad.

San Miguel de Tucumán, 15 de Agosto de 2013

Dra. Graciela I. Cerutti
Cátedra de Garantía de Calidad
de Drogas y Medicamentos

Universidad Nacional de Tucumán
facet
Facultad de Ciencias Exactas y Tecnología

Rama Estudiantil IEEE
Tucumán

THE INSTITUTE OF ELECTRICAL AND ELECTRONICS ENGINEERS

San Miguel de Tucumán 16 de Agosto del 2013

La Rama Estudiantil IEEE Tucumán - Argentina Agradece al:

Tecnóloga Azucarera. Carrera Bocourt, Emilia

Por su disertación acerca de: **"TÉCNICAS ANALÍTICAS"**. Auspiciada por la Facultad de Ciencias Exactas y Tecnología - Universidad Nacional de Tucumán - Argentina.

Ing. Carlos A. Rodríguez MSc
Secretario de Gestión y Extensión
Facultad de Ciencias Exactas y Tecnología - UNT.

Mg. Ing. Gustavo Juárez
Consejero Rama Estudiantil
IEEE Tucumán - Argentina.

Sr. Carlos Agustín Danna
Presidente Rama Estudiantil
IEEE Tucumán - Argentina

XII DIVERSIFICACIÓN 2013
Congreso Internacional sobre Azúcar y Derivados

Certificado de Autor

A: María Elena Díaz de Villegas, Grizel Delgado, Mauricio Ribas, Esmérida Torres, Maribel Saura

Por la presentación del trabajo: Ponencia oral:

IMPLEMENTACIÓN DE UN BIOENSAYO *IN VITRO* COMO INDICADOR DE CALIDAD DEL BIONUTRIENTE FITOMAS E.

en el XII Congreso Internacional sobre Azúcar y Derivados

Dado en el Hotel Nacional de Cuba, La Habana
del 14 al 18 de octubre de 2013

INSTITUTO CUBANO DE INVESTIGACIONES DE LOS DERIVADOS DE LA CAÑA DE AZÚCAR
ICIDCA
DIRECCIÓN GENERAL

Dra. Marianela Cordovés
Secretaria Científica

Universidad Nacional de Tucumán
facet
Facultad de Ciencias Exactas y Tecnología

Rama Estudiantil IEEE
Tucumán

THE INSTITUTE OF ELECTRICAL AND ELECTRONICS ENGINEERS

San Miguel de Tucumán 16 de Agosto del 2013

La Rama Estudiantil IEEE Tucumán - Argentina Agradece al:

MSc. Díaz de Villegas, María Elena

Por su disertación acerca de: **"BIOTECNOLOGIA APLICADA AL DESARROLLO DE BIOPRODUCTOS PARA LA AGRICULTURA EN CUBA"**. Auspiciada por la Facultad de Ciencias Exactas y Tecnología - Universidad Nacional de Tucumán - Argentina.

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Secretario de Gestión y Extensión
Facultad de Ciencias Exactas y Tecnología - UNT.

Mg. Ing. Gustavo Juárez
Consejero Rama Estudiantil
IEEE Tucumán - Argentina.

Carlos A Danna

Sr. Carlos Agustín Danna
Presidente Rama Estudiantil
IEEE Tucumán - Argentina

UNIVERSIDAD NACIONAL DE TUCUMÁN
FACULTAD DE BIOQUÍMICA, QUÍMICA Y FARMACIA
CÁTEDRA DE GARANTÍA DE CALIDAD
DE DROGAS Y MEDICAMENTOS
DEL INSTITUTO DE FARMACIA "Dr. A. F. ROVELLI"

Certificamos que la .....**DRA. MARÍA ELENA DÍAZ DE VILLEGAS**.....

ha participado como **Disertante** en la **Jornada de Actualización "Desarrollo de Bioproductos de Aplicación en Farmacia y Agricultura".**

Organizado por la Cátedra de Garantía de Calidad de Drogas y Medicamentos del Instituto de Farmacia "Dr. A. F. Rovelli" de esta Facultad.

San Miguel de Tucumán, 15 de Agosto de 2013

Dra. Graciela I. Cerutti
Cátedra de Garantía de Calidad
de Drogas y Medicamentos

ICIDCA

Seminario Científico ICIDCA

11-15 de noviembre 2013

Diploma de Participación

Ponente: Mª Elena Díaz de Villegas

Autores: Mª Elena Díaz de Villegas; Grizel Delgado; Aideé Martínez; L. González; M.A. Peña; N. Sánchez; S. Armenteros; E. Carrera; Y. Carrillo; E. Tény; T. Ruiz

Trabajo presentado: Microorganismos eficientes para diversos usos. Experiencias del ICIDCA con el LEBACID

Sesión: Bioproductos y Bioprocesos Fecha: 12 de noviembre 2013

Luis O.Gálvez Taupier
Director General
ICIDCA

VII SEMINARIO INTERNACIONAL DE SANIDAD VEGETAL

El Instituto de Investigaciones de Sanidad Vegetal

otorga el presente

Certificado

A: *María C. Díaz de Villegas, P. Villa, A Bell, C. Torres, J. Hartiney.*

Por su participación como

DELEGADO☐ PONENTE☒ CONFERENCISTA☐ COMITÉ ORGANIZADOR☐

PRESIDENTE DE SALA☐ ASISTENTE☐

En el Seminario Internacional de Sanidad Vegetal
"Por la Sostenibilidad de los Sistemas Agrícolas y el Bienestar de los Agricultores",
celebrado del 7 al 11 de abril de 2014

Dado en el Palacio de Convenciones, La Habana, Cuba.

Instituto de Investigaciones de Sanidad Vegetal — Dirección — INISAV

Dra. Marlene M. Veitía Rubio
PRESIDENTA

INISAV Instituto de Investigaciones de Sanidad Vegetal

MINAG MINISTERIO DE LA AGRICULTURA

syngenta

GBM GRUPO B. M. INC.

PALACIO DE CONVENCIONES DE LA HABANA

CONGRESO INTERNACIONAL LABIOFAM 2014 CON LOS ★

CERTIFICADO DE AUTOR

SE OTORGA A: María Elena Díaz de Villegas

TÍTULO DEL TRABAJO: Evaluación de cepas de colección para la producción del inóculo LB-1 (Microorganismos eficientes)

EN LA MODALIDAD DE:

- ■ CONFERENCIA MAGISTRAL
- ■ CONFERENCIA
- ■ SIMPOSIO
- ☒ TEMA LIBRE
  - X ORAL ..... PÓSTER
- ■ MESA REDONDA
- ■ PRESIDENTE DE SESIÓN
- ■ SECRETARIO DE SESIÓN
- ■ COORDINADOR
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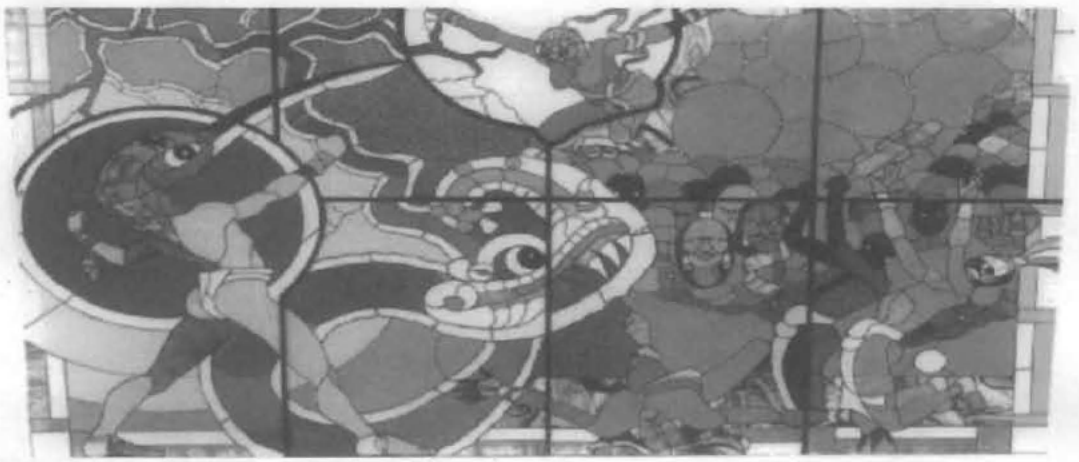
Dirección General LABIOFAM S.A.

ING. ISBEL GONZÁLEZ MARRERO
Presidente del Comité Científico

DR. JOSÉ A. FRAGA CASTRO
Presidente del Comité Organizador

SEPTIEMBRE, DEL 22 AL 25 PALACIO DE CONVENCIONES, LA HABANA



Departamento de Ingeniería Química
hace patente su

RECONOCIMIENTO

al

DRA. MA. ELENA DÍAZ DE VILLEGAS

Por su valiosa exposición con la conferencia

"EVOLUCIÓN Y TENDENCIAS ACTUALES DE LA INGENIERÍA DE PROCESOS"

En el SEMINARIO DEPARTAMENTAL

Celaya, Gto., 10 de noviembre de 2014

Ramiro Rico M.

Dr. Ramiro Rico Martínez
Jefe de Departamento

ICIDCA

Seminario Científico ICIDCA

CERTIFICADO

Autores: Ma Elena Díaz de Villegas, Grizel Delgado, Aidin Martínez, Gisela González, Noylin Sánchez, Emilia Carrera, Silvano Legrá, Silvia Armenteros.

Trabajo presentado: *Evaluación de cepas de colección para la producción del inoculo lb-1 (microorganismos eficientes)*

Comisión: Bioproductos y Bioprocesos

Fecha: 19 de Noviembre de 2014

(X) Presentación oral

( ) Cartel

Dr. Alejandro Abril
Director Gestión del Conocimiento

ICIDCA
Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar
Dirección de Gestión del Conocimiento